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Short communication

Effect of premedication with subcutaneous adrenaline on the pharmacokinetics and immunogenicity of equine whole IgG antivenom in a rabbit model

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ABSTRACT

Subcutaneous administration of a low dose of adrenaline is used to prevent the early adverse reactions (EARs) induced by snake antivenoms. We used a rabbit model to study the effect of premedication with adrenaline on the potential of antivenoms to exert therapeutic effects and to induce late adverse reactions. We found that premedication with adrenaline did not change the heart rate or blood pressure of normal rabbits, but reduced the rise in temperature in rabbits previously sensitized with antivenom. Pharmacokinetic studies suggest that premedication with adrenaline does not affect the ability of the antivenom to exert the initial control of envenomation nor the susceptibility of rabbits to develop recurrence of antigenemia and envenomation. Our results also indicate that it is unlikely that premedication with adrenaline decreases the incidence of late reactions induced by the antivenom administration, although it reduces the extent of early reactions.

The parenteral administration of antivenoms is the clinical strategy to treat snakebite envenomations [1]. Snake antivenoms are formulations of immunoglobulins, or their $F(ab')_2$ or Fab fragments, purified from plasma of animals (e.g. horses or sheep) immunized with snake venoms [2]. After intravenous administration, anti-venom immunoglobulins distribute in the body compartments of the envenomated patient, binding venom toxins, and neutralizing their ability to induce tissue damage or other pathophysiological disturbances [3]. Within the first hours after intravenous administration of antivenoms, some patients develop early adverse reactions (EARs), whose frequency varies between antivenoms [4]. Clinical manifestations of EARs induced by antivenoms include fever, pruritus, urticaria, tachycardia, hypotension, smooth muscle spasms, gastrointestinal symptoms, bronchospasm, respiratory collapse, angioedema, shock and death [1,4]. In order to prevent EARs, some clinical protocols recommend the premedication with 0.25 mg/patient of subcutaneous adrenaline [5–8]. Owing to the vasoactive effects of adrenaline, its subcutaneous administration could affect the pharmacokinetics of other drugs administered simultaneously, as described for ropivacaine and bupivacaine [9,10]. Thus, premedication with adrenaline could affect the pharmacokinetics of antivenoms and consequently their ability to exert therapeutic effects. Moreover, changes in the pharmacokinetics of antivenoms may generate differences in the way they stimulate the immune system of patients to produce antibody responses towards heterologous immunoglobulins, thus affecting their potential to induce late reactions. In this work, a rabbit model was used to study the effect of premedication with adrenaline on the pharmacokinetics and immunogenicity of antivenom formulated with whole equine IgG.

This study was performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the International Guiding Principles for Biomedical Research Involving Animals [11], and meet the ARRIVE guidelines [12]. All procedures involving animals were approved by the Institutional Committee for the Care and Use of Laboratory

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Animals of Universidad de Costa Rica (CICUA). Venom was obtained from twelve healthy, adult specimens of *Oxyuranus scutellatus* (taipan) collected in Papua New Guinea. The antivenom used in this study was the anti-taipan antivenom produced at Instituto Clodomiro Picado [13–15]. This antivenom contained 46 mg/mL of total equine protein [13] and 13 mg/mL of antibodies towards taipan venom [14].

The antipyretic effect of adrenaline was tested in two groups of six New Zealand rabbits (body weight 2.8–3.2 kg) which were sensitized eleven days before the test by the intravenous injection of antivenom at a dose of 138 mg of total equine protein per kg of body weight. At the moment of the test, the rabbits of the control group were subcutaneously injected with saline solution and the rabbits of the test group were injected with 0.02 mg/kg adrenaline 1:1000, diluted in saline solution. Immediately, all the rabbits received an intravenous bolus of antivenom at a dose of 138 mg of total equine protein per kilogram of body weight. Three hours later the rectal temperature of rabbits was recorded. The chronotropic and vasopressor effects of adrenaline were tested in a group of three non-sensitized New Zealand rabbits (body weight 2.8–3.2 kg) which received a subcutaneous injection of 0.02 mg/kg adrenaline 1:1000 diluted in saline solution. The rabbits were maintained in a prone position, and the cardiac electrophysiology and arterial pressure were measured before and 1, 5, 10 and 15 min after adrenaline administration. Heart rate was determined by resting 3-lead electrocardiography and confirmed by echocardiography. Blood pressure measurements were performed with a digital sphygmomanometer using the blood pressure cuff placed on the base of the rabbit tail.

Pharmacokinetic parameters were determined in a group of three New Zealand rabbits (body weight 2.8–3.2 kg) which received a subcutaneous injection of 0.02 mg/kg adrenaline 1:1000, diluted in saline solution, and were immediately injected with an intravenous bolus of antivenom at a dose of 13 mg of anti-taipan antibodies per kilogram of body weight. Another group of three rabbits injected with saline solution instead of adrenaline, and then receiving antivenom, was used as control. Blood samples were collected at 0, 1, 5, 15, 30, 60, 180, 360, 720, 1440, 2880, 10080, 12960 and 20160 min after administration of antivenom. After allowing blood to clot at 20–22 °C, serum was separated by centrifugation and stored at −20 °C until analysis. Equine anti-taipan venom antibodies in rabbit serum were determined by a direct ELISA as described by Navarro and co-workers [16]. Pharmacokinetics parameters were calculated as described by Rojas and co-workers [17].

The effect of adrenaline on the immunogenicity of equine immunoglobulins was tested in the serum samples collected at 20160 min (336 h) after administration of the antivenom in the rabbits used for the pharmacokinetic study. Rabbit antibodies towards equine IgG were determined as described by Navarro et al. [16].

Statistical analyses were performed using the statistical software IBM® SPSS v 22.0 (SPSS, Inc., Chicago, IL, USA). The significance of the differences in the raise of the body temperature was tested by one-way ANOVA, assessing homogeneity of variances by Levene's test. Differences in heart rate and blood pressure were evaluated by repeated measured ANOVA. Differences in the pharmacokinetic parameters were evaluated by *t* test. Differences between groups regarding the titre of anti-equine antibodies were assessed by one-way ANOVA, evaluating equality of variances by Levene's test and using a Tukey HSD post-hoc analysis. Differences within groups regarding the titre of anti-equine antibodies were performed using one-sample *t*-test. For differences in the raise of body temperature, heart rate, blood pressure and titre of anti-equine antibodies, a value of $P < 0.05$ was considered to be significant. In the case of differences in pharmacokinetic parameters, a value of $P < 0.100$ was considered to be significant.

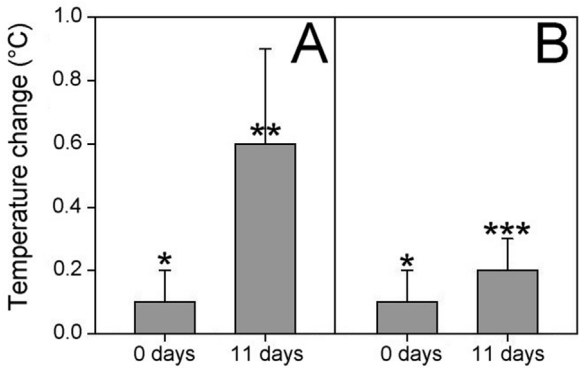

Fig. 1. Effect of premedication with adrenaline on the changes in body temperature induced in rabbits by the intravenous administration of antivenom. *During sensitization (at day 0), antivenom did not induce febrile responses in the rabbits. At this time, differences between the body temperature of rabbits in the control group (A) and the rabbits premedicated with adrenaline (B) were not significant ($F = 0.000$; $df = 1$; $P = 1.000$). **The body temperatures of rabbits in the control group (A) during the challenge (at day 11) were significantly higher than during sensitization ($F = 17.297$; $df = 1$; $P = 0.002$). ***The body temperatures of rabbits premedicated with adrenaline (B) during the challenge (at day 11) were significantly higher than during sensitization ($F = 5.548$; $df = 1$; $P = 0.040$), but significantly lower than the body temperatures of rabbits in the control group during the challenge ($F = 7.757$; $df = 1$; $P = 0.019$). Results are presented as mean ± S. D. (n = 6).

In the test of the antipyretic effect of adrenaline, the first injection of antivenom did not induce a febrile response in the rabbits (Fig. 1), as expected on the basis of the low content of endotoxin in the antivenom (i.e. <8.8 EU/mL; [18]). As a consequence of sensitization, animals developed IgG antibodies towards equine immunoglobulins [18]. Therefore, immediately after the second administration of antivenom, performed 11 days after sensitization, the equine immunoglobulins were recognized by anti-equine antibodies generated in the rabbits, and formed immune complexes capable of inducing pyrogenic responses (Fig. 1A; [27]). When challenged with a second dose of antivenom,

Table 1
Effect of adrenaline[a] on heart rate and blood pressure of rabbits.

Parameter	Time (min)				
	0	1	5	10	15
Heart rate (electrocardiography)	231 ± 15	237 ± 5	244 ± 15	243 ± 6	237 ± 5
Heart rate (echocardiography)	253 ± 18	253 ± 25	247 ± 29	249 ± 10	244 ± 14
Systolic pressure	141 ± 20	157 ± 30	154 ± 19	166 ± 28	155 ± 24
Diastolic pressure	74 ± 14	84 ± 23	90 ± 18	87 ± 10	81 ± 8

[a] Adrenaline 1:1000 was subcutaneously administered at a dose of 0.02 mg/kg of rabbit body weight (n = 3). No significant changes were observed in the heart rate or blood pressure of rabbits within 15 min after the subcutaneous administration ($F = 2.525$; $df = 4$; $P = 0.06$).

Table 2
Effect of adrenaline on the pharmacokinetic parameters of an equine-derived antivenom intravenously administered in a rabbit model.

Pharmacokinetic parameter	Rabbits premedicated with SC adrenaline		Control rabbits	
	Median	90% confidence interval	Median	90% confidence interval
V_z obs (mL)	96	80–114	121	46–240
V_{ss} obs (mL)	90	78–109	99	37–202
Cn_{max} (μg/mL)	446	364–522	476	460–503
t_{max} (h)	5	−33–77	15	2–21
AUC_∞ obs* (μg h mL^{-1})	796055	534754–965422	1064517	1004177–1134948
k_{el}* (h^{-1})	0.0005	0.0003–0.0007	0.0003	0.0001–0.0005
CL obs* (mL/h)	0.049	0.039–0.064	0.037	0.036–0.038
$t_{1/2}$* (h)	1452	860–1793	2272	885–4397
MRT_∞* (h)	1950	1242–2434	2701	1060–5317

(V_z), the apparent volume of distribution during the slowest phase; (V_{ss}), the steady-state distribution volume; (t_{max}), time required to reach the maximum concentration (Cn_{max}); (AUC_∞), the area under the antivenom concentration/time curves at $t=\infty$; ($AUMC_\infty$), the area under the mean curve; k_{el}, elimination rate; (CL), the systemic clearance; ($t_{1/2}$), the half-life of decay during the slowest phase; (MRT_∞), the mean residence time. Results were expressed as median and its 90% confidence interval (n = 4). The significance of the differences between parameters was determined by *t* test. The pharmacokinetic parameters in which differences between rabbits premedicated with SC adrenaline and control rabbits were statistically significant (values of $P < 0.100$) are marked with an asterisk (*).

sensitized rabbits premedicated with adrenaline showed a raise in body temperature (Fig. 1B), but the increment was lower than in rabbits receiving antivenom without premedication with adrenaline (Fig. 1A). This demonstrates that, at this dose, adrenaline had an inhibitory effect of fever. Furthermore, this dose of adrenaline did not induce significant changes in the heart rate or blood pressure of rabbits within 15 min after the subcutaneous administration ($F = 2.525$; df = 4; $P = 0.060$; Table 1). Similar outcomes have been described in clinical studies of snakebite envenomations of human patients premedicated with adrenaline [6,7].

In our experiments, premedication with adrenaline had a negligible effect on the volume of the central compartment (Table 2). Therefore, this intervention did not influence the maximal concentration reached by the antivenom in rabbit plasma, nor did it modify the time required to achieve that concentration (Table 2). These results suggest that premedication with adrenaline does not affect the ability of the antivenom therapy to reduce the concentration of circulating venom and to exert the initial control of the envenomation.

When compared to control animals, rabbits premedicated with adrenaline showed a small increase in the elimination rate and the systemic clearance of antivenom (Table 2). Thus, premedication with adrenaline reduced the half-life of decay during the slowest phase and the mean residence time (Table 2). Consequently, and in spite of the fact that both groups of rabbits received the same antivenom dose, the areas under the antivenom concentration/time curves were slightly lower in rabbits pretreated with adrenaline (Table 2). The relatively limited acceleration of the antivenom elimination induced by the premedication with adrenaline suggests that it is not enough to increase the susceptibility of patients to recurrence of antigenemia or envenomation.

Adrenaline shortened the mean residence time of the antivenom (Table 2). Thus, premedication with adrenaline reduced, albeit not drastically, the time during which the antivenom immunoglobulins stimulate the immune system of the rabbits to induce an antibody response towards these heterologous proteins. Nevertheless, our observations showed that premedication with adrenaline did not induce changes in the titre of antibodies generated in both groups of rabbits towards the equine immunoglobulins of the antivenom ($P > 0.05$; Fig. 2). This result suggests that it is unlikely that premedication with adrenaline decreases the incidence of late reactions, i.e. serum sickness, induced by antivenom administration.

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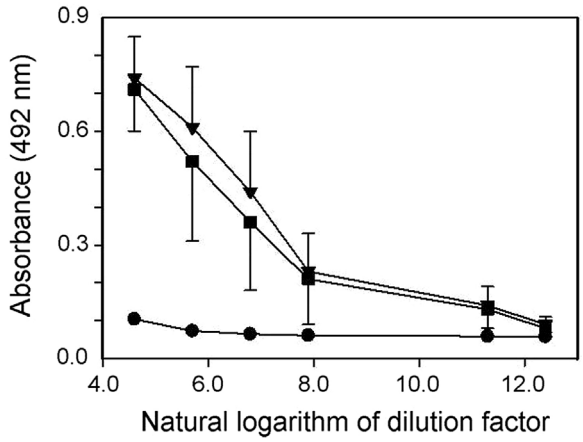

Fig. 2. Effect of premedication with adrenaline on the antibody response of rabbits towards equine immunoglobulins. Differences in the titre of anti-equine antibodies were significant ($F = 3.961$; df = 2, 39; $P = 0.027$). After the Tukey HSD post-hoc analysis, no significant differences ($P = 0.995$) were found between rabbits premedicated with adrenaline and then receiving antivenom (■) and rabbits of the control group, which received antivenom but were not premedicated (▼). However, both groups of animals showed titres significantly higher ($P = 0.034$) than the non-sensitized rabbits (●). Within all groups, the absorbance decreased as the dilution of the sample was higher ($P < 0.001$). Results are presented as mean ± S.D. (n = 3).

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