UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

INSTITUTO DE CIÊNCIAS BÁSICAS DA SAÚDE

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS: BIOQUÍMICA

Efeito da epigalocatequina galato sobre ratos expostos ao modelo de demência pela infusão intracerebroventricular de estreptozotocina

REGINA BIASIBETTI

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Dedico este trabalho
a todas as pessoas que,
como eu, amam o que fazem.

“All You Need Is Love”

The Beatles

IV

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SUMÁRIO

PARTE I

RESUMO

Demência é conceituada como um declínio progressivo nas funções cognitivas e perda grave de memória. A doença de Alzheimer (DA) é a forma mais comum de demência. Dentre as características neuropatológicas, a doença caracteriza-se por perdas seletivas neuronais e sinápticas, principalmente colinérgica, presença de placas neuríticas extracelulares contendo o peptídeo β-amilóide (Aβ) e emaranhados neurofibrilares compostos de formas hiperfosforiladas da proteína tau. Diversos modelos animais têm sido desenvolvidos para investigar a gênese e tratamentos da DA, dentre eles, destaca-se a infusão intracerebroventricular (icv) de estreptozotocina (STZ), que exibe características neuroquímicas e fisiopatológicas semelhantes à DA em roedores, através da dessensibilização dos receptores de insulina, especialmente em hipocampo e córtex cerebral. O objetivo deste trabalho foi avaliar as alterações neurogliais em hipocampo de ratos expostos à estreptozotocina intracerebroventricular, bem como o efeito da epigalocatequina galato (EGCG), um antioxidante contido no chá verde (*Camellia sinensis*), através da medida do conteúdo e secreção da proteína S100B, medida de GFAP, GSH, atividade da AchE, captação de glicose, estresse oxidativo e nitrosativo e análise comportamental. Como resultado, encontramos, nos animais expostos à STZ, declínio cognitivo, alteração da AchE, estresse oxidativo e nitrosativo e alterações neurogliais hipocampais, especialmente relacionadas à captação da glicose, às defesas antioxidantes e à secreção da proteína S100B, a qual pode atuar tanto na sinalização neurônio-astrócito, em condições fisiológicas e patológicas, quanto em diversas doenças neurodegenerativas, incluindo a Doença de Alzheimer. Ainda, observou-se o efeito neuroprotetor da EGCG sobre os animais expostos à STZ icv. A EGCG mostrou-se eficaz na recuperação do déficit cognitivo causado pelo modelo, bem como das alterações neurogliais e do estresse oxidativo/nitrosativo. Por fim, este trabalho confirma o comprometimento cognitivo e o estresse oxidativo presentes no modelo, os quais se somam às alterações funcionais encontradas nos astrócitos. Tais alterações também estão presentes na DA, apontando a interação neuroglial como um importante alvo de estudo na doença e na busca por alternativas terapêuticas.

ABSTRACT

Dementia is defined as a progressive decline in cognitive function and severe memory loss. Alzheimer's disease (AD) is the most common form of dementia. Among the neuropathological features, the disease is characterized by selective neuronal and synaptic loss, mainly cholinergic, the presence of extracellular neuritic plaques containing the β-amyloid peptide (Aβ) and neurofiblrilary tangles composed of hyperphosphorylated forms of tau. Several animal models have been developed to investigate the genesis and treatments of AD, among them stands out the intracerebroventricular (icv) infusion of streptozotocin (STZ), which exhibits neurochemical and pathophysiological features similar to AD in rodents, by desensitization of insulin receptors, especially in the hippocampus and cortex. The aim of this study was to evaluate the neuroglial changes in the hippocampus of rats exposed to icv-STZ, and the effect of (-)epigallocatechin-3-gallate (EGCG), an antioxidant contained in green tea, (*Camellia sinensis*), by measuring the content and secretion of S100B, content of GFAP, GSH, AchE activity, glucose uptake, oxidative and nitrosative stress and behavioral analysis. In animals exposed to STZ, we found a cognitive decline, changes in AchE activity, oxidative and nitrosative stress and hippocampal neuroglial changes, especially related to glucose uptake, antioxidant defenses and secretion of S10B, which can act in the astrocyte-neuron signaling in physiological and pathological conditions, as in several neurodegenerative diseases, including Alzheimer's disease. Furthermore, we observed the neuroprotective effect of EGCG on animals exposed to STZ-icv. EGCG was effective in recovery of cognitive impairment caused by this model, as well as glial changes in oxidative/nitrosative stress. Finally, this work confirms the cognitive impairment and oxidative stress in the model, which add to the functional changes found in astrocytes. Such changes are also present in AD, pointing to neuroglial interaction as an important target of study in the disease and the search for therapeutic alternatives.

LISTA DE ABREVIATURAS

Aβ, peptídeo β-amilóide
AGEs, produtos finais avançados de glicação
apo E, apolipopotreína E
BHE, barreira hemato-encefálica
CAT, catalase
DA, doença de Alzheimer
DCF, diacetato de 2,7-diclofluorescina
ICV, intracerebroventricular
IGF, fator de crescimento semelhante à insulina
iNOS, enzima óxido nítrico sintase induzível
ip, intraperitoneal
GFAP, proteína fibrilar glial ácida
GPx, glutationa peroxidase
GSK 3, glicogênio sintase cinase 3
GluT, transportador de glicose
LAM, labirinto aquático de Morris
LCR, líquido cefalorraquidiano
NAD, nicotinamida adenina dinucleotídeo
NF-κB, fator de transcrição nuclear kappa B
NGF, fator de crescimento neural
NO, óxido nítrico
PARP, poli-ADP-ribose-polimerase
PI3K, fosfatidil inositol-3 cinase
PKB, proteína cinase B/Akt
RAGE, receptor de produtos finais avançados de glicação
SNC, sistema nervoso central
SOD, superóxido dismutase
STZ, estreptozotocina

INTRODUÇÃO

1. Demência

Demência pode ser conceituada como “perda substancial das habilidades intelectuais, memória em especial, de uma maneira severa a ponto de interferir com a vida social, profissional e emocional dos indivíduos” (Rademakers and Rovelet-Lecrux 2009), resultado de um complexo declínio cognitivo devido a uma “disfunção crônica e progressiva da atividade cortical e/ou subcortical” (Ritchie and Lovestone 2002). Tais sintomas se apresentam de uma maneira mais pronunciada do que aqueles observados como conseqüência do envelhecimento, quando todas as funções celulares, de uma maneira geral, estão em declínio. Estima-se que a doença atinge, atualmente, 24 milhões de pessoas, e que esta quantidade irá dobrar a cada 20 anos, sendo que 60% destes indivíduos residem em países em desenvolvimento (Ferri, Prince et al. 2005).

Atualmente, o diagnóstico de um paciente com demência é feito antes mesmo do comprometimento das funções intelectuais e das atividades de vida diária do mesmo, graças a técnicas de imageamento encefálico e marcadores periféricos (Kurz and Lautenschlager 2010). Por outro lado, a confirmação diagnóstica da Doença de Alzheimer (DA), o principal tipo de demência, só pode ser obtida com a análise morfológica *post mortem* do tecido encefálico.

À medida que se sucede um aumento da expectativa de vida da população, aumenta a ocorrência de doenças associadas ao envelhecimento. Previsões da Organização Mundial da Saúde apontam um crescimento elevado, de cerca de

29 milhões de casos de demência em 2020, especialmente DA, entre a população acima de 60 anos (Essink-Bot, Pereira et al. 2002; Haan and Wallace 2004; Langa, Larson et al. 2004). Tal fato torna a doença um grave problema de saúde pública e um enorme custo para o sistema governamental, fazendo com que a busca por alternativas de terapia e prevenção seja extremamente relevante e emergencial.

1.1. Doença de Alzheimer

A DA pode ser classificada em dois tipos: hereditária (tipo I), quando relacionada a diferentes genes como o da proteína precursora amilóide, da apolipopotreína E (apo E) ou das presenilinas; e esporádica (tipo II), a qual todos os indivíduos estão sujeitos na medida em que envelhecem. Fatores como obesidade, hipercolesterolemia e, em especial, diabetes mellitus (disfunções metabólicas) (Qiu, De Ronchi et al. 2007; de la Monte, Longato et al. 2009; Hallschmid and Schultes 2009), hipertensão e aterosclerose (disfunções cardiovasculares (Rocchi, Orsucci et al. 2009) e até mesmo infecções (Holmes and Cotterell 2009) têm sido associados com um aumento na incidência da DA esporádica e sua fisiopatogenia.

Clinicamente, a DA é caracterizada pela ocorrência de uma diminuição da capacidade do indivíduo de formar novas memórias e de lembrar acontecimentos recentes, entre outros distúrbios neuropsiquiátricos como alterações de personalidade e humor (Cummings, Mega et al. 1994; Selkoe 2001). Histopatologicamente há evidências uma extensa perda neuronal, com presença das chamadas placas senis formadas pelo depósito extracelular do peptídeo beta amilóide e por emaranhados neurofibrilares intracelulares

resultantes da deposição anormal de uma proteína associada aos microtúbulos, a proteína tau, hiperfosforilada ou poliubiquitinada (Selkoe 2001; Selkoe 2001; Jalbert, Daiello et al. 2008; Duyckaerts, Delatour et al. 2009). Em áreas relacionadas à cognição e à formação de memórias, como o córtex pré-frontal e o hipocampo, juntamente com suas regiões associadas, ocorrem uma série de eventos que levam à disfunção neuronal, especialmente de neurônios colinérgicos. Deste modo, cria-se um quadro de desequilíbrio neuroquímico onde há sinais de neurodegeneração, neuroinflamação, estresse oxidativo, aumento da sinalização pró-apoptótica, déficit colinérgico, excitotoxicidade glutamatérgica, disfunção mitocondrial e da homeostase do cálcio, com prejuízo da transmissão sináptica e do equilíbrio entre diferentes neurotransmissores (McGeer, Singh et al. 1987; Selkoe 2001; Eikelenboom, Veerhuis et al. 2006).

1.2. Modelo animal de demência induzido pela infusão intracerebroventricular de estreptozotocina

Há diversos modelos animais de demência não-transgênicos que visam mimetizar algumas características neuroquímicas e comportamentais observadas em indivíduos que desenvolveram a DA. Dentre eles, destacam-se a infusão de ibotenato no núcleo basal magnocelular, comprometendo-o, assim como suas eferências colinérgicas (Swarowsky, Rodrigues et al. 2008); a infusão do peptídeo beta amilóide via intracerebroventricular (icv) (Zussy, Brureau et al. 2011); a oclusão permanente das carótidas comuns, causando uma hipoperfusão encefálica crônica (Vicente, Degerone et al. 2009); a infusão icv bilateral da toxina botulínica (Lackovic, Rebic et al. 2009); a infusão

intrahipocampal bilateral (Costa, Tramontina et al. 2011) e icv (Kamat, Tota et al. 2010) de ácido ocadáico e a infusão bilateral icv de estreptozotocina (STZ) (Rodrigues, Biasibetti et al. 2009; Tramontina, Wartchow et al. 2011), modelo utilizado neste trabalho.

A estreptozotocina é um fármaco de ação antibiótica utilizada experimentalmente para induzir diabetes, principalmente em roedores. Para isso, é administrada por uma via sistêmica e age no pâncreas destruindo permanentemente as células beta pancreáticas produtoras de insulina, originando assim, um quadro de diabetes (Baydas, Nedzvetskii et al. 2003). O mecanismo de ação da STZ, consiste em sua captação pelas células beta através de transportadores de glicose (GluT) do tipo 2. Por ser uma nitrosamida metilnitrosureia ligada a uma D-glicose, uma vez metabolizada pela célula, gera N-nitrosureido, o qual causa a fragmentação do DNA celular, seguida da ativação de uma enzima de reparo, a poli-ADP-ribose-polimerase (PARP), a qual consome NAD+ e favorece a formação de radicais livres que acabam promovendo a morte celular (Uchigata, Yamamoto et al. 1982; Hosokawa, Dolci et al. 2001; Szkudelski 2001).

Este modelo tem sido amplamente aceito para experimentação relativa à DA (Hoyer, Lee et al. 2000; Weinstock and Shoham 2004; de la Monte, Longato et al. 2009). Quando administrada via icv na dose de 1-3 mg/kg, a STZ causa uma espécie de dessensibilização dos receptores de insulina (RI) e do IGF (ambos do tipo tirosina cinase) com conseqüentes alterações bioquímicas e fisiopatológicas semelhantes às encontradas na demência do tipo Alzheimer. De uma maneira resumida (figura 1), quando a insulina se liga ao seu receptor

(tanto central quanto periférico), este recruta seu substrato (SRI) no sítio de ancoramento, tornando-o fosforilado nos resíduos de tirosina. Este, por sua vez, torna-se capaz e recrutar várias moléculas sinalizadoras, dentre estas a enzima fosfatidilinositol-3 cinase (PI3K). Esta enzima fosforila o fosfoinotidide da membrana que, por sua vez, ancora a proteína cinase B (PKB), também conhecida como Akt, a qual promove a translocação do receptor de glicose GluT 4 para a membrana plasmática, promovendo maior aporte de glicose nos tecidos dependentes de insulina.

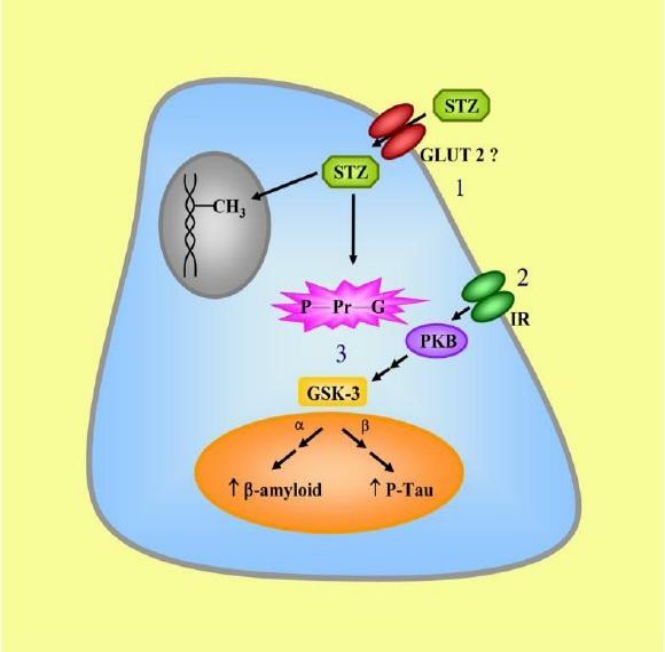

Figura 1: Provável mecanismo de ação da STZ na célula (neurônio e/ou astrócito). 1. Entrada através do transportador de glicose 2 (GLUT2) em neurônio ou astrócito, 2. Ação sobre o receptor de insulina (IR), 3. Consequente comprometimento da via da enzima glicogênicinase 3 (GSK 3) por desequilíbrio da fosforilação/glicação de proteínas no neurônio. Adaptado de Rodrigues et al, 2011; Handbook of Journal of Alzheimer Disease (2011).

Por outro lado, a Akt também modula por fosforilação a atividade da enzima glicogênio sintase cinase 3 (GSK 3) a qual, dependendo de sua isoforma, regula os peptídios β-amilóide (isoforma α) e a fosforilação da proteína tau (isoforma β) (Salkovic-Petrisic, Tribl et al. 2006; Salkovic-Petrisic and Hoyer

2007). É importante ressaltar que a STZ não ultrapassa a barreira hemato-encefálica e sua administração icv não causa comprometimento sistêmico (Duelli, Schrock et al. 1994; Lannert and Hoyer 1998).

2. Astrócitos

Em 1846, Virchow observou pela primeira vez a existência, no SNC, de uma substância intersticial que continha células especiais estelares ou de forma alongada, morfologicamente distintas dos neurônios, a qual denominou de neuroglia. Pertencentes a este grupo, estão as células da microglia, os oligodendrócitos, as células ependimárias e, as mais numerosas células do SNC, os astrócitos.

Os astrócitos possuem um algo grau de plasticidade, cabendo-lhes inúmeras funções, como por exemplo: (1) direcionam e participam das funções das sinapses durante o desenvolvimento; (2) principal fonte de proteínas da matriz extracelular e moléculas de adesão no SNC; (3) produção de fatores tróficos, como S100B; (4) estoque de glicogênio como fonte de reserva energética para o cérebro; (5) participação na barreira hemato-encefálica, mediando o transporte de substâncias entre o sangue e o encéfalo; (6) tamponamento dos níveis de íons, como K^+, Na^+ e lactato, preservando a atividade neuronal; (7) papel crítico na captação e metabolismo de neurotransmissores, como glutamato e gaba; (8) participação na resposta imune cerebral; (9) síntese e liberação de glutationa [para revisão ver (Jessen 2004)].

Os astrócitos se comunicam por junções gap, formando um grande sincício, sendo classificados de acordo com a morfologia e localização em: protoplasmáticos, encontrados na substância cinzenta e fibrosos, encontrados na substância branca (Halassa, Fellin et al. 2007). Seus prolongamentos atingem as sinapses neuronais, e sua contribuição na função sináptica fica ainda mais evidente (Figura 2). Esta situação é denominada sinapse tripartite, onde o astrócito é considerado o 3º elemento constituinte da sinapse (Perea and Araque 2010).

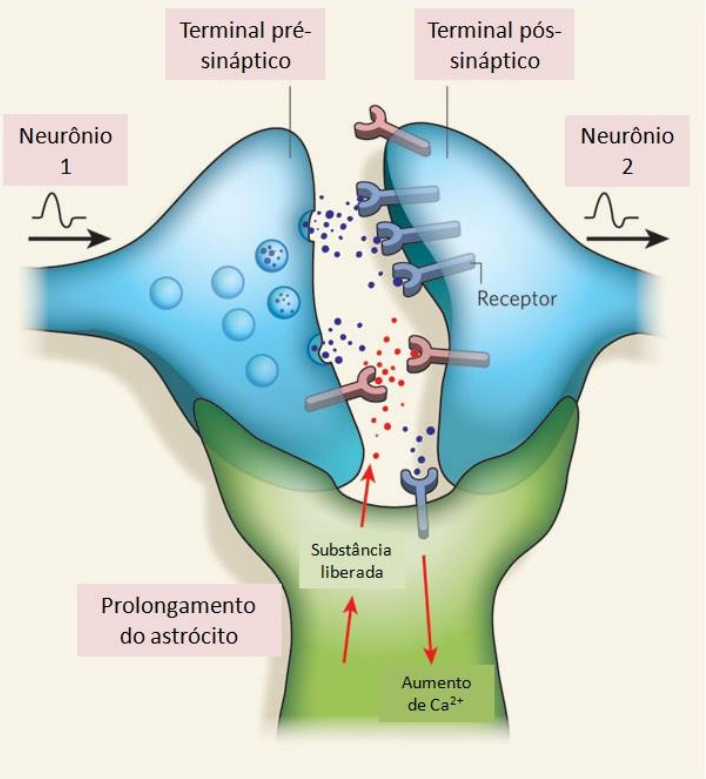

Figura 2: Sinapse tripartite. Adaptada de (Allen and Barres 2009).

Além disso, os astrócitos apresentam receptores para neurotransmissores. Quando ativados, estimulam cascatas de sinalização que acabam por liberar substâncias neuroativas, estabelecendo uma comunicação neuroglial (Allen and Barres 2009).

2.1. Parâmetros para estudo dos astrócitos

A proteína fibrilar glial ácida, GFAP (do inglês *glial fibrilar acidic protein*) é o principal filamento intermediário de astrócitos maduros do SNC. É considerada um dos principais antígenos utilizados para a identificação e estudo do comportamento astrocítico. A ativação glial, em resposta à injúria, envolve mudanças em seu conteúdo. Um aumento de sua expressão no tecido, associado a condições de injúria cerebral, pode ser interpretado como sinal de astrogliose (Eng, Ghirnikar et al. 2000).

Os astrócitos provêem um importante sistema antioxidante para o sistema nervoso através da produção e secreção de glutationa (GSH) (Dringen, 2000). A glutationa é um tripeptídeo (γ-glutamilcisteinilglicina) que atua direta ou indiretamente em muitos processos biológicos (Pope, Milton et al. 2008). Para a efetividade de tal papel antioxidante, é necessário um equilíbrio entre suas formas oxidada (GSSG) e reduzida (GSH), bem como da quantidade sintetizada e do consumo e transporte para outras células (Hirrlinger and Dringen 2010). A GSH age de maneira enzimática ou não enzimática na conversão do peróxido de hidrogênio em água, neutralizando esta espécie reativa.

O comprometimento desse sistema antioxidante pode influenciar na sobrevivência neuronal em certas condições patológicas que envolvem danos oxidativos, como a DA. Há evidências de que o conteúdo de GSH diminui com o envelhecimento (Cudkowicz, Sexton et al. 1999) e de que seu fornecimento e o de precursores aos neurônios estão afetados na DA (Calabrese, Sultana et al. 2006).

A S100B é uma proteína ligante de cálcio, pertencente a uma família de proteínas chamadas S100 (solúvel em 100% de sulfato de amônio), produzida e secretada - principalmente - por astrócitos. Ela possui ações parácrinas e autócrinas, tanto intra quanto extracelulares, sobre neurônios e sobre outros astrócitos (Rothermundt, Peters et al. 2003). Dentre estas ações, estão a regulação da proliferação, diferenciação e morfologia celular, homeostase do Ca^{2+}, fosforilação e transcrição de proteínas, atividade enzimática e metabolismo (Donato 2003; Goncalves, Leite et al. 2008). Tais efeitos são mediados, em parte, pela interação da S100B com o receptor para produtos finais de glicação avançada (RAGE), um receptor multiligante, envolvido na transdução de estímulos inflamatórios e de diversos fatores neurotróficos e neurotóxicos (Donato 2001; Donato 2003).

Episódios isquêmicos, procedimentos cirúrgicos, traumatismos cranianos e distúrbios psiquiátricos provocaram aumento da proteína em soro e no líquido cefalorraquidiano (LCR) (Romner, Ingebrigtsen et al. 2000; Bertsch, Casarin et al. 2001; Robson, Alston et al. 2001; Berger, Pierce et al. 2002; Andreazza, Cassini et al. 2007). Já em doenças neurodegenerativas, existe uma série de controvérsias. Alguns estudos mostram aumento de S100B em análise de tecido *post mortem* de pacientes com Doença de Alzheimer (Van Eldik and Griffin 1994) e esclerose lateral amiotrófica (Migheli, Cordera et al. 1999) e em LCR nos estágios iniciais da doença de Alzheimer (Peskind, Griffin et al. 2001).

Os astrócitos também são conhecidos por estocar glicogênio e prover glicose aos neurônios quando necessário (Lebed, Orlovsky et al. 2008). A glicose é convertida até lactato e este substrato é, então, transportado aos neurônios (Pellerin 2005) e se sabe que nos pacientes em estágio inicial da DA

há uma diminuição da captação glial de glicose (Freemantle, Vandal et al. 2006).

3. Espécies Reativas (ER)

Um radical livre é definido como sendo qualquer espécie química capaz de existência independente e que contenha um ou mais elétrons desemparelhados (Southorn and Powis 1988), situação energeticamente instável que confere alta reatividade a essas espécies. Aproximadamente 5% do oxigênio utilizado na cadeia respiratória mitocondrial não é completamente reduzido a água, sendo convertido a intermediários reativos como radical superóxido ($O_2^{\cdot -}$)**,** radical hidroxila ($OH^{\cdot}$) e peróxido de hidrogênio (H_2O_2) (Cohen 1989; Cadenas and Davies 2000; Turrens 2003). O termo Espécies Reativas de Oxigênio (ERO) inclui, não só os radicais formados pela redução de O_2, mas também alguns não radicais derivados do oxigênio, como o peróxido de oxigênio, o oxigênio *singlet*, o ácido hipocloroso e o ozônio (Halliwell and Gutteridge 2007).

Além das ERO, existem diversas outras espécies radicalares, como os radicais de cloreto, enxofre, brometo, de carbonato e ainda as espécies reativas de nitrogênio, sendo as principais representantes o óxido nítrico ($NO^{\cdot}$) e o peroxinitrito ($ONOO^-$). De uma maneira geral, o termo espécies reativas é utilizado para englobar todas essas espécies.

As ERO são consideradas importantes na sinalização celular, na expressão gênica e no crescimento e sobrevivência celular (Leloup, Casteilla et al. 2011). No entanto, quando há um desbalanço entre os sistemas oxidantes e as defesas antioxidantes (aumento das ER e diminuição das defesas), um quadro

de estresse oxidativo se instala, podendo causar dano celular por oxidar estruturas como proteínas, lipídios e DNA, alterando, também, suas funções biológicas (Droge 2002).

O SNC é particularmente sensível à lipoperoxidação devido ao seu alto conteúdo de ácidos graxos poliinsaturados e ao alto consumo de oxigênio – cerca de 20% do total consumido (Mariani, Polidori et al. 2005; Ferreira, Bonatto et al. 2006). Na DA, há um aumento no nível de ER, sendo responsáveis, em parte, pelo prejuízo cognitivo observado nesses pacientes (Ansari and Scheff 2010). A infusão icv de STZ é capaz de mimetizar este aumento de estresse oxidativo e nitrosativo, tanto por aumentar as ER, quanto por diminuir as defesas antioxidantes (Sharma and Gupta 2002; Rodrigues, Biasibetti et al. 2009; Tramontina, Wartchow et al. 2011).

4. (-)-Epigalocatequina-3-galato

O chá verde é a segunda bebida mais consumida no mundo (Sae-tan, Grove et al. 2011). No leste asiático, esta bebida, obtida da planta *Camellia sinensis,* é consumida há centenas de anos e um crescente número de trabalhos e estudos a respeito de suas propriedades benéficas em doenças cardiovasculares, neurodegenerativas e antitumorais tem sido observado (Ahmed, Wang et al. 2004; Doss, Potta et al. 2005; Ahmed, Pakozdi et al. 2006; Rezai-Zadeh, Arendash et al. 2008; Tanaka, Ishii et al. 2011). Os benefícios potenciais, associados ao consumo de chá verde podem ser atribuídos às propriedades antioxidantes dos polifenóis, particularmente as catequinas. As principais catequinas são a (-)-epigalocatequina-3-galato (EGCG) constituindo de 48-55% dos flavonóides totais, seguida da (-)-epigalocatequina (EGC),

constituindo de 9-12%, (-)-epicatequina-galato (ECG), representando também de 9-12% e da (-)-epicatequina (EC), representando apenas 5-7% dos flavonóides totais. Na figura 3 estão representadas as estruturas das diferentes catequinas.

O típico chá verde, preparado pela infusão de 1 g de folhas da planta *Camellia Sinensis* em 100 mL de água fervente por 3 minutos contém, em média, de 250 a 350 mg de matéria seca, composta por 30 a 42% de catequinas e por 3 a 6% de cafeína (Riemersma, Rice-Evans et al. 2001; Babu and Liu 2008).

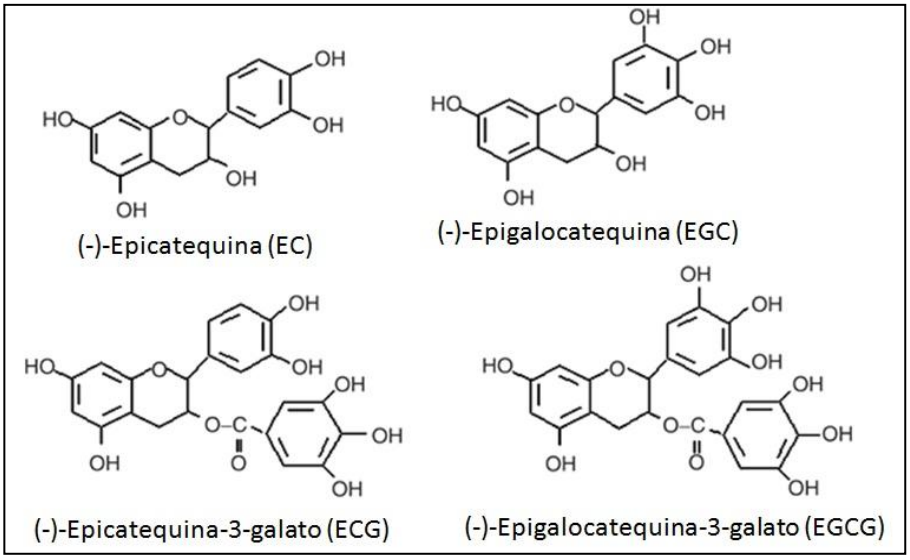

Figura 3: Catequinas constituintes do chá verde.

Sobre a biodisponibilidade dos polifenóis do chá verde, sabe-se que é relativamente baixa e a forma de administração parece ter grande influência. As catequinas podem ser absorvidas no intestino por difusão passiva ou por transportadores (Vaidyanathan and Walle 2001). O pico da concentração plasmática, em humanos, é atingido entre 1,5 e 2,5 horas após o consumo (Nakagawa, Okuda et al. 1997), sendo de aproximadamente 2 μM, o que

representa até 2% da quantidade ingerida (Pietta, Simonetti et al. 1998; Yang, Chen et al. 1998). O mesmo perfil de absorção é encontrado em ratos (Chen, Lee et al. 1997).

A EGCG, por ser a mais prevalente e possuir maior capacidade antioxidante, é alvo de muitos estudos. Ela é capaz de prevenir e/ou reduzir efeitos deletérios das ERO associadas a doenças neurodegenerativas (Weinreb, Amit et al. 2007), além de ser capaz de atravessar a BHE (Chu, Wang et al. 2007; Lin, Wang et al. 2007). Estudos *in vivo* e *in vitro* mostram que a EGCG aumenta as defesas antioxidantes, como a GSH e também a expressão de enzimas como a superóxido dismutase (SOD), glutationa peroxidase (GPx) e catalase (CAT) (Fu, Zheng et al. 2008; Dorchies, Wagner et al. 2009; Sahin, Orhan et al. 2010).

OBJETIVOS

Objetivo geral

Verificar o efeito da (-)-epigalocatequina-3-galato sobre o modelo de demência induzido pela infusão intracerebroventricular de estreptozotocina.

Objetivos específicos

- Avaliar o déficit cognitivo em animais submetidos ao modelo de doença de Alzheimer esporádica por infusão intracerebroventricular de estreptozotocina após 7 semanas.
- Avaliar o estresse oxidativo e nitrosativo no hipocampo.
- Avaliar a captação de glicose e a atividade da enzima acetilcolinesterase no hipocampo.
- Medir o imunoconteúdo de S100B e GFAP no tecido hipocampal, bem como o conteúdo de S100B secretado no LCR.
- Avaliar o efeito da (-)-epigalocatequina-3-galato sobre os parâmetros acima, administrada 2 semanas após a infusão icv de STZ, durante 4 semanas.

PARTE II

CAPÍTULO I

Green tea (-)epigallocatechin-3-gallate reversed oxidative stress and reduces acetylcholinesterase activity in streptozotocin-induced model of dementia

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Green tea (-)epigallocatechin-3-gallate reverses oxidative stress and reduces acetylcholinesterase activity in a streptozotocin-induced model of dementia

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Abstract

Alzheimer's disease (AD) is the most prevalent form of dementia. Intracerebroventricular (ICV) infusion of streptozotocin (STZ) provides a relevant animal model of chronic brain dysfunction that is characterized by long-term and progressive deficits in learning, memory, and cognitive behavior, along with a permanent and ongoing cerebral energy deficit. Many studies with green tea epigallocatechin gallate (EGCG) demonstrate its beneficial effects on cognition and memory. As such, this study evaluated, for the first time, the effects of sub-chronic EGCG treatment of rats that were submitted to ICV infusion of STZ. Male Wistar rats were divided into sham, STZ, sham+EGCG and STZ+EGCG groups. EGCG was administered at a dose of 10 mg/kg/day for 4 weeks per gavage. Learning and memory was evaluated using Morris' Water Maze. Oxidative stress markers and involvement of the nitric oxide system were also evaluated as well as glial parameters including S100B content and secretion and GFAP content, acetylcholinesterase activity (AChE) and glucose uptake. Our results show that EGCG was not able to modify glucose uptake and glutathione content, although cognitive deficit, S100B content and secretion, AChE activity, glutathione peroxidase activity, NO metabolites, and Reactive Oxygen Species content were completely reversed by EGCG administration, confirming the neuroprotective potential of this compound. These findings contribute to the understanding of diseases accompanied by cognitive deficits and the STZ-model of dementia.

Keywords

Acetylcholinesterase - astrocytes – dementia – epigallocatechin gallate – oxidative stress - streptozotocin

Introduction

Dementia can be conceptualized as the chronic and progressive dysfunction of cortical and/or subcortical activity, resulting in a complex cognitive decline [1]. The most common types of dementia are Alzheimer's disease (AD), frontoparietal dementia and vascular dementia [2]. AD is the most prevalent form of dementia comprising 50-70% of all cases, affecting about 40% of individuals over 85 years [3]. Clinically, AD is characterized by a progressive and irreversible cognitive impairment, with significant deficits in the ability of individuals to form new memories and remembering recent events [4-5]. Acetylcholine, a neurotransmitter involved in the regulation of learning and memory functions [6], presents a reduced concentration in the neocortex and hippocampus in AD [7-8].

Histopathologically, the disease is characterized by extensive neuronal loss, mainly of the cholinergic neurons in the hippocampus, the presence of senile plaques and intracellular neurofibrillary tangles [5, 9-11]. Streptozotocin (STZ), obtained from the *Streptomyces* species, is used intrapenitoneally to induce diabetes in rodents at concentrations ranging from 50–100 mg/kg [12]. When STZ is administrated in lateral ventricles, at a concentration 3 mg/kg, it provides a relevant model of chronic brain dysfunction that is characterized by long-term and progressive deficits in learning, memory, and cognitive behavior [13]. Oncoming to the cognitive deficit, occurs an increased production of ROS, augmentation of MDA levels, reductions in glutathione content and decreased glucose uptake are all observed [14-15].

In AD and other aging-related neurodegenerative diseases, recruitment and activation of glial cells occur even before the appearance of pathological and clinical signs of disease [16-17]. Astrocytes are involved in the brain antioxidant defense and

secretion of neurotrophic factors. Glial activation in response to injury stimuli commonly involves changes in glial fibrillary acidic protein (GFAP), S100B protein, synthesis and release of glutathione, and glutamate metabolism. Therefore, the evaluation of glial activation in STZ-induced and other models of dementia is very useful to understand the role of astrocytes in these diseases, as well as to identify possible molecular therapeutic targets. S100B is a calcium-binding protein produced and secreted by astrocytes, involved in the regulation of glucose metabolism, cytoskeleton and proliferation [18]. Extracellular S100B has trophic activity on neurons and glial cells [19].

Epigallocatechin-3-gallate (EGCG) is the major polyphenol in green tea, known for its potent antioxidant property [20]. It has recently attracted the interest as a natural molecule (see [21] for a review) due to its emerging biological activities, such as anti-inflammatory, anti-arthritic, and cancer chemoprevention effects, in a variety of experimental models [22-25]. Although its cellular mechanism is still not established, reports suggest the involvement of nitric oxide and oxidative stress pathways in its protective actions [26-27]. EGCG provides both short- and long-term protection against oxidative stress through a variety of mechanisms [28]. The antioxidant activity of EGCG has been observed in various models; however, it can also act as a pro-oxidant under certain conditions.

The present study has been designed to investigate the effect of EGCG and its possible neuroprotective mechanisms in the STZ-induced dementia model, evaluating spatial cognitive deficit, oxidative stress, acetylcholinesterase activity, glucose uptake and glial alterations in the hippocampus of the rat brain.

Material and methods

Animals

Male Wistar rats (90-days old, weighing 250–320 g) were obtained from our breeding colony (at the Department of Biochemistry, Universidade Federal do Rio Grande do Sul), and were maintained under controlled light and environmental conditions (12 hour light/12 hour dark cycle at a constant temperature of 22 ± 1°C) with free access to food and water. All animal experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996, and following the regulations of the local animal house authorities.

Rats were divided into 4 groups: sham (N = 11), sham-epigallocatechin-3-gallate (N = 10), STZ (N = 11), and STZ-epigallocatechin-3-gallate (N = 11). After behavioral tasks, rats were anaesthetized for CSF puncture and posterior hippocampal slice preparation, aiming to evaluate S100B secretion, S100B and GFAP contents, glucose uptake, acetylcholinesterase activity (AChE), nitrite contents (NO), glutathione contents (GSH), reactive species levels (DCFH), and glutathione peroxidase activity (GPx).

Surgical procedure

Streptozotocin was ICV infused, based on previous studies [29]. Briefly, on the day of the surgery animals were anesthetized with ketamine/xylazine (75 and 10 mg/kg, respectively, i.p.) and placed in a stereotaxic apparatus. A midline sagittal incision was made in the scalp. Burr holes were drilled in the skull on both sides over the lateral ventricles. The lateral ventricles were accessed using the following coordinates [30]: 0.9 mm posterior to bregma; 1.5 mm lateral to sagittal suture; 3.6 mm beneath the surface of the brain. Rats received a single bilateral infusion of 5 μL STZ (3 mg/kg) or vehicle

(Hank's balanced salt solution – HBSS – containing in mM: 137 NaCl; 0.63 Na_2HPO_4; 4.17 $NaHCO_3$; 5.36 KCl; 0.44 KH_2PO_4; 1.26 $CaCl_2$; 0.41 $MgSO_4$; 0.49 $MgCl_2$ and 10 glucose, in pH 7.4) using a Hamilton syringe. After the surgical procedure, rats were placed on a heating pad to maintain body temperature at 37.5 ± 0.5°C and were kept there until recovery from anesthesia. The animals were submitted to behavioral tasks and biochemical analysis at 7 weeks after STZ injection.

Epigallocatechin gallate administration

Epigallocatechin gallate was dissolved in 0.9% NaCl and administered per gavage. The STZ+EGCG and EGCG groups received epigallocatechin gallate (10 mg/kg) on the 15th to 43th day after ICV-infusion of STZ. The treatment lasted 4 weeks, starting at 2 weeks after the surgical procedure.

Cognitive evaluation

At six weeks after surgery (2 weeks resting + 4 weeks of treatment), rats were submitted to training in the Morris water maze [31-32]. The apparatus consisted of a circular pool (180 cm diameter, 60 cm high) filled with water (depth 30 cm; 24± 1°C), placed in a room with consistently-located spatial cues. An escape platform (10 cm diameter) was placed in the middle of one of the quadrants, 1.5 cm below the water surface, equidistant from the sidewall and the middle of the pool. The platform provided the only escape from the water and was located in the same quadrant every trial. Four different starting positions were equally spaced around the perimeter of the pool. On each training day, all four start positions were used once in a random sequence, i.e., four training trials per day. A trial began by placing the animal in the water facing the wall of the pool at one of the starting points. If the animal failed to escape within 60 s it was

gently conducted to the platform by the experimenter. The rat was allowed to stay there for 20 s. The inter-trial interval was 10 min. After each trial, the rats were dried, and returned to their cages at the end of the session. Animals were trained for 5 days. At twenty-four h after the last training session, the rats were submitted to a test session (seven weeks after surgery). Before this session, the submerged platform was removed. The retention test consisted of placing the animals in the water for 1 min. The number of crossings over the original position of the platform and time spent in the target quadrant compared to the opposite quadrant were measured.

Obtaining CSF and hippocampal samples

Animals were anesthetized as described above and then positioned in a stereotaxic holder and CSF was obtained by cisterna magna puncture using an insulin syringe (27 gauge × 1/2" length). CSF was frozen (−20°C) until further analysis [33]. The animals were killed by decapitation, and the brains were removed and placed in cold saline medium with the following composition (in mM): 120 NaCl; 2 KCl; 1 $CaCl_2$; 1 $MgSO_4$; 25 HEPES; 1 KH_2PO_4 and 10 glucose, adjusted to pH 7.4. The hippocampi were dissected and transverse slices of 0.3 mm were obtained using a McIlwain Tissue Chopper. Fresh slices were used for glucose uptake measurements, the rest of the samples were then frozen at −20°C (for measurement of GFAP and S100B) or -80°C (for measurement of AChE, ROS, GPx, GSH and NO).

Chemicals

Epigallocatechin gallate (EGCG) was purchased from Interprise USA Corporation. Streptozotocin, 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES), o-phenylenediamine (OPD), o-phthaldialdehyde (OPA), meta-phosphoric acid, sodium nitrate, nitrate reductase, o-phenylenediamine, and monoclonal anti-S100B antibody were purchased from Sigma. Anti-S100 antibody conjugated with peroxidase and anti-GFAP antibody were from Dako. Peroxidase secondary antibodies were from Amersham. All other chemicals were purchased from local commercial suppliers.

Quantification of S100B and GFAP

S100B content in the hippocampus and CSF was measured by ELISA [34]. Briefly, 50 μL of sample plus 50 μL of Tris buffer were incubated for 2 h on a microtiter plate previously coated with monoclonal anti-S100B (SH-B1). Polyclonal anti-S100B was incubated for 30 min and then peroxidase-conjugated anti-rabbit antibody was added for a further 30 min. A colorimetric reaction with o-phenylenediamine was measured at 492 nm. The standard S100B curve ranged from 0.020 to 10 ng/mL. ELISA for GFAP [35] was carried out by coating the microtiter plate with 100 μL samples containing 30 μg of protein for 24 h at 4°C. Incubation with a rabbit polyclonal anti-GFAP for 2 h was followed by incubation with a secondary antibody conjugated with peroxidase for 1 h, at room temperature; the standard GFAP curve ranged from 0.1 to 7.5 ng/mL.

Acetylcholinesterase activity

Acetylcholinesterase activity was measured using the Ellman method [36]. Briefly, hippocampal slices were homogenized in cold 10 mM Tris-HCl buffer, pH 7.2.

Homogenates were centrifuged at 10 000g for 10 min at 4°C; supernatants used as acetylcholinesterase sources were kept into aliquots and stored at -20°C. Enzyme samples in 20 mM phosphate buffer, pH 7.4, were incubated for 150 s with 0.8 mM acetylthiocholine iodide in the presence of 10 mM 5,5-ditio-bis-(2-nitrobenzóico) (DTNB), for color development (all chemicals from Sigma). Production of the yellow anion of 5-thio-2-nitrobenzoic acid was measured with a SPECTRA max 190, 96-well plate reader, at 415 nm.

Glucose uptake

Glucose uptake was measured in hippocampal slices. Briefly, slices were transferred to 24-well plates and incubated for 30 min at 37°C in a Hank's balanced salt solution (HBSS) containing (in mM): 137 NaCl, 5.36 KCl, 1.26 $CaCl_2$, 0.41 $MgSO_4$, 0.49 $MgCl_2$, 0.63 $Na_2HPO_4.7H_2O$, 0.44 KH_2PO_4, 4.17 $NaHCO_3$ and 5.6 glucose, adjusted to pH 7.4. The assay was started by the addition of 0.1 µCi/mL [2,3-^{3}H]deoxy-D- glucose. Incubation was stopped after 30 min by removal of the medium and rinsing the cells twice with ice-cold HBSS. The slices were then lysed in a solution containing 0.5 M NaOH. Radioactivity was measured in a scintillation counter. Non-specific uptake was determined by using 25 µM cytochalasin B. Final glucose uptake was obtained by subtracting the non-specific uptake of the total uptake to obtain the specific uptake.

Glutathione and NO contents

The glutathione content was determined as described before [37]. Briefly, hippocampal slices were homogenized and assayed in sodium phosphate buffer (0.1 M, pH 8.0) containing 5 mM EDTA and protein was precipitated with 1.7% meta-phosphoric acid. Supernatant was assayed with o-phthaldialdehyde (1 mg/mL of methanol) at room

temperature for 15 min. Fluorescence was measured using excitation and emission wavelengths of 350 and 420 nm, respectively. A calibration curve was performed with standard glutathione solutions (0–500 μM). Glutathione concentrations were expressed as nmol/mg protein. NO metabolites, NO^{-3} (nitrate) and NO^{-2} (nitrite) were determined according to [38]. Briefly, homogenates from one hippocampus were mixed with 25% trichloroacetic and centrifuged at 1 800 g for 10 min. The supernatant was immediately neutralized with 2 M potassium bicarbonate. NO^{-3} was reduced to NO^{-2} by nitrate reductase. The total NO^{-2} in the supernatant was measured by a colorimetric assay at 540 nm, based on the Griess reaction. A standard curve was performed using sodium nitrate (0–50 μM).

Evaluation of Intracellular ROS Production

Intracellular ROS production was detected using the non-fluorescent cell permeating compound, 20-70-dichlorofluorescein diacetate (DCFH-DA). DCFH-DA is hydrolyzed by intracellular esterases to dichlorofluorescin (DCFH), which is trapped within the cell. This non-fluorescent molecule is then oxidized to fluorescent dichlorofluorescin (DCF) by the action of cellular oxidants. Hippocampal slices were homogenized and treated with DCFH-DA (10 μM) for 30 min at 37°C. Following DCFH-DA exposure, the cells were scraped into PBS with 0.2% Triton X-100. The fluorescence was measured in a plate reader (Spectra Max GEMINI XPS, Molecular Devices, USA) with excitation at 485 nm and emission at 520 nm [39].

Glutathione peroxidase activity

GPx activity was measured using the RANSEL kit (Randox Antioxidant Products, UK).

Protein Determination

Protein content was measured by Lowry's method using bovine serum albumin as standard [40].

Statistical analysis

Parametric data from the experiments are presented as means ± standard error and statistically evaluated by two-way analysis of variance, followed by the Tukey's test, assuming $p < 0.05$. The escape latency parameter in the water maze task was evaluated by repeated measures analysis of variance, assuming $p < 0.05$.

Results

Behavioral effects

The Morris water maze was used to evaluate reference memory in the four groups: sham, sham+EGCG, STZ, and STZ+EGCG. In the training sessions, from day 3 onwards, there was a significant increase in the average time to find the platform in the STZ group (escape latency), when compared with the sham group (Fig. 2A) ($F_{(3, 43)} = 7.206$, $p = 0.002$). In the probe trial, the STZ group presented the highest latency to arrive at the original platform location, in relation to all other groups (Fig. 2B) ($F_{(3,43)} = 53.814$, $p = 0.0001$). Moreover, the number of crossings over the previous platform location were significantly lower in the STZ group, compared to all the other groups, showing a clear effect of EGCG treatment on the cognitive decline observed in this model (Fig. 2C) ($F_{(3,43)} = 18.892$, $p = 0.0001$). In addition, the STZ group demonstrated no differences in the time spent in the target quadrant, compared with the opposite quadrant (Student's t test, $p = 0.639$), in contrast to the other groups (Fig. 2D). Note that epigallocatechin-3-gallate "per se" had no effect on the behavior analyzed.

Glucose uptake

A significant decrease in glucose uptake was observed in the STZ-treated group, as compared to the sham group, and this difference was not reversed by EGCG administration (Fig. 3) ($F_{(3, 26)} = 7.823$, $p = 0.012$).

Changes in S100B and GFAP contents

A significant increase in S100B immunocontent ($F_{(3, 30)} = 4.630$, $p = 0.010$) of the hippocampus was observed in STZ-treated rats (Fig. 4A) and this increase was not found in the STZ+EGCG group ($p = 0.913$); this effect in the STZ group was not found in the cerebral cortex (data not shown) and EGCG by itself did not alter the S100B and GFAP contents in either brain region. The hippocampal GFAP content was not changed in any of treatments ($F_{(3, 25)} = 0.021$, $p = 0.998$; Fig. 4B), nor was it significantly higher in the cerebral cortex (data not shown).

Alterations in CSF S100B

A significant decrease in CSF S100B was observed in the STZ-treated group, as compared to the sham group, and this difference was reversed by EGCG administration (Fig. 5) ($F_{(2, 22)} = 4.571$, $p = 0.017$).

Acetylcholinesterase activity

There was a significant rise in enzyme activity in the streptozotocin-treated group ($F_{(3, 35)} = 3.635$, $p = 0.037$). The acetylcholinesterase activity in the streptozotocin-treated rats that received EGCG was not different to that of the sham ($p = 0.993$), showing that

EGCG reversed the augmentation in acetylcholinesterase activity provoked by STZ (Fig. 6).

Oxidative stress in the hippocampus

Glutathione content, glutathione peroxidase activity (Fig. 7), NO production (based on nitrite content) and ROS levels (based on oxidation of DCFH) (Fig. 8) were used as parameters to evaluate a possible hippocampal oxidative and nitrosative stress. The GPx activity was decreased in the STZ-group ($F_{(3, 39)}$ = 12.518, p = 0.0001) and EGCG reversed this effect (p = 0.962) (Fig. 7A). Glutathione content was lower in STZ-treated rats and EGCG did not reverse this decrease (Fig. 7B) ($F_{(3, 33)}$ = 7.450, p = 0.032). We used the technique of DCFH-DA to measure the levels of reactive oxygen species in cells (Fig 8A). There was an increase in ROS in the STZ group ($F_{(3, 33)}$ = 26.707, p = 0.0001) and this augmentation was reverted by EGCG (p = 0.978). Moreover, EGCG exhibited a pro-oxidant effect (p = 0.020). Results show an increase in nitrite content in the STZ group, which was reverted in STZ+EGCG animals (Fig. 8B) ($F_{(3, 37)}$ = 5.706, p = 0.029).

Discussion

Streptozotocin, injected once in rat lateral ventricles, resulted in a persistent and significant deficit in performance in the Morris water maze tests at 7 weeks after the surgery. These results are in agreement with other results demonstrating cognitive impairment after streptozotocin administration (ICV) in rats [41] and intracerebral in mice [42]. The Morris water maze test is used to test spatial memory by observing the latency to reach a hidden platform. As such, a decrease in latency time in repeated trials (4 trials/day for 5 days) demonstrates intact learning and memory function. STZ-treated rats did not show a decline in the latency time, whereas EGCG treatment (10 mg/kg/day

orally for 28 days) of STZ-treated rats decreased the time to reach the hidden platform. Recent reports have shown that treatment with aminoguanidine [41], statins [30] and even non-pharmacological actions, such as exercise [32], exert beneficial effects by preventing or reversing cognitive and biochemical impairment in this model. It is interesting to note that one of the key mechanisms of neuronal death in AD involves damage to energy metabolism [43]. Glucose uptake was evaluated in this work and, not surprisingly, the STZgroup showed a decreased glucose uptake. EGCG was not able to reverse this reduction, probably because it does not interfere in glucose metabolism or pathways. However, even though EGCG did not alter the glucose uptake by hippocampal cells in treated rats, their cognitive behaviour was kept intact, as observed for the learning curve of STZ+EGCG rats.

In the present study, we evaluated the antioxidant capacity of EGCG in the streptozotocin-induced model of dementia, because free radical generation is a major component of neurodegeneration, together with memory impairment in this model [13, 29]. This was demonstrated by the measurement of total reactive oxygen species in the hippocampus by the DCFH-DA technique. A pro-oxidant effect of EGCG was found *per se*, as also described in previous *in vitro* studies, in both tumor cells and normal cells [44-45]. Nevertheless, our results for behavior did not show any prejudicial alterations in learning and memory and, furthermore, EGCG presented an antioxidant activity in the STZ-induced group.

Our study confirmed some findings described previously for the STZ-induced model of dementia: decreased GSH content [30, 41, 46], decreased GPx activity [47-48], and augmentation of nitrite [49-50]. The antioxidant system requires GSH, whose levels diminish when levels of free radicals increase [51]. Glutathione eliminates H_2O_2 and organic peroxides by glutathione peroxidase. GPx activity is decreased in the STZ-

group and EGCG reversed this decline, possibly explaining why GSH was maintained decreased in the STZ+EGCG group. GSH was consumed by the GPx reaction, and stimulated by EGCG. Levels of nitric oxide are increased in the STZ group and, again, EGCG could reverse this augmentation. There is substantial evidence to indicate that the protective effect of EGCG may be partially due to its NO scavenging and Nitric Oxide Synthese (NOS) inhibiting activity [52-53]. Lin and Lin [54] showed that, in addition to the reduction in iNOS expression, tea polyphenols may block peroxynitrite and nitrite production through inhibition of oxidative reactions. These results suggest that EGCG decreases the activity and protein levels of iNOS by reducing the expression of iNOS mRNA and that the reduction could occur through prevention of the binding of nuclear factor-kB to the iNOS promoter, thereby inhibiting the induction of iNOS transcription.

The cholinesterase inhibitors are currently being used for symptomatic treatment of patients with AD [55]. A significant increase was found in acetylcholinesterase activity in the STZ group, as compared to SHAM, and this increase leads to diminished cholinergic transmission due to a decrease in acetylcholine level. EGCG significantly decreased acetylcholinesterase activity, as compared to the STZ group, suggesting that this compound could be useful for reducing the cognitive deficit due to increasing cholinergic communication, such as by some antioxidant compounds [46, 49, 56].

S100B is a calcium-binding protein predominantly expressed and secreted by astrocytes in the vertebrate brain [19, 57] and high levels of brain tissue S100B have been observed in neurodegenerative disorders, including AD [58]. Intracellularly, S100B binds to many protein targets, possibly modulating cell proliferation, cytoskeleton plasticity, and astrocyte energy metabolism (see [59] for a review). S100B

alterations could lead to the downregulation of the glycolytic pathway in intracerebroventricular-injected streptozotocin rats [60]. Protein phosphatase calcineurin appears to be involved in the inflammatory activation of astrocytes in transgenic AD models [61] and S100B is able to stimulate this [62]. Interestingly, we found a significant increment in hippocampal S100B in the STZ group, corroborating previous results [63-64], which was prevented by EGCG administration.

Although the CSF S100B content is not necessarily increased in AD subjects [65], the present study showed that CSF S100B is lower in the STZ group and EGCG administration prevented this decrease. Assuming that extracellular S100B has neurotrophic activity [18, 34], this reduction could indicate impairment in astroglial function in some brain regions in STZ-treated rats. Interestingly, in other dementia models, such as okadaic acid injection [66] and chronic hipoperfusion [64], a decrease in CSF S100B is also observed.

Most reports about oxidative stress and dementia have shown that antioxidant molecules can prevent the oxidative stress and cognitive impairment in the model of STZ-induced dementia. However, our findings indicate that the major green tea catechin EGCG exhibits a beneficial effect even two weeks after the injury, at a low dose, reinforcing its antioxidant role described in several studies. For example, prior treatment with EGCG (30 mg/kg) lowered lipid peroxidation in isoproterenol-induced cardiotoxicity in rats [67]. EGCG can interact with peroxy radicals and inhibit lipid peroxidation [68] and can protect membrane integrity and scavenge lipid radicals generated in the membrane [69]. Moreover, *in vivo* and *in vitro* studies have demonstrated the beneficial role of EGCG in models of dementia, particularly AD. Green and black tea extracts were able to protect cultured hippocampal cells against Aβ-

induced toxicity, as well as provoke reductions in both Abeta1-40 and 1-42 soluble and insoluble forms in "Swedish" mutant amyloid precursor protein overexpressing (APPsw, Tg) mice [70-71]. However, these studies focused on the amyloid precursor protein and on the activity of the enzymes, alpha-, beta- and gamma-secretase, and the accumulation of the beta-amyloid peptide [72-73]. In our model of dementia, induced by STZ, at this stage, accumulation of the beta amyloid peptide was not observed (data not shown).

Conclusions

In summary, our results for ICV-injected STZ rats confirmed the spatial cognitive deficit and oxidative and nitrative stress in this model and demonstrate some astroglial alterations. Our data indicate the beneficial effect of EGCG in reversing the cognitive deficit in this model of dementia. Moreover, we observed its ability to modulate S100B intracellular content and secretion, acetylcholinesterase activity, and antioxidant activity, which are possibly associated with such cognitive deficits. These findings contribute to the understanding of the neuroprotective effects of EGCG.

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References

[1] Ritchie, K.; Lovestone, S. The dementias. *Lancet* **360:**1759-1766; 2002.

[2] Shagam, J. Y. The many faces of dementia. *Radiol Technol* **81:**153-168; 2009.

[3] Ferri, C. P.; Prince, M.; Brayne, C.; Brodaty, H.; Fratiglioni, L.; Ganguli, M.; Hall, K.; Hasegawa, K.; Hendrie, H.; Huang, Y.; Jorm, A.; Mathers, C.; Menezes, P. R.; Rimmer, E.; Scazufca, M. Global prevalence of dementia: a Delphi consensus study. *Lancet* **366:**2112-2117; 2005.

[4] Cummings, J. L.; Mega, M.; Gray, K.; Rosenberg-Thompson, S.; Carusi, D. A.; Gornbein, J. The Neuropsychiatric Inventory: comprehensive assessment of psychopathology in dementia. *Neurology* **44:**2308-2314; 1994.

[5] Selkoe, D. J. Alzheimer's disease results from the cerebral accumulation and cytotoxicity of amyloid beta-protein. *J Alzheimers Dis* **3:**75-80; 2001.

[6] Blokland, A.; Jolles, J. Spatial learning deficit and reduced hippocampal ChAT activity in rats after an ICV injection of streptozotocin. *Pharmacol Biochem Behav* **44:**491-494; 1993.

[7] Kristensen, M. O. [Neurotransmitters in Alzheimer's disease]. *Ugeskr Laeger* **152:**2165-2168; 1990.

[8] Perry, E.; Walker, M.; Grace, J.; Perry, R. Acetylcholine in mind: a neurotransmitter correlate of consciousness? *Trends Neurosci* **22:**273-280; 1999.

[9] Jalbert, J. J.; Daiello, L. A.; Lapane, K. L. Dementia of the Alzheimer type. *Epidemiol Rev* **30:**15-34; 2008.

[10] Duyckaerts, C.; Delatour, B.; Potier, M. C. Classification and basic pathology of Alzheimer disease. *Acta Neuropathol* **118:**5-36; 2009.

[11] Selkoe, D. J. Alzheimer's disease: genes, proteins, and therapy. *Physiol Rev* **81:**741-766; 2001.

[12] Baydas, G.; Nedzvetskii, V. S.; Nerush, P. A.; Kirichenko, S. V.; Yoldas, T. Altered expression of NCAM in hippocampus and cortex may underlie memory and learning deficits in rats with streptozotocin-induced diabetes mellitus. *Life Sci* **73:**1907-1916; 2003.

[13] Lannert, H.; Hoyer, S. Intracerebroventricular administration of streptozotocin causes long-term diminutions in learning and memory abilities and in cerebral energy metabolism in adult rats. *Behav Neurosci* **112:**1199-1208; 1998.

[14] Sharma, M.; Gupta, Y. K. Chronic treatment with trans resveratrol prevents intracerebroventricular streptozotocin induced cognitive impairment and oxidative stress in rats. *Life Sci* **71:**2489-2498; 2002.

[15] Duelli, R.; Schrock, H.; Kuschinsky, W.; Hoyer, S. Intracerebroventricular injection of streptozotocin induces discrete local changes in cerebral glucose utilization in rats. *Int J Dev Neurosci* **12:**737-743; 1994.

[16] Mrak, R. E.; Griffin, W. S. Glia and their cytokines in progression of neurodegeneration. *Neurobiol Aging* **26:**349-354; 2005.

[17] Strohmeyer, R.; Rogers, J. Molecular and cellular mediators of Alzheimer's disease inflammation. *J Alzheimers Dis* **3:**131-157; 2001.

[18] Donato, R. S100: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. *Int J Biochem Cell Biol* **33:**637-668; 2001.

[19] Goncalves, C. A.; Leite, M. C.; Nardin, P. Biological and methodological features of the measurement of S100B, a putative marker of brain injury. *Clin Biochem* **41:**755-763; 2008.

[20] Matsuo, N.; Yamada, K.; Shoji, K.; Mori, M.; Sugano, M. Effect of tea polyphenols on histamine release from rat basophilic leukemia (RBL-2H3) cells: the structure-inhibitory activity relationship. *Allergy* **52:**58-64; 1997.

[21] Williams, R. J.; Spencer, J. P. Flavonoids, cognition, and dementia: Actions, mechanisms, and potential therapeutic utility for Alzheimer disease. *Free Radic Biol Med*; 2011.
[22] Ahmed, S.; Wang, N.; Lalonde, M.; Goldberg, V. M.; Haqqi, T. M. Green tea polyphenol epigallocatechin-3-gallate (EGCG) differentially inhibits interleukin-1 beta-induced expression of matrix metalloproteinase-1 and -13 in human chondrocytes. *J Pharmacol Exp Ther* **308:**767-773; 2004.
[23] Doss, M. X.; Potta, S. P.; Hescheler, J.; Sachinidis, A. Trapping of growth factors by catechins: a possible therapeutical target for prevention of proliferative diseases. *J Nutr Biochem* **16:**259-266; 2005.
[24] Ahmed, S.; Pakozdi, A.; Koch, A. E. Regulation of interleukin-1beta-induced chemokine production and matrix metalloproteinase 2 activation by epigallocatechin-3-gallate in rheumatoid arthritis synovial fibroblasts. *Arthritis Rheum* **54:**2393-2401; 2006.
[25] Tanaka, T.; Ishii, T.; Mizuno, D.; Mori, T.; Yamaji, R.; Nakamura, Y.; Kumazawa, S.; Nakayama, T.; Akagawa, M. (-)-Epigallocatechin-3-gallate suppresses growth of AZ521 human gastric cancer cells by targeting the DEAD-box RNA helicase p68. *Free Radic Biol Med* **50:**1324-1335; 2011.
[26] Aucamp, J.; Gaspar, A.; Hara, Y.; Apostolides, Z. Inhibition of xanthine oxidase by catechins from tea (Camellia sinensis). *Anticancer Res* **17:**4381-4385; 1997.
[27] Guo, Q.; Zhao, B.; Li, M.; Shen, S.; Xin, W. Studies on protective mechanisms of four components of green tea polyphenols against lipid peroxidation in synaptosomes. *Biochim Biophys Acta* **1304:**210-222; 1996.
[28] Yao, K.; Ye, P.; Zhang, L.; Tan, J.; Tang, X.; Zhang, Y. Epigallocatechin gallate protects against oxidative stress-induced mitochondria-dependent apoptosis in human lens epithelial cells. *Mol Vis* **14:**217-223; 2008.
[29] Sharma, M.; Gupta, Y. K. Effect of chronic treatment of melatonin on learning, memory and oxidative deficiencies induced by intracerebroventricular streptozotocin in rats. *Pharmacol Biochem Behav* **70:**325-331; 2001.
[30] Tramontina, A. C.; Wartchow, K. M.; Rodrigues, L.; Biasibetti, R.; Quincozes-Santos, A.; Bobermin, L.; Tramontina, F.; Goncalves, C. A. The neuroprotective effect of two statins: simvastatin and pravastatin on a streptozotocin-induced model of Alzheimer's disease in rats. *J Neural Transm* **118:**1641-1649; 2011.
[31] Morris, R. Developments of a water-maze procedure for studying spatial learning in the rat. *J Neurosci Methods* **11:**47-60; 1984.
[32] Rodrigues, L.; Dutra, M. F.; Ilha, J.; Biasibetti, R.; Quincozes-Santos, A.; Leite, M. C.; Marcuzzo, S.; Achaval, M.; Goncalves, C. A. Treadmill training restores spatial cognitive deficits and neurochemical alterations in the hippocampus of rats submitted to an intracerebroventricular administration of streptozotocin. *J Neural Transm* **117:**1295-1305; 2010.
[33] Silva, M. C.; Rocha, J.; Pires, C. S.; Ribeiro, L. C.; Brolese, G.; Leite, M. C.; Almeida, L. M.; Tramontina, F.; Ziegler, D. R.; Goncalves, C. A. Transitory gliosis in the CA3 hippocampal region in rats fed on a ketogenic diet. *Nutr Neurosci* **8:**259-264; 2005.
[34] Leite, M. C.; Galland, F.; Brolese, G.; Guerra, M. C.; Bortolotto, J. W.; Freitas, R.; Almeida, L. M.; Gottfried, C.; Goncalves, C. A. A simple, sensitive and widely applicable ELISA for S100B: Methodological features of the measurement of this glial protein. *J Neurosci Methods* **169:**93-99; 2008.
[35] Tramontina, F.; Leite, M. C.; Cereser, K.; de Souza, D. F.; Tramontina, A. C.; Nardin, P.; Andreazza, A. C.; Gottfried, C.; Kapczinski, F.; Goncalves, C. A. Immunoassay for glial fibrillary acidic protein: antigen recognition is affected by its phosphorylation state. *J Neurosci Methods* **162:**282-286; 2007.

[36] Ellman, G. L.; Courtney, K. D.; Andres, V., Jr.; Feather-Stone, R. M. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* **7:**88-95; 1961.
[37] Browne, R. W.; Armstrong, D. Reduced glutathione and glutathione disulfide. *Methods Mol Biol* **108:**347-352; 1998.
[38] Hevel, J. M.; Marletta, M. A. Nitric-oxide synthase assays. *Methods Enzymol* **233:**250-258; 1994.
[39] Vieira de Almeida, L. M.; Pineiro, C. C.; Leite, M. C.; Brolese, G.; Leal, R. B.; Gottfried, C.; Goncalves, C. A. Protective effects of resveratrol on hydrogen peroxide induced toxicity in primary cortical astrocyte cultures. *Neurochem Res* **33:**8-15; 2008.
[40] Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J Biol Chem* **193:**265-275; 1951.
[41] Rodrigues, L.; Biasibetti, R.; Swarowsky, A.; Leite, M. C.; Quincozes-Santos, A.; Quilfeldt, J. A.; Achaval, M.; Goncalves, C. A. Hippocampal alterations in rats submitted to streptozotocin-induced dementia model are prevented by aminoguanidine. *J Alzheimers Dis* **17:**193-202; 2009.
[42] Saxena, G.; Singh, S. P.; Agrawal, R.; Nath, C. Effect of donepezil and tacrine on oxidative stress in intracerebral streptozotocin-induced model of dementia in mice. *Eur J Pharmacol* **581:**283-289; 2008.
[43] Salkovic-Petrisic, M.; Hoyer, S. Central insulin resistance as a trigger for sporadic Alzheimer-like pathology: an experimental approach. *J Neural Transm Suppl***:**217-233; 2007.
[44] Hsuuw, Y. D.; Chan, W. H. Epigallocatechin gallate dose-dependently induces apoptosis or necrosis in human MCF-7 cells. *Ann N Y Acad Sci* **1095:**428-440; 2007.
[45] Chung, W. G.; Miranda, C. L.; Maier, C. S. Epigallocatechin gallate (EGCG) potentiates the cytotoxicity of rotenone in neuroblastoma SH-SY5Y cells. *Brain Res* **1176:**133-142; 2007.
[46] Agrawal, R.; Mishra, B.; Tyagi, E.; Nath, C.; Shukla, R. Effect of curcumin on brain insulin receptors and memory functions in STZ (ICV) induced dementia model of rat. *Pharmacol Res* **61:**247-252; 2010.
[47] Javed, H.; Khan, M. M.; Khan, A.; Vaibhav, K.; Ahmad, A.; Khuwaja, G.; Ahmed, M. E.; Raza, S. S.; Ashafaq, M.; Tabassum, R.; Siddiqui, M. S.; El-Agnaf, O. M.; Safhi, M. M.; Islam, F. S-allyl cysteine attenuates oxidative stress associated cognitive impairment and neurodegeneration in mouse model of streptozotocin-induced experimental dementia of Alzheimer's type. *Brain Res* **1389:**133-142; 2011.
[48] Pinton, S.; da Rocha, J. T.; Gai, B. M.; Prigol, M.; da Rosa, L. V.; Nogueira, C. W. Neuroprotector effect of p,p'-methoxyl-diphenyl diselenide in a model of sporadic dementia of Alzheimer's type in mice: contribution of antioxidant mechanism. *Cell Biochem Funct* **29:**235-243; 2011.
[49] Tiwari, V.; Kuhad, A.; Bishnoi, M.; Chopra, K. Chronic treatment with tocotrienol, an isoform of vitamin E, prevents intracerebroventricular streptozotocin-induced cognitive impairment and oxidative-nitrosative stress in rats. *Pharmacol Biochem Behav* **93:**183-189; 2009.
[50] Dhull, D. K.; Jindal, A.; Dhull, R. K.; Aggarwal, S.; Bhateja, D.; Padi, S. S. Neuroprotective Effect of Cyclooxygenase Inhibitors in ICV-STZ Induced Sporadic Alzheimer's Disease in Rats. *J Mol Neurosci*; 2011.
[51] Dringen, R. Metabolism and functions of glutathione in brain. *Prog Neurobiol* **62:**649-671; 2000.
[52] Nagai, K.; Jiang, M. H.; Hada, J.; Nagata, T.; Yajima, Y.; Yamamoto, S.; Nishizaki, T. (-)-Epigallocatechin gallate protects against NO stress-induced neuronal damage after ischemia by acting as an anti-oxidant. *Brain Res* **956:**319-322; 2002.

[53] Tedeschi, E.; Menegazzi, M.; Yao, Y.; Suzuki, H.; Forstermann, U.; Kleinert, H. Green tea inhibits human inducible nitric-oxide synthase expression by down-regulating signal transducer and activator of transcription-1alpha activation. *Mol Pharmacol* **65:**111-120; 2004.
[54] Lin, Y. L.; Lin, J. K. (-)-Epigallocatechin-3-gallate blocks the induction of nitric oxide synthase by down-regulating lipopolysaccharide-induced activity of transcription factor nuclear factor-kappaB. *Mol Pharmacol* **52:**465-472; 1997.
[55] Ellis, J. M. Cholinesterase inhibitors in the treatment of dementia. *J Am Osteopath Assoc* **105:**145-158; 2005.
[56] Fernandez-Bachiller, M. I.; Perez, C.; Gonzalez-Munoz, G. C.; Conde, S.; Lopez, M. G.; Villarroya, M.; Garcia, A. G.; Rodriguez-Franco, M. I. Novel tacrine-8-hydroxyquinoline hybrids as multifunctional agents for the treatment of Alzheimer's disease, with neuroprotective, cholinergic, antioxidant, and copper-complexing properties. *J Med Chem* **53:**4927-4937; 2010.
[57] Marenholz, I.; Heizmann, C. W.; Fritz, G. S100 proteins in mouse and man: from evolution to function and pathology (including an update of the nomenclature). *Biochem Biophys Res Commun* **322:**1111-1122; 2004.
[58] Griffin, W. S.; Sheng, J. G.; Royston, M. C.; Gentleman, S. M.; McKenzie, J. E.; Graham, D. I.; Roberts, G. W.; Mrak, R. E. Glial-neuronal interactions in Alzheimer's disease: the potential role of a 'cytokine cycle' in disease progression. *Brain Pathol* **8:**65-72; 1998.
[59] Donato, R. Intracellular and extracellular roles of S100 proteins. *Microsc Res Tech* **60:**540-551; 2003.
[60] Hoyer, S.; Lannert, H. Long-term abnormalities in brain glucose/energy metabolism after inhibition of the neuronal insulin receptor: implication of tau-protein. *J Neural Transm Suppl***:**195-202; 2007.
[61] Norris, C. M.; Kadish, I.; Blalock, E. M.; Chen, K. C.; Thibault, V.; Porter, N. M.; Landfield, P. W.; Kraner, S. D. Calcineurin triggers reactive/inflammatory processes in astrocytes and is upregulated in aging and Alzheimer's models. *J Neurosci* **25:**4649-4658; 2005.
[62] Leal, R. B.; Frizzo, J. K.; Tramontina, F.; Fieuw-Makaroff, S.; Bobrovskaya, L.; Dunkley, P. R.; Goncalves, C. A. S100B protein stimulates calcineurin activity. *Neuroreport* **15:**317-320; 2004.
[63] Swarowsky, A.; Rodrigues, L.; Biasibetti, R.; Leite, M. C.; de Oliveira, L. F.; de Almeida, L. M.; Gottfried, C.; Quillfeldt, J. A.; Achaval, M.; Goncalves, C. A. Glial alterations in the hippocampus of rats submitted to ibotenic-induced lesion of the nucleus basalis magnocellularis. *Behav Brain Res* **190:**206-211; 2008.
[64] Vicente, E.; Degerone, D.; Bohn, L.; Scornavaca, F.; Pimentel, A.; Leite, M. C.; Swarowsky, A.; Rodrigues, L.; Nardin, P.; de Almeida, L. M.; Gottfried, C.; Souza, D. O.; Netto, C. A.; Goncalves, C. A. Astroglial and cognitive effects of chronic cerebral hypoperfusion in the rat. *Brain Res* **1251:**204-212; 2009.
[65] Peskind, E. R.; Griffin, W. S.; Akama, K. T.; Raskind, M. A.; Van Eldik, L. J. Cerebrospinal fluid S100B is elevated in the earlier stages of Alzheimer's disease. *Neurochem Int* **39:**409-413; 2001.
[66] Costa, A. P.; Tramontina, A. C.; Biasibetti, R.; Batassini, C.; Lopes, M. W.; Wartchow, K. M.; Bernardi, C.; Tortorelli, L. S.; Leal, R. B.; Goncalves, C. A. Neuroglial alterations in rats submitted to the okadaic acid-induced model of dementia. *Behav Brain Res*; 2010.
[67] Devika, P. T.; Stanely Mainzen Prince, P. (-)Epigallocatechin-gallate (EGCG) prevents mitochondrial damage in isoproterenol-induced cardiac toxicity in albino Wistar rats: a transmission electron microscopic and in vitro study. *Pharmacol Res* **57:**351-357; 2008.
[68] Katiyar, S. K.; Mukhtar, H. Tea antioxidants in cancer chemoprevention. *J Cell Biochem Suppl* **27:**59-67; 1997.
[69] Saffari, Y.; Sadrzadeh, S. M. Green tea metabolite EGCG protects membranes against oxidative damage in vitro. *Life Sci* **74:**1513-1518; 2004.

[70] Bastianetto, S.; Yao, Z. X.; Papadopoulos, V.; Quirion, R. Neuroprotective effects of green and black teas and their catechin gallate esters against beta-amyloid-induced toxicity. *Eur J Neurosci* **23:**55-64; 2006.
[71] Rezai-Zadeh, K.; Arendash, G. W.; Hou, H.; Fernandez, F.; Jensen, M.; Runfeldt, M.; Shytle, R. D.; Tan, J. Green tea epigallocatechin-3-gallate (EGCG) reduces beta-amyloid mediated cognitive impairment and modulates tau pathology in Alzheimer transgenic mice. *Brain Res* **1214:**177-187; 2008.
[72] Lee, J. W.; Lee, Y. K.; Ban, J. O.; Ha, T. Y.; Yun, Y. P.; Han, S. B.; Oh, K. W.; Hong, J. T. Green tea (-)-epigallocatechin-3-gallate inhibits beta-amyloid-induced cognitive dysfunction through modification of secretase activity via inhibition of ERK and NF-kappaB pathways in mice. *J Nutr* **139:**1987-1993; 2009.
[73] Lin, C. L.; Chen, T. F.; Chiu, M. J.; Way, T. D.; Lin, J. K. Epigallocatechin gallate (EGCG) suppresses beta-amyloid-induced neurotoxicity through inhibiting c-Abl/FE65 nuclear translocation and GSK3 beta activation. *Neurobiol Aging* **30:**81-92; 2009.

Legends to the figures

Fig. 1. Schematic representation of the experimental protocol. Time is represented in weeks. Surgery corresponds to STZ or vehicle ICV infusion; 4 weeks of treatment with EGCG; cognitive evaluation based on Morris' water maze (MWM) task; cerebrospinal fluid and hippocampi were harvested for biochemical evaluation.

Fig. 2. Cognitive performance of rats submitted to ICV-STZ injection, as evaluated by the Morris water maze test. (A) Performance in the reference memory protocol, based on escape latency. Each point represents the mean ± standard error. *Significant differences were detected by comparing STZ and other groups from day 3 onward group (N = 10-11, repeated measures analysis of variance, $p < 0.05$); (B) Memory in the probe trial of reference memory, as measured by latency to arrive at the original place of platform location. Values are mean ± standard error. *Significantly different from all groups (N = 10-11, two-way ANOVA, followed by Tukey's test, $p < 0.05$); (C) Number of crossings over the platform position. Values are mean ± standard error. [a] Significantly different from all groups; [b] Significantly different from all groups (N = 10-11, two-way

ANOVA, followed by Tukey's test, $p < 0.05$). (D) Time spent (in s) in the target quadrant compared to the opposite quadrant. Values are mean ± standard error. *Significant difference between the times spent in quadrants in each group (N = 10–11, Student's t test, $p < 0.05$).

Fig. 3. Glucose uptake in hippocampal slices of rats submitted to ICV-STZ injection. Adult rats were submitted to ICV injection of STZ and oral EGCG administration. Seven weeks later, hippocampi were dissected out and the glucose uptake assay performed on hippocampal slices. Values are mean ± standard error. [a] Significant difference between Sham and Sham+EGCG group (N = 6-7, two-way ANOVA, followed by Tukey's test, $p < 0.05$).

Fig. 4. GFAP and S100B content in hippocampus of rats submitted to ICV-STZ injection. Adult rats were submitted to ICV injection of STZ. Hippocampi were dissected out and the contents of S100B (panel A) and GFAP (panel B) were measured by ELISA at the seventh week. Values are mean ± standard error of 6-8 rats in each group. *Significantly different from all other groups (two-way ANOVA, followed by Tukey's test, $p < 0.05$).

Fig. 5. S100B levels in the cerebrospinal fluid of rats submitted to ICV-STZ injection. Seven weeks later, cerebrospinal fluid (CSF) was collected by cisterna magna puncture. S100B content was measured by ELISA. Values are mean ± standard error of seven to ten rats in each group. *Significantly different from other groups (two-way ANOVA, followed by Tukey's test, $p < 0.05$).

Fig. 6. Acetylcholinesterase activity in the hippocampus of rats submitted to ICV-STZ injection. Adult rats were submitted to ICV injection of STZ and EGCG oral administration. Seven weeks later, hippocampi were dissected out and homogenized for measurement of acetylcholinesterase activity. Values are mean ± standard error. *Significantly different from all other groups, (N = 8-9, two-way ANOVA, followed by Tukey's test, $p < 0.05$).

Fig 7. Glutathione peroxidase activity and glutathione levels in the hippocampus of rats submitted to ICV-STZ injection. Adult rats were submitted to ICV injection of STZ. Seven weeks later, hippocampi were dissected out and homogenized for measurement of glutathione peroxidase (in panel A) or GSH (in panel B). Values are mean ± standard error. *Significantly different from all other groups; [a] differs from Sham and Sham+EGCG groups. (N = 8-10, two-way ANOVA, followed by Tukey's test, $p < 0.05$).

Fig 8. Reactive Oxygen Species levels and NO production (based on nitrite content) in the hippocampus of rats submitted to ICV-STZ injection. Adult rats were submitted to ICV injection of STZ. Seven weeks later, hippocampi were dissected out and homogenized for measurement of ROS (in panel A) or NO production (in panel B). Values are mean ± standard error. *Significantly different from all other groups; [a] differs from sham group; [b] differs from STZ group; [c] differs from STZ+EGCG group. (N = 8-10, two-way ANOVA, followed by Tukey's test, $p < 0.05$).

Figure 1

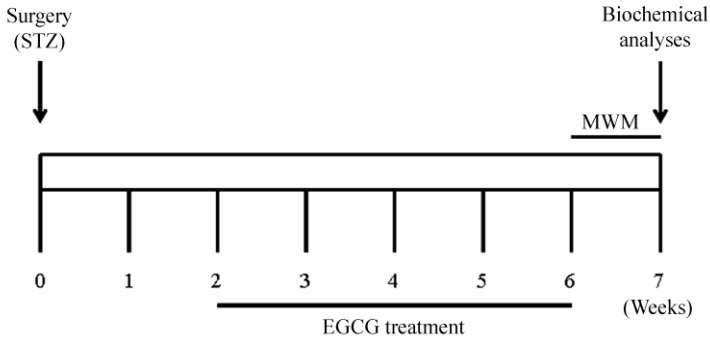


Figure 2

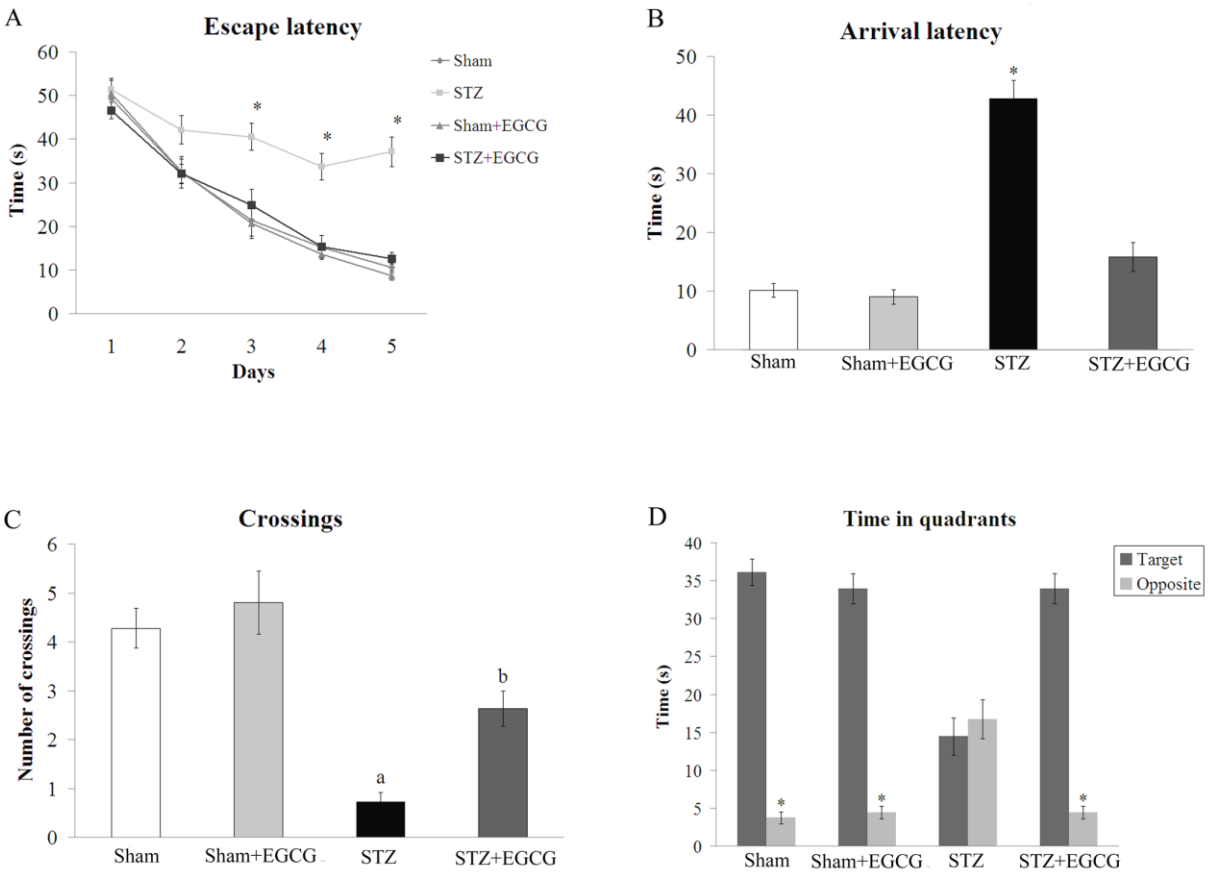


Figure 3

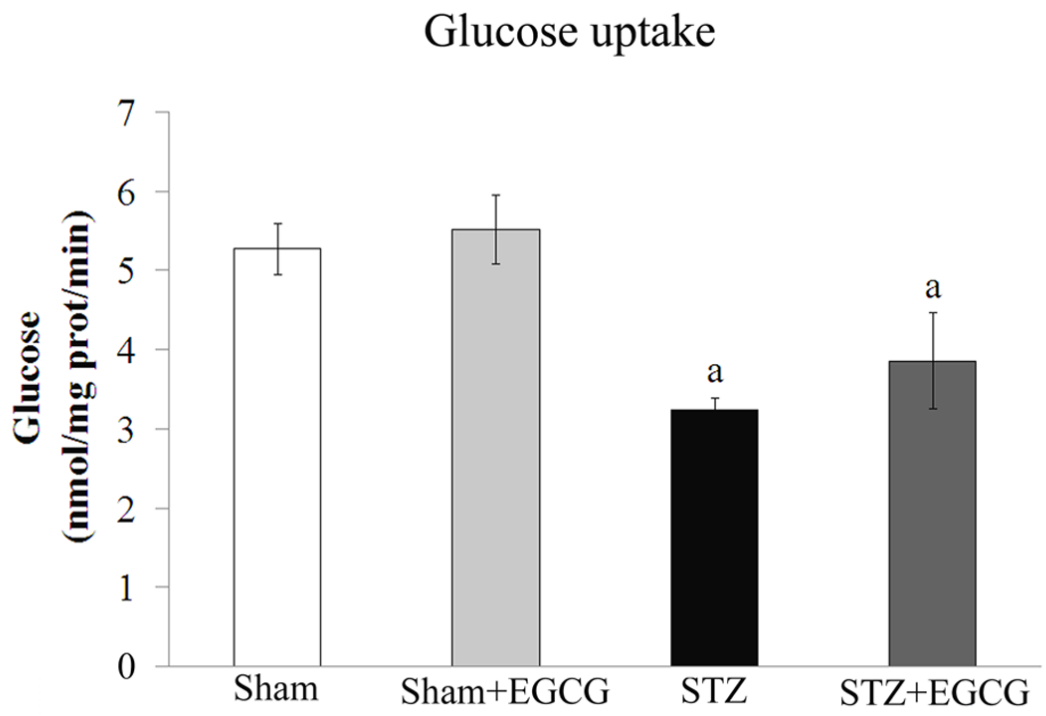

Glucose uptake
Glucose (nmol/mg prot/min)
7
6
5
4
3
2
1
0
a
a
Sham
Sham+EGCG
STZ
STZ+EGCG

Figure 4

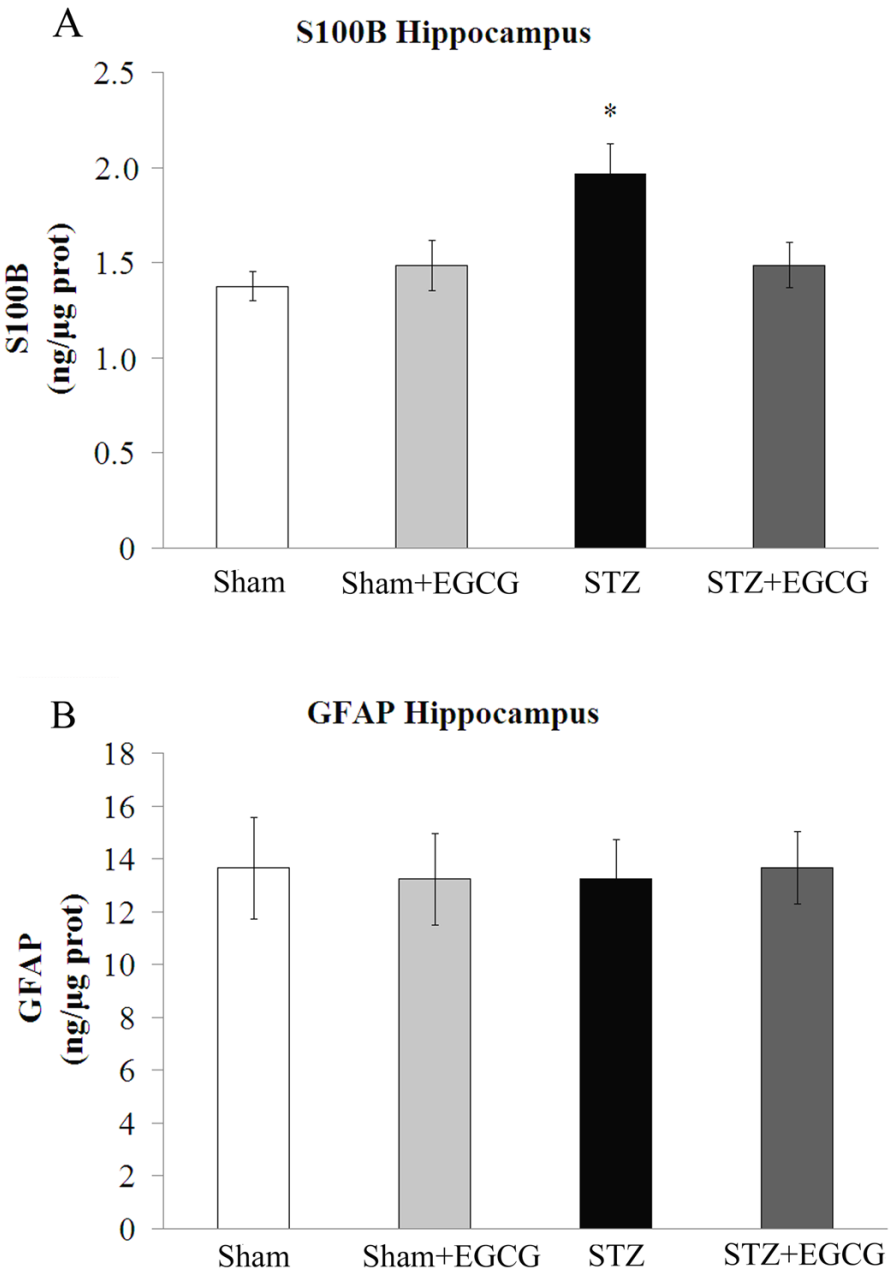

A
S100B Hippocampus
S100B (ng/µg prot)
2.5
2.0
1.5
1.0
0.5
0
*
Sham
Sham+EGCG
STZ
STZ+EGCG
B
GFAP Hippocampus
GFAP (ng/µg prot)
18
16
14
12
10
8
6
4
2
0
Sham
Sham+EGCG
STZ
STZ+EGCG

Figure 5

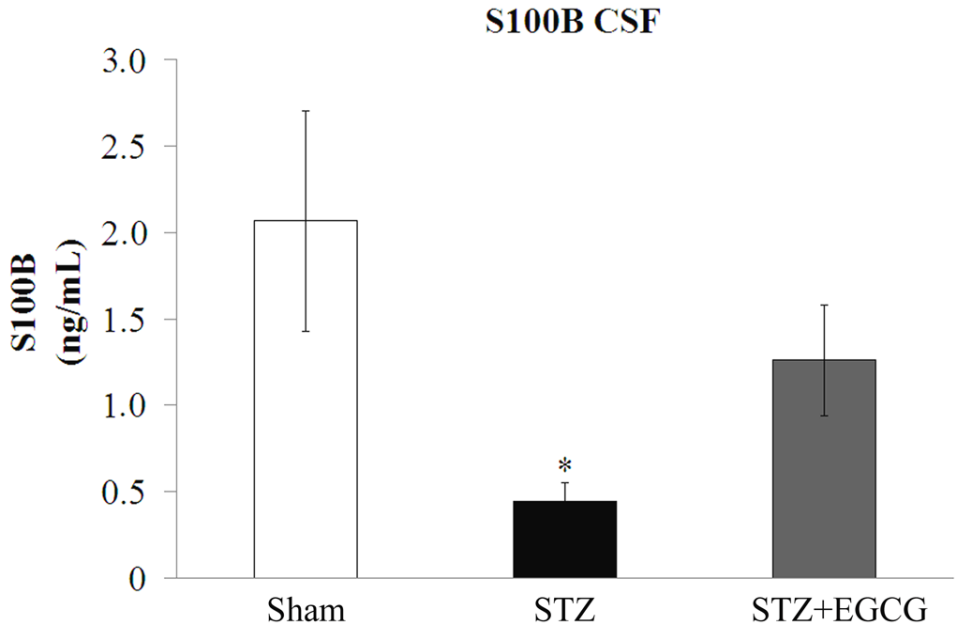


Figure 6

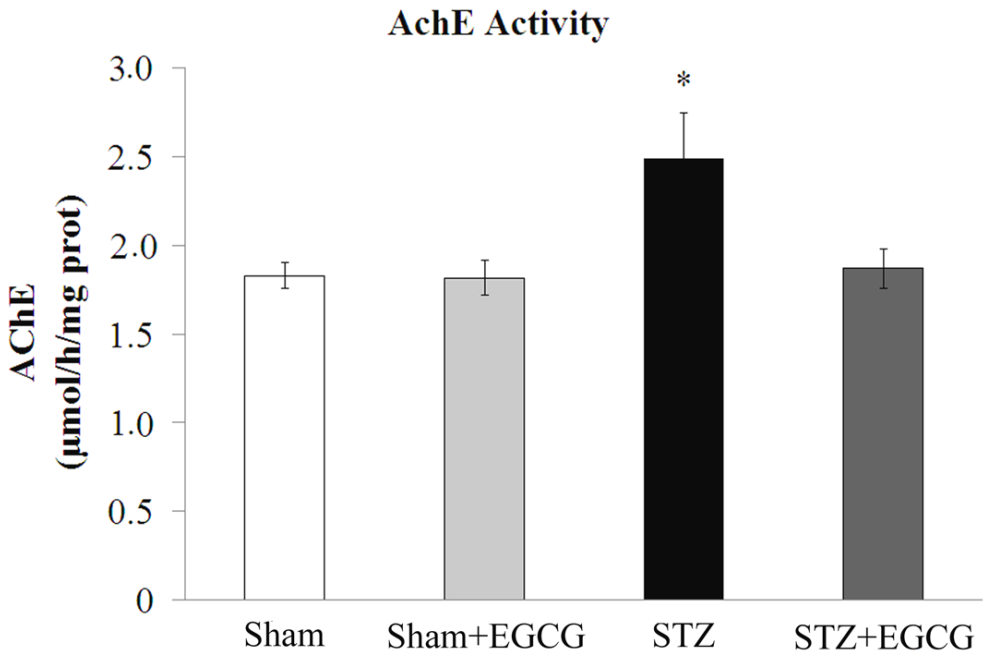


Figure 7

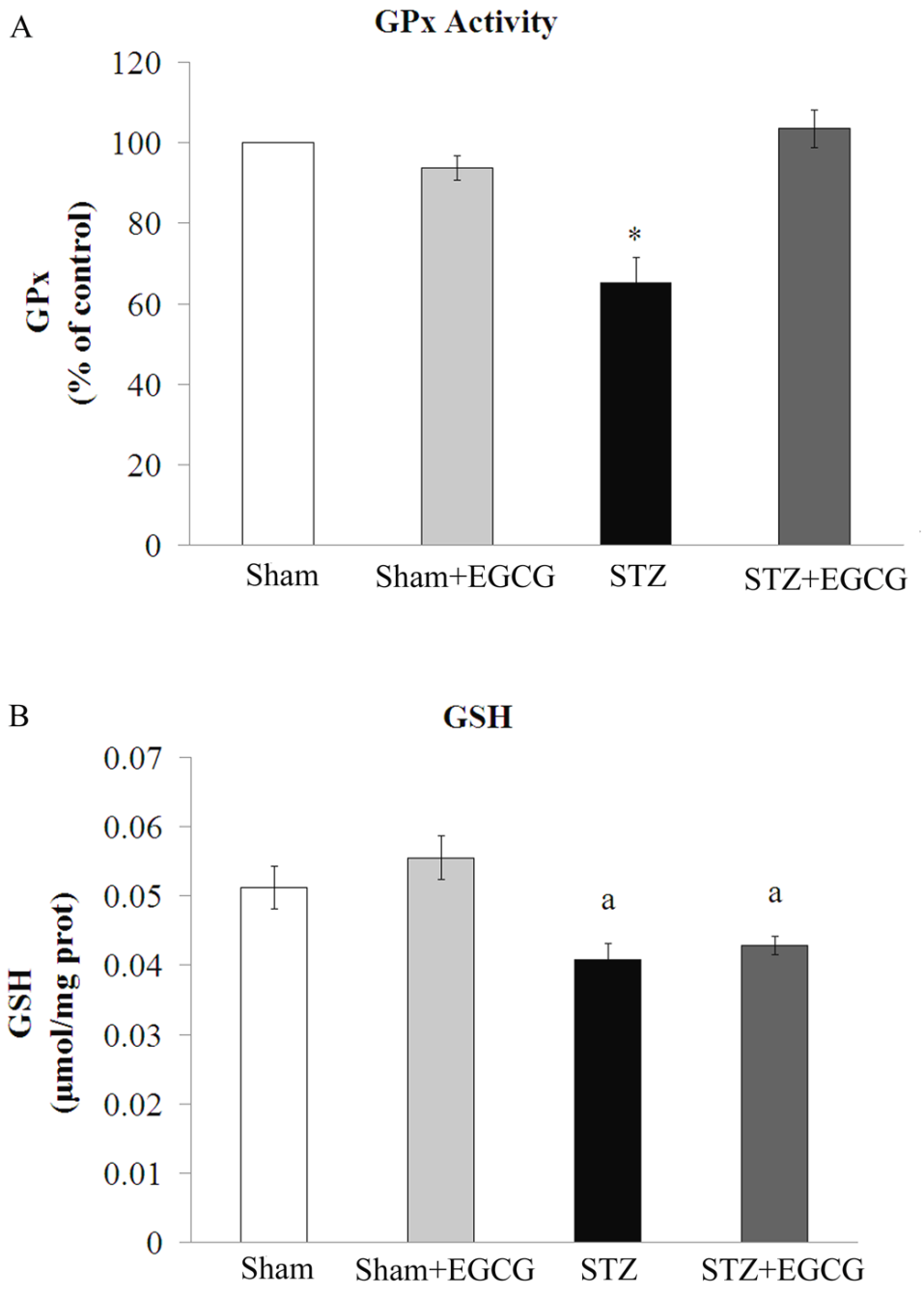
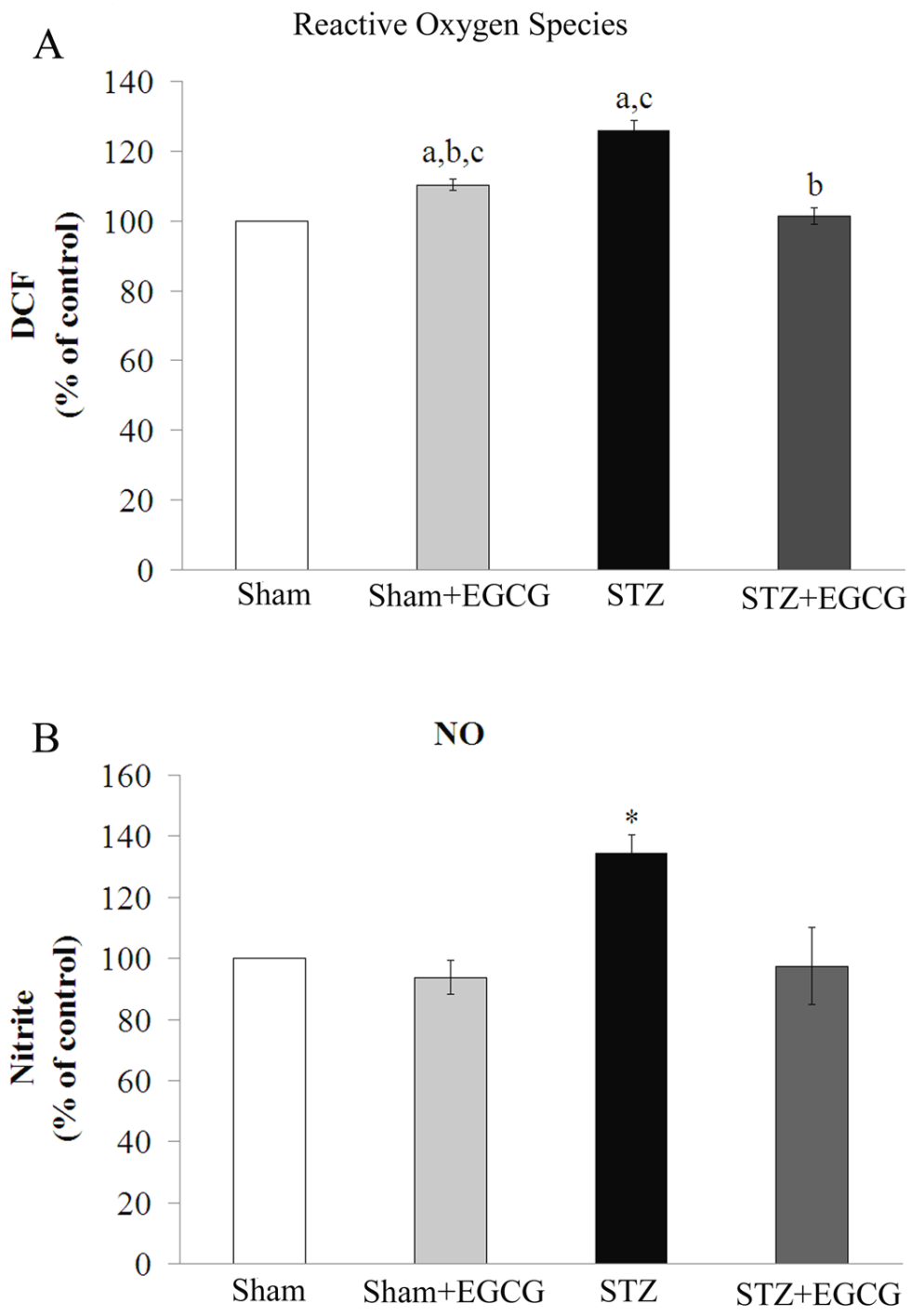

A
GPx Activity
GPx
(% of control)
120
100
80
60
40
20
0
*
Sham
Sham+EGCG
STZ
STZ+EGCG
B
GSH
GSH
(µmol/mg prot)
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
a
a
Sham
Sham+EGCG
STZ
STZ+EGCG

Figure 8


A
Reactive Oxygen Species
DCF
(% of control)
140
120
100
80
60
40
20
0
a,b,c
a,c
b
Sham
Sham+EGCG
STZ
STZ+EGCG
B
NO
Nitrite
(% of control)
160
140
120
100
80
60
40
20
0
*
Sham
Sham+EGCG
STZ
STZ+EGCG

PARTE III

DISCUSSÃO

1. Resumo dos resultados.

1.1. Achados decorrentes da infusão icv de STZ em ratos sete semanas após a cirurgia:

Parâmetro avaliado	Sete semanas após a cirurgia
Aprendizado e memória espacial	Diminuído
Captação de glicose em fatias hipocampais	Diminuída
Atividade da AchE (hipocampo)	Aumentada
Conteúdo de S100B (hipocampo)	Aumentado
Conteúdo de GFAP (hipocampo)	Inalterado
Conteúdo de S100B (LCR/secreção)	Diminuído
Conteúdo de GSH (hipocampo)	Diminuído
Espécies reativas de oxigênio (hipocampo)	Aumento determinado por DCF
Estresse Nitrosativo (hipocampo)	Aumento determinado por níveis de nitritos e nitratos
Atividade da GPx (hipocampo)	Diminuída

1.2. Efeito da epigalocatequina galato sobre o modelo de doença de Alzheimer esporádica por infusão icv de STZ sete semanas após a cirurgia:

Parâmetro avaliado	**Sete semanas após a cirurgia**
Déficit cognitivo avaliado em labirinto aquático de Morris	Reversão
Diminuição na captação de glicose em fatias hipocampais	Não reverte
Aumento da atividade da AchE (hipocampo)	Reversão
Aumento do conteúdo de S100B (hipocampo)	Reversão
Diminuição do conteúdo de S100B (LCR/secreção)	Reversão
Diminuição do conteúdo de GSH (hipocampo)	Não reverte
Aumento das espécies reativas de oxigênio (hipocampo)	Reversão
Aumento do estresse nitrosativo (hipocampo)	Reversão
Diminuição da atividade da GPx (hipocampo)	Reversão

2. Reversão do declínio cognitivo causado pelo modelo de doença de Alzheimer esporádica por infusão icv de STZ.

Vários autores já mostraram que a infusão única e bilateral de STZ via icv na dose de 3 mg/kg foi efetiva na causa do déficit cognitivo avaliado no teste de

memória espacial no Labirinto Aquático de Morris (LAM), de duas a oito semanas após a cirurgia estereotáxica (Prickaerts, Blokland et al. 1995; Pathan, Viswanad et al. 2006; Grunblatt, Salkovic-Petrisic et al. 2007). Os principais achados neuroquímicos justificando o déficit cognitivo encontrado no modelo são a deficiência hipocampal na transmissão colinérgica e na captação de glicose (Pathan, Viswanad et al. 2006; Tiwari, Kuhad et al. 2009; Labak, Foniok et al. 2010). O aumento na atividade da enzima acetilcolinesterase causado pela infusão com STZ foi completamente revertido pela administração sub-crônica de EGCG. Com relação à captação de glicose em fatias hipocampais, os níveis se mantiveram menores do que o grupo sham no grupo STZ+EGCG, mostrando que a EGCG não interfere na captação de glicose neuroglial. Porém este fato não é determinante para o desempenho cognitivo, visto que os animais aprenderam a tarefa e mantiveram um desempenho parecido com o dos grupos sham e sham+EGCG.

3. Reversão do estresse oxidativo e nitrosativo

Tanto na doença de Alzheimer quanto no modelo de DA esporádica, onde há infusão icv de STZ, incluindo os achados deste trabalho, temos o desequilíbrio entre a produção de espécies reativas e as defesas antioxidantes. Uma determinação exata da contribuição de cada fonte de estresse oxidativo é complicada, mas sabe-se que é uma situação multifatorial: formação de AGES (Smith, Taneda et al. 1994), nitração (Good, Werner et al. 1996; Smith, Richey Harris et al. 1997; Williamson, Gabbita et al. 2002; Castegna, Thongboonkerd et al. 2003), peroxidação lipídica (Markesbery and Lovell 1998; Butterfield,

Drake et al. 2001), carbonilação de proteínas (Smith, Taneda et al. 1994; Smith, Rudnicka-Nawrot et al. 1995), causando, obviamente, prejuízo às funções celulares.

Neste trabalho pode-se observar a diminuição do conteúdo de nitritos e nitratos no tecido hipocampal dos ratos que foram infundidos com STZ e que posteriormente receberam EGCG. Há relatos na literatura de que os polifenóis do chá verde são capazes de reduzir a expressão da iNOS e inibir as reações oxidativas (Lin and Lin 1997), sugerindo que a inibição se dá através da prevenção da ligação do fator nuclear-κB ao sítio de ligação.

Diversos trabalhos têm demonstrado o papel antioxidante e antiinflamatório da EGCG (Guo, Zhao et al. 1996; Stewart, Mullen et al. 2005). Sua ação já foi testada em vários tipos celulares, tanto *in vitro* quanto *in vivo*. Em ratos que receberam uma dieta aterogênica, rica em colesterol, a EGCG foi capaz de reduzir a placa aterosclerótica, diminuindo marcadores inflamatórios, como a proteína C reativa, indicando seu potencial antiinflamatório (Ramesh, Geraldine et al. 2010). Kumar & Kumar (Kumar and Kumar 2009) relataram que um pré-tratamento de 14 dias com EGCG atenuou alterações comportamentais, danos oxidativos, disfunção das enzimas do complexo mitocondrial e dano estriatal em animais tratados com ácido 3-nitropropiônico, um modelo de doença de Huntington. Em um recente trabalho de nosso grupo, a EGCG foi capaz de proteger a viabilidade das mitocôndrias expostas ao cádmio (Abib, Peres et al. 2011), um metal que causa lipoperoxidação, entretanto os níveis de tióis não protéicos não se mantiveram iguais aos níveis do controle, resultado parecido com o que obtivemos em relação a não reversão do conteúdo de GSH.

A ação antioxidante da EGCG também pode ser observada em nosso trabalho, através do restabelecimento da atividade da GPx, diminuída no grupo STZ, e diminuição das ERO, cujos níveis se encontravam aumentados no grupo STZ. Neste parâmetro a EGCG teve um efeito pró-oxidante *per se*. Este efeito já havia sido relatado em outro trabalho, porém em uma linhagem de células beta pancreáticas (Suh, Chon et al. 2010). No entanto, quando se observa a curva de aprendizado e o teste no LAM, vê-se que tal efeito não prejudicou o desempenho cognitivo dos animais deste grupo (sham+EGCG).

4. Alterações no imunoconteúdo de GFAP e S100B hipocampais e de S100B no LCR

A proteína S100B possui ações tanto intra quanto extracelulares. Dentro da célula, está envolvida com a regulação da inibição da fosforilação de proteínas, a plasticidade do citoesqueleto, a regulação de enzimas e o controle do crescimento e diferenciação celular [para revisão ver (Donato, Sorci et al. 2009)]. Considerando que o modelo de demência estudado apresenta uma importante redução do metabolismo oxidativo, juntamente com déficit energético encefálico (Lannert and Hoyer 1998), isto pode estar relacionado à alteração no imunoconteúdo de S100B hipocampal encontrada nos animais que receberam STZ via icv. Além disso, o aumento da S100B intracelular tem sido encontrado em análise *post mortem* de tecido cerebral de pacientes com DA (Van Eldik and Griffin 1994). Mais uma vez, a EGCG atenuou este efeito causado pela STZ.

No líquido cefalorraquidiano dos animais infundidos com a STZ houve uma diminuição do imunoconteúdo de S100B e este fato pode ter contribuído para a piora cognitiva destes animais se levarmos em consideração que dentre as funções tróficas extracelulares da S100B estão: a promoção do aumento da sobrevivência neuronal, o estímulo ao crescimento de neuritos e a melhora da função sináptica (Van Eldik and Wainwright 2003).

Quanto à GFAP, não encontramos alteração no imunoconteúdo desta proteína no hipocampo dos animais submetidos à infusão icv de STZ sete semanas após a cirurgia, corroborando com resultados semelhantes do nosso grupo (Rodrigues, Biasibetti et al. 2009; Tramontina, Wartchow et al. 2011). É necessário ressaltar o fato de que houve alteração numa importante proteína astrocítica, a S100B, sem ter havido alteração no tradicional marcador de astrogliose, a proteína de citoesqueleto GFAP. Tal achado também salienta o fato de que a astrogliose, que é uma resposta dos astrócitos a injúrias, pode ser complexa e ampla, devendo ser analisada por diferentes parâmetros.

5. Reversão da atividade da acetilcolinesterase

Clinicamente, o tratamento de escolha para casos brandos a moderados da doença de Alzheimer são os anticolinesterásicos, como donepezil, rivastigmina e galantamina. Estes fármacos são capazes de inibir a enzima que quebra o neurotransmissor acetilcolina, após sua liberação na fenda sináptica, fazendo com que haja um maior tempo de ação deste. Para os casos moderados a severos, os fármacos aprovados são os agonistas glutamatérgicos de

receptores NMDA, como a memantina (Klafki, Staufenbiel et al. 2006; Mangialasche, Solomon et al. 2010).

Vários extratos ou até mesmo moléculas isoladas de plantas têm sido descritas como anticolinesterásicos, como huperzina (Ha, Wong et al. 2011), berberina (Ji and Shen 2011), extrato de *Knema laurina (Akhtar, Lam et al. 2011)* e *Illicium verum* (Bhadra, Mukherjee et al. 2011), 4-fenilcumarinas da planta *Mesua elegans* (Awang, Chan et al. 2010), etc, sendo que muitos destes compostos têm uma atividade antioxidante comprovada. Xiao e colaboradores (Xiao, Chen et al. 2008) observaram que quando a EGCG fora administrada junto com a huperzina, a atividade inibitória sobre a AchE atingiu mais de 90%, e este efeito deve-se principalmente à ligação da EGCG à albumina, fazendo com que a huperzina seja melhor transportada.

No presente trabalho a EGCG foi capaz de diminuir o aumento na atividade da AchE causado pela administração icv de STZ, mostrando ser um composto potencialmente útil para diminuir o déficit cognitivo causado pelo prejuízo na rede colinérgica observado em pacientes e no modelo de DA esporádica aqui utilizado.

CONSIDERAÇÕES FINAIS

Os resultados deste trabalho confirmam o dano cognitivo e o estresse oxidativo e nitrosativo, especificamente em hipocampo, demonstrando, ainda o envolvimento neuroglial no modelo de demência por infusão intracerebroventricular de estreptozotocina, evidenciadas pelas alterações na secreção e no conteúdo da proteína S100B, atividade da AchE e captação de glicose.

É importante ressaltar que

- A maioria dos trabalhos envolvendo modelos de demência por infusão intracerebroventricular de estreptozotocina que utilizam tratamentos farmacológicos, administram os mesmos imediatamente após a infusão da STZ, ou poucos dias depois, tratando seus resultados como prevenção. Neste trabalho, esperou-se duas semanas para início do tratamento, ou seja, a lesão já estava estabelecida e mesmo assim ocorreram efeitos que reverteram o quadro.

- Estudos que relatam o papel benéfico da EGCG em modelos de demência transgênicos e também *in vitro,* descrevem seus resultados com base em propriedades que esta molécula tem em prevenir as células contra a toxicidade do peptídeo β-amilóide e sua agregação e na modulação da atividade das enzimas β-, γ- e α-secretase. Entretanto, neste estágio, a agregação do peptídeo em questão não ocorre e conclui-se que os efeitos benéficos são devido às propriedades antioxidantes, antiinflamatórias e anticolinesterásicas da EGCG.

REFERÊNCIAS BIBLIOGRÁFICAS

(2011). Handbook of animal models in alzheimer's disease. Washington, DC, IOS Press.

Abib, R. T., K. C. Peres, et al. (2011). "Epigallocatechin-3-gallate protects rat brain mitochondria against cadmium-induced damage." Food Chem Toxicol **49**(10): 2618-2623.

Agrawal, R., B. Mishra, et al. (2010). "Effect of curcumin on brain insulin receptors and memory functions in STZ (ICV) induced dementia model of rat." Pharmacol Res **61**(3): 247-252.

Ahmed, S., A. Pakozdi, et al. (2006). "Regulation of interleukin-1beta-induced chemokine production and matrix metalloproteinase 2 activation by epigallocatechin-3-gallate in rheumatoid arthritis synovial fibroblasts." Arthritis Rheum **54**(8): 2393-2401.

Ahmed, S., N. Wang, et al. (2004). "Green tea polyphenol epigallocatechin-3-gallate (EGCG) differentially inhibits interleukin-1 beta-induced expression of matrix metalloproteinase-1 and -13 in human chondrocytes." J Pharmacol Exp Ther **308**(2): 767-773.

Akhtar, M. N., K. W. Lam, et al. (2011). "New class of acetylcholinesterase inhibitors from the stem bark of Knema laurina and their structural insights." Bioorg Med Chem Lett **21**(13): 4097-4103.

Allen, N. J. and B. A. Barres (2009). "Neuroscience: Glia - more than just brain glue." Nature **457**(7230): 675-677.

Andreazza, A. C., C. Cassini, et al. (2007). "Serum S100B and antioxidant enzymes in bipolar patients." J Psychiatr Res **41**(6): 523-529.

Ansari, M. A. and S. W. Scheff (2010). "Oxidative stress in the progression of Alzheimer disease in the frontal cortex." J Neuropathol Exp Neurol **69**(2): 155-167.

Aucamp, J., A. Gaspar, et al. (1997). "Inhibition of xanthine oxidase by catechins from tea (Camellia sinensis)." Anticancer Res **17**(6D): 4381-4385.

Awang, K., G. Chan, et al. (2010). "4-Phenylcoumarins from Mesua elegans with acetylcholinesterase inhibitory activity." Bioorg Med Chem **18**(22): 7873-7877.

Babu, P. V. and D. Liu (2008). "Green tea catechins and cardiovascular health: an update." Curr Med Chem **15**(18): 1840-1850.

Bastianetto, S., Z. X. Yao, et al. (2006). "Neuroprotective effects of green and black teas and their catechin gallate esters against beta-amyloid-induced toxicity." Eur J Neurosci **23**(1): 55-64.

Baydas, G., V. S. Nedzvetskii, et al. (2003). "Altered expression of NCAM in hippocampus and cortex may underlie memory and learning deficits in rats with streptozotocin-induced diabetes mellitus." Life Sci **73**(15): 1907-1916.

Berger, R. P., M. C. Pierce, et al. (2002). "Neuron-specific enolase and S100B in cerebrospinal fluid after severe traumatic brain injury in infants and children." Pediatrics **109**(2): E31.

Bertsch, T., W. Casarin, et al. (2001). "Protein S-100B: a serum marker for ischemic and infectious injury of cerebral tissue." Clin Chem Lab Med **39**(4): 319-323.

Bhadra, S., P. K. Mukherjee, et al. (2011). "Anticholinesterase activity of standardized extract of Illicium verum Hook. f. fruits." Fitoterapia **82**(3): 342-346.

Blokland, A. and J. Jolles (1993). "Spatial learning deficit and reduced hippocampal ChAT activity in rats after an ICV injection of streptozotocin." Pharmacol Biochem Behav **44**(2): 491-494.

Browne, R. W. and D. Armstrong (1998). "Reduced glutathione and glutathione disulfide." Methods Mol Biol **108**: 347-352.

Butterfield, D. A., J. Drake, et al. (2001). "Evidence of oxidative damage in Alzheimer's disease brain: central role for amyloid beta-peptide." Trends Mol Med **7**(12): 548-554.

Cadenas, E. and K. J. Davies (2000). "Mitochondrial free radical generation, oxidative stress, and aging." Free Radic Biol Med **29**(3-4): 222-230.

Calabrese, V., R. Sultana, et al. (2006). "Nitrosative stress, cellular stress response, and thiol homeostasis in patients with Alzheimer's disease." Antioxid Redox Signal **8**(11-12): 1975-1986.

Castegna, A., V. Thongboonkerd, et al. (2003). "Proteomic identification of nitrated proteins in Alzheimer's disease brain." J Neurochem **85**(6): 1394-1401.

Chen, L., M. J. Lee, et al. (1997). "Absorption, distribution, elimination of tea polyphenols in rats." Drug Metab Dispos **25**(9): 1045-1050.

Chu, K. O., C. C. Wang, et al. (2007). "Uptake and distribution of catechins in fetal organs following in utero exposure in rats." Hum Reprod **22**(1): 280-287.

Chung, W. G., C. L. Miranda, et al. (2007). "Epigallocatechin gallate (EGCG) potentiates the cytotoxicity of rotenone in neuroblastoma SH-SY5Y cells." Brain Res **1176**: 133-142.

Cohen, M. V. (1989). "Free radicals in ischemic and reperfusion myocardial injury: is this the time for clinical trials?" Ann Intern Med **111**(11): 918-931.

Costa, A. P., A. C. Tramontina, et al. (2010). "Neuroglial alterations in rats submitted to the okadaic acid-induced model of dementia." Behav Brain Res.

Costa, A. P., A. C. Tramontina, et al. (2011). "Neuroglial alterations in rats submitted to the okadaic acid-induced model of dementia." Behav Brain Res.

Cudkowicz, M. E., P. M. Sexton, et al. (1999). "The pharmacokinetics and pharmaco-dynamics of Procysteine in amyotrophic lateral sclerosis." Neurology **52**(7): 1492-1494.

Cummings, J. L., M. Mega, et al. (1994). "The Neuropsychiatric Inventory: comprehensive assessment of psychopathology in dementia." Neurology **44**(12): 2308-2314.

de la Monte, S. M., L. Longato, et al. (2009). "Insulin resistance and neurodegeneration: roles of obesity, type 2 diabetes mellitus and non-alcoholic steatohepatitis." Curr Opin Investig Drugs **10**(10): 1049-1060.

Devika, P. T. and P. Stanely Mainzen Prince (2008). "(-)Epigallocatechin-gallate (EGCG) prevents mitochondrial damage in isoproterenol-induced cardiac toxicity in albino Wistar rats: a transmission electron microscopic and in vitro study." Pharmacol Res **57**(5): 351-357.

Dhull, D. K., A. Jindal, et al. (2011). "Neuroprotective Effect of Cyclooxygenase Inhibitors in ICV-STZ Induced Sporadic Alzheimer's Disease in Rats." J Mol Neurosci.

Donato, R. (2001). "S100: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles." Int J Biochem Cell Biol **33**(7): 637-668.

Donato, R. (2003). "Intracellular and extracellular roles of S100 proteins." Microsc Res Tech **60**(6): 540-551.

Donato, R., G. Sorci, et al. (2009). "S100B's double life: intracellular regulator and extracellular signal." Biochim Biophys Acta **1793**(6): 1008-1022.

Dorchies, O. M., S. Wagner, et al. (2009). "Protection of dystrophic muscle cells with polyphenols from green tea correlates with improved glutathione balance and increased expression of 67LR, a receptor for (-)-epigallocatechin gallate." Biofactors **35**(3): 279-294.

Doss, M. X., S. P. Potta, et al. (2005). "Trapping of growth factors by catechins: a possible therapeutical target for prevention of proliferative diseases." J Nutr Biochem **16**(5): 259-266.

Dringen, R. (2000). "Metabolism and functions of glutathione in brain." Prog Neurobiol **62**(6): 649-671.

Droge, W. (2002). "Free radicals in the physiological control of cell function." Physiol Rev **82**(1): 47-95.

Duelli, R., H. Schrock, et al. (1994). "Intracerebroventricular injection of streptozotocin induces discrete local changes in cerebral glucose utilization in rats." Int J Dev Neurosci **12**(8): 737-743.

Duyckaerts, C., B. Delatour, et al. (2009). "Classification and basic pathology of Alzheimer disease." Acta Neuropathol **118**(1): 5-36.

Eikelenboom, P., R. Veerhuis, et al. (2006). "The significance of neuroinflammation in understanding Alzheimer's disease." J Neural Transm **113**(11): 1685-1695.

Ellis, J. M. (2005). "Cholinesterase inhibitors in the treatment of dementia." J Am Osteopath Assoc **105**(3): 145-158.

Ellman, G. L., K. D. Courtney, et al. (1961). "A new and rapid colorimetric determination of acetylcholinesterase activity." Biochem Pharmacol **7**: 88-95.

Eng, L. F., R. S. Ghirnikar, et al. (2000). "Glial fibrillary acidic protein: GFAP-thirty-one years (1969-2000)." Neurochem Res **25**(9-10): 1439-1451.

Essink-Bot, M. L., J. Pereira, et al. (2002). "Cross-national comparability of burden of disease estimates: the European Disability Weights Project." Bull World Health Organ **80**(8): 644-652.

Fernandez-Bachiller, M. I., C. Perez, et al. (2010). "Novel tacrine-8-hydroxyquinoline hybrids as multifunctional agents for the treatment of Alzheimer's disease, with neuroprotective, cholinergic, antioxidant, and copper-complexing properties." J Med Chem **53**(13): 4927-4937.

Ferreira, A. R., F. Bonatto, et al. (2006). "Oxidative stress effects on the central nervous system of rats after acute exposure to ultra high frequency electromagnetic fields." Bioelectromagnetics **27**(6): 487-493.

Ferri, C. P., M. Prince, et al. (2005). "Global prevalence of dementia: a Delphi consensus study." Lancet **366**(9503): 2112-2117.

Freemantle, E., M. Vandal, et al. (2006). "Omega-3 fatty acids, energy substrates, and brain function during aging." Prostaglandins Leukot Essent Fatty Acids **75**(3): 213-220.

Fu, Y., S. Zheng, et al. (2008). "Epigallocatechin-3-gallate inhibits growth of activated hepatic stellate cells by enhancing the capacity of glutathione synthesis." Mol Pharmacol **73**(5): 1465-1473.

Goncalves, C. A., M. C. Leite, et al. (2008). "Biological and methodological features of the measurement of S100B, a putative marker of brain injury." Clin Biochem **41**(10-11): 755-763.

Good, P. F., P. Werner, et al. (1996). "Evidence of neuronal oxidative damage in Alzheimer's disease." Am J Pathol **149**(1): 21-28.

Griffin, W. S., J. G. Sheng, et al. (1998). "Glial-neuronal interactions in Alzheimer's disease: the potential role of a 'cytokine cycle' in disease progression." Brain Pathol **8**(1): 65-72.

Grunblatt, E., M. Salkovic-Petrisic, et al. (2007). "Brain insulin system dysfunction in streptozotocin intracerebroventricularly treated rats generates hyperphosphorylated tau protein." J Neurochem **101**(3): 757-770.

Guo, Q., B. Zhao, et al. (1996). "Studies on protective mechanisms of four components of green tea polyphenols against lipid peroxidation in synaptosomes." Biochim Biophys Acta **1304**(3): 210-222.

Ha, G. T., R. K. Wong, et al. (2011). "Huperzine a as potential treatment of Alzheimer's disease: an assessment on chemistry, pharmacology, and clinical studies." Chem Biodivers **8**(7): 1189-1204.

Haan, M. N. and R. Wallace (2004). "Can dementia be prevented? Brain aging in a population-based context." Annu Rev Public Health **25**: 1-24.

Halassa, M. M., T. Fellin, et al. (2007). "The tripartite synapse: roles for gliotransmission in health and disease." Trends Mol Med **13**(2): 54-63.

Halliwell, B. and J. M. C. Gutteridge (2007). Free radicals in biology and medicine. Oxford, Oxford University Press.

Hallschmid, M. and B. Schultes (2009). "Central nervous insulin resistance: a promising target in the treatment of metabolic and cognitive disorders?" Diabetologia **52**(11): 2264-2269.

Hevel, J. M. and M. A. Marletta (1994). "Nitric-oxide synthase assays." Methods Enzymol **233**: 250-258.

Hirrlinger, J. and R. Dringen (2010). "The cytosolic redox state of astrocytes: Maintenance, regulation and functional implications for metabolite trafficking." Brain Res Rev **63**(1-2): 177-188.

Holmes, C. and D. Cotterell (2009). "Role of infection in the pathogenesis of Alzheimer's disease: implications for treatment." CNS Drugs **23**(12): 993-1002.

Hosokawa, M., W. Dolci, et al. (2001). "Differential sensitivity of GLUT1- and GLUT2-expressing beta cells to streptozotocin." Biochem Biophys Res Commun **289**(5): 1114-1117.

Hoyer, S. and H. Lannert (2007). "Long-term abnormalities in brain glucose/energy metabolism after inhibition of the neuronal insulin receptor: implication of tau-protein." J Neural Transm Suppl(72): 195-202.

Hoyer, S., S. K. Lee, et al. (2000). "Inhibition of the neuronal insulin receptor. An in vivo model for sporadic Alzheimer disease?" Ann N Y Acad Sci **920**: 256-258.

Hsuuw, Y. D. and W. H. Chan (2007). "Epigallocatechin gallate dose-dependently induces apoptosis or necrosis in human MCF-7 cells." Ann N Y Acad Sci **1095**: 428-440.

Jalbert, J. J., L. A. Daiello, et al. (2008). "Dementia of the Alzheimer type." Epidemiol Rev **30**: 15-34.

Javed, H., M. M. Khan, et al. (2011). "S-allyl cysteine attenuates oxidative stress associated cognitive impairment and neurodegeneration in mouse model of streptozotocin-induced experimental dementia of Alzheimer's type." Brain Res **1389**: 133-142.

Jessen, K. R. (2004). "Glial cells." Int J Biochem Cell Biol **36**(10): 1861-1867.

Ji, H. F. and L. Shen (2011). "Berberine: a potential multipotent natural product to combat Alzheimer's disease." Molecules **16**(8): 6732-6740.

Kamat, P. K., S. Tota, et al. (2010). "Okadaic acid (ICV) induced memory impairment in rats: a suitable experimental model to test anti-dementia activity." Brain Res **1309**: 66-74.

Katiyar, S. K. and H. Mukhtar (1997). "Tea antioxidants in cancer chemoprevention." J Cell Biochem Suppl **27**: 59-67.

Klafki, H. W., M. Staufenbiel, et al. (2006). "Therapeutic approaches to Alzheimer's disease." Brain **129**(Pt 11): 2840-2855.

Kristensen, M. O. (1990). "[Neurotransmitters in Alzheimer's disease]." Ugeskr Laeger **152**(30): 2165-2168.

Kumar, P. and A. Kumar (2009). "Protective effects of epigallocatechin gallate following 3-nitropropionic acid-induced brain damage: possible nitric oxide mechanisms." Psychopharmacology (Berl) **207**(2): 257-270.

Kurz, A. F. and N. T. Lautenschlager (2010). "The concept of dementia: retain, reframe, rename or replace?" Int Psychogeriatr **22**(1): 37-42.

Labak, M., T. Foniok, et al. (2010). "Metabolic changes in rat brain following intracerebroventricular injections of streptozotocin: a model of sporadic Alzheimer's disease." Acta Neurochir Suppl **106**: 177-181.

Lackovic, Z., V. Rebic, et al. (2009). "Single intracerebroventricular injection of botulinum toxin type A produces slow onset and long-term memory impairment in rats." J Neural Transm **116**(10): 1273-1280.

Langa, K. M., E. B. Larson, et al. (2004). "Out-of-pocket health care expenditures among older Americans with dementia." Alzheimer Dis Assoc Disord **18**(2): 90-98.

Lannert, H. and S. Hoyer (1998). "Intracerebroventricular administration of streptozotocin causes long-term diminutions in learning and memory abilities and in cerebral energy metabolism in adult rats." Behav Neurosci **112**(5): 1199-1208.

Leal, R. B., J. K. Frizzo, et al. (2004). "S100B protein stimulates calcineurin activity." Neuroreport **15**(2): 317-320.

Lebed, Y. V., M. A. Orlovsky, et al. (2008). "Early reaction of astroglial cells in rat hippocampus to streptozotocin-induced diabetes." Neurosci Lett **444**(2): 181-185.

Lee, J. W., Y. K. Lee, et al. (2009). "Green tea (-)-epigallocatechin-3-gallate inhibits beta-amyloid-induced cognitive dysfunction through modification of secretase activity via inhibition of ERK and NF-kappaB pathways in mice." J Nutr **139**(10): 1987-1993.

Leite, M. C., F. Galland, et al. (2008). "A simple, sensitive and widely applicable ELISA for S100B: Methodological features of the measurement of this glial protein." J Neurosci Methods **169**(1): 93-99.

Leloup, C., L. Casteilla, et al. (2011). "Balancing mitochondrial redox signaling: a key point in metabolic regulation." Antioxid Redox Signal **14**(3): 519-530.

Lin, C. L., T. F. Chen, et al. (2009). "Epigallocatechin gallate (EGCG) suppresses beta-amyloid-induced neurotoxicity through inhibiting c-Abl/FE65 nuclear translocation and GSK3 beta activation." Neurobiol Aging **30**(1): 81-92.

Lin, L. C., M. N. Wang, et al. (2007). "Pharmacokinetics of (-)-epigallocatechin-3-gallate in conscious and freely moving rats and its brain regional distribution." J Agric Food Chem **55**(4): 1517-1524.

Lin, Y. L. and J. K. Lin (1997). "(-)-Epigallocatechin-3-gallate blocks the induction of nitric oxide synthase by down-regulating lipopolysaccharide-induced activity of transcription factor nuclear factor-kappaB." Mol Pharmacol **52**(3): 465-472.

Lowry, O. H., N. J. Rosebrough, et al. (1951). "Protein measurement with the Folin phenol reagent." J Biol Chem **193**(1): 265-275.

Mangialasche, F., A. Solomon, et al. (2010). "Alzheimer's disease: clinical trials and drug development." Lancet Neurol **9**(7): 702-716.

Marenholz, I., C. W. Heizmann, et al. (2004). "S100 proteins in mouse and man: from evolution to function and pathology (including an update of the nomenclature)." Biochem Biophys Res Commun **322**(4): 1111-1122.

Mariani, E., M. C. Polidori, et al. (2005). "Oxidative stress in brain aging, neurodegenerative and vascular diseases: an overview." J Chromatogr B Analyt Technol Biomed Life Sci **827**(1): 65-75.

Markesbery, W. R. and M. A. Lovell (1998). "Four-hydroxynonenal, a product of lipid peroxidation, is increased in the brain in Alzheimer's disease." Neurobiol Aging **19**(1): 33-36.

Matsuo, N., K. Yamada, et al. (1997). "Effect of tea polyphenols on histamine release from rat basophilic leukemia (RBL-2H3) cells: the structure-inhibitory activity relationship." Allergy **52**(1): 58-64.

McGeer, E. G., E. A. Singh, et al. (1987). "Sodium-dependent glutamate binding in senile dementia." Neurobiol Aging **8**(3): 219-223.

Migheli, A., S. Cordera, et al. (1999). "S-100beta protein is upregulated in astrocytes and motor neurons in the spinal cord of patients with amyotrophic lateral sclerosis." Neurosci Lett **261**(1-2): 25-28.

Morris, R. (1984). "Developments of a water-maze procedure for studying spatial learning in the rat." J Neurosci Methods **11**(1): 47-60.

Mrak, R. E. and W. S. Griffin (2005). "Glia and their cytokines in progression of neurodegeneration." Neurobiol Aging **26**(3): 349-354.

Nagai, K., M. H. Jiang, et al. (2002). "(-)-Epigallocatechin gallate protects against NO stress-induced neuronal damage after ischemia by acting as an anti-oxidant." Brain Res **956**(2): 319-322.

Nakagawa, K., S. Okuda, et al. (1997). "Dose-dependent incorporation of tea catechins, (-)-epigallocatechin-3-gallate and (-)-epigallocatechin, into human plasma." Biosci Biotechnol Biochem **61**(12): 1981-1985.

Norris, C. M., I. Kadish, et al. (2005). "Calcineurin triggers reactive/inflammatory processes in astrocytes and is upregulated in aging and Alzheimer's models." J Neurosci **25**(18): 4649-4658.

Pathan, A. R., B. Viswanad, et al. (2006). "Chronic administration of pioglitazone attenuates intracerebroventricular streptozotocin induced-memory impairment in rats." Life Sci **79**(23): 2209-2216.

Pellerin, L. (2005). "How astrocytes feed hungry neurons." Mol Neurobiol **32**(1): 59-72.

Perea, G. and A. Araque (2010). "GLIA modulates synaptic transmission." Brain Res Rev **63**(1-2): 93-102.

Perry, E., M. Walker, et al. (1999). "Acetylcholine in mind: a neurotransmitter correlate of consciousness?" Trends Neurosci **22**(6): 273-280.

Peskind, E. R., W. S. Griffin, et al. (2001). "Cerebrospinal fluid S100B is elevated in the earlier stages of Alzheimer's disease." Neurochem Int **39**(5-6): 409-413.

Pietta, P. G., P. Simonetti, et al. (1998). "Catechin metabolites after intake of green tea infusions." Biofactors **8**(1-2): 111-118.

Pinton, S., J. T. da Rocha, et al. (2011). "Neuroprotector effect of p,p'-methoxyl-diphenyl diselenide in a model of sporadic dementia of Alzheimer's type in mice: contribution of antioxidant mechanism." Cell Biochem Funct **29**(3): 235-243.

Pope, S. A., R. Milton, et al. (2008). "Astrocytes protect against copper-catalysed loss of extracellular glutathione." Neurochem Res **33**(7): 1410-1418.

Prickaerts, J., A. Blokland, et al. (1995). "Spatial discrimination learning and choline acetyltransferase activity in streptozotocin-treated rats: effects of chronic treatment with acetyl-L-carnitine." Brain Res **674**(1): 142-146.

Qiu, C., D. De Ronchi, et al. (2007). "The epidemiology of the dementias: an update." Curr Opin Psychiatry **20**(4): 380-385.

Rademakers, R. and A. Rovelet-Lecrux (2009). "Recent insights into the molecular genetics of dementia." Trends Neurosci **32**(8): 451-461.

Ramesh, E., P. Geraldine, et al. (2010). "Regulatory effect of epigallocatechin gallate on the expression of C-reactive protein and other inflammatory markers in an experimental model of atherosclerosis." Chem Biol Interact **183**(1): 125-132.

Rezai-Zadeh, K., G. W. Arendash, et al. (2008). "Green tea epigallocatechin-3-gallate (EGCG) reduces beta-amyloid mediated cognitive impairment and modulates tau pathology in Alzheimer transgenic mice." Brain Res **1214**: 177-187.

Riemersma, R. A., C. A. Rice-Evans, et al. (2001). "Tea flavonoids and cardiovascular health." QJM **94**(5): 277-282.

Ritchie, K. and S. Lovestone (2002). "The dementias." Lancet **360**(9347): 1759-1766.

Robson, M. J., R. P. Alston, et al. (2001). "Jugular bulb oxyhemoglobin desaturation, S100beta, and neurologic and cognitive outcomes after coronary artery surgery." Anesth Analg **93**(4): 839-845.

Rocchi, A., D. Orsucci, et al. (2009). "The role of vascular factors in late-onset sporadic Alzheimer's disease. Genetic and molecular aspects." Curr Alzheimer Res **6**(3): 224-237.

Rodrigues, L., R. Biasibetti, et al. (2009). "Hippocampal alterations in rats submitted to streptozotocin-induced dementia model are prevented by aminoguanidine." J Alzheimers Dis **17**(1): 193-202.

Rodrigues, L., M. F. Dutra, et al. (2010). "Treadmill training restores spatial cognitive deficits and neurochemical alterations in the hippocampus of rats submitted to an intracerebroventricular administration of streptozotocin." J Neural Transm **117**(11): 1295-1305.

Romner, B., T. Ingebrigtsen, et al. (2000). "Traumatic brain damage: serum S-100 protein measurements related to neuroradiological findings." J Neurotrauma **17**(8): 641-647.

Rothermundt, M., M. Peters, et al. (2003). "S100B in brain damage and neurodegeneration." Microsc Res Tech **60**(6): 614-632.

Sae-tan, S., K. A. Grove, et al. (2011). "Weight control and prevention of metabolic syndrome by green tea." Pharmacol Res **64**(2): 146-154.

Saffari, Y. and S. M. Sadrzadeh (2004). "Green tea metabolite EGCG protects membranes against oxidative damage in vitro." Life Sci **74**(12): 1513-1518.

Sahin, K., C. Orhan, et al. (2010). "Epigallocatechin-3-gallate prevents lipid peroxidation and enhances antioxidant defense system via modulating hepatic nuclear transcription factors in heat-stressed quails." Poult Sci **89**(10): 2251-2258.

Salkovic-Petrisic, M. and S. Hoyer (2007). "Central insulin resistance as a trigger for sporadic Alzheimer-like pathology: an experimental approach." J Neural Transm Suppl(72): 217-233.

Salkovic-Petrisic, M., F. Tribl, et al. (2006). "Alzheimer-like changes in protein kinase B and glycogen synthase kinase-3 in rat frontal cortex and hippocampus after damage to the insulin signalling pathway." J Neurochem **96**(4): 1005-1015.

Saxena, G., S. P. Singh, et al. (2008). "Effect of donepezil and tacrine on oxidative stress in intracerebral streptozotocin-induced model of dementia in mice." Eur J Pharmacol **581**(3): 283-289.

Selkoe, D. J. (2001). "Alzheimer's disease results from the cerebral accumulation and cytotoxicity of amyloid beta-protein." J Alzheimers Dis **3**(1): 75-80.

Selkoe, D. J. (2001). "Alzheimer's disease: genes, proteins, and therapy." Physiol Rev **81**(2): 741-766.

Shagam, J. Y. (2009). "The many faces of dementia." Radiol Technol **81**(2): 153-168.

Sharma, M. and Y. K. Gupta (2001). "Effect of chronic treatment of melatonin on learning, memory and oxidative deficiencies induced by intracerebroventricular streptozotocin in rats." Pharmacol Biochem Behav **70**(2-3): 325-331.

Sharma, M. and Y. K. Gupta (2002). "Chronic treatment with trans resveratrol prevents intracerebroventricular streptozotocin induced cognitive impairment and oxidative stress in rats." Life Sci **71**(21): 2489-2498.

Silva, M. C., J. Rocha, et al. (2005). "Transitory gliosis in the CA3 hippocampal region in rats fed on a ketogenic diet." Nutr Neurosci **8**(4): 259-264.

Smith, M. A., P. L. Richey Harris, et al. (1997). "Widespread peroxynitrite-mediated damage in Alzheimer's disease." J Neurosci **17**(8): 2653-2657.

Smith, M. A., M. Rudnicka-Nawrot, et al. (1995). "Carbonyl-related posttranslational modification of neurofilament protein in the neurofibrillary pathology of Alzheimer's disease." J Neurochem **64**(6): 2660-2666.

Smith, M. A., S. Taneda, et al. (1994). "Advanced Maillard reaction end products are associated with Alzheimer disease pathology." Proc Natl Acad Sci U S A **91**(12): 5710-5714.

Southorn, P. A. and G. Powis (1988). "Free radicals in medicine. II. Involvement in human disease." Mayo Clin Proc **63**(4): 390-408.

Stewart, A. J., W. Mullen, et al. (2005). "On-line high-performance liquid chromatography analysis of the antioxidant activity of phenolic compounds in green and black tea." Mol Nutr Food Res **49**(1): 52-60.

Strohmeyer, R. and J. Rogers (2001). "Molecular and cellular mediators of Alzheimer's disease inflammation." J Alzheimers Dis **3**(1): 131-157.

Suh, K. S., S. Chon, et al. (2010). "Prooxidative effects of green tea polyphenol (-)-epigallocatechin-3-gallate on the HIT-T15 pancreatic beta cell line." Cell Biol Toxicol **26**(3): 189-199.

Swarowsky, A., L. Rodrigues, et al. (2008). "Glial alterations in the hippocampus of rats submitted to ibotenic-induced lesion of the nucleus basalis magnocellularis." Behav Brain Res **190**(2): 206-211.

Szkudelski, T. (2001). "The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas." Physiol Res **50**(6): 537-546.

Tanaka, T., T. Ishii, et al. (2011). "(-)-Epigallocatechin-3-gallate suppresses growth of AZ521 human gastric cancer cells by targeting the DEAD-box RNA helicase p68." Free Radic Biol Med **50**(10): 1324-1335.

Tedeschi, E., M. Menegazzi, et al. (2004). "Green tea inhibits human inducible nitric-oxide synthase expression by down-regulating signal transducer and activator of transcription-1alpha activation." Mol Pharmacol **65**(1): 111-120.

Tiwari, V., A. Kuhad, et al. (2009). "Chronic treatment with tocotrienol, an isoform of vitamin E, prevents intracerebroventricular streptozotocin-induced cognitive impairment and oxidative-nitrosative stress in rats." Pharmacol Biochem Behav **93**(2): 183-189.

Tramontina, A. C., K. M. Wartchow, et al. (2011). "The neuroprotective effect of two statins: simvastatin and pravastatin on a streptozotocin-induced model of Alzheimer's disease in rats." J Neural Transm **118**(11): 1641-1649.

Tramontina, F., M. C. Leite, et al. (2007). "Immunoassay for glial fibrillary acidic protein: antigen recognition is affected by its phosphorylation state." J Neurosci Methods **162**(1-2): 282-286.

Turrens, J. F. (2003). "Mitochondrial formation of reactive oxygen species." J Physiol **552**(Pt 2): 335-344.

Uchigata, Y., H. Yamamoto, et al. (1982). "Protection by superoxide dismutase, catalase, and poly(ADP-ribose) synthetase inhibitors against alloxan- and streptozotocin-induced islet DNA strand breaks and against the inhibition of proinsulin synthesis." J Biol Chem **257**(11): 6084-6088.

Vaidyanathan, J. B. and T. Walle (2001). "Transport and metabolism of the tea flavonoid (-)-epicatechin by the human intestinal cell line Caco-2." Pharm Res **18**(10): 1420-1425.

Van Eldik, L. J. and W. S. Griffin (1994). "S100 beta expression in Alzheimer's disease: relation to neuropathology in brain regions." Biochim Biophys Acta **1223**(3): 398-403.

Van Eldik, L. J. and M. S. Wainwright (2003). "The Janus face of glial-derived S100B: beneficial and detrimental functions in the brain." Restor Neurol Neurosci **21**(3-4): 97-108.

Vicente, E., D. Degerone, et al. (2009). "Astroglial and cognitive effects of chronic cerebral hypoperfusion in the rat." Brain Res **1251**: 204-212.

Vieira de Almeida, L. M., C. C. Pineiro, et al. (2008). "Protective effects of resveratrol on hydrogen peroxide induced toxicity in primary cortical astrocyte cultures." Neurochem Res **33**(1): 8-15.

Weinreb, O., T. Amit, et al. (2007). "A novel approach of proteomics and transcriptomics to study the mechanism of action of the antioxidant-iron chelator green tea polyphenol (-)-epigallocatechin-3-gallate." Free Radic Biol Med **43**(4): 546-556.

Weinstock, M. and S. Shoham (2004). "Rat models of dementia based on reductions in regional glucose metabolism, cerebral blood flow and cytochrome oxidase activity." J Neural Transm **111**(3): 347-366.

Williams, R. J. and J. P. Spencer (2011). "Flavonoids, cognition, and dementia: Actions, mechanisms, and potential therapeutic utility for Alzheimer disease." Free Radic Biol Med.

Williamson, K. S., S. P. Gabbita, et al. (2002). "The nitration product 5-nitro-gamma-tocopherol is increased in the Alzheimer brain." Nitric Oxide **6**(2): 221-227.

Xiao, J., X. Chen, et al. (2008). "Investigation of the mechanism of enhanced effect of EGCG on huperzine A's inhibition of acetylcholinesterase activity in rats by a multispectroscopic method." J Agric Food Chem **56**(3): 910-915.

Yang, C. S., L. Chen, et al. (1998). "Blood and urine levels of tea catechins after ingestion of different amounts of green tea by human volunteers." Cancer Epidemiol Biomarkers Prev **7**(4): 351-354.

Yao, K., P. Ye, et al. (2008). "Epigallocatechin gallate protects against oxidative stress-induced mitochondria-dependent apoptosis in human lens epithelial cells." Mol Vis **14**: 217-223.

Zussy, C., A. Brureau, et al. (2011). "Time-course and regional analyses of the physiopathological changes induced after cerebral injection of an amyloid beta fragment in rats." Am J Pathol **179**(1): 315-334.