EFFECTS OF DILAZEPI ON FIBRINOLYTIC SYSTEM IN ANIMALS. I. ENHANCEMENT OF FIBRINOLYTIC ACTIVITY BY ADMINISTERED DILAZEP IN EX VIVO EXPERIMENT

KATSUAKI NAKAJIMA, SUSUMU YAMAMOTO, MASAMI TSUKAMOTO AND MASAHIKO NAGAKURA

Tokyo Research Laboratories, Kowa Company Ltd., Higashimurayama Tokyo, 189, Japan

(Received January 30, 1982)

The effects of dilazep on blood fibrinolytic system were studied in ex vivo experiment. Fibrinolytic activity in plasma was determined by the fibrin plate method at designed times after the administration of dilazep in guinea pigs, rabbits and mongrel dogs. In guinea pigs, fibrinolytic activity of euglobulin (plasminogen activator level, 1.10±0.08 CU/ml in control animals) rose to 1.53±0.24, 1.92±0.13 and 2.35±0.15 CU/ml 2 h after the p.o. administration of 30, 100 and 300 mg/kg of dilazep, respectively. Plasminogen levels in euglobulin were unchanged, however, plasmin inhibitory activities were lowered following the increase of plasminogen activator levels. About 2-fold increase of plasminogen activator level was also observed in rabbits 60 min after the p.o. administration of 300 mg/kg dilazep and returned to the initial level (0.054 CU/ml) within 3 h after the administration. A rapid increase of plasminogen activator level was observed in a case of the i.v. administration of dilazep into rabbits and mongrel dogs. In in vitro experiment, dilazep added to whole blood or plasma of guinea pigs ranging 0.1 to 1000 μg/ml did not reveal any effects on plasminogen activator level or plasmin inhibitory activity in plasma.

Keywords—Dilazep; dipryridamole; dextran sulfate; blood fibrinolytic system; plasminogen activator; ex vivo; in vitro

INTRODUCTION

Thrombus formation is considered to be a potential trigger for the clinically important diseases such as myocardial and cerebral infarction. Anticoagulants have been used to prevent the formation of thrombus,1,2) and recently thrombolytic agents, namely urokinase and streptokinase,3-7) have been used to treat the thromboembolic disorders. However, the activation of the endogenous fibrinolytic system seems to be more desirable and physiological than the activation of plasma plasminogen by the injected exogenous enzymes. Therefore, drugs stimulating synthesis of plasminogen activator in the vessel wall and/or the release of the activators have become of current interest.8,9)

In this paper, we studied about the effect of dilazep, a coronary vasodilator which is known to have an enhancing effect on oxygen dissociation from red blood cells10,11) and an inhibitory effect on platelet aggregation,12,13) on blood fibrinolytic system in animals and found that dilazep caused the increase of plasminogen activator level in plasma.

MATERIALS AND METHODS

Drugs — Dilazep dihydrochloride (Comelian) and sodium dextran sulfate (MDS-T, S content =16 %, Mw =7000) were obtained from Kowa Co., Nagoya. Dipryridamole (Persantin) was purchased from Tanabe Seiyaku Co., Osaka; pentobarbital (Somnopentyl) from Pitman-Moore Inc., Washington Crossing; lidocain hydrochloride (Xylocain) from Fujisawa Pharmaceutical Co., Osaka.

Reagents — Buffer, Veronal-HCl-NaCl, buffer, pH 7.8 was prepared according to Ohta et al.14) Plasminogen-free bovine fibrin-agar-plate
(PK Kowa measuring kit of fibrinolytic activity) was obtained from Kowa Co., Nagoya; urokinase from Mochida Seiyaku Co., Tokyo; plasmin from Daichi Chemical Co., Tokyo.

Animals — Healthy male mongrel dogs weighing about 12 kg, male Japanese white rabbits weighing about 3 kg and male Hartley guinea pigs weighing 340—420 g were used. The animals in each species were on a standard laboratory diet, respectively.

Collection of Blood Sample — Bloods were collected from a vein in the fore-leg of mongrel dog, from the ear vein cannula of rabbits and from the abdominal vein of guinea pigs using plastic syringes containing 3.8 % trisodium citrate solution. The blood was introduced into syringes in a portion of 1 vol of citrate to 9 vol of blood. The plasma was immediately separated by centrifugation at 1500 \( \times g \) for 10 min at 4°C.

Ex Vivo Experiment — Male guinea pigs were given orally 30, 100 and 300 mg/kg of dilazep and/or 300 mg/kg of dextran sulfate dissolved in distilled water, and blood was collected at the designed times under the anesthetized condition by the intraperitoneal injection of 30 mg/kg pentobarbital. Control animals received orally an equal volume of distilled water. Subcutaneous injection of dilazep or dipyriramole were accomplished on the back of guinea pigs. The male mongrel dog was anesthetized by injecting intravenously 30 mg/kg of pentobarbital, and a first control blood was collected. A volume (0.1 ml/kg) of physiological saline, equal to the volume of the drug to be injected, was administered intravenously; 15 min later, a second control blood was collected and 1 mg/kg of dilazep was injected intravenously within 5 s; subsequent blood samples were collected 15, 30 and 45 min after the drug injection. The male rabbit was tied up on a board and anesthetized locally by injecting 2 ml of 1 % lidocaine solution beside the central ear vein; 5 min later, polyethylene cannula (Indwelling tube for infant feeding, Atom Co., Tokyo) was inserted from the central ear vein into the right atrium and immediately a small volume of physiological saline was infused con-

**FIG. 1. Effect of Precipitation pH on Fibrinolytic Activity**

Open marks (○, △, □) represent plasminogen activator level and closed marks (●, △, ■) plasminogen level in each species of animals.

**FIG. 2. Time Course of Plasminogen Activator Level in Guinea Pigs after the p.o. Administration of 300 mg/kg Dilazep**

Mean values (± SE) of 10 animals are given in control (before) and of 3 animals 1, 2, 4 and 6 h after the administration. a) \( p < 0.05 \), b) \( p < 0.001 \).
continuously to prevent the inflow of the blood into the cannula. The first control blood was collected from the ear vein cannula and the test drugs were administered orally or intravenously; subsequent blood samples were collected from the ear vein cannula at designed times.

**In Vitro Experiment** — Dilazep was added to whole blood or plasma collected from control guinea pigs at final concentrations ranging from 0.1 to 1000 μg/ml and incubated for 15 min at 37°C. After the incubation, whole blood was centrifuged at 1500×g for 10 min at 4°C to separate plasma and used in the assay of fibrinolytic activity.

**Method of Assay for Fibrinolytic Activity** — Fibrinolytic activity was assayed by the fibrin plate method with plasminogen-free bovine fibrin-agar-plate according to Abe *et al.* Optimum pH of the euglobulin preparation was studied in a range of pH 4.6 to 6.6. The euglobulin fraction of the plasma was precipitated from a mixture of 0.5 ml of plasma and 9.5 ml of distilled water by adjusting the pH to designated pH with 1% acetic acid and left standing for 30 min in an ice-cold bath. The precipitate was separated by centrifugation at 1500×g for 15 min at 4°C and redissolved in equal volume of Veronal-HCl-NaCl buffer to plasma used. Five microliters of the redissolved euglobulin was introduced into 3-mm well cut in the fibrin plate and the plate was incubated for 20 h at 37°C to assay for plasminogen activator level. After the incubation, the diameter of lysis zone surrounding the sample well was measured and given the plasmin activity (casein units/ml, CU/ml) by comparing with a standard curve obtained from authentic plasmin. The plasminogen level (urokinase-activated euglobulin containing 500

![Graph 3](image)

**FIG. 3. Effect of Dilazep on Plasminogen Activator Level in Guinea Pigs**

Each bar represents mean ± SE of plasminogen activator level of euglobulin fraction. Open bars refer to values 2 h after the p.o. administration, shadowed bars 30 min after the s.c. administration. a) *p* < 0.05, b) *p* < 0.001.

![Graph 4](image)

**FIG. 4. Comparison of Fibrinolytic Response after the Administration of Dilazep, Dipyridamole and Dextran Sulfate in Guinea Pigs**

Each bar represents mean ± SE of plasminogen activator level of euglobulin fraction. Open bars refer to values 2 h after the p.o. administration, shadowed bars 30 min after the s.c. injection. a) *p* < 0.001.
IU/ml urokinase at the final concentration) and plasmin inhibitory activity in plasma (mixture prepared from the same portion of plasma and authentic plasmin) were also assayed by the fibrin plate method described in previous paper.20)

Fig. 1 shows the recovery of fibrinolytic activity of the euglobulin fraction of mongrel dogs, rabbits and guinea pigs, respectively. The fibrinolytic activity of euglobulin (plasminogen activator level) rose with pH and reached the maximum at pH 5.6–6.0 in mongrel dogs. The activity of urokinase-activated euglobulin (plasminogen level) appeared as the same pattern as plasminogen activator. In rabbit euglobulin fraction, both of the highest fibrinolytic activities, the plasminogen and plasminogen activator, were observed at pH 5.8–6.2. In guinea pig euglobulin, the highest fibrinolytic activity of plasminogen activator level was observed at pH 5.8–6.2, and of plasminogen level at pH 5.2–6.2. Those results indicated that the optimal pH for isolation of euglobulin were present at pH 5.8 in mongrel dogs, and at pH 6.0 in rabbits and guinea pigs.

RESULTS

Fibrinolytic Response in Guinea Pigs

The time course of plasminogen activator levels in guinea pigs is shown in Fig. 2. Plasminogen activator level was increased by oral administration of 300 mg/kg dilazep 1–2 h after the administration and returned to control level within 4 h.

Fig. 3 exhibits that dilazep orally administered into guinea pigs exerted the increase of plasminogen activator level in a dose dependent fashion, 1.53±0.24, 1.92±0.13 and 2.35±0.15 CU/ml in doses of 30, 100 and 300 mg/kg, respectively. In subcutaneous administration of dilazep, the enhancement of fibrinolytic activity was also observed. The plasminogen activator level rose to 2.06±0.22 or 2.77±0.29 CU/ml 30 min after the injection of 20 or 50 mg/kg.

Data of the comparative studies for a few drugs in guinea pigs are given in Fig. 4. Dipyridamole had no effects on the plasminogen activator level 30 min after the s.c. administration of 50 mg/kg. Dextran sulfate exerted a potent enhancing effect on fibrinolytic activity. The plasminogen activator level rose to 2.60±0.08 CU/ml 2 h after the p.o. administration of 300 mg/kg.

Fibrinolytic Response in Mongrel Dogs

Plasminogen activator level of the first control plasma and the second control plasma went unchanged, however, a significant increase of

---

**FIG. 5.** Time Course of Plasminogen Activator Level in Mongrel Dogs after the i.v. Injection of 1 mg/kg Dilazep

Values are expressed as mean of two experiments.

**FIG. 6.** Time Course of Plasminogen Activator Level in Rabbits after the p.o. Administration of 300 mg/kg Dilazep

Values are expressed as mean of two experiments.
plasminogen activator level was observed 15 min after the i.v. administration of 1 mg/kg dilazep and returned to initial level within 60 min (Fig. 5).

Fibrinolytic Response in Rabbits

Fig. 6 shows the time course of plasminogen activator level in rabbits receiving oral dosing of 300 mg/kg dilazep. Control level of plasminogen activator (0.054 CU/ml) rose to 0.091 and 0.101 CU/ml 30 and 60 min after the p.o. administration and returned to initial level within 3 h. When 1 mg/kg dilazep was additionally injected intravenously 5 h after the p.o. administration, a transient increase of plasminogen activator was observed.

Fig. 7 shows the data of the i.v. administration of dilazep or dipyridamole into rabbits. Plasminogen activator level rose to twice the initial level 5 min after the injection of 1 mg/kg dilazep, decreased gradually and returned to initial level within 60 min after the injection. A slight increase of plasminogen activator level was observed 5 min after the injection of 300 μg/kg dilazep. However, plasminogen activator level was unchanged in case of the i.v. administration of 3 mg/kg dipyridamole.

Fibrinolytic Response in Vitro Experiment

Representative data obtained in in vitro experiment are given in Fig. 8. Added dilazep ranging from 0.1 to 1000 μg/ml at final concentrations to whole blood or plasma of guinea pigs exerted no effects on plasminogen activator level. Plasmin inhibitory activity against 6 CU/ml plasmin did not change by dilazep added into whole blood or plasma.

DISCUSSION

The effect of dilazep, a coronary vasodilator, on blood fibrinolytic system was investigated in experimental animals. A significant increase of plasminogen activator level was observed in guinea pigs in a dose dependent fashion 120 min after the p.o. administration of dilazep ranging 30 to 300 mg/kg. The enhancement of fibrinolytic activity was also observed in cases of the s.c. injection of dilazep into guinea pigs, the p.o.

FIG. 7. Effect of Dilazep and Dipyridamole injected intravenously into Rabbits on Plasminogen Activator Level

Solid circles and solid lines represent time course of plasminogen activator level after the injection of 1 mg/kg dilazep (mean±SE, n=4), closed circles and broken lines 300 μg/kg dilazep (mean, n=2) and open diamonds 3 mg/kg dipyridamole (mean, n=2). a) p < 0.05, b) p < 0.01.

FIG. 8. Effect of Added Dilazep to Guinea Pig Whole Blood or Plasma, at Different Final Concentrations, on Plasminogen Activator Level and Plasmin Inhibitory Activity

Upper graph: plasminogen activator level in euglobulin fraction after the addition of dilazep to whole blood (△) or plasma. (●). Lower graph: plasmin inhibitory activity against 6 CU/ml plasmin after the addition of dilazep to whole blood (△) or plasma (●).
administration into rabbits and the i.v. administration into rabbits and mongrel dogs. In all animals tested, plasminogen levels were unchanged, however, plasmin inhibitory activity in plasma was lowered with the increase of plasminogen activator (unpublished observation). These results suggest that the enhancing effect of dilazep on fibrinolytic system is not a discrepancy induced by different species of animals or different administering routes of the drug. Dilazep added to whole blood of guinea pigs in vitro had no effects on plasminogen activator level and plasmin inhibitory activity. It is, therefore, considered that dilazep does not act directly on blood fibrinolytic enzymes as shown in the mode of action of urokinase and streptokinase.\textsuperscript{16-18} Holemans\textsuperscript{19} reported that the injection of vasoactive drugs, epinephrine or histamine, into unanesthetized dogs produced an increase of fibrinolytic activity and the maximal effect was observed 5 min after the injection. The data of the i.v. administered dilazep into unanesthetized rabbits in our present study corresponded to his observation. Thus, dilazep may cause the liberation of plasminogen activator from vascular bed as described in the possible mode of action of vasoactive drugs.\textsuperscript{19} However, dipyridamole, a representative coronary vasodilator, exerted on effects on blood fibrinolytic system in our present study.

On the other hand, dextran sulfate which was reported to reduce the plasmin inhibitory activity in plasma\textsuperscript{20,21} exerted a potent enhancing effect on fibrinolytic system in guinea pigs. It is therefore not deniable that dilazep might cause an indirectly decrease of plasmin inhibitors in bloodstream. Further studies, however, are required to establish the mode of action of dilazep on blood fibrinolytic system, and the data will be published in succeeding papers.

REFERENCES


16) G.H.Barlow: Urinary and kidney cell plasminogen activator (urokinase).


17) L.Summaria, I.G.Boreisha, L.Arزادon and


