

SERUM KINETICS OF *Calloselasma rhodostoma* (MALAYAN PIT VIPER) VENOM COMPONENTS IN A RABBIT

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ABSTRACT: The serum kinetics of *Calloselasma rhodostoma* (Malayan pit viper) venom – specifically two of its components, the major hemorrhagin (rhodostoxin) and a thrombin-like enzyme – was examined in a rabbit by double-sandwich enzyme-linked immunosorbent assay (ELISA). The animal received intramuscularly a 1.0-mg/kg dose of *C. rhodostoma* venom. The venom level in serum peaked 12 hours after the injection, followed by a gradual decline and finally reached low rates 72 hours after administration. The serum kinetic profile of venom components, however, did not correspond to the profile of the whole *C. rhodostoma* venom. The serum levels of the *C. rhodostoma* thrombin-like enzyme increased slowly and peaked only 48 hours post-injection. Then both thrombin-like enzyme and rhodostoxin remained at relatively high levels 72 hours after administration. Data suggest that various venom components bind to tissue at the injection site with different affinities and that conjugated venom components were continuously released into circulation at different rates. The prolonged high serum levels of both thrombin-like enzyme and hemorrhagin are consistent with the clinical picture of prolonged clotting deficiency in severe cases of *C. rhodostoma* envenomation. Our results also suggest that since venom components are being released into and eliminated from the circulation at different rates, the “average composition” of the venom antigen in the circulation changes over time. This implies that data from ELISA quantification of antigen levels from serum venom employing “whole venom” as reagent must be interpreted with care.

KEY WORDS: *Calloselasma rhodostoma* venom, thrombin-like enzyme, hemorrhagin, serum kinetics, rabbit, ELISA.

CONFLICTS OF INTEREST: There is no conflict.

FINANCIAL SOURCE: Government of Malaysia.

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INTRODUCTION

Recently, ELISA has been employed in studies on serum kinetics of snake venom or venom components in mice, rabbits, dogs as well as in humans (1-7). The information on serum kinetics of envenomated victims is important to the development of a personalized modality of treatment, in which the dose and frequency of antivenom administration are based on the amount of venom injected and on the kinetics of venom distribution in the body (1, 4, 6). We report herein our investigation of the serum kinetics of *Calloselasma rhodostoma* (Malayan pit viper) venom and its two main toxins (the hemorrhagic rhodostoxin and thrombin-like enzyme) in a rabbit intramuscularly injected with sublethal dose of this venom.

C. rhodostoma venom was obtained from Latoxan (Rosans, France). Micro-ELISA plates were purchased from Nunc (Denmark). IgG-horseradish peroxidase conjugate (HRP) was from Bio-Rad Laboratories (USA). All chemicals and reagents utilized were of analytical grade (Sigma Chemical Company or Merck). *C. rhodostoma* venom hemorrhagin (called rhodostoxin) and thrombin-like enzyme were isolated as described in previous studies (8, 9). Protein concentration was determined by the Lowry method (10). Preparation of rabbit antisera against *C. rhodostoma* venom and the two venom components (rhodostoxin and thrombin-like enzyme) was carried out according to Tan and Ponnudurai (9). Double sandwich ELISA was accomplished following modifications by Harmon and Pawlik (11). Microtiter plates were coated overnight at 4°C with either purified IgG antibodies against the venom or venom components or IgG from preimmune serum (control). The plates were washed four times with PBS-Tween solution and the venom or venom components were added and incubated for two hours. After washing, IgG-HRP conjugate was added and incubated for another two hours, followed by addition of the substrate solution. Ten minutes later, the reaction was stopped by adding sulfuric acid.

One rabbit (2 kg) received intramuscularly a non-lethal dose of *C. rhodostoma* venom (1 mg/kg) 1 cm deep in its hind leg. The animal was bled (1 to 1.5 mL) in the marginal ear vein at the following time intervals: 0.25; 0.5; 1; 2; 4; 6; 12; 24; 48; 72; 96; 120; 144; 168 and 192 hours. Pre-injection serum was used as control. Dilutions (1:5 to 1:10) of obtained sera were assayed in triplicate for *C. rhodostoma* venom and venom components by double sandwich ELISA. Appropriate dilutions of *C. rhodostoma* venom (1.5 to 50 ng/mL) or venom components (0.5 to 12.5 ng/mL) were employed to construct the respective standard curves. The concentration of the

snake venom or venom components from the test serum was then read from the appropriate standard curves.

There was a progressive increase in absorption of *C. rhodostoma* venom from the inoculation site into the blood stream. The venom antigen levels in serum peaked (42.6 ng/mL) 12 hours after the injection and then gradually declined. Antigens were not detectable 192 hours post-injection (Figure 1 – A). *C. rhodostoma* venom antigen concentrations found in rabbit serum ranged from a few ng/mL to 47 ng/mL, and are of the same order of magnitude as those reported in sera of envenomated patients. For example, plasma venom levels of patients bitten by *C. rhodostoma* varied from 175 ng/mL (patients with non-clotting blood) to 35 ng/mL (patients with no gross clotting abnormalities) (12).

Barral-Netto *et al.* (1) reported that in mice injected with *Bothrops jararaca* venom, the venom antigen peaked after 15 minutes. Thereafter serum levels of venom dropped progressively and venom was not detectable 24 hours after injection. Similar pharmacokinetics was reported in rats injected with *Bothrops erythromelas* venom (13). A recent study that utilized dogs injected with *Crotalus durissus terrificus* venom, however, indicated that blood venom levels remained high 48 hours after intoxication (6). It is therefore obvious that the rate of venom clearance from blood circulation differs from species to species. However, all the results (including our findings) indicated that there was a fast absorption of venom from the inoculation site, followed by slow clearance of venom from circulation, which is indicative of a slow diffusion rate of venom components to other tissues. Our results also undoubtedly indicated that venom components bind to tissues at the injection site and that conjugated components were continuously released into circulation.

The *C. rhodostoma* thrombin-like enzyme antigen levels in the rabbit serum also increased progressively after the injection and peaked at of 2.5 ng/mL, 48 hours after injection (Figure 1 – B). Afterwards, its levels decreased slowly and was negligible five days after the venom administration. This serum kinetic profile is similar to that of thrombin-like enzyme in rats reported by Zhao *et al.* (14). On the other hand, serum kinetics of rhodostoxin exhibited a biphasic profile (Figure 1 – C). Serum rhodostoxin levels were high (1.3 ng/mL) 15 minutes post-injection, indicating a rapid absorption of venom components. However, levels had dropped slightly to 0.9 ng/mL at one hour, and then progressively increased and reached a peak (1.9 ng/mL) 12 hours after the inoculation. After that, serum rhodostoxin levels gradually decreased,

becoming negligible 120 hours after the injection. The cause of this biphasic profile of rhodostoxin is not clear. Observation of prolonged high serum levels of both thrombin-like enzyme and hemorrhagin is consistent with the clinical symptom of prolonged clotting defect in victims of severe *C. rhodostoma* envenomation (15).

It is interesting to note that, using double-sandwich ELISA technique, the serum kinetics profiles of *C. rhodostoma* venom components do not correspond to that of *C. rhodostoma* venom antigen. While both serum venom and rhodostoxin levels peaked 12 hours post-injection, serum thrombin-like enzyme was maximized 48 hours post-injection. Also, while only a very small amount of injected venom antigen appeared to remain in blood circulation at 72 hours post-injection, Figure 1 (B and C) shows that the thrombin-like enzyme and hemorrhagin levels in serum were both still relatively high at this moment. Presumably, the thrombin-like enzyme and hemorrhagin have higher affinity for tissue at the inoculation site and are therefore released into the circulation at a slower rate.

Our results therefore show that the various venom components have different rates of release into and clearance from the circulation, which implies that the “average composition” of “venom antigen” in the circulation of the victim is changing over time after venom inoculation. This indicates that results obtained from the usual practice of using antibodies against “whole” venom as reagents in ELISA for serum venom antigen quantification must be interpreted with care.

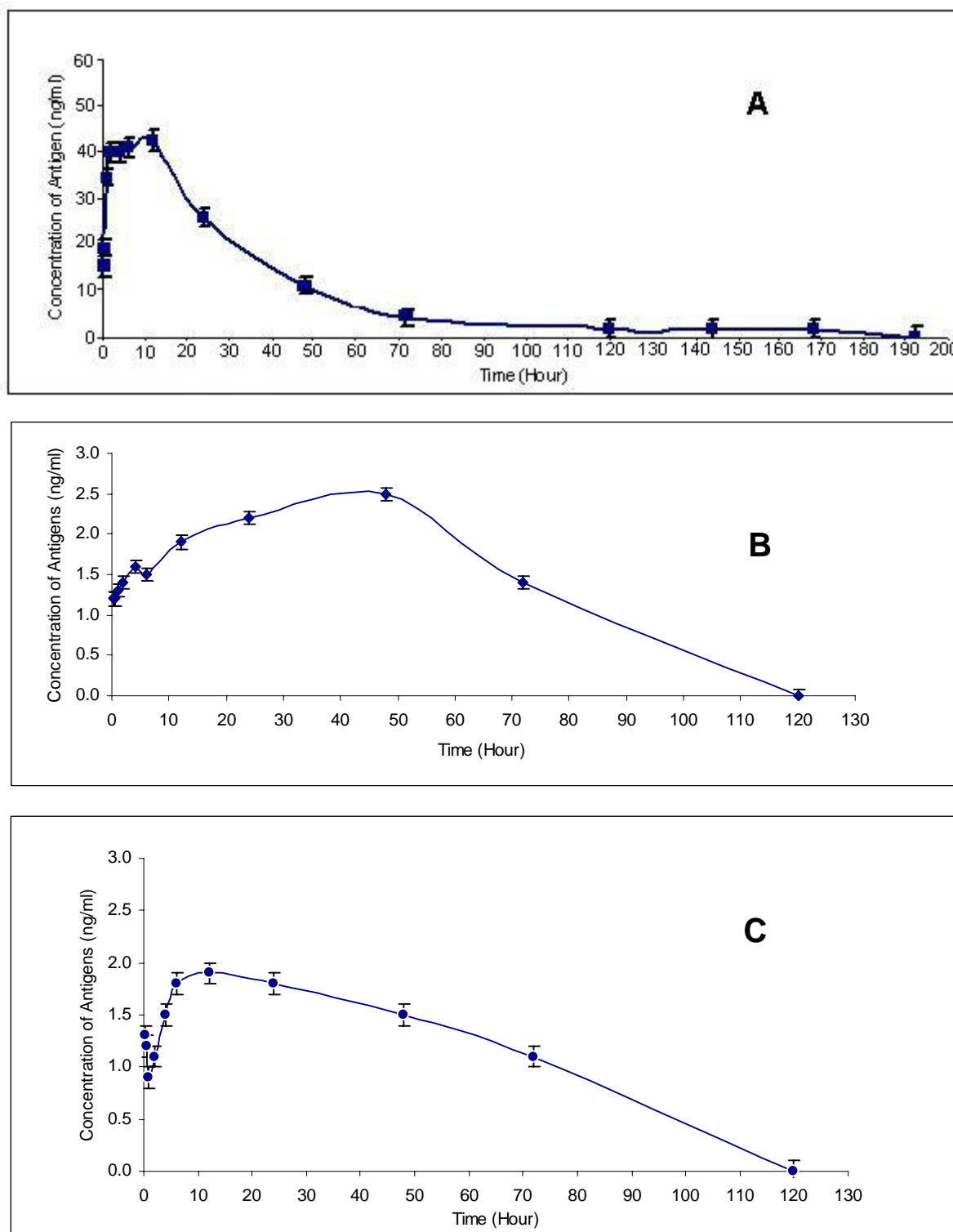


Figure 1. Serum kinetics of (A) *Calloselasma rhodostoma* venom and venom components (B: thrombin-like enzyme; C: rhodostoxin) in a rabbit following a single intramuscular injection of a non-lethal dose of the venom.

ACKNOWLEDGEMENTS

This work was supported by research grants IRPA-03-07-04-097 and 06-02-03-1001 from the Government of Malaysia.

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