MORPHOLOGY COAGULATION STUDIES IN GUINEA PIGS FOLLOWING INJECTION OF VIPERA RUSSELLI VENOM

Pages with reference to book, From 63 To 66

Salem G. Memon, Fazal Khan, Naeem-A-Jafarey (Dept of patholgy Basic Medical Sciences Institute, Jinnah Postgraduatc Medical Center, Karachi.)

Abstract

The studies on the morphology of lesions produced and the blood coagulation disturbances after Russell viper envenomation were studied in guinea pigs. The study showed very little morphological changes but the coagulation studies revealed disturbances in coagulation mechanism. The exact pathological process operating in Russell viper emvenomation needs further studies including the histochemical and ultrastructural studies. At present one can only infer that the consumption coagulonathy is the main pathologic change leading to severe bleeding disorders and the death of the animals (JPMA 30:63, 1980).

Introduction

Snake bite is a public health problem in many countries of the world including Pakistan. It is estimated that about 30,000 to 40,000 deaths occur annually around the globe due to snake bites (Swaroop and Grab. 1954). In Pakistan the data collected at Liaquat Medical College Hospital, Hyderabad and Lady Reading Hospital, Peshawar show that during the summer months the majority of emergency admissions in these hospitals are due to snake bites.

These studies were planned to provide baseline data on the morphology of lesions and blood coagulation defects in the experimental animals. Ultimately it is planned to study the efficacy of different immunization procedures.

Material and Methods

SNAKE VENOM: Dried lyopholized Russell viper venom (R.V.V.) was obtained from the National Health Laboratories, Islamabad. The venom was reconstituted in phosphate Buffer saline (pH. 7.6) and suitable dilutions were made from this stock solution.

A minimum lethal dose, (M.L.D.) from a guinea-pig weighing 350-400 Grams, was found to be 0.66 mg of R.V.V.

EXPERIMENTAL ANIMALS. Guinea pigs of either sex weighing 350-400 Grams were used as experimental animals. The animals were obtained from the animal house of National Health Laboratories Islamabad.

SYSTEM ENVENOMATION OF ANIMALS The guinea pigs were divided in two groups. Group-A animals were divided in four sub-groups of 4 animals each. Each sub-group was given 1 MLD. RVV subcutaneously and then sacrificed at intervals of 6, 12, 18 and 24 hours. A control group of 4 animals was given only phosphate Buffer Saline subcutaneously.

Group-B animals were divided in two major sub-groups, B 1 and B 2. Sub-group B-1 animals were further sub divided in four sub sets of 5 animals each (20 guinea pigs) and given 1 MLD, RVV; while sub group B-2 (20 animals) were given 1/2 MLD of RVV. Blood was withdrawn at intervals of 6. 12. 18 and 24 hours for coagulation studies according to the following schedule (Table I).

Table 1: Coagulation Studies

Group	Treatment	Hours after Injection of R.V.V.	No of animals in each group
A.	1 M.L.D. of R.V.V.	6	4
	=	12	4
	=	18	4
	=	24	4
	Control	—	4
B1	1 M.L.D. of R.V.	6	5
	=	12	5
	=	18	5
	=	24	5
	Control	—	5
B2	½ M.L.D. of R.V.V.	6	5
	=	12	5
	=	18	5
	=	24	5
	Control	—	5
	Total Animals		70

Blood was also withdrawn from a control group of 5 animals.

AUTOPSY: Animals of category-A were sacrificed and thorough macroscopic search done for gross lesions. Representative blocks were taken from brain, heart, lung, kidneys, liver, spleen and adrenals stained with Hea-matoxylin eosin and Phosphotungstic Acid Haematoxylin (P.T.A.H.).

COAGULATION STUDIES

The following coagulation studies were done on each sample of blood withdrawn from animals of group-B: -

(1) Prothrombin time (P.T.)
(2) Activated partial Thromboplastin time (A.P.T.T.)
(3) Thrombin time (T.T)
(4) Clotting time (C.T.)
(5) Platelet count (P.C)
(6) Plasma Fibrinogen level (Fib. Level)

Results and Observations

In group-A animals both macroscopic and microscopic studies revealed very meagre changes. The only observable change being a congestion of vital organs which was propor-tional to the time between envenomation and sacrifice. A few degenerative changes like ballooning of liver cells, degeneration of Hippocampus cells and fibrinoid degeneration of small blood vessels of lung were observed. Adrenal glands showed minor degrees of haemorrhage. Sludging of RBC's was observed in the blood vessels of many organs including the heart.

Table II: Coagulation Studies After Injection ½ M.L.D R.V.V.

Time after Test	6 hours	12 hours	18 hours	24 hours	Control
P.T	42.8 Seconds	35 Seconds	34.6 Seconds	28.2 Seconds	22 Seconds
A.P.T.T	51.2 =	48.6 =	40.8 =	40.2 =	22.8 =
T.T	24.2 =	21.6 =	12 =	7.4 =	5.2 =
C.T	600 =	567.5 =	593 =	358.6 =	179 =
Platelet Count	1,59,000/c.m.m	1,49,000/c.m.m	1,37,000/c.m.m	1,35,000/c.m.m	2,44,000/c.mm
Plasma fibrinogen	114.4 mg/100 ml	196.0 mg/100 ml	220 mg/100 m l	212mg/100 mm	250 mg/100 ml

Table III: Coagulation Studies After Injection of 1 M.L.D R.V.V.

Time after Test	6 hours	12 hours	18 Hours	24 hours	Control
PT	113.4 Seconds	No clot in 3 Minutes	Noclotin3Minute	147 Seconds	22 Seconds
APTT	155.6 =	= = =	= = =	147 =	27.8 =
T.T	60.8 =	= = =	= = =	92 =	05.2 =
C.T	No clot in½ hours	No clot in ½ hour	No clot in ½ hour	No clot in ½ hours	179.0 =
Platelet count	96,800/c.m.m	93,500/c.m.m	86,700/c.m.m	1,16,500/c.m.m	2,44,000/ per c.mm
Plasma fibrinogen	37.4 mg/100ml	No clot	No clot	40 mg/100 ml	250 mg/ 100 ml

Group-B animals treated with RVV showed prolongation of PT, APTT, TT and CT, the platelet count was reduced considerably and fibrinogen levels were markedly decreased. Prothrombin time in the control animals was found to be 22 + 0.94 seconds. In group B-2 animals the PT was prolonged to 42.8 + 1.11 seconds at 6 hours but started decreasing afterward. In group B-l animals the PT noted at 6 hours

was 113.4 + 6.52 seconds. No clot was observed in 3 minutes in 12 and 18 hours samples, but two animals showed a PT of less than 3 minutes after 24 hours (mean value of 147 seconds).
Group B-2 animals showed prolongation of APTT to 51.2 ± 2.89 seconds (as against 27.8 + 0.86 seconds in the controls) at 6 hours; it however continued to decrease after 6 hours till 24 hours. Group B-l animals showed prolongation of APTT to 155.6 ± 6.88 seconds at 6 hours. No clot was observed at 3 minutes 12, 18. and 24 hours.
The TT increased to 42.2 + 1.85 seconds (as against 5.2 + 0.37 seconds in controls) at 6 hours but started reducing afterwards. Group B-l animals showed the TT to be 60.8 - 10.11 seconds at 6 hours while no clot was observed at 3 minutes after 12 and 18 hours. However at 24 hours three animals in the group B-l had a CT less than 3 minutes-the mean being 92 seconds.
The clotting time in animals of group B-2 increased 3-4 fold 600 ± 34.89 seconds as against 180 seconds in the control animals but continually decreased at 12. 18 and 24 hours. There was a marked increase in the CT in animals of group B-l that is the blood did not coagulate at 6 hours. However clotting was noted in two animals with mean CT. value of 25 minutes and 7 seconds.
The PC/Cumm in both the groups B-l and B-2 reduced markedly and this trend continued till 24 hours.

Discussion

The results of morphological changes were meagre and disappointing, the only observed changes being a mild to moderate hea-morrhage in different part of the body. Few degenerative changes were also seen in the liver and the cells of hippocampal gyrus.
Coagulation studies showed changes of PI, APTT and CT which were prolonged, while PC/cumm and plasma fibrinogen level were found to be reduced. The prolongation of PT is in line with the findings of Forbes et al (1969) and Mohammad et al (1971) who carried out same studies in experimental animals on snake venoms other than R.V.V. Chan and Reid (1964), Hashmi et al (1969). Philips et al (1973). Bhargava et al (1976) studied human snake bite cases and found similar results. The measurement of prothrombin activity of the plasma is a good index for monitoring the course of envenomation process. The time at which antivenin therapy should be terminated can also be determined by this test (Chavarria et al. 1970).
In the present studies there was a prolongation of APTT in the guinea pigs after the injection of RVV in both 1/2 MLD, I MLD treated groups. Similar observations have been made by Philips et al (1973) who studied the effect of puff adder venom on the coagulation mechanism using rats as experimental animals. Increase in APTT indicates that one or more of ten clotting factors XII, XI, IX, VIII, X V, II and I are deficient or affected. The prolongation of APTT with normal PT would indicate the possibility of the deficiency of clotting factors exclusively in the intrinsic (PATHWAY) namely factors XII. XI, IX or VIII. APTT is an important and comprehensive test which helps to determine a pro-coagulant deficiency.
The Thrombin lime was also found to be increased in the present study supporting the observations of Mohammad et al (1969), Mac-kay et al (1969) who performed such studies in experimental animals with a different venom. Reid et al (1963). Sezi et al (1972), Mitrakul (1973) did similar studies in human snake bite cases. The snakes however were other than the Russell viper.
The present study also showed prolongation of clotting time in the experimental animals injected with R.V.V. The blood did not coagulate, even after 30 minutes in animals injected with 1 MLD.
In only two animals of this group the blood coagulated in less than 30 minutes when the sample was taken after 24 hours. These observations are also in accord with those of Mohammad et al (1969) and Mackay et al (1970) who performed similar studies in experimental animals and Reid et al (1963), Weiss et al (1969). Fainaru et al (1970). Bhargava et al (1976) who performed such studies in human beings. The snake venoms however both in experimental animals and human beings were other than

R.V.V.
In the envenomated guinea pigs platelet count was found to be considerably reduced as compared to the controls which is in line with the observations of others (Rechnic et al., 1962; Philips et al., 1973). Similar results have also been shown for different snake venoms in human beings (Chan and Reid. 1964; Hashmi et al., 1969; Sezi et al., 1972).
Present results indicate that fibrinogen levels appreciably decreased after R.V.V. envenomation. In the animals administered 1 MLD, no fibrinigen could be detected at 12 and 18 hours. However, it started reappearing at 24 hours in some animals. The findings are supported by the work of others (Rechnic et al., 1962; Mohammad et al., 1969; Philips et al., 1973) who have reported hypofibrinoge nemia and afibrinogenemia in experimental animals injected with various snake venoms. Studies in man (Chan and Reid, 1964; Hashmi et al., 1969; Bhargava et al., 1977) have shown similar results. The results with PT, APTT, TT and CT together with marked reduction of fibrinogen and plate-lets strongly support the contention that disseminated intravascular coagulation (DIC) occurs after RVV envenomation and that the over all picture seems to be consumption coagulopathy resulting in defibrination syndrome.

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