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THE EFFECT OF TRANEXAMIC ACID ON THE FIBRINOLYTIC SYSTEM DURING ANAPHYLAXIS IN RABBITS; THE IMPORTANCE OF THE FIBRINOLYTIC SYSTEM DURING ANAPHYLAXIS

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We previously reported (Shimaya *et al.* (1992) *Enzyme*, **46**, 204) that a rapid and strong increase of plasminogen activator (PA) was induced during anaphylaxis, and that the main plasma fibrinolytic enzyme which increased in the anaphylaxis group was shown to be tissue-type plasminogen activator (t-PA). Anaphylaxis was induced in rabbits by giving BSA after t-AMCHA injection. 44% of those rabbits died within 3 h after BSA injection. In the dead group, the euglobulin fibrinolytic activity (EFA) could not be detected by the plasminogen-rich fibrin plate method and the t-PA activity, using the natural substrate plasminogen, did not rise significantly reaching a peak at 10–15 min. However, the EFA and t-PA activity increased significantly in the surviving group. A significant prolongation of the activated partial thromboplastin time (APTT) and the prothrombin time (PT) was observed during anaphylaxis in both groups. These findings suggest that increased PA activity during anaphylaxis is an important defense mechanism against the rapid increase in the blood coagulation system.

KEY WORDS: Tranexamic acid, Plasminogen activator, Anaphylaxis, Fibrinolysis, Rabbit

INTRODUCTION

Recently, much attention has been focussed on the interrelation between the antigen-antibody (Ag-Ab) reaction, blood coagulation and fibrinolytic systems, but the detailed interrelation still remains unclear. Numerous reports have suggested that the Ag-Ab reaction can activate the blood coagulation system. Kaplan *et al.*[1] found that the Ag-Ab reaction can activate Factor XII (Hageman Factor). Further studies also suggested that the Ag-Ab reaction can activate the blood coagulation system via the kallikrein system. It has been shown that activated Factor XII or kallikrein activates the fibrinolytic system.

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We have performed experiments on the *in vitro* Ag-Ab reaction in serum, and measured the activities of various enzymes[2], especially the complement[3,4], in terms of the hydrolysis of synthetic amino acid esters. Our results indicate that the *in vitro* Ag-Ab reaction does not activate plasminogen directly, and the complement system may be involved in the participation of the activating mechanism.

We established a rabbit model of anaphylaxis, which is the most dramatic and severe of all Ag-Ab reactions[5]. We previously reported that a rapid and strong increase of plasminogen activator (PA) was induced during anaphylaxis[5]. The pathophysiological significance of the increased PA activity during anaphylaxis is still unknown.

Tranexamic acid (trans-4-(aminomethyl) cyclohexanecarboxylic acid, t-AMCHA) is a fibrinolysis inhibitor, which acts by preventing plasminogen binding to the lysine site on fibrin, and thereby interferes with plasminogen activation[6]. Clinically, t-AMCHA was observed to be of benefit in gastrointestinal bleeding, subarachnoid hemorrhage[7] and, more recently, in the treatment of hereditary angioneurotic edema[8].

In the present study, after t-AMCHA was administered to rabbits and inhibited the fibrinolytic system, anaphylactic shock was induced by injecting BSA. All the rabbits not pretreated with t-AMCHA (anaphylaxis group) survived. On the other hand, 44% of the rabbits pretreated with t-AMCHA (t-AMCHA group) were dead within 3 h. We have measured the activity of the enzymes in the fibrinolytic and blood coagulation systems during anaphylaxis.

MATERIALS AND METHODS

Reagents and Chemicals

BSA was from Gibco Life Technologies Inc., Grand Island, N. Y., USA; Freund's complete adjuvant from DIFCO Laboratories, Detroit, Mich., USA; bovine fibrinogen from Sigma Chemical Co., St. Louis, Mo., USA and Miles Inc., Kankakee, Il., USA; Ortho brain thromboplastin and Ortho Activated Thrombofax from Ortho Diagnostics, Raritan, N.J., USA; and the synthetic amide substrate, H-D-Val-Leu-Lys-pNA (S-2251) from Kabi Diagnostica Co., Ltd., Sweden. All other chemicals were obtained from commercial sources and were of the highest grade available.

Human immunoglobulin[9] and Glu-plasminogen[10] were purified from human pooled plasma.

Animals and Sensitization

Twenty-four New Zealand white rabbits (Kyudo Co., Kumamoto, Japan) weighing 2.5–4.1 kg were sensitized by a single intracutaneous injection of 2 mg BSA dissolved in 2 ml of Freund's complete adjuvant[11]. The anti-BSA antibody protein in the antiserum was measured by reversed single radial immunodiffusion (RSRID)[12]. An early rise in anti-BSA antibody level could be detected at 2 weeks, and the level remained elevated at 6 weeks. However, the anti-BSA antibody level was not elevated in all rabbits. Animals whose anti-BSA antibody level was increased, were used for the experiments at 3–6 weeks after sensitization. IgG type reagin antibody was found in this

anti-BSA antibody by the Henson and Cochrane method[11] employing the acute passive cutaneous anaphylactic (PCA) reaction.

Anaphylaxis

The animals were anesthetized by giving an intraperitoneal injection of ethylcarbamate (urethane) (1.0 g/kg). They were sedated with intermittent doses of 0.1 to 0.25 g/kg urethane. A 4-Fr ATOM disposable indwelling tube (ATOM Co., Tokyo, Japan) was inserted into the femoral artery after cutdown and further advanced so as to enter the abdominal aorta. This tube was kept filled with saline solution containing 3.8% sodium citrate. The catheter was used for blood sampling and for monitoring the systemic blood pressure by means of a pressure transducer (Nihon Koden Co., Tokyo, Japan). After the line placement and a 60-min stabilization period, baseline measurements and sampling were made. Anaphylaxis was induced by injecting 10 mg BSA (dissolved in 1 ml of saline solution) into the artery. Control animals received the same amount of human immunoglobulin in the same manner.

t-AMCHA Treatment

Anaphylaxis was induced by giving BSA 5 min after a 30 mg/kg t-AMCHA injection, as described by Schneider[13].

Samples

5 ml blood samples were obtained via the catheter at 1, 5, 10, 15, 30, 60, and 90 min after the injection of BSA or human immunoglobulin. All samples were collected in plastic syringes containing 3.8% sodium citrate (9:1 vol/vol) and immediately transferred to plastic tubes that were kept on ice. The materials were centrifuged at 3000 rpm and 4°C for 15 min. The plasma was immediately separated from the red blood cells, and aliquots were stored at –70°C. Euglobulin fractions were prepared by titration of 20-fold diluted (with distilled water) plasma with 0.25% (v/v) acetic acid until an end-point pH of 5.2 was reached. After the euglobulins had been precipitated by centrifugation, they were dissolved in a buffer containing 0.137 M barbital sodium and 0.063 M NaCl adjusted to pH 7.38 with 0.1 M NaOH. The euglobulin fractions were stored at –70°C.

Assay methods

(1) Euglobulin fibrinolytic activity (EFA). EFA was determined by the plasminogen-rich fibrin plate method of Astrup and Müllertz[14]. EFA is expressed as the lysis area (mm^2) on the fibrin plate.

(2) Tissue-type PA (t-PA) activity. The t-PA activity in the plasma euglobulin fractions was assayed by Verheijen's method using cyanogen bromide (CBrN) fragments of fibrinogen, human Glu-plasminogen, and the chromogenic substrate, H-D-Val-Leu-Lys-pNA[15]. The CBrN-fibrinogen fragments considerably enhanced the sensitivity and specificity of the method due to specific stimulation of the plasminogen activation

only by t-PA. The results are expressed as the percentage in t-PA activity, as described by Krishnamurti *et al.*[16].

(3) The activated partial thromboplastin time (APTT). The APTT of plasma was determined according to the method of Sibley *et al.*[17]. 0.1 ml of citrated plasma was activated at 37°C for 3–5 min in contact with 0.1 ml of Ortho Activated Thrombofax. The clotting time was determined after recalcification with 0.1 ml of 0.02 M $CaCl_2$. Duplicate APTT determinations were performed on each plasma.

(4) The prothrombin time (PT). The PT was determined by the method of Pinckard *et al.*[18]: 0.2 ml of prewarmed (37°C) Ortho Brain Thromboplastin was added to 0.1 ml of the test plasma at 37°C and the clotting time was determined. Duplicate PT determinations were performed on each plasma.

Data analysis

The results of the experiments are given as the means ±SEM. Statistical analysis was performed by Student's *t* test on paired samples.

TABLE I

Effect of t-AMCHA on anaphylaxis induced in rabbits

Treatment group	surviving (N)	dead (N)	total (N)
control	6	0	6
anaphylaxis	8	0	8
tranexamic acid	5	4	9

Control group; the rabbits given human immunoglobulin injection.
Anaphylaxis group; the rabbits given only BSA injection.
Tranexamic acid group; the rabbits given BSA injecction 5 min after tranexamic acid administration.

RESULTS

As shown in Table 1, 100% of the rabbits in the anaphylaxis group survived. 44% of the rabbits in the t-AMCHA group were dead within 3 h after BSA injection. The rabbits in the t-AMCHA group was divided into two groups; the dead and surviving groups.

Figures 1A and 1B show the time course of the systolic and diastolic pressure. The systolic and diastolic pressure in the dead group decreased gradually during anaphylaxis, and reached 35% of the normal value at 90 min after the BSA injection. The arterial pressure in the dead group decreased significantly at 60 and 90 min after the BSA injection ($p < 0.05$) in comparison with those in the surviving group. However, both pressures in the surviving group fell rapidly within 1 min after the BSA injection, and reached a minimum value at 15 min ($p < 0.05$), but returned to about 72% of the normal value after 30 min. This data in the surviving group agrees with that of the anaphylaxis group.

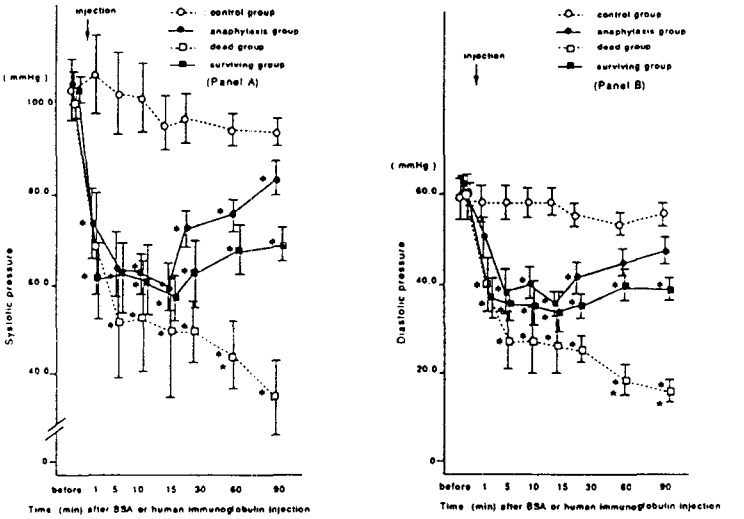

FIGURE 1 Systolic (Panel A) and diastolic (Panel B) arterial blood pressure during anaphylaxis. The death group; the rabbits in the tranexamic acid group which were dead within 3 h after BSA injection. The surviving group; the rabbits in the tranexamic acid group which were alive. Values are the means ±SEM. (*$p < 0.05$ vs. control; *$p < 0.05$ vs. surviving group)

Figure 2 shows the time course of EFA as evaluated by the plasminogen-rich fibrin plate method. EFA could not be detected before giving BSA or in the dead and control group at any point. In the surviving group, an early and moderate rise in EFA level was observed at 1 min after BSA injection, and the value peaked at 10 min, but EFA could not be detected after 30 and 90 min. The level in the surviving group was lower than that of the anaphylaxis group.

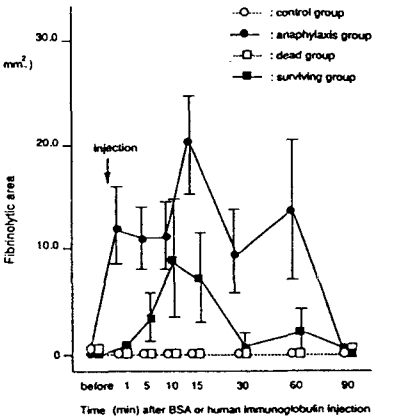

FIGURE 2 Time course of the euglobulin fibrinolytic activity (EFA) during anaphylaxis. EFA was determined by the standard fibrin plate method. Values are the means ±SEM.

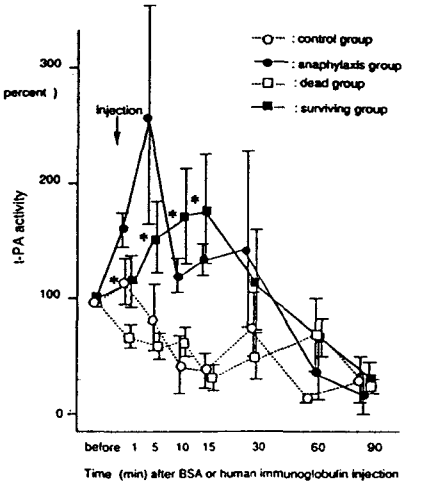

FIGURE 3 Time course of t-PA activity during anaphylaxis. The t-PA activity was assayed by the method of Verheijen *et al.*[15] using Glu-plasminogen in the presence of CNBr-fibrinogen fragments. The results are expressed as the increase in t-PA activity in percent, as described by Krishnamurti *et al.*[16]. Values are the means ±SEM. (*$p < 0.05$ vs. dead group)

As described by us previously[5], the main plasma fibrinolytic enzyme which increased in anaphylaxis was proved by zymography to be t-PA. The t-PA activity was measured from the conversion of the natural substrate Glu-plasminogen to plasmin in the presence of CBrN-fibrinogen fragments. As shown in Figure 3, the t-PA activity did not rise significantly in the dead group at any point. The t-PA activity in the surviving group rose significantly at 1, 5, 10, 15 min after BSA injection ($p < 0.05$) in comparison with those in the dead group. The level in the surviving group was lower than that for the anaphylaxis group. Because the plasma sample contained t-AMCHA, it was impossible to measure the PAI activity of the plasma. It was concluded that t-AMCHA completed inhibited the fibrinolytic system in the dead group, whereas it only partially inhibited it in the surviving group.

We measured the blood coagulation activity. Figure 4 shows the time course of the APTT. The significant prolongation of the APTT was observed in the dead, surviving, and anaphylaxis groups ($p < 0.05$). This level of the APTT at 90 min after BSA injection in the dead group was higher than that for the surviving group.

Figure 5 shows the time course for the PT. Significant prolongation of the PT was observed in the dead, surviving, and anaphylaxis groups ($p < 0.05$). The PT in the dead group did not rise significantly at any point after the BSA injection in comparison with those in the surviving group.

Although the data are not shown, we performed control experiments on the *in vitro* Ag-Ab reaction in the plasma before carrying out anaphylaxis *in vivo*. 0.022 mg BSA were added to plasma samples obtained from sensitized rabbits in the same manner. The samples were incubated at 37°C for 1, 5, 10, 15, 30, 60, and 90 min. EFA could not be detected in any of the samples using the plasminogen-rich fibrin plate method.

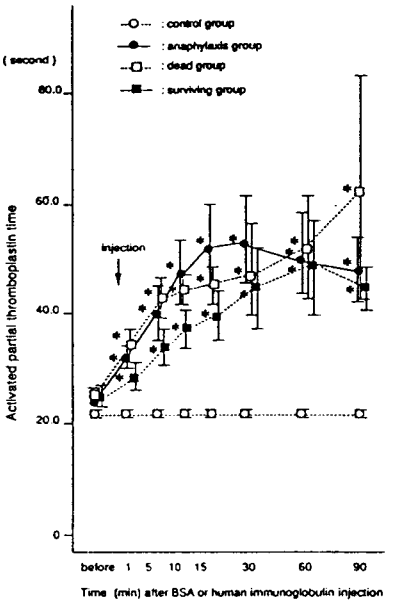

FIGURE 4 Time course of the activated partial thromboplastin time (APTT) during anaphylaxis. The APTT of plasma was measured according to the method of Sibley *et al.*[17]. Values are the means ±SEM. (*$p < 0.05$ vs. control)

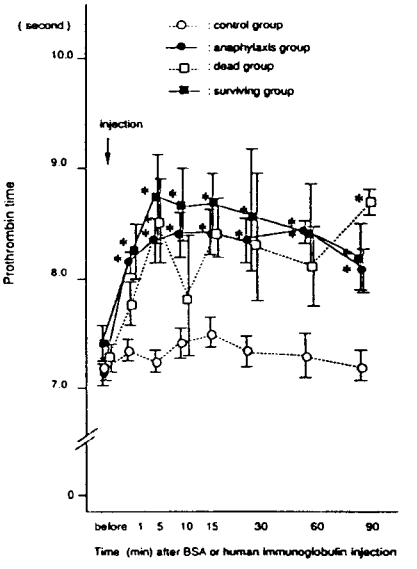

FIGURE 5 Time course of the prothrombin time (APTT) during anaphylaxis. The PT of plasma was measured according to the method of Pinckard *et al.*[18]. Values are the means ±SEM. (*$p < 0.05$ vs. control)

DISCUSSION

Some serine proteases are well known to act as triggers of PA release[19,20]. Among them, thrombin has been reported to be one of the most powerful triggers of all[21]. In the present study, we found significant prolongation of the APTT and PT in the anaphylaxis and t-AMCHA groups. Two possibilities could explain such a prolongation of the APTT and PT: a consumption of the coagulation factors, and an increase of the various inhibitors. As reported by us previously[5], the amidolytic activities with the thrombin substrate (H-D-Phe-Pig-Arg-pNA) and the plasma kallikrein substrate (H-D-Pro-Phe-Arg-pNA) rose significantly in comparison with those in the control group. This data suggests that the prolongation of the APTT and PT depended on the consumption of the coagulation factors. This explanation appears to be supported by other studies[2,17]. We found not only an increase of PA activity, but also an increase of thrombin-like enzyme and plasma kallikrein-like enzyme at the same time during anaphylaxis[5]. These findings suggest that the increase of PA might result from a fibrinolytic reaction via the blood coagulation and kallikrein systems. We assume that a vasoamine release from mast cell might activate the blood coagulation system, causing the fibrinolytic and complement systems to interact in a very complex manner.

Sundsmo and Wood[22] found that activated Factor B (Bb), the central serine esterase of the alternative pathway of complement activation, could activate plasminogen in an experimental system containing purified plasminogen and Bb. Takada *et al.*[23] reported that t-AMCHA inhibited 92% of the complement activity 10 min after being injected into humans. The t-AMCHA dose in the study of Takada *et al.*[23] was 66% of that in our study. It is assumed that t-AMCHA inhibited the complement activity in our study. t-AMCHA administration inhibited the increase in EFA and t-PA activity at 30 and 60 min after the BSA injection. It would seem that the fibrinolytic system via the complement system could participate in the mechanism of this increased fibrinolytic activity. However, we performed experiments on the *in vitro* Ag-Ab reaction in the plasma, and a rise in EFA was not detected under the experimental conditions employed. Discrepancies existed between the *in vivo* and *in vitro* experiments undertaken in the study of Takada *et al.*[23]. It is assumed that the increase in PA cannot be explained only by the plasma component containing complement. A recent study by Hamilton *et al.*[24], reported that deposit of the terminal complement protein C5b-9 (membrane attack complex) on a cultured endothelial surface resulted in a 2-3-fold increase in endothelial prothrombinase (an enzyme complex which converts prothrombin into thrombin) activity. It is considered that there is a possibility that *in vivo*, the complement, blood coagulation and fibrinolytic systems could interrelate, more complexly and closely, through the vascular endothelium.

In our *in vivo* study, rabbits were injected with t-AMCHA, a specific protease inhibitor to plasmin, which is considered as one of the chemical mediators. Within 3 h, the mortality rate in the t-AMCHA group was unexpectedly 44%. The effects on EFA and t-PA activity confirmed that t-AMCHA completely inhibited the fibrinolytic system in the dead group, whereas t-AMCHA partially inhibited the fibrinolytic system in the surviving group. As parameters of blood coagulation, APTT and PT in both groups rose significantly in comparison with those in the control group. However the

APTT and PT in the dead group did not rise significantly in comparison with those in the surviving group. These findings suggest that the increased fibrinolytic system during anaphylaxis is not detrimental but a defence mechanism against the rapid increase of blood coagulation activity.

Although the data is not shown, we observed a few pathohistological changes between the dead and surviving groups. Pathohistologically the cause of the rabbits' death could not be concluded. Avikainen *et al.*[25] detected significant changes in thrombocyte and coagulation factors, but could not pathohistologically observe the fibrin thrombi in any tissues in haemorrhagic and traumatic shock. The absence of fibrin thrombi has been explained by a rapid pre- and post-mortem fibrinolysis, as reported previously[25,26]. Avikainen *et al.*[27] also could not detect increased fibrin in the lungs, liver, kidneys and spleen in rabbit haemorrhagic and traumatic shock, using I^{125} fibrinogen, in spite of preventing fibrinolysis with t-AMCHA.

Considering the unexpected result that the rabbits died within 3 h as t-AMCHA completely inhibited the fibrinolytic activity, it was evident that increased PA activity during anaphylaxis is the important defence mechanism against the rapid increase in blood coagulation. Hence, more attention should be paid to the blood coagulation and fibrinolytic activities during anaphylaxis in humans.

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