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Effect of suramin on myotoxicity of some crotalid snake venoms

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Abstract

We investigated the protective effect of suramin, an enzyme inhibitor and an uncoupler of G protein from receptors, on the myotoxic activity in mice of different crotalid snake venoms (*A.c. laticinctus*, *C.v. viridis*, *C.d. terrificus*, *B. jararacussu*, *B. moojeni*, *B. alternatus*, *B. jararaca*, *L. muta*). Myotoxicity was evaluated *in vivo* by injecting *im* the venoms (0.5 or 1.0 mg/kg) dissolved in physiological saline solution (0.1 ml) and measuring plasma creatine kinase (CK) activity. Two experimental approaches were used in mice (N = 5 for each group). In protocol A, 1 mg of each venom was incubated with 1.0 mg suramin (15 min, 37ºC, *in vitro*), and then injected *im* into the mice at a dose of 1.0 mg/kg (*in vivo*). In protocol B, venoms, 1.0 mg/kg, were injected *im* 15 min prior to suramin (1.0 mg/kg, *iv*). Before and 2 h after the *im* injection blood was collected by orbital puncture. Plasma was separated and stored at 4ºC for determination of CK activity using a diagnostic kit from Sigma. Preincubation of some venoms (*C.v. viridis*, *A.c. laticinctus*, *C.d. terrificus* and *B. jararacussu*) with suramin reduced (37-76%) the increase in plasma CK, except for *B. alternatus*, *B. jararaca* or *L. muta* venoms. Injection of suramin after the venom partially protected (34-51%) against the myotoxicity of *B. jararacussu*, *A.c. laticinctus* and *C.d. terrificus* venom, and did not protect against *C.v. viridis*, *L. muta*, *B. moojeni*, *B. alternatus* or *B. jararaca* venoms. These results show that suramin has an antimyotoxic effect against some, but not all the North and South American crotalid snake venoms studied here.

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- Myotoxicity
- Creatine kinase
- Suramin

Myonecrosis can be a significant consequence of poisoning by snake venom components named myotoxins, and many antivenins are ineffective in antagonizing the activity of these myotoxins (1-4). We have previously shown that heparin and other polyanions inhibit the increase of creatine kinase (CK) release induced by crotalid venoms in mice (5-8). We ascribed this antagonism to the formation of acid-base complexes between the basic myotoxins of the venoms and the polyanion compounds. There are reports suggesting that some heparinoids exhibit various biological functions (9,10) and among them, suramin, one of the oldest heparinoids, represents an important target molecule (11,12). Suramin is a polysulfonated naphthylamine-amine-benzamide derivative (Figure 1) that has been widely used for more than fifty years in the treatment of onchocerciasis and trypanosomiasis (12-14). There are several reports showing that suramin has

anti-enzymatic activity by binding basic proteins and it also inhibits the coupling of various metabotropic autonomic receptors to the G proteins. Among the targets, are the G protein subunits in which suramin inhibits guanosine diphosphate release (13,14). Suramin blocks cell surface binding of various growth factors and increases tissue glycosaminoglycans (12-14). There are also studies showing that suramin can inhibit the neurotoxic effect of ß-bungarotoxin and partially inhibit some pharmacological effects of *Cerastes vipera* venom (15,16).

In the present study we investigated the antagonism or protection of the myotoxic effect of some North and South American snake venoms by this polysulfated substance. Experiments were performed with *Bothrops jararacussu* and *B. jararaca* venoms obtained from Instituto Vital-Brazil (Niterói, RJ, Brazil), *B. alternatus*, *B. moojeni*, and *Crotalus durissus terrificus* venoms from the Instituto Butantan (São Paulo, SP, Brazil), whereas *C. viridis viridis* (prairie rattlesnake) and *Agkistrodon contortrix laticinctus* (broad-banded copperhead) venoms were obtained from the Venom Research Laboratory (Oklahoma State University, Stillwater, OK, USA). *Lachesis muta* venom and suramin were purchased from Sigma (St. Louis, MO, USA). All other compounds were of analytical grade.

Venoms, whose quantity is reported as mg protein and whose protein concentration was estimated as described by Fuly et al. (17), were dissolved in physiological saline solution (PSS; 0.1 ml) and injected *im* into the thigh of Swiss mice (20-25 g). Venom was injected at a dose of 1.0 mg/kg, except for *C.d. terrificus* venom or *B. jararaca*, which was injected at the dose of 0.5 mg/kg. Previous studies showed that *im* injection of 0.1 ml PSS has no effect on plasma CK levels (5-8).

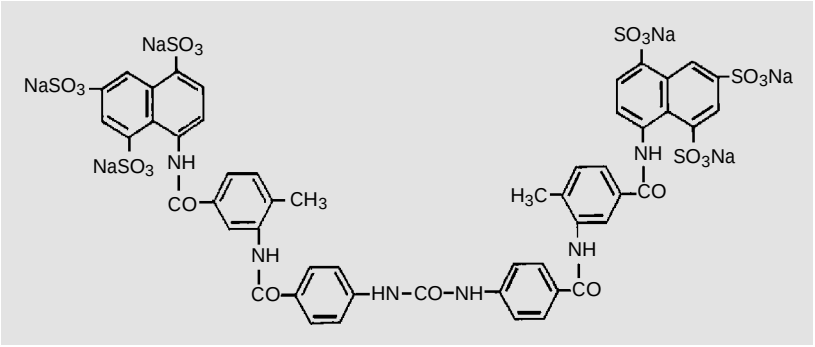

Figure 1. Structure of suramin, a polysulfonated naphthylurea (molecular weight 1429).

Two experimental protocols were used. In protocol A, 1.0 mg/ml of suramin and 0.5-1.0 mg venom/ml were incubated together for 15 min at 37ºC (*in vitro*), and then injected *im*, 1 mg venom/kg, into each animal (*in vivo*). In protocol B, each venom was administered *im* to the animals 15 min before the intravenous injection of suramin (1.0 mg/kg). In both protocols the final volume of venom injected, alone or plus suramin, was 0.1 ml. The venom doses were selected based on previous studies (5-8). The animals were lightly anesthetized with diethyl ether immediately before and 2 h after venom injection for blood collection according to the guidelines for care and use of laboratory animals (18). Plasma was separated by centrifugation and stored at 4ºC for subsequent determination of CK activity. The procedure for the measurement of CK activity has been described previously (1,5-8). Enzyme activity is reported as international units per liter (U/l), where 1 U is the amount that catalyzes the transformation of 1 µmol of substrate at 25ºC.

Figure 2 shows that *im* injections of all crotalid crude venoms induced a significant increase in plasma CK activity compared to PSS. *B. jararacussu* venom injection increased CK levels from 71.16 ± 7.44 (mean ± SEM, N = 5) to 5384.8 ± 187.4 U/l (N = 5) at 2 h. For all the other venoms intermediate values were obtained. Preincubation of the venom with suramin significantly ($P<0.05$) inhibited the increase in plasma CK activity induced by *B. jararacussu*, *B. moojeni*, *C.d. terrificus*, *C.v. viridis* and *A.c. laticinctus* venoms. However, suramin did not offer

protection against the myotoxicity of *B. alternatus*, *L. muta* (Figure 2) or *B. jararaca* venom (data not shown). Protocol B showed that suramin significantly ($P<0.05$) inhibited the increase in plasma CK activity induced by *B. jararacussu*, *C.d. terrificus* and *A.c. laticinctus* venoms and did not antagonize the myotoxicity of *B. alternatus*, *B. moojeni*, C.*v. viridis*, *L. muta* (Figure 2) or *B. jararaca* venom (data not shown). It is interesting to note that, in protocol B, suramin was less effective in antagonizing the myotoxic effect of some venoms which present very active hemorrhagic toxins in their composition (18-20).

In summary, these data show that suramin protects against the myotoxic effect of some (*B. jararacussu*, *C.d. terrificus* and *A.c. laticinctus*), but not all (*C.v. viridis*, *B. moojeni* and *L. muta*) venoms in both protocols. Some closely related venoms (*B. alternatus* and *B. jararaca*) sometimes gave opposite results. The present results extend previous observations showing that polysulfated substances neutralize the toxicity of some crotalid venoms, which have been reported to contain basic myotoxic phospholipases A_2 (PLA_2; 2,4,8,18). These myotoxic PLA_2 present in some crotalid venoms are either Asp-49 catalytically active variants or Lys-49, enzymatically inactive homologues (4,18). However, suramin offered no protection against the myotoxicity of *Bothrops* venoms (*B. alternatus* or *B. jararaca*) whose study did not reveal basic myotoxic PLA_2 (18). Like heparin, suramin did not protect against the myotoxicity of *L. muta* venom, in agreement with our previous data (17). These results indicate that suramin has a significant antivenom effect against some crotalid snake venoms. It partially reproduces the antimyotoxic effect previously described for other polysulfated substances (5,6,8) and deserves further study to evaluate its antimyotoxic activity and therapeutic potential as an antivenin.

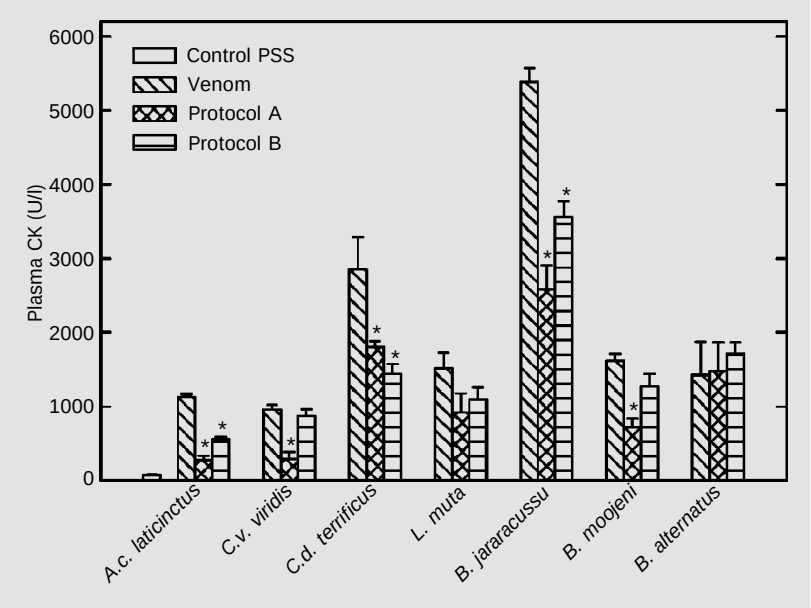

Figure 2. Effect of intramuscular injection of crotalid venoms on plasma creatine kinase (CK) activity and effect of treatment with suramin. Suramin (1.0 mg/kg) was administered according to two protocols: protocol A, preincubation *in vitro* with each venom; protocol B, 15 min *iv* administration of suramin after *im* injection of venom. Plasma CK activity of the physiological saline solution (PSS) control group was 71.16 ± 7.44 U/l (N = 5). The animals received the venom at the dose of 1 mg/kg, except for a group that received *C.d. terrificus* crude venom at the dose of 0.5 mg/kg. The values reported are means ± SEM. *$P<0.05$ for the difference between the control (venom alone) and the values for each of the two protocols for suramin treatment (ANOVA).

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