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MAY 77 E WERLE, I TRAUTSCHOLD

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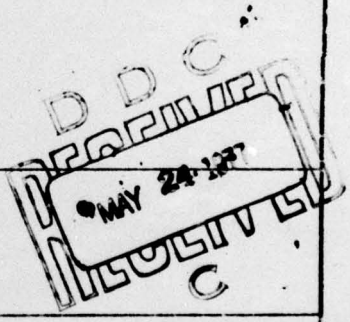

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Determination of the kallikrein inactivator "Trasylol^{R}" in the blood and organs and its distribution after injections

by

E. Werle and I. Trautschold

From: Clinical Chemistry Department of the Surgical Clinic of the University of Munich (Director: Prof. E. Werle, M.D.)

Summary

This paper describes A method ~~is described~~ suitable for clinical use for the quantitative determination of the kallikrein inactivator (Trasylol) in the blood after parenteral administration. Its applicability is demonstrated based on animal experiments.

Kallikrein inactivator from bovine parotid glands is administered parenterally in the form of Trasylol* for various indications (1). This disclosed the need for a method of determination of the kallikrein inactivator in blood and tissue fluids. So far, quantitative determination was only possible with a relatively broad margin of error for pure inactivator solutions, by measuring the kallikrein inactivating effect on the blood pressure of anesthetized dogs. The qualitative determination is based on the fact that Trasylol is capable of inhibiting fibrinolysin (plasmin). This inhibition can be seen on the thrombelastogram (1d).

* Trasylol is the brand name of the Farbenfabrik Bayer Company.

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The new photometric method developed by us for the determination of kallikrein activity and its inactivators (2)** has proved to be eminently usable in a somewhat modified form for the determination of free inactivator in the blood, body fluids, tissues and urine after the administration of Trasylol. It has been established that upon deproteination of whole blood or organ homogenates mixed with Trasylol by means of perchloric acid, the inactivator remains quantitatively in the supernatant and after neutralization and precipitation of the perchlorate without loss, it can still be detected therein in a concentration of 0.1 kallikrein inactivator units/ml. The inactivator is not decomposed by the blood enzymes even after a prolonged period of incubation at 37°; therefore it can still be quantitatively demonstrated in blood samples that have been left to stand for several hours. Citrated arterial whole blood was mixed with 10 kallikrein inactivator units per ml blood and after 60 min incubation at 37° the inactivator distribution was investigated in the plasma and erythrocytes. Almost the entire inactivator activity was found in the deproteinated plasma, while the erythrocytes contained only traces of inactivator.

In order to test the applicability of the method we studied the distribution of Trasylol after intravenous, intramuscular and intraperitoneal injection and after continuous infusion

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** It is based on the capacity of kallikrein to split benzoyl-arginine ethylester esterolytically. The course of the splitting can be followed on extinction measurements at 254 mμ.

into the blood and organs of dogs and rats. The experiments were carried out on anesthetized dogs. The total dose used was 100 kallikrein inactivator units/kg body weight.

Method and evaluation

Five ml blood was drawn at given intervals after the Trasylol injections, it was mixed with 5.0 ml 6% cold perchloric acid and mixed thoroughly. This was followed by brief centrifugation and an aliquot portion (V_1) of the clear supernatant was neutralized with 2n KOH (V_2). After standing briefly at 0° the liquid was poured off the $KClO_4$ precipitate and this neutralized supernatant was incubated with a known quantity of kallikrein (V_4) for 30 min at 37° and pH 8.0 for determination of the inactivator (V_3). The determination of the kallikrein activity with and without inactivator, and therewith of the inhibition (H), is measured on the splitting of benzoylarginine ethylester by kallikrein, which has been described elsewhere***. The inactivator concentration should be so selected that inhibition is less than 50%, since inhibition is only proportional to the inactivator concentration up to 50%. The kallikrein inactivator unit is the quantity of inactivator which is capable of inactivating 2 kallikrein units by half in 1 hour at pH 8 and 37°. The quantity of inactivator in the blood is calculated according to the

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The inactivator contained in the deproteinated solutions can also be detected based on the fact that it is capable of inhibiting the proteolytic effect of trypsin (3).

following formula:

$$\frac{V_2 \cdot H \cdot V_4}{V_1 \cdot V_3 \cdot 50} = \text{kallikrein inactivator units/ml blood}$$

V_1 = volumes for neutralization in ml

V_2 = volumes after neutralization in ml

V_3 = volumes for incubation with kallikrein in ml

V_4 = kallikrein quantity used in kallikrein units

H = inhibition %

To calculate the inactivator content/g tissue in organ homogenates, the result must be multiplied by the dilution factor with which the tissue was homogenized. The behavior of the Trasylol level in the blood is shown in Fig. 1 after various routes of administration. According to the figure, immediately after intravenous injection of the Trasylol quantity cited above there is, for instance, a blood level of ca. 15 kallikrein inactivator units/ml, which dropped within 1 hour to 4 kallikrein inactivator units/ml. Similar values were found upon repetition of the experiments. Three intramuscular injections were administered into the thigh musculature. In the first 30 min after administration Trasylol was not detectable in the blood. After 1 hour a maximum of ca. 3.5 kallikrein inactivator units/ml was reached. Two hours after the injection there only remained 1 kallikrein inactivator unit/ml, that is to say the decrease was more rapid than after intravenous injection. If the free inactivator quantity active for 5 hours after intravenous injection is

mixed with the blood and taken as equal to 100, after an intramuscular injection only approximately 30% has become active.

Conditions are considerably better after intraperitoneal injection. There is an immediate rise in the inactivator concentration in the blood with a maximum of 3 kallikrein inactivator units/ml within 90 min. Decrease takes place slowly. The 5-hour value is still only 1.5 kallikrein inactivator units/ml. The active portion of the inactivator after intraperitoneal injection is ca. 75%.

Inactivator was infused at a constant rate for 82 min. Equilibrium between supply and elimination set in within 30 min with ca. 3.5 kallikrein inactivator units/ml, which was maintained until the end of the infusion; thereafter the inactivator level dropped again relatively rapidly. The degree of activity compared to intravenous injections was ca. 65%.

Of all routes of administration the intravenous appears to be the most suitable, since it gives rise to an immediate high Trasylol level which does not drop more rapidly than after the other routes of injection. The drop in the inactivator content of the blood after a Trasylol infusion was observed in 3 patients.

Patient 1 received 25,000 units of Trasylol infused within 30 min on 2 successive days. No Trasylol could be detected in the blood 24 hours after the last infusion.

Patient 2 received an infusion of 25,000 units on the

first day and 50,000 units on the second day. The drop in the inactivator content is shown in Table 1.

Samples of the 24-hour urine of both patients, whose urinary excretion was normal, were devoid of Trasylol.

We were also able to demonstrate the clinical application of the method on blood samples of a patient with severe acute pancreatitis who was treated with Trasylol* (Table 1). It was found that the drop in the blood of patient 3 took place considerably more slowly than in patient 2 or in animal experiments, and that 24 hours after the injection there still remained a remnant of inactivator, the size of which was a function of the prior dose, actively circulating in the blood. A urine sample of the patient on the third day of Trasylol administration was devoid of inactivator. Possibly this delayed elimination of Trasylol from the blood was due to an existing renal insufficiency.

For temporary orientation concerning the time spent by the inactivator in the organism we have tested the inactivator content of urine in dog experiments and found only small quantities, while in certain organs, such as the liver and kidneys, there was an accumulation.

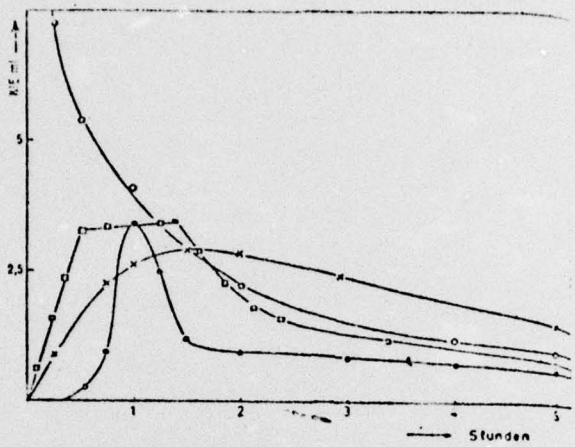
The same Trasylol dose was administered a second time to 2 experimental dogs a few hours after the first one. After the second injection, intravenous in one dog and intramuscular

* We are indebted to Prof. M.M. Forrell, M.D., IInd University Clinic for Internal Medicine, Munich, for the blood samples.

in the second, the rate of elimination of inactivator from the blood had not changed in comparison to conditions after the first injection.

Very high doses were injected to study inactivator excretion in the urine, so that despite a possible enzymatic degradation of the inactivator, excretion should still be detectable. For this 15,000 kallikrein inactivator doses were injected twice intravenously to rats on successive days. The blood level after one such injection was only 250 kallikrein inactivator units/ml blood ca. 5 min after the injection. Thus in the rat the inactivator disappears quickly from the blood. The 24-hour urine (ca. 10 ml) of animals that had received 30,000 kallikrein inactivator units contained up to 500 kallikrein inactivator units/ml. Shortly after the injection the organs of the animals showed a high inactivator content, which was the highest in the kidneys with 4,000 kallikrein inactivator units/g compared to 600 kallikrein inactivator units/g liver and 450 kallikrein inactivator units/g spleen. It follows from this that the excretion of intact inactivator, at least in the rat, takes place via the kidney. Since the inactivator quantity does not rise, it appears that it is partially decomposed in the organism.

We are indebted to Miss R. Zill for conscientious assistance.



Figures 1

Fig. 1. Concentration of kallikrein inactivator (Trasylol) in the blood of anesthetized dogs after administration of 1000 kallikrein inactivator units/kg body weight. ___o___o___= intravenous, ___●___●___= intramuscular, ___x___x___= intraperitoneal, ___ ___ ___ = infusion.

Tabelle 1

Inaktivatorgehalt im Serum von Patienten nach Verabreichung hoher Dosen Trasylol

Patient Nr.	Tag	Verabreichte Inaktivatordosis i. v.	Inaktivatorgehalt/ml Serum		
			Vor Infusion	Nach Infusion	1 Std. nach Infusion
2	1	25 000	0	3	1,5
	2	50 000	0	7,5	3,5
	3	0	0,2	—	—
3	1	15 000	0	2	
	2	30 000	0,75	5	
	3	45 000	1,1	10	
	4	60 000	2,4	19,5	
	5	0	—	—	
	6	0	4,4	—	

Tables 1.

Table 1. Inactivator content in the serum of patients after administrration of high doses of Trasylol. Key: 1. -. 2. Patient no. 3. Day. 4. Intravenous dose of inactivator administered. 5. Inactivator content/ml serum. 6. Before infusion. 7. After infusion. 8. One hour after infusion.

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p. 775

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Address: Prof. E. Werle, M.D. and Dr. I. Trautschold, Chirurigische Universitäts-Klinik, Nussbaumstrasse 20, Munich 15, W. Germany.