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COMPARATIVE NEPHROTOXICITY OF NATIVE OR Co-60 GAMMA RAYS IRRADIATED CROTOXIN IN MICE

André Moreira Rocha[1], Josefa M Nascimento-Rocha[2], Astério Souza Magalhães Filho[2], Glaucie J Alves[1], Raquel da Silva Aires[1], Thompson O Turíbio[1], Gabriela O Coelho Thomazi [1], Patrick J Spencer[1], Nanci do Nascimento[1]

[1] Instituto de Pesquisas Energéticas e Nucleares (IPEN / CNEN - SP)
Av. Professor Lineu Prestes 2242
05508-000 São Paulo, SP
andrerocha@usp.br

[2] Instituto Tocantinense Presidente Antônio Carlos Porto (ITPAC/Porto)
Rua 02 Quadra 07 S/N Jardim dos Ipês
77500-000 Porto Nacional, TO
0304@prof.itpacporto.com.br

ABSTRACT

Snake venoms are complex mixtures of proteins and peptides with a wide spectrum of physiological targets such as the blood coagulation and cardiovascular systems and the motor end plate among others. Acute renal failure is a common complication in accidents with the South American rattlesnake. The toxin involved in this pathology is the crotoxin, a major component of the venom in terms of concentration and toxicity. Snake venoms, when irradiated with ^{60}Co gamma rays present a significant decrease in toxicity while the immunogenic properties of its components are preserved. The use of irradiated venom is an attractive alternative for antisera production since it might reduce the appearance of renal lesions improving the welfare and lifespan of those animals employed on antivenom production. At the present work, we have compared the effects of native and irradiated crotoxin on the mice renal function. Tubular lesions were observed in all the samples from the animal group injected with native crotoxin. Animals injected with the irradiated toxin presented alteration only after 30 minutes and 1 hour after injection. These data suggest that the onset of the renal lesions is delayed and that the severity of the lesions might be lower when using irradiated crotoxin.

1. INTRODUCTION

Snake bites accidents affect at least five million people around the world, with 2.7 million of serious injuries and 125 thousands deaths [1]. In Brazil, there are 365 species of snakes, aggregated in ten families, with 75 genera. Sixteen percent (59) of those species are capable to inject venoms, being considered of special medical attention due to the need of serotherapy.

In Brazil, these accidents are mainly caused by *Bothrops* snakes, being ninety percent of the cases, with low lethality (0.31%). Accidents involving *Crotalus* snakes occurs in 7.7% of the cases but with greater mortality (~2%). Other snakes, as *Lachesis* (1.4%) or coral snakes (0.4%) are also cause of those accidents, with low mortality [2]. The causes of those accidents are related to multiple factors, as climate, raining or dry seasons, environment invasion during agricultural expansion or wood extraction activities, occurring mostly in adult males, at work age (15 to 49 y old), and the bite occurring mostly in unprotected lower limbs [3].

Snake venoms are complex protein mixtures with extensive enzymatic and biologic activities. Those proteins comprise at least 95% of the content of dry venoms, comprising enzymes, non-enzymatic toxins and nontoxic proteins. They cause several physiological, hematological and neurological effects in the human host [4].

The venom of *Crotalus durissus terrificus* (*C. d. terrificus*), Brazilian rattlesnake, is mainly composed by crotoxin, crotamine, convulxin and gyroxin. Crotoxin represents the main component, 40 to 60% of dry weight, and it is composed by two subunits, crotapotin and A_2 phospholipase, being responsible for the most of toxic neuropathological and physiological effects [5].

Beside to neuro-blocking effects, the most common physiopathological consequence of rattlesnake bite is the acute renal injury. Kidney concentration of the venom occurs, with almost double concentration, as compared to concurrent serum levels, due to the renal excretion of this toxin [6]. The main toxic activity is dependent of A_2 phospholipase, that promotes acute inflammatory changes in the renal tubules, with the participation of acute phase proteins, platelet activating factor, endothelin, eicosanoids and other authacoids, as kynins and catecholamines [7]. The main therapy to those accidents is the use of specific antiserum, containing antibodies that could block the toxins activity. Those antisera are produced in horses and must be available quick for therapy, due to neurological effects of the toxin [8].

The production of those antisera is attained by immunizing animals with the toxins and this effect is dependent of both venom immunogenicity and toxicity. The immunogen must induce a good humoral immune response in the host, with the less significant toxicity [9].

For reducing the toxicity of rattlesnake venoms, several chemical procedures were attempted as chelating agents, formalin, glutaraldehyde, iodine, tanin, carboxymethil cellulose, aside to physical treatments as heat, X rays or UV light. Most of those chemical modification methods give poor results, probably due to chemical radical introduction in the toxin.

Gamma irradiated detoxified venom induces a good immune response without adding new chemical radicals to the toxin [9, 10].

Despite those good results, the use of irradiated venom in substitution to the native one during the immunization process could cause some renal effects in the animals employed for antiserum production, resulting in low mean life and low efficiency. The goal of the present work is to study the kidney histology in mice inoculated with native or ^{60}Co irradiated *C. d. terrificus* venom, looking for histological renal involvement during this process.

2. METHODS

Crotoxin, the main toxin of *C. d. terrificus* venom, was purified by chromatography and dissolved in sterile acidified saline at 2 mg/mL. Aliquotes were irradiated with 2 kGy of ^{60}Co in a Gammacell 220 (Atomic Energy Canada Ltd) with dose rate of 1,031 kGy/h, at room temperature and in the presence of oxygen, with control crotoxin aliquots maintained in the same conditions without irradiation.

Groups of five female Balb/c mice obtained from the IPEN colony received 10 ug (one LD50% dose) of native or irradiated crotoxin by i.p. route. Each group was killed at regular intervals after toxin injection, from 0.5 to 48 hrs. Mice were killed by anesthesia with ketamin and xylazine. Kidneys were obtained by dissection and fixed in at least 20 volume of formaldehyde 4% in phosphate buffered saline pH 7.2, with two fixative changes at 4^{o} C by 24 hrs. All animal procedures were previously approved by Ethics Committee for Use of Animals models and (Protocol n. º 129/13A CEUA-IPEN/SP)

Routine histological paraffin embedding and microtomy was performed at ITPAC Porto Nacional Research Lab. Slices containing 5 μm sections were stained by Haematoylin Eosin stains and mounted for microscopy and digital imaging. We used a light microscope (BIOVAL, Mod. L1000) equipped with ocular digital camera OPTCAM®. Representative fields at 40X objective were digitalized in each experimental time and toxin injected. Two independent observers trained in kidney histology analyzed each section in a blind format analyzing the presence and semi quantitative intensity of renal alterations, defined as tubular damage, hemorrhages and vascular alterations or glomerular damage. Data were analyzed using statistical approaches using Microsoft Excel 2007®.

3. RESULTS AND DISCUSSION

We performed the toxicity of irradiated crotoxin in groups of five mice, as described in Methods, using non irradiated (native) crotoxin as standard crotoxin and PBS injected mice as control. Histology was performed as described and the representative field was digitalized after analysis by two independent observers. Control mice presented normal renal cortex, with easily identified proximal and distal tubes, and glomeruli with normal structure (Figure 1).

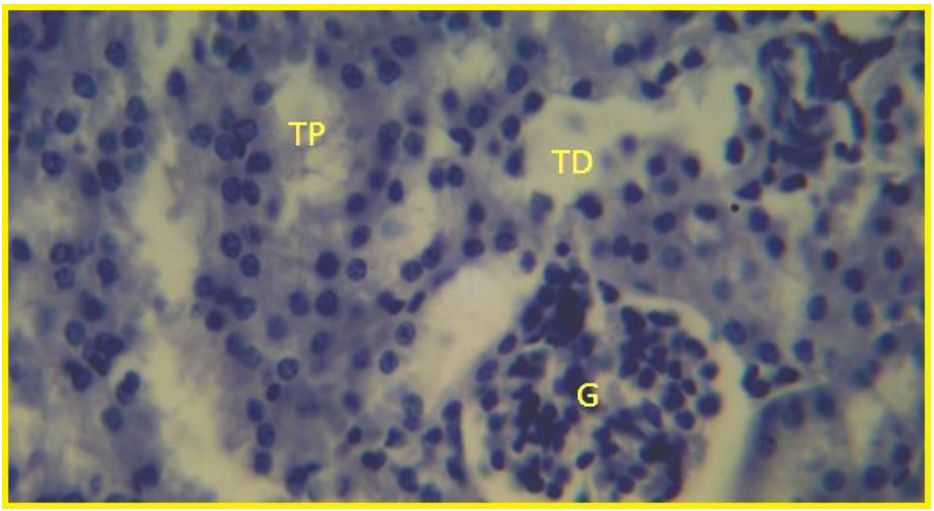

Figure 1: Renal cortex of control mice, showing Proximal tubule (TP), Distal tubule (TD) and Glomerulus (G) with normal cellularity and appearance, without distention and without vascular dilatation or hemorrhages (HE – 40X).

Kidneys from animals injected with native Crotoxin presented alterations at early times as shown in Figure 2.

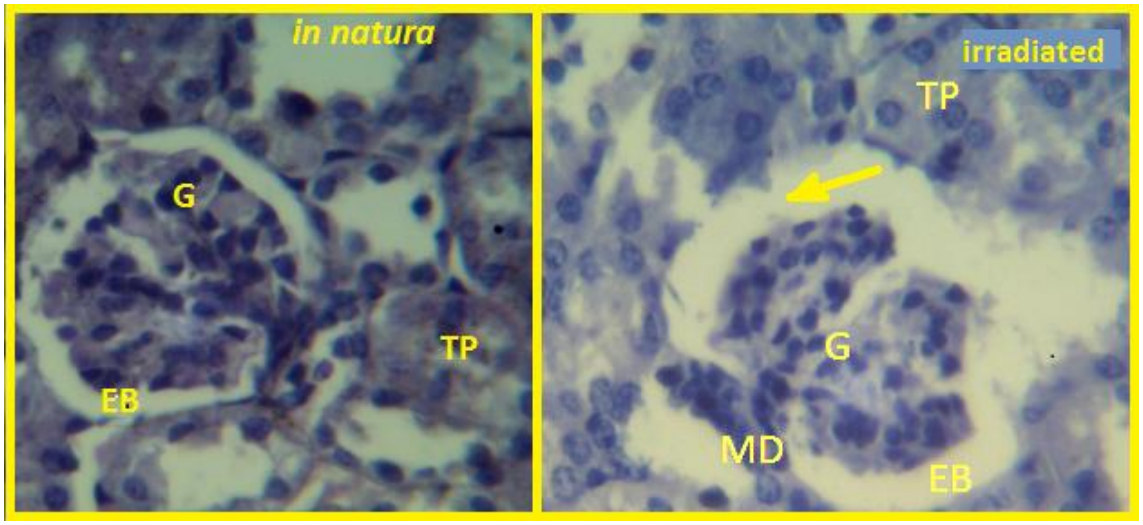

Figure 2 – Kidneys from mice injected with native (*in natura*) or irradiated crotoxin, after 0.5 h post injection. (HE – 40X). TP – Proximal tubule; G – Glomerulus; MD – Dense Macule; EB – Bowman space. Note the degenerative changes in proximal tubule of native toxin injected animals. Bowman space dilatation (arrow) in kidney from mice injected with irradiated crotoxin.

Renal tubules from mice injected with native crotoxin showed degenerate tubular cells. Kidneys from mice injected with irradiated toxin presented only dilated bowman spaces and no tubular alterations. Subsequent figures show the progressive histological changes after 1 hour post injection (Figure 3).

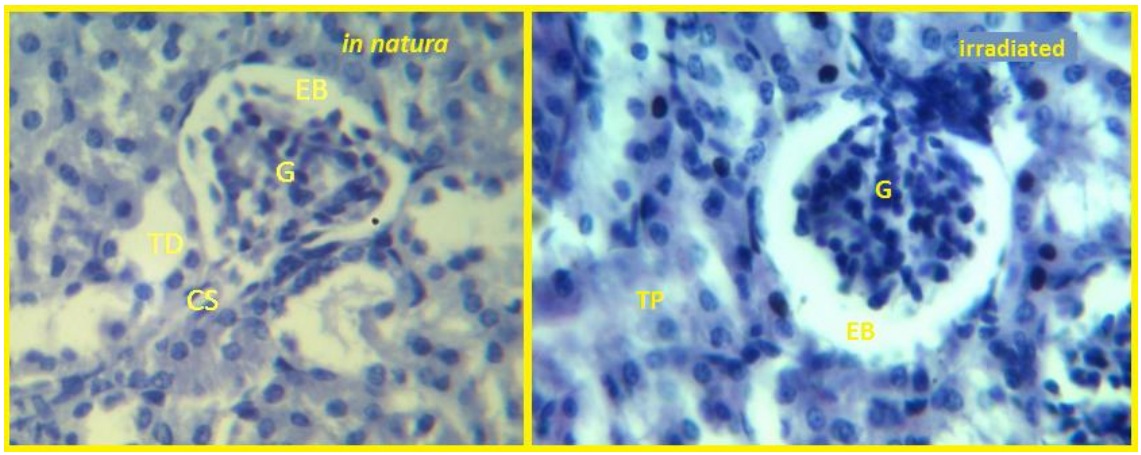

Figure 3: Evolutive changes in renal tubule after 1h of injection. Note the normal glomeruli in both toxins but more evident tubular changes in native toxin (HE – 40X).

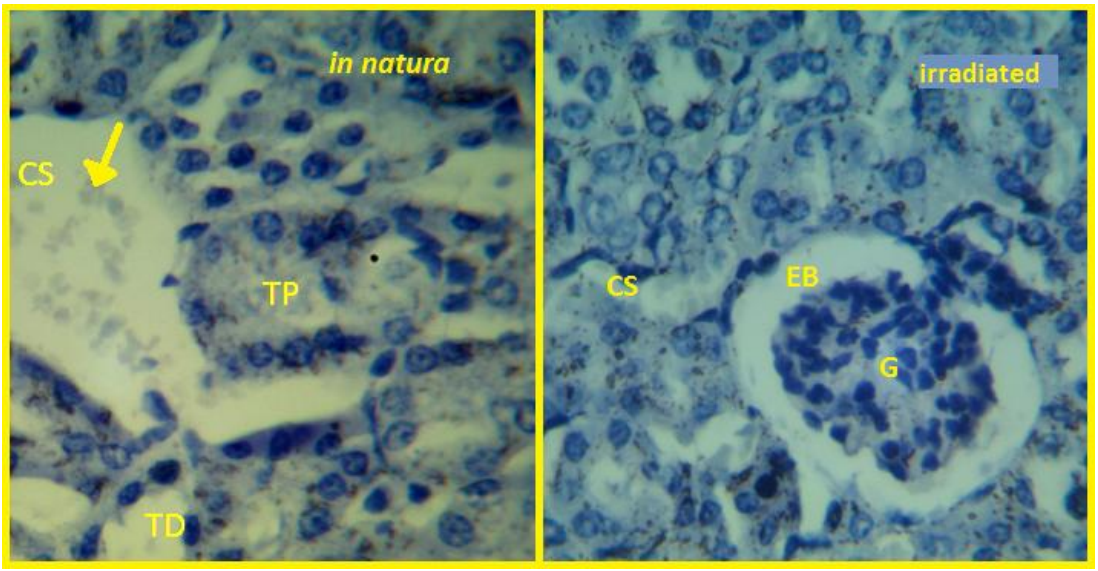

Figure 4: Alterations after 24 h of the injection. Note the presence of hemorrhage foci in native toxin injected mice (arrow).

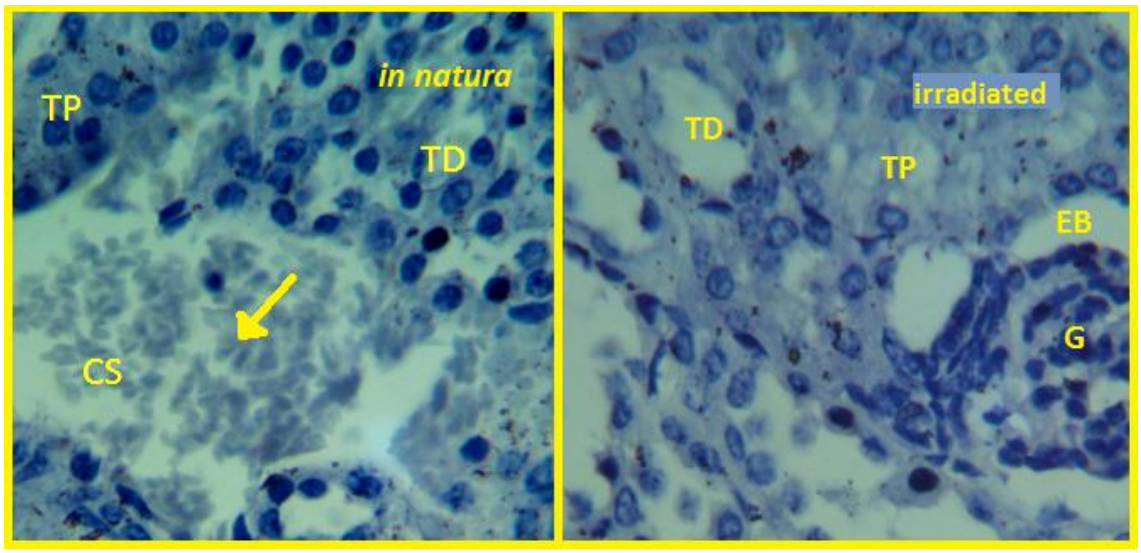

Figure 5: More evident hemorrhage in other kidney sample from mice injected with native crotoxin. Observation after 24 h post injection (arrow) (HE – 40X).

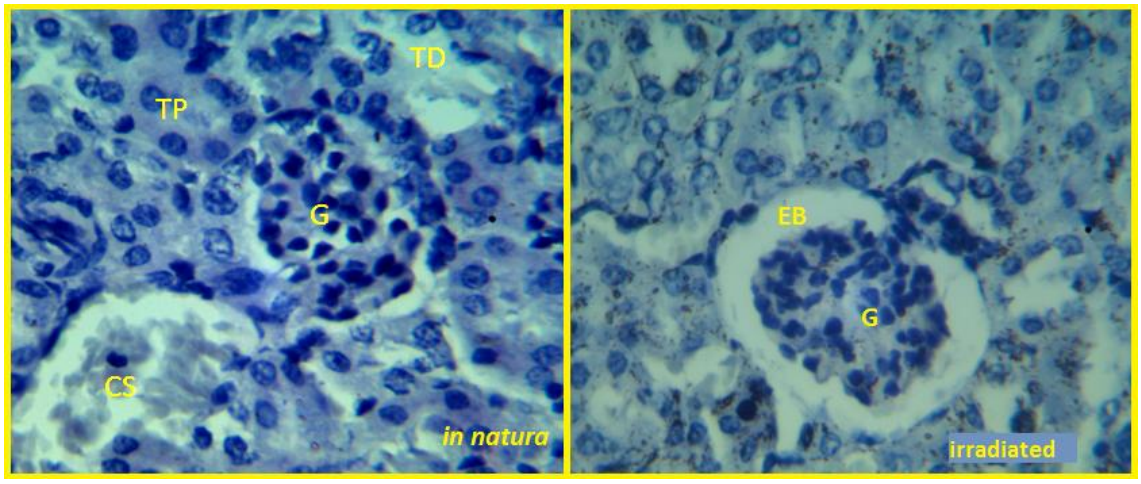

Figure 6: Progressive changes after 48 h. of the injection of toxins where most of the lesions are controlled. (HE – 40X).

As could be seen in those sequential figures (Figure 4, 5 and 6), there are evolving changes in the kidneys related to tubular injury and hemorrhagic phenomena. Kidney lesions, analyzing the frequency of events in 40 fields of renal cortex and those data could be seen in Figure 7. Most of the alterations were more frequent in animals injected with native crotoxin when compared with the lower frequencies in those animals that were injected with irradiated toxin.

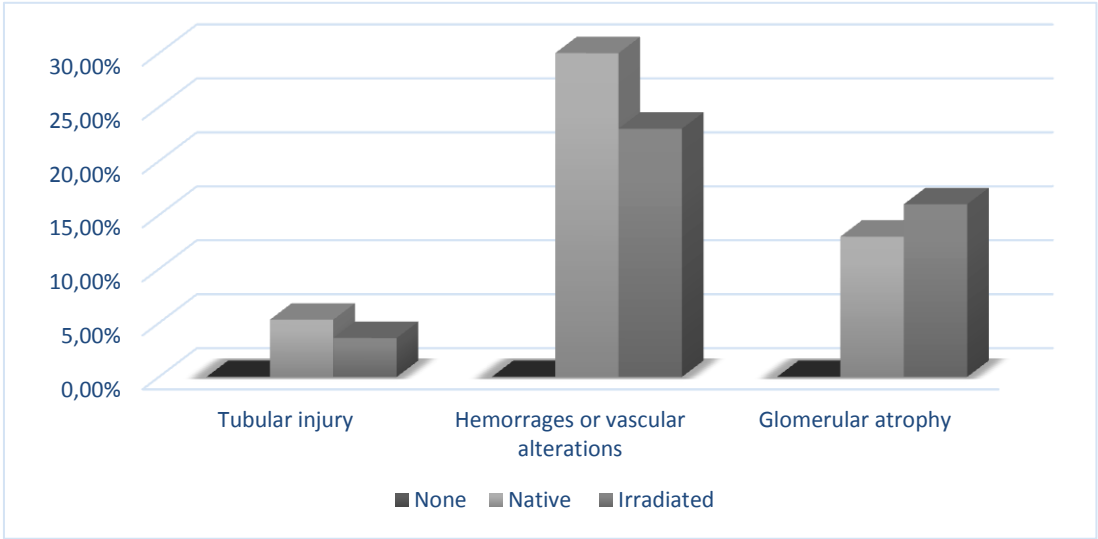

Figure 7: Morphological analyses (%) of histological slices in mice injected with native or irradiated crotoxin.

In the present study it was conducted comparative histologic study of kidneys from mice inoculated with native or ^{60}Co irradiated crotoxin.

The nephrotoxicity of the venom can be indirect, because it causes rhabdomyolysis leading to myoglobinuria, causing tubular obstruction by myoglobin [11].

Kidney damage can vary; however, involve all structures, usually presenting tubular necrosis caused by ischemia and / or direct nephrotoxic action and glomerulonephritis [12].

Histologically, acute tubular necrosis is the predominant lesion, presenting dilated tubules, cellular necrosis, interstitial edema and inflammatory cell infiltration [12].

Kidney morphological changes occurred more frequently in groups of animals inoculated with non-irradiated crotoxin which presented vascular changes (29.92%) and tubular changes (5.28%). These changes were less present in those animals that had received irradiated crotoxin (vascular change: 22.88%; tubular changes 3.52%).

The average of histopathological changes remained higher in animals receiving non-irradiated crotoxin (15.8%) than in animals inoculated with irradiated crotoxin (14.1%).

Whereas antiophidic sera production process that are used after ophidic accidents, equine animals are inoculated with venom snakes, and it is significant to use ^{60}Co irradiated snake venoms, as this study looked at presence of less histopatological changes in renal cortex animals inoculated with irradiated poison, and this would entail in a better quality of animals submitted to an antivenom production process.

4. CONCLUSIONS

This study allowed viewing, by optical microcopy, the histopathologic differences caused by native and ^{60}Co irradiated snake venoms. It was found through the interpretation of histological and quantitative analysis, in percentage, that was less frequent histopathological changes in kidneys of animals inoculated with irradiated venom.

The results also suggest that ionizing radiation should be used to detoxify snake venoms to antisera production, since the venom becomes less toxic improving welfare in these animals.

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