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Lipoic acid effects on renal function, aminopeptidase activities and oxidative stress in *Crotalus durissus terrificus* envenomation in mice

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ABSTRACT

Crotalus durissus terrificus envenomation has been associated with direct nephrotoxicity, rhabdomyolysis, hyperuricemia, urinary hypoosmolality, alterations in aminopeptidase activities (AP) and oxidative stress. This study evaluated the effects of lipoic acid (LA) on renal function, lethality, AP and GSSG/GSH in mice injected with *C. d. terrificus* venom (*vCdt*). The doses and routes of administration of LA and *vCdt* promoted no systemic myotoxicity. LA did not alter significantly the lethality of *vCdt*. In nonenvenomed, LA induced hypercreatinemia, urinary hyperosmolality, decrease of urinary urea and creatinine, increase of protein in plasma and in soluble fraction (SF) and decrease in membrane-bound fraction (MF) of cortex and medulla. Decreased levels of all AP (except neutral-AP in MF-medulla) were also induced by LA in nonenvenomed. LA associated with *vCdt* decreased plasma osmolality, hematocrit, urinary uric acid, but increased urinary and SF-medullar protein. LA mitigated the increase of protein in SF-cortex and corrected hyperuricemia, GSSG/GSH and protein in MF-cortex and MF-medulla, as well as decreased plasma neutral AP and acid AP in MF-medulla of envenomed mice. Data suggest that LA contributes to the solubilization/remotion of proteins in MF with impairment of most AP, but it could be beneficial for the treatment of the direct nephrotoxicity of *vCdt*.

1. Introduction

Among the main complications of *Crotalus durissus terrificus* bites is acute renal failure (ARF) (Amaral et al., 1986; Rezende et al., 1989; Ribeiro et al., 1998; Brasil, 2001; Monteiro et al., 2001; Azevedo-Marques et al., 2003; Castro et al., 2004; Pinho et al., 2005), with prevalence of 0.5–14% (França and Málaque, 2003). The pathogenesis of ARF induced by *C. d. terrificus* venom (*vCdt*) is multifactorial. It has been associated to direct nephrotoxicity (in isolated and perfused kidneys, thus in the absence of systemic factors) (Monteiro et al., 2001), as well as to rhabdomyolysis, shock and hemolysis (Pinho et al., 2000). Glomeruli of animals treated with crude venom or with crotoxin present high content of protein, indicating direct nephrotoxicity effect or increase of vascular permeability. Renal tissue can release mediators, including prostaglandins and histamine, which are related to the increase of capillary permeability, hypotension, metabolic acidosis and shock (Pinho et al., 2000; Monteiro et al., 2001). Moreover, acute tubular necrosis caused by crude venom or crotoxin (Pinho et al., 2000; Monteiro et al., 2001) can be detected in

Abbreviations: AP, aminopeptidase; APA, acid aminopeptidase; APB, basic aminopeptidase; APN, neutral aminopeptidase; ARF, acute renal failure; CAP, cystyl aminopeptidase; CK, creatine kinase; DPPIV, dipeptidyl peptidase IV; GSH, reduced glutathione; GSSG, oxidized glutathione; LA, lipoic acid; LDH, lactate dehydrogenase; MF, membrane-bound fraction; PAP, pyroglutamyl aminopeptidase; PIP, prolyl iminopeptidase; SF, soluble fraction; *vCdt*, venom of *Crotalus durissus terrificus*.

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renal biopsy or necropsy (Amaral et al., 1986; Azevedo-Marques et al., 2003). The onset of necrosis may occur by systemic toxic conditions and dehydratation, and mainly by the direct nephrotoxicity of venom (Amaral et al., 1986; Azevedo-Marques et al., 2003), as well as rhabdomyolysis, which is caused by the myotoxicity of *vCdt* (Monteiro et al., 2001). Recently, Yamasaki et al. (2008) suggested that hyperuricemia (which seems to occur before glomerular injury, since hypercreatinemia did not occur in all envenomed mice) and urinary hypoosmolality (together with hypercreatinemia and slight decrease of plasma urea and increased hematocrit) are important characteristics of ARF induced by *C. d. terrificus* in mice. Moreover, in the kidney this venom caused medullar and cortical oxidative stress and generalized decrease of several aminopeptidase (AP) activities in soluble and membrane-bound fractions in cortex, increase of basic AP (APB) and decrease of prolyl iminopeptidase (PIP) in medullar soluble fraction and decrease of acid AP (APA) and dipeptidyl peptidase IV (DPPIV) in medullar membrane-bound fraction (Yamasaki et al., 2008). Ala-(Leu-Gly)-AP, detected in urine or serum shortly after tubular injury, has also been suggested to contribute to prediction of ARF and need for renal replacement therapy (Trof et al., 2006). For example, the early detection of Ala-AP in septic acute kidney injury is preceded by overt kidney failure (Bagshaw et al., 2007). Alterations of some APs have been also related to renal dysfunction caused by cyclosporin A (Marinho et al., 2006).

Lipoic acid (LA) has been proposed as therapeutical agent for treatment or prevention of diabetes, polyneuropathy, cataract, neurodegeneration and nephropathies (Takaoka et al., 2002; Amudha et al., 2007a,b) and has been studied mainly by its antioxidant properties (Winiarska et al., 2008). The efficacy of LA has been attributed to the unique properties of lipoate/dihydrolipoate system to scavenger reactive oxygen species and to stimulate the synthesis of other antioxidants, as glutathione (Bilska et al., 2007).

To understand the renal effects of LA, and mechanisms and consequences of nephrotoxicity effects of *vCdt*, as well as to evaluate the possible application of LA as coadjuvant in the serotherapy of this envenomation, the present work measured the effects of LA in classical parameters of renal function, lethality, AP activities and oxidative stress in mice with or without acute dysfunction caused by *vCdt*.

2. Material and methods

2.1. Preparation of venom solution

Venom solution was prepared using 1.0 mg of lyophilized venom (provided by the Instituto Butantan) in 6.25 mL of sterile 0.9% NaCl, under mild mixing, for 10 min, at 4 °C and, then, centrifuged at 20,927×*g* for 10 min at 4 °C. The pellet was discarded and the supernatant was aliquoted and stored at −20 °C until the use, when it was diluted to the adequate concentration for administration, in sterile phosphate buffered saline (PBS) ($Na_2HPO_4 \cdot 7H_2O$, 19,3 g/L; $NaH_2PO_4 \cdot H_2O$, 3.9 g/L; NaCl, 8.77 g/L; pH 7.4).

The same lot of venom was used throughout this study.

2.2. Preparation of lipoic acid

Lipoic acid (DL-alpha-lipoic acid) (Sigma, USA) was dissolved in absolute ethanol (50 mg/mL) in the moment of use. Immediately, this solution was diluted (1:5) in PBS (LA solution). The adopted dose was 2 mg/20 g body mass, in a maximum volume of 0.2 mL of LA solution, *per oral* (po). In order to evaluate its effects on ARF, this dose was administered 2 h after the envenomation. LA at this dose and route was effective against nephrotoxicity induced by chloroquine, when compared with other doses (0.2 and 0.6 mg/20 g body mass) (Murugavel and Pari, 2004). Intraperitoneal injection of LA at this same dose attenuated the ischaemia/reperfusion-induced increases in blood urea nitrogen, plasma concentrations of creatinine and fractional excretion of sodium (Takaoka et al., 2002).

2.3. Animals, treatments and urine collection

Adult male Swiss mice, weighing 18–20 g, provided by the Animal Facility of the Instituto Butantan, were maintained in polyethylene cages (inside length × width × height = 56 × 35 × 19 cm) with food and water *ad libitum*, in a container with controlled temperature of 25 °C, relative humidity of 65.3 ± 0.9% and 12 h:12 h photoperiod light: dark (lights on at 6:00 am).

Animals and research protocols used in this study are in agreement with the COBEA (Brazilian College of Animal Experimentation) and were approved by the Ethics Committee of the Instituto Butantan (492/08).

Venom solution was injected intraperitoneally (ip) at a dose of 80%LD50, as determined by Yamasaki et al. (2008), in a maximum volume of 0.2 mL.

Animals were divided into four groups, which received: (1) 0.2 mL of PBS ip and, after 2 h, 0.2 mL of PBS po (control ip + po); (2) LA, adopted dose and via as aforementioned (see Preparation) (LA); (3) 80%LD50 *vCdt*, in a maximum volume of 0.2 mL ip and, after 2 h, subdivided and treated as follows: (3A) animals (not treated) (*vCdt*); (3B) animals treated with LA, adopted dose and via as aforementioned (see Preparation) (*vCdt* + LA).

Immediately after treatments, each group was placed in appropriate metabolic cages for urine collection, which was performed 24 h after venom injection. Pooled urine was centrifuged at 2564×*g*, for 5 min, at 4 °C; the supernatant was stored at −80 °C, for the appropriate procedures, and the pellet was discarded.

Immediately after urine collection, animals were anesthetized for blood and kidneys collection.

2.4. Lethality

It was evaluated 24 h after administration of venom or administration of vehicle or AL alone.

2.5. Obtaining kidneys and plasma, and measurement of hematocrit

The animals were anesthetized with xylazin (Calmiun, Agener União, Brazil) 0.1% and ketamine (Cetamin, Syntec, Brazil) 1% (ip, 0.1 mL/10 g body mass). Then, the blood was