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GENOTOXIC EFFECT OF ALKALOIDS

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Because of the increased use of alkaloids in general medical practice in recent years, it is of interest to determine genotoxic, mutagenic and recombinogenic response to different groups of alkaloids in prokaryotic and eucaryotic organisms. Reserpine, boldine and chelerythrine did not show genotoxicity response in the SOS-Chromotest whereas skimmianine showed genotoxicity in the presence of a metabolic activation mixture. Voacristine isolated from the leaves of Ervatamia coronaria *shows* in vivo *cytostatic and mutagenic effects in* Saccharomyces cerevisiae *hapioids cells. The* Rauwolfia *alkaloid (reserpine) was not able to induce reverse mutation and recombinational mitotic events (crossing-over and gene conversion) in yeast diploid strain XS2316.*

Key words: alkaloids – SOS-Chromotest – *Saccharomyces cerevisiae* – mutation – recombination

Alkaloids are a group of natural products of great interest in Medicine. The best known alkaloids show pharmacological properties as suitable drugs in medical practice. It has been suggested that alkaloids can be considered potentially mutagenic, since the therapeutic action of certain alkaloids is related to their interaction with DNA (Beljanski & Beljanski, 1982) and the mutagenic and carcinogenic effects are due to this interaction (Miller & Miller, 1981). This is important for the estimate of long-term risks in view of the well-established correlation between mutagtenicity and carcinogenicity (McCann et al., 1975).

Several studies have indicated that most of the chemical mutagens are also potential inducers of mitotic recombination (Murthy, 1979; Kunz et al., 1980). In somatic tissues, it has been proposed that mitotic recombination, which can lead to homozygosity of recessive alleles, could be involved in the promotional stage of carcinogenesis (Kinsella & Radman, 1978). The purpose of the study was to analyse if indole (voacristine and reserpine), furoquinoleine (skimmianine), benzophenantridine (chelerythrine) and aporphine (boldine) alkaloids are able to induce the genotoxic, mutagenic and recombinogenic effects in procaryotic and eucaryotic cells.

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MATERIALS AND METHODS

Strains – *Escherichia coli* PQ37 (F–, thr leu his-4 pyr-D thi galE galK lac-169 sri300: : tn 10 rpoB rpsL uvrA rfa trp: :MUC sulA: :Mud (Ap, Iac) c-ts), was used for SOS-Chromotest (Quillardet et al., 1985).

The diploid *Saccharomyces cerevisiae* strain XS2316 has been previously described (Machida & Nakai, 1980) and its genotype is as follows:

$$\frac{+}{\text{ade}} \circ \frac{\text{leu1-1 trp5-48 + + + a}}{\text{leu 1-12 + cyh2 met13 lys5-1 } \alpha} \frac{\text{his1-1}}{\text{his1-1}}$$

The haploid repair-proficient RAD+ strain XV-185-14C (Mat a ade2-1 arg4-17 his1-7 lys1-1 trp5-48 hom3-10) was used for mutagenesis (Melo et al., 1986).

Chemicals – The indole alkaloid voacristine was isolated as a major product from the leaves of *Ervatamia coronaria* (Stapf) (Apocynaceae) collected in Pernambuco/Brazil (Melo et al., 1986). Reserpine was obtained from Merck Darmanstadt West Germany. Skimmianine and chelerythrine were isolated from roots of *Zanthoxylum sp.* (Von Poser et al., 1988). The aporphine alkaloid boldine was purchased from Sigma Chemical Co., USA.

SOS-Chromotest – The SOS-Chromotest was performed according to Quillardet et al. (1985). To carry out SOS-Chromotest the alkaloids were diluted in spectrophotometric dimethylsulphoxide (DMSO) grade.

Microsomal fraction – The microsomal fraction S9 was prepared from livers of Sprague-Dawleys rats pretreated with a polychlorinated biphenyl mixture (Aroclor 1254) as described by Maron & Ames (1983). The S9 mix for metabolic activation was prepared according to Quillardet et al. (1985).

Irradiation procedures – A suspension of cells (2×10^7/ml) was irradiated with differents UV doses at 254 nm, as described previously by Henriques & Moustacchi (1980).

8-Methoxypsoralen + UVA treatment – The 8-Methoxypsoralen (8-MOP) (Sigma, St. Louis, USA) was used as positive control in the determination of recombinational events in yeast. A suspension of cells (2×10^7/ml) was incubated for 20 min at 4 °C, with 5×10^{-5} of 8-MOP, and irradiated with 365 nm source (UVA) described by Averbeck & Moustacchi (1975) and Henriques & Moustacchi (1980).

Test of the induction of mitotic recombinational and mutational events in yeast cells – A suspension of cells (4×10^7 in exponential phase of growth was incubated for 20 h at 30 °C with various concentrations of different alkaloids. The media, growth condition and determination of mutagenic and recombinogenic activities were previously described by Machida & Nakai, 1980; Melo et al., 1986 and Von Poser et al., 1980.

RESULTS AND DISCUSSION

The Table I shows that reserpine, chelerytrine and boldine alkaloids were not able to induce

TABLE I

Response to reserpine, skimmianine, chelerytrine and boldine in the SOS-Chromotest with metabolization. The S9 mix was induced with Aroclor 1254

Agent	Dose (ng/test)	β-galactosidase (B) (Unit)	Alkaline Phosphatase (P) (Unit)	B/P	Induction factor
Aflotoxin B1[a]	0	167	1578	0.106	1
	5	443	1029	0.272	2.57
	20	1018	1617	0.629	5.93
Reserpine	0	208	2956	0.070	1
	$1X10^2$	217	2956	0.073	1.04
	$2.5X10^3$	209	2555	0.082	1.17
	$5X10^3$	225	2700	0.083	1.18
	$1X10^4$	245	3029	0.081	1.16
	$2X10^4$	229	2573	0.089	1.27
Skimmianine	0	167	1578	0.106	1
	$5X10^3$	209	1644	0.127	1.20
	$1X10^4$	456	1644	0.277	2.61
	$2X10^4$	612	1644	0.372	3.51
	$4X10^4$	855	1748	0.498	4.61
Chelerytrine	0	167	1578	0.106	1
	$2.3X10^4$	235	1841	0.128	1.21
	$2.8X10^4$	214	1709	0.125	1.18
	$5.75X10^4$	240	1845	0.130	1.23
	$1.15X10^5$	320	2098	0.182	1.43
Boldine	0	230	952	0.230	1
	$5X10^2$	200	937	0.213	0.93
	$1X10^3$	169	952	0.203	0.88
	$5X10^3$	174	1020	0.171	0.74
	$7.5X10^4$	191	990	0.193	0.84
	$1X10^4$	165	984	0.168	0.73

a: positive control

TABLE II

Induction of crossing-over (+/cyh 2) and gene conversion (leu 1-1/ leu 1-12) in diploid strain XS 2316 of *Saccharomyces cerevisiae* after reserpine and boldine treatment in exponential phase of growth

Agent	Dose (J/m^2)	Concentration ($\mu g/ml$)	% Survival	Crossing-over 10^5 survivors	Gene conversion 10^5 survivors
UV-radiation[a] (254 mm)	0		100 (480)[b]	47.9 (023)[b]	6.25 (03)[b]
	15		99 (476)	302 (144)	168 (80)
Reserpine		0	100 (1008)	51.7 (522)	7.5 (75)
		150	099 (0996)	55.7 (555)	7.5 (75)
		200	091 (0915)	59.6 (546)	7.2 (66)
		250	093 (0942)	57.0 (582)	5.6 (57)
		300	079 (0789)	51.8 (414)	5.6 (45)
8.MOP[a] + UVA	0		100 (932)[b]	123.4 (115)	14.7 (138)
	2000		100 (934)	390.0 (373)	80.5 (771)
Boldine		0	100 (960)	137.5 (132)	12.5 (12)
		100	98 (940)	222.3 (210)[e]	76.6 (72)[e]
		125	99 (948)	244.7 (232)[c]	54.8 (52)[e]
		150	112 (1076)	250.9 (270)[c]	59.5 (64)[e]
		200	86 (824)	273.1 (225)[d]	45.1 (40)[d]

The frequency of crossing-over and gene conversion was determined by dividing the numbers of prototrophs per ml by number of cells per ml.
a: positive control; *b:* number in parenthesis, actual number of colonies scored, three plates for each dose; *c:* genetic frequencies significant at the 1% level; *d:* genetic frequencies significant at the 2% level; *e:* genetic frequencies significant at the 5% level.

SOS functions when tested by the SOS-Chromotest in the presence of a metabolic activation mixture. However, the furoquinoleine alkaloid skimmianine was able to show genotoxic activity, inducing the SOS function. In the absence of metabolization all the four alkaloids tested did not show any genotoxic activity (Von Poser et al., 1988, 1990; Moreno, 1990).

Recently, Quillardet et al. (1985) demonstrated a strong correlation between induction of the SOS-Chromotest and indirect mutagenesis as measured by the Ames Test (Maron & Ames, 1983), confirming the validity of using the SOS-Chromotest as a short-term assay for determination of mutagenic and potentially oncogenic products. Thus, from this observation, it may be inferred that, with the exception of the skimmianine, the reserpine, chelerytrine and boldine alkaloids are not genotoxic and mutagenic in *E. coli*.

Considering that the yeast exponentially growing cells normally present high level of cytochrome P-450 metabolic activating system (Zimmermann et al., 1984) the search for mutagenic and recombinogenic effects of reserpine and boldine was carried out in exponential cultures of yeast diploid cells. The Table II shows that at the doses applied, these two alkaloids are non toxic. It can also be observed that the frequency of convertants (LEU+) and recombinants (CYH+) induced by reserpine was close to that for spontaneous rate. The frequency of convertants (LEU+) induced by boldine were highly altered, showing a clear convertogenic effect of this alkaloid. The induction of crossing-over in the exponentially growing diploid yeast cells treated with boldine was slightly altered (Table II). These alkaloids are not able to induce nuclear reserve mutation in yeast cells (Von Poser et al., 1990; Moreno, 1990). It is important to recall that there is a high correlation between gene conversion and gene mutation. In general, all the chemicals that are convertogenic are also mutagenic in the same system or in other systems (Murthy, 1979). Moreover, the fact that gene conversion is not mutagen-specific, differently from reverse-mutation system, reduces the chances of false negatives (Murthy, 1979). Hence, it can be suggested that reserpine is not mutagenic and recombinogenic whereas the boldine is able to induce the recombinational events is an eukaryotic organism such as Saccharomyces cerevisiae.

Recently we have shown that voacristine, an indole alkaloid which presents dose-dependent cytostatic and cytotoxic effects on cultures in repair proficient and deficient strains of Saccharomyces cerevisiae (Melo et al., 1986) is able to induce efficient nuclear reverse mutation in yeast wild-type (Table III) and repair mutant strain (Melo et al., 1986).

TABLE III

Mutagenic effect of Voacristine on exponentially growing cells of the wild-type yeast strain (RAD^+ XV-185-4C)

Concentration of Voacristine (μg/ml)	% survival RAD^+	LYS^+ revertants/10^8 survivors RAD^+
0	100	1.0 ± 0.2[a]
25	–	–
50	70	1.1 ± 0.1
100	49.4	8.5 ± 3.4
150	11.1	15.6 ± 1.8
200	0.8	205.1 ± 52.2

a: mean ± S.D.

Based in the relationship between mutagenicity and/or recombinogenicity and carcinogenicity, the results show a probable long-term risk of the use of phytotherapics which contain the furoquinoleine skimmianine, the indole voacristine and the aporphine boldine alkaloids. As a consequence of these observation, it appears to be of interest to examine the mutagenic and/or recombinogenic response of alkaloids that belong to other groups and are also largely used in folk medicine. From this kind of study we could estimate the actual risk-benefit relationship represented by the use of natural products as phytotherapics to human health.

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