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EFFECTS OF GAMMA RADIATION ON SNAKE VENOMS

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ABSTRACT

Ionizing radiation is able to detoxify several venoms, including snake venoms, without affecting significantly their immunogenic properties. Inn order to elucidate this phenomena, we conceived a comparative pharmacological study between native and irradiated (2,000 Gy) crotoxin, the main toxin of the South American rattlesnake *Crotalus durissus terrificus* . Crotoxin was isolated and purified by molecular exclusion chromatography, pI precipitation and, susbequentely submitted to irradiation. Gel filtration of the irradiated toxin resulted in some high molecular weight aggregates formation. Crotoxin toxicity decreased two folds after irradiation, as determined by LD_{50} in mice. Native and irradiated crotoxin biodistribution ocured in the same general manner, with renal elimination. However, in contrast to irradiated crotoxin, the native form was initially retained in kidneys. A later concentration (2-3 hr) appeared in phagocytic mononuclear cells rich organs (liver and spleen) and neural junction rich organs (muscle and brain).

KEYWORDS

Crotoxin; gamma radiation; biodistribution.

INTRODUCTION

Ophidic accidents are an important public health problem in Brazil, with 20,000 cases occuring annually. Thirteen percent of these accidents are caused by the Brazilian rattlesnake *Crotalus durissus terrificus*. The bitten patients present high mortality if quick serotherapic treatment is not established (Ribeiro et al., 1990).

Ionizing radiation promotes modification on biomolecules, including proteins. Among these modifications, structural alterations and loss of biological activity have been observed. However, although inactive, irradiated proteins preserve their immunogenic properties. This fact has been explored by many investigators to produce atoxic immunogens for antisera production. In previous experiments, Nascimento et al. observed a decrease of snake venom components toxicity, following irradiation.

C. d. terrificus venom presents many pharmacologically active compounds, including crotoxin, the dominant toxic component. This 23 Kd protein has phospholipase A2 and indirect hemolytic activities (Rubsamen et al., 1971), acting on neuromuscular junctions, with both pre and postsynaptic effects (Vital Brazil, 1972; Vital Brazil, 1976). It is composed by two subunits, one with enzymatic activity, the phospholipase, and the other, called crotapotin, determinant of neural action (Bon et al, 1979). Despite the importance of understandig the toxin kinetic, crotoxin biodistrbution has been poorly studied. Using dogs as experimental model, Lomba et al. (1969) observed a tricompartimental model fit for crotoxin, with concentration of the toxin in muscle and kidneys, wich is diverted to spleen if antiserum is concomitantely administrated. On the other hand, the organ distribution of crotoxin was dependent of crotapotin, wich is responsible for the relative concentration of the toxin in brain and muscle and also for the renal excretion (Habermann et al., 1972). These authors

investigated mainly the interaction of the toxin subunits, without a descriptive analysis of the crotoxin subunits biodistribution.

In the present work, we intend to compare the biodistribution of native and irradiated crotoxin in mice organs, in order to elucidate if its low toxicity after irradiation is related to possible changes in irradiated toxin binding.

MATERIAL & METHODS

Crotoxin purification

C. d. terrificus crude venom (150 mg) was submitted to molecular sieving chromatography on Sephadex G-75, according to the method described by Aird & Kaiser (1985). The tubes containing crotoxin were pooled, freeze-dried and the toxin was then further purified by isoelectrical point precipitation (pI=4.7).

Irradiation of crotoxin

A 2mg/ml in 150 mM NaCl sample of pure crotoxin was irradiated with 2,000 Gy in a ^{60}Co gamma source (Gammacell 220, Atomic Energy of Canada), with a dose rate of 540 Gy/hr. This irradiated material was then resubmitted to chromatography as described above.

Toxicity determination

The toxicity of the native and irradiated forms of the toxin was evaluated by lethal dose 50% (LD_{50}) and calculated according to the Spearman-Karber method, as preconized by the World Health Organization (WHO, 1981). Briefly,groups of 5 mice each were injected with serial dilutions of either native or irradiated crotoxin. Survival was then recorded for 24 hours.

Biodistribution of native and irradiated crotoxin

Both Native and irradiated crotoxin (5μg of each) were radioiodinated according to the method of Hunter & Greenwood (1962). The toxin and the free iodine were separated by Sephadex G-100 chromatography. Mice (10/group) weighting 30 g were intravenously injected with $2x10^6$ cpm of radiolabelled native or irradiated toxin, both of them presenting a specific ativity higher than 0,28 cpm/μg. The animals were sacrified (under ether anesthesy) at different time intervals and perfused. Blood samples were obtained by orbital plex bleeding and, after perfusion, kidneys, spleen, liver, muscle and brains were carefully removed and individually weighted. The samples radioactivity was counted and expressed as counts/mg organ. The statistical analysis was performed by ANOVA and Kruskall-Wallis tests.

RESULTS AND DISCUSSION

Irradiation of crotoxin

While the highly purified native crotoxin elution profile resulted in one single peak (data not shown), the chromatography of irradiated crotoxin resulted in two peaks (figure 1), one corresponding to crotoxin, and a second one, eluting in the high molecular weight region, consisting of agregated crotoxin.

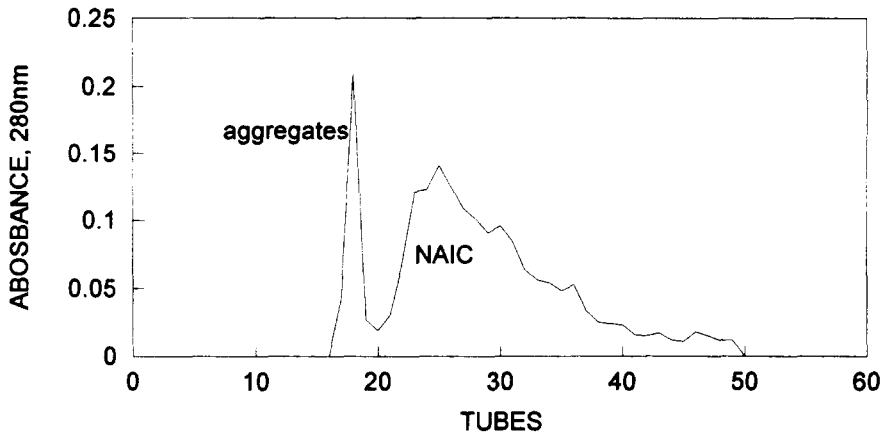

Figure 1: Chromatographic profile of 15 mg irradiated crotoxin on Sephadex G-100.

Toxicity

After irradiation, we could observe a decrease of toxicity of at least two folds, when compared to the native form of the toxin (table 1).

Biodistribution

The results for native and irradiated crotoxin are shown in figures 2 and 3 respectively.Both forms of the toxin present a similar biodistribution pattern, exception made to the kidneys wich present no initial retention for irradiated crotoxin. Liver and spleen, both phagocytic mononuclear cells rich organs, showed a slow elimination of crotoxin with a slightly lower amount of irradiated toxin present in these tissues, when compared with the irradiated one.

Table 1: Comparative toxicity of native and irradiated crotoxin

SAMPLE	LD_{50} (μg/g)
Native crotoxin	0.06
Irradiated crotoxin	0.11

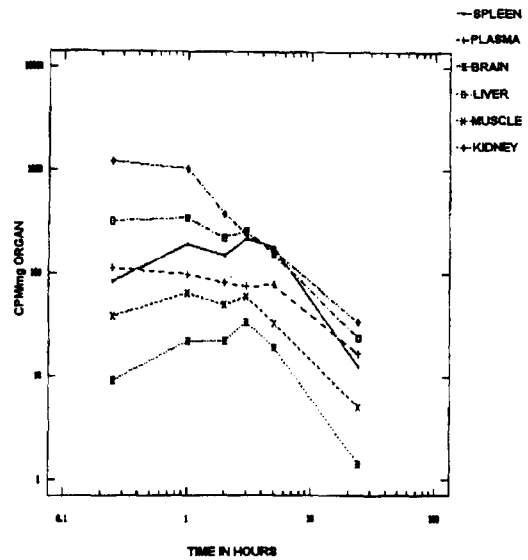

Figure 2: Biodistribution pattern of ^{125}I labeled native crotoxin in mice at different times.

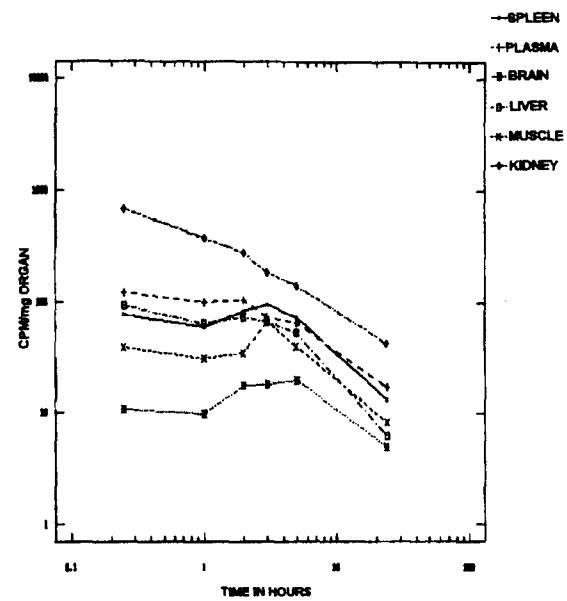

Figure 3: Biodistribution pattern of ^{125}I labeled irradiated crotoxin in mice at different times.

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