EFFECTS OF Co^{60} GAMMA RADIATION ON THE IMMUNOGENIC AND ANTIGENIC PROPERTIES OF *Bothrops jararacussu* VENOM .

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ABSTRACT

Ionizing radiation has been successfully employed to attenuate animals toxins and venoms for immunizing antisera producing animals. However, the radiation effects on antigenicity and immunogenicity have not yet been elucidated. In the present work, we investigated the effects of gamma rays on the antigenic and immunogenic behaviour of *Bothrops jararacussu* venom. Venom samples (2mg/ml in 150 mM NaCl) were irradiated with 500, 1000 and 2000 Gy of ^{60}Co gamma rays. These samples were submitted to antigen capture ELISA on plates coated with commercial bothropic antiserum. Results suggest a loss of reactivity of the 1000 and 2000 Gy irradiated samples. Antibodies against native and 2000 Gy irradiated venoms were produced in rabbits. Both sera were able to bind native venom with a slightly higher titer for anti-irradiated serum. These data suggest that radiation promoted structural modification on the antigen molecules. However since the antibodies produced against irradiated antivenom were able to recognize native venom, there must have been preservation of some antigenic determinants. It has already been demonstrated that irradiation of proteins leads to structural modifications and unfolding of the molecules. Our data suggest that irradiation led to conformational epitopes destruction with preservation of linear epitopes and that the response against irradiated venom may be attributed to these linear antigenic determinants.

I. INTRODUCTION

Ionizing radiation promotes changes on proteic toxins leading to modifications on their biological activities [1]. These modifications include decrease of enzymatic activity, loss or decay of toxicity as well as alterations on primary, secondary and conformational structure[1]. As a result of these changes, the immunological behaviour of the irradiated molecules may be modified. These features have been explored by many authors to attenuate toxins [2, 3, 4], intending to produce an atoxic immunogen using irradiated highly lethal proteins. Others authors also demonstrated that the radiation-induced modifications may lead to an increase of the immunogenic properties of proteic antigens [2]. Snake venoms are employed as immunogens for ophidic antisera production. However, animals exposed to the highly active toxins and enzymes of those substances frequently undergo adverse reactions and severe pain, affecting the productivity. Our group has been working on snake venom irradiation, intending to decrease its toxicity while preserving its immunogenicity in order to improve antisera production. Among the Brazilian venomous snakes, the genus *Bothrops* is the prevalent on what refers to accidents incidence, resulting mainly in local effects and, in severe cases, systemic alterations involving hypovolemic shock and acute renal failure as a consequence of myonecrosis and accumulation of myoglobin in renal tubules[4]. Myonecrosis can result from two different mechanisms. One occurring in all bothropic envenomations, resulting from ischemia and tissue damage, while the other is a consequence of myotoxins acting specifically on muscle cells [5] . These toxins are specie-specific and are not neutralized by commercial bothropic antiserum, although myotoxic venoms are included in the pool of antigens employed for antisera production [6]. Some authors suggest that this might be due to a low immunogenicity of these components [7]. The aim of this work was to evaluate the effects of ionizing radiation upon *Bothrops jararacussu* venom, one of the most myotoxic venoms included in this genus, investigating the toxic and immunogenic properties of the venom in its native and irradiated forms.

II. MATERIAL AND METHODS

Venom. *Bothrops jararacussu* venom, in crystalized form was purchased from Instituto Butantan and was kept at -20°C prior to use.

Irradiation. Native venom samples (2 mg/ml in 150 mM NaCl) were irradiated with 500, 1000 and 2000 Gy, with a dose rate of 450 Gy/h in a ^{60}Co gammacell source (Atomic Agency of Canada ltd.), at room temperature and in the presence of atmospheric oxygen.

Toxicity. The toxicity of native and irradiated samples was assessed by 50% lethal dose (LD_{50}) and

calculated by the Spearman-Karber method, according to the World Health Organization recommendations [8]. Each group consisted of 5 male Swiss mice (20 ±2 g).The highest amount of venom injected in each animal was of 300 µg and the serial dilution factor was of 1.3.

Immunization. Male New Zealand rabbits (3 months) were inoculated with 500 µg of either native or 2.000 Gy irradiated venom intradermally, following a classical immunization schedule. Briefly, animals were initially injected with 500 µg of native or irradiated venom in Freund's complete adjuvant, followed by boosters of venom plus Freund's incomplete adjuvant (first booster) or venom in phosphate buffer. The boosters were given with 15 days intervals.

Enzyme Linked Immunosorbent Assay (ELISA). The antibodies titers in the immunized rabbits were determined by this method. 96 wells plates were coated with 100 µl of 10 µg/ml native venom in pH 9.6 carbonate buffer and blocked with 1% bovine albumin. Antisera were tested in quadruplicate, starting from a 1:400 dilution and using a 2 fold dilution factor. Bound antibodies were detected by horseradish peroxidase coupled antirabbit IgG antibodies.

Radial double Immunodifusion. Four isometrically disposed wells (Ø=2mm) were punched on agarose coated microscope slides and filled with 10µl (2mg/ml) of native or 2000 Gy irradiated venom or antinative or antirradiated sera (1/4). Slides were incubated 24h at 37°C, washed and stained with Coomassie Blue.

Antigen Capture ELISA: Microplates were coated with 10µg/well of commercial bothropic antiserum (Instituto Butantan). After blocking and washing, 1.5, 3, 6 or 30µg of native or irradiated (500, 1000, 2000 Gy) samples were added to the wells and kept for 2h at 37°C in humid chamber. After this, the washed plate was assayed by adding rabbit anticrotalic serum which reacts specifically with the myotoxic fraction of *Bothrops jararacussu* venom. The reaction was developed as previously described.

III. RESULTS

Toxicity: The toxicity decreased in a dose-dependent manner (Table 1).

TABLE 1. Toxicity of Native and Irradiated Venom

Sample	LD_{50} (µg/ animal)	Confidence interval (95%)
native	80.7	63.53-102.50
500 Gy	77	59.41-100.74
1000 Gy	184	144.90-236.01
2000 Gy	>300	

Enzyme Linked Immunosorbent Assay (ELISA): Both groups responded the same manner, presenting a similar IgG titer (Figure 1). However, in the titration portion of the curve, the serum raised against irradiated venom presented a slightly higher antibody titer.

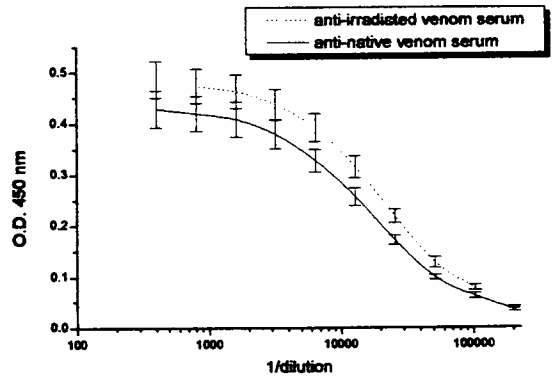

Figure 1. ELISA Titration of the Antibodies Raised Against Native or Irradiated Venom.

Radial double Immunodifusion: Antibodies raised against native venom recognized only the venom in its native form, while the antibodies induced by irradiated venom reacted against both native and irradiated forms of the antigens (Figure 2).

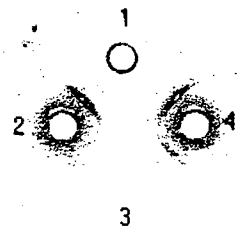

Figure 2. Double Radial Imunodifusion . 1-Native Venom. 2-Antinative Venom serum. 3-Irradiated Venom. 4-Antirradiated Venom serum.

Antigen capture ELISA: Native and 500Gy irradiated venom showed similar dose-dependent reactivity pattern, while 1000 and 2000 Gy irradiated venom presented no reactivity (Figure 3).

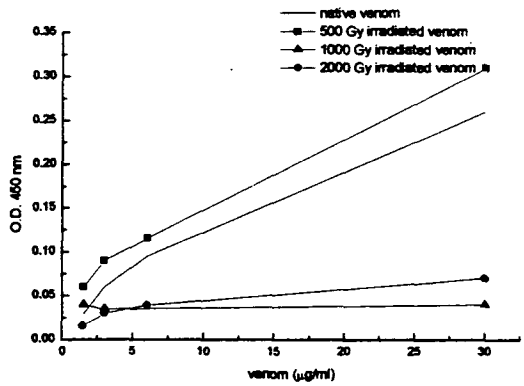

Figure 3. Antigen capture ELISA of native and irradiated venom

IV. DISCUSSION

Our results show that ionizing radiation promotes several changes on the venom, decreasing its toxicity as well as modifying its immunological properties. Toxicity was attenuated in a dose-dependent manner, with a decrease of more than 3.2 folds for the sample irradiated with 2000 Gy. As reported by other authors [2, 3], exposure to radiation led to formation of high molecular weight aggregates (data not shown). However, these toxic and structural modifications did not affect quantitatively the antibody response, as evidenced by ELISA assay against native venom, suggesting preservation of common antibodies-inducing epitopes on both forms of the venom. Immunodiffusion assay indicates a different behaviour of native and 2000 Gy irradiated venom when assayed against the used antibodies. Antiserum against native venom recognized only native venom , while antiserum against 2000Gy irradiated venom was able to recognize both forms of the antigens. Two hypothesis can be considered to justify these reactivity patterns: 1- The 2000 Gy irradiated venom could contain concomitantly native and radiation-neoformed epitopes inducing Igs against both forms of the venoms. 2- Radiation could lead to conformational modifications of the venom components, preserving mainly primary structure, resulting in linear epitopes. Since primary structure is shared by native and irradiated venom components, antirradiated venom antibodies could recognize both forms of the venom. Sandwich ELISA assay showed a lack of immunoreactivity for the samples irradiated with 1000 and 2000 Gy, while native and 500 Gy irradiated samples were recognized, suggesting destruction of the anti-native venom antibodies binding sites. Taken together, these results suggest that irradiated venom induces antibodies able to recognize native venom but that these antibodies are directed to epitopes that do not induce antibodies in unirradiated venoms.

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