BIOCHEMICAL, IMMUNOLOGICAL AND PHARMACOLOGICAL ANALYSIS OF THE RATTLESNAKE VENOM IRRADIATION PRODUCTS

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ABSTRACT

Crotoxin, the most important toxin from *Crotalus durissus terrificus*, venom after being submited to a 2,000Gy dose of gamma radiation displayed an attenuated toxicity, preserving its immunological properties. Aggregates formation was also observed. Recent studies indicate a relationship between increasing aggregates formation and increasing the radiation dose. These aggregates seem to be responsible for the loss toxicity of crotoxin. In the present work, *Crotalus durissus terrificus* venom was irradiated with increasing doses of ^{60}Co gamma rays in order to optimize aggregates formation, associating attenuated toxicity and maintenance of immunogenicity excluding the crotoxin purification step. Results from gel filtration chromatography of native and irradiated with 2,000, 3,000, 5,000, 10,000 and 12,000 Gy venoms indicate an increasing of the amount of aggregates formed. The aggregates resulting showed no toxicity up to 2,5μg/g. These data suggest that toxicity is being attenuated by high radiation doses, like when irradiating crotoxin, and that the aggregates formed are the responsible for this attenuation.

INTRODUCTION

Accidents involving snakes are frequent in our country and serotherapy is the most efficient treatment (2). However, the current antisera production does not reach the desirable amount, in part, as a consequence of the high toxicity of the antigens employed in immunization. Our group has been using ionizing radiation to detoxify venoms in order to improve antisera production (3,4) since previous works with irradiated crotoxin from *Crotalus durissus terrificus* showed that the aggregates formed during irradiation are the main cause of detoxification (5).

These aggregates are atoxic, do not present any enzimatic activity and its formation occurs in a dose dependent manner (5). In order to omit the toxin purification step, the total crude venom was irradiated with different doses, trying to find the dose that induces high amounts of aggregates, suitable to generate neutralizing antibodies.

PROCEDURES AND RESULTS

Venom Irradiation

The venom was dissolved in saline solution (0,15M NaCl adjusted pH 3,0 with concentrated HCl) at a concentration of 2mg/ml, as determinated by the Bradford method (1). This solution was irradiated with 2,000, 3,000, 5,000, 10,000 and 12,000Gy dose using gamma rays derived from a ^{60}Co Source Gammacel 220 (Atomic Energy Agency of Canada Ltd.) in in the presence of O_2 at room temperature.

Isolation of aggregates

After irradiation the venom was passed over a column (2,5x44cm) Sephadex G-100 equilibrated and eluted with 0,1M acetic acid (pH3,0). Absorbance was determinated at 280 nm and fractions corresponding to aggregated and non aggregated irradiated venom (NAIV) were pooled and lyophilized (Fig.2 - 6). Non irradiated crude venom was submited to gel filtration as control (Fig.1).

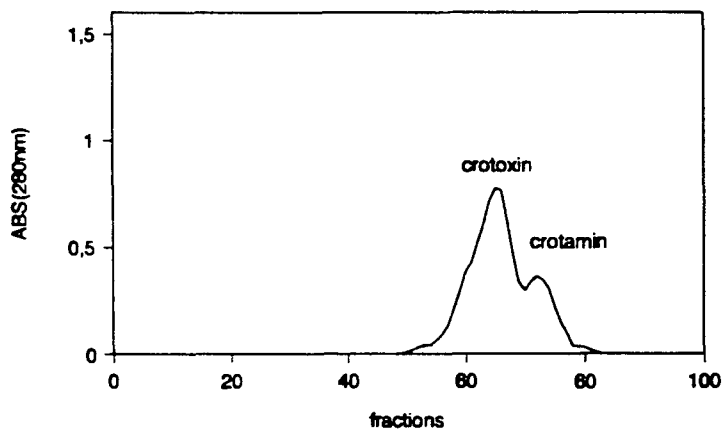


FIGURE 1 - Crude venom elution on Sephadex G100 (44x2,5cm); 100mM acetic acid.

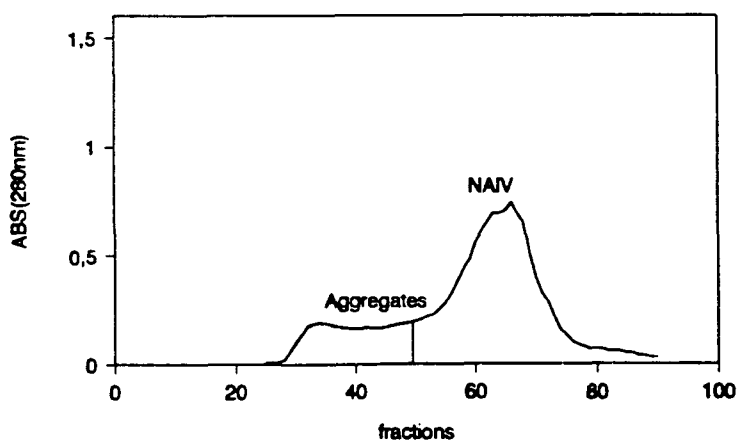


FIGURE 2 - Irradiated venom with 2,000Gy dose using Sephadex G100 (44x2,5cm);100mM ác. acético.

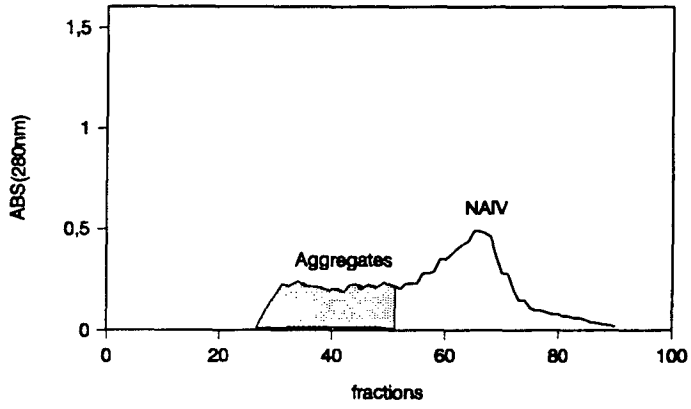


FIGURE 3 - Irradiated venom with 3,000Gy dose using Sephadex G100 (44x2,5cm);100mM acetic acid.

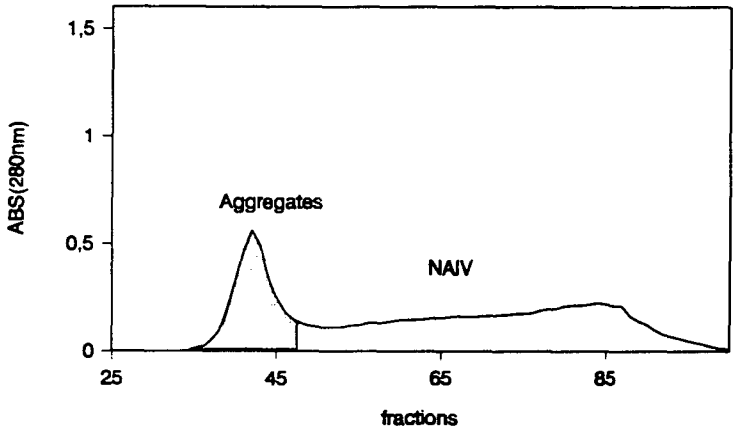


FIGURE 4 - Irradiated venom with 5,000Gy dose using Sephadex G100 (44x2,5cm);100mM acetic acid

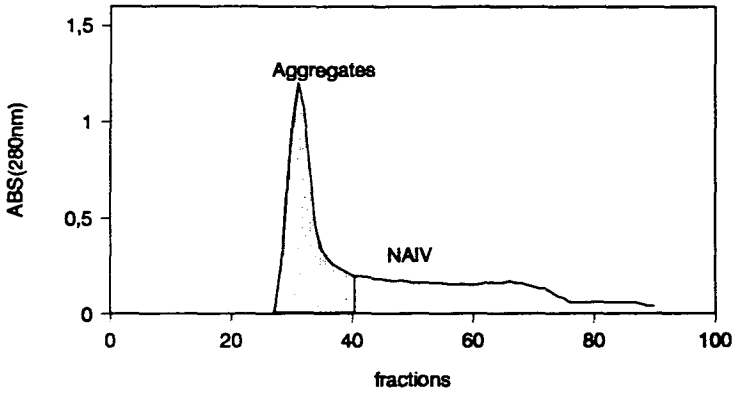


FIGURE 5 - Irradiated venom with 10,000Gy dose using Sephadex G100 (44x2,5cm);100mM acetic acid.

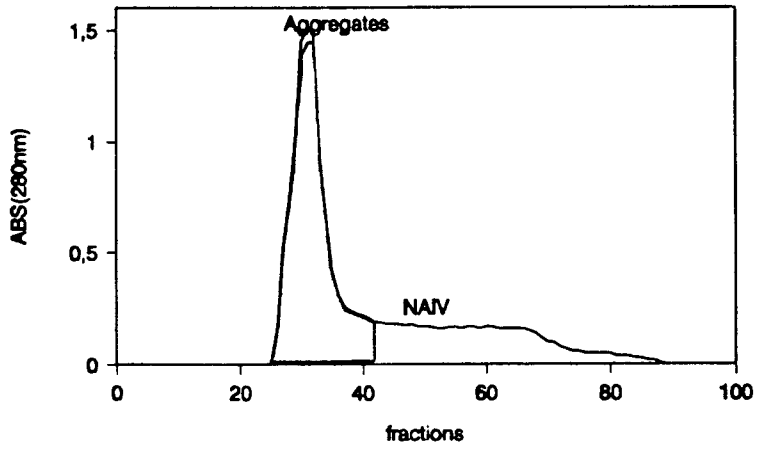


FIGURE 6 - Irradiated venom with 12,000Gy dose using Sephadex G100 (44x2,5cm);100mM acetic acid.

Relative area of peaks

The percentage of aggregates generated during irradiation was calculated through whole integration of each value of fractions absorbance. The results are shown in table 1.

TABLE 1 - Relative area of peaks.

Irradiation dose	% of aggregates
2,000Gy	11
3,000Gy	36
5,000Gy	28
10,000Gy	39
12,000Gy	53

Lethality Assays

Lyophilized aggregated and non aggregated material were dissolved in saline solution (0,15M NaCl), containing 1% of bivine serum albumin, and injections were made i.p. in 20-40g mice. Survival was determined after 24hs. Toxicity assays for various samples are shown in table 2.

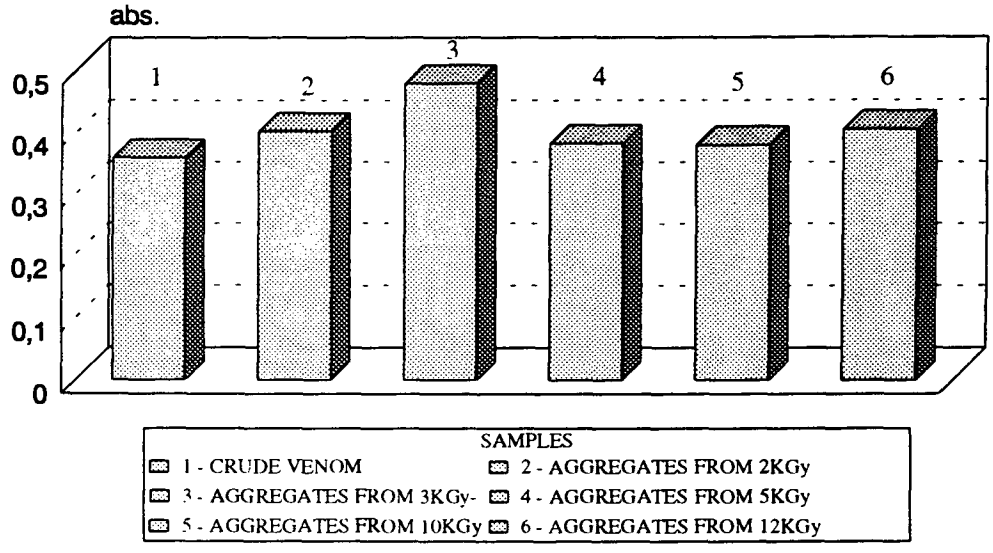
TABLE 2 - Toxicity assay (DL_{50}).

Fraction	DL_{50} (µg/g)
Aggregates from 2,000Gy irradiated venom	>2,5
Aggregates from 3,000Gy irradiated venom	>2,5
Aggregates from 5,000Gy irradiated venom	>2,5
Aggregates from 10,000Gy irradiated venom	>2,5
Aggregates from 12,000Gy irradiated venom	>2,5
Non Aggregates from 2,000Gy irradiated venom	0,44
Non Aggregates from 12,000Gy irradiated venom	>0,75
Native Crude Venom	0,09

Production of antibodies

Mice tested with lethality assay were bled after 30 days of injection to obtain anti-sera. Antibody concentration was determined by an enzyme linked immunoassay (ELISA-Fig.7). The results showed that aggregates from 3,000Gy dose of crude venom are more efficient to generate antibody than non irradiated venom.

FIGURE 7 - Immunogenic capacity of Aggregates from different radiation doses (dilution 1/50)



DISCUSSION

Irradiaton of venom by gamma rays results in high molecular weight products, the aggregates. The amount of these aggregates formation occured in a dose dependent manner and they are virtually non toxic to mice (relative crude venom).

The aggregates from irradiated venom with 3000Gy dose showed effective to be used to obtain high amounts of immunogens.

AKNOWLEDGEMENTS

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