

## EFFECT OF PRETREATMENT WITH VENOM OF *Apis mellifera* BEES ON THE YIELD OF GAMMA-RAY INDUCED CHROMOSOME ABERRATIONS IN HUMAN BLOOD LYMPHOCYTES

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### ABSTRACT

Venom of the honey bee *Apis mellifera* induced a protective effect against the induction of dicentric chromosomes by gamma radiation (2.0 Gy) in human peripheral blood lymphocytes when the cultures were treated with 0.00015  $\mu$ l venom/1 ml medium 6 h before irradiation. In cultures to which the venom was added immediately before irradiation with 0.25, 1.0 and 2.0 Gy, no significant differences in number of dicentric chromosomes induced was observed when compared to cultures submitted to irradiation only. The venom did not induce clastogenic effects nor did it increase the frequency of sister chromatid exchanges.

### INTRODUCTION

The venom of the honey bee is a complex mixture of several chemical agents with pharmacological and biochemical activities. Studies to determine its chemical composition were begun by Langer (1887) and later continued by Neumann and Habermann (1954), who showed that the biological activity of the venom is due to the presence of several peptides and proteins. The venom components can be separated into two fractions by dialysis. The non-dialyzable fraction contains the enzymes phospholipase A and hyaluronidase, together with other proteins with known enzymatic

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activity. The dialyzable fraction contains low molecular weight peptides, histamine, inorganic ions and other components, in addition to 80% water (Dotimas and Hider, 1987).

It has long been believed that bee venom is beneficial in certain cases of rheumatoid arthritis (Beck, 1935). Using rat paw edema as a model, Winter *et al.* (1962) and Billingham *et al.* (1969) confirmed the anti-inflammatory activity of the venom, which appears to be associated only with the peptide denoted 401. When the dose-response curve for the venom was compared with that obtained for hydrocortisone, peptide 401 was found to be approximately 100-fold more active. The authors concluded that the anti-inflammatory activity of peptide 401 is not the result of its ability to lyse mast cells and that these two effects reflect distinct pharmacological properties. The anti-inflammatory activity of peptide 401 was later confirmed by others (Hanson *et al.*, 1974).

Bee venom also has a radioprotective action on mice exposed to lethal X-ray doses (Shipman and Cole, 1967; Kanno *et al.*, 1970).

In addition to whole venom, some of the venom components have been tested separately for radioprotective action. Ginsberg *et al.* (1968) performed a series of experiments in which they administered mellitin subcutaneously to mice 24 h before irradiation (850 R). These investigators noted that the animals survived up to 30 days after a dose of 60 mg/kg body weight, although good protection was achieved with doses of approximately 5 mg/kg.

Another component tested was histamine. The two terminal histamine peptides detected in bee venom, alanyl-glycylglutaminylglycylhistamine (procamine) and alanyl-glycylpropylalanylglutaminylglycylhistamine, release histamine *in vivo* by being slowly hydrolyzed and it is suggested that both probably release chelating copper ions ( $\text{Cu}^{2+}$ ) (Peck and O'Connor, 1974). Thus these compounds probably also contribute to the radioprotective properties of natural venom.

In view of the above observations, the objective of the present study was to evaluate the radioprotective action of *Apis* venom against chromosome aberrations induced by gamma rays *in vitro* in human peripheral blood lymphocytes.

## MATERIAL AND METHODS

### *Venom collection*

Fresh *Apis mellifera* venom was collected immediately before each experiment. The bees were immobilized by freezing and their stings were removed together with the venom sac and the acid gland responsible for venom production. The venom was

collected into graduated capillary tubes, diluted in distilled water and added to the human peripheral blood lymphocyte cultures at a concentration of 0.00015  $\mu\text{l/ml}$  culture medium.

### *Culture irradiation*

A cobalt-60 source of gamma radiation of the Department of Genetics, Faculty of Medicine of Ribeirão Preto, USP, was used. The dose rate was 1.30 Gy/h, at a distance of 1 m from the source.

The culture flasks were taken to the cobalt source and irradiated for 7.5 min. Three different doses of radiation were used: 0.25 Gy (Experiment A), 1.0 Gy (Experiment B) and 2.0 Gy (Experiments C and D). In Experiments A, B and C, both the venom and the radiation were administered at the beginning of culture, whereas in Experiment D venom was added at the beginning and the culture was irradiated 6 h later.

### *Technique of human peripheral blood lymphocyte culture*

Blood samples were obtained from healthy non-smokers aged 25 to 35 years with no history of radiotherapy or chemotherapy. Lymphocyte rich plasma (1 ml) was added to each culture flask containing 80% RPMI-1640 medium (Gibco), 20% normal human serum, phytohemagglutinin (4% in relation to the total volume, prepared at the Cytogenetics Laboratory of the Faculty of Medicine of Ribeirão Preto), streptomycin (0.01 mg/ml, Ceme) and penicillin (0.5 mg/ml, Fontoura Wyeth S.A.).

Each experiment (A, B, C and D) had four classes of treatment; venom (0.00015  $\mu\text{l/ml}$  culture medium), radiation (0.25, 1.0 and 2.0 Gy), venom plus radiation and control.

After the treatment, the cultures were incubated for 48 h for analysis of chromosome aberrations and for 72 h for analysis of sister chromatid exchanges (SCE). Colchicine was added during the last 95 min. Fixation, slide preparation and staining were carried out by the technique of Moorhead *et al.* (1960).

In the additional test performed to determine the effect of venom on frequency of SCEs, the cultures were made in parallel with those of Experiment A, using plasma from six of the seven individuals involved and adding 10  $\mu\text{g/ml}$  bromodeoxyuridine (Sigma) in addition to the venom. The material was stained by the fluorescence plus Giemsa technique (Perri and Wolff, 1974; Korenberg and Freedlender, 1974). Approximately three drops of Hoeschst 33258 Calbiochem-Behring Corp (0.05  $\mu\text{g/ml}$  Hanks solution) was applied to fresh slides which were covered with coverslips and exposed to UV light for 50 min at a distance of 30 cm.

Analysis of chromosome aberrations and SCE were done on coded slides and metaphase figures were drawn schematically.

## RESULTS

### *Action of Apis venom associated with different doses of gamma radiation in terms of induction of chromosome aberrations*

In the tests for the determination of the radioprotective effect of *A. mellifera* bee venom on human peripheral blood lymphocyte cultures, a series of preliminary experiments was first performed to determine the amount of venom to be used per culture, since high concentrations, fully inhibited cell growth. The best results were obtained with 0.00015  $\mu$ l of venom per ml culture medium.

Table I presents the distribution of different types of chromosome aberrations observed in the cultures. The student "t" test (Sokal and Rohlf, 1969) was used for statistical analysis.

In experiments A, B and C, there were no significant differences. In experiment D, in which venom was added and the cells irradiated 6 h later with 2.0 Gy, there was a significant reduction in the frequency of chromosome aberrations.

The types of aberrations detected were fragments, chromatid and isochromatid breaks, triradial figures, translocations, rings and dicentrics (Table I).

When only dicentrics and rings were considered (Tables II and III), a significant difference was observed between R and VR in experiment D.

### *Action of Apis venom in terms of SCE induction*

Venom was found not to be a clastogenic agent. No significant variation in the number of SCEs was detected (Table IV).

## DISCUSSION

Chromosome aberrations are considered to be highly sensitive biological indicators of the effects of ionizing radiation and are also possibly related to the induction of carcinogenesis and mutagenesis. Human lymphocytes represent a valuable test system for radio-cytogenetic studies and the frequency of aberrations induced in this system may act as indicators of exposure to radiation *in vivo* (Sasaki, 1969; Bender, 1971; Lloyd and Purrott, 1981).

A dose-effect curve for irradiation can be constructed by analyzing the frequency of dicentrics in lymphocytes irradiated *in vitro* (Bender and Gooch, 1962; Abbatt, 1971) since lymphocytes have been shown to have the same radiosensitivity when irradiated *in vivo* and *in vitro* (Clemenger and Scott, 1973).

Table I - Distribution of different chromosome aberration types observed in experiments A, B, C and D where the lymphocytes were treated with bee venom (0.00015 µl/ml medium) and exposed to different doses of gamma radiation.

Treatment	Number of individuals	Number of cells analysed		Breaks		Fragments	Triradial figures <sup>(a)</sup> Translocations <sup>(b)</sup>	Dicentric + Rings	Frequency of aberrations (%)
		Total	With aberrations	C	IC				
Control (A)	7	350	6	6	0	0	0	0	1.7
Venom	7	350	8	3	4	0	2 <sup>(b)</sup>	0	2.57
0.25 Gy	7	350	15	8	3	2	1 <sup>(b)</sup>	6	5.7
Venom + 0.25 Gy	7	350	12	6	2	0	1 <sup>(b)</sup>	4	3.7
Control (B)	5	500	4	3	0	2	0	0	1.0
Venom	5	500	4	4	0	0	0	0	0.8
1.0 Gy	5	460	42	5	0	37	8 <sup>(b)</sup>	19	15.0
Venom + 1.0 Gy	5	461	37	6	0	40	8 <sup>(b)</sup>	11	14.1
Control (C)	5	500	4	6	0	0	0	0	1.2
Venom	5	500	7	4	0	4	0	0	1.6
2.0 Gy	5	477	101	26	7	90	1 <sup>(a)</sup>	35	33.3
Venom + 2.0 Gy	5	486	119	31	4	117	2 <sup>(a)</sup>	33	38.5
Control (D)	6	600	28	30	0	0	0	0	5.0
Venom	6	600	23	18	4	5	0	0	4.5
6 h + 2.0 Gy	6	600	194	39	5	187	1 <sup>(a)</sup>	142	62.3
Venom (6 h) + 2.0 Gy	6	600	144	22	6	171	3 <sup>(a)</sup>	106	51.3

C: chromatid-type aberration; IC: isochromatid-type aberration.

Table II - Number of dicentric + rings/100 cells analysed/blood sample from six individuals treated with bee venom 6 h before 2 Gy gamma radiation.

Individual	Without venom	With venom
1	36	25
2	12	14
3	23	21
4	20	14
5	22	11
6	29	21

Table III - Mean differences, "t" values and significance level obtained by the "t" test applied in experiments A, B, C and D, considering the dicentrics plus rings (R x VR - comparison between the treatments with radiation and venom + radiation).  $\alpha$  - 5%.

Experimental condition	R x VR		
	Differences (d)	"t" Values (t)	Significance level (P)
A (n - 7)	0.005	0.195	50% < P < 90%
B (n - 5)	0.016	0.546	50% < P < 90%
C (n - 5)	0.006	0.204	50% < P < 90%
D (n - 6)	0.073	2.604	2% < P < 5%

Table IV - SCE frequency and mean SCE number per cell in human peripheral blood lymphocyte cultures treated with 0.00015  $\mu$ l bee venom/ml medium\*.

Treatment	Individuals							M + SE/cell
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>	Total	
Control	221	158	157	112	164	130	942	7.85 $\pm$ 1.70
Venom	197	225	138	136	186	159	1051	8.76 $\pm$ 1.52

\*Twenty metaphases/individual/treatment were analyzed.

Several factors may influence the induction of chromosome aberrations in lymphocytes, such as individual variability (Bochkov and Pilosov, 1968; Sasaki *et al.*, 1970; Bianchi *et al.*, 1982) and methodological variables (Bukton and Evans, 1973; Liniecki *et al.*, 1973), culture time appearing to be the most important among the latter (Takahashi *et al.*, 1979; Pohl-Ruling *et al.*, 1983). When analysis is performed during the second or third division after induction, some of the unstable aberrations may be eliminated. Thus, the culture time used for all of the present experiments was 48 h, which guarantees that cells are analysed predominantly in the first cycle.

No radioprotective activity was detected in cultures treated with venom + radiation, together. However, in Experiment D in which venom was added to the culture 6 h before irradiation with 2.0 Gy, the frequency of dicentrics + rings was significantly

lower in cultures treated with venom + radiation than in those treated with radiation only, demonstrating the radioprotective action of the venom (Tables I and II). Thus, pretreatment time had an effect on the radioprotective response, in agreement with the results obtained by our group (Varanda *et al.*, 1992) when analyzing bone marrow cells of Wistar rats treated *in vivo* with venom. We observed a reduced frequency of cells with chromosome aberrations when the animals were treated with venom 24 h before exposure to radiation (3.0 or 4.0 Gy). The radioprotective effect was not observed when the animals were treated with venom only 1 h before irradiation, or after irradiation. Dicentric formation involves breaks and subsequent chromosome rearrangements. Their decreased frequency may have resulted from a greater repair activity, probably related to the action of the venom, which, according to Hyre and Smith (1986), interferes with T and B lymphocyte function in mice.

Thus, we conclude that bee venom has a radioprotective effect against the formation of dicentrics in cell cultures treated 6 h before irradiation. This protection may be attributed to a stimulation of the repair system. It was also clear that the venom itself has no clastogenic activity, nor did it induce an increase in the frequency of SCEs at the concentration used. SCE analysis is a very sensitive method for monitoring damage induced in DNA (Perry and Evans, 1975; Latt *et al.*, 1981).

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## RESUMO

O veneno da abelha *Apis mellifera* tem sido considerado como um agente terapêutico potencialmente valioso, com atividade radioprotetora e anti-inflamatória *in vivo*. Essa toxina mostrou um efeito protetor contra cromossomos dicêntricos induzidos por radiação gama (2,0 Gy) em linfócitos do sangue periférico humano em cultura. Esse efeito foi observado somente quando as culturas foram tratadas com o veneno (0,00015 µl/ml de meio) 6 horas antes da irradiação (2,0 Gy). Nas culturas em que o veneno foi adicionado imediatamente antes da irradiação com 0,25; 1,0 e 2,0 Gy, não foram observadas diferenças significativas no número de cromossomos dicêntricos induzidos, quando comparado com as culturas submetidas a irradiação somente. O veneno não apresentou efeito clastogênico e nem aumentou a frequência de trocas entre cromátides irmãs.

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