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STUDY OF IRRADIATED TOXINS WITH ^{60}Co GAMMA RAYS: IMMUNE SYSTEM BEHAVIOR

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ABSTRACT

Ionizing radiation has been successfully employed to modify the immunological properties of biomolecules. Very promising results were obtained when crude animal venoms, as well as isolated toxins, were treated with ^{60}Co gamma rays, yielding toxoids with good immunogenicity. The achievement of modified antigens with lower toxicity and preserved or improved immunogenicity can be useful. Ionizing radiation has been proven to be a powerful tool to attenuate snake venoms toxicity without affecting and even increasing their immunogenic properties. However, little is known about the modifications that irradiated molecules undergo and even less about the immunological response that such antigens elicit. In the present work, we have investigated the immunological behavior of bothropstoxin-I, a K49 phospholipase, before and after irradiation. Structural modifications of the toxin were investigated by SDS-PAGE. Isogenic mice were immunized with either the native or the irradiated toxin. The circulating antibodies were isotyped and titrated by ELISA. According to our data, irradiation promoted structural modifications in the toxin, characterized by higher molecular weight forms of the protein (aggregates and oligomers). Our data indicate that irradiated toxins were immunogenic and the antibodies elicited by them were able to recognize the native toxin in ELISA. These results indicate that irradiation of toxic proteins can promote significant modifications in their structures, but still retain many of the original antigenic and immunological properties of native proteins. Also, our data indicate that the irradiated protein induced higher titers of IgG2a and IgG2b, suggesting that Th1 cells were predominantly involved in the immune response.

1. INTRODUCTION

Annually 20,000 cases of snakebites are reported in Brazil [1] and the sorotheraphy is the only effective method applicable, since administered in time, with adequate dose and route [2].

Some snake's species present venoms with low immunogenicity and high toxicity, being required the development of techniques that increase the immune response and reduce the toxicity of the venoms. Ionizing radiation consists of electromagnetic waves resulting from nuclear transitions. It can interact with biomolecules in two ways: directly, when the radiation hits the molecule, or indirectly when free radicals are generated and these react with the target molecule. Radiation promotes changes in enzymatic, pharmacological and immunological properties of proteins; the two latter being more radioresistent [3,4,5].

Radiation has been successfully employed to modify biomolecules, reducing or abolishing their biological activity without affecting their immunogenic properties [6] at this, this methodology could be used to produce toxoids and vaccines.

The immune system is constituted by cells and molecules highly specialized on the combat of infectious agents, where there are two kinds of immune response: innate and adaptative. [7,8]. In the present work, we evaluated the effects on immune system of irradiated bothropstoxin-1 (Bthx-1), a K49 phospholipase, as a model to further characterize the immune response against irradiated proteins.

2. MATERIALS AND METHODS

2.1. Animals

B10.PL isogenic mice were obtained from the animal housing facility of IPEN/CNEN/SP and maintained in sterilized isolators and absorbent media, with food and water *ad libitum*. The manipulation of these animals before or during the experiments was according to the "Principles of Laboratory Animal Care" (NIH publ. N^{o} 86-23, revised in 1985) and to the "Principles of Ethics in Animal Experimentation" (COBEA – Colégio Brasileiro de Experimentação Animal).

2.2. Proteins irradiation

Bothropstoxin-1 was dissolved in 0,15 M NaCl to a final concentration of 2 mg/mL. This solution was irradiated with a 2000 Gy dose using gamma rays derived from a ^{60}Co source (Gamma Cell, Atomic Agency of Canada Ltd) at room temperature and in the presence of atmospheric O_2, with a 5170 Gy/h dose rate.

2.3. SDS-PAGE

Purified bothropstoxin-1, native or irradiated, were submitted to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing and not-reducing conditions.

2.4. Antibodies production

Specific anti-native or anti-irradiated bothropstoxin-1 antibodies were obtained by immunizing B10.PL mice, with the proteins in its native or irradiated forms, following a classical immunization protocol [9]. Blood samples were collected and after centrifugation,

the plasma was separated and frozen until moment of use. Presence of specific antibodies in plasma samples was investigated by ELISA.

3. RESULTS AND DISCUSSION

According to our data, SDS – PAGE profiles of the proteins shows that γ-irradiation causes breakdown of polypeptide chains resulting in formation of aggregated higher molecular weight molecules (Figure 1). This SDS-PAGE allowed to identify structural modifications after the irradiation process (^{60}Co). The BTHX-1 irradiated did not present dissociation of the subunities, even in the presence of the reducer agent. As observed by another group [10] the proteins can be converted in high molecular aggregates due to the generation of cross-linking reactions inter-proteins, hydrophobic and electrostatic interactions and formation of disulfide bridges.

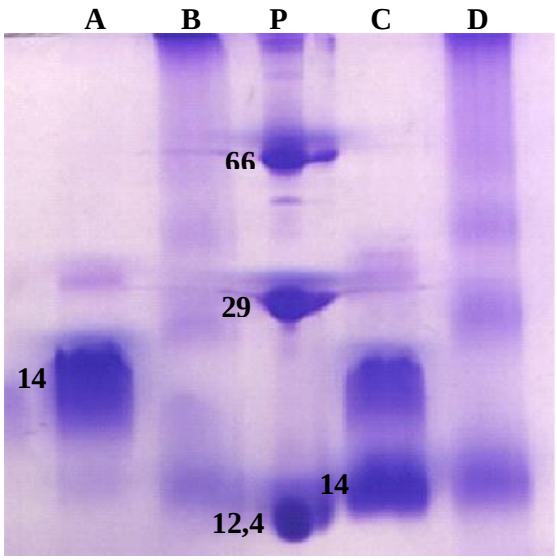

Figure 1. SDS-PAGE profile of bothropstoxin-1. (A) Bothropstoxin-1 native and not-reduced; (B) Bothropstoxin-1 irradiated and not-reduced; (C) Bothropstoxin-1 native and reduced; (D) Bothropstoxin-1 irradiated and reduced. (P) Molecular weight marker.

Sera of animals immunized with the native and irradiated proteins were analyzed in order to evaluate levels of IgG (Figures 2 e 3), as well as to quantify specific isotypes (Figure 3). While the native proteins induced a predominance of polarization to Th2 response, the irradiated molecules apparently promoted a switch towards a Th1 pattern.

The antibodies produced by immunization against irradiated toxin were capable to recognize the native form of the toxin. The same phenomena was observed by BAPTISTA [11]. The native BTHX-1 presented higher titers to IgG 1, which indicates an immune response of type Th2 predominance (humoral immunity). BREWER *et al.* [12] observed this relation in macrophage depletion assay. However, the irradiated BTHX-1 produces higher titers for IgG2a and IgG2b, it indicates a predominance of Th1 cells response which is involved with cellular immunity [7].

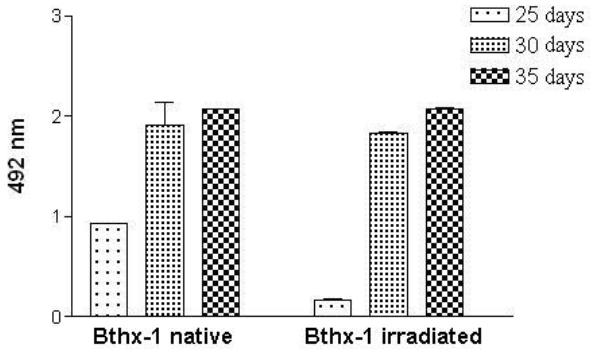

Figure 2. Enzyme Linked Immunosorbent Assay of the antibodies raised against native and irradiated bothropstoxin-1 samples. Plasma dilution: 1/800.

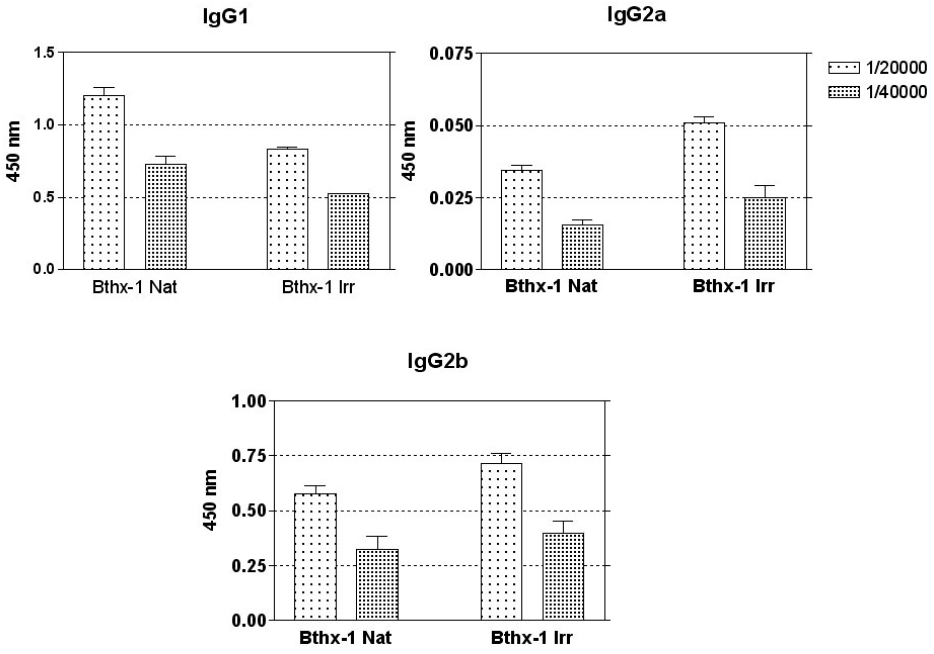

Figure 3 - Enzyme Linked Immunosorbent Assay isotyping (IgG1, IgG2a and IgG2b) of the antibodies raised against native and irradiated bothropstoxin-1 (BTHX-1) samples.

4. CONCLUSIONS

These results indicate that irradiation of toxic proteins can promote significant modifications in their structures, but still retain many of the original antigenic and immunological properties of native proteins. Our results indicate a potential use of detoxified proteins as antigens for immunization.

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