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EFFECTS OF PROLONGED POISONING BY RUSSELL'S VIPER VENOM ON BLOOD COAGULATION, PLATELETS AND FIBRINOLYSIS

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SUMMARY: The effects of Russell's viper venom on blood coagulation, platelets and the fibrinolytic enzyme system were studied in rabbits after injecting repeated doses of 0.05 MLD of the venom. Thrombocytopenia was the earliest change to appear. It was followed by rise in serum fibrinogen degradation products and prolongation of prothrombin time, activated partial thromboplastin time and thrombin time indicating a progressive consumption coagulopathy and activation of fibrinolysis. Red blood cell morphology was unchanged during the first three weeks; whereas the fragmentation appeared after the fourth week and it increased in severity with further envenomations, i.e. when chronic DIC was established.

INTRODUCTION

The venomous reptiles of the Indo-Pakistan subcontinent are the Russell's Viper (RV), *Echis carinatus* (family: *Viperidae*), Cobra and Kraits (family: *Elapidae*) (1). Cobra venom is rich in neurotoxins and it causes death due to respiratory paralysis. On the other hand, Russell's viper venom (RVV) is rich in hemorrhagins and cytotoxins, therefore it produces coagulation defects (2). Reports on coagulation changes in victims of RV bite and experimental venom work are conflicting (3). This is because in most of the experimental work high doses (2, 1 and 0.5 MLD) of venom were used and the animals died within 24 hr (4,5), whereas most of the patients receive smaller quantities of venom during bite. In addition, a prolonged defibrination lasting for about four weeks was also observed in patients of viper bites by Reid (6) and Visudhiphan and colleagues (7). They postulated that depot of procoagulant toxin persists at the site of bite and minute concentrations are released off and on, resulting in prolonged defibrination. This type of chronic snake venom poisoning may also be observed amongst snake charmers and workers in research laboratories who do milking of snakes.

The purpose of the present study was to demonstrate the effects of repeated small doses (0.05 MLD) of RVV on coagulation and the fibrinolytic enzyme system.

MATERIALS AND METHODS

Snake venom: Lyophilized Russell's viper venom was obtained from National Institute of Health, Islamabad. The venom was reconstituted in phosphate buffered saline (pH 7) in such a way that one ml of diluted fluid contained 2.6 mg of crude RVV. A minimal lethal dose (MLD) for a rabbit weighing 1.5 kg was found to be 2.6 mg. The dilutions of venom were made with normal saline just before injection.

Animals: A total of 16 local domestic rabbits were used as experimental animals. The average weight of the animals at the commencement of the experiment was 1.5 kg.

Injection schedule: 1) *Experimental group.* Eight male animals were included in this group. Each of them received 10-18 doses of 1:20 dilution of RVV at intervals of 3 days. Six animals died after receiving 10-16 doses of the venom; whereas the remaining two animals were killed at the end of the experiment, when they had received 18 doses of RVV. Blood samples were taken for hematological determinations after 24 hr, 3 days and then at weekly intervals until the experiment was terminated at the end of the eighth week.

2) *Control group.* Eight male rabbits were included in this group. Each animal received 1.0 ml of physiological saline through the intramuscular route at the time of envenomation of each experimental animal. Blood samples were drawn at the time they were obtained from the animals in the experimental group so that the coagulation studies could also be carried out together.

3) *Investigations.* Platelets were counted by the visual method according to Dacie and Lewis (8). The peripheral smears were studied for red blood cell morphology after staining with Giemsa stain. Reticulocytes were stained with brilliant cresyl blue and counted by use of an oil immersion lens according to Dacie and Lewis (8). Prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), and fibrinogen levels were determined according to Dacie and Lewis (8) to evaluate the coagulative activity of the venom.

Fibrinogen degradation products (FDPs) were estimated by the latex agglutination technique (Thrombo-wellcotest of B. Wellcome), which employs antibody to human FDPs. As the sensitivity of the reagent was so adjusted that in the presence of FDPs at a concentration of 2 μg per ml or higher the latex particles clumped together giving macroscopic agglutination. Therefore, double dilutions of serum were made with glycine-saline buffer and each dilution was tested. The agglutination pattern at the end of the test period indicated the presence of at least 2 $\mu\text{g}/\text{ml}$ FDPs in the respective dilution. We used the reagent for measuring FDPs in rabbit sera without introducing any conversion factor.

RESULTS

During the first week, the platelet counts in the experimental animals mostly remained within the normal limits. The counts of the experimental groups were 280 to 300 $\times 10^3/\mu\text{l}$; whereas in control animals 290 to 400 $\times 10^3/\mu\text{l}$. Then there was a gradual fall in the platelet count and it ranged between 140 to 180 $\times 10^3/\mu\text{l}$ at the end of the 2nd week and 140 to 160 $\times 10^3/\mu\text{l}$ at the end of the 3rd week (Fig. 1). The maximum fall was observed at the end of the 4th week when the platelet counts were 70 to 80 $\times 10^3/\mu\text{l}$ (Fig. 1). Later there was a gradual rise in the platelet counts, which were 70 to 90 $\times 10^3/\mu\text{l}$ at the end of the 5th week, 90 to 100 $\times 10^3/\mu\text{l}$ at the end of the 6th week, 140 to 160 $\times 10^3/\mu\text{l}$ at the end of the 7th week and 120 to 140 $\times 10^3/\mu\text{l}$ at the end of the 8th week (Fig. 1). The counts in control animals remained between 290 to 400 $\times 10^3/\mu\text{l}$.

Fig. 1. Changes in platelet counts, fibrinogen level and FDPs in rabbits receiving repeated doses of 0.05 MLD of RVV.

Fig. 2. Changes in PT, APTT and TT in rabbits receiving repeated doses of 0.05 MLD of RVV.

No abnormality in the red blood cell morphology was seen during the first three weeks of the experiment. At the end of the fourth week, a mild degree of crenation of red blood cells appeared. The morphological changes increased progressively and at the end of the 8th week, the blood smears showed a marked

Fig. 3. Photomicrograph showing fragmentation of red blood cells of rabbits receiving repeated doses of 0.05 MLD of RVV. Giemsa x480.

degree of crenation of red cells, fragmentation and anisocytosis (Fig. 3). Red blood cell morphology remained normal in the control animals. No significant difference in the reticulocyte count was observed in the experimental and control animals (Table I).

Prothrombin time was within the normal limits during the first week of experiment when it ranged from 14 to 15 sec in experimental animals (Fig. 2). It was significantly prolonged in the subsequent samples and maximum prolongation was seen at the end of the 8th week when it was found to be 19 to 20 sec (Fig. 2). In the control animals, it remained 9 to 11 sec (Fig. 2).

Activated partial thromboplastin time remained within the normal limits during the first three weeks of the experiment when it was from 27 to 35 sec in the experimental group and 25 to 32 sec in the control animals (Fig. 2). It was slightly prolonged in the samples taken at the end of the 4th, 5th, 6th and 7th weeks when it was found to be from 34 to 36, 30 to 34, 35 to 38 and 34 to 38 sec respectively (Fig. 2). Maximum prolongation was seen in the samples taken at the end of the 8th week when it was 40 to 46 sec (Fig. 2). Similarly TT was almost normal during the first three weeks of the experiment when it ranged from

Table I. *Changes in RBC morphology and reticulocyte counts in rabbits receiving repeated doses of 0.05 MLD of RVV*

	24 hr	3 days	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	7 wk	8 wk	Control
RBC morphology	NN	NN	NN	NN	NN	Frag.+	Frag.+	Frag.+	Frag.++	Frag.++	NN
Reticulocyte count %	0.5-1.0	0.2-1.0	1-2	1-2	1-2	2-3	2-3	2-4	2-3	2-3	2-3

Frag.: Fragmentation, NN: Normochromic and normocytic.

8 to 11 sec in the experimental groups (Fig. 2) and 7 to 8 sec in the control animals (Fig. 2). Then there was a gradual prolongation of TT in the subsequent samples. Maximum prolongation was seen at the end of the 8th week when it was 15 to 19 sec (Fig. 2).

The fibrinogen level was from 378 to 406 mg/dl during the first three weeks, in experimental animals. This was followed by a gradual fall in the fibrinogen contents and it ranged between 210 and 300 mg/dl in the subsequent samples (Fig. 1). It remained between 385 and 415 mg/dl in control animals throughout the experiment (Fig. 1).

Serum FDPs were normal only in the first two samples. Then there was a gradual rise in the level of serum FDPs, and at the end of the 3rd week it ranged between 12 and 18 μ g/ml (Fig. 1); whereas in control animals it was 2 to 5 μ g/ml (Fig. 1). In the subsequent samples serum FDPs were 40 μ g/ml (Fig. 1).

DISCUSSION

Effects of Russell's Viper venom have been studied by many workers both in patients of RV bite (1,9,10,11), and in experimental models (4,5,12).

In the present study, there was progressive thrombocytopenia in experimental animals and lowest counts were observed at the end of the 4th week; whereas in the subsequent samples thrombocytopenia was not so severe. In the experimental models produced by Aung-Khin et al. (4), Memon et al. (5) and Rehman, Hayee and Nagi (13), very high doses (2 MLD to 0.5 MLD) of RVV were used resulting in a marked degree of thrombocytopenia that lead to the death of the animals within 48 hr. However, the results of the present experiment are more in accordance with what were observed by Rehman et al. (13) in rabbits receiving 0.1 MLD of RVV. Platelet counts in patients of RV bite studied by Chugh and associates (1) varied

from 60 to 200 x 10³/μl. Reid, Chan and Thean (14), Bhargawa et al. (15), Devaraj (10) and Mahasandana et al. (9) have also noted low platelet counts after viper bites.

The reticulocyte counts remained within normal limits in our experimental animals. As this parameter has not been included previously in animal experiments, the studies on human patients show that majority of them have normal reticulocyte counts (1,14).

The red blood cells in the peripheral smears showed a significant fragmentation in all the animals in the present study. On the other hand, Aung-Khin et al. (4) and Memon et al. (5) could not observe such change in their experimental animals. This could be due to both a short survival time after envenomation and the production of acute DIC which is associated with severe thrombocytopenia and hypofibrinogenemia (16). A similar observation was made in a previous study by Rehman et al. (13) in the animals receiving 0.5 MLD and 1 MLD of RVV; whereas fragmentation of RBCs was noticed in the animals injected with repeated doses of 0.1 MLD of RVV. Chugh et al. (1), Bhargava et al. (15) and Gomperts and Demetriou (17) also observed fragmentation of red blood cells in their patients of RV bite. Bick (18) and Matsuda (19) described red cell fragmentation in 36 to 50% of their cases of DIC which were mostly chronic.

The fibrinogen levels showed a moderate fall in the envenomated rabbits. This is in agreement with the observations made by different workers (4,5). In human studies, the fibrinogen levels have also been reported to be variable with some levels falling as low as 9.0 mg/dl (1,20,21).

Serum FDPs levels were significantly raised in the animals in the present study, indicating an activation of the fibrinolytic enzyme system. Other workers have also studied the effects of RVV on the fibrinolytic system in patients of RV bite (1,21). They reported FDPs from 5 to 160 μg/ml in patients of RV poisoning. Memon et al. (5) failed to study effects of RVV on fibrinolysis. Rehman et al. (13) observed a significant rise in serum FDPs in their experimental animals injected with 1 MLD, 0.5 MLD and 0.1 MLD of RVV. Aung-Khin et al. (4) also reported the fibrinolytic activity of RVV by observing the shortening of the euglobulin clot lysis time. However, Mittrakul (12) demonstrated by performing an in vitro study that RVV has no direct fibrinolytic activity. The in vivo activation of fibrinolysis seems to be secondary to intravascular clotting as the RVV has no direct fibrinolytic activity.

The results of this experiment show that a picture of progressive consumption coagulopathy is produced after repeated injections of smaller doses of RVV. In vivo coagulant and fibrinolytic activities of RVV have been confirmed. In addition, fragmentation of red blood cells has been successfully reproduced in this experimental model.

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