Effects of heparin on Russell's Viper Venom envenomated experimental rabbits

Than-Than[1], *Soe-Soe*[2], *and Khin-Ei-Han*[2]

1.– Pathology Department, Institute of Medicine Mandalay, and

2- Pathology Research Division, Department of Medical Research

Effects of heparin were studied on 20 Russell's Viper Venom (RVV) envenomated rabbits. After injection with LD 100 dose of RVV, the experimental animals were divided into four groups. Group I rabbits were left untreated; in Group II antivenom treatment was given ; in GroupIII antivenom plus heparin was administered intravenously and in Group IV heparin alone was given. Effectiveness of heparin therapy was assessed by comparing the changes in the blood screening tests of haemostasis such as prothrombin time (PT), activated partial thromboplastin time (APTT) and thrombin time (TT), survival rate, degree of congestion and haemorrhage in the kidneys, presence or absence of fibrin deposition and acute tubular necrosis in the kidneys of different groups of animals. Results showed no significant differences in the blood tests and fibrin deposition in the kidneys. However, there is an increase in the survival rate, as well as reduction in the severity of congestion, haemorrhage and acute tubular necrosis of the kidneys in the rabbits treated with specific antivenom (ASV) plus heparin (Group III) over that treated with antivenom alone (Group II) and heparin alone (Group IV).

INTRODUCTION

It has been well established that RVV contains activators to coagulation factors V and X (1,2) which may be responsible for the initiation of disseminated intravascular coagulation (DIC) (3,4). Since the action of heparin (5) is to inactivate products of coagulation including activated factor X and thrombin, it may halt or slow down the process of DIC, and preserve organs' function by limiting fibrin deposition in vital organs. Hence it is thought that heparin therapy would be most beneficial in experimental Russell's viper venom envenoming.

The present study is aimed to observe whether heparin confers any added advantage in experimentally envenomated rabbits treated with specific antivenom (ASV).

MATERIALS AND METHODS

Twenty Japanese white rabbits weighing 2.5-3.5 kg were used. Baseline blood samples were taken from the marginal ear veins of all the rabbits. After intramuscular injection with LD 100 dose of RVV (dried crystal obtained from the Biological Laboratories of Myanmar Pharmaceutical Industry, (MPI)), blood samples were collected at 1 hr, 3 hrs, 6 hrs, 12 hrs, and 24 hrs respectively. The experimental animals were then divided into four groups each group comprising of 5 animals, and were treated as follows: -

Group I - The rabbits were left untreated after venom injection.

Group II - Half an hour after venom injection an appropriate dose (ranging from 0.75 ml- 1.05 ml) of specific anti-venom produced by MPI was administered intravenously. This dose is calculated for every rabbit based on the findings (6) that 1 ml of specific antivenom could neutralize 2 mg of RVV.

Group III- Specific antivenom (as for Group

II) plus heparin 20 units per kg was injected intravenously.

Group IV-Treated with heparin only (dosage same as Group III).

All the blood samples were screened for prothombin time (PT), activated partial thromboplastin time (APTT) and thrombin time (TT)) using standard methods (7).

The experimental animals were observed closely, survival times noted, and autopsy performed immediately after death; those that survived beyond 24 hours were sacrificed. Kidneys from all the animals were fixed in buffered formalin and processed for light microscopy using standard procedure.

Effectiveness of heparin therapy was assessed by comparing among the following different experimental groups.

1. Changes in screening tests of haemostasis
2. Survival rate
3. Histopathological changes in the kidney with special emphasis on fibrin deposition, haemorrhage, congestion and acute tubular necrosis

RESULTS

Results of Screening tests of haemostasis

Results of all the groups are shown in Fig 1. PT, TT and APTT of untreated rabbits (GroupI) were non-clotting at 6 hours (clotting times > 120 seconds for PT and TT and > 180 for APTT. Rabbits treated with antisnake venom (Group II) showed slight prolongation of some of the screening tests within 3-6 hours which reverted back to normal at 12 hours. Rabbits treated with both antisnake venom and heparin (Group III) revealed prolongation of screening tests at 6 hours which , however, returned to normal at 12 hours. Rabbits treated with heparin alone (Group IV) displayed earlier prolongation of the screening tests at 1 hour which became non-clotting at 12 hours until 24 hours.

Results of survival rate

The survival rate of the rabbits between the groups were compared (Table 1). In Group I, as expected all rabbits died within 24 hours (4½ - 23hrs). In Group II, where specific antivenom therapy was given, 2 rabbits (40%) survived up to 24 hours. In the group treated with both antivenom and heparin (Group III), 3 rabbits (60%) survived up to 24 hrs. The survival rate of Group IV was similar to that of Group II. Thus the results showed improvement in the survival rate of rabbits treated with antivenom and heparin in comparison to those treated with antivenom alone and heparin alone.

Table 1. Survival Rate (%)

Group	% Survival up to 24 hours
Group I	0%
Group II	40%
Group III	60%
Group IV	40%

Results of histopathological changes

Histopathological changes in the kidneys were examined with emphasis on presence or absence of fibrin deposition, haemorrhage, severity of congestion and morphological features of acute tubular necrosis.

Fibrin deposits were found either in the renal vessels or inside the glomerular tuft in 3 rabbits (60%) of the untreated group (Group I), as well as that of the group treated with heparin onlv (Group IV). In

the groups treated with ASV (Group II) and ASV plus heparin (Group III) 40% of the rabbits exhibited fibrin deposits in the kidneys. On the other hand it was noted that degree of haemorrhage and congestion was markedly reduced in the group treated with ASV plus heparin in comparison to the group treated with ASV only. Features of acute tubular necrosis (Fig 2-7) were seen in all (100%) the experimental animals of groups I, II and IV while it was found in 4 rabbits (89%) in the Group III treated with ASV plus heparin.

DISCUSSION

The results of the coagulation parameter did not reveal any significant differences between the groups. The earlier development of prolongation of the screening tests and longer duration of non-clotting state in heparin-treated group may be due to the anticoagulant effect of heparin. Increase in the survival rate in Group III may be due to the additive beneficial effect of heparin.

The percentage of fibrin deposits present in Group II and III were the same. This may indicate that heparin in the dosage used in the study may bot be enough to abolish or prevent DIC.

The most striking histological findings of the kidneys were the notable decrease in congestion and haemorrhage in Group III. The reason is not definitely known, however it is speculated that enhanced removal of the activated clotting products under the influence of the heparin may be one of the reasons.

Effects of heparin on DIC due to snake bite (8,9) have been studied by many workers and opinions differ as to its effectiveness. Studies by Warrell et al (8), on cases bitten by Echis carinatus showed no beneficial effects. However, Weiss et al (9) demonstrated beneficial effects of heparin on their cases bitten by Echis carinatus.

Ahuja et al (10) showed neutralizing action of heparin on procoagulant action of RVV, both *in vivo and in vitro* studies. In vitro studies of Malayan pit viper venom by Chan et al(11) showed that high doses of heparin was required to counteract the effects of the venom.

In conclusion, our results showed beneficial effects of heparin in terms of survival rate and histopathological changes in the kidneys. However, the coagulation parameters and fibrin deposits did not reveal any difference. Results of the clinical trial of heparin in Russell's viper bite victims has been reported elsewhere (Myint Lwin et al, Departmental report, 1988).

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