EFFECT OF ANTIPLASMIN ON AFIBRINOGENEMIA CAUSED BY INTRAVENOUS INJECTION OF VIPER (Vipera lebetina) VENOM

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Intravenous injection of 1 ml of a 0.01% solution of venom from the viper Vipera lebetina into rats causes a sharp increase in the thrombin content in the blood, leading to massive intravascular blood clotting and death of the animals. If the venom is injected in smaller doses (0.5 ml of a 0.001% solution), the thrombin formed is neutralized through the protective reactions of the anticlotting system, accompanied by increased fibrinolysis and increased circulation of anticoagulants. The afibrinogenemia following injection of viper venom (0.5 ml of a 0.001% solution) is largely prevented by preliminary (before injection of the venom) and abolished by subsequent (after injection of the venom) administration of antiplasmin (30-60 mg).

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Investigations have shown that if the venom of vipers, especially of Vipera lebetina, enters the blood stream of animals and man a severe afibrinogenemia develops, leading to partial or total loss of the clotting power of the blood and to its high fibrinolytic activity [1]. To correct the high, pathological fibrinolysis and to restore the clotting power of the blood in poisoning with snake venom, the substance ε-AKK has been successfully used [1].

In the present investigation the substance used for this purpose was the natural inhibitor of fibrinolysin, known as antiplasmin. In a previous investigation antiplasmin abolished the pathologically high fibrinolysis developing in animals after intravenous injection of tissue thromboplastin [4].

EXPERIMENTAL METHOD

Experiments were carried out on albino rats of both sexes weighing 170-190 g. The venom of V. lebetina was obtained from the Tashkent Snake Nursery and used as a solution in 0.85% NaCl. The fibrinogen preparation (fraction 1 "0") was prepared by Cohn's method and purified by Blomback's method. Thrombin, purified from plasminogen, was prepared by Niewiarowski's method [11], and antiplasmin by Loomis's method [9].

The fibrinogen concentration was determined by Bidwell's method and expressed in mg% [7]. The fibrinolytic activity of the blood was determined by Astrup's method in fibrin disks heated for 30 min to 82-84° [6].

TABLE 1. Thrombin Activity in Blood after Intravenous Injection of V. lebetina Venom (0.5 ml of 0.001% solution)

Experimental conditions	No. of animals	Thrombin activity (in sec) after injection		
		1 min	5 min	15 min
Injection of viper venom	15	181±16	187±30	280±33
Injection of physiological saline	12	450±22 <0,01	445±70 <0,01	476±24 <0,01

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TABLE 2. Fibrinogen Content and Fibrinolytic Activity of Blood and Thrombin Time in Animals Receiving Antiplasmin before Intravenous Injection of V. lebetina Venom

Experimental conditions	No. of animals	Fibrinogen (in mg%)	Fibrinolytic activity (in %)	Thrombin time (in sec)
Injection of viper venom	16	43±10	182±14	169±29
Injection of antiplasmin + viper venom	17	150±18 $P_{I-II}<0,01$	127±11 $P_{I-II}<0,01$	43±7 $P_{I-II}<0,01$
Injection of physiological saline	16	240±27 $P_{II-III}<0,02$	100±11 $P_{II-III}>0,05$	12±2 $P_{II-III}<0,01$

The activity (or concentration) of thrombin in the blood was determined by Glueck's method [8] and expressed as clotting time of the reacting mixture (or in units of thrombin).

The substances were injected into the jugular vein. Blood was taken from the same vessel for testing. The results were analyzed by the statistical method of quantitative estimation of a pharmacological effect [2].

EXPERIMENTAL RESULTS

The experiments in vitro first showed that the preparation of viper venom has no fibrinolytic activity, does not contain even traces of thrombin, and has no significant thromboplastic activity (shortens the recalcification time of normal plasma from 81 to 19 sec; the concentration of venom for these tests was 0.001%).

In the experiments of series I, intravenous injection of 1 ml 0.01% viper venom solution caused death of the animals in the first 10 min after injection. At autopsy massive thrombi were found in the chambers of the heart, in the lungs and the large and small blood vessels. If the dose of venom was reduced to 0.5 ml of a 0.001% solution, as a rule neither death of the experimental animals nor thrombus formation was observed.

The venom of V. lebetina, which possesses thromboplastic properties, can lead to thrombin formation if it enters the blood stream [10]. Determination of the activity (or concentration) of thrombin in the circulating blood (Table 1) showed that it is formed in largest amounts during the first minute after injection of viper venom. If the venom was injected in a dose of 1 ml of 0.01% solution, the taking of blood with anticoagulant during the first minute after injection was accompanied by clotting of the blood in the syringe; this demonstrated the formation of large quantities of thrombin.

After injection of 0.5 ml of a 0.001% solution of venom the quantity of thrombin formed in the first minute, recorded on the scale for low thrombin concentrations, was $0.3 \cdot 10^{-5}$ unit (or 181 sec when measured as clotting time of the reacting mixture). The content of thrombin found 5 and 15 min after injection of venom was reduced through its inactivation, but not down to the physiological level. This indicates that the character of thrombin neutralization after injection of venom and tissue thromboplastin is different in character [5]. Thrombin formed by the action of venom stays in the blood stream longer. The concentration of fibrinogen also was lower (126 ± 13 mg%) 5 min after injection of the viper venom (0.4 ml of 0.001% solution), but the fibrinolytic activity (166 ± 13%) and anticoagulant activity (thrombin time 229 ± 33 sec) were increased in these animals. In the control animals these indices were 238 ± 17 mg%, 100 ± 11%, and 12 ± 2%, respectively. These changes described are protective in character and directed against the thrombus-forming action of the venom. This reaction is analogous to the protective reaction of the anticlotting system in response to direct injection of thrombin or thromboplastin into healthy animals [3].

In the next experiments antiplasmin was used to correct the afibrinogenemia developing in the animals following administration of viper venom. Intravenous injection of antiplasmin was given 2-3 min before injection of the venom (0.5 ml of a 0.001% solution). Blood was taken 5 min after injection of the poison.

The results given in Table 2 show that preliminary injection of antiplasmin largely, but not completely, prevented the development of hypofibrinogenemia.

In subsequent experiments antiplasmin was injected twice (3 and 10 min after injection of the viper venom), when the animals had already developed hypofibrinogenemia. The results demonstrate that by giving two injections of antiplasmin, the fibrinogen concentration in the experimental animals could be kept at a higher level than in control animals receiving an injection of venom only (114 ± 25 mg% in the experimental animals, 47 ± 8 mg% in the controls; $P < 0.02$). The fibrinolytic activity of the blood and the thrombin time of the experimental animals were considerably reduced compared with those in the control rats (fibrinolytic activity in the former 183 ± 14%, in the latter 137 ± 12%; $P < 0.02$; thrombin time 149 ± 17 and 300 ± 27 sec, respectively; $P < 0.01$; in normal animals, fibrinogen content 240 ± 27 mg%, fibrinolytic activity 100 ± 11%, thrombin time 12 ± 2 sec).

Injection of antiplasmin into the animals (either preliminarily or after injection of V. lebetina venom) largely, but not completely, prevented or corrected the afibrinogenemia..

LITERATURE CITED

1. Z. S. Barkagan, Probl. Gematol., No. 8, 10 (1965).
2. M. L. Belen'skii, Elements of Quantitative Evaluation of a Pharmacological Effect [in Russian], Riga (1959).
3. B. A. Kudryashov and P. D. Ulitina, Dokl. Akad. Nauk SSSR, 120, No. 3, 677 (1958).
4. V. E. Pastorova and B. A. Kudryashov, Byull. Éksperim. Biol. i Med., No. 2, 64 (1967).
5. V. E. Pastorova and B. A. Kudryashov, Probl. Gematol., No. 6, 16 (1968).
6. T. Astrup and S. Müllertz, Arch. Biochem., 40, 346 (1952).
7. E. Bidwell, Biochem. J., 55, 497 (1953).
8. H. J. Glueck, J. Appl. Physiol., 6, 650 (1954).
9. E. C. Loomis, A. Ryder, and C. Georye, Arch. Biochem., 20, 444 (1949).
10. L. Nahas et al., Thrombos. Diasthes. Haemorrh. (Stuttgart), 12, 355 (1964).
11. S. Niewiarowski, Krzepniecie Krwi. Warsaw (1960), p. 125.