MECHANISM OF PROLONGED HYPOCOAGULATION FOLLOWING INTRATRACHEAL INJECTION OF HEPARIN

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The creation of long-lasting, moderate hypocoagulation is an essential therapeutic component in the treatment of various diseases whose course is complicated by the DBC syndrome [1, 4]. However, this effect is difficult to achieve by parenteral injection of heparin because of the rapid elimination of the substance from the blood stream and the risk of various complications following multiple injections.

The least traumatic method of achieving the required effect of heparin is by administering it through the lungs [9]. Experimental studies, confirmed by clinical observations, have shown that even a single intrapulmonary injection of heparin can induce long-lasting (up to 14 days), moderate hypocoagulation [9-13]. The mast cell population, which stores the preparation after intratracheal injection, promoting its prolonged action on the blood clotting system, exerts a stabilizing action on the heparin concentration in the blood stream [2, 5, 6]. Meanwhile, the character of interaction of heparin entering the blood stream via the lungs with blood clotting factors has not been studied. This makes interpretation of changes in integral coagulation tests difficult and limits the indications for the use of this safe and highly effective method of heparin administration for therapeutic and prophylactic purposes.

The aim of this investigation was to compare the effect of heparin, injected intratracheally and intravenously, on isolated factors of blood clotting and to examine relations between the response of the clotting system and the state of the mast cell population.

EXPERIMENTAL METHOD

Experiments were carried out on male rats, divided into three groups: animals of group 1 received heparin intratracheally in a dose of 12,500 U/kg body weight (volume of heparin injected 0.5 ml), those of group 2 received heparin intravenously in a dose of 500 U/kg, and animals of group 3 (control) received 0.9% NaCl solution intratracheally (volume of NaCl solution injected 0.5 ml). Blood plasma was obtained by centrifugation (2000*g*, 5 min). The coagulability of the blood was assessed by the thromboelastograms (TEG), obtained on apparatuses obtained from the firm of "Hellige" (West Germany). Activity of the blood clotting factors was determined with the aid of a Schnitger und Gross coagulometer (Switzerland), using reagents and techniques of the firm Dia Med (Switzerland). The plasma heparin concentration was determined with the aid of a diagnostic kit based on the chromogenic substrate and technique of the firm Boehringer–Mannheim (West Germany). The molecular weight of the heparin preparation was established by gel-filtration (column length 0.85 m, diameter 16 mm, sorbent Sephacril G-200, eluant 0.9% NaCl, rate of elution 35 ml/h).

Specimens [5] were prepared from a suspension of mast cells obtained from peritoneal washings [14]. Depending on weakening of the degree of metachromasia and the number of granules visible in the cell, four types of mast cells were distinguished [3].

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TABLE 1. Changes in Blood Heparin Concentration after Intravenous and Intratracheal Administration

Time after administration, h	Administration of preparation		
	intravenous, of preparation	intratracheal USP U/ml	control USP U/ml
2	0,182±0,042	0,284±0,124*	0,104±0,004
5	0,040±0,008	0,316±0,086*	0,084±0,020
24	0,034±0,018	0,125±0,020*	0,083±0,012

Legend. *$p < 0.05$, $n = 6$.

EXPERIMENTAL RESULTS

A significant lengthening of the "r + k" parameter of the TEG (from 2.9 ± 1.5 min in the control to 8.0 ± 4.8 min) was observed 2 h after intravenous injection of the heparin preparation, evidence of preservation of the hypocoagulation effect at the time of observation. Significant differences in the TEG parameters from the control were no longer present 5 and 24 h after intravascular injection ("r + k" = 2.5 ± 0.7 min).

Complete noncoagulability of the blood was recorded in 30% of observations made 2 h after intratracheal injection of heparin, and in the remaining cases profound hypocoagulation was observed ("r + k" over 9 min) A maximum of the anticoagulant effect was noted 5 h after instillation, when the blood of 100% of the rats did not clot. After 24 h only a weak hypocoagulation effect was observed ("r + k" = 4.5 ± 2.2 min).

Throughout the time of investigation a significantly raised plasma heparin level was detected after intratracheal administration of the drug (Table 1).

Investigations of activity of blood clotting factors showed that as early as 2 h after insufflation of heparin through the trachear, profound inhibition of the factors of the external and internal ways of thrombin formation took place. At the same times after intravenous injection of heparin, mainly factors of the internal path were affected, Activity of the majority of factors was restored 5 h after intravenous injection of heparin, when activity of factors IX, XI, and XII was significantly increased. After intratracheal injection, at this time only partial recovery of the factors of the prothrombin complex was observed, and inactivation of the factors of the "inner path" was even stronger, with the exception of factor V (Fig. 1).

The hypocoagulation effect 24 h after intratracheal insufflation of heparin is realized through continuing inhibition of factors of the inner path of coagulation. After intravenous injection, complete restoration of activity and partial hyperactivation of the factors studied took place.

We know that, compared with unfractionated heparin, its low-molecular-weight fractions have a weaker effect on combined activity of factors VIII, IX, XI, and XII, determined by the APTT test, but at the same time, their effect on activity of factor X is stronger [8].

The USSR/CIS commercial heparin which we used is a polydispersed preparation consisting of a mixture of molecules with mol. wt. ranging from $4 \cdot 10^3$ to $4 \cdot 10^4$ daltons (D). The bulk of the preparation consists of molecules with mol wt. of about $(1\text{-}2) \cdot 10^4$ D.

During the first few hours after their introduction into the lungs, low-molecular-weight heparin fractions passed through the air–blood barrier, after which larger molecules enter the blood stream. Inactivation of factor X is therefore most marked 2 h after intratracheal insufflation of heparin. Inhibition of factors VIII, IX, XI, and XII, observed after 5 h, may be associated with delayed entry of the heavier heparin molecules into the blood stream. When combined with AT-III molecules they cause prolonged inhibition of the above-mentioned clotting factors, which exhibit increased sensitivity to the heparin–AT-III complex.

The intensity of inhibition of activity of the clotting factors clearly coincides with the blood heparin time course and indicates the formation of depots of this anticoagulant after intratracheal insufflation. The mast cell population is able to store and secrete heparin. Analysis of the response of the mast cells to intravenous and intratracheal administration confirms this conclusion (Table 2).

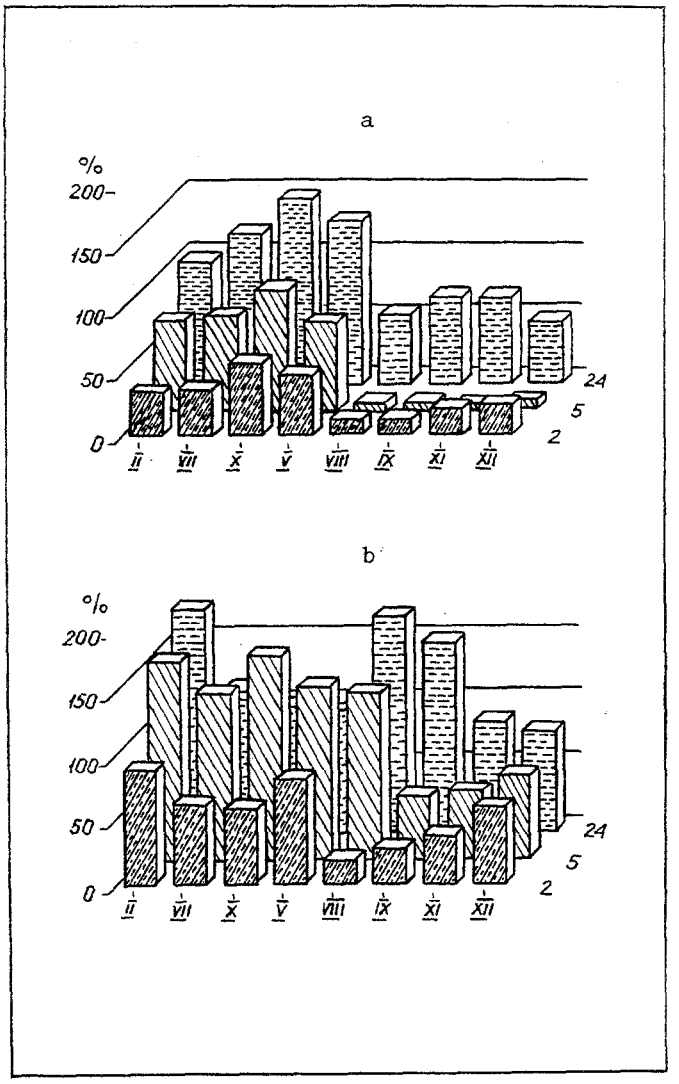

Fig. 1. Changes in activity of blood clotting factors (in %) 2, 5, and 24 h after intratracheal (a) insufflation of heparin preparation (12,500 U/kg) and after intravenous injection (b) (500 U/kg).

Saturation of the cells with heparin clearly takes place very rapidly. Only 10 min after its intravenous injection, significant "darkening" of the mast cell population is observed, indicating uptake of heparin from the blood by these cells. The same effect was found in a study of the mast cell population of film preparations of the mesentery [3] In a study with radioactive labeling, accumulation of heparin in mast cells in the peritoneal cavity was recorded as early as 1 min after intravenous injection of heparin [7].

Staining of the mast cell population returned to normal 24 h after intravenous injection.

An essential distinguishing feature of intratracheal insufflation of hepatin is the increase in saturation of the mast cells with heparin, which proceeded steadily throughout the period of investigation, evidence of its prolonged existence in the mast cells (Table 2). This confirms the existing view that mast cells are involved in the redistribution and storage of heparin by the cell pool, maintaining a prolonged hypocoagulation effect [2, 5, 6].

The mechanism of the action of heparin on blood coagulability after intravenous and intratracheal administration thus differs. Unlike intravenously injected heparin, which inactivates all clotting factors for a short time, intratracheal insufflation gives a prolonged hypocoagulation effect on account of inhibition of activity of the factors involved in the internal path of thrombin formation. The duration of the hypocoagulating action of heparin, administered by the intratracheal route, and the character of inhibition of the coagulation factors, correspond to a change in the metachromatic staining properties of the mast cell population.

TABLE 2. Changes in Degree of Saturation of Mast Cell Population by Heparin after Intravenous and Intratracheal Administration of Substances (results of psychochemical analysis, in %)

Time after administration	Type of cells	Mode of administration			
		intravenous		intratracheal	
		NaCl	heparin	NaCl	heparin
10 min	1	37	44*	32	51**
	2	30	19*	32	30
	3	25	28	11	9
	4	7	7	21*	9*
24 h	1	25	19	30	67**
	2	28	21	35	18**
	3	27	33	29	18*
	4	16	24	1	1

Legend. *$p < 0.05$ Compared with NaCl, **$p < 0.05$ compared with the other mode of administration, $n = 6$; data shown in median form.

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