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DETOXIFICATION OF SNAKE VENOM USING IONIZING RADIATION

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It is generally recognized that energy absorbed from ionizing radiation (gamma rays) can inactivate biological material in two ways. A direct effect occurs when the primary event, i. e., ionization, is produced in the molecule itself. This is the case when a compound is irradiated in dry state. When a compound is irradiated in a solution, the indirect effect joins the direct(9).

Since water is the most abundant constituent of biological material, it is important to consider the species produced by excitation and ionization of water itself, and the reaction of these species with the target molecules of biological importance.

This indirect effect results from the reactions among the studied molecules and the products of radiation interaction with water or other solvents. Highly reactive compounds, the so-called free radicals, which are formed undergo many reactions among themselves, with the dissolved gas, and with other molecules in the solution. With water, the excitation is less important than ionization which is followed within picoseconds by the formation of free hydroxyl radicals and hydrated electrons(2,4,16).

Alexander & Hamilton(1) showed that irradiation of proteins has revealed damage to aminoacid side chains, production of new groups, splitting of peptide bonds and formation of intramolecular and intermolecular cross-links.

With these results, it would be possible to use of ionizing radiation to change those protein molecules in order to improve some of their properties according to the necessity.

On the other hand, it is recognized that venoms in general are poorly immunogenic, yet fairly toxic(15). This causes problems because serotherapy is the treatment of choice in snakebite envenomations, and horse antivenom availability is dependent upon immunogenicity.

☑ **KEY WORDS:** Detoxification of snake venom, ionizing radiation, gamma rays.

To improve antivenom production and extend the useful life of immunized horses effort has been devoted to decrease chronic venom toxicity(5,6,7,8,11,12,17,18, 20,22).

Thus, once snake venoms are rich in proteins, Lauhatirananda et al.(15) and Salafranca(21) tested the effects of ionizing radiation on cobra venom. They reported that irradiated venoms presented low toxicity, and that when the samples were in a dry state, the suitable irradiation dose to attenuate toxicity was higher than that used to samples in aqueous form.

In addition, Kankonkar et al.(14) showed that in *Naja naja* venom solution, the irradiation dose to detoxification is dependent upon solution concentration, while Baride et al.(3) found some changes in biochemical properties of *Naja naja*, *Bungarus cheruleus*, *Echis carinatus* and *Vipera russelli* observing aggregation of venom proteins.

Few years later, Herrera et al.(13), using gamma rays to detoxify *Lachesis muta* and *Bothrops atrox* venom, observed that enzymatic activities have different sensibility among themselves under irradiation conditions.

Based on these studies and considering that around 20,000 cases/year of envenomations involving snakes are notified in Brazil, gamma rays have been employed in our laboratory to detoxify Brazilian snake venoms in order to improve antivenom production.

Murata et al.(19) irradiated *Crotalus durissus terrificus* venom with gamma rays using different doses and found that 2,000 Gy was a good compromise in irradiation dosage for venoms solution, which promoted significant venom detoxification, yet maintained many of

the venom original immunological properties, as tested in mice, rabbits and horses.

Following these studies Guarnieri (10), used gamma rays to detoxify *Bothrops jararaca* venom. As found to *Crotalus durissus terrificus* venom, 2,000 Gy was the necessary dose to get the detoxification with maintenance of immunological properties observed through results of immunodiffusion, immunoblotting, immunoprecipitation, immunization of mice and rabbits and neutralization tests.

In addition, it was observed through proteolytic, hemorrhagic, coagulant and edema-forming activities, gel filtration and electrophoresis some conformational and structural alterations, protein aggregate formation and attenuation of tested activities. Furthermore, the animals immunized with irradiated venom presented no cutaneous lesion which is very common when *Bothrops jararaca* venom is injected.

More recently, Nascimento (manuscript submitted to Toxicon) has worked with crotoxin, main toxin of *Crotalus durissus terrificus* venom, in order to study the effects of gamma rays on purified toxin. Irradiation of crotoxin resulted in an aggregation and a generation of lower molecular weight breakdown products.

The high molecular weight aggregates were isolated by gel filtration and its immunological, biological and biochemical properties were analyzed.

The aggregates presented no toxicity, no phospholipase activity and no ability to promote creatine kinase (CK) release into muscle tissue. On the other hand, these aggregates were highly antigenic and were able to induce antibody formation in mice which cross-reacted with non irradiated crotoxin. In addition, mice immunized with aggregated immunogen survived a challenge of 15 LD_{50} of non irradiated crotoxin.

When biodistribution experiments were developed in mice, using labeled crotoxin with 125I, it could be observed that irradiated crotoxin was poorly retained in tested organs (liver, muscle, spleen, kidneys, lungs, heart and brain), mainly in kidneys where no retention was observed.

Nascimento suggests that this results indicate ionizing radiation as a good tool in the detoxification process, highlighting out the aggregates as the ideal immunogen to be used during the immunization process to get snake antivenom without damage to the serum-productor animals.

Considering these promising results, other snake venoms have been studied in our laboratory such as *Bothrops jararacussu, Lachesis muta* venom and some experiments also were made with bee venom with good results of attenuation of toxicity when submitted to the effects of gamma rays.

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