

## USE OF A KALLIKREIN INHIBITOR IN THE TREATMENT OF URTICARIA AND HEREDITARY ANGIONEUROTIC EDEMA

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Patients with hereditary angioneurotic edema are known to lack an inhibitor of kallikrein which normally prevents the release of kinins (4, 17). The kinins released cause an increase of the capillary permeability with formation of edema. Why the edema is localized to certain areas is not known. Patients with chronic urticaria show an increased reactivity to injected kallikrein which might also be caused by a decrease of kallikrein inhibitor (14).

Frey and Kraut (8) observed that normal blood inhibited kallikrein. It was later shown that plasma contained two kallikrein inhibitors, and a third with a lower molecular weight than those in plasma was isolated from the lung, spleen, liver, lymphatic glands and parotid gland (9). An inhibitor purified from the parotid gland and the lung of cattle is commercially available.<sup>1</sup> It has a molecular weight of approximately 6500 and is built up of 58 amino acids, the sequence of which has been recently clarified (Fig. 1). In addition to kallikrein this product (TR.) also inactivates trypsin, chymotrypsin and plasmin (9). It is used clinically mainly to counteract fibrinolysis in abruptio placentae and in extracorporeal circulation, in conditions with hypercoagulability, and in acute pancreatitis. Its clinical significance has been

discussed at a symposium in 1965 (11).

In this investigation we have studied the clinical effect of TR. in the treatment of patients with urticaria and hereditary angioneurotic edema. We have also investigated its ability to inhibit skin reactions to kallikrein, bradykinin and histamine.

### Material and Methods

*Drugs used:* Histamine hydrochloride<sup>2</sup> 0.1 mg/ml. Synthetic bradykinin<sup>3</sup> 0.1 mg/ml. Kallikrein.<sup>4</sup> The dry powder containing kallikrein 40 U, thiomersalsodium 0.02 mg and sodium chloride 3.44 was dissolved in 1 ml of saline. The kallikrein inhibitor TR.<sup>1</sup> 20,000 KIU/ml (kallikrein inhibiting units according to Werle, ref. 9 p. 7).

*Patients:* The patients and their diagnoses are listed in table 1. The number of patients Nos. refer to those used in an earlier investigation where the clinical data and the skin reactions to histamine, bradykinin and kallikrein in urticaria were described (14). The clinical picture of patients A and B with hereditary angioneurotic edema has also been published elsewhere (15).

*Experimental procedure:* Intradermal injections of 0.1 ml of histamine, bradykinin

<sup>1</sup> Commercial name: Trasylol®. In the following abbreviated TR. It was kindly supplied by Bayer Ab, Leverkusen, Germany.

<sup>2</sup> Vitrum AB, Stockholm, Sweden.

<sup>3</sup> BRS 640. Kindly supplied by Sandoz, Stockholm, Sweden.

<sup>4</sup> Padutin®. Kindly supplied by Bayer AG, Leverkusen, Germany.

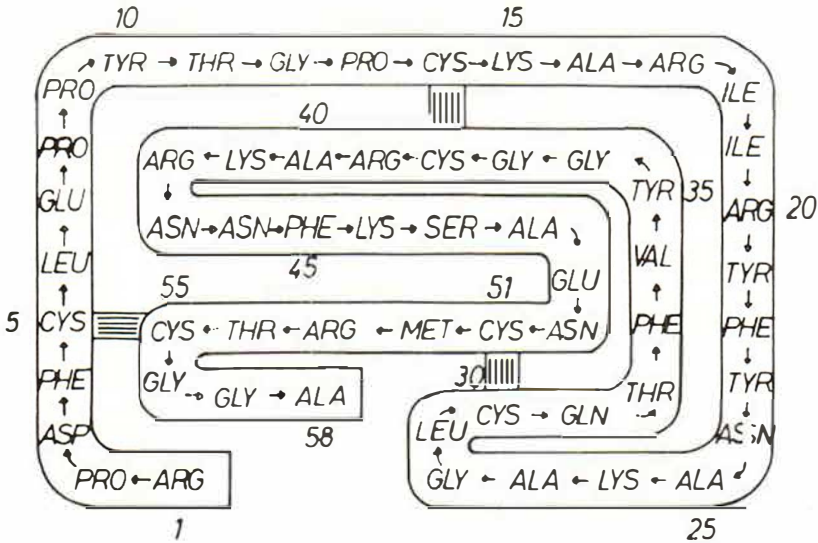


Fig. 1. Primary structure of Trasylol (from Anderer and Hörnle, ref. 2).

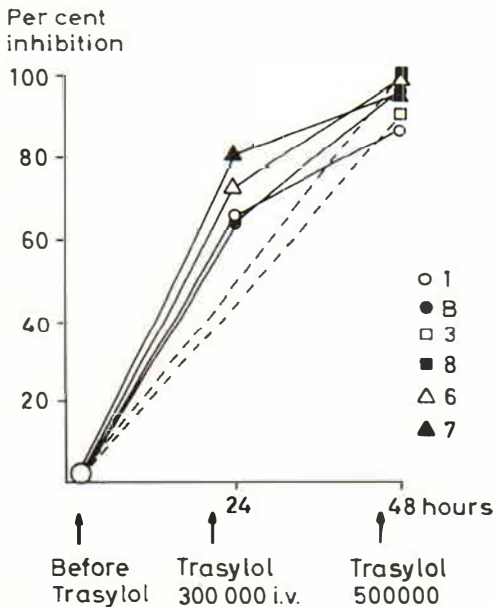


Fig. 2. Trasylol inhibition of kallikrein induced edema. The curves show the Trasylol induced percentual decrease of the edematous area seen 5 hours after intradermal injections of kallikrein. The figures refer to the patient's number.

and kallikrein were given on the volar side of the forearm. The area of the erythema and of the wheal was estimated by measuring the diameters and calculating that they were regular ellipses. All measurements

were made by the authors, at 20 minutes and 1, 2, 5 and 24 hours after the injections.

On the following day 300,000 KIU of TR. was given as an intravenous infusion in 300 ml of saline during 3 hours. Intracutaneous tests as described above were then repeated in most patients. On the third day 6 of the patients were given an intravenous infusion of 500,000 KIU of TR. in 500 ml saline during 3-4 hours. Intracutaneous tests were repeated and care taken to avoid skin areas used previously. Freshly prepared solutions of kallikrein and TR. were used throughout.

Results

*Chronic urticaria:* Six patients with chronic urticaria were treated with intravenous infusions of TR. Patient No. 2 received 300,000 KIU within 3 hours which immediately stopped her itching and the urticarial wheals disappeared for 5 days. Itching wheals then reappeared. She was given the same dose of TR. and again the effect was prompt but five days later new urticarial lesions appeared. The other patients received 800,000 KIU of TR. within 2 days.

Their urticarial reactions disappeared within 24 hours after the start of TR. in-

Table 1. Patients treated with Trasylol

Pat. no.	Type of urticaria	Sex	Age	Area of edema 5 hours after i. c. inj. of kallikrein before Trasylol-infusion	Dose of Trasylol within 48 hours	Time after Trasylol with no urticaria	Side effects of Trasylol infusion
1	chronic	f	58	1509	800,000	90 days	Trombophlebitis at the site of first infusion
2	chronic	f	25	1965	a) 300,000 b) 300,000	5 days 5 days	None None
3	chronic	f	44	1981	800,000	5 days	Flushing, orthostatism, perspiration
6	chronic	f	48	4950	800,000	10 days	Flushing, hypersalivation, orthostatism, perspiration
7	chronic	f	63	7070	800,000	30 days	Flushing
10	chronic	f	49	1415	800,000	14 days	Flushing, perspiration
16	acute	f	34	393	300,000	no effect	More urticaria develops
28	cold	m	21	177	300,000	no effect	None
A	Her. ang. edema	f	49	7540	a) 700,000 b) <500	42 days —	None Anaphylactic shock
B	Her. ang. edema	f	25	1650	800,000	21 days	Metallic taste, orthostatism, swelling of abdomen, perspiration

fusions but reappeared within 5 to 90 days (Table 1). In patient No. 3 a throat infection during the premenstrual period may have contributed to the relapse and in patient No. 6 it was provoked by molds. The side effects appearing during or shortly after the infusion are listed in Table 1.

After treatment with TR. the cutaneous reaction to kallikrein became normal. The inhibition of the infiltrate appearing 5 hours after injection of kallikrein is shown in Figure 2. The inhibition is stronger after the second infusion of TR. The kallikrein reaction at 24 hours was inhibited in the same way. When the urticaria reappeared

the reaction to kallikrein was about the same as before treatment with TR. The same abnormal reaction to kallikrein as before treatment with TR. was, however, also seen in three patients before their urticaria had exacerbated. Injection of histamine and of bradykinin caused, in addition to the regular immediate response, a delayed reaction after 3-10 hours in patients Nos. 1, 3 and 7. The delayed response was not seen after the treatment with TR. but could be reproduced when the urticaria reappeared. No definite effect on the immediate response to histamine or bradykinin was observed after TR.

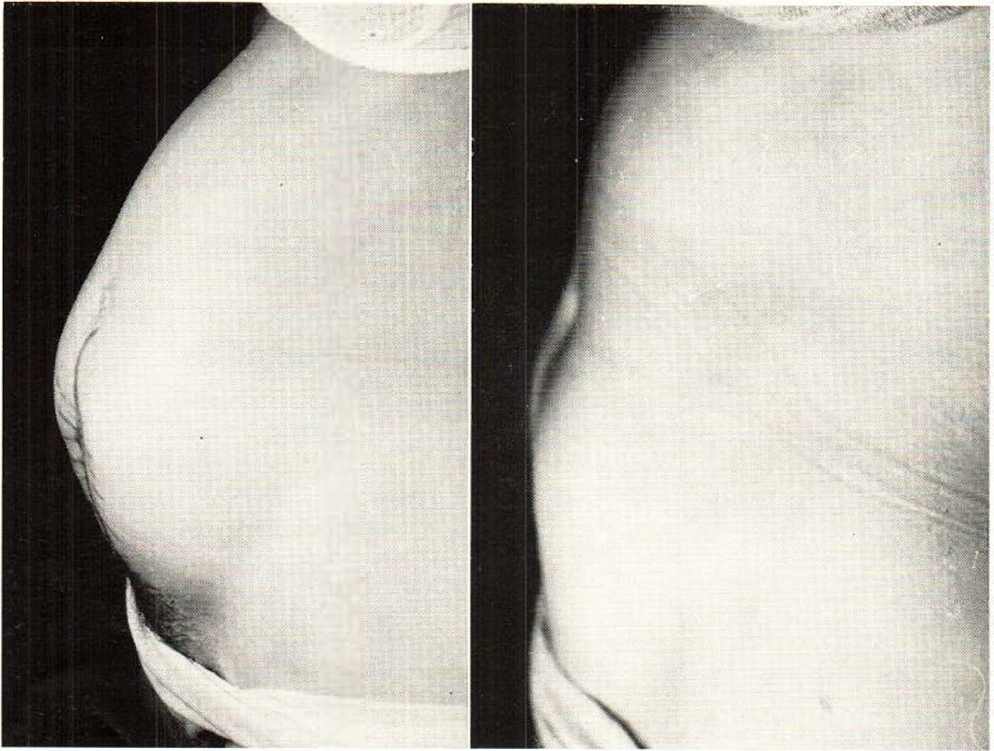


Fig. 3.

Left. Abdominal swelling 3 hours after termination of second infusion of Trasylol.

Right. Twenty-four hours later the swelling has disappeared.

*Acute and cold urticaria:* Intravenous infusion of 300,000 KIU of TR. was given to one patient with acute urticaria and one patient with cold urticaria. In the first patient more urticaria appeared during and after the infusion. The patient with cold urticaria showed the same degree of reaction to ice-cubes before and after infusion of TR. The reactions to kallikrein were unchanged after treatment with TR. in both patients.

*Hereditary angioneurotic edema:* Patient A received 100,000 KIU of TR. during an attack of pharyngeal and laryngeal edema. The patient felt immediately that the swelling stopped and the edema of the pharynx was seen to disappear within a few hours. No side effects were noted. On the following 4 days two additional infusions of 300,000 KIU each were given. After the infusions she still lacked the

C'1-esterase inhibitor in serum. The patient was then in good condition and had been without symptoms for 6 weeks when she got a minor attack of edema in the skin. She now showed a strong reaction to kallikrein at 5 and 24 hours. We therefore decided to treat her again with TR. An infusion containing 1000 KIU/ml was started slowly. About ten seconds after its start the patient felt a stinging sensation in the lips and tongue. She had probably received less than 500 KIU when the infusion was stopped. During the following minutes her lips became tense, she had paresthetic pricklings in the hands and feet and complained of thirst. Her blood pressure was 14c/80, and her pulse regular, 70 per minute. A few minutes later she became pale, the blood pressure was not measurable and the pulse was palpable only over the femoral artery. A swelling of the chest and especially of the

abdomen was evident. She was unconscious for 4 minutes and had spontaneous passage of urine and faeces. She recovered during treatment with oxygen, intravenous infusion of epinephrine, metaraminol bitartrate and corticosteroids.

*Patient B.* This daughter of patient A had prodromes of her disease in the form of restlessness and dysphoria. Antihistamines had been tried during the previous week without effect. She had a strong and tender reaction to kallikrein. Treatment with TR. was therefore decided on. During the first infusion of 300,000 KIU she felt a metallic taste and also felt warm for a few hours after the infusion, otherwise there were no side effects. On the following day 500,000 KIU of TR. was infused during 3 hours. About 1 hour after termination of the infusion she began to sweat and to have an orthostatic reaction. At the same time her abdomen started to swell. During the following hours the circumference of her waist increased by 15 cm (Fig. 3). She had had previous attacks of abdominal swelling but not of the same degree and they had always been combined with pains and signs of constipation or diarrhoea. She now lost her appetite during the first hours but was otherwise able to walk about in fairly good condition. The abdominal edema had almost disappeared the following day. She also felt more relaxed and the depression and restlessness had disappeared. Her increased reaction to intradermally injected kallikrein was normal after treatment. Before treatment with TR. there were also delayed reactions to intradermally injected histamine and bradykinin. After TR. the reactions to histamine and bradykinin also became normal. Her C'1-esterase activity was increased as before infusion and she still lacked the inhibitor. Three weeks after the infusions of TR. she had an abdominal attack, which was said to be less painful than usual.

*Intracutaneous tests with TR.:* After the severe anaphylactic reaction in patient A we decided to test the sensitivity of the patients to TR. 4-6 weeks after they had been treated. TR. was given intradermally in increasing concentrations beginning with

0.002 KIU in 0.1 ml saline. The dose was then increased 10 times for each 10 minutes until 200 KIU per wheal had been given. Doses of 2-200 KIU caused a slight erythema for 15-30 minutes in all patients. Patient no. 6 complained of nausea after the last injection of 200 KIU and hypersalivation and a drop in blood pressure were noted. She also had anorexia and had to lie down during the following hours. The next day she had completely recovered. In the other patients with chronic urticaria the test did not produce any general effects.

### Discussion

Our patients with chronic urticaria had suffered from daily eruptions of this condition for a long time. We therefore believe that the disappearance of the urticaria in these patients was not due to a temporary remission but was caused by the infusion of TR. The patients with hereditary angioneurotic edema often had periods of 1-2 weeks without symptoms. The effect of the treatment is therefore more difficult to judge but there was no doubt about the good influence on the acute laryngo-pharyngeal edema in patient A. She was convinced that the infusion had alleviated her condition. Thus, despite the fact that she had suffered from a most severe anaphylactic reaction to TR. she wanted her daughter to receive the same treatment. Our indication for the treatment with TR. was the strong reactions to kallikrein. No curative effect was obtained in two patients with acute and cold urticaria, respectively, where the reactions to kallikrein were normal. TR. has not been used previously for the treatment of urticaria although Nugent and Atendido (20), when describing a patient treated for pancreatitis, mentioned that he also had an urticarial exanthema which disappeared. Frey *et al.* in their recent monograph on kallikrein mention one patient with hereditary angioneurotic edema which had improved when treated with TR. by Werle (9 p. 241).

The half life of TR. in blood is short. When injected intravenously in rats the half life in blood was found to be only 30-60 minutes (9, 11). After an initial accumulation in the liver it is passed to the kidney and slowly broken down (9, 11). After one week the tissue level of TR. would probably not be sufficiently high to explain why some patients are free from symptoms for a longer period. One may therefore speculate on other possibilities such as an inactivation or depletion of precursor to kallikrein. This should mean, on the other hand, that at this time the patients should again have an increased reaction to intracutaneous injection of kallikrein. The patients were not regularly tested one week after treatment with TR. so that we do not know for what length of time the reaction to kallikrein is suppressed. On a few occasions tests with kallikrein were performed during the first week, with normal results. This can be explained by the presence of residual TR. in the tissues. On several occasions tests were performed weeks after treatment but before urticarial exacerbation. The reaction to kallikrein was then again abnormal. This means—if our theory is correct—that for several weeks there should still be a depletion or inactivation of kallikrein precursor. The mechanism of this is not clear.

Donaldson found *in vitro* that TR. in high concentrations (625 U/ml) partially inhibited the generation of C'1-esterase in the presence of Hageman factor, but did not block the esterolytic activity of activated C'1-esterase (6). In our patients with hereditary angioneurotic edema TR. did not influence the level of C'1-esterase inhibitor or C'1-esterase activity. The patients with urticaria had normal levels of C'1-esterase inhibitor. The inhibitors of kallikrein and C'1-esterase occurring in serum therefore are probably different although they have much in common (16).

The side effects produced by TR. in the form of hypersalivation, flushing, a feeling of warmth and, later, increased perspiration and a temporary orthostatic fall in blood pressure were regarded as being of minor importance. They might possibly be due to

liberation of histamine. The same mechanism is possible as an explanation for the abdominal swelling seen in patient B and the increased number of wheals and increased itching during infusion of TR. in the patient with acute urticaria. More dangerous are the allergic reactions. The incidence is said to be low (12), though reports on such reactions have been made. The antigenic properties may be neglected if the drug is only given during a short period. A survey of the literature reveals that an anaphylactic shock can occur among patients who have received repeated high doses of TR. We have found 8 cases of severe anaphylactic reactions in the literature (1, 3, 10, 13, 18, 21, 22). In these reports it is often mentioned that the patients reacted because earlier treatment had been given. It is difficult, however, to obtain information as to how often this occurs. The fact that over 100,000 infusions have been given with only 31 cases of reported hypersensitivity (12) does not necessarily mean that the incidence of such reactions is low when repeated doses are given over a long period.

Intracutaneous tests with TR. have been tried for the detection of hypersensitivity (19). Bumm *et al.* (3) found intracutaneous tests to be of no value since the erythema was the same in controls as in a patient who had developed an anaphylactic reaction during treatment with TR. From our experience we also consider such tests to be valueless. The injected dose might, however, be used to provoke anaphylaxis. Thus one of our patients with chronic urticaria developed minor but definite signs of anaphylaxis after intradermal injection of 200 KIU of TR. but she had no more erythema or wheal formation than non-sensitive subjects. It has been suggested that C'1-esterase also generates the formation of anaphylatoxin (5) with subsequent release of histamine. It is not known whether this ability might be more pronounced in anaphylactic reactions in patients with hereditary angioneurotic edema than in others. If such a mechanism is involved it might aggravate the anaphylactic shock produced by TR.

This indicates the need for being very

restrictive and cautious when treating patients with repeated doses. More experience is certainly required before this interesting substance can be generally recommended for the treatment of chronic urticaria and hereditary angioneurotic edema.

## SUMMARY

Intravenous infusions of a commercial kallikrein inhibitor Trasylol (TR.) stopped the daily urticarial eruptions in 6 patients with chronic urticaria and it was possibly also effective in the treatment of 2 patients with hereditary angioneurotic edema. The patients were free from urticarial symptoms for 5-90 days. Treatment with antihistamines and corticosteroids had had little or no effect. Previously they had strongly increased edematous reaction to intradermally injected kallikrein which became normal after treatment with the kallikrein inhibitor. Some of the patients also showed delayed reactions to intradermal injections of histamine and bradykinin after 3-10 hours, which disappeared after infusion of TR. The findings indicate that the kallikrein-kinin system might be involved in these delayed reactions. TR. did not influence the level of C'1-esterase inhibitor. In two patients with acute and cold urticaria respectively and with normal kallikrein reactions TR. treatment appeared to have no effect. The side effects noted during or shortly after the infusion were hypersalivation, flushing, orthostatic fall in blood pressure and increased perspiration, all of which might have been due to a histamine releasing effect. A severe anaphylactic reaction in one patient with hereditary angioneurotic edema (who had also been treated 6 weeks earlier) stopped further trials with repeated infusions for a long period. Intracutaneous tests with TR. in an attempt to predict an anaphylactic reaction were of no value. Thus one of our patients developed minor but definite signs of anaphylaxis after intradermal injection of 200 KIU of TR. but no more local reaction than the controls. More experience is needed before this inhibitor can be generally used as a

treatment for chronic urticaria and hereditary angioneurotic edema.

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