UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE FARMÁCIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS FARMACÊUTICAS

Avaliação *in vivo* do dano oxidativo a biomoléculas e da inflamação em pacientes portadores de Doenças do Ciclo da Ureia: aspectos clínicos e bioquímicos na hiperamonemia

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Orientadora: Profª. Drª. Carmen Regla Vargas

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“*O sucesso nasce do querer, da determinação e persistência em se chegar a um objetivo. Mesmo não atingindo o alvo, quem busca e vence obstáculos, no mínimo fará coisas admiráveis*.”

José de Alencar

RESUMO

As Doenças do Ciclo da Ureia (DCU) são erros inatos do metabolismo (EIM) que cursam com uma deficiência total ou parcial na atividade das enzimas ou transportadores envolvidos no ciclo da ureia, o qual é responsável pela transformação e excreção da amônia tóxica. As DCU podem causar danos neurológicos irreversíveis, levando ao óbito principalmente neonatos, seu desfecho clínico é grave e de rápida progressão, necessitando de diagnóstico urgente e acurado. Estudos demonstram que metabólitos tóxicos e estresse oxidativo podem estar envolvidos em sua fisiopatologia. Portanto, no capítulo I deste trabalho, objetivamos investigar o perfil clínico e laboratorial de pacientes DCU, a fim de auxiliar profissionais na rápida identificação dos sinais e sintomas e manejo da doença. Trinta pacientes portadores de DCU foram diagnosticados, incluindo portadores de deficiência de ornitina transcarbamilase (n=11), deficiência de argininosuccinato sintetase 1 (n=10), deficiência de arginase (n=5), deficiência de carbamoilfosfato sintetase (n=2) e deficiência de argininosuccinato liase (n=2). Os níveis de amônia e ácido orótico encontravam-se aumentados no diagnóstico. Os principais sinais e sintomas apresentados incluíram níveis alterados de consciência, encefalopatia hepática aguda, convulsões, falta de apetite, vômitos, coma e estresse respiratório, com taxa de óbito de 20%. No capítulo II, buscando investigar a fisiopatologia envolvida, avaliamos *in vivo* o dano oxidativo a biomoléculas e inflamação. Verificamos níveis de espécies reativas de nitrogênio (ERN) diminuídos nos pacientes, além do aumento de espécies reativas ao ácido tiobarbitúrico (TBARS) e proteínas carboniladas, indicando envolvimento da via de síntese do óxido nítrico (NO) e dano oxidativo a proteínas e lipídeos. As citocinas pró-inflamatórias IL-6, IL-8, interferon-γ e TNF-α e anti-inflamatória IL-10 encontravam-se aumentadas, indicando um estado pró-inflamatório nas DCU. O presente trabalho é pioneiro em delinear perfil oxidativo e inflamatório em pacientes DCU, além de demonstrar o importante papel da hiperamonemia em seu grave desfecho.

Palavras-chave: Erros inatos do metabolismo; doenças do ciclo da ureia; hiperamonemia; estresse oxidativo; inflamação.

ABSTRACT

Urea Cycle Diseases (UCD) are inborn errors of metabolism (IEM) that result in a total or partial deficiency in the activity of enzymes or transporters involved in the urea cycle, which is responsible for the transformation and excretion of toxic ammonia. UCD can cause irreversible neurological damage, leading mostly neonates to death, clinical outcomes are severe and of rapid progression, requiring urgent and accurate diagnosis. Studies demonstrate that toxic metabolites and oxidative stress may be involved in its pathophysiology. Therefore, in chapter I of this work, we aim to investigate the clinical and laboratorial profile of UCD patients, in order to assist professionals in the rapid identification of signs and symptoms and disease management. Thirty UCD patients were diagnosed, including ornithine transcarbamylase deficiency (n=11), argininosuccinate synthetase 1 deficiency (n=10), arginase deficiency (n=5), carbamoylphosphate synthetase deficiency (n=2) and argininosuccinate lyase deficiency (n=2). Ammonia and orotic acid levels were increased at diagnosis. The main signs and symptoms presented included altered levels of consciousness, acute hepatic encephalopathy, seizures, lack of appetite, vomiting, coma and respiratory distress, with a death rate of 20%. In chapter II, aiming to investigate the pathophysiology involved, we evaluated in vivo oxidative damage to biomolecules and inflammation. We found decreased levels of reactive nitrogen species (RNS) in patients, in addition to an increase in thiobarbituric acid reactive species (TBARS) and carbonyl proteins, indicating involvement of the nitric oxide (NO) synthesis pathway and oxidative damage to proteins and lipids. The pro-inflammatory cytokines IL-6, IL-8, interferon-γ and TNF-α and the anti-inflammatory IL-10 were increased, indicating a pro-inflammatory state in UCD. The present work is a pioneer in outlining the oxidative and inflammatory profile in UCD patients, in addition to demonstrating the important role of hyperammonemia in the severe clinical outcome.

Keywords: Inborn errors of metabolism; urea cycle diseases; hyperammonemia; oxidative stress; inflammation.

SUMÁRIO

1. INTRODUÇÃO

1.1. Erros Inatos do Metabolismo

Os erros inatos do metabolismo (EIM) são um grupo de doenças genéticas caracterizadas pela síntese alterada de uma proteína, geralmente uma enzima, prejudicando sua atividade de forma parcial ou total. Esta alteração resulta no comprometimento de rotas metabólicas, resultando no acúmulo de substratos e de seus derivados, assim como na diminuição da síntese dos produtos. Os EIM constituem uma parcela importante dentre a totalidade de doenças genéticas da infância, apresentando alta taxa de morbidade e mortalidade em crianças, sendo assim extremamente necessário um diagnóstico e tratamento precoce (SCRIVER *et al.,* 2001).

Com base em sua fisiopatologia, Saudubray e Charpentier (2001) classificam os EIM em três grandes grupos. São estes: a) distúrbios na síntese ou degradação de moléculas complexas, que incluem as doenças lisossômicas de depósito e peroxissomais; b) doenças envolvendo déficit de energia, tais como as doenças de depósito de glicogênio, defeitos de gliconeogênese e defeitos de oxidação de ácidos graxos, acidemias lácticas congênitas e doenças mitocondriais de cadeia respiratória; e c) erros inatos do metabolismo intermediário, como as aminoacidopatias, as acidemias orgânicas, **as doenças do ciclo da ureia** e as intolerâncias aos açucares.

1.2. Doenças do Ciclo da Ureia

As doenças do ciclo da ureia (DCU) são EIMs relacionados ao processo de excreção de amônia do organismo. A produção de amônia ocorre em todos os tecidos do corpo durante o metabolismo de uma variedade de compostos, sendo produzida principalmente pelo catabolismo de aminoácidos e outros compostos nitrogenados, através do processo de transaminação seguido de desaminação, a partir de aminas biogênicas, de grupos amino de bases nitrogenadas como purinas e pirimidinas, e no intestino, pela flora bacteriana intestinal através da ação da urease sobre a ureia (ROMÉRO-GOMEZ *et al.,* 2009). Os níveis de amônia circulantes no organismo devem-se manter baixos

e controlados, uma vez que este composto é tóxico ao sistema nervoso central (SNC). O ciclo da ureia (Figura 1) é responsável pela transformação de amônia em ureia, sua forma não-tóxica, que é então eliminada através das vias urinárias. Defeitos nos genes que codificam as enzimas e transportadores envolvidos no ciclo da ureia resultam em bloqueio desta via, culminando em intoxicação decorrente do acúmulo de amônia (hiperamonemia) e de metabólitos intermediários ao ciclo (BRUSILOW *et al.*, 2001; WEINER *et al.,* 2013; HERMAN *et al.,* 2018).

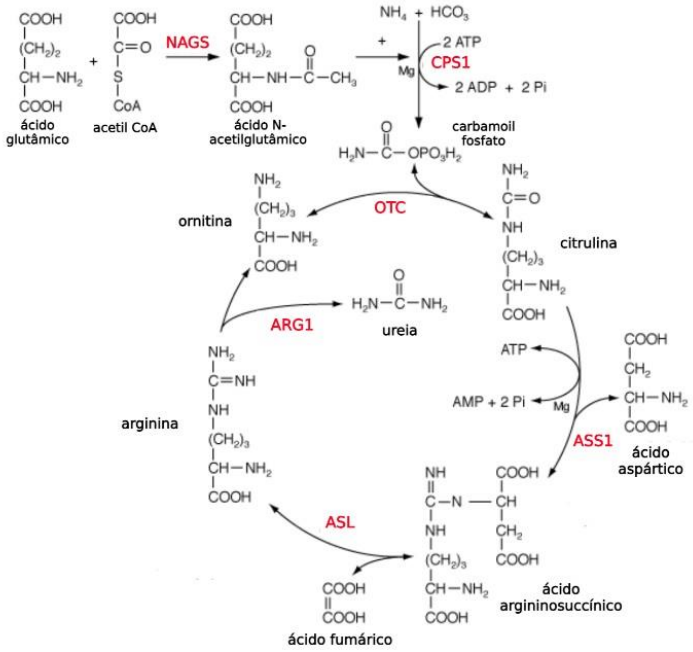

Figura 1. Ciclo da ureia. NAGS: N-acetilglutamato sintase; CPS1: carbamoilfosfato sintetase; OTC: ornitina transcarbamilase; ASS1: argininosuccinato sintetase 1; ASL: argininosuccinato liase; ARG1: arginase 1. (Adaptado de: Brusilow, S. W.; Horwich, A. L.: Urea Cycle Enzimes. In: Scriver C. R.; Beaudet, A. L.; Sly, W. S.; Valle, D. (eds.): The Metabolic and Molecular Bases of Inherited Disease. 7th ed. New York: McGraw-Hill, 1995).

As DCU compreendem um grupo de oito doenças, cada uma correspondente à respectiva enzima ou transportador deficiente do ciclo. São estas: deficiência de N-Acetilglutamato sintase (DNAGS); deficiência de carbamoil fosfato sintetase 1 (DCPS1); deficiência de ornitina transcarbamilase (DOTC); deficiência de argininosuccinato sintetase (DASS1); deficiência de

argininosuccinato liase (DASL); deficiência de arginase (DARG1); deficiência de transportador de citrina (DCITRIN); deficiência do transportador de ornitina translocase (DORNT1).

Os mecanismos envolvidos na toxicidade presente nas DCU permanecem desconhecidos. Ainda assim, existem alguns estudos que nos ajudam a compreender ao menos em parte o que ocorre nestas deficiências. Atualmente, sabe-se que as anormalidades e sintomas que ocorrem nos pacientes com EIM, provavelmente são ocasionadas por acúmulo de metabólitos tóxicos. As DCU em específico apresentam o acúmulo de amônia como denominador comum, assim como o acúmulo de metabólitos intermediários. Os principais efeitos destas descompensações são a disfunção mitocondrial e o estresse oxidativo (EREZ *et al.,* 2011; PARMEGGIANI & VARGAS, 2018).

Nos últimos anos muito tem se falado sobre o papel dos radicais livres na fisiopatologia de diversas doenças humanas, especialmente em doenças neurodegenerativas, como Alzheimer e Parkinson. Estudos já evidenciaram o papel do estresse oxidativo - caracterizado pelo desequilíbrio entre as defesas antioxidantes e substâncias pró-oxidantes no organismo - (HALLIWELL e GUTERRIDGE, 2001) na fisiopatologia de alguns EIM, entre eles as aminoacidopatias, uma vez que o acúmulo de metabólitos tóxicos que acontece nessas doenças pode levar a um aumento excessivo na produção de espécies reativas (BARSCHAK *et al.,* 2006; WAJNER *et al*., 2004).

Considerando que pacientes DCU apresentam extensos danos cerebrais com comprometimento neurológico e que a fisiopatologia envolvida nestas doenças ainda não é totalmente compreendida, faz-se necessário investigar e confirmar a presença de certos mecanismos, em específico o envolvimento do estresse oxidativo e inflamação com intuito de auxiliar na descoberta de novas estratégias terapêuticas, auxiliando a busca por terapias adjuvantes e tratamento destes pacientes. Neste contexto, esta dissertação permitiu investigar e gerar dados originais publicados e encaminhados a publicação em revistas internacionais, bem como publicar artigo de revisão sobre o assunto na literatura internacional.

1.3 Amônia e Ciclo da Ureia

A amônia é uma importante fonte de nitrogênio para a síntese de aminoácidos e auxilia na regulação do pH, sendo a produção de amônia e sua excreção, o principal mecanismo pelo qual o rim produz bicarbonato. Na natureza, a amônia existe como NH_3 e na forma iônica de amônia como íon amônio (NH_4^+), esta última sendo mais prevalente sob condições fisiológicas. Entretanto, seu acúmulo no organismo não é benéfico, causando elevada toxicidade e sendo passível de ultrapassar membranas celulares, incluindo a barreira hematoencefálica (WALKER *et al*., 2009). O ciclo da ureia foi o primeiro ciclo metabólico a ser descoberto, cinco anos antes da descoberta do ciclo do ácido cítrico (KREBS E HENSELEIT, 1932). Esta transformação ocorre principalmente no fígado e em menor quantidade nos rins. Primeiramente a amônia e o bicarbonato são condensados pela enzima carbamoil fosfato sintetase 1 (CPS1). A atividade da CPS1 depende do cofator N-acetilglutamato (sintetizado pela N-acetilglutamato sintase - NAGS). O carbamoil fosfato é posteriormente ligado à ornitina para formar citrulina pela ação da ornitina transcarbamilase (OTC). A citrulina atravessa a mitocôndria para o citosol em um transportador de ornitina (ORNT1) e é então ligada ao aspartato (dependente do transportador de citrina (CITRIN) para atingir o citosol), originando argininosuccinato pela atividade da argininosuccinato sintetase 1 (ASS1). A argininosuccinato liase (ASL) cliva este composto em arginina, que finalmente é hidrolisada pela arginase (ARG1) formando ornitina e ureia. A ornitina é restaurada para reentrar no ciclo (dependente do transportador de ornitina (ORNT1) para atingir a mitocôndria) e a ureia é liberada na corrente sanguínea, para finalmente chegar aos rins e ser excretada na urina. Todas as DCU possuem caráter autossômico recessivo, com exceção da mais prevalente, a deficiência de ornitina transcarbamilase, que possui herança ligada ao X.

A incidência geral das DCU é estimada entre 1:35.000 a 1:52.000 nascidos vivos (SUMMAR *et al.,* 2013; NETTESHEIM *et al*., 2017).

As DCU geralmente apresentam-se como encefalopatia hepática (HE) neonatal causada pela hiperamonemia, tendo como sinais clínicos mais

comuns vômito, convulsões, acidose metabólica e coma, podendo evoluir para morte, ou resultar em incapacidade neurológica e motora irreversível (BURGARTD *et al.,* 2016; HABERLE *et al.,* 2019).

1.4 Classificação das Doenças do Ciclo da Ureia

1.4.1 *Deficiência de N-acetilglutamato sintase (DNAGS; OMIM 237310)*

A N-Acetilglutamato Sintase (NAGS) é a enzima responsável pela catalise de glutamato e acetil-CoA em N-acetilglutamato na mitocôndria, sendo este cofator da enzima CPS1. Assim, quando a NAGS se encontra deficiente, a reação de conversão da amônia à carbamoil fosfato torna-se prejudicada, limitando a etapa inicial do ciclo da ureia; portanto, a DNAGS resulta em uma deficiência funcional da CPS1. Esta doença resulta em uma hiperamonemia primária concomitante a uma elevação dos níveis de glutamina e diminuição de citrulina e arginina plasmáticas. Pacientes podem desenvolver quadros hiperamonêmicos tanto no período neonatal quanto ao longo da vida. O uso do carbamilglutamato como análogo de NAG tem sido utilizado na clínica, porém deve-se observar a resposta do organismo a este, uma vez que, em alguns casos o medicamento não demonstrou total eficiência.

1.4.2 *Deficiência de carbamoilfosfato sintetase 1 (DCPS1; OMIM 237300)*

A deficiência completa ou parcial da enzima carbamoil sintetase 1 (CPS1) resulta em uma não-conversão ou conversão parcial da amônia tóxica do organismo em carbamoil fosfato, como descrito anteriormente. Assim, indivíduos com esta doença desenvolvem rapidamente hiperamonemia no período neonatal. Estes pacientes também estão sujeitos a crises hiperamonêmicas ao decorrer da infância e vida adulta, tendo quadro clínico e laboratorial idênticos aos apresentados na DNAGS. Análises bioquímicas podem ser altamente sugestivas de DCPS1, no entanto, testes moleculares são frequentemente necessários para fazer este diagnóstico, assim como é essencial o diagnóstico diferencial da DNAGS. Outros sintomas incluem insuficiência hepática aguda, imaturidade hepática e coma neonatal, frequentemente sendo necessário a realização de um transplante de emergência (KESKINEN *et al.,* 2008).

1.4.3 *Deficiência de ornitina transcarbamilase (DOTC; OMIM 311250)*

A deficiência da enzima ornitina transcarbamilase (DOTC) impossibilita a conversão do complexo carbamoil fosfato e ornitina à citrulina, resultando em aumento de glutamina e alanina plasmáticos e diminuição de citrulina. Os pacientes portadores de DOTC desenvolvem rapidamente hiperamonemia no período neonatal e, posteriormente, correm o risco de apresentarem episódios repetidos durante a vida. Esta doença pode causar uma grande toxicidade hepática. O gene da OTC está localizado no cromossomo X; portanto, a maioria das pessoas gravemente afetadas são indivíduos do sexo masculino. A DOTC é a DCU mais comum e apresenta a maior taxa de mortalidade em neonatos.

1.4.4 *Deficiência de argininosuccinato sintetase 1 ou citrulinemia tipo I (DASS1; OMIM 215700)*

Na deficiência de arginisuccinato sintetase 1 (DASS1) não há a transformação do complexo aspartato/citrulina à arginissuccinato no citosol, portanto, indivíduos que possuem esta deficiência apresentam hiperamonemia grave no período neonatal. O diagnóstico é quase direto, pois o quadro clínico do paciente caracteriza-se pela combinação de altos níveis de amônia sanguínea e/ou elevada glutamina plasmática juntamente a níveis aumentados de citrulina. A doença pode se apresentar acompanhada de falha hepática total ou parcial. Ao contrário de CPS1, NAGS e OTC, esta enzima é distribuída por todo o corpo e está envolvida na produção de NO. A citrulinemia tipo 1 apresenta um variado espectro clínico, podendo apresentar uma forma neonatal aguda, de início tardio mais leve, sem sintomas e/ou hiperamonemia, ou durante a gravidez e pós-parto, com mulheres apresentando sintomas graves. Recém-nascidos com a forma neonatal aguda tipicamente parecem normais ao nascimento, mas, à medida que a amônia se acumula no organismo, tornam-se progressivamente letárgicos, com vômitos, convulsões e perda de consciência. A forma de início tardio, que é menos comum, está associada a dores de cabeça intensas, perda parcial da visão, fala arrastada, problemas com equilíbrio e coordenação muscular (ataxia), problemas de

comportamento e letargia. Os níveis elevados de amônia no sangue geralmente ocorrem após jejum prolongado, durante uma doença ou infecção ou após refeições com alto teor de proteína. Alguns indivíduos com mutações genéticas que causam a citrulinemia tipo 1 nunca experimentam sinais e sintomas e só passam a descobrir serem portadores da doença após um irmão ou irmã ser diagnosticado (WAISBREN, 2019).

1.4.5 *Deficiência de argininosuccinato liase (DASL; OMIM 207900)*

A acidúria argininosuccínica é ocasionada pela deficiência total ou parcial da enzima argininosuccinato liase (ASL), comprometendo a transformação do argininosuccinato citosólico em arginina, ocorrendo uma grande elevação de argininosuccinato em plasma e urina, assim como diminuição de arginina. Uma vez que o argininosuccinato é livremente excretado na urina, eliminando assim 4 nitrogênios, a hiperamonemia é tipicamente menos grave neste distúrbio em relação à outras DCU. Esta desordem é frequentemente marcada por hepatomegalia crônica e elevação das transaminases hepáticas. Testes moleculares para ASL ou medida da atividade da enzima são úteis para confirmação diagnóstica (NAGAMANI *et al*., 2012).

1.4.6 *Deficiência de arginase 1 ou argininemia (DARG1; OMIM 207800)*

A argininemia é uma desordem metabólica ocasionada pela deficiência da enzima arginase 1 (DARG1), responsável por catalisar a conversão de arginina em ureia e ornitina, já na etapa final do ciclo. O aumento de arginina plasmática frequentemente se encontra superior a 300 µmol/L. Diferente das demais DCU, não é característico da argininemia apresentar-se em período neonatal e sim na infância, entre 2 e 4 anos de idade. Estes indivíduos frequentemente apresentam atraso no desenvolvimento, paraparesia espástica progressiva, retardo mental, atraso no crescimento e episódios ocasionais de hiperamonemia.

1.4.7 *Deficiência no transportador de citrina ou citrulinemia tipo II (DCITRIN; OMIM 603471/605814)*

Esta DCU diminui a disponibilidade de aspartato que entra no ciclo da ureia para se complexar à citrulina e ser transformado em argininosuccinato pela enzima ASS1, ocorrendo uma deficiência funcional do ciclo. Este distúrbio geralmente apresenta-se na forma mais tardia, ocorrendo na adolescência ou na idade adulta com episódios recorrentes de hiperamonemia e sintomas neuropsiquiátricos.

1.4.8 *Deficiência de ornitina translocase ou síndrome de hiperornitinemia-hiperamonemia-homocitrulinúria (HHH) (DORNT1; OMIM 238970)*

O defeito na translocase de ornitina resulta no transporte diminuído de ornitina para a mitocôndria, com acúmulo de ornitina no citoplasma e redução de ornitina intramitocondrial, causando ureogênese comprometida, hiperamonemia, e acidúria orótica. As concentrações de ornitina no plasma são extremamente altas e a altos níveis de homocitrulina são encontrados na urina dos pacientes. A maioria dos afetados apresenta hiperamonemia intermitente acompanhada de vômitos, letargia e coma (em casos extremos). O crescimento é anormal e o desenvolvimento intelectual é afetado, assim como há o surgimento de comprometimento motor como a espasticidade. Também são encontrados disfunção hepática e coagulopatia frequentemente.

1.5 Diagnóstico

Inicialmente os sintomas clínicos característicos de DCU devem ser identificados, podendo manifestar-se de forma aguda, crônica ou intermitente, a qualquer idade. Os sinais e sintomas de cada uma das DCU são inespecíficos, entretanto, sabe-se que a amônia elevada é o denominador comum entre o grupo, portanto, estas doenças devem ser imediatamente suspeitadas caso o paciente apresente quadro de encefalopatia hepática causada pela hiperamonemia, principalmente em neonatos.

Assim, a dosagem de amônia é o exame laboratorial primordial indicado quando há suspeita de DCU, e uma dosagem baixa em neonatos sintomáticos

pode excluir a presença destas doenças. Imediatamente após a confirmação da hiperamonemia, a dosagem de aminoácidos no plasma, dosagem de acilcarnitinas em sangue ou plasma, dosagem de ácidos orgânicos na urina, e dosagem de ácido orótico na urina são realizados, a fim de identificar metabólitos alterados e realizar o diagnóstico diferencial entre as DCU, assim como excluir a possibilidade de hiperamonemias neonatais secundárias a outros EIM, como acidúrias orgânicas (acidúria propiônica (PA), acidúria metilmalônica (MMA) e acidúria isovalérica (IVA)) e defeitos da oxidação de ácidos graxos (deficiências de acil-CoA desidrogenase de cadeia média (MCAD), 3-hidroxiacil-CoA desidrogenase de cadeia longa (LCHAD), e acil-CoA desidrogenase de cadeia muito longa (VLCAD)) (BRAISSANT *et. al.,* 2013).

A análise molecular é o método mais indicado para fins confirmatórios devido a sua alta sensibilidade em frente à testes de medida de atividade enzimática. Testes pré-natais podem ser realizados após a identificação de um paciente índice e subsequente gravidez, auxiliando pais e médicos no manejo peri- e pós-natal dos pacientes. Neste caso, as amostras de escolha e análises recomendadas diferem de acordo com a DCU investigada (HABERLE *et al.,* 2019).

Painéis analíticos validados para diagnóstico de DCU vem sendo alvo de muita investigação nos últimos anos. A triagem neonatal para doenças como DASS1, DASL e DCITRIN se baseiam na detecção de aumento de citrulina, com ou sem elevação dos níveis de argininosuccinato. Outras DCU ainda possuem baixa sensibilidade em sua detecção na triagem neonatal, uma vez que, a ornitina, por exemplo, pode se demonstrar normal durante os primeiros dias de vida em neonatos (síndrome HHH) e casos de DARG1 são muito raros. A detecção de aminoácidos como a glutamina, devido a sua baixa instabilidade, e diminuição de citrulina, devido à sua baixa sensibilidade e especificidade, se mostram complexas, dificultando assim o diagnóstico de DNAGS, DCPS1 e DOTC. (STEPIEN *et al.,* 2019; VERNON *et al.,* 2015; MERRITT *et al.,* 2018). A rápida progressão das doenças e elevada taxa de óbito também contribui para seu difícil diagnóstico.

1.6 Tratamento

A principal abordagem terapêutica nas DCU consiste na tentativa urgente de normalizar os altos níveis de amônia responsáveis pelos graves desfechos clínicos nestas doenças. Para tanto, o tratamento consiste na administração intravenosa ou oral de drogas retiradoras de amônia do organismo, tais como, benzoato de sódio, fenilacetato de sódio e fenilbutirato de sódio. O benzoato de sódio é um composto metabolicamente ativo que se conjuga primeiramente a Coenzima A para formar benzoil-CoA. A benzil-CoA por sua vez, se conjuga com a glicina na mitocôndria celular do fígado e rins, estimulando a reposição de glicina e, consequentemente, o consumo de nitrogênio. Assim ocorre a formação de ácido hipúrico, composto rapidamente excretado pelos rins, via filtração glomerular e secreção tubular. Um mol de hipurato elimina um mol de nitrogênio. O mecanismo de ação do fenilacetato de sódio consiste em sua conjugação com a glutamina por acetilação para formar a fenilacetilglutamina, também seguramente excretada pelos rins. O fenilbutirato de sódio é rapidamente hidrolisado por meio de lipases pancreáticas a fenilacetato (ativo), configurando-se como seu pró-fármaco. A fenilacetiglutamina promove a excreção de dois moles de azoto residual. Pacientes portadores de DNAGS podem ser tratados com N-carbamilglutamato (NCG), o qual possui função análoga ao cofator NAG, ativando assim a enzima CPS1 e, consequentemente, o restante do ciclo. A DNAGS é a única DCU que pode ser efetivamente tratada por um medicamento (ENNS *et al.,* 2007; DE LAS HERAS *et al.,* 2017).

Além disso, uma dieta restrita com baixo teor de proteínas e suplementação de aminoácidos é necessária para reduzir ou normalizar os níveis de aminoácidos alterados pelo bloqueio do ciclo. A suplementação de arginina (100-250 mg/kg/dia) é essencial em DCU que comprometam a síntese deste aminoácido (DCPS1, DNAGS, DOTC, DASS1 e DASL) (HABERLE *et al.,* 2019) e a citrulina pode ser fornecida (100–200 mg/kg/dia) para portadores de DCPS1 ou DOTC na tentativa de aumentar a depuração de nitrogênio pelo ciclo da ureia (SUMMAR, 2001).

Procedimentos como a diálise também servem como alternativa para eliminar rapidamente a amônia em excesso. O transplante hepático é recomendado em pacientes portando DCU severa e não-responsiva aos

tratamentos convencionais e risco de desenvolver insuficiência hepática. O procedimento tem a capacidade de restaurar os níveis de amônia, prevenindo novas crises hiperamonêmicas e aumentar a taxa de sobrevivência dos pacientes, quando realizado nos primeiros meses de vida. Neonatos portadores de DASS1 com níveis superiores a 360 µmol/L de amônia, e outras DCU apresentaram considerável melhoria no quadro clínico pós-transplante, principalmente neurológico. Entretanto, a literatura aponta casos em que pacientes transplantados portadores de DCU consideradas mais severas, como a DOTC e DCPS1, com níveis de amônia superiores a 360 µmol/L, não apresentaram melhoria no quadro neurológico, sugerindo o envolvimento de outros fatores no mecanismo toxicológico destas doenças (KIDO *et al.,* 2021a, b).

1.7 Manifestações clínicas

O acúmulo de amônia pode ser desencadeado por diversos fatores nas DCU, como passagem da vida intrauterina para extrauterina, infecções, febre, vômitos, diminuição de energia e de ingestão de proteína, catabolismo e involução do útero durante período pós-parto, quimioterapia e altas doses de corticoide, exercício físico intenso e prolongado, aumento inesperado na ingesta de carga proteica e por alguns medicamentos, principalmente valproato e L-asparaginase/pegaspargase (MCMORRIS *et al.,* 2021). Além disso, a hiperamomenia pode ocorrer em qualquer período da vida, na forma neonatal ou tardia. Uma vez que a encefalopatia se instaura, os danos neurológicos começam a surgir, sendo a sua extensão diretamente proporcional aos níveis de amônia circulantes. Fatores como estágio maturacional cerebral do indivíduo afetado e tempo de exposição à amônia tóxica também influenciam na extensão do dano. Assim, sinais e sintomas como acidose metabólica, alteração de consciência, dificuldade de alimentação, vômitos, ataxia, convulsões e coma podem aparecer de imediato, culminando em uma elevada taxa de mortalidade. A hiperamonemia crônica pode causar confusão, letargia, cefaleia, dificuldades de aprendizado e dano cognitivo, aversão a proteínas, dores abdominais, *failure to thrive*, hepatomegalia, sintomas psiquiátricos como hiperatividade, alterações de humor, mudanças de comportamento,

agressividade, entre outros. A hiperamonemia causa graves déficits cognitivos e motores, que parecem ser linearmente proporcionais à sua extensão e duração. Heterozigotas de DOTC geralmente são assintomáticas, mas apresentam fenótipo variável. Além disso, observa-se que algumas manifestações clínicas podem ser consideradas específicas para certas DCU, tais como, alterações capilares (tricorrexis nodosa) na DASL, diplegia espástica progressiva na DARG1 e síndrome HHH na DORNT1 (HABERLE *et al.,* 2019).

1.8 Fisiopatologia

Sabe-se que a hiperamonemia é responsável por provocar a ativação de uma vasta gama de mecanismos tóxicos cerebrais, entretanto, a fisiopatologia deste grupo de doenças ainda permanece incerta. As hipóteses atuais se baseiam principalmente em: 1) desregulação osmótica cerebral causada pelo acúmulo de glutamina (BRUSILOW, 2010); 2) ativações de receptor de glutamato/NMDA com estimulação da via do óxido e consequente lesão excitotóxica (FELIPO *et al.,* 2002); e 3) comprometimento do metabolismo energético causando déficit energético, levando a efeitos a longo prazo, como uma falha na mielinização normal e liberação de hormônios tróficos (BRAISSANT, 2010). As alterações neuropatológicas nas DCU são semelhantes às da encefalopatia hepática e encefalopatia hipóxico-isquêmica, além disso há o comprometimento da substância branca, refletindo em dano aos astrócitos (BRAISSANT, 2010).

1.9 Radicais Livres

Radical livre (RL) define-se como uma estrutura química com um ou mais elétrons desemparelhados ocupando um orbital molecular ou atômico sozinho, o que confere uma alta reatividade e instabilidade à molécula. A fim de emparelhar seu orbital, estas moléculas são capazes de combinar-se de forma inespecífica com proteínas, lipídeos e DNA, promovendo danos celulares e a perda de sua função (HALLIWELL & GUTTERIDGE, 2015). Estes radicais podem ser formados endogenamente por diferentes mecanismos em ambas as condições fisiológicas e patológicas, por reações redox ou por processos de catálise enzimática (subprodutos do metabolismo aeróbio) bem como por

fatores exógenos, tais como drogas, poluentes do ar, por absorção de radiação (ultravioleta ou visível) e agentes químicos (HALLIWELL, 1994).

São chamados de espécies reativas de oxigênio (EROS) os compostos derivados do oxigênio que não são propriamente radicalares, mas que produzem radical livre, tais como: radicais superóxido (O_2^-), hidroxila (OH^-) ou a peróxido de hidrogênio (H_2O_2), sendo este último chamado de espécie reativa e não radical, por não possuir um elétron desemparelhado (HALLIWELL & GUTTERIDGE, 2015). Além das ERO, existem as espécies reativas de nitrogênio, como o óxido nítrico ($NO^{\cdot-}$), que é um radical livre gerado pela enzima óxido nítrico sintase nos sistemas biológicos (GIUVILI *et al.,* 1998).

O organismo humano, a fim de evitar os danos celulares ocasionados pela formação de radicais livres, produz defesas antioxidantes intrínsecas capazes de inativar as espécies reativas (HALLIWELL & GUTTERIDGE, 2015). Estas defesas podem ser enzimáticas, como por exemplo: superóxido dismutase, catalase e glutationa peroxidase ou não-enzimáticas, que compreendem as proteínas ligantes de metal (transferrina, ferritina etc.), as vitaminas (E, A, C etc.), glutationa, albumina, entre outras (HALLIWELL E GUTTERIDGE, 2015). Quando há um desequilíbrio entre a capacidade antioxidante e as espécies reativas formadas, em favor destas últimas, ocorre o estresse oxidativo (EO), tendo como principais consequências a lipoperoxidação das membranas celulares, a oxidação de proteínas e a lesão ao DNA/RNA celular, podendo causar mutações. Vários estudos têm demonstrado que o estresse oxidativo participa da fisiopatologia de alguns EIM, como aminoacidopatias, acidemias orgânicas e doenças peroxissomais (VARGAS, 2004; BARSCHAK *et al.,* 2006) e desempenha um papel importante em doenças neurodegenerativas, uma vez que o cérebro é um órgão extremamente suscetível à ação dos radicais livres.

1.10 Defesas Antioxidantes

Os sistemas biológicos desenvolveram mecanismos de defesa antioxidante, bem como sistemas de reparo, capazes de converter e inativar as ER (HALLIWELL, 1994). Qualquer substância que, quando presente em baixas concentrações em relação a um substrato oxidável, significativamente diminui

ou previne a oxidação desse substrato pode ser classificada como antioxidante. Estas defesas podem ser enzimáticas e não enzimáticas, agindo de forma a diminuir ou remover a formação de ER (ação de sequestro, conhecida como *scavenger*) (HALLIWELL & GUTTERIDGE, 2015).

Fazem parte das defesas antioxidantes enzimáticas a catalase (CAT), superóxido dismutase (SOD) e glutationa peroxidase (GPx) (Matés *et al*., 1999). Localizada principalmente nos peroxissomos, a CAT é uma hemoproteína presente em todas as células de mamíferos, catalisando a dismutação do peróxido de hidrogênio à água e oxigênio, prevenindo a formação de $OH^{\bullet}$. A SOD é uma metaloenzima presente em todos os organismos aeróbios, que catalisa a dismutação de $O_2^{\bullet -}$ reduzindo-o a H_2O_2, o qual é menos reativo e pode ser degradado por outras enzimas, como a CAT ou GPx. A GPx é uma seleno-enzima, encontrada em todos os tecidos animais, e representa a principal defesa mitocondrial contra o H_2O_2, uma vez que essas organelas, em geral, não possuem CAT. A ação da GPx consiste na oxidação da glutationa (GSH) a glutationa oxidada (GSSG), reduzindo o H_2O_2 a H_2O (HALLIWELL & GUTTERIDGE, 2015). A GSSG consiste em duas GSH ligadas por ponte dissulfeto que pode ser convertida novamente a GSH pela enzima glutationa redutase (GR) com a utilização de NADPH como coenzima (Halliwell, 2006). A GPx é considerada um dos principais sistemas de defesa antioxidante do organismo (HALLIWELL & GUTTERIDGE, 2015; MATÉS *et al*., 1999).

Os antioxidantes não enzimáticos, que apresentam direta ou indiretamente, capacidade de defesa antioxidante, podem ser representados por moléculas sintetizadas no próprio organismo, como a bilirrubina, ácido úrico, melatonina e glutationa, ou por compostos obtidos a partir da dieta, como as vitaminas A, C e E, flavonóides, carotenóides, polifenóis, selênio e L-carnitina (LC) (SALVADOR & HENRIQUES, 2004). O mecanismo de atuação desses antioxidantes é bastante diverso, podendo envolver a remoção do oxigênio presente no meio, o sequestro de ERO ou de seus precursores, inibição da formação de ER, entre outros (HALLIWELL & GUTTERIDGE, 2015).

1.11 Estresse Oxidativo e Inflamação

O EO vem sendo observado na fisiopatologia de alguns EIM, como aminoacidopatias, acidemias orgânicas e doenças peroxissomais (SITTA *et al*., 2009; BARSCHAK *et al*., 2006; VARGAS *et al*., 2004) e desempenha um papel importante em doenças neurodegenerativas, uma vez que o cérebro é um órgão extremamente suscetível à ação dos RL, devido ao seu baixo conteúdo de defesas antioxidantes, a alta concentração de neurotransmissores auto-oxidáveis, ao alto conteúdo de lipídeos poliinsaturados, ao alto consumo de oxigênio e ao alto conteúdo de ferro em algumas áreas particulares (HALLIWELL & GUTTERIDGE, 2015).

O EO ocorre quando há um desequilíbrio entre a capacidade antioxidante e as espécies reativas formadas, em favor destas últimas. Ocorrendo o EO o organismo pode reagir de duas maneiras: adaptando-se ou sofrendo dano celular. Em caso de EO brando, o organismo pode reagir através do aumento da produção de defesas antioxidantes, tentando com isto restabelecer o equilíbrio pró-oxidante/antioxidante. Mas caso o EO seja severo, pode levar a danos irreversíveis, como a morte celular. Este desequilíbrio pró-oxidante/antioxidante pode causar danos em diversas biomoléculas, podendo resultar na lipoperoxidação das membranas celulares, oxidação de proteínas e lesão ao DNA/RNA celular (HALLIWELL & GUTTERIDGE, 2015).

A inflamação é uma defesa do organismo contra agentes agressores de origem química, física ou biológica, caracterizada por ser uma resposta inespecífica a uma agressão (TILLEY et al., 2001), sendo, portanto, a resposta mais precoce quando há uma lesão tissular ou quadro infeccioso. Os processos inflamatórios são uma desordem complexa envolvendo diferentes células e componentes moleculares (MURIACH *et al.,* 2014). A resposta inflamatória encontra-se intimamente relacionada com as defesas antioxidantes e com o estresse oxidativo. Espécies reativas de oxigênio/nitrogênio (ERO/ERN) são capazes de alterar diferentes vias de sinalização, estimulando a liberação de citocinas e outros mediadores pró-inflamatórios. Da mesma maneira, a sustentação de estados inflamatórios pode promover um aumento do EO (OKUN *et al.,* 2011; HIGDON *et al.,* 2012; OLSEN *et al.,* 2015). Além disso, o desequilíbrio da resposta inflamatória vem sendo demonstrado em

alguns EIM, como os achados referente ao aumento da produção de citocinas pró-inflamatórias em pacientes com a doença da Urina do Xarope do Bordo (MESCKA *et al.,* 2015), adrenoleucodistrofia ligada ao X (MARCHETTI *et al.,* 2018a) e MPS tipo IVA (DONIDA *et al.,* 2015) encontrados recentemente.

1.12 Estresse oxidativo, inflamação e DCU

Acredita-se que o estresse oxidativo nas DCU esteja relacionado principalmente ao desequilíbrio na produção de óxido nítrico (NO), uma vez que as espécies reativas de nitrogênio (ERN) podem ser produzidas em qualquer local onde o ânion superóxido esteja presente. Além disso, o NO é amplamente distribuído em todos os tecidos devido ao seu importante papel no controle da pressão arterial e função dos vasos. A produção deste metabólito é feita pela enzima óxido nítrico sintase (NOS), a qual utiliza oxigênio molecular, NADPH e arginina como substrato. A principal via reguladora desta enzima, além do Ca^{2+} e da calmodulina, é a disponibilidade de arginina. Portanto, considerando que esta esteja acumulada na deficiência de arginase 1, por exemplo, pode haver o consequente aumento da atividade da NOS e em mais NOS ativas consumindo NADPH, sendo assim, é compreensível que as ERN possam participar do dano encontrado nesta DCU. Modelos animais de deficiência de arginase, demonstraram desenvolvimento de severa esteatose hepática relacionada a um desequilíbrio entre a NOS e arginina (NAVARRO *et al.,* 2015), e trabalhos ainda mais recentes relataram que a disfunção da NOS pode ser crucial na progressão de doenças no fígado e aumento da resistência vascular associada à hipertensão portal e fibrose (PERSICO *et al*., 2017).

Estudos já demonstraram aumento da produção de superóxido e diminuição da atividade de enzimas oxidantes em cérebros de ratos infundidos com NH_4^+, e subsequente prevenção, tanto pela inibição da NOS mediada pela nitroarginina quanto por antagonistas do receptor NMDA, sugerindo que o estresse oxidativo induzido por NH_4^+ é dado, pelo menos em parte, pelo aumento da formação de NO através da ativação excessiva do receptor NMDA (KOSENKO, 1998; 1999). De acordo com essa hipótese, a produção de radicais livres é aumentada em culturas primárias de astrócitos expostos a

NH_4^+ (MURTHY *et al.,* 2001), o que pode levar à formação de peroxinitritos altamente tóxicos e, sugere-se, que a morte neuronal e glial decorra da depleção secundária de ATP, aumento de radicais e estresse oxidativo (RODRIGO *et al.,* 2009). Além disso, a produção de NO induzida por NH_4^+ pode inibir a glutamina sintase, potencializando as consequências da hiperamonemia no SNC (ROSE & FELIPO, 2005). Outros estudos também sugerem que a amônia possa alterar a permeabilidade da barreira hematoencefálica (BHE) por meio de um mecanismo envolvendo aumento do NO e estresse oxidativo no endotélio microcapilar do cérebro (SKOWRONSKA *et al.,* 2012).

Nagasaka *et al.* (2004) sugerem o envolvimento de mecanismos complexos na DOTC no que diz respeito a produção e metabolização do NO e em sua função na dinâmica redox do organismo, ao demonstrar a partir de dois estudos, que os pacientes com esta deficiência podem apresentar tanto um aumento, quanto uma diminuição da NO e de seus derivados, independente da forma na qual a arginina seja suplementada.

Estudos demonstraram que a citrulina afeta o desequilíbrio da resposta antioxidante celular, relevante nas deficiências de ASS1 de ASL, as quais são caracterizadas pelo alto e leve aumento da citrulina plasmática, respectivamente (DELWING *et al*., 2008). Da mesma forma que a arginina e outros metabólitos relacionados à DARG1, também induzem o desequilíbrio oxidativo, demonstrando uma diminuição nas atividades de defesas enzimáticas e não-enzimáticas e aumento dos parâmetros de dano oxidativo. Estes estudos ainda demonstraram que algumas das alterações podem ser prevenidas com a administração de antioxidantes exógenos, incluindo NG-nitro-1-arginina metil éster (I-NAME) e vitaminas, comprovando que EROs são responsáveis por, pelo menos, alguns dos efeitos danosos em pacientes com deficiências de ASS1, ASL, ARG1 e citrina. Dados obtidos em um estudo de 2016 (HUEMER *et. al*.), utilizando 19 pacientes deficientes de arginase 1, comparados com controles de idade e sexo semelhantes, demonstraram diminuição da capacidade antioxidante no plasma total, e aumento de níveis de nitrato no plasma, sugerindo um estado pró-oxidante favorecido. Entretanto,

neste mesmo estudo, as medidas de isoprostanos, produtos da oxidação proteica e 8-hidroxi-2-deoxiguanosina continuaram inalteradas, sugerindo um padrão inespecífico de resposta aos agentes oxidantes ou mesmo a ausência de estresse oxidativo nesta DCU, fazendo-se necessário a realização de estudos mais aprofundados para confirmação destas hipóteses.

Estudos *in vivo* e *in vitro* realizados por Zanatta *et al*., (2013; 2015; 2016) demonstraram que a ornitina induz o dano oxidativo lipídico e diminui as defesas antioxidantes, principalmente a GSH. E alguns destes efeitos foram prevenidos com a administração de antioxidantes exógenos, fortalecendo a ideia de que o EROs e o estresse oxidativo participam do desfecho clínico da síndrome HHH. A homocitrulina, presente na DORNT1, também pode induzir alterações nos parâmetros de estresse oxidativo, mesmo que em menor intensidade.

Um estudo realizado por Hussein, investigando a suscetibilidade ao estresse oxidativo em pacientes infantis com EIM (incluindo pacientes com DARG1) após serem submetidos a transplante de fígado, demonstrou maior propensão destes ao estresse oxidativo. No geral, estes pacientes tendiam a um estado pró-inflamatório, devendo-se levar em consideração que inflamação e estresse oxidativo são dois fatores bastante conectados (SANCHEZ *et al.,* 2015; DANDEKAR *et al*., 2015; LUGRIN *et al.,* 2014).

Nagasaka *et al*. também trabalharam com pacientes portadores de DCITRIN em 2009. Os resultados demonstraram que a 8-hidroxi-2-deoxiguanosina e produtos da peroxidação lipídica estavam aumentados nas amostras de urina destes pacientes, enquanto a vitamina E estava diminuída, mas acompanhada de um aumento da atividade das enzimas antioxidantes SOD e catalase nos eritrócitos, evidenciando a presença de um desequilíbrio redox em resposta a produção aumentada de ERO/ERN e consequente dano molecular. Além disso, estes pacientes não estavam em estado descompensado ou hiperamonêmico, o que sugere que estas alterações aconteçam inclusive em pacientes assintomáticos.

2. OBJETIVOS

2.1 Objetivo Geral

O objetivo geral deste trabalho foi traçar o perfil clínico e laboratorial de pacientes brasileiros portadores de Doenças do Ciclo da Ureia, bem como avaliar *in vivo* o dano oxidativo a biomoléculas e inflamação nestes pacientes.

2.2 Objetivos específicos

a) Traçar o perfil clínico e laboratorial de pacientes DCU, através de um estudo transversal, retrospectivo e descritivo.
b) Investigar níveis de amônia, ácido orótico e aminoácidos em pacientes.
c) Avaliar a presença de dano nitrosativo em pacientes DCU, através da dosagem de nitrito e nitrato.
d) Avaliar a presença de dano oxidativo a lipídeos em pacientes DCU, através da dosagem de espécies reativas ao ácido tiobarbitúrico (TBARS);
e) Avaliar a presença de dano oxidativo a proteínas em pacientes DCU, através da dosagem de conteúdo de carbonilas;
f) Avaliar o perfil inflamatório em pacientes DCU, através da medida de níveis de citocinas IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, interferon-γ e TNF- α.

3. RESULTADOS

Os resultados desta dissertação serão apresentados na forma de capítulo/artigo científico.

3.1 CAPÍTULO I - Clinical findings of patients with hyperammonemia affected by urea cycle disorders with hepatic encephalopathy

Artigo científico publicado no periódico IJDN – International Journal of Developmental Neuroscience (fator de impacto = 2,457).

Clinical findings of patients with hyperammonemia affected by urea cycle disorders with hepatic encephalopathy

Running title: Findings of hyperammonemic UCD patients

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ABSTRACT

Background: Urea Cycle Disorders (UCD) are a group of genetic diseases caused by deficiencies in the enzymes and transporters involved in the urea cycle. The impairment of the cycle results in ammonia accumulation, leading to neurological dysfunctions and poor outcomes to affected patients.

Aim of the study: To investigate and describe UCD patients principal clinical and biochemical presentations to support professionals on urgent diagnosis and quick management, aiming better outcomes for patients.

Methods: We explored medical records of thirty patients diagnosed in a referral center from Brazil to delineate UCD clinical and biochemical profile.

Results: Patients demonstrated a range of signs and symptoms, such as altered levels of consciousness, acute encephalopathy, seizures, progressive loss of appetite, vomiting, coma, and respiratory distress, in most cases combined with high levels of ammonia, which is an immediate biomarker, leading to an UCD suspicion. The most prevalent UCD detected were ornithine transcarbamylase deficiency (11), followed by citrullinemia type I (10), hyperargininemia (5), carbamoyl phosphate synthetase 1 deficiency (2) and argininosuccinic aciduria (2). Clinical symptoms were highly severe, being the majority developmental and neurological disabilities, with 23% of death rate. Laboratory analysis revealed high levels of ammonia (mean ± SD: 860 ± 470 μmol/L; reference value: ≤ 80 μmol/L), hypoglycemia, metabolic acidosis, and high excretion of orotic acid in the urine (except in CPS1 deficiency).

Conclusions: We emphasize the need of urgent identification of UCD clinical and biochemical conditions, and immediate measurement of ammonia, to enable the correct

diagnosis and increase the chances of patient's survival, minimizing neurological and psychomotor damage caused by hepatic encephalopathy.

Keywords: Urea cycle disorders; hyperammonemia; inborn errors of metabolism; hepatic encephalopathy.

1. Introduction

Urea cycle disorders (UCD) are inborn errors of metabolism caused by deficiencies in the enzymes and transporters involved in the urea cycle, leading to a malfunction. The urea cycle (Figure 1) is responsible for the detoxification of ammonia in the human body, a toxic metabolite largely produced and mostly resulting from the amino acids and other nitrogenated compounds catabolism. Ammonia is metabolized to urea through a cyclic and enzymatically controlled mechanism that occurs in the liver, being non-toxic and excreted in the urine. If the ammonia detoxification pathway is impaired, accumulation of the cycle secondary metabolites and hyperammonemia may occur, characterizing an UCD (1,2,3).

In UCD, a total or partial defect of one of the six enzymes or two transporters involved in the urea cycle causes a blockage in the urea formation and excretion. The urea cycle diseases are N-acetylglutamate synthase deficiency (NAGSD), carbamoyl phosphate synthetase 1 deficiency (CPS1D), ornithine transcarbamylase deficiency (OTCD), argininosuccinate synthase 1 deficiency (ASS1D, CTLN1D or citrullinemia type 1), argininosuccinate lyase deficiency (ASLD or argininosuccinic aciduria), arginase 1 deficiency (ARG1D or hyperargininemia), ornithine transporter deficiency (ORNT1D) and citrin transporter deficiency (CTLN2D or citrullinemia type 2) (4).

With exception of OTCD, which is X-linked inherited, all other UCD's are inherited in an autosomal recessive manner and the estimated overall incidence is found between 1:35,000 to 1:52,000 live births (5, 6).

Neuropsychiatric complications in UCD are caused by hepatic encephalopathy (HE). HE genesis is hyperammonemic related, acting along with several neurotoxins and different factors, and being associated with hepatocellular insufficiency, resulting from acute or chronic liver disease. Serum ammonia levels higher than 150 μM in

neonates and 80 μM in adults are considered abnormal. In high concentrations, ammonia has serious neurotoxic effects since its ionized form can cross the blood-brain barrier (BBB) (6). Studies addressing the mechanisms involved in severe neurological damage presented by UCD patients demonstrate that ammonia causes several dysfunctions in varied and complex neurological systems, such as: swelling of astrocytes (cells responsible for detoxifying cerebral ammonia) (8,9); changes in neurotransmitters (10,11); increased production of free radicals and nitric oxide (NO) (12); transition of mitochondrial permeability (13,14,15); activation of factors that stimulate inflammation (16,17,18,19) and alterations in mitochondrial energy metabolism (12). HE presented by UCD patients may result in severe cerebral edema and irreversible brain damage and its extent is directly proportional to ammonia levels, ammonia exposure time and maturational brain stage (20). Although some studies have shown inconsistencies in the correlation between ammonia levels and the severity of this metabolic condition, there is strong evidence that ammonia is the primary factor responsible for development of HE (21). However, it should be noted that high ammonia levels do not necessarily cause HE and, on the other hand, normal ammonia levels do not preclude its presence (22,23). The exact neurological mechanisms presented in UCD have not yet been fully unraveled, but all the above-cited factors seem to act synergistically.

Individuals presenting acute hyperammonemic crisis may experience vomiting, progressive lack of appetite, altered levels of consciousness, seizures and coma, a clinical picture very similar to sepsis in newborns (24,25). The chronic presentation is more frequent in UCD late onset phenotypes, and is characterized by the presence of cognitive, psychological, and intellectual damage, although symptoms can be widely variable (26,27). However, each one of the urea cycle disorders can present

symptomatic peculiarities and different levels of severity (20). OTC deficiency, the most common urea cycle disorder, is generally more severe and lethal in male neonates due to the X-linked inheritance character, although females can present clinical features as acute as males (28,29). ASL deficiency can cause increased frailty in the capillary structure (trichorrhexis nodosa), which is a very specific alteration of this deficiency (30).

When UCD symptoms are suspected, it is essential to measure blood ammonia immediately, which can show levels greater than 500 μM/L in neonates in acute crises. However, the absence of hyperammonemia in adults does not exclude an UCD. Subsequently, it is essential to perform amino acid analysis in blood, as well as urine orotic and organic acids investigation to outline the clinical subtype (26,31). Enzyme activity tests and mutation analysis can be performed for confirmatory purposes (24).

Most countries do not include UCD in their neonatal screening programs, and, to date, only part of this group of diseases has a validated analytical panel, most due to difficulties to determine important urea cycle amino acids such as citrulline and glutamine. In addition, many patients presenting UCD, especially neonates in hyperammonemic crisis, require immediate diagnosis (0-24 hours of life) (32,33).

Immediately after the UCD diagnosis, patients begin a long therapeutic journey, which consists of medications and a low-protein diet. In this case, it is essential to monitor the balance between dietary restriction and the lack of nutrients due to diet, being necessary supplementation with formulas containing amino acids (arginine in UCD that compromise the synthesis of this amino acid, and citrulline in OTCD and CPS1D, to stimulate nitrogen clearance). Vitamins are also administrated to avoid possible malnutrition and consequent delays in development and patient's growth. Administration of scavenger drugs is essential, both for the control of acute

hyperammonemia, and for the long-term maintenance of ammonia levels in the body. Combined therapy of sodium phenylacetate and sodium benzoate, which through conjugation with glutamine and glycine, respectively, promote the excretion of phenylacetylglutamine and hippurate directly in the urine, is currently used. And phenylbutyrate (metabolic precursor of phenylacetate) can also be administered as a safe monotherapy for UCD treatment (34,37,36). N-carbamylglutamate (NCG) is an analog of NAG, used as an activator of CPS1 in cases of NAGSD, stimulating ureagenesis (37). Other hyperammonemia treatments include dialysis, antibiotics to reduce enteric ammonia production and, in some cases, after proving the need and real benefits, liver transplant is indicated (26). The unspecific clinical presentation in UCD, especially in neonates, is the major challenge for an early diagnosis, and consequently it is difficult to improve survival.

Considering the severity of this group of diseases, the aim of this study was to describe the clinical and laboratorial features of patients diagnosed with UCD in the Genetics Medical Service of Hospital de Clínicas de Porto Alegre (SGM-HCPA), to better elucidate these profiles.

2. Materials and methods

A cross-sectional, retrospective, and descriptive study was performed, which included all patients diagnosed with UCD at the Genetics Medical Service of Hospital de Clínicas de Porto Alegre (SGM-HCPA), from January 2001 to January 2022.

We analyzed medical records, such as description data (age, sex, date of birth, origin, parental consanguinity), clinical data (clinical features presented before the UCD diagnosis), biochemical profile (ammonia, urinary orotic acid, blood amino acid) and drug therapy information.

All tests necessary for the diagnosis of UCD were performed at the present reference center, as described below:

2.1. Determination of blood ammonia levels

Blood ammonia measurement was performed using a commercial Multigent Ammonia Ultra kit for quantitative enzymatic determination in plasma, in which ammonia in the presence of glutamate dehydrogenase (GDH) combines with α-ketoglutarate and NADH to produce glutamate and NAD^+. The decrease in absorbance (due to the conversion of NADH to NAD^+) at 340 nm is proportional to the ammonia concentration in the examined plasma. Enzymatic reactions were performed using the Architect C4000 equipment.

2.2. Determination of creatinine in the urine

Prior to the measurement of orotic acid, correction of urine volume was performed, based on their respective urinary creatinine value. Creatinine measurements were performed using the Jaffé method adapted for microplates (Labtest commercial kit).

2.3. Determination of orotic acid in the urine

Based on creatinine concentration, a urine volume equivalent to 200 mg of creatinine was required. This volume was transferred to previously identified glass tubes, and purified water was added to complete 1 ml. Concomitantly, a calibration curve was prepared, using a solution containing 1 mg % of orotic acid in increasing volumes and water (total volume: of 1 mL) along with a reaction blank. Two mL of citrate buffer was added to all tubes (curve, tests and blank). Afterwards, 0.5 mL of brominated water was

added to the curve tubes and samples (except to the reaction blank). All tubes were homogenized and left to rest for 1 min. In the next step, 1 mL of 5% ascorbic acid was added to all tubes, followed by an incubation for 5 min at 40 ºC under agitation. Finally, 2 ml of 2.5% P-dimethylaminobenzaldehyde (P-DABA) were added to all tubes, followed by agitation and incubation for 10 min at 40 ºC. Absorbances were read at 480 nm in a SpectraMax M2 spectrophotometer (38).

2.4. Determination of amino acids in blood by liquid chromatography-mass spectrometry (LC/MS/MS)

Using samples of dry blood spots collected on filter paper, the analysis of amino acids by liquid chromatography coupled to a tandem mass spectrometer (LC/MS/MS) was performed. A 3 mm diameter circle was cut from the filter paper and transferred to an eppendorf, to which, 100 µL of the standard solution of amino acids labeled with deuterium (H^2) was added. Afterwards, samples were homogenized for 30 min and the supernatant evaporated with nitrogen at 50 °C. Afterwards, 60 µL of 3N HCl-butanol were added for the derivatization of the samples, which were finally incubated at 65 °C for approximately 15 min. 100 µL of acetonitrile/milliQ water/formic acid matrix (50/50/0.1%) were used to dilute the sample that underwent centrifugation, and finally 30 µL were injected into the equipment, which had a mobile phase composed of acetonitrile/water milli Q/formic acid (50/50/0.25%). Amino acids were analyzed as butyl esters using Alliance 2695 liquid chromatograph (Waters) coupled to a Quattro Micro MS/MS mass spectrometer (Perkin Elmer). The source and desolvation temperatures were 120 ºC and 300 ºC, respectively. Input, collision, and output powers were 3V, 16V, and 2V, respectively. Amino acids were monitored by MRM (multiple monitoring reaction), with parent ion scan 85 (m/z) or neutral loss scan 102 (m/z).

Concentrations were determined by the response of each analyte to its respective internal standard. Results were reported in µM/L (39).

2.5. Determination of amino acids in plasma by high-performance liquid chromatography (HPLC)

Amino acid levels in plasma were measured by HPLC (Schimadzu Corp.) using a reversed-phase column (ODS 25 cm x 4.6 mm x 5 µm). A sample deproteinization step was performed, using 200 µL of methanol and subsequent centrifugation of the mixture. The supernatant was aliquoted and 40 µL was added to 10 µL of internal standard (homocysteic acid), along with 50 µL of 4% mercaptoethanol. Subsequently, 200 µl of o-phthaldialdehyde were added to the mixture for derivatization. Finally, 20 µL were injected into the HPLC equipment and detection was performed by fluorescence (40).

3. Results

Since its implementation until January 2022, Genetics Medical Service of Hospital de Clínicas de Porto Alegre performed 572 diagnoses including aminoacidopathies and organic acid disorders, with only 30 patients (5,24%) presenting urea cycle disorders. The present study evaluated the laboratorial profile of the 30 patients in question, through the analysis of their respective medical records.

Individuals diagnosed at the reference center are from different regions of Brazil. As for UCD patients, 11 (36.6%) individuals were from the South region, followed by 8 (26.7%) from the Southeast, 8 (26.7%) from the Northeast, 2 (6.7%) from the Midwest and one patient (3.3%) from the North of the country.

Among the 30 UCD patients, 19 (63.3%) were male and 11 (36.6%) were female. Patients presented the first signs and symptoms and received clinical and laboratory support in an age that ranged from 0 days of life to 33 years old. In this group, OTCD and ASS1D were the most frequent diagnosis, representing an occurrence of 11 (36.7%) and 10 (33.3%) individuals for each group, respectively. ARG1D had the third highest frequency, with a total of 5 patients (16.6%), followed by CPS1D and ASLD with 2 (6,7%) patients for each group. There were no cases of NAGS or citrine and ornithine transporters deficiency. Twelve patients (40%) presented signs and symptoms of UCD before the first month of life. In hospital survival data was collected, showing 7 (23.3%) deaths due to the severe disease outcomes (3 ASS1D, 2 CPS1D, 1 OTCD, 1 ASLD). The occurrence of consanguinity between parents was reported in 5 cases (16.6%), and 9 of them (30%) reported the occurrence of close family members death after the appearance of similar symptoms. One of the UCD patients underwent gene mutation research, which demonstrated a 3-base pair deletion of exon 9 at position c.929_931delAAG p.Glu310del, described in the literature as characteristic of patients with late OTCD, confirming the diagnosis (Table 1).

The analysis showed a great variability of signs and symptoms among patients, and these findings are shown in table 2. At least 50% of the group had vomiting and seizures and 29% of the total progressed to coma, 86% of these diagnosed with OTCD.

Blood ammonia, the primary biomarker investigated when UCD is suspected, was reported in medical records, being the majority extremely high (mean ± SD: 860 ± 470 μmol/L; reference value: ≤ 80 μmol/L). In addition, levels of orotic acid in the urine of 13 patients (43.3%) were measured, and 10 (77%) were shown to be high (Table 3).

Amino acid analysis performed by LC/MS/MS and HPLC showed increased citrulline in all patients diagnosed with citrullinemia type 1, as well as increased glycine, alanine, and glutamine in most of this group. Patients with OTCD had lowered levels of citrulline, and the amino acids alanine and glutamine were shown to be generally increased. All individuals diagnosed with hyperargininemia had elevated arginine levels. Both patients with CPS1D demonstrated decreased citrulline and increased glutamine levels in tandem and HPLC analyses, respectively. The prevailing laboratory findings of the two ASLD patients were increased citrulline and glutamine, with one of them also presenting decrease in several other amino acids, such as leucine, isoleucine, ornithine, methionine, and phenylalanine. The complete description of the altered amino acids for each UCD patient and their mean concentrations are found in tables 4 and 5, respectively. The UCD diagnosis was primarily given by the results of amino acid measurements by HPLC or LC/MS/MS.

After diagnosis, UCD patients started drug treatment with scavenger drugs, sodium benzoate and sodium phenylbutyrate, and to complement the recommended treatment, 6 patients in the group (20%) started low-protein diet. Other drugs were administrated to treat signs and symptoms and 5 patients (16.6%) started antibiotic therapy, probably due to the similarity of the clinical picture with neonatal sepsis. The study also showed that 8 patients (26.6%) started treatment with some class of anticonvulsant to treat symptoms (Table 6).

4. Discussion

The present study compiled 30 cases of UCD, diagnosed in a reference center in southern of Brazil, with individuals from all regions of the country. Studies addressing UCD in Brazil are still scarce, making it difficult to obtain more accurate information about these group of diseases.

UCD represents only a small portion of diagnosis when compared to the large group of existing aminoacidopathies and organic acid disorders. In Brazil, till the present day, none of UCD are included in the neonatal screening program, generating a scarcity of studies in the area, due to a lack of an algorithm that includes appropriate cutoff values for the validation of a method for diagnosis. Another determining factor in the difficulty of UCD diagnosis is the great variability of their signs and symptoms, which are very similar to other pathologies, especially in the neonatal period. Neonatal sepsis, a pathology whose symptomatic characteristics are temperature instability, respiratory stress, and hyperventilation, for example, is very similar to the first signs of hyperammonemia (26).

In this scenario, we must recognize the probable existence of many undiagnosed or underdiagnosed UCD patients, a factor that is aggravated by the rapid and severe development of the crisis and the consequent high mortality rate.

Data referring to the frequency of UCD indicates a higher occurrence of OTCD, ASLD and ASS1D. In our study, in accordance with international literature (6), we verified a high frequency of OTCD and ASS1D. At the same time, no cases of NAGSD and disorders related to mitochondria transporters involved in urea synthesis, such as ORNT1D and citrullinemia type 2, were reported.

OTC deficiency, unlike other UCD, has its gene located on the X chromosome and, therefore, when present in males, it usually manifests a very severe neonatal condition. OTCD is characterized by an increase in the amino acids glutamine and alanine, and a decrease in plasma citrulline (41,42). In our group of patients, a woman heterozygous and asymptomatic for OTCD was diagnosed at the age of 33 years old, after her two male children were diagnosed with the disease. At the time, the patient was pregnant with a girl, and her daughter is also an asymptomatic carrier of OTCD.

This case of late diagnosis is the reason for the high mean age presented for OTCD patients in Table 1. Although most OTCD female carriers are healthy, it is important to mention that the phenotypes are variable, and women may also show the most severe form. Table 4 and 5, shows the complete amino acid profile of our patients.

Individuals affected by ASS1D have a deficiency in the cytosolic enzyme argininosuccinate synthetase 1 due to pathogenic variants in the ASS1 gene located on chromosome 9q34.11. With the occurrence of hyperammonemia crisis in the first 28 days of life, signs and symptoms are highly severe. The majority of our ASS1D patients (54.5%) was diagnosed before the first month of life. However, in the late-onset form, the phenotypic characteristics can be much more variable. The clinical laboratorial picture showed hyperammonemia and high levels of plasma glutamine and citrulline (Table 4), as well described in the literature (43,44,45). Convulsive crises and metabolic acidosis were very common symptoms, both indicative of severe metabolic crisis.

In 2012, Carvalho (46) described the clinical and neurological profile of 16 patients diagnosed with hyperargininemia from four regions of Brazil, demonstrating spastic paraparesis as the most relevant clinical outcome for these patients. A similar result was found in our study, in which this clinical outcome was recurrent in most patients in the corresponding group. The mean age at diagnosis also showed some similarity, being slightly later when compared to most cases discussed in the literature (1-4 years old) (Table 1). Hyperammonemia in these cases is less frequent than in other UCD (Table 3). However, patients may present with neonatal and/or recurrent hyperammonemic crisis. Patients who presented CPS1D showed a characteristic biochemical profile similar to that previously reported in the literature, which consists of primary hyperammonemia followed by high levels of glutamine concomitant with decreased levels of plasma citrulline and arginine (47). Only 2 cases of CPS1D were

reported in this study, with very high severity and death informed in both cases. Literature indicates that deficiencies in proximal enzymes of the urea cycle, such as CPS1D and OTCD, are considered to cause more aggressive hyperammonemia and therefore high mortality rate (48).

The high blood ammonia levels described in Table 3 correlate with the severe signs and symptoms presented by our patients. Survivors of hyperammonemic crisis have been shown to develop disabilities that correlate with the number, severity, and duration of the hyperammonemic episode. Levels above 500 µm/L lead to very serious complications and reduce the chances of survival for patients. However, this condition can still be reversed if diagnosed early and with the help of intense treatment (49). The toxicity mechanism caused by the ammonia brain increase has been studied and there is strong evidence that it is due to the accumulation of glutamine in astrocytes due primarily to osmotic action, dysfunctional and increased mitochondrial permeability, oxidative damage, and disturbance in various neurological systems (50,51).

High concentrations of orotic acid in the urine can occur in several genetic metabolic diseases, however the importance of this parameter in the diagnosis of UCD is undeniable, being an excellent marker of OTCD. Due to the urea cycle disturbance presented in OTCD, the intramitochondrial accumulated carbamoyl phosphate can diffuse into the cytosol and stimulate the de novo biosynthesis of pyrimidines pathway, resulting in increased concentrations of orotic acid, which can be found in the patient's urine. Orotic acid levels should also be used to differentiate OTCD from other UCD with similar symptoms, such as CPS1D and NAGSD, which have normal orotic acid excretion (Table 3). Therefore, this analysis, along with the verification of hyperammonemia, become the first indicators of the existence of an UCD (52,53).

The rate of patients from our study who reported cases of a possible UCD and subsequent death in the family was considerable, with three of the patients in the group being siblings, culminating in the death of the oldest brother. In the case of a first diagnosis of a hereditary disease in the family, the importance of genetic counseling is reinforced. Thus, parents have the choice of going ahead with some next pregnancy or choosing not to take risks, going in search of other means of parenthood such as adoption, for example.

In the present study, amino acid analysis by HPLC and LC/MS/MS were essential to delineate and differentiate the profile of each UCD. These analyzes, despite having high costs when compared to other detection methods, are essential to obtain the best possible delineation of biochemical parameters, allowing the correct diagnosis.

One patient went to mutation analysis for confirmatory diagnosis, which was performed in another country. Molecular analysis for confirmatory diagnosis in UCD is important, however, identifying and reversing hyperammonemic crisis in a short period of time is the most important factor in UCD, since amino acids and orotic and arginosucccinic acids profiles are very elucidative for diagnosis. However, in the long term, molecular analysis proves to be of great value in genetic counseling or even in cases of prenatal diagnosis.

UCD patients were on a variety of drug therapies to treat signs and symptoms, before and after diagnosis. Once diagnosed, administration of scavenger drugs to control toxic ammonia levels, such as sodium benzoate and sodium phenylbutyrate, and low-protein diet started. One of the patients was under valproic acid therapy before the diagnosis. Valproic acid induces hyperammonemia as documented in several case reports (54,55) exacerbating hyperammonemic crises present in UCD and triggering hepatic encephalopathy (HE) (56), therefore, its use must be immediately stopped. In

addition, it is essential to monitor frequently ammonia levels in patients presenting hyperammonemia and HE in use of valproic acid or sodium valproate, to unmask an underlying error of metabolism, such as UCD (57).

HE is a brain disturbance associated with high prevalence of toxin levels in cerebral parenchyma. Ammonia is the main agent associated to HE, being extremely neurotoxic when increased in patients with advanced liver diseases. The exact mechanism involved in HE pathogenesis still unknown, but previous studies suggest that high levels of ammonia in brain triggers a cascade of events that work together to cause the syndrome. The reaction between ammonia and glutamate by glutamine synthetase in astrocytes protects brain against hyperammonemia, but excessive levels of ammonia originate intracellular osmotic disequilibrium, causing astrocyte swelling and consequently brain edema (8,9). The leading hypothesis to the brain edema caused in HE is attributed to the perturbance in the ammonia cerebral detoxification system and, therefore, the accumulation of glutamine in astrocytes. Brain edema and intracranial pressure are considered the most life-threatening abnormality in severe acute HE, often developing seizures, hypoxic injury, and ultimately neuronal death. Several other complex mechanisms are associated with the occurrence of HE, including disturbance in neurotransmitters (downregulation of glutamate receptors, increased GABAergic signaling and an excessive depolarization of NMDA receptors) causing neurocognitive decline (10,11); disturbance of energy status caused by compromised tricarboxylic acid cycle (TCA) (α-ketoglutarate is depleted due to the detoxification reactions between ammonia and α-ketoglutarate to form glutamate, and subsequently glutamine), and glucose and potassium metabolism; oxidative and nitrative damage (12); cerebral blood flow; disturbance transition of mitochondrial permeability (13,14,15) and activation of factors that stimulate inflammation (16,17,18,19).

5. Conclusions

Only 30 urea cycle disorders cases were identified among 572 diagnoses of aminoacidopathies and organic acid disorders, probably due to the lack of diagnostic suspicion or difficulties in differential diagnosis in the various states of Brazil, considering that UCD symptoms typically begin during the first few days of life with extreme severity and high lethality. We emphasize the importance of ammonia levels in patients presenting clinical conditions that suggest UCD to avoid the severe outcomes caused by hepatic encephalopathy (HE). HE toxicity mechanisms is still poorly understood, but it is believed that several factors combined contribute to the severe clinical manifestations. HE manifestations is completely reversible if hyperammonemia is treated early, reinforcing the needing of an urgent diagnosis and management of the ammonia excessive levels. Therefore, it is essential for clinicians a better understanding of the principal clinical symptoms presented by affected patients, allowing a special and urgent care. As demonstrated in our work, altered levels of consciousness, acute encephalopathy, seizures, progressive loss of appetite, vomiting, coma, and respiratory distress, combined with high levels of ammonia, which is an immediate biomarker, are symptoms suggestive of hyperammonemic crises in UCD and, therefore, these diseases should be investigated immediately. Thus, the need of neonatal screening to establish an accurate laboratorial profile is indispensable. Late diagnosis of hyperammonemia can have severe and often lethal consequences for the patient and only early identification and appropriate treatment will provide a better prognosis.

6. Declaration of interests

The authors declare the absence of conflict of interests.

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8. References

1. H. Herman, E. Shambaugh, Urea biosynthesis I. The urea cycle and relationships acid cycle. 20 (2018) 83–7. https://doi: 10.1093/ajcn/30.12.2083
2. S.W. Brusilow, A.L. Horwich, Urea cycle enzymes, in: C.R. Scriver, D. Beaudet, D. Valle, W.S. Sly (Eds.), The Metabolic and Molecular and Bases of Inherited Disease. 8th edn. New York: McGraw-Hill, 2001, 1909–1961. https://doi: 10.1036/ommbid.395
3. I.D. Weiner, J.W. Verlander, Renal ammonia metabolism and transport, Compr Physiol. 3 (2013) 201–20. https://doi: 10.1002/cphy.c120010.
4. M.L. Summar, N.A. Mew, Inborn Errors of Metabolism with Hyperammonemia: Urea Cycle Defects and Related Disorders, Pediatr Clin North Am. 65 (2018) 231–46. https://doi: 10.1016/j.pcl.2017.11.004.
5. M.L. Summar, S. Koelker, D. Freedenberg, C. Le Mons, J. Haberle, H.S. Lee, B. Kirmse, The incidence of urea cycle disorders, Mol Genet Metab. 110 (2013) 179–80. https://doi: 10.1016/j.ymgme.2013.07.008.
6. S. Nettesheim. et al. Incidence, disease onset and short-term outcome in urea cycle disorders-cross-border surveillance in Germany, Austria and Switzerland. Orphanet. J. Rare Dis. 12 (2017) 111. https://doi.org/10.1186/s13023-017-0661-x
7. S. Dasarathy, R.P. Mookerjee, V. Rackayova, V. Rangroo Thrane, B. Vairappan, P. Ott, C.F. Rose, Ammonia toxicity: from head to toe?, Metab Brain Dis. 32 (2017) 529-538. https://doi.org/10.1007/s11011-016-9938-3
8. A. Martinez-Hernandez, K.P. Bell, M.D. Norenberg, Glutamine synthetase: glial localization in brain, Science. 195 (1977) 1356-8. doi: 10.1126/science.14400.
9. L. Hertz, H.R. Zielke, Astrocytic control of glutamatergic activity: astrocytes as stars of the show, Trends Neurosci. 27 (2004) 735-43. https://doi.org/10.1016/j.tins.2004.10.008
10. G. Marcaida, V. Felipo, C. Hermenegildo, M.D Miñana, S. Grisolía, Acute ammonia toxicity is mediated by the NMDA type of glutamate receptors, FEBS Lett. 296 (1992) 67-8. https://doi.org/10.1016/0014-5793(92)80404-5
11. G. Marcaida, M.D. Miñana, M. Burgal, S. Grisolía, V. Felipo, Ammonia prevents activation of NMDA receptors by glutamate in rat cerebellar

neuronal cultures, Eur J Neurosci 7 (1995) 2389-96. https://doi.org/10.1111/j.1460-9568.1995.tb01036.x

12. P. Ott, H. Vilstrup, Cerebral effects of ammonia in liver disease: current hypotheses, Metab Brain Dis 29 (2014) 901-11. https://doi.org/10.1007/s11011-014-9494-7

13. K.V. Rama Rao, A.R. Jayakumar, D.M. Norenberg, Ammonia neurotoxicity: role of the mitochondrial permeability transition, Metab Brain Dis. 18 (2003) 113-27. https://doi.org/10.1023/A:1023858902184

14. A.P. Halestrap, K.Y. Woodfield, C.P Connern, Oxidative stress, thiol reagents, and membrane potential modulate the mitochondrial permeability transition by affecting nucleotide binding to the adenine nucleotide translocase, J Biol Chem. 272 (1997) 3346-54. https://doi.org/10.1074/jbc.272.6.3346

15. A.J. Kowaltowski, R.F. Castilho, A.E. Vercesi, Mitochondrial permeability transition and oxidative stress, FEBS Lett. 495 (2001) 12-5. https://doi.org/10.1016/S0014-5793(01)02316-X

16. A.P Sinke, A.R. Jayakumar, K.S. Panickar, M. Moriyama, P.V. Reddy, M.D. Norenberg, NFkappaB in the mechanism of ammonia-induced astrocyte swelling in culture, J Neurochem. 106 (2008) 2302-11. https://doi.org/10.1111/j.1471-4159.2008.05549.x

17. P. Marchetti, M. Castedo, S.A. Susin, N. Zamzami, T. Hirsch, A. Macho, A. Haeffner, F. Hirsch, M. Geuskens, G. Kroemer, Mitochondrial permeability transition is a central coordinating event of apoptosis, J Exp Med 184 (1996) 1155-60. https://doi.org/10.1084/jem.184.3.1155

18. A. Bowie, L.A. O'Neill, The interleukin-1 receptor/Toll-like receptor superfamily: signal generators for pro-inflammatory interleukins and microbial products, J Leukoc Biol. 67 (2000) 508-14. https://doi.org/10.1002/jlb.67.4.508

19. J.M. Kyriakis, J. Avruch, Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation, Physiol Rev. 81 (2001) 807-69. https://doi.org/10.1152/physrev.2001.81.2.807

20. C. Bachmann, Mechanisms of hyperammonemia, Clin Chem Lab Med. 40 (2002) 653–62. doi: 10.1515/CCLM.2002.112

21. A.R. Jayakumar, M.D. Norenberg, Hyperammonemia in Hepatic Encephalopathy, J Clin Exp Hepatol, 8 (2018), 272-280. doi: 10.1016/j.jceh.2018.06.007

22. O. Riggio, G. Mannaioni, L. Ridola et al., "Peripheral and splanchnic indole and oxindole levels in cirrhotic patients: a study on the pathophysiology of hepatic encephalopathy," American Journal of Gastroenterology, 105 (2010) 1374– 1381

23. L. Noiret, S. Baigent, and R. Jalan, Arterial ammonia levels in cirrhosis are determined by systemic and hepatic hemodynamics, and by organ function: a quantitative modelling study, Liver International, 34 (2014) 45-55

24. V. J. Leonard, A.A.M. Morris, Urea cycle disorders, Seminars in Neonatology. 7 (2002) 27–35. doi:10.1053/siny.2001.0085

25. J. Rocha, S. Sequeira, A. Cabral, M. Ferreira Almeida, Consenso para o Tratamento Nutricional das Doenças do Ciclo da Ureia, Acta Pediátrica Port. 40 (2009)175– 84. https://doi.org/10.25754/pjp.2009.4495

26. J. Häberle, A. Burlina, A. Chakrapani, M. Dixon, D. Karall, M. Lindner, H. Mandel, D. Martinelli, G. Pintos-Morell, R. Santer, A. Skouma, A. Servais, G. Tal, V. Rubio, M. Huemer, C. Dionisi-Vici, Suggested guidelines for the diagnosis and management of urea cycle disorders: First revision, J Inherit Metab Dis. (2019) 1–39. https://doi: 10.1002/jimd.12100

27. S.E. Waisbren, A.K. Stefanatos, T.M.Y. Kok, B. Hismi, Neuropsychological attributes of urea cycle disorders: a systematic review of the literature, J Inherit Metab Dis. (2019) 121-46. https://doi: 10.1002/jimd.12146.

28. U. Teufel, J. Weitz, C. Flechtenmacher, V. Prietsch, J. Schmidt, G.F. Hoffmann, S. Kolker, G. Engelmann, High urgency liver transplantation in ornithine transcarbamylase deficiency presenting with acute liver failure, Pediatr Transplant. 15 (2011) 110-5. https://doi: 10.1111/j.1399-3046.2009.01171.x.

29. F. Scaglia, Q. Zheng, W.E. O'Brien, J. Henry, J. Rosenberger, P. Reeds, B. Lee, An integrated approach to the diagnosis and prospective management of partial ornithine transcarbamylase deficiency, Pediatrics 109 (2002) 150-152. https://doi.org/10.1542/peds.109.1.150

30. S.C.S. Nagamani, A. Erez, B. Lee, Argininosuccinate lyase deficiency, Genet Med. 14 (2012) 501–7. https://doi: 10.1038/gim.2011.1.

31. K.M. Stepien, T. Geberhiwot, C.J. Hendriksz, E.P. Treacy, Challenges in diagnosing and managing adult patients with urea cycle disorders, J Inherit Metab Dis. (2019) 1136-1146. https://doi: 10.1002/jimd.12096.

32. H.J. Vernon, Inborn errors of metabolism: Advances in diagnosis and therapy, JAMA Pediatr. 169 (2015) 778–82. https://doi: 10.1001/jamapediatrics.2015.0754.

33. J. L. Merritt, L.L. Brody, G. Pino, P. Rinaldo, Newborn screening for proximal urea cycle disorders: Current evidence supporting recommendations for newborn screening, Mol Genet Metab. 124 (2018) 109–13. https://doi: 10.1016/j.ymgme.2018.04.006.

34. D. Bartholomew, R. Reichel, S. Brusilow, Prospective Diagnosis and Treatment of Urea Cycle Disorders, Pediatr Res. 21 (1987) 288-289. https://doi: 10.1016/s0022-3476(05)83044-6.

35. J. De Las Heras, L. Aldámiz-Echevarría, M.L. Martínez-Chantar, T.C. Delgado, An update on the use of benzoate, phenylacetate and phenylbutyrate ammonia scavengers for interrogating and modifying liver nitrogen metabolism and its implications in urea cycle disorders and liver disease, Expert Opin Drug Metab Toxicol. 13 (2017) 439–48. https://doi: 10.1080/17425255.2017.1262843

36. G.M. Enns, S.A. Berry, G.T. Berry, W.J. Rhead, S.W. Brusilow, A. Hamosh, Survival after treatment with phenylacetate and benzoate for urea-cycle disorders, N Engl J Med. 356 (2007) 2282–92. https://doi.org/10.1080/17425255.2017.1262843

37. N. Ah Mew, L. Caldovic, N-acetylglutamate synthase deficiency: an insight into the genetics, epidemiology, pathophysiology, and treatment, Appl Clin Genet 4 (2011) 127-35. https://doi.org/10.2147/TACG.S12702

38. A.M. Glasgow, A new method for measuring urinary orotic acid, Am J Clin Pathol. 77 (1982) 452–5. https://doi: 10.1093/ajcp/77.4.452.

39. M. Rashed, M. Bucknall, D. Little, A. Awad, M. Jacob, M. Alamoudi, M. Almattar, P.T. Ozand, Screening blood spots for inborn errors of metabolism by electrospray tandem mass spectrometry with a microplate batch process and a

computer algorithm for automated flagging of abnormal profiles, Clin Chem. 43 (1997) 1129-1141.

40. M.H. Joseph, C.A. Marsden, Amino acids and sample peptides, in: Lim CK (ed), HPLC of Small Peptides. IRL Press, Oxford, 1986, pp. 13-27.

41. J.H. Lee, G.H. Kim, H.W. Yoo, C.K. Cheon, OTC gene in ornithine transcarbamylase deficiency: Clinical course and mutational spectrum in seven korean patients, Pediatr Neurol. 51 (2014) 354-359. https://doi: 10.1016/j.pediatrneurol.2014.03.029.

42. P. Burgard, S. Kölker, G. Haege, M. Lindner, G.F. Hoffmann, Neonatal mortality and outcome at the end of the first year of life in early onset urea cycle disorders—review and meta-analysis of observational studies published over more than 35 years, J Inherit Metab Dis. 39 (2016) 219–29. https://doi: 10.1007/s10545-015-9901-1.

43. N. Shibata, Y. Hasegawa, K. Yamada, H. Kobayashi, J. Purevsuren, Y. Yang, V.C. Dung, N.N Khanh, I.C. Verma, S. Bijarnia-Mahay, D.H. Niu, G.F. Hoffman, Y. Shigematsu, T. Fukao, S. Fukuda, T. Taketani, S. Yamaguchi, Diversity in the incidence and spectrum of organic acidemias, fatty acid oxidation disorders, and amino acid disorders in Asian countries: Selective screening vs. expanded newborn screening, Mol Genet Metab Reports. 26 (2018) 5–10. https://doi: 10.1016/j.ymgmr.2018.05.003.

44. E.Y. Yoshitoshi-Uebayashi, T. Toyoda, K. Yasuda, M. Kotaka, K. Nomoto, K. Okita, K. Yasuchika, S. Okamoto, N. Takubo, T. Nishikubo, T. Soga, S. Uemoto, K. Osafune, Modelling urea-cycle disorder citrullinemia type 1 with disease-specific iPSCs, Biochem Biophys Res Commun. 486 (2017) 613–9. https://doi: 10.1016/j.bbrc.2017.03.037.

45. M. Zielonka, S. Kölker, F. Gleich, N. Stützenberger, S.C.S. Nagamani, A.L. Gropman, G.F. Hoffmann, S.F. Garbade, R. Posset, Early prediction of phenotypic severity in Citrullinemia Type 1, Ann Clin Transl Neurol. 6 (2019) 1858–71. https://doi: 10.1002/acn3.50886.

46. D.R. Carvalho, J.M. Brum, C.E. Speck-Martins, F.D. Ventura, M.M.M. Navarro, K.E.F.A Coelho, D. Portugal, R. Pratesi, Clinical features and neurologic progression of hyperargininemia, Pediatr Neurol. 46 (2012) 369–74. https://doi: 10.1016/j.pediatrneurol.2012.03.016

47. C. Diez-Fernandez, J. Häberle, Targeting CPS1 in the treatment of Carbamoyl phosphate synthetase 1 (CPS1) deficiency, a urea cycle disorder, Expert Opin Ther Targets. 21 (2017) 391–9. https://doi: 10.1080/14728222.2017.1294685.

48. N. Ah Mew, L. Krivitzky, R. McCarter, M. Batshaw, M. Tuchman, Clinical outcomes of neonatal onset proximal versus distal urea cycle disorders do not differ, J Pediatr. 162 (2013) 324-9 https://doi: 10.1016/j.jpeds.2012.06.065

49. C. Unsinn, A. Das, V. Valayannopoulos, E. Thimm, S. Beblo, A. Burlina, V. Konstantopoulou, S. Mayorandan, P. de Lonlay, J. Rennecke, J. Derbinski, G. F. Hoffmann, J. Haberle, Clinical course of 63 patients with neonatal onset urea cycle disorders in the years 2001-2013, Orphanet J Rare Dis. 11 (2016) 1–11. https://doi: 10.1186/s13023-016-0493-0.

50. V. Walker, Ammonia toxicity and its prevention in inherited defects of the urea cycle, Diabetes Obes Metab. 11 (2009) 823–35. https://doi: 10.1111/j.1463-1326.2009.01054.x.

51. B. Parmeggiani, C.R. Vargas, Oxidative stress in urea cycle disorders: Findings from clinical and basic research, Clin Chim Acta. 477 (2018) 121–6. https://doi: 10.1016/j.cca.2017.11.041.

52. C. Salerno, C. Crifò, Diagnostic value of urinary orotic acid levels: Applicable separation methods, J Chromatogr B Anal Technol Biomed Life Sci. 781 (2002) 57–71. https://doi:10.1016/S1570-0232(02)00533-0

53. P.K. Held, C.A. Haynes, V.R. De Jesús, M.W. Baker, Development of an assay to simultaneously measure orotic acid, amino acids, and acylcarnitines in dried blood spots, Clin Chim Acta. 436 (2014) 149–54. https://doi: 10.1016/j.cca.2014.05.016

54. R. Aiyer, M Seide, RG Stern, Valproic acid induced hyperammonemia in a long-time treated patient, Case Rep Psychiatry. (2016) 2016:6242314. https://doi: 10.1155/2016/6242314

55. T. McMorris, A. Chu, L. Vu, A. Bernardini. Hyperammonemia in patients receiving valproic acid in the hospital setting: A retrospective review. Ment Health Clin. 11 (2021) 243-247. doi: 10.9740/mhc.2021.07.243.

56. J. Wu, J. Li, W. Jing, X. Tian, X. Wang. Valproic acid-induced encephalopathy: A review of clinical features, risk factors, diagnosis, and treatment. Epilepsy &

behavior: E&B. 120 (2021) 107967. https://doi.org/10.1016/j.yebeh.2021.107967

57. S. Mehta, S. Tayabali, R. Lachmann. Valproate-induced hyperammonemia - uncovering an underlying inherited metabolic disorder: a case report. J Med Case Reports. 12 (2018) 134. https://doi.org/10.1186/s13256-018-1666-3

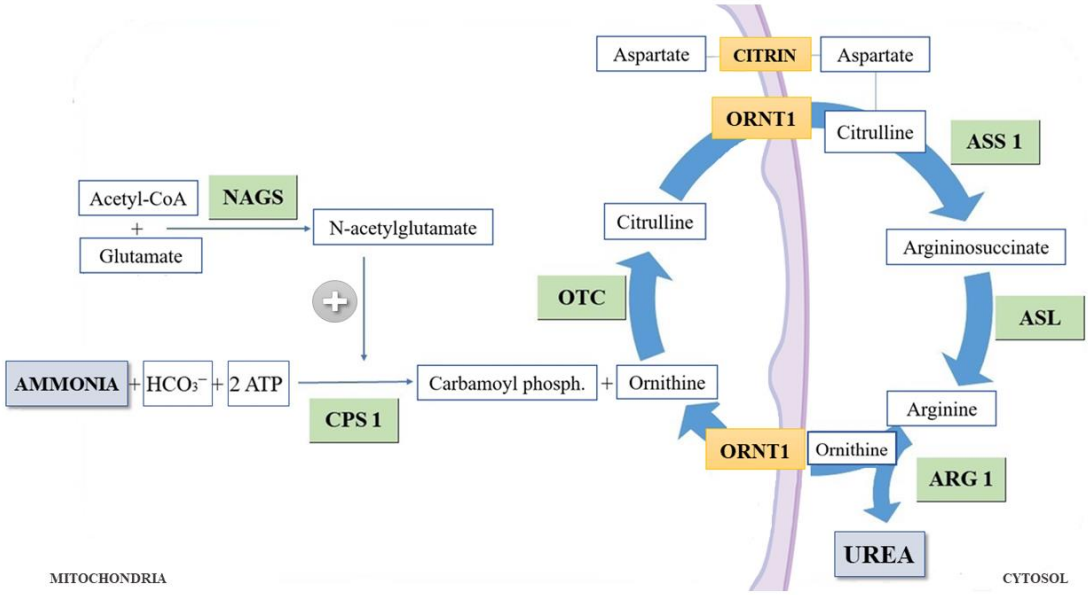

Figure 1. The urea cycle. In the liver, ammonia and bicarbonate are condensed by carbamoyl phosphate synthetase 1 (CPS1). CPS1 activity depends on the cofactor N-acetylglutamate (which is synthesized by N-acetylglutamate synthase - NAGS). The carbamoyl phosphate is subsequently bonded to ornithine to form citrulline by the action of ornithine transcarbamylase (OTC). Citrulline crosses mitochondria to cytosol in an ornithine transporter (ORNT1) and is then bonded to aspartate (which is dependent on the citrine transporter (CITRIN) to reach the cytosol), originating argininosuccinate by argininosuccinate synthase 1 (ASS1) activity. Argininosuccinate lyase (ASL) cleaves this compound into arginine that is finally hydrolyzed by arginase (ARG1) into ornithine and urea. Ornithine is restored to re-enter the urea cycle and urea is excreted by kidneys.

Table 1. Characteristics of UCD patients. Frequency, age, gender, parental consanguinity, and death of UCD patients diagnosed in a referral hospital in Brazil.

Variable	OTCD (n = 11)	ASS1D (n = 10)	ARG1D (n = 5)	CPS1D (n = 2)	ASLD (n = 2)
% of UCD diagnosis	36.7%	33.3%	16.6%	6.7%	6.7%
Age of diagnosis (months)*	62.4 ± 117	25.33 ± 58.3	7.4 ± 2.24	0.59 ± 0.16	0.46 ± 0.23
Gender:					
Males	63.6%	60%	40%	100%	50%
Females	13.7%	40%	60%	-	50%
Parental consanguinity	NI	30%	17%	NI	NI
Death cases	9.0%	30%	NI	100%	50%

NI: not informed.

*Values are expressed as mean ± SD.

Table 2. Profile of UCD patients. Gender, age, signs and symptoms, parental consanguinity, cases in family and death of UCD patients diagnosed in a referral hospital in Brazil.

UCD	Patient	Gender	Age of diagnosis (in months)	Clinical symptoms	Consanguinity	Previous UCD in family members	In hospital survival
OTCD	1*	M	17	Hypoactivity Somnolence Ataxia Vomiting Coma	No	Yes	No
OTCD	2*	M	8	Altered consciousness Vomiting Coma	No	Yes	Yes
OTCD	3*	F	394	Assimptomatic	No	Yes	Yes
OTCD	4*	F	82	Assimptomatic	No	Yes	Yes
OTCD	5	M	16	Hypoactivity Hypoglycemia Metabolic acidosis Vomiting Convulsive seizures Coma	No	Yes	Yes
OTCD	6	M	<1	Hypoactivity Metabolic acidosis Convulsive seizures Coma	No	Yes	Yes
OTCD	7#	M	13	Hepatomegaly Growth retardated Neuropsychomotor retardation Neurological regression Cognitive deficit Vomiting Convulsive seizures	No	Yes	Yes
OTCD	8	M	<1	Refusal to feed Somnolence Hypoglycemia Respiratory acidosis Vomiting Coma	No	No	Yes
OTCD	9	M	<1	Fetal macrosomia Tachydyspnea Respiratory insuficiency	No	No	Yes

OTCD	10	F	24	Feeding difficulty Psychomotor alteration Vomiting Coma	No	No	Yes
OTCD	11	F	133	Seizures Coma	No	No	Yes
ASS1D	12	F	<1	Hypotony Hypoactivity Convulsive seizures Metabolic acidosis	No	No	Yes
ASS1D	13	M	<1	Ketonuria Increased lactate Metabolic acidosis	No	No	No
ASS1D	14	F	187	NI	No	No	Yes
ASS1D	15	M	<1	Respiratory discomfort Increased lactate Hyperkalemia Convulsive seizures Metabolic acidosis	Yes	No	No
ASS1D	16	F	1.3	Altered consciousness Vomiting Seizures	No	No	Yes
ASS1D	17	M	<1	Hyperproteinorrhachia Plateletopenia Hypoglycemia Cerebral edema Vomiting Metabolic acidosis	Yes	No	No
ASS1D	18	M	39	Seizures	No	No	Yes
ASS1D	19	M	23	Hypotony Psychomotor retardation Cerebral hypoxia Convulsive seizures	No	No	Yes
ASS1D	20	M	<1	Acute encephalopathy Liver failure	No	No	Yes
ASS1D	21	F	1.2	Hypoglycemia Metabolic acidosis Coma	Yes	Yes	Yes
ARG1D	22	F	96	Microcephaly Convulsive seizures	No	No	Yes
ARG1D	23	F	72	Spastic paraparesis	No	No	Yes

				Convulsions			
ARG1D	24	F	84	Cyanosis Reduced brain activity Speech and gait regression	Yes	No	Yes
ARG1D	25	M	120	Behavioral change Agitation Anxiety Spastic paraparesis Convulsive seizures	No	No	Yes
ARG1D	26	M	72	Hyperactivity Learning difficulty Spastic paraparesis	No	No	Yes
CPS1D	27	M	<1	Brain alteration Convulsive seizures	No	Yes	No
CPS1D	28	M	<1	Hypoactivity Hepatomegaly Apnea Metabolic acidosis Convulsive seizures Coma	No	No	No
ASLD	29	F	<1	Poor feeding Oliguria Seizure	Yes	No	Yes
ASLD	30	M	<1	Feeding difficulty Vomiting Convulsive seizures Coma	No	No	Yes

*Members of the same family.
[#]Molecular diagnosis.

Table 3. Biomarkers of UCD patients. Mean values of ammonia and orotic acid in UCD patients diagnosed in a referral hospital in Brazil.

Biomarker	OTCD	ASS1D	ARG1D	CPS1D	ASLD	Reference values
Ammonia (µM/L)	724 ± 528 (n = 4)	1464 (n = 1)	<5 (n = 1)	549 ± 88 (n = 2)	1142 ± 365 (n = 2)	≤ 80
Orotic acid (µg/mg of creatinine)	158.2 ± 141 (n = 5)	206 ± 12.2 (n = 3)	107 ± 102 (n =2)	5.1 ± 0.5 (n = 2)	60 (n = 1)	≤ 14

Values are expressed as mean ± SD.

Table 4. Amino acids profile of UCD patients diagnosed in a referral hospital in Brazil.

UCD	Patient	Amino acid profile in blood
OTCD	1*	Citrulline ↓ Alanine ↑
OTCD	2*	Citrulline ↓ Glutamine ↑ Alanine ↑
OTCD	3*	Citrulline ↓
OTCD	4*	Citrulline ↓
OTCD	5	Citrulline ↓ Ornithine ↑ Alanine ↑ Glutamine ↑
OTCD	6	Citrulline ↓
OTCD	7[#]	Citrulline ↓ Alanine ↑ Lysine ↑
OTCD	8	Citrulline ↓ Alanine ↑ Glutamine ↑ Glycine, threonine and arginine ↑ Glycine↑
OTCD	9	Citrulline ↓ Alanine ↑ Glutamine ↑
OTCD	10	Citrulline ↓ Glycine ↑ Glutamine ↑
OTCD	11	Citrulline ↓ Glycine ↑
ASS1D	12	Citrulline ↑ Glutamine ↑ Alanine ↑ Tyrosine↓
ASS1D	13	Citrulline ↑
ASS1D	14	Citrulline ↑
ASS1D	15	Citrulline ↑ Methionine ↑ Alanine ↑
ASS1D	16	Citrulline ↑
ASS1D	17	Citrulline ↑ Glycine ↑ Alanine ↑ Methionine ↑ Glycine, threonine and arginine ↑
ASS1D	18	Citrulline ↑ Alanine ↑ Glutamine ↑

		Glycine, threonine and arginine ↑
ASS1D	19	Citrulline ↑ Glycine, threonine and arginine ↑
ASS1D	20	Citrulline ↑ Alanine ↑ Glutamine ↑
ASS1D	21	Citrulline ↑
ARG1D1	22	Arginine ↑ Glycine, threonine and arginine ↑
ARG1D	23	Arginine ↑ Glycine, threonine and arginine ↑
ARG1D	24	Arginine ↑
ARG1D	25	Arginine ↑
ARG1D	26	Arginine ↑
CPS1D	27	Citrulline ↓ Tyrosine ↓ Glutamine ↑
CPS1D	28	Citrulline ↓ Glutamine ↑ Alanine ↑ Lysine ↑
ASLD	29	Citrulline ↑ Glutamine ↑
ASLD	30	Citrulline ↑ Glutamine ↑ Ornithine ↓ Leucine ↓ Isoleucine ↓ Methionine ↓ Phenylalanine ↓ Tyrosine ↓

*Members of the same family.
[#]Molecular diagnosis.

Table 5. Alterations in amino acids concentrations in UCD patients diagnosed in a referral hospital in Brazil.

Altered amino acid	OTCD	ASS1D	ARG1D	CPS1D	ASLD	Reference values (μM/L)
Citrulline	3.01 ± 1.45 (n=11)	562.95 ± 554 (n=10)	NR	1.44 ± 0.52 (n=2)	89.3 ± 66 (n=2)	5.60 – 16.26 < 30 days 8.19 – 20.11 > 30 days
Glutamine	1589 ± 413 (n=5)	3541 ± 4929 (n=3)	NR	1110 ± 359 (n=2)	1104 ± 436 (n=2)	376 – 709 < 30 days 246 - 823 > 30 days
Arginine	NR	NR	254 ± 168 (n=5)	NR	NR	4.29 – 22.9 < 30 days 2.63 – 26.5 > 30 days
Alanine	915 ± 456 (n=6)	1468 ± 1758 (n=5)	NR	759 (n=1)	Normal ranges	131 – 710 < 30 days 143 - 547 > 30 days
Ornithine	766 (n=1)	NR	NR	NR	11.5 (n=1)	48 – 211 < 30 days 22 – 163 > 30 days
Lysine	377 (n=1)	NR	NR	586 (n=1)	NR	92 - 325 < 30 days 52 - 284 > 30 days
Glycine + threonine + arginine	786 (n=1)	2307 ± 596 (n=3)	940 ± 10 (n=2)	NR	NR	87 – 647 < 30 days 117 - 707 > 30 days
Glycine	782 ± 79 (n=3)	831 (n=1)	NR	NR	NR	136 - 387 < 30 days 111 – 263 > 30 days
Methionine	NR	48.4 ± 16 (n=2)	NR	NR	6.13 (n=1)	10 – 60 < 30 days 9 – 47 > 30 days
Tyrosine	NR	207 (n=1)	NR	2.3 (n=1)	33.7 (n=1)	55 – 147 < 30 days 22 - 200 > 30 days
Phenylalanine	NR	NR	NR	NR	36 (n=1)	38 – 137 < 30 days 31 - 91 > 30 days
Leucine	NR	NR	NR	NR	34.13 (n=1)	48 – 160 < 30 days 47 - 216 > 30 days
Isoleucine	NR	NR	NR	NR	24 (n=1)	26 – 91 < 30 days 31 - 107 > 30 days

Amino acid values are expressed as mean ± SD.
NR (normal range) - amino acids levels inside the reference values.

Table 6. Therapy of UCD patients in addition to scavenger drugs. Medical and dietary therapy of UCD patients diagnosed in a referral hospital in Brazil before and after diagnosis.

UCD	Patient	Drugs in use	Diet
ASS1D	1	Phenobarbital, domperidone	Not informed
ASS1D	2	Phenobarbital	Not informed
ASS1D	3	AZT, phenobarbital, oxacillin and amikacin, dobutamine, L-carnitine, L-arginine	Low-protein
ASS1D	4	L-carnitine	Low-protein
ASS1D	5	Valproic acid, analgesics, folic acid and L-arginine	Low-protein
ASS1D	6	Phenobarbital	Not informed
OTCD	7	Antibiotic, glucose serum	Not informed
OTCD	8	Phenobarbital, lactulone, metronidazole	Low-protein
OTCD	9	Ampicillin, gentamicin	Not informed
OTCD	10	Ampicillin, gentamicin, dopamine and dobutamine	Not informed
OTCD	11	Carbamazepine, L-arginine and ornithine	Hygh-calorie and low-proteic
ARG1D	12	Phenobarbital	Low-protein

O texto completo da seção 3.2 CAPÍTULO II, que no texto completo da dissertação defendida ocupa o intervalo de páginas compreendido entre as páginas 79 – 108, foi suprimido por tratar-se de manuscrito em preparação para publicação em periódico científico. Consta de um estudo avaliando o perfil oxidativo e inflamatório em pacientes portadores de DCU. Através de dosagens de biomarcadores em amostras de plasma destes pacientes, verificou-se uma diminuição de espécies reativas de nitrogênio, apontando um forte envolvimento da via de síntese de óxido nítrico na fisiopatologia destas doenças. A presença de dano oxidativo a proteínas e lipídeos foi demonstrada através da verificação do aumento de espécies reativas ao ácido tiobarbitúrico (TBA-RS) e proteínas carboniladas, respectivamente. Ademais, citocinas pró- e anti-inflamatórias encontravam-se aumentadas nestes indivíduos, indicando a ocorrência de um estado pró-inflamatório nas DCU.

4. DISCUSSÃO

Os erros inatos do metabolismo (EIM) são doenças metabólicas hereditárias que cursam com a interrupção de uma via metabólica, devido a deficiência na atividade de enzimas, ocasionando assim uma falha na síntese, degradação, armazenamento e transporte de moléculas no organismo (SCRIVER *et al.,* 2001). Os EIM se classificam conforme a área do metabolismo afetada, sendo um destes grupos compostos pelos EIM intermediários, doenças que culminam com intoxicação aguda ou crônica, demonstrando relação evidente com a ingesta alimentar (SAUDUBRAY & CHARPENTIER, 2001), no qual estão inseridas as DCU.

Atualmente, há uma escassez de estudos delineando o perfil dos pacientes brasileiros portadores de DCU, e neste contexto, o presente trabalho objetivou ampliar os conhecimentos relacionados às suas principais características e elucidar com maior clareza o perfil dos mesmos, auxiliando todo o corpo hospitalar responsável no rápido reconhecimento de sinais e sintomas, principais biomarcadores, e manejo da doença (Capítulo I), assim como, compreender melhor o envolvimento do estresse oxidativo e inflamação, investigando parâmetros de dano nitrosativo, peroxidação lipídica, dano oxidativo à proteínas e perfil de inflamação, na fisiopatologia destas doenças (Capítulo II).

Sabe-se que a suspeita clínica é o primeiro passo para se diagnosticar um EIM, entretanto, a ausência de sintomas clínicos específicos nestas doenças muitas vezes dificulta seu diagnóstico (SCHWARTZ *et al*., 2008). O baixo conhecimento dos profissionais responsáveis pelo manejo destes pacientes também influencia na ausência de um diagnóstico acurado, muitas vezes levando uma grande parcela de EIMs a um diagnóstico errôneo (SOUZA *et al*., 2007).

Na década de 90, a espectrometria de massas em tandem (LC/MS/MS) possibilitou um grande avanço no diagnóstico de doenças hereditárias

relacionadas ao metabolismo de aminoácidos, oxidação de ácidos graxos e acidemias orgânicas, através da análise de aminoácidos e acilcarnitinas em papel filtro (MATERN, 2002), possibilitando o diagnóstico de múltiplos EIM simultaneamente e em questão de minutos (CARPENTER E WILEY, 2002), e proporcionando assim um grande salto nos Programas de Triagem Neonatal (PTN) do mundo todo.

Entretanto, até o presente momento, certos grupos de doenças hereditárias ainda não possuem painel analítico completamente validado devido à baixa especificidade e sensibilidade na detecção de determinados analitos cruciais à suspeita diagnóstica.

No caso das DCU, aminoácidos determinantes no diagnóstico de DNAGS, DCPS1 e DOTC, como baixos níveis de citrulina e instabilidade da glutamina (CAVICCHI *et al.,* 2009), podem acarretar muitos falsos positivos e negativos, e, portanto, muitos países ainda não realizam sua triagem neonatal. Já a DASL, DASS1 e DCITRIN se baseiam na detecção de altos níveis de citrulina, com ou sem aumento de ácido argininosuccínico e representam a parcela de DCU mais triadas ao redor do mundo, entretanto, falsos positivos podem ocorrer, uma vez que, algumas doenças como a deficiência de dihidrolipoamida desidrogenase (HAVIV *et al.,* 2014) e a deficiência de piruvato carboxilase (RABIER *et al.,* 2006) também podem cursar com aumento de citrulina. Triagem para doenças como DARG1 e DORN1 continuam a ser avaliadas, uma vez que a arginina (CEDERBAUM *et al.,* 1982) e a ornitina (HALL *et al.,* 2014) podem estar em níveis normais nos primeiros dias de vida destes pacientes. Além disso, muitos pacientes com DCU, especialmente neonatos em crise, requerem diagnóstico imediato, pois o surgimento e grave manifestação da doença ocorre logo nas primeiras horas de vida.

O Capítulo I desta dissertação demonstra o perfil clínico e laboratorial de pacientes portadores de Doença do Ciclo da Ureia diagnosticados no Hospital de Clínicas de Porto Alegre, um centro de referência no diagnóstico de doenças genéticas no Brasil. Desde sua implementação até janeiro de 2022 foram diagnosticados 30 pacientes portadores de DCU, provenientes de todas as regiões do Brasil. Não houve casos de diagnóstico de DNAGS, e deficiência

de transportadores de citrina e ornitina, devido à demasiada raridade destas doenças. Na maioria dos casos os pacientes eram provenientes de regiões mais centralizadas do Brasil como Sul e Sudeste, o que pode inferir uma possível influência de fatores socioeconômicos na taxa de diagnósticos destas doenças. O grupo de pacientes demonstrou taxa de mortalidade considerável (23,3%), salientando a importância da urgência na identificação destas doenças para um manejo precoce, visando o melhor prognóstico possível ao paciente. Os sinais e sintomas mais frequentes nos pacientes foram estresse respiratório, níveis alterados de consciência, falta de apetite, convulsões, vômito, encefalopatia aguda e coma, na maioria dos casos combinados com níveis elevados de amônia, o principal biomarcador destas doenças. A sepse neonatal é o diagnóstico errôneo mais comum em pacientes com manifestações precoces de DCU. Além disso, outras doenças que cursam com o aumento de amônia também podem gerar diagnósticos incorretos (LEONARD& MORRIS, 2006; ELLAWAY, 2002) tais como, acidemias orgânicas e defeitos na oxidação de ácidos graxos mitocondriais (RIBAS *et al.,* 2021). Portanto, uma vez que a hiperamonemia é confirmada, além das dosagens de aminoácidos e ácido orótico, se faz também necessária a dosagem de acilcarnitinas e ácidos orgânicos para diagnóstico diferencial entre EIMs.

O perfil de aminoácidos dos pacientes foi característico de cada DCU. Pacientes portadores de DASS1 e DASL cursavam com aumento de citrulina, estando os níveis deste aminoácido consideravelmente mais elevados em DASS1 em comparação à DASL. O diagnóstico diferencial entre estas duas doenças podem ser realizadas através da detecção do ácido argininosuccínico, presente somente em pacientes DASL (WILCKEN *et al.,* 2004). A DCITRIN possui quadro laboratorial e clínico semelhante à DASS1, e, portanto, a pesquisa de gene é indicada para sua diferenciação (GAO *et al.,* 2003). Portadores de DOTC apresentaram níveis diminuídos de citrulina além de elevada taxa de ácido orótico. Diversos EIM cursam com aumento deste biomarcador, entretanto, no caso de suspeita de DCU, a dosagem deste composto se torna essencial, uma vez que pode ser usada para diferenciar

DOTC e DCPS1, estando elevada apenas na DOTC (GRUNEWALD *et al.,* 2004). Pacientes com DARG1 apresentaram níveis elevados de arginina, ao oposto de outras DCU, as quais possuem a síntese deste aminoácido prejudicada, necessitando de suplementação (BRUSILOW & BATSHAW, 1979). Não houve casos de diagnóstico de DNAGS, entretanto vale salientar que esta doença possui perfil clínico e laboratorial idêntico à DCPS1, apresentando hiperamonemia primária e níveis de glutamina aumentados, assim como citrulina e arginina diminuídas e ácido orótico normal, sendo imprescindível a análise enzimática ou molecular para seu diagnóstico diferencial quando se há suspeita (SANCHO-VAELLO *et al.,* 2016; RAPP *et al.,* 2002), uma vez que a DNAGS é a única DCU com possibilidade de cura total, a partir da administração do carbamilglutamato (GUFFON *et al.,* 2005). A síndrome HHH cursa com aumento de amônia, ornitina, e homocitrulina na urina, frequentemente acompanhado à elevados níveis de ácido orótico e sua confirmação pode ser realizada através de identificação de mutação (SALVI *et al.,* 2001) ou por avaliação da ornitina marcada isotopicamente e incorporada em proteína de fibroblastos de pele cultivados (ou tecido hepático) (SHIH *et al*., 1982).

A amônia média apresentada pelos pacientes deste estudo demonstrou-se dez vezes maior que o valor de referência normal (média ± SD: 860 ± 470 µmol/L; valor de referência: ≤ 80 µmol/L), este fator, associado à tenra idade dos pacientes, sendo grande parte menor de 30 dias de vida, corrobora com os graves desfechos clínicos encontrados.

Nosso estudo também demonstrou o perfil clínico de uma família portadora de DOTC, composta por mãe, dois filhos meninos e uma filha menina, na qual as duas integrantes femininas heterozigotas, por serem apenas carreadoras do gene deficiente, se apresentam assintomáticas. Em contrapartida, os meninos apresentavam grave sintomatologia no momento do diagnóstico, tendo um deles vindo à óbito. Na maioria dos casos, pacientes heterozigotas não apresentam perfil clínico e laboratorial sugestivo de DOTC, podendo apresentar perfil de aminoácidos normal, e, portanto, fazendo-se necessário o diagnóstico molecular para confirmação diagnóstica. Entretanto,

cabe salientar que, apesar da herança ligada ao X da doença, a literatura relata diversos casos de mulheres que apresentam quadro clínico tão grave quanto aos apresentados por homens afetados (TEUFEL *et al.,* 2012; SCAGLIA *et al.,* 2002).

Por fim, cabe salientar que de forma pioneira nossos resultados apresentam um perfil completo de pacientes portadores de DCU brasileiros, um estudo necessário para auxiliar médicos e laboratoristas no diagnóstico urgente e acurado deste grupo de doenças.

A toxicidade dos metabólitos acumulados contribui para a fisiopatologia das DCU, levando ao grave dano neurológico causado pelos altos níveis de amônia ao cérebro, responsável por provocar déficits neurológicos e dano cerebral, principalmente em neonatos. A apresentação clínica depende e se correlaciona com os níveis de amônia ao qual o cérebro foi exposto e por quanto tempo, assim como pela maturidade do sistema nervoso central do indivíduo afetado.

Os mecanismos envolvidos na toxicidade da hiperamonemia ao cérebro ainda não estão completamente elucidados, mas sabe-se que a mesma interfere em diversas vias cerebrais de maneira altamente complexa, contribuindo para um sinergismo fisiopatológico entre todas estas vias. A investigação da fisiopatologia da hiperamonemia se torna ainda mais desafiadora quando está associada a encefalopatia hepática, bastante comum em pacientes com DCU, uma vez que os mecanismos tóxicos da amônia se confundem com aqueles causados pelo distúrbio hepático.

Sabe-se que a detoxificação da amônia cerebral é realizada principalmente através da ação da glutamina sintase, formando glutamina, e que este aminoácido se encontra aumentado no fluído extracelular de pacientes com dano hepático (TOFTENG *et al.,* 2022) e em modelos animais de hiperamonemia (BRAISSANT *et al.,* 2013). Entretanto, a circulação de níveis exacerbados de amônia causa uma sobrecarga neste sistema, se tornando insuficiente para prevenir o acúmulo do composto tóxico (COOPER *et al.,* 2012; DESJARDINS *et al.,* 2012).

O edema cerebral causado pelo desequilíbrio osmótico devido ao aumento de concentração de NH_4^+ cerebral, acarreta o surgimento do inchaço dos astrócitos e de lesões simétricas no parênquima (GROPMAN *et al.,* 2007). O edema também é capaz de aumentar a pressão intracranial e consequentemente resultar em hérnia uncal e compressão do tronco cerebral (BUTTERWORTH, 1998). Com o inchaço dos astrócitos, canais de cloreto liberam osmólitos, na tentativa de reduzir o gradiente osmótico presente. Vias de neurotransmissores também são afetadas pela hiperamonemia tanto aguda como crônica (BACHMANN *et al.,* 2004). O glutamato proveniente dos neurônios glutamatérgicos se liga aos receptores N-metil-D-aspartato (NMDA) e AMPA nos neurônios pós receptores, promovendo a entrada exacerbada de eletrólitos (Na^+ K^+, Ca^{2+}). A ativação de receptores NMDA pode aumentar a produção de óxido nítrico e consequentemente cGMP, sendo a ativação excessiva destes receptores responsável por morte celular excitotóxica (BRAISSANT *et al.,* 2013; FELIPO *et al.,* 2002). Os neurotransmissores GABAérgicos, aumentando a corrente cloreto GABA-induzida, podem inativar bombas de extrusão de cloreto neuronais. Há também um aumento na ligação de agonistas do GABA e componentes benzodiazepínicos modulatórios (HA *et al.,* 1996). Altos níveis de amônia também podem aumentar a produção de neuroesteroides, sendo estes produzidos pelos astrócitos e micróglia em resposta a ativação de receptores na membrana mitocondrial externa (CAGNIN *et al.,* 2006). Os sistemas serotoninérgicos e colinérgicos também são influenciados pelo aumento de amônia cerebral, aumentando a liberação de 5-hidroxitriptamina (5HT) (FELIPO, 2002), podendo ser um dos responsáveis pela presença de falta de apetite e vômitos nestas doenças (BACHMANN *et al.,* 2004), e aumentando a atividade da acetilcolinesterase (AChE) na falha hepática aguda (RATNAKUMARI *et al.,* 1994).

A amônia também aumenta a expressão de GLUT1, responsável por levar glicose ao cérebro (FELIPO, 2002). Altos níveis de amônia foram capazes de inibir a piruvato desidrogenase e estimular glicólise em ratos com falha hepática aguda (BRUSILOW *et al.,* 2010; RAMA RAO *et al.,* 2012). O aumento de lactato cerebral também é observado em várias doenças que cursam com hiperamonemia (BRUSILOW *et al.,* 2010; BUTTERWORTH, 1998; FELIPO,

2002; BOSOI *et al.,* 2014). A glutamina também se demonstrou aumentada em estudos relacionando animais com hiperamonemia e em estágios de coma na encefalopatia, sugerindo alta do fluxo anaplerótico associado à detoxificação da amônia (ZWINGMANN *et al.,* 2007).

Distúrbios energéticos também ocorrem na hiperamonemia. Uma grande parcela do 2-oxoglutarato proveniente do ciclo do ácido cítrico é utilizada para suprir o glutamato necessário para detoxificar amônia cerebral, estando o 2-oxoglutarato aumentado em cérebros de animais com severa encefalopatia hepática. Este composto pode ser reposto em parte pela produção anaplerótica de oxalacetato, até que haja uma saturação desta via. Astrócitos expostos a altos níveis de amônia podem induzir a transição da permeabilidade mitocondrial, consequentemente causando morte celular (FELIPO, 2002; Rama RAO *et al.,* 2012).

A literatura apresenta fortes indicativos de que dano oxidativo e dano nitrosativo possuem papel importante na fisiopatologia de doenças que cursam com hiperamonemia (RAMA RAO *et al.,* 2012). A amônia e a glutamina aumentam a produção de espécies reativas de oxigênio pelos astrócitos, e superóxido e peroxidação lipídica também pode estar aumentadas em casos de hiperamonemia (KOSENKO *et al.,*1997; KOSENKO *et al.,* 2003; JAYAKUMAR *et al.,* 2004; SATHYASAIKUMAR *et al.,* 2007), provavelmente devido a alterações nas atividades de moléculas protetoras à EROs, tais como, redução de glutationa, e aumento na expressão e atividade da catalase e glutationa peroxidase (KOSENKO *et al.,*1997; KOSENKO *et al.,* 2003; JAYAKUMAR *et al.,* 2004; SATHYASAIKUMAR *et al.,* 2007; LICHTER-KONECKI, 2008). A transição da permeabilidade mitocondrial, possivelmente mediada pelo aumento de Ca^{2+} intracelular, assim como o aumento de produção de EROs pela cadeia respiratória, xantinas e aldeído oxidases podem ser os principais causadores desta condição (KOSENKO *et al.,* 2003; Rama RAO E NORENBERG, 2012). A hiperamonemia também pode estimular a síntese de óxido nítrico neuronal (nNOS), através da ativação de receptores NMDA nos astrócitos. Este efeito foi revertido pelo uso de nitroarginina, reduzindo seus efeitos tóxicos em cérebros de ratos (LICHTER-KONECKI *et al.,* 2008; BRAISSANT *et al.,* 2010; FELIPO *et al.,* 2002). Estudos envolvendo

animais com depleção de arginina (presente em algumas DCU), demonstraram uma atividade da NOS e NO reduzida, o que pode ser explicado dado o papel da arginina como precursor na síntese de NO (LICHTER-KONECKI *et al.,* 2008).

Na busca de um melhor compreendimento da complexa fisiopatologia das DCU, foram realizadas neste trabalho uma série de análises utilizando amostras de plasmas de pacientes portadores de DCU, no intuito de avaliar a presença de dano oxidativo e inflamação, uma vez que, se presentes, podem proporcionar uma nova via estratégica para a testagem de tratamentos adjuvantes nestas doenças, tais como, o uso de antioxidantes e anti-inflamatórios.

Foram realizadas dosagem de níveis de nitrito e nitrato, espécies reativas de ácido tiobarbitúrico (TBARS), conteúdo de proteínas carboniladas e determinação de níveis de citocinas (IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, interferon-γ e TNF-α) nestes pacientes. Importante ressaltar que a literatura dispõe de poucos estudos correlacionando fluídos biológicos de pacientes DCU com dano oxidativo e inflamação.

Como citado anteriormente, o EO vem sendo proposto como um dos mecanismos responsáveis pelo dano cerebral presente nas DCU. Ultimamente, o papel do EO em doenças neurodegenerativas tem recebido muita atenção, visto que o cérebro é extremamente suscetível à ação de espécies reativas, devido ao alto consumo de oxigênio, ao alto conteúdo de lipídeos poliinsaturados, ao alto conteúdo de ferro e ao baixo conteúdo de defesas antioxidantes em algumas áreas particulares do cérebro (Halliwell, 1996).

Desta forma, nosso estudo demonstrou níveis de nitrito e nitrato diminuídos em pacientes DCU, quando comparados a indivíduos saudáveis, inferindo uma baixa produção destes analitos em nosso grupo de pacientes e reforçando o envolvimento da via de síntese do óxido nítrico nestas doenças.

As RNS vem sendo alvo de estudos envolvendo as DCU, uma vez que, muitos aminoácidos que se encontram alterados neste grupo de doenças se relacionam diretamente com a via de síntese do óxido nítrico (NO). O NO

desempenha vários papéis fisiológicos, incluindo manutenção do tônus muscular, funções de neurotransmissores e mediações em defesas celulares. NO também interage com sistemas mitocondriais para regular respiração celular e aumentar a produção de RNS, ativando mecanismos de sobrevivência ou morte celular (MORI *et al*., 2004; MONCADA *et al.,* 2006). A síntese deste composto se dá pela ação do óxido nítrico sintase (NOS), que converte arginina e O_2 em citrulina e NO (ANGGARD *et al.,* 1994).

Heterozigotas portadoras de DOTC demonstraram níveis diminuídos de nitrito e nitrato urinários, tendo estes aumentado significativamente com a administração de arginina, entretanto, este aumento não foi o suficiente para atingirem valores normais. Os níveis destes metabólitos foram correlacionados com as apresentações clínicas destes pacientes, uma vez que, NO possui efeito estimulante sob a vasodilatação, migraína e age como neurotransmissor (NAGASAKA *et al.,* 2004). Estudos subsequentes do mesmo grupo de pesquisa correlacionaram a produção de nitrito e nitrato (metabólitos da NO) e dimetilarginina assimétrica (ADMA), um inibidor endógeno da NOs, em pacientes portadores de DOTC, DASS1 e DASL sob reposição oral de arginina, sugerindo que a reposição de arginina é eficaz na manutenção de nitrito e nitrato em níveis normais ou supranormais em pacientes com DOTC e DASL e não demonstrando o mesmo efeito em DASS1, atribuindo este fato ao aumento de ADMA nestes pacientes (NAGASAKA *et al.,* 2009). Além disso, já foi demonstrado que altos níveis de amônia podem estimular a ativação de receptores NMDA, aumentando níveis intracelulares de Ca^{2+} e a atividade dos NOs (MORI *et al.,* 2000; BRAISSANT *et al*., 2010) causando uma superestimulação da via do NO e, portanto, aumentando a produção de RNS e consequentemente causando lesões oxidativas (PARIHAR *et al.,* 2012; GHAFOURIFAR *et al*., 1999; RICHTER *et al.,* 1999). Ademais, estudos envolvendo a DARG1 demonstraram correlação positiva entre dano nitrativo e aumento dos níveis de arginina (SCAGLIA *et al.,* 2004) e as DCU que não cursam com níveis aumentados de arginina, apresentaram baixa produção de NO, devido à diminuição de seu substrato. Estes níveis se demonstraram ainda

mais diminuídos em DCU que cursam com aumento de citrulina (DASS1 e DASL) (BRAISSANT *et al.,* 2010).

Neste sentido, para melhor entender a presença de dano oxidativo, realizamos a dosagem de espécies reativas ao ácido tiobarbitúrico (lipoperoxidação) e proteínas carboniladas em amostras de plasma do grupo DCU em questão, ambas se encontrando aumentadas quando comparadas aos controles.

O malondialdeído quando presente em fluídos biológicos indica a geração de radicais livres e processo de lesão (HALLIWELL *et al.,* 2007). A lipoperoxidação pode induzir alterações na estrutura lipídica da membrana, reduzindo sua seletividade para transporte iônico e sinalização, prejudicando sua permeabilidade e transporte celular podendo resultar em morte celular devido à liberação de enzimas hidrolíticas e formação de produtos citotóxicos (ANDRADE JUNIOR *et al*., 2005; SILVA E FERRARI, 2011). Estudos anteriores utilizando animais demonstraram que a peroxidação lipídica parece ser secundária à excitotoxicidade (KOSENKO *et al.,* 1999; PRESTES *et al.,* 2006), sugerindo que seus efeitos estejam presentes apenas *in vivo*. A hiperamonemia aguda também leva a peroxidação lipídica em fígado e cérebro de ratos (O'CONNOR *et al.,* 1990) sendo associada com aumento de transaminases e diminuição de defesas antioxidantes, como GPx, GSH, SOD e CAT (SUBRAMANIAN *et al*., 2007). Modelos animais de hiperamonemia também apresentaram altos níveis de malondialdeído (MDA) (CHRONI *et al.,* 2006; CARBONERO-AGUILAR *et al*., 2011), e esta espécie também está comprovadamente presente na doença hepática crônica e doença hepática crônica associada à encefalopatia hepática (LEMBERG *et al*., 2007; STARFI *et al.,* 2020; POMACU *et al.,* 2021; MONTES-CARLOS, *et al*., 2020; MOKERJEE *et al*., 2007; GIMENEZ-GARZO *et al*., 2015; ZHENG *et al.,* 2016). O conteúdo de carbonilas plasmáticas também se demonstrou elevado nos pacientes de nosso estudo, inferindo a presença de dano oxidativo proteico. A aderência de grupos carbonila à proteína é muito difícil de reverter devido ao alto peso dos agregados formados e esta alteração pode levar proteínas, enzimas e receptores ao mau funcionamento, perturbando células normais do

metabolismo (STADMAN & LEVINE, 2003; HALLIWELL & GUTTERIDGE, 2015). Nosso achado corrobora com estudos em humanos que detectaram o aumento de proteínas carboniladas na doença hepática aguda e doença hepática aguda associada a encefalopatia hepática (VIDELA *et al.,* 2004; POMACU *et al*., 2021; MONTES-CARLOS *et al*., 2020; GIMENEZ-GARZO *et al*., 2015). Além disso, diversos estudos já demostraram presença de dano oxidativo a proteínas em doenças metabólicas (RIBAS *et al.,* 2010).

Para determinar o perfil inflamatório de nossos pacientes, foi realizada medida de um painel de citocinas (IL-1β, lL-2, IL-4, IL-6, IL-8, IL-10, interferon-γ e TNF-α). A inflamação é caracterizada por ser uma resposta inespecífica a uma agressão, considerada, portanto, uma defesa do organismo contra agentes agressores de origem química, física ou biológica (TILLEY *et al.,* 2001), sendo, portanto, a resposta mais precoce quando há uma lesão tissular ou quadro infeccioso. Os processos inflamatórios são, de certa forma, uma desordem complexa envolvendo diferentes células e componentes moleculares (MURIACH *et al*., 2014). A resposta inflamatória encontra-se intimamente relacionada com as defesas antioxidantes e com o estresse oxidativo. Espécies reativas de oxigênio/nitrogênio (ERO/ERN) são capazes de alterar diferentes vias de sinalização, estimulando a liberação de citocinas e outros mediadores pró-inflamatórios. Da mesma maneira, a sustentação de estados inflamatórios pode promover um aumento do EO (OLSEN *et al.,* 2015; MARINHO *et al.,* 2014; HIGDON *et al*., 2012; OKUN *et al.,* 2011).

Assim, observou-se que os pacientes com DCU apresentaram aumento dos níveis de citocinas pró-inflamatórias IL-6, IL-8, interferon-γ and TNF-α, em relação aos controles, indicando um estado pró-inflamatório nesses pacientes. Nesse contexto, um estudo anterior mostrou aumento das citocinas inflamatórias IL-6 e TNF-α em soro de pacientes com encefalopatia hepática moderada (SRIVASTAVA *et al*., 2011). Outro estudo utilizando culturas de astrócitos indicou que o aumento de citocinas pró-inflamatórias, tais como TNF-α, IL-1β, IL-6, e IFN-γ, e amônia induziram o aumento da permeabilidade da mitocôndria (ALVAREZ *et al.,* 2011), culminando em aumento da disfunção mitocondrial, e sendo um importante fator na fisiopatologia da encefalopatia

hepática e hiperamonemia. Neste contexto, destaca-se também que o aumento de citocinas pró-inflamatórias pode estar relacionado a uma produção excessiva de espécies reativas, contribuindo para a inflamação (LUGRIN, 2014). Devido a sua ingestão insuficiente ou a alta demanda de consumo, pacientes com doenças metabólicas herdadas e inflamatórias geralmente apresentam níveis reduzidos de compostos antioxidantes, podendo ser devido aos altos níveis de ERO ativadas por células imunocompetentes (MESCKA *et al.,* 2015; MANGGE *et al.,* 2014; MUÑOZ & COSTA, 2013).

Outro fator a ser levado em consideração é a ativação e elevação da NOS nas DCU, a qual promove a geração de NO e consequentemente o O_2 e NO produzidos podem resultar em danos nitrosativos e oxidativos (JOZSEF *et al.,* 2006; LUCAS & MAES, 2013). O NF-κB, fator de transcrição responsável pela expressão de numerosos genes associados com a resposta imune inata, também é afetada pela resposta oxidativa. O NF-κB tem um importante papel na resposta pró-inflamatória, principalmente pela ativação de macrófagos, que secretam citocinas importantes, como citocinas inflamatórias IL-1, IL-6, IL-12 e TNF-alfa e anti-inflamatórios IL-10 e IL-13. Além disso, devido ao surgimento de doença hepática, ou até mesmo decorrente da produção exacerbada de espécies reativas de oxigênio, citocinas pró-inflamatórias podem ser liberadas, ativando também uma Sindrome de Resposta Inflamatória Sistêmica (SRIS).

Neste contexto, também vale ressaltar que os astrócitos, células cerebrais mais afetadas na hiperamonemia, também fazem parte da linhagem dos macrófagos e podem secretar um repertório completo de citocinas e fatores neurotróficos aos neurônios. Os astrócitos também possuem papel central na formação da barreira hematoencefálica e no controle do tônus cerebrovascular. Portanto, é concebível que qualquer disfunção nestas células, irá acarretar alteração na secreção de células inflamatórias, assim como, sua ativação derivada de uma possível neuroinflamação, irá impactar na função da barreira hematoencefálica e no tônus cerebrovascular.

Em nosso estudo também foram avaliados os níveis das citocinas anti-inflamatórias IL-4 e IL-10 e nós encontramos os níveis de IL-10 aumentados nestes pacientes. No entanto, não foram encontradas diferenças significativas

entre as concentrações de IL-4 nos grupos testados. Assim, o aumento dos níveis de IL-10, que atua nos macrófagos ativados, reduzindo os efeitos das citocinas IL-1, TNF-alfa, IL-6 e IL-8, inibindo a produção de radicais livres de oxigênio (SOMMER *et al.,* 2010), pode representar uma tentativa de reparar a resposta à inflamação.

Em conjunto, os dados apresentados nesta dissertação demonstram e reforçam a necessidade de um rápido diagnóstico para pacientes portadores de DCU, a fim de se obter um melhor prognóstico, além de apontar o envolvimento do estresse oxidativo e da inflamação como mecanismos tóxicos nestas doenças. Apesar das limitações presentes em nosso trabalho, como pequeno número de pacientes, nossos resultados apontam novas vias terapêuticas a serem exploradas, como por exemplo, o uso de antioxidantes e anti-inflamatórios como adjuvantes no tratamento destes pacientes.

5. CONCLUSÕES

A partir dos resultados obtidos nessa dissertação de mestrado, foi possível concluir:

a) O perfil de pacientes portadores de DCU brasileiros é bastante característico, sendo sua apresentação clínica altamente severa e prejudicial, e potencialmente letal aos pacientes, principalmente quando não diagnosticados e tratados com a devida urgência;
b) A amônia é um biomarcador essencial no diagnóstico das DCU, sendo recomendado a sua dosagem sempre que houver suspeita de EIM;
c) Os níveis de nitrito e nitrato estão diminuídos em pacientes DCU, comprovando o envolvimento destas doenças com a via de síntese do óxido nítrico, uma vez que as DCU cursam com alterações em aminoácidos cruciais para sua síntese. A diminuição de metabólitos instáveis da NO (nitrito e nitrato) em pacientes DCU não significa a ausência de dano nitrosativo, podendo este estar agindo em diversas vias cerebrais complexas e contribuindo para a fisiopatologia destas doenças;
d) O dano oxidativo a lipídeos e proteínas está presente nas DCU, demonstrado através da dosagem de altos níveis de espécies reativas ao ácido tiobarbitúrico (TBARS) e proteínas carboniladas, respectivamente, no plasma destes pacientes e, portanto, comprovando a presença de peroxidação lipídica e o envolvimento do dano oxidativo na fisiopatologia destas doenças;
e) Um estado pró-inflamatório ocorre nas DCU, sendo demonstrado pelas dosagens aumentadas das citocinas IL-6, IL-8, interferon-γ e TNF-α no plasma destes pacientes, e apontando um envolvimento importante deste processo na fisiopatologia destas doenças;
f) É possível inferir que, devido ao favorável estado pró-inflamatório presente nestas doenças, também ocorre a tentativa de reparação deste

sistema, demonstrado através dos elevados níveis da citocina anti-inflamatória IL-10 no plasma destes pacientes.

6. PERSPECTIVAS

Pretendemos dar continuidade a este trabalho realizando análises adicionais, incluindo a dosagem de outros marcadores de estresse oxidativo e avaliando *in vitro* efeitos de antioxidantes em pacientes DCU.

Considerando o exposto, as perspectivas deste trabalho são:

a) Avaliar as defesas antioxidantes enzimáticas (através da medida da atividade das enzimas CAT, SOD, GPx e GR) e não enzimáticas (através da quantificação de GSH) em eritrócitos;

b) Avaliar as defesas antioxidantes não enzimáticas (Status Antioxidante Total –TAS – e Reatividade Antioxidante Total – TAR) no plasma e na urina;

c) Avaliar o dano ao DNA (pelo ensaio cometa com a adição de enzimas e pela dosagem de 8-OHdG em urina);

d) Avaliar o efeito *in vitro* de suplementação com antioxidantes.

7. REFERÊNCIAS

ALVAREZ, V.M.; RAMA RAO, K.V.; BRAHMBHATT, M.; NORENBERG, M.D. Interaction between cytokines and ammonia in the mitochondrial permeability transition in cultured astrocytes. Journal of Neuroscience Research. 89(12) 2028–2040. 2011.

ANGGARD, E. Nitric oxide: mediator, murderer, and medicine. Lancet. 343:1199-206. 1994.

BACHMANN, C.; BRAISSANT, O.; VILLARD, A.M.; BOULAT, O.; HENRY, H. Ammonia toxicity to the brain and creatine. Mol. Genet. Metab. 81 (Suppl. 1) S52–S57. 2004.

BARSCHAK, A.G.; et al. Evidence that oxidative stress is increased in plasma from patients with maple syrup urine disease. Metab Brain Dis. 21:279-286. 2006.

BOSOI, C.R.; ZWINGMANN, C.; MARIN, H.; PARENT-ROBITAILLE, C.; HUYNH, J.; TREMBLAY, M.; et al. Increased brain lactate is central to the development of brain edema in rats with chronic liver disease. J. Hepatol. 60(3) 554–560. 2014.

BRAISSANT, O. Current concepts in the pathogenesis of urea cycle disorders, Mol. Genet. Metab. 100(Suppl. 1)S3–S12. 2010

BRAISSANT, O.; MCLIN, V.A.; CUDALBU, C. Ammonia toxicity to the brain. J Inherit Metab Dis. 36(4):595–612. 2013.

BRUSILOW, S.W.; BATSHAW, M.L. Arginine therapy of argininosuccinase deficiency. Lancet. 1(8108):124-127. 1979.

BRUSILOW, S.W.; HORWICH, A.L. Urea cycle enzymes. In: Scriver CR, Beaudet D, Valle D, Sly WS eds. The Metabolic and Molecular and Bases of Inherited Disease. 8th edn. New York: McGraw-Hill, 1909–1961. 2001

BRUSILOW, S.W.; KOEHLER, R.C.; TRAYSTMAN, R.J.; COOPER, A.J.L.; Astrocyte glutamine synthetase: importance in hyperammonemic syndromes and potential target for therapy. Neurotherapeutics. 7(4)452–470. 2010.

BURGARD, P.; KÖLKER, S.; HAEGE, G.; LINDNER, M.; HOFFMANN, G.F. Neonatal mortality, and outcome at the end of the first year of life in early onset urea cycle disorders—review and meta-analysis of observational studies published over more than 35 years. J Inherit Metab Dis. 39(2):219–29. 2016.

BUTTERWORTH, R.F. Effects of hyperammonaemia on brain function, J. Inherit. Metab. Dis. 21 (Suppl. 1)6–20. 1998.

CAGNIN, A.; TAYLOR-ROBINSON, S.D.; FORTON, D.M; BANATI, R.B. In vivo imaging of cerebral “peripheral benzodiazepine binding sites” in patients with

hepatic encephalopathy, Gut 55(4) 547–553. 2006

CARBONERO-AGUILAR, P.; et al. Brain biomolecules oxidation in portacaval-shunted rats Liver Int. 31 (7)964-969. 2011.

CARPENTER, K.; WILEY, V. Application of tandem mass spectrometry to biochemical genetics and newborn screening. Clin Chim Acta. 322:110. 2002.

CAVICCHI, C.; MALVAGIA, S.; LA MARCA, G., et al. Hypocitrullinemia in expanded newborn screening by LC-MS/MS is not a reliable marker for ornithine transcarbamylase deficiency. J Pharm Biomed Anal. 49(5):1292-1295. 2009.

CEDERBAUM, S.D.; MOEDJONO, S.J.; SHAW, K.N.; CARTER, M.; NAYLOR, E.; WALZER, M. Treatment of hyperargininaemia due to arginase deficiency with a chemically defined diet. J Inherit Metab Dis. 5(2):95-99. 1982.

CHRONI, E.; et al. Brain oxidative stress induced by obstructive jaundice in rats. J. Neuropathol Exp Neurol. 65(2)193-98. 2006.

COOPER, A.J.L The role of glutamine synthetase and glutamate dehydrogenase in cerebral ammonia homeostasis. Neurochem. Res. 37(11)2439–2455. 2012.

DANDEKAR, A.; MENDEZ, R.; ZHANG, K. Cross talk between ER stress, oxidative stress, and inflammation in health and disease. Methods Mol. Biol. 1292: 205–214. 2015.

DE ANDRADE JÚNIOR, D.R.; DE SOUZA, R.B.; DOS SANTOS, S.A.; DE ANDRADE. Os radicais livres de oxigênio e as doenças pulmonares. J Bras Pneumol 31:60–68. 2005.

DE LAS HERAS, J.; ALDÁMIZ-ECHEVARRÍA, L.; MARTÍNEZ-CHANTAR, M.L.; DELGADO, T.C. An update on the use of benzoate, phenylacetate and phenylbutyrate ammonia 19 scavengers for interrogating and modifying liver nitrogen metabolism and its implications in urea cycle disorders and liver disease. Expert Opin Drug Metab Toxicol. 13(4):439–48. 2017.

DELWING, D.; DELWING, D.; BAVARESCO, C.S.; WYSE, A.T. (2008) Protective efect of nitric oxide synthase inhibition or antioxidants on brain oxidative damage caused by intracerebroventricular arginine administration. Brain Res. 1193:120–127. 2008.

DESJARDINS, P.; DU, T.; JIANG, W.; PENG, L.; BUTTERWORTH, R.F. Pathogenesis of hepatic encephalopathy and brain edema in acute liver failure: role of glutamine redefined, Neurochem. Int. 60 (7)690–696. 2012.

DONIDA, B.; et al. Oxidative stress and inflammation in mucopolysaccharidosis type IVA patients treated with enzyme replacement therapy. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease. 1852(5)1012–1019. 2015.

ELLAWAY, C.J.; WILCKEN, B.; CHRISTODOULOU, J. Clinical approach to inborn errors of metabolism presenting in the newborn period. J Paediatr Child Health. 38(5):511-517. 2002.

ENNS, G.M.; BERRY, S.A.; BERRY, G.T.; RHEAD, W.J.; BRUSILOW, S.W.; HAMOSH, A. Survival after treatment with phenylacetate and benzoate for urea-cycle disorders. N Engl J Med. 356(22):2282–92. 2007.

FELIPO, V. BUTTERWORTH, R.F. Neurobiology of ammonia, Prog. Neurobiol. 67(4)259–279. 2002.

GAO, H.Z.; et al. Identification of 16 novel mutations in the argininosuccinate synthetase gene and genotype-phenotype correlation in 38 classical citrullinemia patients. Human Mutation. 22(1)24–34. 2003.

GIMENEZ-GARZO, C.; et al. Is cognitive impairment in cirrhotic patients due to increased peroxynitrite and oxidative stress? Antioxidants Redox Signal. 22(10)871-877. 2007.

GROPMAN, A.L; SUMMAR, M.; LEONARD, J.V. Neurological implications of urea cycle disorders. J. Inherit. Metab. Dis. 30(6)865–869. 2007.

GRUNEWALD, S.; FAIRBANKS, L.; GENET, S.; et al. How reliable is the allopurinol load in detecting carriers for ornithine transcarbamylase deficiency? J Inherit Metab Dis. 27(2):179-186. 2004.

GUFFON, N.; SCHIFF, M.; CHEILLAN, D.; WERMUTH, B.; HÄBERLE, J.; VIANEYSABAN, C. Neonatal hyperammonemia: the N-carbamoyl-Lglutamic acid test. J Pediatr. 147(2):260-262. 2005.

HA, J.H.; BASILE, A.S. Modulation of ligand binding to components of the GABAA receptor complex by ammonia: implications for the pathogenesis of hyperammonemic syndromes, Brain Res. 720 (1–2)35–44. 1996.

HÄBERLE, J.; BURLINA, A.; CHAKRAPANI, A.; DIXON, M.; KARALL, D.; LINDNER, M., et al. Suggested guidelines for the diagnosis and management of urea cycle disorders: First revision. J Inherit Metab Dis. 1–39. 2019.

HALL, P.; MARQUARDT, G.; MCHUGH, D.; et al. Open postanalytical tools improve performance of newborn screening by tandem mass spectrometry. Genet Med. 16(12):889-895. 2014.

HALLIWELL, B. Free radicals, antioxidants and human disease: curiosity, cause or consequence? The Lancet. 344:721-724. 1994.

HALLIWELL, B.; GUTTERIDGE, J.M.C. Oxidative stress: adaptation, damage, repair senescence and death. In: Halliwell B, Gutteridge JMC (eds) Free radicals in biology and medicine. Oxford University Press, Oxford. 2015.

HAVIV, R.; ZEHARIA, A.; BELAICHE, C.; HAIMI COHEN, Y.; SAADA, A. Elevated plasma citrulline: look for dihydrolipoamide dehydrogenase deficiency. Eur J Pediatr. 173:243-245. 2014.

HERMAN, H.; SHAMBAUGH, E. Urea biosynthesis I. The urea cycle and relationships acid cycle. 20:83–7. 2018.

HIGDON, A.; et al. Cell signalling by reactive lipid species: new concepts and molecular mechanisms. The Biochemical Journal. 442(3)453–464. 2012.

HUEMER, M.; CARVALHO, D.R.; BRUM, J.M.; UNAL, O.; COSKUN, T.; WEISFELD-ADAMS, J.D.; SCHRAGER, N.L.; SCHOLL-BURGI, S.; SCHLUNE, A.; DONNER, M.G.; HERSBERGER, M.; GEMPERLE, C.; RIESNER, B.; ULMER, H.; HABERLE, J.; KARALL, D. Clinical phenotype, biochemical profile, and treatment in 19 patients with arginase 1 deficiency, J. Inherit. Metab. Dis. 39 (3)331–340. 2016.

JAYAKUMAR, A.R.; RAMA RAO, K.V.; SCHOUSBOE, A.; NORENBERG, M.D. Glutamine induced free radical production in cultured astrocytes. Glia. 46(3)296–301. 2004.

KESKINEN, P.; SIITONEN, A.; SALO, M. Hereditary urea cycle diseases in Finland. Acta paediatrica. 97(10), 1412–1419. 2008.

KIDO, J.; MATSUMOTO, S.; HÄBERLE, J.; NAKAJIMA, Y.; WADA, Y.; MOCHIZUKI, N.; MURAYAMA, K.; LEE, T.; MOCHIZUKI, H.; WATANABE, Y.; HORIKAWA, R.; KASAHARA, M.; NAKAMURA, K. Long-term outcome of urea cycle disorders: report from a nationwide study in Japan. J Inherit Metab Dis. 44(4):826–837. 2021a.

KIDO. J.; MATSUMOTO, S.; HÄBERLE, J.; INOMATA, Y.; KASAHARA, M.; SAKAMOTO, S.; HORIKAWA, R.; TANEMURA, A.; OKAJIMA, H.; SUZUKI, T.; NAKAMURA, K. Role of liver transplantation in urea cycle disorders: report from a nationwide study in Japan. J Inherit Metab Dis. 2021b.

KOSENKO, E.; KAMINSKY, Y.; KAMINSKY, A.; VALENCIA, M.; LEE, L.; HERMENEGILDO, C.; FELIPO, V. Superoxide production and antioxidant enzymes in ammonia intoxication in rats. Free Radic Res. 27(6):637–644. 1997.

KOSENKO, E.; VENEDIKTOVA, N.; KAMINSKY, Y.; MONTOLIU, C.; FELIPO, V. Sources of oxygen radicals in brain in acute ammonia intoxication in vivo. Brain Res. 981 (1–2)193–200. 2003.

KREBS, H. A.; HENSELEIT, K. Studies on urea formation in the animal organism. Hoppe-Seylers Z. Physiol. Chem. 210:33-66. 1932.

LEMBERG, A.; et al. Involvement of serum apolipoprotein AI and B100 and lecithin cholesterol acyl transferase in alcoholic cirrhotics. Ann. Hepatol, 6(4)227-232. 2007

LEONARD, J.V.; MORRIS, A.A. Diagnosis and early management of inborn errors of metabolism presenting around the time of birth. Acta Paediatr. 95(1):6-14. 2006.

LICHTER-KONECKI, U. Profiling of astrocyte properties in the hyperammonaemic brain: shedding new light on the pathophysiology of the

brain damage in hyperammonaemia, J. Inherit. Metab. Dis. 31(4)492–502. 2008.

LUGRIN, J.; ROSENBLATT-VELIN, N.; PARAPANOV, R.; LIAUDET, L. The role of oxidative stress during inflammatory processes, Biol. Chem. 395 (2) 203–230. 2014.

MARCHETTI, D.P.; et al. Inflammatory profile in X-linked adrenoleukodystrophy patients: Understanding disease progression. Journal of Cellular Biochemistry. 119(1)1223-1232. 2018a.

MARINHO, H.; et al. Hydrogen peroxide sensing, signaling and regulation of transcription factors. Redox biology. 2:535–562. 2014.

MATERN, D. Tandem mass spectrometry in newborn screening. Endocrinologist. 12:507. 2002.

MATÉS, J.M.; PEREZ-GÓMEZ, C.; CASTRO, I.N. Antioxidant enzymes and human diseases. Clin Biochem. 32(8):5595-5603. 1999.

MERRITT, J.L.; BRODY, L.L.; PINO, G.; RINALDO, P. Newborn screening for proximal urea cycle disorders: Current evidence supporting recommendations for newborn screening. Mol Genet Metab. 124(2):109–13. 2018.

MESCKA, C.P.; et al. Investigation of inflammatory profile in MSUD patients: benefit of L-carnitine supplementation. Metabolic Brain Disease. 30(5)1167-74. 2015.

MOHAMED, M.; ESSA.; SUBRAMANIAN, P. Temporal variations of lipid peroxidation products, antioxidants and liver marker enzymes in experimental hyperammonemic rats, Biological Rhythm Research. 38(4)327-332. 2007.

MONCADA, S.; HIGGS, E.A. The discovery of nitric oxide and its role in vascular biology. Br J Pharmacol. 147:S193-201. 2006.

MONTES-CORTES, D.H.; et al. Characterization of oxidative stress and ammonia according to the different grades of hepatic encephalopathy. Dig. Dis. 38 (3)240-250. 2020.

MOOKERJEE, R.P.; et al. Neutrophil dysfunction in alcoholic hepatitis superimposed on cirrhosis is reversible and predicts the outcome. Hepatology. 46 (3) 831-840. 2007.

MORI, M.; GOTOH, T. Arginine metabolic enzymes, nitric oxide and infection. J Nutr. 134:2820S-5S. 2004.

MURIACH, M.; et al. Diabetes and the brain: oxidative stress, inflammation, and autophagy. Oxidative medicine and cellular longevity, 2014:102-158. 2014.

MURTHY, C.R.; RAMA RAO, K.V.; BAI, G.; NORENBERG, M.D. Ammonia induced production of free radicals in primary cultures of rat astrocytes. J Neurosci Res. 66(2):282–288. 2001.

NAGASAKA, H.; et al. Nitric oxide synthesis in ornithine transcarbamylase deficiency: possible involvement of low no synthesis in clinical manifestations of urea cycle defect. J Pediatr. 145(2):259-62. 2004.

NAGASAKA, H.; OKANO Y.; TSUKAHARA, H.; SHIGEMATSU, Y.; MOMOI, T.; YORIFUJI, J.; MIIDA, T.; OHURA, T.; KOBAYASHI, K.; SAHEKI, T.; HIRANO, K.; TAKAYANAGI, M.; YORIFUJI, T. Sustaining hypercitrullinemia, hypercholesterolemia and augmented oxidative stress in Japanese children with aspartate/glutamate carrier isoform 2-citrin-deficiency even during the silent period, Mol. Genet. Metab. 97(1)21–26. 2009.

NAGAMANI, S. C.; EREZ, A.; LEE, B. Argininosuccinate lyase deficiency. Genetics in medicine: official journal of the American College of Medical Genetics. 14(5), 501–507. 2012.

NETTESHEIM, S.; et al. Incidence, disease onset and short-term outcome in urea cycle disorders-cross-border surveillance in Germany, Austria and Switzerland. Orphanet. J. Rare Dis. 12:111. 2017.

OCONNOR, J.E.; AND COSTELL, M. New roles of carnitine metabolism in ammonia cytotoxicity. In Cirrhosis, hepatic encephalopathy, and ammonium toxicity. (eds. S. Grisolia, V. Felipo and M. D. Mifiana), Plenum Press, New York. 183-195. 1990.

OKUN, E.; GRIFFIOEN, K.J.; MATTSON, M.P. Toll-like receptor signaling in neural plasticity and disease. Trends in Neurosciences. 34(5)269–281. 2011.

OLSEN, R. K.J.; CORNELIUS, N.; GREGERSEN, N. Redox signalling and mitochondrial stress responses; lessons from inborn errors of metabolism. Journal of Inherited Metabolic Disease. 38(4)703–719. 2015.

POMACU, M.M.; et al. Interrelation of inflammation and oxidative stress in liver cirrhosis. Exp. Ther. Med. 21(6)602. 2021.

RABIER, D.; TOUATI, G.; CHADEFAUX-VEKEMANS, B.; DE LONLAY, P.; SAUDUBRAY J. Pyruvate Carboxylase Deficiency : Metabolic Characteristics and New Neurological Aspects. Ann Neurol. 59:121-127. 2006.

RAMA RAO, K.V.; NORENBERG, M.D. Brain energy metabolism and mitochondrial dysfunction in acute and chronic hepatic encephalopathy, Neurochem. Int. 60 (7)697–706. 2012.

RAPP, B.; HÄBERLE, J.; LINNEBANK M.; et al. Genetic analysis of carbamoylphosphate synthetase I and ornithine transcarbamylase deficiency using fibroblasts. Eur J Pediatr. 160(5):283-287. 2001.

RATNAKUMARI, L.; QURESHI, I.A.; BUTTERWORTH, R.F. Evidence for cholinergic neuronal loss in brain in congenital ornithine transcarbamylase deficiency, Neurosci. Lett. 178 (1)63–65. 1994.

ROMERO-GÓMEZ, M.; JOVER, M.; GALÁN, J.J.; RUIZ, A. Gut ammonia production and its modulation. Metab Brain Dis 24(1):147–157. 2009.

SALVADOR, M.; HENRIQUES, J.A.P. Radicais livres e a resposta celular ao estresse oxidativo. 1ª edição. Canoas: Editora Ulbra. P.13-110. 2004.

SALVI, S.; SANTORELLI, F.M.; BERTINI, E.; et al. Clinical and molecular findings in hyperornithinemia-hyperammonemia-homocitrullinuria syndrome. Neurology. 57(5):911-914. 2001.

SANCHEZ, A.; CALPENA, A.C.; CLARES, B. Evaluating the oxidative stress in inflammation: role of melatonin, Int. J. Mol. Sci. 16 (8)16981–17004. 2015

SANCHO-VAELLO, E.; MARCO-MARIN, C.; GOUGEARD, N.; et al. Understanding N-acetyl-L-glutamate synthase deficiency: mutational spectrum, impact of clinical mutations on enzyme functionality, and structural considerations. Hum Mutat. 37(7):679-694. 2016.

SATHYASAIKUMAR, K.V.; SWAPNA, I.; REDDY, P.V.; MURTHY, C.R.; DUTTA, A.; SENTHILKUMARAN, B.; et al. Fulminant hepatic failure in rats induces oxidative stress differentially in cerebral cortex, cerebellum and pons medulla, Neurochem. Res. 32 (3)517–524. 2007.

SAUDUBRAY, J.M.; CHARPENTIER, C. Clinical Phenotypes: Diagnosis/Algorithms. In: SCRIVER, C.R.; SLY, W.A.; BEAUDET, A.L.; VALLE, D. The Metabolic and Molecular Bases of Inherited Disease, New York, McGraw-Hill. 2015.

SCAGLIA, F.; ZHENG, Q.; O'BRIEN, W.E.; HENRY, J.; ROSENBERGER, J.; REEDS, P.; LEE, B. An integrated approach to the diagnosis and prospective management of partial ornithine transcarbamylase deficiency, Pediatrics. 109:150-152. 2002.

SCRIVER, C.R.; SLY, W.A.; BEAUDET, A.L.; VALLE, D. The Metabolic and Molecular Bases of Inherited Disease. 2001.

SHIH, V.E.; MANDELL, R.; HERZFELD, A. Defective ornithine metabolism in cultured skin fibroblasts from patients with the syndrome of hyperornithinemia, hyperammonemia and homocitrullinuria. Clin Chim Acta.118(2–3):149-157. 1982.

SILVA, W.J.M.; FERRARI, C.K.B. Metabolismo mitocondrial, radicais livres e envelhecimento. Rev Bras Geriatr e Gerontol. 14:441–451. 2011.

SITTA, A.; et al. Effect of short- and long-term exposition to high phenylalanine blood levels on oxidative damage in phenylketonuric patients. Int J Dev Neurosci. 27:243–247. 2009

SKOWROŃSKA, M.; ZIELIŃSKA, M.; WÓJCIK-STANASZEK, L.; RUSZKIEWICZ, J.; MILATOVIC, D.; ASCHNER, M.; ALBRECHT, J. Ammonia increases paracellular permeability of rat brain endothelial cells by a mechanism encompassing oxidative/nitrosative stress and activation of matrix metalloproteinases. Journal of neurochemistry. 121(1)125–134. 2012.

SOUZA, C.N.; MARTINS, A.M.; D'ALMEIDA, V.; SILVA, L.C.S. Triagem urinária para erros inatos do metabolismo em crianças com atraso no desenvolvimento. Revista Paraense de Medicina. Belém. 21:223-28. 2007.

SRIVASTAVA, S.K.; YADAV, S.K.; YACHHA, M.A.; THOMAS, V.A.; SARASWAT, GUPTA, R.K. Pro-inflammatory cytokines are raised in extrahepatic portal venous obstruction, with minimal hepatic encephalopathy. Journal of Gastroenterology and Hepatology. 26(6)979–986. 2011.

STEPIEN, K.M.; GEBERHIWOT, T.; HENDRIKSZ C.J.; TREACY, E.P. Challenges in diagnosing and managing adult patients with urea cycle disorders. J Inherit Metab Dis. 1136-1146. 2019.

SUMMAR, M. L. Current strategies for the management of neonatal urea cycle disorders. J Pediatr. 138(1Suppl):S30–S39. 2001.

SUMMAR, M.L.; KOELKER, S.; FREEDENBERG, D.; LE MONS, C.; HABERLE, J.; LEE, H.S.; KIRMSE, B. The incidence of urea cycle disorders. Mol Genet Metab. 110: 179–80. 2013.

TEUFEL, U.; WEITZ, J.; FLECHTENMACHER, C.; PRIETSCH, V.; SCHMIDT, J.; HOFFMANN, G.F.; KOLKER, S.; ENGELMANN, G. High urgency liver transplantation in ornithine transcarbamylase deficiency presenting with acute liver failure, Pediatr Transplant. 15:110-5. 2011.

TILLEY, S. L.; COFFMAN, T.M.; KOLLER, B. H. Mixed messages: modulation of inflammation and immune responses by prostaglandins and thromboxanes. The Journal of clinical investigation. 108(1)15–23. 2001.

TOFTENG, F.; HAUERBERG, J.; HANSEN, B.A.; PEDERSEN, C.B.; JØRGENSEN, L.; LARSEN, F.S. Persistent arterial hyperammonemia increases the concentration of glutamine and alanine in the brain and correlates with intracranial pressure in patients with fulminant hepatic failure. J. Cereb. Blood Flow Metab. 26 (1)21–27. 2006.

VARGAS, C.R.; et al. Evidence that oxidative stress in increased in patients with X-linked adrenoleukodistrophy. Biochim Biophys Acta. 1688:26-32. 2004.

VERNON, H.J. Inborn errors of metabolism: Advances in diagnosis and therapy. JAMA Pediatr. 169:778–82. 2015.

VIDELA, L.A.; et al. Oxidative stress-related parameters in the liver of non-alcoholic fatty liver disease patients. Clin. Sci. 106 (3)261-268. 2004.

WAISBREN, S.E.; STEFANATOS, A.K.; OZTURK-HISMI, B. Neuropsychological attributes of urea cycle disorders: A systematic review of the literature. J Inherit Metab Dis. 42(6):1176-1191. 2019.

WALKER, V. Ammonia toxicity and its prevention in inherited defects of the urea cycle. Diabetes, Obes Metab. 11(9):823–35. 2009.

WEINER, I.D.; VERLANDER, J.W. Renal ammonia metabolism and transport. Compr Physiol. 3(1):201–20. 2013.

WILCKEN, B. Problems in the management of urea cycle disorders. Mol Genet Metab. 81(suppl 1):S86-S91. 2004

ZANATTA, A.; VIEGAS, C.M.; TONIN, A.M.; BUSANELLO, E.N.; GRINGS, M.; MOURA, A.P.; LEIPNITZ, G.; WAJNER, M. Disturbance of redox homeostasis by ornithine and homocitrulline in rat cerebellum: a possible mechanism of cerebellar dysfunction in HHH syndrome. Life Sci. 93 (4)161–168. 2013.

ZANATTA, A.; VIEGAS, C. M.; HICKMANN, F. H.; DE OLIVEIRA MONTEIRO, W.; SITTA, A.; DE MOURA COELHO, D.; VARGAS, C. R.; LEIPNITZ, G.; WAJNER, M. Ornithine In Vivo Administration Disrupts Redox Homeostasis and Decreases Synaptic Na(+), K (+)-ATPase Activity in Cerebellum of Adolescent Rats: Implications for the Pathogenesis of Hyperornithinemia-Hyperammonemia-Homocitrullinuria (HHH) Syndrome. Cellular and molecular neurobiology. 35(6), 797–806. 2015.

ZANATTA, A.; RODRIGUES, M.D.; AMARAL, A.U.; SOUZA, D.G.; QUINCOZES-SANTOS, A.; WAJNER, M. Ornithine and homocitrulline impair mitochondrial function, decrease antioxidant defenses and induce cell death in menadione-stressed rat cortical astrocytes: potential mechanisms of neurological dysfunction in HHH syndrome. Neurochem. Res. 41 (9)2190–2198. 2016.

ZHENG, W.; et al. Increased serum malondialdehyde levels are associated with grey matter volume loss in patients with non-alcoholic cirrhosis. Quant. Imag. Med. Surg. 9(2)230. 2019.

ZWINGMANN, C. The anaplerotic flux and ammonia detoxification in hepatic encephalopathy. Metab. Brain Dis. 22 (3–4)235–249. 2007.

8. ANEXOS

8.1 Anexo 1 - Termo de consentimento livre e esclarecido: indivíduos controle

PARA NÃO PORTADORES DE DCU

Nº do projeto GPPG ou CAAE: 43072821.4.0000.5327

Título do Projeto: Avaliação *in vivo* do dano oxidativo a biomoléculas e da inflamação em pacientes portadores de Desordens do Ciclo da Ureia e *in vitro* do dano ao DNA induzido pelos metabólitos acumulados nesta patologia

Você está sendo convidado(a) a participar de uma pesquisa cujo objetivo é investigar a causa da toxicidade apresentada em um grupo de doenças genéticas chamadas Desordens do Ciclo da Ureia (DCU), e entender o mecanismo de moléculas que se encontram em excesso no organismo destes pacientes. Esta pesquisa está sendo realizada pelo Serviço de Genética Médica do Hospital de Clínicas de Porto Alegre (HCPA).

Se você aceitar o convite, sua participação na pesquisa envolverá a coleta de aproximadamente 5mL de sangue (o equivalente a uma colher de chá), os quais serão coletados juntamente às amostras que serão utilizadas para seus testes de acompanhamento, solicitadas rotineiramente pelo seu médico. Também será realizado o acesso ao seu prontuário para coleta de informações referentes a exames de rotina realizados, por isso solicitamos a sua autorização para este acesso.

Você está sendo convidado(a) a participar deste estudo como controle, ou seja, como não portador(a) de DCU. Os resultados dos testes realizados pelo grupo controle serão comparados com os resultados obtidos a partir de amostras de portadores da doença.

Os possíveis riscos ou desconfortos decorrentes da participação na pesquisa são os mesmos envolvidos em uma coleta de sangue para exames laboratoriais de rotina, assim como o possível risco de quebra de confidencialidade de dados. No

entanto, os pesquisadores farão o possível para que isto não ocorra, utilizando um sistema de códigos para identificar os pacientes.

Você não terá benefício direto ao participar deste estudo, porém, poderá contribuir para o aumento do conhecimento sobre o assunto estudado, e, se aplicável, poderá beneficiar futuros pacientes com DCU. O material coletado será única e exclusivamente utilizado para fins do projeto de pesquisa, sendo reservado ao indivíduo participante da pesquisa o acesso às mesmas.

A participação na pesquisa é totalmente voluntária, ou seja, não é obrigatória. Caso você decida não participar, ou ainda, desistir da participação, não haverá nenhum prejuízo ao atendimento que a pessoa recebe ou possa vir a receber na instituição.

Não está previsto nenhum tipo de pagamento pela participação na pesquisa e você não terá nenhum custo com respeito aos procedimentos envolvidos.

Caso ocorra alguma intercorrência ou dano, resultante da participação na pesquisa, você receberá todo o atendimento necessário, sem nenhum custo pessoal.

Os dados coletados durante a pesquisa serão sempre tratados confidencialmente. Os resultados serão apresentados de forma conjunta, sem a identificação dos participantes, ou seja, o seu nome não aparecerá na publicação dos resultados.

Os pesquisadores responsáveis pelo estudo (Profa. Dra. Carmen Regla Vargas e a mestranda Franciele Fátima Lopes) estarão à disposição para o esclarecimento de qualquer dúvida durante todo o andamento da pesquisa, no serviço de Genética Médica do HCPA localizado no 3º andar, Fone: 3359.8011.

Ainda, para maiores informações, você pode contatar o Comitê de Ética em Pesquisa do HCPA, através do telefone (51) 33597640, das 8h às 17h, de segunda à sexta-feira (email: cep@hcpa.edu.br).

Esse Termo é assinado em duas vias, sendo uma para o participante e outra para os pesquisadores.

Nome do participante da pesquisa

_____

Assinatura

_____

Nome do pesquisador que aplicou o Termo

_____

Assinatura

Local e Data: _____

8.2 Anexo 2 - Termo de consentimento livre e esclarecido: pacientes DCU

PARA PORTADORES DE DCU

Nº do projeto GPPG ou CAAE: 43072821.4.0000.5327

Título do Projeto: Avaliação *in vivo* do dano oxidativo a biomoléculas e da inflamação em pacientes portadores de Desordens do Ciclo da Ureia e *in vitro* do dano ao DNA induzido pelos metabólitos acumulados nesta patologia

Você está sendo convidado(a) a participar de uma pesquisa cujo objetivo é investigar a causa da toxicidade apresentada em um grupo de doenças genéticas chamadas Desordens do Ciclo da Ureia (DCU), e entender o mecanismo de moléculas que se encontram em excesso no organismo destes pacientes. Esta pesquisa está sendo realizada pelo Serviço de Genética Médica do Hospital de Clínicas de Porto Alegre (HCPA).

Se você aceitar o convite, sua participação na pesquisa envolverá a coleta de aproximadamente 5mL de sangue (o equivalente a uma colher de chá), os quais serão coletados juntamente às amostras que serão utilizadas para seus testes de acompanhamento, solicitadas rotineiramente pelo seu médico. Também será realizado o acesso ao seu prontuário para coleta de informações referentes a exames de rotina realizados, por isso solicitamos a sua autorização para este acesso.

Você está sendo convidado(a) a participar deste estudo como paciente, ou seja, por ser portador(a) de DCU.

Os possíveis riscos ou desconfortos decorrentes da participação na pesquisa são os mesmos envolvidos em uma coleta de sangue para exames laboratoriais de rotina, assim como o possível risco de quebra de confidencialidade de dados. No entanto, os pesquisadores farão o possível para que isto não ocorra, utilizando um sistema de códigos para identificar os pacientes.

Você não terá benefício direto ao participar deste estudo, porém, poderá contribuir para o aumento do conhecimento sobre o assunto estudado, e, se aplicável, poderá beneficiar futuros pacientes. O material coletado será única e exclusivamente utilizado para fins do projeto de pesquisa, sendo reservado ao indivíduo participante da pesquisa o acesso às mesmas.

A participação na pesquisa é totalmente voluntária, ou seja, não é obrigatória. Caso você decida não participar, ou ainda, desistir da participação, não haverá nenhum prejuízo ao atendimento que você recebe ou possa vir a receber na instituição.

Não está previsto nenhum tipo de pagamento pela participação na pesquisa e você não terá nenhum custo com respeito aos procedimentos envolvidos.

Caso ocorra alguma intercorrência ou dano, resultante da participação na pesquisa, você receberá todo o atendimento necessário, sem nenhum custo pessoal.

Os dados coletados durante a pesquisa serão sempre tratados confidencialmente. Os resultados serão apresentados de forma conjunta, sem a identificação dos participantes, ou seja, o seu nome não aparecerá na publicação dos resultados.

Os pesquisadores responsáveis pelo estudo (Profa. Dra. Carmen Regla Vargas e a mestranda Franciele Fátima Lopes) estarão à disposição para o esclarecimento de qualquer dúvida durante todo o andamento da pesquisa, no serviço de Genética Médica do HCPA localizado no 3º andar, Fone: 3359.8011.

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Esse Termo é assinado em duas vias, sendo uma para o participante e outra para os pesquisadores.

_____

Nome do participante da pesquisa

_____

Assinatura

_____

Nome do pesquisador que aplicou o Termo

_____

Assinatura

Local e Data: _____

8.3 Anexo 3 - Carta de aprovação do comitê de ética do hospital de clínicas de porto alegre

HOSPITAL DE CLÍNICAS DE PORTO ALEGRE

Grupo de Pesquisa e Pós Graduação

Carta de Aprovação

Projeto

2020/0562

Pesquisadores:

CARMEN REGLA VARGAS

BIANCA GOMES DOS REIS	VITORIA VOLFART DA ROCHA	JESSICA LAMBERTY FAVERZANI
FRANCIELE FÁTIMA LOPES	ANGELA SITTA	DANIELLA DE MOURA COELHO

Número de Participantes: 20

Título: Avaliação in vivo do dano oxidativo a biomoléculas e da inflamação em pacientes portadores de Desordens do Ciclo da Ureia e in vitro do dano ao DNA induzido pelos metabólitos acumulados nesta patologia

Este projeto foi APROVADO em seus aspectos éticos, metodológicos, logísticos e financeiros para ser realizado no Hospital de Clínicas de Porto Alegre.
Esta aprovação está baseada nos pareceres dos respectivos Comitês de Ética e do Serviço de Gestão em Pesquisa.

- Os pesquisadores vinculados ao projeto não participaram de qualquer etapa do processo de avalição de seus projetos.

- O pesquisador deverá apresentar relatórios semestrais de acompanhamento e relatório final ao Grupo de Pesquisa e Pós-Graduação (GPPG).

07/05/2021

Assinado digitalmente por:
PATRICIA ASHTON PROLLA
Grupo de Pesquisa e Pós-graduação
06/06/2021 19:35:19

Impresso do sistema AGHUse-Pesquisa por CRISTIAN FIDALGO CABRAL em 07/05/2021 16:46:04

8.4 Anexo 4 – Artigo de revisão publicado em revista internacional (Cellular and molecular neurobiology - JCR: 4,231) intitulado: Hyperammonemia in inherited metabolic diseases

Cellular and Molecular Neurobiology
https://doi.org/10.1007/s10571-021-01156-6

REVIEW PAPER

Hyperammonemia in Inherited Metabolic Diseases

Graziela Schmitt Ribas[1,2] · Franciele Fátima Lopes[2] · Marion Deon[2] · Carmen Regla Vargas[1,2]

Received: 28 June 2021 / Accepted: 10 October 2021

Abstract
Ammonia is a neurotoxic compound which is detoxified through liver enzymes from urea cycle. Several inherited or acquired conditions can elevate ammonia concentrations in blood, causing severe damage to the central nervous system due to the toxic effects exerted by ammonia on the astrocytes. Therefore, hyperammonemic patients present potentially life-threatening neuropsychiatric symptoms, whose severity is related with the hyperammonemia magnitude and duration, as well as the brain maturation stage. Inherited metabolic diseases caused by enzymatic defects that compromise directly or indirectly the urea cycle activity are the main cause of hyperammonemia in the neonatal period. These diseases are mainly represented by the congenital defects of urea cycle, classical organic acidurias, and the defects of mitochondrial fatty acids oxidation, with hyperammonemia being more severe and frequent in the first two groups mentioned. An effective and rapid treatment of hyperammonemia is crucial to prevent irreversible neurological damage and it depends on the understanding of the pathophysiology of the diseases, as well as of the available therapeutic approaches. In this review, the mechanisms underlying the hyperammonemia and neurological dysfunction in urea cycle disorders, organic acidurias, and fatty acids oxidation defects, as well as the therapeutic strategies for the ammonia control will be discussed.

Keywords Ammonia · Hyperammonemia · Urea cycle disorders · Organic acidurias · Defects of fatty acids oxidation

Ammonia Metabolism and Detoxification

Ammonia is an important source of nitrogen for cellular synthesis of proteins and essential compounds, being also important to the pH homeostasis. Ammonia is generated in the intestines from protein digestion and deamination by urease-positive bacteria and microbial deaminase (Stewart and Smith 2007; Bosoi and Rose 2009; Romero-Gómez et al. 2009). Several tissue metabolic reactions produce ammonia in the organism, such as glutamate dehydrogenase, glutaminase, and AMP deaminase activities. Glutaminase has a crucial role in the ammonia production since it converts glutamine to glutamate and ammonia in the intestines, kidneys, and brain (Cooper and Plum 1987; Butterworth 2002; Dasarathy et al. 2017).

Part of the ammonia produced by renal deamidation of glutamine in the proximal tubules is excreted in the urine (50%); however, the kidney can also liberate ammonia to blood (Weiner et al. 2015). Ammonia reabsorption is increased in situations of metabolic alkalosis and/or hypokalemia. Some hormones as mineralocorticoids and glucocorticoids may also impair the ammonia excretion by the kidney (Tchan 2018; Eguchi et al. 2021; Weiner and Verlander 2013). Besides, muscle protein metabolism, particularly during exercise, illness, and sepsis, contributes to the ammonia formation, which is incorporated in the alanine structure to be transported to the liver via the systemic circulation (Cooper and Plum 1987; Graham and MacLean 1992; van Hall et al. 1995).

In most extrahepatic tissues, ammonia reacts with glutamate by the action of the enzyme glutamine synthetase, leading to glutamine formation, which is transported to the liver. Hepatocytes have specific enzymes of the urea cycle (UC) that convert ammonia in the nitrogenous urea product, which is released into the blood to be excreted by the

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kidneys. This enzymatic cycle begins with the mitochondrial reaction between ammonia and bicarbonate (HCO_3^-), catalyzed by carbamylphosphate synthase 1 (CPS1), to form carbamylphosphate. CPS1 is activated by *N*-acetylglutamate (NAG), which is produced from glutamate and acetyl-CoA by *N*-acetyl glutamate synthase (NAGS). In the sequence, ornithine transcarbamylase (OTC) produces citrulline from carbamylphosphate and ornithine. Citrulline is then converted to the amino acid arginine in the cytosol of the hepatocytes through the reactions catalyzed by argininosuccinate synthase 1 (ASS1) and argininosuccinate lyase (ASL). In the final step, arginase cleaves arginine into urea and regenerates the ornithine, making it possible to restart the cycle (Fig. 1) (Walker 2009, 2014).

Therefore, urea cycle function is fundamental to maintain low serum concentrations of ammonia (50–150 µM in preterm neonates, 50–75 µM in term neonates, and lower than 50 µM in adults) (Braissant et al. 2013). Impaired ammonia hepatic detoxification [e.g., liver cirrhosis, acute liver failure, portosystemic shunting, urea cycle disorders (UCD) and UC suppressors drugs] can result in toxic blood levels of ammonia (Felipo and Butterworth 2002; Häberle 2013a, b). In the presence of hepatic dysfunction, hyperammonemic episodes are usually provoked by infections, intestinal bleeding, constipation, surgery, glucocorticoids, excessive use of diuretics or catabolic states associated with high protein degradation (Vilstrup et al. 2014).

Ammonia can circulate as ammonium ion (NH_4^+) or as a small gaseous fraction (NH_3). The ionized form predominates at physiological pH and can cross cellular membranes, including blood–brain barrier (BBB) (Sørensen 2013; Dasarathy et al. 2017). Brain is not able to convert NH_4^+ to urea, so NH_4^+ is maintained at low levels by the astrocytic glutamine synthetase activity (Suárez et al. 2002; Cagnon and Braissant 2007). However, severe liver diseases result in toxic accumulation of ammonia leading to a clinical condition known as hepatic encephalopathy (HE) characterized by neuropsychiatric alterations and coma (Fiati Kenston et al. 2019). The extent of brain damage depends on its maturation stage and on the magnitude and duration of hyperammonemia.

Prolonged hyperammonemia or ammonia blood levels between 200 and 500 µM, especially during the two first years of life, usually induce irreversible brain damage (Bachmann 2003; Enns 2008; Msall et al. 1984; Tuchman et al. 2008; Uchino et al. 1998). Brain abnormalities are frequently observed in neonates and infants with severe hyperammonemia, such as cortical atrophy, ventricular enlargement, demyelination, or gray and white matter hypodensities, which are related with severe cognitive impairment, seizures, and cerebral palsy (Enns 2008; Gropman et al. 2007; Tuchman et al. 2008). In these patients, the origin of hyperammonemia can be associated with genetic diseases, such as UCD, classical organic acidurias (OA), fatty acids oxidation defects (FAOD), pyruvate carboxylase deficiency, tyrosinemia type I, glycogen storage disease and others (Summar and Mew 2018). Early detection and correction of hyperammonemia is crucial for the patient survival and a better prognosis.

The mechanisms underlying the hyperammonemia and neurological dysfunction in UCD, OA, and FAOD, as well

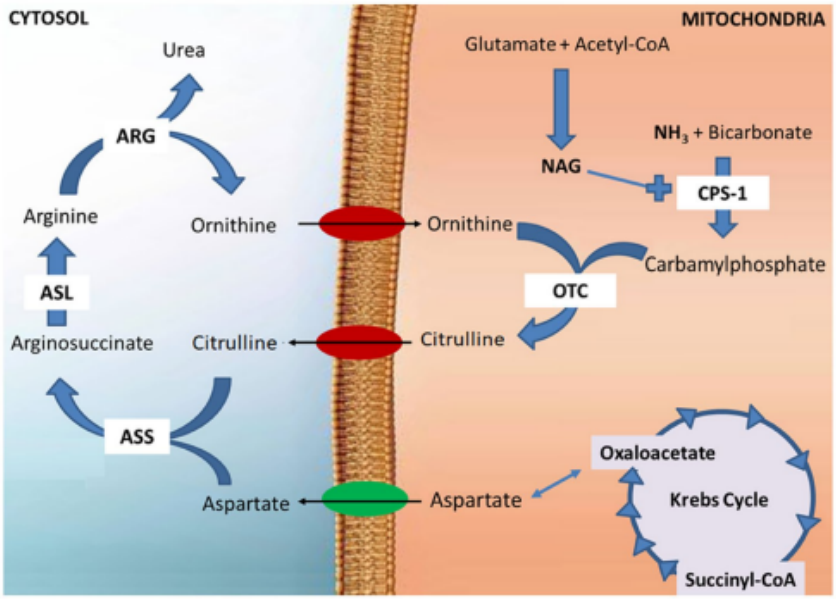

Fig. 1 Urea cycle reactions. The urea cycle initiates in the mitochondria of hepatocytes from the reaction between ammonia and bicarbonate to form carbamylphosphate, which requires *N*-acetylglutamate (NAG) as an allosteric activator. Carbamylphosphate reacts with ornithine to produce citrulline that leaves the mitochondria and originates arginine in the cytosol. Arginine is cleaved by arginase 1 (ARG), restoring the ornithine and forming urea which is eliminated by kidneys. *CPS1* carbamylphosphate synthase; *OTC* ornithine transcarbamylase; *ASS* argininosuccinate synthase; *ASL* argininosuccinate lyase

as the therapeutic strategies for the ammonia control will be discussed in this article review article. In view of the seriousness of these diseases due to the production of toxic metabolites in individuals affected, in order to elucidate the pathophysiological mechanisms of brain dysfunction found in these patients, the present work aimed to review the subject, emphasizing the consequences of toxic metabolism, mainly to the brain tissue. In addition, we intend to address the possible therapeutic targets and approaches.

Mechanisms of Brain Ammonia Toxicity

Ammonia has a central role in the development of brain disturbances during acute or chronic liver diseases and ammonia concentrations above than 150 µmol/L were related with the occurrence of cerebral edema (Bernal et al. 2007; Clemmesen et al. 1999). Ammonia can enter brain cells through K^+ ion channels and transporters (Na^+/K^+ ATPase, H^+/K^+ and $Na^+/K^+/Cl^-$ co-transporters) (Moser 1987; Kelly et al. 2009) or through aquaporin-8 channel (Liu et al. 2006; Saparov et al. 2007) or other ammonia specific transporters (Bakouh et al. 2006). Since astrocytes have a high affinity for potassium (Marcaggi et al. 2004; Bosoi and Rose 2009; Rangroo Thrane et al. 2013), ammonia can compromise astrocyte potassium buffering, resulting in altered brain electrolytic homeostasis (Alger and Nicoll 1983; Brookes and Turner 1993; Marcaggi et al. 2004; Rangroo Thrane et al. 2013).

Many toxic brain effects caused by ammonia excess are related with the accumulation of glutamine in astrocytes. The reaction between ammonia and glutamate by glutamine synthetase in astrocytes protects brain against hyperammonemia; however, this detoxification system creates an osmotic stress in the cells, leading to astrocyte swelling (Hertz and Zielke 2004). This mechanism is related with brain edema and was confirmed in a large number of studies (Gregorios et al. 1985a,b; Ganz et al. 1989; Willard-Mack et al. 1996; Tanigami et al. 2005; Cagnon and Braissant 2007; Jayakumar et al. 2008; Butterworth et al. 2009; Rangroo Thrane et al. 2012). However, astrocyte swelling does not explain all the neurotoxic effects of ammonia and it has been proposed that cerebral aquaporins can regulate the water fluxes, modifying the osmotic effects induced by glutamine (Rama Rao et al. 2010).

Glutamine is taken up by astrocytic mitochondria where it is converted into glutamate and ammonia, which induces changes in pH and in cell membrane potential, rise of intracellular Ca^{2+} levels, inhibition of several bioenergetic pathways and alterations in protein phosphorylation state of various ion channels, transporters, and enzymes (Busa and Nuccitelli 1984; Norenberg 1998; Cudalbu 2013; Rose et al. 2005; Wang et al. 2012; Rangroo Thrane et al. 2013). Ammonia accumulation can disrupt mitochondria metabolism, leading to an excessive production of free radicals (Kosenko et al. 1997; Murthy et al. 2001; Sinke et al. 2008; Norenberg et al. 2009) and nitric oxide (NO) through citrulline-NO cycle (Braissant et al. 1999; Bachmann et al. 2004; Zielinska et al. 2011). These events associated with an increase in mitochondrial Ca^{2+} levels result in the opening of a specific permeability transition pore in the inner mitochondrial membrane, leading to the induction of mitochondrial permeability transition (MPT). MPT provokes a collapse of the mitochondrial inner membrane potential and impairs oxidative phosphorylation and ATP synthesis, leading to the loss of the mitochondria and cell death (Rama Rao et al. 2003; Rama Rao and Norenberg 2012; Albrecht and Norenberg 2006; Jayakumar et al. 2004; Halestrap et al. 1997; Kowaltowski et al. 2001). Ammonia-induced MPT and oxidative stress can also activate the nuclear factor-κB (NF-κB), stimulating an inflammatory cascade that aggravates the neurological damage (Sinke et al. 2008; Marchetti et al. 1996; Bowie and O'Neill 2000; Kyriakis and Avruch 2001).

Ammonia brain toxicity has been also attributed to alterations in the brain mitochondrial energy metabolism, demonstrated by inhibition of α-ketoglutarate dehydrogenase (Lai and Cooper 1986) and other tricarboxylic acid cycle (TCA) enzymes, such as pyruvate dehydrogenase and isocitrate dehydrogenase (Zwingmann et al. 2003). In addition, the detoxification reactions between ammonia and α-ketoglutarate to form glutamate, and subsequently glutamine, cause α-ketoglutarate depletion that compromises Krebs cycle activity (Braissant et al. 2013). Ammonia stimulates glycolysis in astrocytes, as well as it inhibits pyruvate oxidation, resulting in an increased production of brain lactate (Ott et al. 2005; Dam et al. 2013; Bosoi et al. 2014; Zwingmann et al. 2003). High blood and brain lactate levels were verified in patients with HE and hyperammonemia and were associated with increased intracranial pressure and brain edema, the main neurological complications of acute liver failure (Tofteng and Larsen 2004).

Alterations in the neurotransmission also represent an important mechanism of neurological damage induced by hyperammonemia. Astrocytes provide glutamine to the neurons, where it is deaminated into glutamate which initiates an excitatory signal through the activation of the *N*-methyl-D-aspartate (NMDA) receptor. This process is mediated by pH and calcium alterations induced by astrocyte swelling that induce glutamate release from astrocytes, as well as inhibit the reuptake of this neurotransmitter. As consequence, glutamate causes an excessive depolarization of NMDA receptors, inducing alterations in NO metabolism and in Na/K-ATPase activity, that result in mitochondrial dysfunction, oxidative stress, and neuronal apoptosis (Ott and Vilstrup 2014). In animal models of acute liver failure,

the NMDA receptor stimulation and ATP depletion were associated with convulsions and elevated mortality, which were ameliorated by treatment with MK-801, which is a NMDA receptor blocker (Marcaida et al. 1992, 1995). Glutamine is also used by the neurons for the γ-aminobutyric acid (GABA) synthesis, an inhibitory neurotransmitter. After its receptor binding, GABA can be cleared from the synaptic cleft by the astrocytes, where it is recycled into glutamine. Elevated glutamine concentrations in the astrocytes impair this cycle, compromising the GABAergic neurotransmission (Ott and Vilstrup 2014). Clinical evidence reinforces this mechanism since GABA receptor agonists, like barbiturates and benzodiazepines, aggravate the symptoms of HE (Goulenok et al. 2002).

In addition to astrocytes, other cell types have been implicated in the pathophysiology of HE and hyperammonemia (Butterworth 2011). Microglia are immune cells in the brain that release multiple pro-inflammatory mediators when activated (Ransohoff and Cardona 2010). This microglial activation can induce alterations in brain–blood barrier (BBB) permeability leading to brain edema (Jiang et al. 2009a,b; Rangroo Thrane et al. 2012). Therefore, multiple mechanisms can act synergistically to explain the neurological manifestations induced by hyperammonemia, which makes more complex the understanding of these events.

Inherited Disorders Associated with Hyperammonemia

Urea Cycle Disorders (UCD)

Hyperammonemia crisis in UCD patients is the most important clinical hallmark. These diseases occur due to a defect in any of the six enzymes and two transporters involved in the urea cycle pathway, responsible for the detoxification of ammonia in the human body. The enzymes involved in the cycle are located in the mitochondria (CPS1, OMIM #608307; OTC, OMIM #300461) and in the cytosol (ASS, OMIM #603470; ASL, OMIM #608310; ARG, OMIM #608313) and the conversion of ammonia to urea is performed mainly in the liver and to a much lesser extent in the kidney (Brusilow and Maestri 1996; Scriver et al. 2001).

Neonates presenting UCD usually have a total lack or very reduced enzyme activity or a deficient transporter function, whereas late-onset UCD patients still have some residual activity. Individuals affected by this group of diseases, especially neonates, may present severe encephalopathy, which is linearly proportional to the levels and time of the excessive ammonia circulating exposure (Häberle et al. 2019). Usually, newborns exhibit the primary symptoms after the first 24 h of life, when the hyperammonemic crisis can be stimulated by events like the passage from intra to extrauterine life, catabolic events, protein overload, and administration of certain drugs (Häberle et al. 2018). Children who have hyperammonemia in the neonatal period have poor cognitive, adaptive, and behavioral developments (Kido et al. 2012). Late-onset hyperammonemia can be triggered by the same neonatal form events, as for example, protein catabolism, liver failure, exogenous intoxications, portosystemic shunting, "Reye syndrome", and circumstances that improve ammonia or nitrogen supply in human organism.

Primary therapy consists in removing the extra nitrogen from the body, dietary restriction with low protein intake and amino acids supplementation. Liver transplantation (LT) provides a practical cure for UCD but does not revert preexistent neurological damage and despite the efficiency of cure, the need is always evaluated due to the high risks of survival (Soria et al. 2019).

The encephalopathy presented by UCD patients is the main responsible for the high death rate in these diseases. Ammonia penetrates the BBB through diffusion mechanisms (Cooper et al. 1985) and astrocytic cells are responsible for the brain detoxification, performing the conversion of ammonia and glutamate into glutamine, by the action of the enzyme glutamine synthetase (GS), which is excreted without causing any damage (Auron and Brophy 2012). Studies show that individuals with acute liver failure show a swelling in astrocytic cells (Kato et al. 1992), as well as cerebral edema, increasing intracranial pressure. The swelling in astrocytes contributes to several neurological diseases, such as encephalopathy, epilepsy, migraine, among others (Bemeur et al. 2016; Sepherinezad et al. 2020). When ammonia is elevated in the brain, glutamate concentrations also increase and it causes alterations in NMDA receptors, which seems to be responsible for neuronal damage in acute and chronic hyperammonemia (Cooper 2001). The mechanisms involved in this process are still poorly understood, but it is believed that the increase in ammonia circulation has an important role.

Carbamoylphosphate Synthetase 1 Deficiency (CPS1D)

CPS1D is an autosomal recessive disease with an estimated prevalence of 1/1,300,000 live births. The enzyme carbamylphosphate synthase is responsible for catalyzing the synthesis of carbamoyl phosphate from bicarbonate and ammonia, characterizing the first enzymatic reaction of the urea cycle and the entry of ammonia in this pathway (Diez-Fernandez and Häberle 2017). To achieve the active conformation, CPS1 requires NAG as an allosteric activator and the lack of NAGS activity is limiting for the urea cycle, characterizing another UCD. CPS1D and NAGS deficiencies cannot be differentiated just by following biochemical profile and molecular testing is necessary to make the distinction (Nassogne 2005). CPS1D patients have a biochemical

picture characterized by an increase in ammonia and glutamine and a decrease in plasma citrulline and arginine. Untreated hyperammonemia in CPS1D can trigger severe cases of encephalopathy, seizures, coma, and death, as well as severe and irreversible psychomotor disorders.

N-acetylglutamate Synthase Deficiency (NAGSD)

The enzyme NAGS catalyzes the formation of NAG from *N*-acetyl-CoA and glutamate, which acts as an allosteric activator of the enzyme CPS1, the first enzyme limiting in urea cycle. NAGSD is an autosomal recessive disease with an estimated incidence of 1/3,500,000–7,000,000 live births (Summar et al. 2013). NAGSD is characterized by an increase in ammonia, glutamine, and glutamate in plasma, and a decrease in citrulline levels. This deficiency is the only UCD that can be effectively treated by a drug. *N*-carbamylglutamate (NCG) as well as NAG can function as activating co-factor of CPS1 and the oral administration can restore ureagenesis and normalize ammonia levels in affected patients (Bachman et al. 1982; Guffon et al. 1995; Morris 1998; Ah Mew and Caldovic 2011). If untreated, hyperammonemia can provoke neurological deterioration that appears within a few hours to a few months of life (Peoch et al. 2020).

Ornithine Transcarbamylase Deficiency (OTCD)

A partial or total lack of OTC activity originates OTCD, the most frequent UCD, an inherited metabolic disease with an estimated incidence of 1/56,500 live births (Summar et al. 2013). This enzyme is responsible for the formation of citrulline from carbamylphosphate and ornithine. OTCD, unlike the other UCD, is inherited in a X-linked manner and when manifested in neonatal-onset form, patients rapidly develop cerebral edema, seizures, and coma, resulting in high rates of mortality and morbidity. Usually, male patients manifest the most aggressive form of the disease because they have only one X chromosome. The phenotype presented by heterozygous women is quite variable, ranging from classical severe form to asymptomatic (Scaglia et al. 2002). High protein intake and/or infectious damage can trigger acute decompensations on asymptomatic individuals (Nassogne et al. 2005).

The biochemical hallmarks of OTCD are elevated ammonia, glutamine, and alanine, and low levels of arginine and citrulline in plasma accompanied by elevated urinary orotic acid. The encephalopathy caused by the exacerbate levels of ammonia in OTCD compromises motor and mental ability irreversibly and individuals frequently show developmental delays, attention deficit hyperactivity disorder, and cognitive function deficits (Lichter-Konechi et al. 2016).

Liver transplantation may be an alternative for OTCD patients who do not respond to conventional treatment with nitrogen scavenger drugs, diet and amino acid supplementation, and with good metabolic outcome and survival rate (Kim et al. 2013). However, due to the severity, the long-term prognosis for early-onset OTCD patients is poor, and the mortality rate for this disease is still extremely high (Brassier et al. 2015).

Argininosuccinate Synthetase 1 Deficiency (ASS1D)

ASS1 catalyzes the condensation of citrulline and aspartate into argininosuccinate. The poor function of this enzyme causes a defect in the urea cycle, triggering a clinical condition known as ASS1D or citrullinemia type 1 (Häberle et al. 2003; Engel et al. 2009) which is an autosomal recessive disease with an incidence approximately of 1/250,000 live births. Outcomes for citrullinemia type 1 are highly variable and the disease can be presented as neonatal ("classic" form), late onset ("non-classic" form), without any symptoms or hyperammonemia, and postpartum form. ASS1D biochemical profile shows low plasma arginine, elevated plasma and urine citrulline levels, and orotic aciduria. Neonatal hyperammonemic crisis provokes progressively encephalopathy, causing lethargy, poor feeding, vomiting, seizure, and coma, which often leads to death (Häberle et al. 2003).

Citrin (Aspartate–Glutamate Carrier) Deficiency (CTLN2)

Citrin transporter (liver-type aspartate–glutamate carrier isoform 2) is responsible for the exchange of mitochondrial and cytosolic glutamate to provide aspartate supply for argininosuccinate synthetase in the urea cycle. This protein also has an important role in aerobic glycolysis, gluconeogenesis and synthesis of proteins and nucleotides (Saheki et al. 2005). Citrin deficiency occurs with a SLC25A13 gene defect, the human citrin encoding gene (Saheki and Kobayashi 2002).

CTLN2 is an autosomal recessive inherited disorder, presented as three different clinical phenotypes age-dependent: (i) neonatal intrahepatic cholestasis due to citrin deficiency (NICCD); (ii) adult form with recurrent hyperammonemia and neuropsychiatric manifestations (citrullinemia type 2, CTLN2); and (iii) childhood form with failure to thrive and dyslipidemia caused by citrin deficiency (FTTDCD). Citrin deficiency was first described in Asiatic population but currently it is observed in all ethnic groups (Dimmock et al. 2009; Lu et al. 2005). The prevalence of citrullinemia type 2 is still unknown. Hyperammonemic form usually manifests suddenly, in 20–50-year-old adults, who may or may not have a prior history of NICCD or FTTDCD. High levels of ammonia and plasma citrulline are suggestive of CTLN2, as well as

increases in pancreatic secretory trypsin inhibitor (PSTI) (Tsuboi et al. 2001). Clinical manifestations are mainly neuropsychiatric symptoms and include disorientation, delirium, aggression, delusion, loss of memory, and coma that can lead to death by brain edema (Saheki et al. 2005).

Argininosuccinate Lyase Deficiency (ASLD)

The enzyme ASL cleaves argininosuccinic acid to produce arginine and fumarate. Enzyme activity defects lead to ASLD that can manifest on the neonatal or late onset form. Usually, the clinical symptoms appear after the first 24 h of life, with newborns developing lethargy, seizures, coma, and death if this UCD is not recognized and the hyperammonemia is not properly treated. ASLD is the second most common UCD, with a prevalence of 1/70,000 live births and an autosomal recessive inherited pattern. Argininosuccinic acid (ASA) is the laboratorial finding hallmark with urinary concentrations greater than > 10,000 µmol/g of creatinine (normal range 0–1 µmol/g of creatinine). Elevated plasma ammonia and citrulline also are found in patient biochemical profiles. Neurocognitive deficits are a quite common outcome in ASLD with an increased incidence when compared to other UCD (Tuchmann et al. 2008). ASLD patients also demonstrate severe neurological complications regardless of hyperammonemic crisis frequency presented in life, including in patients who did not present the metabolic crisis at all, contrasting with other UCD.

Arginase 1 Deficiency (ARG1D)

Arginase 1 catalyzes the hydrolysis of arginine to ornithine and urea, the final step of ureagenesis. Urea (nontoxic) is then transported and excreted in the urine by kidney function. Ornithine is recycled to continue the cycle for further rounds of urea production (Jackson et al. 1986). The reduced activity of arginase 1 characterizes ARG1D or hyperargininemia, a rare autosomal inborn error of metabolism, with an estimated incidence of 1/950,000 (Summar et al. 2013). Elevated plasma arginine, spasticity, loss of mental and motor skills, failure to thrive and seizures are the most important biochemical and clinical findings. When compared with other UCD, hyperammonemia presented by patients with ARG1D is less frequent and severe, and neonatal presentations are rarely reported (Sin et al. 2015). When present, hyperammonemia is linked to be the cause of ataxia, which is rare and intermittent. The poor neurological and developmental outcomes in ARG1D patients seem to be associated with the accumulated arginine levels (Delwing et al. 2008).

Mitochondrial Ornithine Transporter Deficiency (ORNT1D)

Ornithine transporter deficiency (ORNT1D) is a rare autosomal recessive UCD, also known as hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome with approximately 100 cases reported in literature (Martinelli et al. 2015). When this transporter fails, an inadequate quantity of ornithine is provided to the mitochondrial OTC enzymatic reaction and it provokes the accumulation of cytoplasmic ornithine and lysine. The biochemical hallmarks of this disease are the high levels of ammonia and ornithine in plasma, as well as the high excretion of homocitrulline in the urine, although exceptions have been described since some infants cannot excrete significant amounts and hypoproteic diet helps to reduce this elimination (Valle and Simmel 2001). As in other UCD, ORNT1D patients present hyperammonemia with subsequent neurological abnormalities development. As with ARG1D, this disease shows childhood spastic paraparesis as a differential clinical symptom from other UCD, which manifests a little bit later than in ARG1D.

Organic Acidurias (OA)

OA are inborn errors of intermediary metabolism caused by genetic mutations that compromise the activity of specific enzymes involved in the catabolism of amino acids and additional substrates (odd-chain fatty acids, cholesterol and nucleotides). They represent more than 65 different diseases which are mainly inherited in an autosomal recessive manner and affect in conjunct 1 in 3000 live births (Villani et al. 2017; Wajner 2019). Clinical presentation is variable but neonates or infants typically manifest acute and potentially life-threatening metabolic decompensation, which is triggered by conditions that accelerate catabolism, such as prolonged fasting, vomiting, fever, hemorrhage, or infections (Kölker et al. 2013).

Newborn patients affected with OA usually manifest vomiting, convulsions, lethargy, and hypotonia, which are accompanied by severe laboratorial alterations such as metabolic acidosis, elevated blood lactate, ketonuria, thrombocytopenia, hypoglycemia, and hyperammonemia (Filipowicz et al. 2006; Kölker 2015; Häberle et al. 2018; Wajner 2019). Patients who survive the metabolic decompensation crises can present permanent neurological sequelae that cause mental and motor delays. Furthermore, chronic systemic complications such as pancreatitis, cardiomyopathy, and renal failure can be life-threatening (Deodato et al. 2006; Nizon et al. 2013; Thomas 2015).

The metabolic block caused by enzymatic deficiency leads to the accumulation of characteristic organic acids in tissues and biological fluids, depending on the affected metabolic pathway. This metabolites accumulation is related to the pathophysiology of these diseases and allows

the diagnosis through the analysis of the urinary organic acids profile by gas chromatography coupled to mass spectrometry (GC/MS) (Wajner et al. 2019, 2020).

Classical OA comprehend three types of inherited disorders of branched-chain amino acids: propionic aciduria (PA; MIM# 606054), methylmalonic aciduria (MMA; MIM# 251000), and isovaleric aciduria (IVA; MIM# 243500), which are caused by deficiency of propionyl-CoA carboxylase, methylmalonyl-CoA mutase, and isovaleryl-CoA dehydrogenase, respectively (Ogier De Baulny and Saudubray 2002; Dionisi-Vici et al. 2006). In each one of these diseases, characteristic organic acids accumulate: propionic acid in PA, methylmalonic acid in MMA and isovaleric acid in IVA, as well as their acylglicine and acylcarnitine derivatives (Wajner 2019).

Hyperammonemia is a common finding of these classical OA during metabolic decompensation and it may result from inhibitory effects exerted by the accumulated organic acids in the urea and Krebs cycles (Filipowicz et al. 2006; Häberle et al. 2018). Isovaleryl-CoA, methylmalonyl-CoA, and propionyl-CoA, which are the main metabolites of IVA, PA, and MMA, respectively, may inhibit NAGS and, consequently, limit the CPS1 reaction (Kasapkara et al. 2011; Dercksen et al. 2014). In addition, due to the impaired synthesis of succinyl-CoA in PA and MMA (Fig. 2), glutamine catabolism is accelerated to produce α-ketoglutarate, in an attempt to improve the Krebs cycle activity. Therefore, these metabolic effects can result in chronic hyperammonemia usually accompanied by low glutamine levels (Thomas 2015; Filipowicz et al. 2006; Häberle et al. 2018; Summar and Mew 2018).

Secondary carnitine deficiency that occurs in most organic acidurias can also contribute to hyperammonemia, since low carnitine levels have been associated with a suppressed expression of urea cycle enzymes (Horiuchi et al. 1992; Tomomura et al. 1997). Carnitine supplementation was able to reduce hyperammonemia in patients with carnitine deficiency due to the antiepileptic treatment with valproic acid, reinforcing the important role exerted by this molecule on the blood ammonia control (Maldonado et al. 2017).

There is increasing evidence suggesting that hyperammonemia plays an important role in the neurological dysfunction observed in OA, exerting synergistic toxic effects by accumulated organic acids on cell metabolism (Viegas et al. 2014). Several in vivo and in vitro animal studies have demonstrated that the organic acids accumulated in OA may induce inhibitory effects on the energy metabolism and on the respiratory chain, oxidative stress, and inflammation, resulting in cellular damages that probably contribute to the clinical manifestations of these diseases (Wajner 2019; Wajner et al. 2020). With regards to MMA, it was demonstrated that rats co-injected with ammonia and methylmalonic acid present increased lipid peroxidation in cerebral cortex (Marisco et al. 2003), as well as significant reduction of glutathione (GSH) and sulfhydryl concentrations in cortex and striatum, suggesting that ammonia and methylmalonic acid act in conjunct to promote redox disturbances in the cells (Viegas et al. 2014). Complementing these findings, Royes et al. (2016) showed that ammonia potentiated the duration of convulsive episodes in mice injected with methylmalonic acid and exacerbated the reduction of the mitochondrial membrane potential induced by this organic

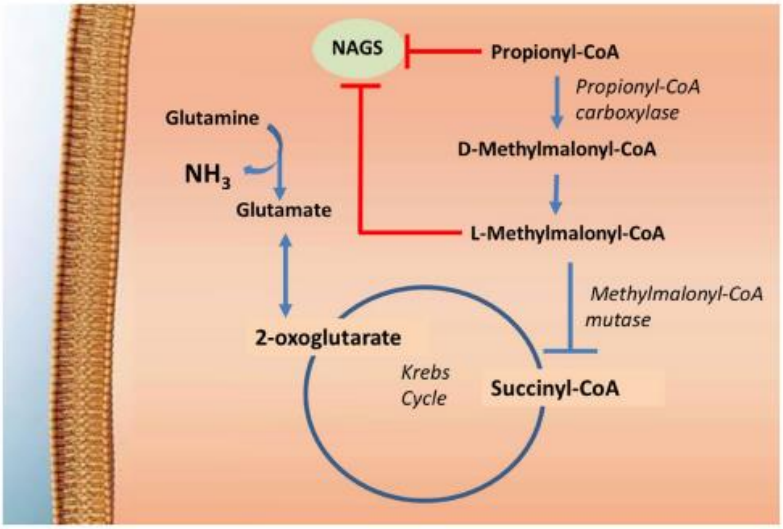

Fig. 2 Mechanisms of hyperammonemia in MMA and PA. Methylmalonyl-CoA and propionyl-CoA, the main metabolites of MMA and PA, respectively, exert inhibitory effects on N-acetyl glutamate synthase (NAGS). Besides, the enzymatic deficiencies involved in these disorders harm the synthesis of succinyl-CoA, therefore favoring the degradation of glutamine to produce 2-oxoglutarate. The consequence of these metabolic events is a high formation of ammonia which accumulates in blood of patients

acid, demonstrating that ammonia aggravates mitochondrial dysfunction in this disorder.

Fatty Acids Oxidation Defects (FAOD)

Fatty acids oxidation (FAO), which the main part takes place in the mitochondria, is essential for energy supply in all tissues, particularly to some organs such as the heart, skeletal muscle, liver, and kidneys. Energy production from fatty acids becomes crucial in periods of catabolic stress related to increased muscular activity, fasting or febrile illness, when most of glucose reserves are consumed by glycolysis. Therefore, fatty acids are used as an alternative energy source when glucose is not available. Fatty acid oxidation comprehends four components: the carnitine cycle, the ß-oxidation cycle, the electron transfer path, and the synthesis of ketone bodies. The mitochondrial β-oxidation cycle consists of four sequential reactions catalyzed by flavin adenine dinucleotide-dependent acyl-CoA dehydrogenases, 2-enoyl-CoA hydratases, nicotinamide adenine dinucleotide-dependent L-3-hydroxyacyl-CoA dehydrogenases, and 3-ketoacyl-CoA thiolases. Each cycle of mitochondrial fatty acid β-oxidation produces one molecule of acetyl-CoA (Scriver et al. 2001; Vishwanath 2016).

FAOD are a group of inborn errors of metabolism (IEM) in which the cells are unable to transport specific molecules or there are deficiencies in enzymes involved in FAO. They are autosomal recessive genetic defects, with an estimated combined incidence of 1:9000 (Kang et al. 2018). In FAOD, the defects can affect any part of the mitochondrial β-oxidation pathway, such as the plasma membrane functions, mitochondrial fatty acid transport or the short-, medium- or long-chain fatty acid β-oxidation pathways. The more prevalent disorders of this group in the clinical practice are medium-chain acyl-CoA dehydrogenase (MCAD), long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD), and very long-chain acyl-CoA dehydrogenase (VLCAD) deficiencies (Ribas and Vargas 2020).

The clinical findings are highly variable with a heterogeneous clinical phenotype at various ages of onset, from neonate to adulthood. There may be a variety of symptoms ranging from mild liver dysfunction, cardiomyopathy, and/or skeletal myopathy to severe liver disease with a recurrent Reye-like syndrome. They include hypoketotic hypoglycemia, lethargy that can lead to coma, seizures, muscle weakness, manifested generally in the neonatal period and after exercise. Cardiac and hepatic alterations also frequently occur. The central nervous system is often affected due to the severity of the hypoglycemia episodes or hyperammonemia, which can be exacerbate under stressors such as fasting, infections, and physical exercise conditions (Houten et al. 2016).

The clinical presentation manifested by these disorders are mainly due to the failure of the metabolism in maintaining a normal energy supply, although toxicity of the accumulating metabolites is growing in importance (Wajner and Amaral 2015). The acetyl-CoA molecules, produced by each cycle of FAO, are the immediate sources of the ketone bodies, therefore a malfunction of any phase of the β-oxidation cascade or the ketone body pathway will produce an insufficient utilization of fatty acids as an energy source. Since gluconeogenesis alone is not sufficient to fulfill the total energy request, the patient will lead up to fasting hypoglycemia. Deficient long-chain and medium-chain enzymes as well as defects of ketogenesis will culminate in hypoketotic hypoglycemia and increased plasma free fatty acid levels. Disorders of short-chain enzymes and of ketone body utilization cause ketotic hypoglycemia. A lack of acetyl-CoA, as observed in the fatty acid oxidation defects, has further outcome: NAG is not produced in adequate amounts, as a result the patient will present hyperammonemia (Fig. 3). Besides, due to the accumulation of fatty acids, hepatic steatosis may develop. This combination of symptoms is usually designated "Reye-like syndrome" (Scriver et al. 2001; Blau et al. 2014).

Hyperammonemia is an important feature of many IEM and is common in patients presenting hypoglycemia, encephalopathy, and liver dysfunction (such as 'Reye-like' illness) (Häberle 2013a, b). Severe hyperammonemia is particularly well known in complete deficiencies of carnitine acylcarnitine translocase (CACT deficiency, OMIM #212138), carnitine palmitoyltransferase II (neonatal CPT II deficiency, OMIM #608836), and multiple acyl-CoA dehydrogenase (MADD, OMIM #231680) (Olpin 2013).

Carnitine cycle defects include a group of inherited disorders in which different enzymes that help to transport the long-chain fatty acids across mitochondrial membranes are deficient causing impairments in transport of the fatty acids across mitochondrial membranes. As a result of those defects, long-chain fatty acid oxidation is damaged, leading to increment of unoxidized fatty acyl-CoA molecules in cytosol and urea cycle impairment by reduced acetyl-CoA availability and enzymatic inhibition of CPS1 (Fig. 3) (Blau et al. 2014; Häberle 2013a, b).

In most of defects of carnitine metabolism, hyperammonemic decompensations have been often reported as mild but can be severe in single patients, especially those with a defect of CACT and neonatal carnitine palmitoyltransferase II (CPT II) deficiency. CACT deficiency was first reported in 1992 and many patients died with a chronic progressive liver failure and persistent nonreversible hyperammonemia or because of hypertrophic cardiomyopathy and septal heart defects (Rinaldo et al. 2002). CPT II deficiency (neonatal CPT II deficiency, OMIM #608836) results from mutations that either compromise translocation and processing or

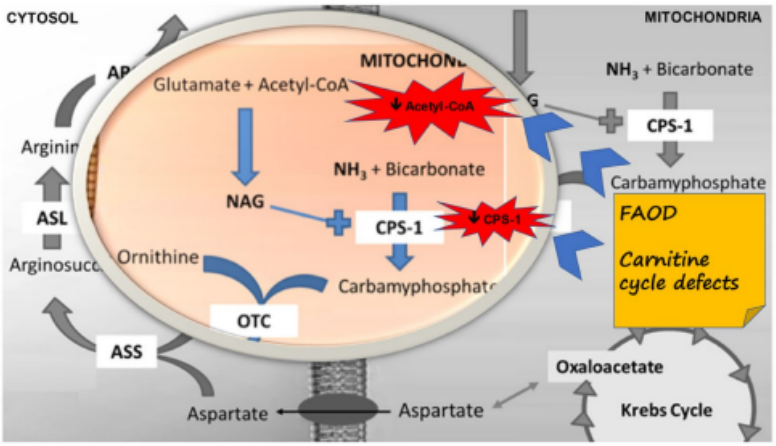

Fig. 3 Suggested mechanisms of hyperammonemia in fatty acid oxidation disorders (FAOD) based on Fig. 1. In FAOD and carnitine cycle defects, the reduced acetyl-CoA availability (reduced availability of *N*-acetylglutamate (NAG) precursors) and the enzymatic inhibition of carbamylphosphate synthase 1 (CPS1; first enzyme of the urea cycle, which transforms ammonia to carbamylphosphate) impair the urea cycle detoxification capacity. The result of these restrictions processes can contribute to hyperammonemia in FAOD

yield a mature enzyme with no measurable residual activity. These affected patients present coma within a few days of birth due to hypoglycemia and hyperammonemia, which may overreach 1000 µmol/L (Rinaldo et al. 2002; North et al. 1995). The phenotype for this severe disorder involves multiple organs and congenital anomalies and often results in neonatal death. Besides, it is important to highlight that severe nutritional carnitine deficiency can have the same inhibition effect on urea cycle, resulting in hyperammonemic encephalopathy as detected in single patients (Limketkai and Zucker 2008).

Multiple acyl-CoA dehydrogenase deficiency, also known as Glutaric Aciduria type 2 (GA-II), with a worldwide prevalence of 1:200,000, results from the deficiency of electron transfer flavoprotein (ETF alfa and beta) or electron transfer flavoprotein/ubiquinone oxidoreductase (ETF/QO) that transport the electrons from β-oxidation to the respiratory chain. MADD may be accompanied by various symptoms including vomiting, hypotonia, hyperammonemia, hepatomegaly, renal cysts, myopathy, and an odor of sweaty feet and congenital anomalies. Clinical presentation includes three phenotypes: (a) neonatal with congenital anomalies; (b) neonatal without congenital anomalies (severe forms) whose manifestation generally occurs within the first 48 h of life and might be fatal; (c) mild and late clinical form that usually appear in the first months until adult life with broad clinical spectrum and better prognosis (Scriver et al. 2001; Olpin 2013).

The most frequent fatty acid oxidation disorder, medium-chain acyl-CoA dehydrogenase deficiency (MCADD, OMIM #201450), is caused by pathogenic variations in the ACADM gene that encodes the flavoenzyme MCAD (Scriver et al. 2001). Patients with MCADD can be asymptomatic or can present a severe phenotype that may lead to coma or sudden death in the first years of life, characterized by metabolic acidosis, lactic acidemia, hypoketotic hypoglycemia, vomiting, seizures, and lethargy, being provoked by fasting or catabolic stress. Part of these symptoms can be explained by the deficient conversion of fatty acids into acetyl-CoA, which is a substrate for the Krebs cycle. Hepatomegaly and hyperammonemia are also observed in patients with MCADD, contributing to the disruption of brain functions and development of encephalopathy. Myopathy, hypotonia, chronic muscle weakness, and rhabdomyolysis are also common findings in the acute episodes (Anderson et al. 2020; Rinaldo et al. 2002). Hyperammonemia is generally milder, but there are exceptions, such as an adult with MCADD who presented with plasma ammonia concentration of 390 µmol/L, mild hypoglycemia, and cardiac arrhythmias (Feillet et al. 2003). In MCADD, while many symptoms can be assigned to the accumulation of toxic long-chain or medium-chain acylcarnitines, hyperammonemia is probably caused due to the lack of acetyl-CoA resulting from acylcarnitines degradation blockade (Corkey et al. 1988). Moreover, long- chain fatty acyl-CoAs, specifically palmitoyl-CoA, have been described to induce fatty acylation of an active site cysteine residue of CPS1 and successfully hinder CPS1 activity (Fig. 3) (Corvi et al. 2001). This fatty acylation must be considered as part of a strategy from the body during starvation to save nitrogen; however it might be fatal particularly in (very) long-chain acyl-CoA oxidation defects (Häberle 2013a, b).

Even though some OA and FAOD may also cause hyperammonemia, high ammonia levels are much less commonly seen in FAOD (Blau et al. 2014; Olpin 2013). Ammonia concentrations are normal between metabolic decompensation, except in the severe defects mentioned above (Häberle 2013a, b).

It is probable, therefore, that various mechanisms contribute to the hyperammonemia in FAOD (Fig. 3), as in the

OA. In defects of carnitine metabolism, disruption of the carnitine cycle results in the accumulation of unoxidized fatty acyl-CoA esters in the cytosol, which are believed to inhibit the urea cycle. The principal mechanism, however, is probably that FAOD reduce the production of acetyl- CoA. This leads to reduced synthesis of NAG and, therefore, hinders urea cycle function, as described for OA and NAGS deficiency (Häberle 2013a, b).

Hyperammonemia Treatment and New Therapeutic Perspectives

Hyperammonemia is a potentially fatal condition, therefore the acute episodes must be immediately corrected to avoid irreversible neurological sequelae in affected patients (even before a definitive diagnosis). Several strategies can be used to correct or control ammonia levels in the blood, that include ammonia removal by dialysis, use of ammonia scavengers [like sodium benzoate (SB), sodium phenylacetate (SP), sodium phenylbutyrate (NaPB) or glycerol phenylbutyrate (GPB)], supplementation with specific amino acids (arginine and citrulline) to stimulate the residual activity of the urea cycle, use of antibiotics (neomycin) and lactulose to reduce the ammonia production by enteric bacteria and nutritional interventions, such as limited protein intake associated with a hypercaloric glucose solution ingestion to enhance anabolism (Auron and Brophy 2012).

Dialysis is the ideal method for rapid ammonia removal and can be performed by hemodialysis and/or continuous renal replacement therapy. Due to the need of specialized centers to perform these procedures and possible complications that can occur in the neonates (thrombosis and bleeding), peritoneal dialysis can be an alternative, especially in patients with less severe forms of hyperammonemia (Schaefer et al. 1999; Arbeiter et al. 2010).

Ammonia scavengers are a therapeutic option in hyperammonemic patients with normal renal function. SP and SB are intravenously administered (250 mg/kg of each drug), while NaPB and GPB can be orally administered. SP acts increasing the renal excretion of glutamine in the form of phenylacetylglutamine, while SB combines with glycine to eliminate hippuric acid in the urine, stimulating the glycine replacement and, consequently, the nitrogen consumption. Although SP and SB are able to maintain normal levels of plasma ammonia, they can cause secondary biochemical alterations, such as potassium depletion (in case of SP use), as well as metabolic acidosis, hypernatremia, and hyperbilirubinemia (with SB infusion) (Summar 2001; Auron and Brophy 2012; Häberle et al. 2019). This way, NaPB and GPB represent a safe alternative for both adult and pediatric patients and positive results in the chronic management of UCD were related in different clinical trials (Lichter-Konecki et al. 2011; Smith et al. 2013). NaPB undergoes liver β-oxidation to phenylacetate after absorption. GPB is an ester pro-drug of phenylbutyrate which is hydrolyzed by pancreatic lipases releasing the active molecule (phenylacetate) which is absorbed more slowly when compared with NaPB. Besides ammonia control, long-term treatment (12 months) with GPB also significantly improved neurocognitive function in pediatric UCD patients (Diaz et al. 2013).

Arginine supplementation (100–250 mg/kg/day) is essential in UCD that compromise the synthesis of this amino acid (CPS1, NAGS, OTC, ASS, and ASL deficiencies). However, when administered in large amounts it can accumulate and to induce nitric oxide production, which is a potent vasodilator and can lead to symptomatic hypotension (Batshaw et al. 2001; Häberle et al. 2019). Likewise, citrulline can be provided (100–200 mg/kg/day) for CPS or OTC deficient patients in an attempt to enhance nitrogen clearance by the urea cycle (Summar 2001). Hyperammonemic patients with propionic or methylmalonic acidurias can be treated with NCG, which is a *N*-acetylglutamate analog that helps to increase the NAGS activity (Filippi et al. 2010). Besides, in PA and MMA patients, L-carnitine treatment (50–150 mg/kg/day) has also an essential role, not only to improve the ammonia control through stimulation of urea cycle enzymes, but mostly to prevent secondary carnitine deficiency and to stimulate the renal excretion of the accumulated organic acids (Di Donato et al. 1984; Ogier de Baulny and Saudubray 2002). Acetyl-L-carnitine, a pro-drug of L-carnitine, was able to reduce ammonia concentrations and to improve cognitive functions in patients with HE (Malaguarnera et al. 2011). In addition to all these properties, L-carnitine exerts antioxidant and anti-inflammatory effects that can possibly improve or prevent neurological damages induced by ammonia excess, needing further investigation (Ribas et al. 2014; Kazak and Yarim 2017).

The disaccharide lactulose is not metabolized in the small intestine and is converted in lactic and acetic acids into the colon. The resultant pH alteration reduces the growth of enteric bacteria that metabolize urea to ammonia and, due to the stimulation of peristalsis, fecal ammonia loss is increased (Vince and Burridge 1980; Weber et al. 1987). The aminoglycoside neomycin eliminates proteolytic bacteria that produce ammonia, therefore contributing to the hyperammonemia reduction; however, severe adverse effects like ototoxicity and renal tubular toxicity can occur, limiting its long-term therapeutic use (Leise et al. 2014; Matoori and Leroux 2015).

In this context, considering the mechanisms related with brain damage induced by ammonia, new approaches have been tested in an attempt to provide neuroprotective effects, including memantine administration (an antagonist of the NMDA receptor) to prevent ammonia-induced

excitotoxicity, as well as the use of L-ornithine-L-aspartate (LOLA) to enhance the hepatic urea synthesis and the muscular glutamine synthetase activity. However, although oral and intravenous LOLA therapy reduced blood ammonia and improved cognitive function in HE patients, some works have not confirmed these benefits, therefore LOLA is not currently recommended for hyperammonemia treatment (Rodrigo et al. 2009; Acharya et al. 2009; Monfort et al. 2009; Kircheis 2016).

Liver transplantation (LT) has been indicated for patients with severe urea cycle disorders that can result in liver failure and cannot be satisfactorily controlled by conventional therapies. When performed in the first months of life, LT may increase the survival rate of the patients, as well as it reduces baseline blood ammonia levels in patients with UCD, then preventing recurrent hyperammonemia attacks. However, newborn patients with OTCD and CPS1D with initial blood ammonia levels ≥ 360 µmol/L may not achieve normal neurodevelopmental, suggesting that other factors than blood ammonia are involved in the brain damage, which are not influenced by hemodialysis or LT (Kido et al. 2021a,b). For newborns with ASSD (even with initial blood ammonia levels ≥ 360 µmol/L) or other UCD with plasma ammonia levels lower than 360 µmol/L, LT was very effective in the general condition improvement of the patients, including neurodevelopmental outcome (Kido et al. 2021b).

Due to the substantial risks for peri- and post-operative complications, as well as the limited availability of liver donors, hepatocyte transplantation was introduced in 1997 in an attempt to prevent metabolic alterations in patients with different inborn errors of metabolism (Strom et al. 1997; Meyburg et al. 2010). Although a reduction of hyperammonemic crises and an improvement of neurodevelopment have been observed with this procedure (Kido et al. 2018; Meyburg and Hoffman 2010; Leonard and McKiernan 2004), several factors limit its use in the clinical practice. The need for invasive procedures to perform portal infusions, as well as the risk of extrahepatic spread of transplanted hepatocytes (mostly to the lung) led to an increased interest in the use of liver stem-like cells (HLSCs) with hepatic differentiation capability (Spada et al. 2020). In 2017, it was reported that HLSC-derived extracellular vesicles were able to correct ureagenesis in an in vitro model of ASS1D (Herrera Sanchez et al. 2017). More recently, a phase I clinical study (Spada et al. 2020) showed that HLSCs administered percutaneously into liver parenchyma of 3 patients with neonatal-onset hyperammonemia due to ASLD or MMA was safe and able to prevent ammonia decompensation. However, more studies are still necessary to better evaluate the long-term effectiveness of this treatment.

Finally, gene therapy has emerged as a promising approach for patients with inherited disorders, since the introduction of a gene capable of encoding a functional enzyme of the urea cycle, can restore the cycle activity avoiding long-term hyperammonemia. Preclinical studies showed that the intravenous administration of adeno-associated virus2/8 coding for OTC was able to restore the enzymatic activity and to control ammonia levels in a mouse model of OTC deficiency (Ye et al. 1996; Wang et al. 2012; Moscioni et al. 2006; Brunetti-Pierri et al. 2008). In 2002, a pilot study in patients with OTCD showed that the infusion of E1- and E4-deleted vector based on adenovirus type 5 and containing human OTC cDNA resulted in transgene expression in hepatocytes of 7 of 17 subjects, although metabolic correction has not been observed at the tested doses (Raper et al. 2002). Besides, the 18th patient recruited in this study died due to a systemic inflammatory response that resulted in disseminated intravascular coagulation and multi-organ failure.

In relation to ASS1D, it was shown that mice treated with adenoviral vectors expressing ASS1 lived significantly longer than untreated animals, although they have not presented an improved clinical outcome (Patejunas et al. 1998). Besides, measurements of the flux of nitrogen from orally administered $15NH_4$ to [15N]urea revealed that the systemic administration of a adenoviral vector expressing human ASS resulted in a partial correction of the enzyme defect (Lee et al. 1999). Promising findings were also described for argininosuccinic aciduria, since the neonatal administration of a codon-optimized human ASL gene packaged within adeno-associated virus serotype 8 led to an increased survival and metabolic correction in a mouse model of this disease (Ashley et al. 2018). In mice knockout for arginase 1, gene therapy improved hyperammonemia and prolonged the animal's survival (Truong et al. 2019). Taken together, these preliminary findings show that significant therapeutic effects can be obtained with minimal enzymatic activity; however, the safety and clinical efficacy of gene therapy still needs to be better investigated so that we can conclude about the cost–benefit of this treatment.

Final Considerations

A considerable amount of evidence demonstrates the seriousness of inherited metabolic diseases that course with hyperammonemia, such as urea cycle diseases, mitochondrial fatty acid beta-oxidation defects, and some organic acidemias, especially propionic, methylmalonic, and isovaleric aciduria. Among these, the diseases of the urea cycle are certainly the ones that are more severe and can quickly lead to death if not diagnosed and treated early. Furthermore, in metabolic diseases that progress with hyperammonemia, adherence to treatment is essential for a good prognosis, considering that high brain levels of ammonia lead to tissue damage and severe neurological and cognitive

manifestations. The therapy used to reduce ammonia and the mechanisms involved in its toxicity (altered brain electrolytic homeostasis, accumulation of glutamine in astrocytes, free radical production, mitochondrial energy metabolism, altered NMDA and GABA neurotransmission) which can act synergistically to explain the neurological manifestations induced by hyperammonemia are discussed in this review. The diagnosis of inborn errors of metabolism that lead to hyperammonemia presupposes the measurement of metabolites in biological fluids and, sometimes, enzymatic assays and molecular tests, which must be carried out quickly. Ammonia levels are critical to diagnosis and should nevertheless precede this laboratory investigation. It should be noted that in addition to the current therapies of protein dietary restriction and supplementation with SB, SP, or NaPBA, the development of new therapeutic strategies aiming to decrease the high ammonia levels may be beneficial in the future to ameliorate disease progression and improve neurological symptomatology in inherited metabolic disorders.

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References

Acharya SK, Bhatia V, Sreenivas V, Khanal S, Panda SK (2009) Efficacy of L-ornithine L-aspartate in acute liver failure: a double-blind, randomized, placebo-controlled study. Gastroenterology 136(7):2159–2168. https://doi.org/10.1053/j.gastro.2009.02.050

Ah Mew N, Caldovic L (2011) *N*-acetylglutamate synthase deficiency: an insight into the genetics, epidemiology, pathophysiology, and treatment. Appl Clin Genet 4:127–135. https://doi.org/10.2147/TACG.S12702

Albrecht J, Norenberg MD (2006) Glutamine: a Trojan horse in ammonia neurotoxicity. Hepatology 44(4):788–794. https://doi.org/10.1002/hep.21357

Alger BE, Nicoll RA (1983) Ammonia does not selectively block IPSPs in rat hippocampal pyramidal cells. J Neurophysiol 49(6):1381–1391. https://doi.org/10.1152/jn.1983.49.6.1381

Anderson DR, Viau K, Botto LD, Pasquali M, Longo N (2020) Clinical and biochemical outcomes of patients with medium-chain acyl-CoA dehydrogenase deficiency. Mol Genet Metab 129:13–19. https://doi.org/10.1016/j.ymgme.2019.11.006

Arbeiter AK, Kranz B, Wingen AM, Bonzel KE, Dohna-Schwake C, Hanssler L, Neudorf U, Hoyer PF, Büscher R (2010) Continuous venovenous haemodialysis (CVVHD) and continuous peritoneal dialysis (CPD) in the acute management of 21 children with inborn errors of metabolism. Nephrol Dial Transpl 25(4):1257–1265. https://doi.org/10.1093/ndt/gfp595

Ashley SN, Nordin JML, Buza EL, Greig JA, Wilson JM (2018) Adeno-associated viral gene therapy corrects a mouse model of argininosuccinic aciduria. Mol Genet Metab 125:241–250. https://doi.org/10.1016/j.ymgme.2018.08.013

Auron A, Brophy PD (2012) Hyperammonemia in review: pathophysiology, diagnosis, and treatment. Pediatr Nephrol 27(2):207–222. https://doi.org/10.1007/s00467-011-1838-5

Bachmann C (2003) Long-term outcome of patients with urea cycle disorders and the question of neonatal screening. Eur J Pediatr 162(Suppl 1):S29-33. https://doi.org/10.1007/s00431-003-1347-z

Bachmann C, Colombo JP, Jaggi K (1982) N-acetylglutamate synthetase (NAGS) deficiency: diagnosis, clinical observations and treatment. Adv Exp Med Biol 153:39–45. https://doi.org/10.1007/978-1-4757-6903-6_6

Bachmann C, Braissant O, Villard AM, Boulat O, Henry H (2004) Ammonia toxicity to the brain and creatine. Mol Genet Metab 81(Suppl 1):S52–S57. https://doi.org/10.1016/j.ymgme.2003.10.014

Bakouh N, Benjelloun F, Cherif-Zahar B, Planelles G (2006) The challenge of understanding ammonium homeostasis and the role of the Rh glycoproteins. Transfus Clin Biol 13(1–2):139–146. https://doi.org/10.1016/j.tracli.2006.02.008

Batshaw ML, MacArthur RB, Tuchman M (2001) Alternative pathway therapy for urea cycle disorders: twenty years later. J Pediatr 138(1 Suppl):S46-54. https://doi.org/10.1067/mpd.2001.111836

Bemeur C, Cudalbu C, Dam G, Thrane AS, Cooper AJ, Rose CF (2016) Brain edema: a valid endpoint for measuring hepatic encephalopathy? Metab Brain Dis 31:1249–1258. https://doi.org/10.1007/s11011-016-9843-9

Bernal W, Hall C, Karvellas CJ, Auzinger G, Sizer E, Wendon J (2007) Arterial ammonia and clinical risk factors for encephalopathy and intracranial hypertension in acute liver failure. Hepatology 46(6):1844–1852. https://doi.org/10.1002/hep.21838

Blau N, Duran M, Gibson KM, Dionisi-Vici C (2014) Physician's guide to the diagnosis, treatment and follow-up of inherited metabolic diseases. Springer, Berlin

Bosoi CR, Rose CF (2009) Identifying the direct effects of ammonia on the brain. Metab Brain Dis 24(1):95–102. https://doi.org/10.1007/s11011-008-9112-7

Bosoi CR, Zwingmann C, Marin H, Parent-Robitaille C, Huynh J, Tremblay M, Rose CF (2014) Increased brain lactate is central to the development of brain edema in rats with chronic liver disease. J Hepatol 60(3):554–560. https://doi.org/10.1016/j.jhep.2013.10.011

Bowie A, O'Neill LA (2000) The interleukin-1 receptor/Toll-like receptor superfamily: signal generators for pro-inflammatory interleukins and microbial products. J Leukoc Biol 67(4):508–514. https://doi.org/10.1002/jlb.67.4.508

Braissant O, Honegger P, Loup M, Iwase K, Takiguchi M, Bachmann C (1999) Hyperammonemia: regulation of argininosuccinate synthetase and argininosuccinate lyase genes in aggregating

cell cultures of fetal rat brain. Neurosci Lett 266(2):89–92. https://doi.org/10.1016/S0304-3940(99)00274-8
Braissant O, McLin VA, Cudalbu C (2013) Ammonia toxicity to the brain. J Inherit Metab Dis 36(4):595–612. https://doi.org/10.1007/s10545-012-9546-2
Brassier A, Gobin S, Arnoux JB et al (2015) Long-term outcomes in ornithine transcarbamylase deficiency: a series of 90 patients. Orphanet J Rare Dis 10:58. https://doi.org/10.1186/s13023-015-0266-1
Brookes N, Turner RJ (1993) Extracellular potassium regulates the glutamine content of astrocytes: mediation by intracellular pH. Neurosci Lett 160(1):73–76. https://doi.org/10.1016/0304-3940(93)9002-7
Brunetti-Pierri N, Clarke C, Mane V, Palmer DJ, Lanpher B, Sun Q, O'Brien W, Lee B (2008) Phenotypic correction of ornithine transcarbamylase deficiency using low dose helper-dependent adenoviral vectors. J Gene Med 10(8):890–896. https://doi.org/10.1002/jgm.1218
Brusilow SW, Maestri NE (1996) Urea cycle disorders: diagnosis, pathophysiology, and therapy. Adv Pediatr 43:127–170
Busa WB, Nuccitelli R (1984) Metabolic regulation via intracellular pH. Am J Physiol 246(4 Pt 2):R409–R438. https://doi.org/10.1152/ajpregu.1984.246.4.R409
Butterworth RF (2002) Pathophysiology of hepatic encephalopathy: a new look at ammonia. Metab Brain Dis 17(4):221–227. https://doi.org/10.1023/A:1021989230535
Butterworth RF (2011) Neuroinflammation in acute liver failure: mechanisms and novel therapeutic targets. Neurochem Int 59(6):830–836. https://doi.org/10.1016/j.neuint.2011.07.014
Butterworth RF, Norenberg MD, Felipo V, Ferenci P, Albrecht J, Blei AT (2009) Members of the ISHEN commission on experimental models of HE. Experimental models of hepatic encephalopathy: ISHEN guidelines. Liver Int 29(6):783–788. https://doi.org/10.1111/j.1478-3231.2009.02034.x
Cagnon L, Braissant O (2007) Hyperammonemia-induced toxicity for the developing central nervous system. Brain Res Rev 56:183–197. https://doi.org/10.1016/j.brainresrev.2007.06.026
Clemmesen JO, Larsen FS, Kondrup J, Hansen BA, Ott P (1999) Cerebral herniation in patients with acute liver failure is correlated with arterial ammonia concentration. Hepatology 29(3):648–653. https://doi.org/10.1002/hep.510290309
Cooper AJ (2001) Role of glutamine in cerebral nitrogen metabolism and ammonia neurotoxicity. Ment Retard Dev Disabil Res Rev 7:280–286. https://doi.org/10.1002/mrdd.1039
Cooper AJ, Plum F (1987) Biochemistry and physiology of brain ammonia. Physiol Rev 67(2):440–519. https://doi.org/10.1152/physrev.1987.67.2.440
Cooper AJL, Mora SN, Cruz NF, Gelbard AS (1985) Cerebral ammonia metabolism in hyperammonemic rats. J Neurochem 44(6):1716–1723. https://doi.org/10.1111/j.1471-4159.1985.tb07159.x
Corkey BE, Hale DE, Glennon MC, Kelley RI, Coates PM, Kilpatrick L, Stanley CA (1988) Relationship between unusual hepatic acyl coenzyme A profiles and the pathogenesis of Reye syndrome. J Clin Invest 82:782–788. https://doi.org/10.1172/JCI113679
Corvi MM, Soltys CL, Berthiaume LG (2001) Regulation of mitochondrial carbamoyl-phosphate synthetase 1 activity by active site fatty acylation. J Biol Chem 276:45704–45712. https://doi.org/10.1074/jbc.M102766200
Cudalbu C (2013) In vivo studies of brain metabolism in animal models of hepatic encephalopathy using ^{1}H magnetic resonance spectroscopy. Metab Brain Dis 28(2):167–174. https://doi.org/10.1007/s11011-012-9368-9
Dam G, Ott P, Aagaard NK, Vilstrup H (2013) Branched-chain amino acids and muscle ammonia detoxification in cirrhosis. Metab Brain Dis 28(2):217–220. https://doi.org/10.1007/s11011-013-9377-3
Dasarathy S, Mookerjee RP, Rackayova V, Rangroo Thrane V, Vairappan B, Ott P, Rose CF (2017) Ammonia toxicity: from head to toe? Metab Brain Dis 32(2):529–538. https://doi.org/10.1007/s11011-016-9938-3
Delwing D, Delwing D, Bavaresco CS, Wyse AT (2008) Protective effect of nitric oxide synthase inhibition or antioxidants on brain oxidative damage caused by intracerebroventricular arginine administration. Brain Res 1193:120–127. https://doi.org/10.1016/j.brainres.2007.11.052
Deodato F, Boenzi S, Santorelli FM, Dionisi-Vici C (2006) Methylmalonic and propionic aciduria. Am J Med Genet C Semin Med Genet 142C(2):104–112. https://doi.org/10.1002/ajmg.c.30090
Dercksen M, Ijlst L, Duran M, Mienie LJ, van Cruchten A, vander Westhuizen FH, Wanders RJ, (2014) Inhibition of N-acetylglutamate synthase by various monocarboxylic and dicarboxylic short-chain coenzyme A esters and the production of alternative glutamate esters. Biochim Biophys Acta 1842:2510–2516. https://doi.org/10.1016/j.bbadis.2013.04.027
Di Donato S, Rimoldi M, Garavaglia B, Uziel G (1984) Propionylcarnitine excretion in propionic and methylmalonic acidurias: a cause of carnitine deficiency. Clin Chim Acta 139(1):13–21. https://doi.org/10.1016/0009-8981(84)90187-6
Diaz GA, Krivitzky LS, Mokhtarani M, Rhead W, Bartley J, Feigenbaum A, Longo N, Berquist W, Berry SA, Gallagher R, Lichter-Konecki U, Bartholomew D, Harding CO, Cederbaum S, McCandless SE, Smith W, Vockley G, Bart SA, Korson MS, Kronn D, Zori R, Merritt JL (2013) Ammonia control and neurocognitive outcome among urea cycle disorder patients treated with glycerol phenylbutyrate. Hepatology 57:2171–2179. https://doi.org/10.1002/hep.26058
Diez-Fernandez C, Häberle J (2017) Targeting CPS1 in the treatment of Carbamoyl phosphate synthetase 1 (CPS1) deficiency, a urea cycle disorder. Expert Opin Ther Targets 21(4):391–399. https://doi.org/10.1080/14728222.2017.1294685
Dimmock D, Maranda B, Dionisi-Vici C, Wang J, Kleppe S, Fiermonte G, Bai R, Hainline B, Hamosh A, O'Brien WE, Scaglia F, Wong LJ (2009) Citrin deficiency, a perplexing global disorder. Mol Genet Metab 96(1):44–49. https://doi.org/10.1016/j.ymgme.2008.10.007
Dionisi-Vici C, Deodato F, Röschinger W, Rhead W, Wilcken B (2006) "Classical" organic acidurias, propionic aciduria, methylmalonic aciduria and isovaleric aciduria: long-term outcome and effects of expanded newborn screening using tandem mass spectrometry. J Inherit Metab Dis 29(2–3):383–389. https://doi.org/10.1007/s10545-006-0278-z
Eguchi K, Izumi Y, Yasuoka Y, Nakagawa T, Ono M, Maruyama K, Matsuo N, Hiramatsu A, Inoue H, Nakayama Y, Nonoguchi H, Lee HW, Weiner ID, Kakizoe Y, Kuwabara T, Mukoyama M (2021) Regulation of Rhcg, an ammonia transporter, by aldosterone in the kidney. J Endocrinol 249(2):95–112. https://doi.org/10.1530/JOE-20-0267
Engel K, Höhne W, Häberle J (2009) Mutations and polymorphisms in the human argininosuccinate synthetase (ASS1) gene. Hum Mutat 30(3):300–307. https://doi.org/10.1002/humu.20847
Enns GM (2008) Neurologic damage and neurocognitive dysfunction in urea cycle disorders. Semin Pediatr Neurol 15(3):132–139. https://doi.org/10.1016/j.spen.2008.05.007
Feillet F, Steinmann G, Vianey-Saban C et al (2003) Adult presentation of MCAD deficiency revealed by coma and severe arrythmias. Intensive Care Med 29(9):1594–1597. https://doi.org/10.1007/s00134-003-1871-3
Felipo V, Butterworth RF (2002) Neurobiology of ammonia. Prog Neurobiol 67:259–279. https://doi.org/10.1016/S0301-0082(02)00019-9

Fiati Kenston SS, Song X, Li Z, Zhao J (2019) Mechanistic insight, diagnosis, and treatment of ammonia-induced hepatic encephalopathy. J Gastroenterol Hepatol 34(1):31–39. https://doi.org/10.1111/jgh.14408

Filipowicz HR, Ernst SL, Ashurst CL, Pasquali M, Longo N (2006) Metabolic changes associated with hyperammonemia in patients with propionic acidemia. Mol Genet Metab 88:123–130. https://doi.org/10.1016/j.ymgme.2005.11.016

Filippi L, Gozzini E, Fiorini P, Malvagia S, la Marca G, Donati MA (2010) N-carbamylglutamate in emergency management of hyperammonemia in neonatal acute onset propionic and methylmalonic aciduria. Neonatology 97(3):286–290. https://doi.org/10.1159/000255168

Ganz R, Swain M, Traber P, DalCanto M, Butterworth RF, Blei AT (1989) Ammonia-induced swelling of rat cerebral cortical slices: implications for the pathogenesis of brain edema in acute hepatic failure. Metab Brain Dis 4(3):213–223. https://doi.org/10.1007/BF01000297

Goulenok C, Bernard B, Cadranel JF, Thabut D, Di Martino V, Opolon P, Poynard T (2002) Flumazenil vs. placebo in hepatic encephalopathy in patients with cirrhosis: a meta-analysis. Aliment Pharmacol Ther 16(3):361–372. https://doi.org/10.1046/j.1365-2036.2002.01191.x

Graham TE, MacLean DA (1992) Ammonia and amino acid metabolism in human skeletal muscle during exercise. Can J Physiol Pharmacol 70(1):132–141. https://doi.org/10.1139/y92-020

Gregorios JB, Mozes LW, Norenberg MD (1985a) Morphologic effects of ammonia on primary astrocyte cultures. II. Electron microscopic studies. J Neuropathol Exp Neurol 44(4):404–414. https://doi.org/10.1097/00005072-198507000-00004

Gregorios JB, Mozes LW, Norenberg LO, Norenberg MD (1985b) Morphologic effects of ammonia on primary astrocyte cultures. I. Light microscopic studies. J Neuropathol Exp Neurol 44(4):397–403. https://doi.org/10.1097/00005072-198507000-00003

Gropman AL, Summar M, Leonard JV (2007) Neurological implications of urea cycle disorders. J Inherit Metab Dis 30(6):865–879. https://doi.org/10.1007/s10545-007-0709-5

Guffon N, Vianey-Saban C, Bourgeois J, Rabier D, Colombo JP, Guibaud P (1995) A new neonatal case of N-acetylglutamate synthase deficiency treated by carbamylglutamate. J Inherit Metab Dis 18:61–65. https://doi.org/10.1007/BF00711374

Häberle J (2013a) Clinical and biochemical aspects of primary and secondary hyperammonemic disorders. Arch Biochem Biophys 536:101–108. https://doi.org/10.1016/j.abb.2013.04.009

Häberle J (2013b) Clinical and biochemical aspects of primary and secondary hyperammonemic disorders. Arch Biochem Biophys 536(2):101–108. https://doi.org/10.1016/j.abb.2013.04.009

Häberle J, Pauli S, Schmidt E, Schulze-Eilfing B, Berning C, Koch HG (2003) Mild citrullinemia in Caucasians is an allelic variant of argininosuccinate synthetase deficiency (citrullinemia type 1). Mol Genet Metab 80(3):302–306. https://doi.org/10.1016/j.ymgme.2003.08.002

Häberle J, Chakrapani A, Ah Mew N, Longo N (2018) Hyperammonaemia in classic organic acidaemias: a review of the literature and two case histories. Orphanet J Rare Dis 13(1):219. https://doi.org/10.1186/s13023-018-0963-7

Häberle J, Burlina A, Chakrapani A, Dixon M, Karall D, Lindner M, Mandel H, Martinelli D, Pintos-Morell G, Santer R, Skouma A, Servais A, Tal G, Rubio V, Huemer M, Dionisi-Vici C (2019) Suggested guidelines for the diagnosis and management of urea cycle disorders: first revision. J Inherit Metab Dis 42(6):1192–1230. https://doi.org/10.1002/jimd.12100

Halestrap AP, Woodfield KY, Connern CP (1997) Oxidative stress, thiol reagents, and membrane potential modulate the mitochondrial permeability transition by affecting nucleotide binding to the adenine nucleotide translocase. J Biol Chem 272(6):3346–3354. https://doi.org/10.1074/jbc.272.6.3346

Herrera Sanchez MB, Previdi S, Bruno S, Fonsato V, Deregibus MC, Kholia S et al (2017) Extracellular vesicles from human liver stem cells restore argininosuccinate synthase deficiency. Stem Cell Res Ther 8:176. https://doi.org/10.1186/s13287-017-0628-9

Hertz L, Zielke HR (2004) Astrocytic control of glutamatergic activity: astrocytes as stars of the show. Trends Neurosci 27(12):735–743. https://doi.org/10.1016/j.tins.2004.10.008

Horiuchi M, Kobayashi K, Tomomura M, Kuwajima M, Imamura Y, Koizumi T, Nikaido H, Hayakawa J, Saheki T (1992) Carnitine administration to juvenile visceral steatosis mice corrects the suppressed expression of urea cycle enzymes by normalizing their transcription. J Biol Chem 267(8):5032–5035

Houten SM, Violante S, Ventura FV, Wanders RJ (2016) The biochemistry and physiology of mitochondrial fatty acid β-oxidation and its genetic disorders. Annu Rev Physiol 78:23–44. https://doi.org/10.1146/annurev-physiol-021115-105045

Jackson MJ, Beaudet AL, O'Brien WE (1986) Mammalian urea cycle enzymes. Annu Rev Genet 20:431–464. https://doi.org/10.1146/annurev.ge.20.120186.002243

Jayakumar AR, Rama Rao KV, Schousboe A, Norenberg MD (2004) Glutamine-induced free radical production in cultured astrocytes. Glia 46(3):296–301. https://doi.org/10.1002/glia.20003

Jayakumar AR, Liu M, Moriyama M, Ramakrishnan R, Forbush B 3rd, Reddy PV, Norenberg MD (2008) Na–K–Cl Cotransporter-1 in the mechanism of ammonia-induced astrocyte swelling. J Biol Chem 283(49):33874–33882. https://doi.org/10.1074/jbc.M804016200

Jiang W, Desjardins P, Butterworth RF (2009a) Hypothermia attenuates oxidative/nitrosative stress, encephalopathy and brain edema in acute (ischemic) liver failure. Neurochem Int 55(1–3):124–128. https://doi.org/10.1016/j.neuint.2009.02.007

Jiang W, Desjardins P, Butterworth RF (2009b) Direct evidence for central proinflammatory mechanisms in rats with experimental acute liver failure: protective effect of hypothermia. J Cereb Blood Flow Metab 29(5):944–952. https://doi.org/10.1038/jcbfm.2009.18

Kang E, Kim YM, Kang M, Heo SH, Kim GH, Choi IH, Choi JH, Yoo HW, Lee BH (2018) Clinical and genetic characteristics of patients with fatty acid oxidation disorders identified by newborn screening. BMC Pediatr 18(1):103. https://doi.org/10.1186/s12887-018-1069-z

Kasapkara CS, Ezgu FS, Okur I, Tumer L, Biberoglu G, Hasanoglu A (2011) N-carbamylglutamate treatment for acute neonatal hyperammonemia in isovaleric acidemia. Eur J Pediatr 170(6):799–801. https://doi.org/10.1007/s00431-010-1362-9

Kato M, Hughes RD, Keays RT, Williams R (1992) Electron microscopic study of brain capillaries in cerebral edema from fulminant hepatic failure. Hepatology 15:1060–1066. https://doi.org/10.1002/hep.1840150615

Kazak F, Yarim GF (2017) Neuroprotective effects of acetyl-L-carnitine on lipopolysaccharide-induced neuroinflammation in mice: involvement of brain-derived neurotrophic factor. Neurosci Lett 658:32–36. https://doi.org/10.1016/j.neulet.2017.07.059

Kelly T, Kafitz KW, Roderigo C, Rose CR (2009) Ammonium-evoked alterations in intracellular sodium and pH reduce glial glutamate transport activity. Glia 57(9):921–934. https://doi.org/10.1002/glia.20817

Kido J, Nakamura K, Mitsubuchi H, Ohura T, Takayanagi M, Matsuo M, Yoshino M, Shigematsu Y, Yorifuji T, Kasahara M, Horikawa R, Endo F (2012) Long-term outcome and intervention of urea cycle disorders in Japan. J Inherit Metab Dis 35(5):777–785

Kido J, Matsumoto S, Mitsubuchi H, Endo F, Nakamura K (2018) Early liver transplantation in neonatal-onset and moderate urea cycle disorders may lead to normal neurodevelopment.

Metab Brain Dis 33(5):1517–1523. https://doi.org/10.1007/s11011-018-0259-6
Kido J, Matsumoto S, Häberle J, Nakajima Y, Wada Y, Mochizuki N, Murayama K, Lee T, Mochizuki H, Watanabe Y, Horikawa R, Kasahara M, Nakamura K (2021a) Long-term outcome of urea cycle disorders: report from a nationwide study in Japan. J Inherit Metab Dis 44(4):826–837. https://doi.org/10.1002/jimd.12384
Kido J, Matsumoto S, Häberle J, Inomata Y, Kasahara M, Sakamoto S, Horikawa R, Tanemura A, Okajima H, Suzuki T, Nakamura K (2021b) Role of liver transplantation in urea cycle disorders: report from a nationwide study in Japan. J Inherit Metab Dis. https://doi.org/10.1002/jimd.12415
Kim IK, Niemi AK, Krueger C, Bonham CA, Concepcion W, Cowan TM, Enns GM, Esquivel CO (2013) Liver transplantation for urea cycle disorders in pediatric patients: a single-center experience. Pediatr Transpl 17(2):158–167. https://doi.org/10.1111/petr.12041
Kircheis G (2016) Current state of knowledge of hepatic encephalopathy (Part V): clinical efficacy of L-ornithine-L-aspartate in the management of HE. Metab Brain Dis 31(6):1365–1367. https://doi.org/10.1007/s11011-016-9912-0
Kölker S, Burgard P, Sauer SW, Okun JG (2013) Current concepts in organic acidurias: understanding intra- and extracerebral disease manifestation. J Inherit Metab Dis 36:635–644. https://doi.org/10.1007/s10545-013-9600-8
Kölker S, Garcia-Cazorla A, Valayannopoulos V, Lund AM, Burlina AB, Sykut-Cegielska J, Wijburg FA, Teles EL, Zeman J, Dionisi-Vici C, Barić I, Karall D, Augoustides-Savvopoulou P, Aksglaede L, Arnoux JB, Avram P, Baumgartner MR, Blasco-Alonso J, Chabrol B, Chakrapani A, Chapman K, Saladelafont I (2015) The phenotypic spectrum of organic acidurias and urea cycle disorders. Part 1: the initial presentation. J Inherit Metab Dis 38:1041–1057. https://doi.org/10.1007/s10545-015-9839-3
Kosenko E, Kaminsky Y, Kaminsky A, Valencia M, Lee L, Hermenegildo C, Felipo V (1997) Superoxide production and antioxidant enzymes in ammonia intoxication in rats. Free Radic Res 27(6):637–644. https://doi.org/10.3109/10715769709097867
Kowaltowski AJ, Castilho RF, Vercesi AE (2001) Mitochondrial permeability transition and oxidative stress. FEBS Lett 495(1–2):12–15. https://doi.org/10.1016/S0014-5793(01)02316-X
Kyriakis JM, Avruch J (2001) Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. Physiol Rev 81(2):807–869. https://doi.org/10.1152/physrev.2001.81.2.807
Lai JC, Cooper AJ (1986) Brain alpha-ketoglutarate dehydrogenase complex: kinetic properties, regional distribution, and effects of inhibitors. J Neurochem 47(5):1376–1386. https://doi.org/10.1111/j.1471-4159.1986.tb00768.x
Lee B, Dennis JA, Healy PJ, Mull B, Pastore L, Yu H, Aguilar-Cordova E, O'Brien W, Reeds P, Beaudet AL (1999) Hepatocyte gene therapy in a large animal: a neonatal bovine model of citrullinemia. Proc Natl Acad Sci USA 96(7):3981–3986. https://doi.org/10.1073/pnas.96.7.3981
Leise MD, Poterucha JJ, Kamath PS, Kim WR (2014) Management of hepatic encephalopathy in the hospital. Mayo Clin Proc 89(2):241–253. https://doi.org/10.1016/j.mayocp.2013.11.009
Leonard JV, McKiernan PJ (2004) The role of liver transplantation in urea cycle disorders. Mol Genet Metab 81(Suppl 1):S74–S78. https://doi.org/10.1016/j.ymgme.2003.08.027
Lichter-Konecki U, Diaz GA, Merritt JL 2nd, Feigenbaum A, Jomphe C, Marier JF, Beliveau M, Mauney J, Dickinson K, Martinez A, Mokhtarani M, Scharschmidt B, Rhead W (2011) Ammonia control in children with urea cycle disorders (UCDs); phase 2 comparison of sodium phenylbutyrate and glycerol phenylbutyrate. Mol Genet Metab 103(4):323–329. https://doi.org/10.1016/j.ymgme.2011.04.013
Lichter-Konecki U, Caldovic L, Morizono H, Simpson K (2016) Ornithine transcarbamylase deficiency. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Mirzaa G, Amemiya A (eds) GeneReviews. University of Washington, Seattle, pp 1993–2021
Limketkai B, Zucker S (2008) Hyperammonemic encephalopathy caused by carnitine deficiency. J Gen Intern Med 23(2):210–213. https://doi.org/10.1007/s11606-007-0473-0
Liu E, MacKenzie T, Dobyns EL, Parikh CR, Karrer FM, Narkewicz MR, Sokol RJ (2006) Characterization of acute liver failure and development of a continuous risk of death staging system in children. J Hepatol 44(1):134–141. https://doi.org/10.1016/j.jhep.2005.06.021
Lu YB, Kobayashi K, Ushikai M, Tabata A, Iijima M, Li MX et al (2005) Frequency and distribution in East Asia of 12 mutations identified in the SLC25A13 gene of Japanese patients with citrin deficiency. J Hum Genet 50:338–346. https://doi.org/10.1007/s10038-005-0262-8
Malaguarnera M, Vacante M, Motta M, Giordano M, Malaguarnera G, Bella R, Nunnari G, Rampello L, Pennisi G (2011) Acetyl-L-carnitine improves cognitive functions in severe hepatic encephalopathy: a randomized and controlled clinical trial. Metab Brain Dis 26(4):281–289. https://doi.org/10.1007/s11011-011-9260-z
Maldonado C, Guevara N, Silveira A, Fagiolino P, Vázquez M (2017) L-Carnitine supplementation to reverse hyperammonemia in a patient undergoing chronic valproic acid treatment: a case report. J Int Med Res 45(3):1268–1272
Marcaggi P, Jeanne M, Coles JA (2004) Neuron-glial trafficking of NH4+ and K+: separate routes of uptake into glial cells of bee retina. Eur J Neurosci 19(4):966–976. https://doi.org/10.1111/j.0953-816X.2004.03165.x
Marcaida G, Felipo V, Hermenegildo C, Miñana MD, Grisolía S (1992) Acute ammonia toxicity is mediated by the NMDA type of glutamate receptors. FEBS Lett 296(1):67–68. https://doi.org/10.1016/0014-5793(92)80404-5
Marcaida G, Miñana MD, Burgal M, Grisolía S, Felipo V (1995) Ammonia prevents activation of NMDA receptors by glutamate in rat cerebellar neuronal cultures. Eur J Neurosci 7(12):2389–2396. https://doi.org/10.1111/j.1460-9568.1995.tb01036.x
Marchetti P, Castedo M, Susin SA, Zamzami N, Hirsch T, Macho A, Haeffner A, Hirsch F, Geuskens M, Kroemer G (1996) Mitochondrial permeability transition is a central coordinating event of apoptosis. J Exp Med 184(3):1155–1160. https://doi.org/10.1084/jem.184.3.1155
Marisco Pda C, Ribeiro MC, Bonini JS, Lima TT, Mann KC, Brenner GM, Dutra-Filho CS, Mello CF (2003) Ammonia potentiates methylmalonic acid-induced convulsions and TBARS production. Exp Neurol 182(2):455–460. https://doi.org/10.1016/S0014-4886(03)00113-4
Martinelli D, Diodato D, Ponzi E, Monné M, Boenzi S, Bertini E, Fiermonte G, Dionisi-Vici C (2015) The hyperornithinemia-hyperammonemia-homocitrullinuria syndrome. Orphanet J Rare Dis 10:29. https://doi.org/10.1186/s13023-015-0242-9
Matoori S, Leroux JC (2015) Recent advances in the treatment of hyperammonemia. Adv Drug Deliv Rev 90:55–68. https://doi.org/10.1016/j.addr.2015.04.009
Meyburg J, Hoffmann GF (2010) Liver, liver cell and stem cell transplantation for the treatment of urea cycle defects. Mol Genet Metab 100(Suppl 1):S77-83. https://doi.org/10.1016/j.ymgme.2010.01.011
Monfort P, Cauli O, Montoliu C, Rodrigo R, Llansola M, Piedrafita B, El Mlili N, Boix J, Agustí A, Felipo V (2009) Mechanisms of cognitive alterations in hyperammonemia and hepatic encephalopathy: therapeutical implications. Neurochem Int 55(1–3):106–112. https://doi.org/10.1016/j.neuint.2009.01.021
Morris AA, Richmond SW, Oddie SJ, Pourfarzam M, Worthington V, Leonard JV (1998) N-acetylglutamate synthetase deficiency:

favourable experience with carbamylglutamate. J Inherit Metab Dis 21(8):867–868. https://doi.org/10.1023/A:1005478904186
Moscioni D, Morizono H, McCarter RJ, Stern A, Cabrera-Luque J, Hoang A, Sanmiguel J, Wu D, Bell P, Gao GP, Raper SE, Wilson JM, Batshaw ML (2006) Long-term correction of ammonia metabolism and prolonged survival in ornithine transcarbamylase-deficient mice following liver-directed treatment with adeno-associated viral vectors. Mol Ther 14(1):25–33. https://doi.org/10.1016/j.ymthe.2006.03.009
Moser H (1987) Electrophysiological evidence for ammonium as a substitute for potassium in activating the sodium pump in a crayfish sensory neuron. Can J Physiol Pharmacol 65(2):141–145. https://doi.org/10.1139/y87-028
Msall M, Batshaw ML, Suss R, Brusilow SW, Mellits ED (1984) Neurologic outcome in children with inborn errors of urea synthesis. Outcome of urea-cycle enzymopathies. N Engl J Med 310(23):1500–1505. https://doi.org/10.1056/NEJM198406073102304
Murthy CR, Rama Rao KV, Bai G, Norenberg MD (2001) Ammonia-induced production of free radicals in primary cultures of rat astrocytes. J Neurosci Res 66(2):282–288. https://doi.org/10.1002/jnr.1222
Nassogne MC, Héron B, Touati G, Rabier D, Saudubray JM (2005) Urea cycle defects: management and outcome. J Inherit Metab Dis 28(3):407–414. https://doi.org/10.1007/s10545-005-0303-7
Nizon M, Ottolenghi C, Valayannopoulos V, Arnoux JB, Barbier V, Habarou F, Desguerre I, Boddaert N, Bonnefont JP, Acquaviva C, Benoist JF, Rabier D, Touati G, de Lonlay P (2013) Long-term neurological outcome of a cohort of 80 patients with classical organic acidurias. Orphanet J Rare Dis 8:148. https://doi.org/10.1186/1750-1172-8-148
Norenberg MD (1998) Astroglial dysfunction in hepatic encephalopathy. Metab Brain Dis 13(4):319–335. https://doi.org/10.1023/A:1020688925901
Norenberg MD, Rama Rao KV, Jayakumar AR (2009) Signaling factors in the mechanism of ammonia neurotoxicity. Metab Brain Dis 24(1):103–117. https://doi.org/10.1007/s11011-008-9113-6
North KN, Hoppel CL, De Girolami U, Kozakewich HP, Korson MS (1995) Lethal neonatal deficiency of carnitine palmitoyltransferase II associated with dysgenesis of the brain and kidneys. J Pediatr 127(3):414–420. https://doi.org/10.1016/S0022-3476(95)70073-0
Ogier De Baulny H, Saudubray JM (2002) Branched-chain organic acidurias. Semin Neonatol 7:65–74. https://doi.org/10.1053/siny.2001.0087
Olpin SE (2013) Pathophysiology of fatty acid oxidation disorders and resultant phenotypic variability. J Inherit Metab Dis 36:645–658. https://doi.org/10.1007/s10545-013-9611-5
Ott P, Vilstrup H (2014) Cerebral effects of ammonia in liver disease: current hypotheses. Metab Brain Dis 29(4):901–911. https://doi.org/10.1007/s11011-014-9494-7
Ott P, Clemmesen O, Larsen FS (2005) Cerebral metabolic disturbances in the brain during acute liver failure: from hyperammonemia to energy failure and proteolysis. Neurochem Int 47(1–2):13–18. https://doi.org/10.1016/j.neuint.2005.04.002
Patejunas G, Lee B, Dennis JA, Healy PJ, Reeds PJ, Yu H, Frazer M, Mull B, Warman AW, Beaudet AL, O'Brien WE (1998) Evaluation of gene therapy for citrullinaemia using murine and bovine models. J Inherit Metab Dis 21(Suppl 1):138–150. https://doi.org/10.1023/a:1005322010854
Peoc'h K, Damaj L, Pelletier R, Lefèvre C, Dubourg C, Denis MC, Bendavid C, Odent S, Moreau C (2020) Early care of N-acetyl glutamate synthase (NAGS) deficiency in three infants from an inbred family. Mol Genet Metab Rep 22:100558. https://doi.org/10.1016/j.ymgmr.2019.100558
Rama Rao KV, Norenberg MD (2012) Brain energy metabolism and mitochondrial dysfunction in acute and chronic hepatic encephalopathy. Neurochem Int 60(7):697–706. https://doi.org/10.1016/j.neuint.2011.09.007
Rama Rao KV, Jayakumar AR, Norenberg DM (2003) Ammonia neurotoxicity: role of the mitochondrial permeability transition. Metab Brain Dis 18(2):113–127. https://doi.org/10.1023/A:1023858902184
Rama Rao KV, Jayakumar AR, Tong X, Curtis KM, Norenberg MD (2010) Brain aquaporin-4 in experimental acute liver failure. J Neuropathol Exp Neurol 69(9):869–879. https://doi.org/10.1097/NEN.0b013e3181ebe581
Rangroo Thrane V, Thrane AS, Chang J, Alleluia V, Nagelhus EA, Nedergaard M (2012) Real-time analysis of microglial activation and motility in hepatic and hyperammonemic encephalopathy. Neuroscience 220:247–255. https://doi.org/10.1016/j.neuroscience.2012.06.022
Rangroo Thrane V, Thrane AS, Wang F, Cotrina ML, Smith NA, Chen M, Xu Q, Kang N, Fujita T, Nagelhus EA, Nedergaard M (2013) Ammonia triggers neuronal disinhibition and seizures by impairing astrocyte potassium buffering. Nat Med 19(12):1643–1648. https://doi.org/10.1038/nm.3400
Ransohoff RM, Cardona AE (2010) The myeloid cells of the central nervous system parenchyma. Nature 468(7321):253–262. https://doi.org/10.1038/nature09615
Raper SE, Yudkoff M, Chirmule N et al (2002) A pilot study of in vivo liver-directed gene transfer with an adenoviral vector in partial ornithine transcarbamylase deficiency. Hum Gene Ther 13:163–175. https://doi.org/10.1089/10430340152712719
Ribas GS, Vargas CR (2020) Evidence that oxidative disbalance and mitochondrial dysfunction are involved in the pathophysiology of fatty acid oxidation disorders. Cell Mol Neurobiol. https://doi.org/10.1007/s10571-020-00955-7
Ribas GS, Vargas CR, Wajner M (2014) L-carnitine supplementation as a potential antioxidant therapy for inherited neurometabolic disorders. Gene 533(2):469–476. https://doi.org/10.1016/j.gene.2013.10.017
Rinaldo P, Matern D, Bennett MJ (2002) Fatty acid oxidation disorders. Annu Rev Physiol 64:477–502. https://doi.org/10.1146/annurev.physiol.64.082201.154705
Rodrigo R, Cauli O, Boix J, ElMlili N, Agusti A, Felipo V (2009) Role of NMDA receptors in acute liver failure and ammonia toxicity: therapeutical implications. Neurochem Int 55(1–3):113–118. https://doi.org/10.1016/j.neuint.2009.01.007
Romero-Gómez M, Jover M, Galán JJ, Ruiz A (2009) Gut ammonia production and its modulation. Metab Brain Dis 24(1):147–157. https://doi.org/10.1007/s11011-008-9124-3
Rose C, Kresse W, Kettenmann H (2005) Acute insult of ammonia leads to calcium-dependent glutamate release from cultured astrocytes, an effect of pH. J Biol Chem 280(22):20937–20944. https://doi.org/10.1074/jbc.M412448200
Royes LF, Gabbi P, Ribeiro LR, Della-Pace ID, Rodrigues FS, de Oliveira Ferreira AP, da Silveira Junior ME, da Silva LR, Grisólia AB, Braga DV, Dobrachinski F, da Silva AM, Soares FA, Marchesan S, Furian AF, Oliveira MS, Fighera MR (2016) A neuronal disruption in redox homeostasis elicited by ammonia alters the glycine/glutamate (GABA) cycle and contributes to MMA-induced excitability. Amino Acids 48(6):1373–1389. https://doi.org/10.1007/s00726-015-2164-1
Saheki T, Kobayashi K (2002) Mitochondrial aspartate glutamate carrier (citrin) deficiency as the cause of adult-onset type II citrullinemia (CTLN2) and idiopathic neonatal hepatitis (NICCD). J Hum Genet 47(7):333–341. https://doi.org/10.1007/s10038020046
Saheki T, Kobayashi K, Iijima M, Moriyama M, Yazaki M, Takei Y, Ikeda S (2005) Metabolic derangements in deficiency of

citrin, a liver-type mitochondrial aspartate-glutamate carrier. Hepatol Res 33(2):181–184. https://doi.org/10.1016/j.hepres.2005.09.031

Saparov SM, Liu K, Agre P, Pohl P (2007) Fast and selective ammonia transport by aquaporin-8. J Biol Chem 282(8):5296–5301. https://doi.org/10.1074/jbc.M609343200

Scaglia F, Zheng Q, O'Brien WE, Henry J, Rosenberger J, Reeds P, Lee B (2002) An integrated approach to the diagnosis and prospective management of partial ornithine transcarbamylase deficiency. Pediatrics 109(1):150–152. https://doi.org/10.1542/peds.109.1.150

Schaefer F, Straube E, Oh J, Mehls O, Mayatepek E (1999) Dialysis in neonates with inborn errors of metabolism. Nephrol Dial Transpl 14:910–918. https://doi.org/10.1093/ndt/14.4.910

Scriver C, Beaudt A, Sly W, Valle D (2001) The metabolic and molecular bases of inherited disease. McGraw-Hill, New York

Sepehrinezhad A, Zarifkar A, Namvar G, Shahbazi A, Williams R (2020) Astrocyte swelling in hepatic encephalopathy: molecular perspective of cytotoxic edema. Metab Brain Dis 35(4):559–578. https://doi.org/10.1007/s11011-020-00549-8

Sin YY, Baron G, Schulze A, Funk CD (2015) Arginase-1 deficiency. J Mol Med (berl) 93(12):1287–1296. https://doi.org/10.1007/s00109-015-1354-3

Sinke AP, Jayakumar AR, Panickar KS, Moriyama M, Reddy PV, Norenberg MD (2008) NFkappaB in the mechanism of ammonia-induced astrocyte swelling in culture. J Neurochem 106(6):2302–2311. https://doi.org/10.1111/j.1471-4159.2008.05549.x

Smith W, Diaz GA, Lichter-Konecki U, Berry SA, Harding CO, McCandless SE, LeMons C, Mauney J, Dickinson K, Coakley DF, Moors T, Mokhtarani M, Scharschmidt BF, Lee B (2013) Ammonia control in children ages 2 months through 5 years with urea cycle disorders: comparison of sodium phenylbutyrate and glycerol phenylbutyrate. J Pediatr 162(6):1228–1234. https://doi.org/10.1016/j.jpeds.2012.11.084

Sørensen M (2013) Update on cerebral uptake of blood ammonia. Metab Brain Dis 28(2):155–159. https://doi.org/10.1007/s11011-013-9395-1

Soria LR, Ah Mew N, Brunetti-Pierri N (2019) Progress and challenges in development of new therapies for urea cycle disorders. Hum Mol Genet 28(R1):R42–R48. https://doi.org/10.1093/hmg/ddz140

Spada M, Porta F, Righi D, Gazzera C, Tandoi F, Ferrero I, Fagioli F, Sanchez MBH, Calvo PL, Biamino E, Bruno S, Gunetti M, Contursi C, Lauritano C, Conio A, Amoroso A, Salizzoni M, Silengo L, Camussi G, Romagnoli R (2020) Intrahepatic administration of human liver stem cells in infants with inherited neonatal-onset hyperammonemia: a phase I study. Stem Cell Rev Rep 16(1):186–197. https://doi.org/10.1007/s12015-019-09925-z

Stewart CA, Smith GE (2007) Minimal hepatic encephalopathy. Nat Clin Pract Gastroenterol Hepatol 4(12):677–685. https://doi.org/10.1038/ncpgasthep0999

Strom SC, Fisher RA, Thompson MT, Sanyal AJ, Cole PE, Ham JM, Posner MP (1997) Hepatocyte transplantation as a bridge to orthotopic liver transplantation in terminal liver failure. Transplantation 63(4):559–569. https://doi.org/10.1097/00007890-199702270-00014

Suárez I, Bodega G, Fernández B (2002) Glutamine synthetase in brain: effect of ammonia. Neurochem Int 41(2–3):123–142. https://doi.org/10.1016/S0197-0186(02)00033-5

Summar M (2001) Current strategies for the management of neonatal urea cycle disorders. J Pediatr 138(1 Suppl):S30–S39. https://doi.org/10.1067/mpd.2001.111834

Summar ML, Mew NA (2018) Inborn errors of metabolism with hyperammonemia: urea cycle defects and related disorders. Pediatr Clin North Am 65(2):231–246. https://doi.org/10.1016/j.pcl.2017.11.004

Summar ML, Koelker S, Freedenberg D, Le Mons C, Haberle J, Lee HS, Kirmse B (2013) The incidence of urea cycle disorders. Mol Genet Metab 110(1–2):179–180. https://doi.org/10.1016/j.ymgme.2013.07.008

Tanigami H, Rebel A, Martin LJ, Chen TY, Brusilow SW, Traystman RJ, Koehler RC (2005) Effect of glutamine synthetase inhibition on astrocyte swelling and altered astroglial protein expression during hyperammonemia in rats. Neuroscience 131(2):437–449. https://doi.org/10.1016/j.neuroscience.2004.10.045

Tchan M (2018) Hyperammonemia and lactic acidosis in adults: differential diagnoses with a focus on inborn errors of metabolism. Rev Endocr Metab Disord 19(1):69–79. https://doi.org/10.1007/s11154-018-9444-5

Thomas JA (2015) Organic acidemias. In: Bernstein LE, Helm JR, Rohr F (eds) Nutrition management of inherited metabolic disease: lessons from metabolic university. Springer International Publishing, Switzerland, pp 187–201

Tofteng F, Larsen FS (2004) The effect of indomethacin on intracranial pressure, cerebral perfusion and extracellular lactate and glutamate concentrations in patients with fulminant hepatic failure. J Cereb Blood Flow Metab 24(7):798–804. https://doi.org/10.1097/01.WCB.0000125648.03213.1D

Tomomura M, Tomomura A, Musa DA, Horiuchi M, Takiguchi M, Mori M, Saheki T (1997) Suppressed expression of the urea cycle enzyme genes in the liver of carnitine-deficient juvenile visceral steatosis (JVS) mice in infancy and during starvation in adulthood. J Biochem 121(1):172–177. https://doi.org/10.1093/oxfordjournals.jbchem.a021562

Truong B, Allegri G, Liu XB, Burke KE, Zhu X, Cederbaum SD, Häberle J, Martini PGV, Lipshutz GS (2019) Lipid nanoparticle-targeted mRNA therapy as a treatment for the inherited metabolic liver disorder arginase deficiency. Proc Natl Acad Sci USA 116(42):21150–21159. https://doi.org/10.1073/pnas.1906182116

Tsuboi Y, Fujino Y, Kobayashi K, Saheki T, Yamada T (2001) High serum pancreatic secretory trypsin inhibitor before onset of type II citrullinemia. Neurology 57(5):933. https://doi.org/10.1212/WNL.57.5.933

Tuchman M, Lee B, Lichter-Konecki U, Summar ML, Yudkoff M, Cederbaum SD, Kerr DS, Diaz GA, Seashore MR, Lee HS, McCarter RJ, Krischer JP, Batshaw ML (2008) Urea cycle disorders consortium of the rare diseases clinical research network. Cross-sectional multicenter study of patients with urea cycle disorders in the United States. Mol Genet Metab 94(4):397–402. https://doi.org/10.1016/j.ymgme.2008.05.004

Uchino T, Endo F, Matsuda I (1998) Neurodevelopmental outcome of long-term therapy of urea cycle disorders in Japan. J Inherit Metab Dis 21(Suppl 1):151–159. https://doi.org/10.1023/A:1005374027693

Valle D, Simell O (2001) The hyperornithinemias. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular basis of inherited disease, 8th edn. McGraw Hill, New York, pp 1857–1896

Van Hall G, van der Vusse GJ, Söderlund K, Wagenmakers AJ (1995) Deamination of amino acids as a source for ammonia production in human skeletal muscle during prolonged exercise. J Physiol 489:251–261. https://doi.org/10.1113/jphysiol.1995.sp021047

Viegas CM, Zanatta Â, Grings M, Hickmann FH, Monteiro WO, Soares LE, Sitta Â, Leipnitz G, de Oliveira FH, Wajner M (2014) Disruption of redox homeostasis and brain damage caused in vivo by methylmalonic acid and ammonia in cerebral cortex and striatum of developing rats. Free Radic Res 48(6):659–669. https://doi.org/10.3109/10715762.2014.898842

Villani GR, Gallo G, Scolamiero E, Salvatore F, Ruoppolo M (2017) "Classical organic acidurias": diagnosis and pathogenesis. Clin Exp Med 17:305–323. https://doi.org/10.1007/s10238-016-0435-0

citrin, a liver-type mitochondrial aspartate-glutamate carrier. Hepatol Res 33(2):181–184. https://doi.org/10.1016/j.hepres.2005.09.031

Saparov SM, Liu K, Agre P, Pohl P (2007) Fast and selective ammonia transport by aquaporin-8. J Biol Chem 282(8):5296–5301. https://doi.org/10.1074/jbc.M609343200

Scaglia F, Zheng Q, O'Brien WE, Henry J, Rosenberger J, Reeds P, Lee B (2002) An integrated approach to the diagnosis and prospective management of partial ornithine transcarbamylase deficiency. Pediatrics 109(1):150–152. https://doi.org/10.1542/peds.109.1.150

Schaefer F, Straube E, Oh J, Mehls O, Mayatepek E (1999) Dialysis in neonates with inborn errors of metabolism. Nephrol Dial Transpl 14:910–918. https://doi.org/10.1093/ndt/14.4.910

Scriver C, Beaudt A, Sly W, Valle D (2001) The metabolic and molecular bases of inherited disease. McGraw-Hill, New York

Sepehrinezhad A, Zarifkar A, Namvar G, Shahbazi A, Williams R (2020) Astrocyte swelling in hepatic encephalopathy: molecular perspective of cytotoxic edema. Metab Brain Dis 35(4):559–578. https://doi.org/10.1007/s11011-020-00549-8

Sin YY, Baron G, Schulze A, Funk CD (2015) Arginase-1 deficiency. J Mol Med (berl) 93(12):1287–1296. https://doi.org/10.1007/s00109-015-1354-3

Sinke AP, Jayakumar AR, Panickar KS, Moriyama M, Reddy PV, Norenberg MD (2008) NFkappaB in the mechanism of ammonia-induced astrocyte swelling in culture. J Neurochem 106(6):2302–2311. https://doi.org/10.1111/j.1471-4159.2008.05549.x

Smith W, Diaz GA, Lichter-Konecki U, Berry SA, Harding CO, McCandless SE, LeMons C, Mauney J, Dickinson K, Coakley DF, Moors T, Mokhtarani M, Scharschmidt BF, Lee B (2013) Ammonia control in children ages 2 months through 5 years with urea cycle disorders: comparison of sodium phenylbutyrate and glycerol phenylbutyrate. J Pediatr 162(6):1228–1234. https://doi.org/10.1016/j.jpeds.2012.11.084

Sørensen M (2013) Update on cerebral uptake of blood ammonia. Metab Brain Dis 28(2):155–159. https://doi.org/10.1007/s11011-013-9395-1

Soria LR, Ah Mew N, Brunetti-Pierri N (2019) Progress and challenges in development of new therapies for urea cycle disorders. Hum Mol Genet 28(R1):R42–R48. https://doi.org/10.1093/hmg/ddz140

Spada M, Porta F, Righi D, Gazzera C, Tandoi F, Ferrero I, Fagioli F, Sanchez MBH, Calvo PL, Biamino E, Bruno S, Gunetti M, Contursi C, Lauritano C, Conio A, Amoroso A, Salizzoni M, Silengo L, Camussi G, Romagnoli R (2020) Intrahepatic administration of human liver stem cells in infants with inherited neonatal-onset hyperammonemia: a phase I study. Stem Cell Rev Rep 16(1):186–197. https://doi.org/10.1007/s12015-019-09925-z

Stewart CA, Smith GE (2007) Minimal hepatic encephalopathy. Nat Clin Pract Gastroenterol Hepatol 4(12):677–685. https://doi.org/10.1038/ncpgasthep0999

Strom SC, Fisher RA, Thompson MT, Sanyal AJ, Cole PE, Ham JM, Posner MP (1997) Hepatocyte transplantation as a bridge to orthotopic liver transplantation in terminal liver failure. Transplantation 63(4):559–569. https://doi.org/10.1097/00007890-199702270-00014

Suárez I, Bodega G, Fernández B (2002) Glutamine synthetase in brain: effect of ammonia. Neurochem Int 41(2–3):123–142. https://doi.org/10.1016/S0197-0186(02)00033-5

Summar M (2001) Current strategies for the management of neonatal urea cycle disorders. J Pediatr 138(1 Suppl):S30–S39. https://doi.org/10.1067/mpd.2001.111834

Summar ML, Mew NA (2018) Inborn errors of metabolism with hyperammonemia: urea cycle defects and related disorders. Pediatr Clin North Am 65(2):231–246. https://doi.org/10.1016/j.pcl.2017.11.004

Summar ML, Koelker S, Freedenberg D, Le Mons C, Haberle J, Lee HS, Kirmse B (2013) The incidence of urea cycle disorders. Mol Genet Metab 110(1–2):179–180. https://doi.org/10.1016/j.ymgme.2013.07.008

Tanigami H, Rebel A, Martin LJ, Chen TY, Brusilow SW, Traystman RJ, Koehler RC (2005) Effect of glutamine synthetase inhibition on astrocyte swelling and altered astroglial protein expression during hyperammonemia in rats. Neuroscience 131(2):437–449. https://doi.org/10.1016/j.neuroscience.2004.10.045

Tchan M (2018) Hyperammonemia and lactic acidosis in adults: differential diagnoses with a focus on inborn errors of metabolism. Rev Endocr Metab Disord 19(1):69–79. https://doi.org/10.1007/s11154-018-9444-5

Thomas JA (2015) Organic acidemias. In: Bernstein LE, Helm JR, Rohr F (eds) Nutrition management of inherited metabolic disease: lessons from metabolic university. Springer International Publishing, Switzerland, pp 187–201

Tofteng F, Larsen FS (2004) The effect of indomethacin on intracranial pressure, cerebral perfusion and extracellular lactate and glutamate concentrations in patients with fulminant hepatic failure. J Cereb Blood Flow Metab 24(7):798–804. https://doi.org/10.1097/01.WCB.0000125648.03213.1D

Tomomura M, Tomomura A, Musa DA, Horiuchi M, Takiguchi M, Mori M, Saheki T (1997) Suppressed expression of the urea cycle enzyme genes in the liver of carnitine-deficient juvenile visceral steatosis (JVS) mice in infancy and during starvation in adulthood. J Biochem 121(1):172–177. https://doi.org/10.1093/oxfordjournals.jbchem.a021562

Truong B, Allegri G, Liu XB, Burke KE, Zhu X, Cederbaum SD, Häberle J, Martini PGV, Lipshutz GS (2019) Lipid nanoparticle-targeted mRNA therapy as a treatment for the inherited metabolic liver disorder arginase deficiency. Proc Natl Acad Sci USA 116(42):21150–21159. https://doi.org/10.1073/pnas.1906182116

Tsuboi Y, Fujino Y, Kobayashi K, Saheki T, Yamada T (2001) High serum pancreatic secretory trypsin inhibitor before onset of type II citrullinemia. Neurology 57(5):933. https://doi.org/10.1212/WNL.57.5.933

Tuchman M, Lee B, Lichter-Konecki U, Summar ML, Yudkoff M, Cederbaum SD, Kerr DS, Diaz GA, Seashore MR, Lee HS, McCarter RJ, Krischer JP, Batshaw ML (2008) Urea cycle disorders consortium of the rare diseases clinical research network. Cross-sectional multicenter study of patients with urea cycle disorders in the United States. Mol Genet Metab 94(4):397–402. https://doi.org/10.1016/j.ymgme.2008.05.004

Uchino T, Endo F, Matsuda I (1998) Neurodevelopmental outcome of long-term therapy of urea cycle disorders in Japan. J Inherit Metab Dis 21(Suppl 1):151–159. https://doi.org/10.1023/A:1005374027693

Valle D, Simell O (2001) The hyperornithinemias. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular basis of inherited disease, 8th edn. McGraw Hill, New York, pp 1857–1896

Van Hall G, van der Vusse GJ, Söderlund K, Wagenmakers AJ (1995) Deamination of amino acids as a source for ammonia production in human skeletal muscle during prolonged exercise. J Physiol 489:251–261. https://doi.org/10.1113/jphysiol.1995.sp021047

Viegas CM, Zanatta Â, Grings M, Hickmann FH, Monteiro WO, Soares LE, Sitta Â, Leipnitz G, de Oliveira FH, Wajner M (2014) Disruption of redox homeostasis and brain damage caused in vivo by methylmalonic acid and ammonia in cerebral cortex and striatum of developing rats. Free Radic Res 48(6):659–669. https://doi.org/10.3109/10715762.2014.898842

Villani GR, Gallo G, Scolamiero E, Salvatore F, Ruoppolo M (2017) "Classical organic aciduria": diagnosis and pathogenesis. Clin Exp Med 17:305–323. https://doi.org/10.1007/s10238-016-0435-0

Vilstrup H, Amodio P, Bajaj J, Cordoba J, Ferenci P, Mullen KD, Weissenborn K, Wong P (2014) Hepatic encephalopathy in chronic liver disease: 2014 Practice Guideline by the American Association for the Study of Liver Diseases and the European Association for the Study of the Liver. Hepatology 60(2):715–735. https://doi.org/10.1002/hep.27210

Vince AJ, Burridge SM (1980) Ammonia production by intestinal bacteria: the effects of lactose, lactulose and glucose. J Med Microbiol 13(2):177–191

Vishwanath V (2016) Fatty acid beta-oxidation disorders: a brief review. Ann Neurosci 23:51–55. https://doi.org/10.1159/000443556

Wajner M (2019) Neurological manifestations of organic acidurias. Nat Rev Neurol 15(5):253–271. https://doi.org/10.1038/s41582-019-0161-9

Wajner M, Amaral AU (2015) Mitochondrial dysfunction in fatty acid oxidation disorders: insights from human and animal studies. Biosci Rep 36:e00281. https://doi.org/10.1042/BSR20150240

Wajner M, Sitta A, Kayser A, Deon M, Groehs AC, Coelho DM, Vargas CR (2019) Screening for organic acidurias and aminoacidopathies in high-risk Brazilian patients: eleven-year experience of a reference center. Genet Mol Biol 42(1 suppl 1):178–185. https://doi.org/10.1590/1678-4685-GMB-2018-0105

Wajner M, Vargas CR, Amaral AU (2020) Disruption of mitochondrial functions and oxidative stress contribute to neurologic dysfunction in organic acidurias. Arch Biochem Biophys 696:108646. https://doi.org/10.1016/j.abb.2020.108646

Walker V (2009) Ammonia toxicity and its prevention in inherited defects of the urea cycle. Diabetes Obes Metab 11(9):823–835. https://doi.org/10.1111/j.1463-1326.2009.01054.x

Walker V (2014) Ammonia metabolism and hyperammonemic disorders. Adv Clin Chem 67:73–150. https://doi.org/10.1016/bs.acc.2014.09.002

Wang L, Wang H, Morizono H, Bell P, Jones D, Lin J, McMenamin D, Yu H, Batshaw ML, Wilson JM (2012) Sustained correction of OTC deficiency in spf (ash) mice using optimized self-complementary AAV2/8 vectors. Gene Ther 19(4):404–410. https://doi.org/10.1038/gt.2011.111

Weber FL Jr, Banwell JG, Fresard KM, Cummings JH (1987) Nitrogen in fecal bacterial, fiber, and soluble fractions of patients with cirrhosis: effects of lactulose and lactulose plus neomycin. J Lab Clin Med 110(3):259–263

Weiner ID, Verlander JW (2013) Renal ammonia metabolism and transport. Compr Physiol 3(1):201–220. https://doi.org/10.1002/cphy.c120010

Weiner ID, Mitch WE, Sands JM (2015) Urea and ammonia metabolism and the control of renal nitrogen excretion. Clin J Am Soc Nephrol 10(8):1444–1458. https://doi.org/10.2215/CJN.10311013

Willard-Mack CL, Koehler RC, Hirata T, Cork LC, Takahashi H, Traystman RJ, Brusilow SW (1996) Inhibition of glutamine synthetase reduces ammonia-induced astrocyte swelling in rat. Neuroscience 71(2):589–599. https://doi.org/10.1016/0306-4522(95)00462-9

Ye X, Robinson MB, Batshaw ML, Furth EE, Smith I, Wilson JM (1996) Prolonged metabolic correction in adult ornithine transcarbamylase-deficient mice with adenoviral vectors. J Biol Chem 271(7):3639–3646. https://doi.org/10.1074/jbc.271.7.3639

Zielińska M, Ruszkiewicz J, Hilgier W, Fręśko I, Albrecht J (2011) Hyperammonemia increases the expression and activity of the glutamine/arginine transporter y+ LAT2 in rat cerebral cortex: implications for the nitric oxide/cGMP pathway. Neurochem Int 58(2):190–195. https://doi.org/10.1016/j.neuint.2010.11.015

Zwingmann C, Chatauret N, Leibfritz D, Butterworth RF (2003) Selective increase of brain lactate synthesis in experimental acute liver failure: results of a [H-C] nuclear magnetic resonance study. Hepatology 37(2):420–428. https://doi.org/10.1053/jhep.2003.50052