PROTECTIVE ACTION OF HEPARIN AND EDTA AGAINST RUSSELL'S VIPER VENOM

By

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It has been reported that proteolytic enzymes in snake venoms contribute to their toxicity either by their necrotic action or their strong coagulant action. The correlation between proteolytic activity and the coagulant action has been reported by different workers (Kellaway, 1939; Kellner and Robertson, 1954). Anticoagulants like heparin have been reported to neutralize Russell's viper venom *in vitro* by Ahuja *et al.* (1946) and *in vivo* to the extent of 1.5 CLD (Certainly Lethel Dose) by Rao and Rao (1957). Since ethylenediaminetetraacetic acid (EDTA) is known for its anticoagulant action and also for its marked antiproteolytic activity (Deutsch and Diniz, 1955), we decided to study if it had a protective action against Russell's viper venom by itself or in combination with heparin.

METHOD

The toxicity experiments were carried out by diluting Russell's viper venom with normal saline to give concentrations between 0.02 and 0.005 mg/ml. 0.5 ml. of the solutions of different dilutions were injected intravenously. A group of six mice weighing 20 g. each was used for each observation as well as for the control. The highest dilution which killed all the six mice within 24 hours was taken as containing one CLD in 0.5 ml. The sample of Russell's viper venom used in the present investigation was found to have a CLD of 1:120,000 i.e. 0.0083 mg. Similarly, the toxicity tests for heparin and EDTA were carried out and 200 I. U. of heparin in 0.5 ml and 0.5 ml of 0.1 percent sodium salt of EDTA intravenously and 0.5 ml of 1.0% EDTA subcutaneously were chosen for the study of their protective action against Russell's viper venom. These were roughly one half their lethal dose. Controls with venom, heparin and EDTA were included.

RESULTS AND CONCLUSION

It was found that EDTA alone had no protection against Russell's viper venom if injected intravenously in a mixture with one lethal dose of Russell's viper venom. It was, however, found that if EDTA is injected subcutaneously (0.5 ml of 1.0 per cent solution) followed by Russell's viper venom intravenously after 30 minutes, it could protect mice against 1.5 CLD of the venom as evident in Table I.

TABLE I

Protective action of EDTA.

Dilution of venom injected 30 min. after injecting 0.5 ml of 1% EDTA subcutaneously.	CLD of venom in 0.5 ml	Number of mice out of six surviving after 24 hours
1/120,000	1.0	6
1/80,000	1.5	5
1/60,000	2.0	0

Table II gives the results obtained with a combination of EDTA with heparin. A mixture of heparin (200 I. U.), sodium salt of EDTA (0.5 mg.) and different quantities of venom in 0.5 ml of normal saline were incubated at 37°C for 30 minutes before injecting intravenously into white mice weighing about 20 g.

TABLE II.

Protective action of heparin and EDTA against Russell's viper venom injected intravenously in mice

COMPOSITION OF MIXTURE INJECTED			Number of mice out of six surviving after 24 hours
Heparin	EDTA	CID of venom	
–	–	1.0	0
200 I. U.	–	–	6
–	0.5 mg.	–	6
200 I. U.	0.5 mg.	1.0	6
200 I. U.	0.5 mg.	1.5	6
200 I. U.	0.5 mg.	2.0	6
200 I. U.	0.5 mg.	2.5	4
200 I. U.	0.5 mg.	3.0	2
200 I. U.	0.5 mg.	4.0	0

It can be seen from Table I that EDTA (5 mg) alone protects mice upto 1.5 CLD of Russell's viper venom if given subcutaneously before giving the dose of the venom. In combination with heparin it protects mice upto 2.0 CLD if given intravenously with the venom (Table II)

It follows therefore that a combination of EDTA and heparin is slightly more effective than either heparin alone or EDTA alone. This protection, however, cannot be considered of practical value as antivenins can neutralize over 100 lethal doses of such venoms per ml of antivenin (Kulkarni and Rao, 1955).

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