

Clastogenic activity of integerrimine determined in mouse micronucleus assays

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ABSTRACT

The pyrrolizidine alkaloid integerrimine, obtained from *Senecio brasiliensis*, was tested by acute dosing at two concentrations (18.75 and 37.5 mg/kg), and at different times, to establish its ability to induce micronuclei in mouse erythrocytes. This alkaloid was able to increase the frequency of micronucleated polychromatic erythrocytes in both, bone marrow and peripheral blood erythrocytes.

INTRODUCTION

The pyrrolizidine alkaloids (PAs) are natural toxins found in many plant genera of wide geographic distribution (McLean, 1970). The species *Senecio brasiliensis* (Sprengel) Less., belonging to the family *Compositae*, is very common in the south-central region of Brazil. *S. brasiliensis* Less. var. *tripartitus* contains two pyrrolizidine alkaloids, the most important of which is integerrimine (Motidome and Ferreira, 1966). This alkaloid is toxic to rats and mice (Moraes, 1952). Integerrimine is mutagenic to *Drosophila melanogaster* (Paula-Ramos and Marques, 1978), to *Tradescantia* (Chies, 1983), to *Aspergillus nidulans* (Rocha and Azevedo, 1986) and to *Saccharomyces cerevisiae* (Paula-Ramos *et al.*, 1991). It is also known to induce mitotic anomalies in *Allium cepa* (Chies, 1983).

Integerrimine has antimitotic effects, with induction of megalocytosis in rat liver parenchymal cells (Nardi *et al.*, 1980; Gimmler and Nardi, 1982), and induces abortions and fetal malformations in mice (Kvitko and Gimmler, 1986). Integerrimine does not induce detectable structural or numerical chromosome

aberrations in mouse spermatocytes at diakinesis/metaphase I, after exposure of the cells in the pre-leptotene state (Gimmler-Luz *et al.*, 1987). However, it elicits structural chromosome aberrations in mouse bone marrow cells (Gimmler-Luz *et al.*, 1990). The relatively low potency of the clastogenicity of integerrimine in mouse bone marrow cells can be confirmed by the micronucleus (MN) assay as described by Schmid (1976). Bone marrow clastogens can also increase the frequency of MN in polychromatic erythrocytes (PCE) of peripheral blood (MacGregor *et al.*, 1980). Schlegel and MacGregor (1982) provide a strong evidence that micronucleated normochromatic erythrocytes (NCE) are accumulated in peripheral blood in mice. Consequently we analyzed the micronuclei frequency in PCE of bone marrow, and in PCE and NCE of peripheral blood.

MATERIAL AND METHODS

Inbred BALB/c male and female mice, aged 12-16 weeks, were used. The animals were housed in a room with controlled temperature and light, in the animal house facilities at Instituto de Biociências, Universidade Federal do Rio Grande do Sul. All

animals received commercial standard mouse cube diet (Nuvilab, CR1, Moinho Nuvipal Ltda., Curitiba, PR, Brazil) and water *ad libitum*.

The animals were injected intraperitoneally (*ip*) with a single acute dose of 18.75 or 37.5 mg/kg integerrimine, whose chemical formula was deduced by Klásek (1973) (Figure 1). These dose levels increase chromosomal aberrations in mouse bone marrow cells (Gimmler-Luz *et al.*, 1990). The mouse LD₅₀/24 h was established by Moraes (1952) as 100 mg/kg. The negative control group received an *ip* injection of the vehicle (0.1% acetic acid solution). The positive control groups were dosed by *ip* injection with 40 mg/kg cyclophosphamide (CP). The application volume was 10 ml/kg of body weight.

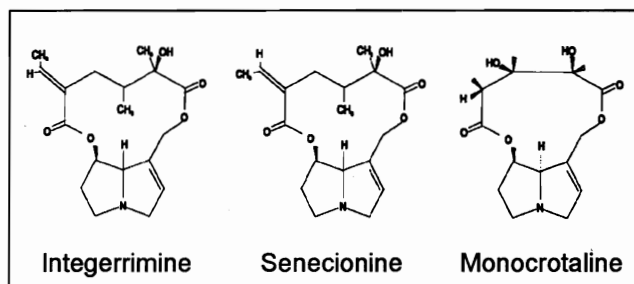


Figure 1 - Chemical structure of the pyrrolizidine alkaloid integerrimine, of its geometric isomer senecionine (cf. Klásek, 1973) and of monocrotaline (cf. Hincks *et al.*, 1991).

Mice were killed at 24, 48 or 72 h after the treatment, and bone marrow and peripheral blood samples were collected. Other mice were sampled at 2, 4, 7, 14 and 21 days after treatment, by a small incision of the caudal vessel, to evaluate the genotoxicity profile in PCE and NCE of peripheral blood. The bone marrow cells were prepared as described by Salamone *et al.* (1980). A drop of peripheral blood was mixed with a drop of fetal calf serum and smeared on a microscope slide, air dried, fixed in methanol, and stained with May Grünwald-Giemsa solution. The slides were coded and analyzed blind. Two thousand PCE from bone marrow and at least 1000 PCE from peripheral blood were analyzed for the presence of MN. The ratio of PCE to NCE in the bone marrow was determined among 500 erythrocytes. Three thousand NCE were analyzed for the presence of micronucleated cells in peripheral blood. Data were analyzed for statistical significance using the Mann-Whitney test.

RESULTS AND DISCUSSION

Integerrimine increased the frequency of micronucleated PCE (mPCE) in bone marrow at both doses tested (Table I). Increase of mPCE frequencies at 24 and 48 h showed gender differences, similar to those

Table I - Results of micronucleus analyses in bone marrow polychromatic erythrocytes, after integerrimine treatment.

Dose (mg/kg)	Sample time ^a	No. of animals			Sex	No. of analyzed PCE	mPCE/1000 PCE (individual data)	Means ± SD	PCE/NCE ± SD
		T	S	A					
N. Control		4	4	4	M	8,000	3.5, 3, 4.5, 3.5	3.6 ± 1.30	0.86 ± 0.42
		5	5	5	F	10,000	5, 5.5, 4.5, 0, 1	3.0 ± 2.71	1.10 ± 0.52
PA (18.75)	24 h	5	5	4	M	8,000	5, 8, 8.5, 4.5	6.5 ± 2.38*	0.50 ± 0.16
		5	5	5	F	10,000	1, 4, 4, 1, 2	2.4 ± 1.52	0.48 ± 0.23
	48 h	5	5	5	M	10,000	6, 5, 4, 2.5, 5	4.6 ± 1.67	0.65 ± 0.17
		5	5	5	F	10,000	4, 8, 5, 6, 4	5.4 ± 1.67*	0.52 ± 0.18
	72 h	5	4	4	M	8,000	8, 4, 4, 4	5.0 ± 2.00*	0.70 ± 0.18
		5	5	5	F	10,000	2.5, 6, 4, 5, 3.5	4.2 ± 1.48	0.60 ± 0.43
PA (37.50)	24 h	5	5	4	M	8,000	5.5, 2.3, 5.5, 3	4.0 ± 1.67	0.79 ± 0.14
		5	5	5	F	10,000	8.5, 4.5, 3.5, 3.5, 7.6	5.5 ± 2.37*	0.78 ± 0.16
	48 h	5	5	5	M	10,000	6, 6, 6.5, 8.5, 7	6.8 ± 1.30*	0.85 ± 0.44
		5	5	5	F	10,000	0.5, 3.5, 4.5, 3, 5	3.3 ± 1.75	0.76 ± 0.38
	72 h	5	4	4	M	8,000	2.5, 3, 2.5, 3.5	2.9 ± 0.48	0.54 ± 0.28
		5	5	5	F	10,000	5, 3, 2, 3, 6	3.8 ± 1.64	0.55 ± 0.20
P. Control	24 h	9	9	9	M	18,000	23, 19, 31, 18, 28.19, 34.5, 23, 22.3	24.2 ± 5.76*	0.61 ± 0.22
		7	7	7	F	14,000	33, 16, 18, 13.5, 20, 40, 27.3	24.0 ± 9.78*	0.59 ± 0.19
	48 h	5	5	5	M	10,000	15.5, 17, 13, 9.5, 11.5	13.3 ± 3.01*	0.31 ± 0.06*
		5	5	5	F	10,000	15, 12.5, 6.5, 11, 8	10.6 ± 3.42*	0.39 ± 0.30*

^aSample time in hours (h) or days (d) after the treatment; PCE = polychromatic erythrocytes; mPCE = micronucleated PCE; NCE = normochromatic erythrocytes; N. control = negative control, treated with 0.1% acetic acid in distilled water; PA = integerrimine; P. Control = positive control, treated with 40 mg/kg of cyclophosphamide; T = treated; S = surviving; A = analyzed; SD = Standard deviation; * = different from negative control at P value of 0.05 (P < 0.05).

observed for chromosome breaks after 12 and 24 h (Gimmler-Luz *et al.*, 1990). Gender differences in the MN assay responses have been encountered before for some other chemicals (Mavournin *et al.*, 1990; Kliesch and Adler, 1992).

Since no significant differences in the MN frequency were found in peripheral blood of negative control groups (one killed and the other used for the sequential analysis), the data were pooled into a single group (Tables II and III). For the same reason, the results obtained 48 h after integerrimine treatment were pooled.

In peripheral blood the increment in mPCE frequency was observed 24 h after the lowest dosage, and 24 h and 48 h after the highest dosage (Table II). These results show that the mPCE can be detected 24 h

after integerrimine treatment in both tissues: bone marrow and peripheral blood.

The significant increase of micronucleated NCE (mNCE) detected in peripheral blood 24 h after exposure to integerrimine (37.5 mg/kg) is unexpected. Only the males presented increased mNCE frequency in peripheral blood on the 4th day, and also on the 21st day after treatment (Table III). Since only one value for the 4th day (5.7) was greater than the highest negative control value (3.7), we believe the NCE assay can be considered negative. When adult male mice are fed with diets including pure monocrotaline or *Crotalaria spectabilis* seeds, the frequencies of mPCE increase in peripheral blood after three days, and become established at a plateau, with continuous feeding (MacGregor *et al.*, 1990).

Table II - Results of peripheral blood polychromatic erythrocyte micronucleous analyses after integerrimine treatment.

Dose (mg/kg)	Sample time ^a	No. of animals			Sex	No. of analyzed PCE	mPCE/1000 PCE (individual data)	Means ± SD	PCE/NCE ± SD
		T	S	A					
N. Control		9	9	8	M	24,000	3, 3, 1, 3.5, 2.5, 1, 1.5, 0	1.9 ± 1.24	55.20 ± 17.98
		10	10	10	F	25,000	1, 1, 2, 2, 1, 0, 1, 0.5, 1, 1	1.0 ± 0.60	76.20 ± 26.18
PA (18.75)	24 h	5	5	4	M	8,000	8.6, 4.6, 7.5, 7.5	7.1 ± 1.71*	66.00 ± 42.70
		5	5	4	F	6,000	1, 1, 2, 2	1.5 ± 0.58	46.00 ± 12.98*
	48 h	11	11	10	M	17,000	3, 2.5, 2.5, 2, 3, 1, 1, 1, 3, 1	2.0 ± 0.91	53.82 ± 22.71
		10	10	10	F	14,000	1.5, 2.5, 1, 3, 1, 0, 4.2, 1, 1	1.7 ± 1.18	60.38 ± 24.24
	72 h	5	4	4	M	5,000	2, 1, 4, 4	2.3 ± 1.99	61.50 ± 20.61
		5	5	5	F	5,000	2, 2, 1, 1, 1	1.4 ± 0.55	52.60 ± 12.78
	4 d	6	6	6	M	6,000	0, 3, 1, 0, 1, 5	1.7 ± 1.97	75.33 ± 18.99
		5	5	5	F	5,000	4, 1, 1, 3, 4	2.6 ± 1.52*	73.20 ± 22.95
	7 d	6	6	4	M	4,000	1, 0, 1, 0	0.5 ± 0.58	87.80 ± 61.11
		5	5	4	F	4,000	0, 1, 0, 0	0.3 ± 0.50	56.40 ± 12.99
	14 d	6	6	5	M	5,000	4, 4, 2, 1, 1	2.4 ± 1.52	55.20 ± 30.24
		5	5	4	F	4,000	2, 1, 1, 2	1.5 ± 0.58	43.40 ± 28.17
	21 d	6	6	5	M	5,000	0, 0, 1, 0, 0	0.2 ± 0.45	61.17 ± 15.00
		5	5	5	F	5,000	2, 0, 0, 0, 1, 0	0.6 ± 0.89	53.25 ± 2.99
PA (37.50)	24 h	5	4	4	M	8,000	3.5, 7.5, 1.5, 6.5	4.7 ± 2.92*	41.80 ± 8.66
		5	5	5	F	5,000	6, 6, 2, 2, 7	4.6 ± 2.41*	57.80 ± 32.97
	48 h	12	12	10	M	14,000	5.5, 4, 7, 7, 10, 6, 4, 10, 7, 11	7.2 ± 2.47*	46.60 ± 23.86
		11	11	11	F	15,000	2.5, 5, 6, 6.5, 6.5, 7, 12, 4, 5, 5, 1	5.5 ± 2.81*	50.50 ± 13.16
	72 h	5	4	4	M	7,000	4.5, 3.5, 4, 2	3.5 ± 1.27	38.00 ± 10.12
		5	5	5	F	7,000	3, 1, 4, 1.5, 5	2.9 ± 1.67*	45.70 ± 5.86*
	4 d	7	6	6	M	6,000	3, 2, 3, 2, 3, 3	2.7 ± 0.52	99.00 ± 25.18*
		6	6	6	F	5,000	2, 1, 6, 0, 0, 1	1.7 ± 2.25	93.80 ± 12.00
	7 d	7	6	5	M	5,000	1, 0, 2, 5, 6	2.8 ± 2.59	29.00 ± 1.73*
		6	6	4	F	4,000	1, 1, 1, 2	1.3 ± 0.50	37.00 ± 9.86*
	14 d	7	6	5	M	5,000	0, 1, 3, 1, 2	1.4 ± 1.14	63.40 ± 19.76
		6	6	5	F	5,000	1, 4, 3, 2, 1	2.2 ± 1.30	49.20 ± 19.03
	21 d	7	6	5	M	5,000	2, 0, 0, 0, 0	0.4 ± 0.89	63.50 ± 15.72
		6	6	6	F	6,000	0, 0, 0, 0, 1, 0	0.2 ± 0.41	47.80 ± 7.86*
P. Control	24 h	5	5	5	M	5,000	9, 10, 9, 5, 12	9.0 ± 2.55*	
		5	5	5	F	9,000	10, 9, 9, 9.5, 10	9.5 ± 0.50*	
	48 h	5	5	5	F	5,000	23, 35, 24, 26, 29	27.5 ± 4.83*	46.20 ± 12.40
		5	5	5	F	5,000	13, 19, 22, 12, 21	17.4 ± 4.62*	37.40 ± 13.26*

For abbreviations see Table I.

Table III - Results of the peripheral blood normochromatic erythrocyte micronucleous analyses after integerrimine treatment.

Dose (mg/kg)	Sample time ^a	No. of animals			Sex	No. of analyzed PCE	mNCE/1000 NCE (individual data)	Means \pm SD
		T	S	A				
N. Control		9	9	9	M	84,000	3.7, 0, 0, 1.3, 1.4, 1.1, 1.2, 0.8, 1	1.2 \pm 1.08
		10	10	10	F	78,000	1, 3, 0, 0.3, 2.5, 0.9, 0.9, 0.7, 1.3, 1.3	1.2 \pm 0.92
PA (18.75)	24 h	5	5	4	M	12,000	2.7, 0.3, 1, 2	1.5 \pm 1.06
		5	5	5	F	15,000	1.3, 1, 0.7, 0.3, 0.3	0.7 \pm 0.44
	48 h	11	11	10	M	30,000	1.7, 2.7, 3, 1.3, 0.7, 0, 0, 0, 0.3, 0.3	1.0 \pm 1.13
		10	10	10	F	30,000	1, 0, 0.7, 1, 0, 0.7, 1.7, 0, 0.3, 1.3	0.7 \pm 0.59
	72 h	5	4	4	M	12,000	0.7, 1.3, 2.3, 3.3	1.9 \pm 1.14
		5	5	5	F	15,000	0.3, 0, 1, 0.3, 1	0.5 \pm 0.45
	4 d	6	6	6	M	18,000	2.3, 2, 2.7, 2.6, 2.7, 3	2.6 \pm 0.35*
		5	5	5	F	15,000	1.7, 2.3, 0.7, 1, 1	1.3 \pm 0.65
	7 d	6	6	6	M	18,000	0.7, 1.7, 1.7, 2.3, 0.7, 0.3	1.2 \pm 0.78
		5	5	5	F	15,000	0.3, 0.7, 1, 0.3, 0.7	0.6 \pm 0.30
	14 d	6	6	6	M	18,000	0.7, 0, 0.7, 1.3, 0.3, 1	0.7 \pm 0.47
		5	5	5	F	15,000	1.3, 0, 0.3, 0, 0.3	0.4 \pm 0.54
	21 d	6	6	6	M	18,000	3.7, 2.7, 2.3, 2.3, 3.3, 2.3	2.8 \pm 0.60*
		5	5	5	F	15,000	1, 0.7, 1.7, 0.7, 1	1.0 \pm 0.41
PA (37.50)	24 h	5	4	4	M	12,000	1.3, 3, 3, 1.7	2.3 \pm 0.38*
		5	5	5	F	15,000	2.7, 2.3, 2.3, 2, 1.3	2.1 \pm 0.52*
	48 h	12	12	9	M	27,000	1, 0.7, 0.3, 1, 1.7, 1, 2, 1.7, 0.7	1.1 \pm 0.56
		11	11	11	F	33,000	0.3, 0.7, 0, 0, 0, 1.3, 1.3, 1.3, 1.7, 1.7, 1.3	0.9 \pm 0.69
	72 h	5	4	4	M	12,000	0.3, 0.3, 1.7, 0.7	0.8 \pm 0.66
		5	5	5	F	15,000	0, 0.7, 1, 1, 0.7	0.7 \pm 0.41
	4 d	7	6	6	M	18,000	3.6, 3.6, 5.7, 4, 3.6, 2.7	3.9 \pm 0.99*
		6	6	6	F	18,000	1.7, 1.3, 2.7, 0.7, 0.7, 3	1.7 \pm 0.98
	7 d	7	6	4	M	12,000	2, 1.3, 1, 1	1.3 \pm 0.47
		6	6	6	F	18,000	1.7, 0, 0, 0.3, 0, 0	0.3 \pm 0.68
	14 d	7	6	5	M	15,000	0.3, 2, 0, 1, 1.3	0.9 \pm 0.80
		6	6	6	F	18,000	1.7, 1.3, 1.7, 0, 0.7, 1	1.1 \pm 0.65
	21 d	7	6	6	M	18,000	1.3, 1, 2.3, 2.7, 2.3, 1.7	1.9 \pm 0.66*
		6	6	6	F	18,000	0.7, 0.7, 1, 1.3, 1.3, 1	1.0 \pm 0.23
P. Control	24 h	5	5	5	M	15,000	2.7, 2.3, 1.3, 2, 2.7	2.2 \pm 0.58*
		5	5	5	F	15,000	2.3, 1, 0, 1, 2	1.3 \pm 0.92

For abbreviations see Table I.

Our results suggest that MN analysis in PCE from bone marrow and from peripheral blood can be considered equivalent assays. Considering our data and the results of MacGregor *et al.* (1990), it seems that the evaluation of mNCE of peripheral blood is best determined after continuous exposure.

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RESUMO

A ação clastogênica de duas concentrações (18,75 e 37,5 mg/kg) do alcalóide pirrolizidínico integerrimina, obtido do *Senecio brasiliensis*, foi testada em camundongos após diferentes tempos do tratamento agudo. Após este tratamento, o alcalóide foi capaz de induzir aumento na frequência de eritrócitos policromados micronucleados presentes tanto na medula óssea quanto no sangue periférico.

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