

Effects of Therapeutic Doses of Heparin on Thrombolysis With Tissue-Type Plasminogen Activator in Rabbits

By Giancarlo Agnelli, Claudia Pascucci, Benilde Cosmi, and Giuseppe G. Nenci

The objective of the study was to evaluate the ability of heparin to enhance the thrombolytic effect of recombinant tissue type plasminogen activator (rt-PA) and to prevent thrombus growth during and after thrombolysis with rt-PA. In the thrombolysis studies, three groups of rabbits were infused with rt-PA at a dose of 0.5 mg, 1 mg, or 2.5 mg over 3 hours, respectively. Rabbits in each group were randomized to receive, in addition to rt-PA, heparin, 20 or 60 antifactor Xa U/kg/h, or saline over 6 hours. The three doses of rt-PA produced the same extent of thrombolysis both in the two groups treated with heparin ($34\% \pm 6\%$, $52\% \pm 7\%$, and $79\% \pm 8\%$ in the lower dose group; $39\% \pm 6\%$, $49\% \pm 4\%$, and $81\% \pm 6\%$ in the higher dose group) and in the group treated with saline ($37\% \pm 4\%$, $47\% \pm 5\%$, and $84\% \pm 7\%$). In the thrombus growth inhibition studies 0.5 mg of rt-PA was infused over 3 hours in each rabbit. In addition, the rt-PA-treated rabbits were randomized to receive heparin, 20 or 60 antifactor Xa U/kg/h over 6 hours, or saline. At the end of infusion, no

statistically significant differences in thrombus growth were found in three groups of rabbits ($54.8 \pm 7.4 \mu\text{g}$ and $52.4 \pm 12.1 \mu\text{g}$ in the low and high dose of heparin groups, respectively, and $59.4 \pm 10.4 \mu\text{g}$ in the saline group). In different experiments rabbits were randomized to receive heparin, 60 antifactor Xa U/kg/h, or saline at the end of the rt-PA infusion. In these experiments heparin inhibited thrombus growth more efficiently than saline ($41.1 \pm 6.5 \mu\text{g}$ and $58.7 \pm 12.9 \mu\text{g}$, respectively, $P < .05$). In vitro experiments confirmed that heparin is unable to prevent fibrin accretion on the clots during lysis with rt-PA while both D-Phe-Pro-Arg-CH₂-Cl (PPACK) and hirudin are able to prevent the accretion of fibrin. We conclude that the data obtained in these animal models do not support the concomitant use of heparin and rt-PA. However, heparin could be used successfully after rt-PA to inhibit thrombus growth.

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TISSUE-TYPE plasminogen activator (t-PA) is the most extensively evaluated agent among the second generation plasminogen activators, a family of natural human proteins with thrombolytic activity. t-PA differs from the first generation plasminogen activators, streptokinase and urokinase, as it binds to fibrin and its plasminogenolytic activity is enhanced in the presence of fibrin.¹ As a result, t-PA has been claimed to be able to produce a selective thrombolysis without producing a plasma proteolytic state. Experiments in rabbits have shown that t-PA is less hemorrhagic than streptokinase when the two drugs are infused at equithrombolytic doses.² Recombinant t-PA (rt-PA) has proved to be an effective thrombolytic agent in myocardial infarction,³⁻⁵ unstable angina,⁶ pulmonary embolism,⁷ and peripheral arterial thrombosis⁸ but the advantages over streptokinase, as far as bleeding is concerned, have not been clearly demonstrated.

rt-PA is generally infused concomitantly with heparin. The additional use of heparin is aimed at preventing vascular reocclusion after rt-PA. Indeed, heparin has been reported both to enhance the fibrinolytic potential in human volunteers⁹ and to reduce the size of the thrombus in patients with deep-vein thrombosis.¹⁰ However, the ability of heparin to potentiate the thrombolysis induced by rt-PA is controversial. Cercek et al showed that lysis of femoral artery thrombi

in dogs was markedly increased by the combination of heparin and rt-PA compared with either agent alone, probably as the consequence of the prevention of new fibrin formation and its incorporation in the thrombus during lysis.¹¹ Stassen et al observed only an insignificant or marginal potentiation by heparin of the thrombolysis induced by rt-PA in a rabbit jugular vein thrombosis model.¹² In a canine experimental model of coronary thrombolysis, Mickelson et al showed that rt-PA interacted favorably with heparin yielding more frequent and complete reperfusion.¹³ More recently, Van Ryn-McKenna et al found that heparin decreased the thrombolytic potential of rt-PA in a rabbit jugular vein model.¹⁴ Topol et al,¹⁵ in a randomized, controlled trial specifically designed to evaluate the role of heparin in the thrombolysis with rt-PA, found that the addition of intravenous heparin to rt-PA did not significantly affect early coronary patency rates.

The aim of this study was to evaluate the effect of heparin on thrombolysis with rt-PA in a rabbit jugular vein thrombosis model. The specific objectives of the study were to evaluate the ability of heparin to enhance the thrombolytic effect of rt-PA and to prevent thrombus growth during and after thrombolysis with rt-PA. Attention was paid to select doses of heparin able to produce therapeutic antifactor Xa levels.

MATERIALS AND METHODS

Materials. Human ¹²⁵I-fibrinogen (FIBI-125 C, 90% clottable, specific activity 7.1 MBq/mg) was purchased from Sorin Biomedica, Saluggia, Italy. Preliminary studies performed in our laboratory did not show any difference in this experimental model using rabbit and human labeled fibrinogen. Bovine thrombin (RHT 19, lot no. 506009 A) used to produce experimental thrombi was obtained from Istituto Behring, L'Aquila, Italy. The chromogenic substrate (S2251, H-D-Val-Leu-Lys-pNa 2HCl, lot no. 87697 51) and the human plasmin (25 casein U/bottle, lot no. 87703 51) used in the plasminogen and α -2-antiplasmin activity assays and the chromogenic substrate (S-2222, Bz-Ile-Glu(-OR)-Gly-Arg-pNA-HCl, lot no. 87012 51)

From the Istituto di Semeiotica Medica, Università di Perugia, Perugia, Italy.

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Address reprint requests to Giancarlo Agnelli, MD, Istituto di Semeiotica Medica, Università di Perugia, via Enrico dal Pozzo, 06100 Perugia, Italy.

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used in the anti-Xa activity assay were obtained from KabiVitrum AB, Stockholm, Sweden. Sodium pentobarbital was obtained from Farmitalia-Carlo Erba, Milan, Italy. Recombinant hirudin (r-hirudin, CGP 39393) with a specific activity of 11,496 antithrombin units (ATU)/mg used in the in vitro experiments was obtained from Ciba-Geigy, Basle, Switzerland, and manufactured by Ciba-Geigy and Plantorgan Werk, FRG. D-Phe-Pro-Arg-CH₂-Cl (PPACK), also used in the in vitro experiments, was obtained from Calbiochem, Frankfurt, FRG.

All animal studies were performed with New Zealand rabbits and conformed to the guiding principles of the American Physiological Society and the International Committee on Thrombosis and Haemostasis.¹⁶

Thrombolysis model. The thrombolytic activity of the different treatments was assessed by the lysis of standard sized, preformed ¹²⁵I-fibrinogen-labeled thrombi produced in the external jugular veins of rabbits (weight 2.5 ± 0.1 kg), as previously described.² White New Zealand rabbits of both sexes were anesthetized by the injection of sodium pentobarbital (30 mg/kg) via the marginal ear vein. Additional pentobarbital was administered when needed to maintain anesthesia. Through a paramedial incision of the neck, the jugular veins and the right carotid artery were exposed. The right carotid artery was then cannulated with a 1.5-mm-diameter polyethylene cannula for blood sampling. The two jugular veins were isolated and cleared over a distance of 2 cm and small side branches were ligated. Each jugular segment was emptied of blood and blood flow was temporarily occluded both proximally and distally by two angiostaths, 1.5 cm apart. A 10-cm length 3.0 TiCron braided polyester suture, presoaked in a collagen solution, was introduced lengthwise in the lumen of the isolated jugular vein for a distance of 4 cm to avoid the embolization of the thrombus.

One hundred and fifty microliters of citrated rabbit blood containing 5 μL of ¹²⁵I-fibrinogen (10 μCi, ~500,000 cpm) was then aspirated in a 1-mL tuberculin syringe containing 50 μL (5 IU) of thrombin and 10 μL of CaCl₂ (0.25 mol/L). These substances were quickly mixed and injected in the isolated segment of the jugular vein via a 25-gauge needle. Injection of air bubbles was carefully avoided. In all instances the thrombi formed quickly. Thrombi were allowed to age for 30 minutes and then both vessel clamps were removed and blood flow restored. The restoration of blood flow was continually checked. Urea solubility and the uniform labeling with ¹²⁵I-fibrinogen of the thrombi were as described previously.²

Evaluation of the thrombolytic activity. The extent of thrombolysis was calculated 30 minutes after the end of the infusion as the difference between the radioactivity originally incorporated in the thrombus and that remaining in the vein segment. Thrombolysis was expressed as a percentage of the original radioactivity. Efforts were made to avoid overestimation of the thrombolysis, a common pitfall of this animal model. The cotton swabs, syringes, and needles were kept for radioisotope counting. The amount of radioactivity delivered to the clot was calculated by subtracting the radioactivity remaining in the cotton swabs, syringes, and needles from the original amount of radioactivity in the syringe. To avoid overestimation of the thrombolysis due to the liberation into the blood of radioactive fibrinogen not incorporated in the thrombus at the moment of clamp removal, a 1-mL sample was drawn immediately before and immediately after the removal of the clamps to measure the radioactivity. The amount of fibrinogen not incorporated in the thrombus was evaluated as total blood radioactivity, assuming a theoretical blood volume of 60 mL/kg body weight. The total blood radioactivity was also subtracted from the original amount of radioactivity in the syringe. In each experiment, at least two rabbits were treated with saline.

Thrombus growth model. The effectiveness of two doses of heparin in preventing thrombus extension during thrombolysis with

rt-PA was assessed as their ability to inhibit the accretion of ¹²⁵I-fibrinogen onto autologous nonradioactive thrombi preformed in the jugular vein of rabbits. Standard sized nonradioactive thrombi were produced as described for the thrombolysis experiments. Fifteen minutes after the thrombi were formed, each animal was injected with 10 μCi of ¹²⁵I-fibrinogen. Five minutes later, each rabbit was injected with the treatments under study. At the end of the treatment infusion, the venous segments containing the thrombi were tied off, split open longitudinally, and the remaining thrombi removed. The specific activity of the whole blood fibrinogen was estimated from the mean of the blood samples collected at hourly intervals throughout the infusion. The ratio of the radioactivity of the thrombus to the circulating fibrinogen radioactivity was used to quantify the thrombus growth, which was then expressed as micrograms of ¹²⁵I-fibrinogen accreted onto the thrombus. In the thrombus growth studies, t-PA-induced thrombolysis was assessed through thrombus wet weight by comparing the results with those of the saline controls.

Treatments. Single chain recombinant t-PA (Actilyse), batch number 7016, was obtained from Boehringer Ingelheim, Florence, Italy. Clinical grade unfractionated heparin with a specific activity of 145 U/mg both in United States pharmacopoeia (USP) and antifactor Xa units and a mean molecular weight of 16,000 D, range 4,000 to 30,000 D was Liquepine (Hoffman-La Roche, Basel, Switzerland).

Treatment schedules. In the thrombolysis studies rabbits were infused with rt-PA at a total dose of 0.5 mg, 1 mg, or 2.5 mg over 3 hours. Rabbits in each group also received heparin, 20 or 60 anti-Xa U, or saline over 6 hours. As a control, other rabbits received only saline or the two doses of heparin for 6 hours. Treatment with rt-PA and heparin or saline started at the same time. The size of thrombi was evaluated at the end of saline or heparin infusions.

In the thrombus growth inhibition studies, saline and heparin, at the doses of 20 and 60 antifactor Xa U, were infused over 6 hours with and without rt-PA. rt-PA was infused at a dose of 0.5 mg over 3 hours. Treatments with heparin or saline and rt-PA started at the same time. The ¹²⁵I-fibrin accretion was evaluated at the end of saline or heparin infusions. In different experiments, rabbits were infused over 3 hours with 0.5 mg of rt-PA. At the end of the infusion a group of rabbits was killed and the thrombus growth evaluated. The remaining rabbits were randomized to receive 3 additional hours of either heparin, 60 antifactor Xa U, or saline. rt-PA and heparin were infused systemically via marginal ear vein contralateral to the jugular vein thrombus using a constant rate infusion pump. In both the thrombolysis and thrombus growth experiments, 10% of the rt-PA or heparin was injected as a bolus dose. The bolus doses of heparin and rt-PA were diluted in 5 mL of saline. Doses of rt-PA and heparin to be infused with the pump were diluted in a total volume of 20 mL. In the thrombolysis studies, the doses of rt-PA were selected because they are known to induce a thrombolysis ranging between 30% and 90%. In the thrombus growth study, the dose of rt-PA was selected because it is known to induce 30% thrombolysis. In both studies, the two doses of heparin were selected because they are known to produce plasma antifactor Xa level at the low and high limit of the therapeutic range, respectively. These schedules were selected because they reproduce, within limits, the schedules adopted in the most important clinical trials with t-PA.

Hemostatic assay. Before treatments and every hour of treatment, samples of arterial blood of 4.5 mL were drawn into trisodium citrate (final concentration 0.012 mol/L; whole blood, 9 parts: citrate, 1 part) for the assays of activated partial thromboplastin time, thrombin clotting time, fibrinogen, plasminogen, α-2-antiplasmin, and anti-Xa activity. Blood samples for fibrinogen assay were collected on aprotinin, 200 kallikrein inhibitors units/mL blood, to inhibit fibrinogenolysis in vitro. Fibrinogen assay was performed by

the clotting rate assay of Claus.¹⁷ Plasminogen was assessed according to Friberger and Knos¹⁸ and α -2-antiplasmin according to Teger-Nielsson et al.¹⁹ Results of fibrinogen and α -2-antiplasmin were expressed as a percentage of pretreatment values. Plasma anti-Xa activity was assessed by the chromogenic method described by Teien et al.²⁰

In vitro studies. In vitro studies were performed to determine the mechanisms contributing to the lack of antithrombotic effect of heparin when administered concomitantly with rt-PA. These in vitro experiments used PPACK, which inhibits both t-PA and thrombin, and hirudin, which inhibits thrombin. Both thrombolysis and inhibition of fibrin accretion on preformed thrombi were studied. Standard clots from 300 μ L of rabbit plasma were obtained after clotting citrated plasma with thrombin in the presence of CaCl₂ (final concentration of thrombin, 0.5 U/mL of plasma; final concentration of CaCl₂, 0.025 mol/L). Clots were stabilized for 2 hours at room temperature. For both thrombolysis and fibrin accretion inhibition studies, the clots were suspended in 2 mL of normal rabbit citrated plasma. In the thrombolysis experiments, radioactive clots were used and the lysis was measured by the decrease in the radioactivity originally incorporated in the clot and confirmed by counting the radioactivity in the supernatant. In the fibrin accretion inhibition experiments, nonradioactive rabbit clots were produced and then immersed in rabbit normal plasma containing radioactive fibrinogen (0.1 μ Ci/mL plasma). The deposition of new fibrin on the clot was assessed by removing the clot from the suspension and counting the radioactivity absorbed on the clot after accurate washing. The evaluation of both thrombolysis and inhibition of fibrin accretion on the thrombi was made after 2 hours of incubation at room temperature with gentle rocking. Both thrombolysis and fibrin accretion inhibition studies were performed in normal rabbit citrated plasma either containing or not containing 800 ng/mL of rt-PA in the presence of saline (for control), heparin (0.75 IU/mL, final concentration), r-hirudin (1.5 μ g/mL, final concentration), or PPACK (final concentration 2 μ mol/L). Heparin, r-hirudin, and PPACK were used at concentrations causing the same prolongation of thrombin clotting time (using bovine thrombin at 5 U/mL) when added to normal plasma. In confirming experiments, doses of PPACK (2 μ mol/L), heparin (1.5 IU/mL), and hirudin (1.5 μ g/mL) producing the same extent of inhibition of fibrin accretion (25% of saline control, 40 μ g of radioactive fibrinogen) in absence of rt-PA were first identified. These doses were then tested in the same model in the presence of rt-PA.

Statistical analysis. The results are expressed as mean \pm standard deviation. Statistical analysis was performed by Student *t*-test for unpaired values, using one-tailed significance tables and taking $P < 0.01$ to indicate a significant difference.

RESULTS

Thrombolytic studies. The results of the thrombolytic studies are shown in Fig 1. Infusion of saline produced 11% \pm 3% thrombolysis ($n = 16$). The two doses of heparin ($n = 12$ for each dose) did not produce significant thrombolysis (12% \pm 3% and 14% \pm 4%, respectively). Infusion of rt-PA with and without heparin produced a dose-dependent thrombolysis. The three doses of rt-PA ($n = 12$ for each dose) produced 34% \pm 6%, 52% \pm 7%, and 79% \pm 8% thrombolysis in rabbits treated with heparin, 20 antifactor Xa/kg/h and 39% \pm 6%, 49% \pm 4%, and 81% \pm 6% thrombolysis in rabbits treated with heparin, 60 antifactor Xa/kg/h. In rabbits treated with rt-PA and saline ($n = 12$) thrombolysis was 37% \pm 4%, 47% \pm 5%, and 84% \pm 7%. Thus, no differences in thrombolysis were observed among the groups of rabbits

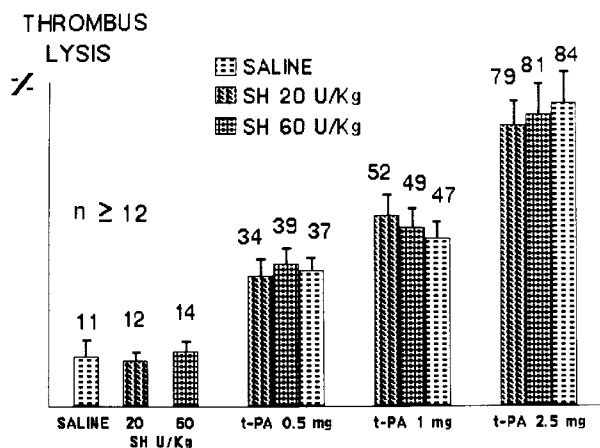


Fig 1. Thrombolytic effects of saline, the two doses of heparin (SH) and t-PA plus saline, and the two doses of heparin.

treated with the two doses of heparin and saline receiving the same dose of rt-PA.

Thrombus growth studies. The results of the thrombus growth studies are shown in Figs 2, 3, 4, and 5. Infusion of saline was followed by the accretion of 65.1 \pm 5.9 μ g of ¹²⁵I-fibrin on the artificial preexisting thrombi ($n = 16$) (Fig 2). Treatment with the two doses of heparin reduced the accreted fibrin to 46.0 \pm 6.4 μ g and 22.0 \pm 4.9 μ g, respectively ($n = 12$ for each dose) (Fig 2). As expected, the infusion of 0.5 mg of rt-PA produced 30% to 40% thrombolysis on a weight basis. Nevertheless, the rabbits treated with rt-PA and saline showed a fibrin accretion onto the thrombus similar to the saline-treated rabbits, rt-PA being unable to prevent fibrin accumulation on the residual thrombus. Treatment with rt-PA (3 hours) and saline (6 hours) ($n = 16$) resulted in an accretion of 59.4 \pm 10.4 μ g of radioactive fibrinogen at the end of the saline infusion (Fig 3). The concomitant administration of rt-PA (3 hours) and the two doses of heparin (6 hours) ($n = 12$ for each dose) produced an accretion of 54.8 \pm 7.4 μ g and 52.4 \pm 9.1 μ g of radioactive fibrin on the preexisting thrombi (Fig 3). Thus, heparin was not able to prevent thrombus growth when infused with rt-PA.

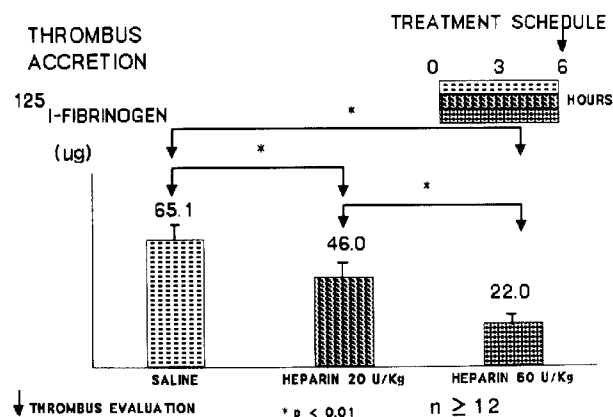


Fig 2. Effect of saline and the two doses of heparin (SH) on fibrin accretion on the experimental thrombi.

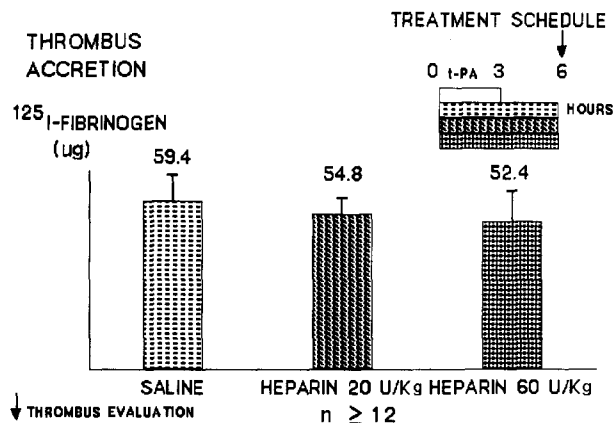


Fig 3. Effect of t-PA infusion (3 hours) plus saline and the two doses of heparin (6 hours) on fibrin accretion on the experimental thrombi.

The results of the experiments where rabbits were treated with 0.5 mg of rt-PA over 3 hours, and partly killed and partly randomized to heparin or saline (3 additional hours) are shown in Figs 4 and 5. After the 3 hours of rt-PA treatment, some rabbits were killed (n = 12). Accreted onto the thrombi was 33.7 ± 5.2 μg of ¹²⁵I-fibrin (saline 35.0 ± 3.1 μg, n = 14; rt-PA plus heparin 31.1 ± 6.1 μg, n = 14; heparin 16.4 ± 1.8 μg, n = 12; Fig 4). In the rabbits randomized to receive an additional 3-hour course of saline (n = 14) or heparin (n = 14), 58.7 ± 8.9 μg and 41.9 ± 6.5 μg radioactive fibrin was accreted on the thrombi (P < .01) (Fig 5). Thus, infused after rt-PA, heparin was more effective than saline in preventing fibrin accretion. However, during heparin treatment a 25% radioactive fibrin accretion onto the thrombi was observed (41.9 μg v 33.7 μg, P < .01).

Hemostatic assays. In rabbits treated with the lower dose of heparin, the anti-Xa level ranged between 0.24 and 0.32 U/mL. In rabbits treated with the higher dose of heparin, the anti-Xa ranged between 0.42 and 0.52 U/mL. As expected, activated thromboplastin time and thrombin clotting time were prolonged in the heparin treated rabbits (1.5 times and 2 times in the low and high dose heparin

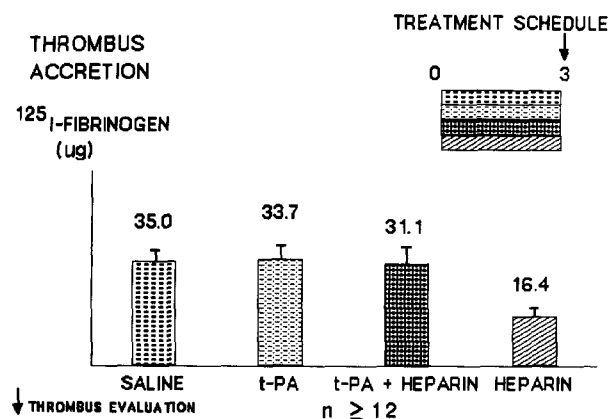


Fig 4. Effect of 3-hour infusion of saline, rt-PA, rt-PA plus heparin, and heparin on fibrin accretion on the experimental thrombi.

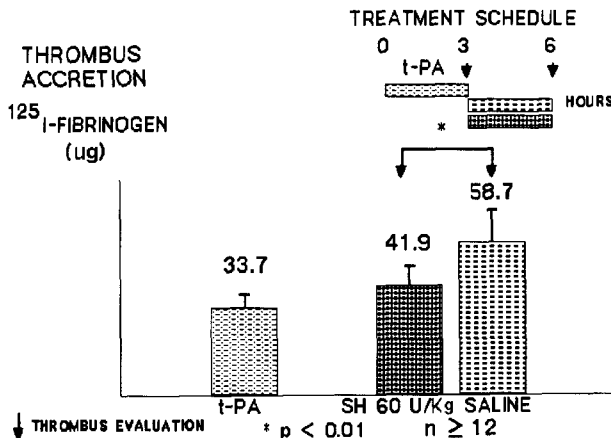


Fig 5. Effect of t-PA infusion (3 hours) followed by saline and heparin (3 hours) on fibrin accretion onto the experimental thrombi.

groups, respectively). Fibrinogen, plasminogen, and α-2-antiplasmin assays showed a statistically significant reduction only after the highest dose of rt-PA infused in the thrombolysis experiments (Table 1). Fibrinogen, α-2-antiplasmin, and plasminogen assays did not show statistically significant differences between the groups treated with heparin and saline receiving the same dose of rt-PA.

In vitro experiments. The results of the in vitro experiment are shown in Tables 2 and 3. In the in vitro thrombolysis experiments, heparin, PPACK, and r-hirudin did not produce any thrombolysis. Heparin and r-hirudin did not enhance the thrombolytic activity of rt-PA. PPACK was effective in inhibiting the thrombolysis induced by rt-PA. In the in vitro fibrin accretion inhibition experiments performed in absence of rt-PA, r-hirudin and PPACK were both more effective than heparin (P < .001). The effective thrombolysis induced by rt-PA was accompanied by a higher accumulation of radioactive fibrin onto the thrombi (296 ± 34 μg v 161 ± 29 μg, P < .001). r-Hirudin and PPACK were able to prevent fibrin accretion onto the thrombi observed in the presence of rt-PA while heparin was not (P < .001). No statistically significant difference between r-hirudin and PPACK was observed (48 ± 6 μg v 43 ± 5 μg). The three doses of r-hirudin, PPACK, and heparin producing the same extent of inhibition of fibrin accretion (40 μg of radioactive fibrinogen) in absence of rt-PA gave different results in presence of t-PA. On the thrombi incubated with r-hirudin, PPACK, and heparin, 49 ± 4 μg, 47 ± 5 μg, and 156.2 ± 19 μg of radioactive fibrinogen were accreted, respectively (P < .001 when both r-hirudin and PPACK were compared with heparin).

DISCUSSION

Biochemical studies on the interaction of heparin with fibrinolysis components,²¹ more recently including t-PA,²²⁻²⁵ have provided controversial results in view of the possible clinical application of this association. Human antithrombin III-heparin complex was shown to efficiently inhibit human plasmin.²¹ More recent studies have shown that heparin is able to both increase the activation of plasminogen by t-PA²²

Table 1. Effect of the Three Doses of rt-PA Infused Along With Heparin (H) and Saline (S) on Plasma Fibrinogen, Plasminogen, and α -2-Antiplasmin (third-hour samples)

	rt-PA (0.5 mg)		rt-PA (1 mg)		rt-PA (2.5 mg)	
	H	S	H	S	H	S
Fibrinogen (%)	98 \pm 4	96 \pm 5	94 \pm 6	95 \pm 7	70 \pm 7*	68 \pm 8*
Plasminogen (%)	94 \pm 9	93 \pm 8	82 \pm 9	86 \pm 9	66 \pm 8*	63 \pm 9*
α -2-antiplasmin	94 \pm 4	92 \pm 6	80 \pm 9	82 \pm 9	59 \pm 8*	56 \pm 9*

Data are expressed as percentage of the pretreatment level (means \pm SD).

* $P < .01$ when compared with the pretreatment values.

and reduce the stimulation of t-PA activity by fibrin,^{22,23,25} heparin, and fibrin competing for the same binding sites on plasminogen and/or t-PA.²³ In contrast, Fry and Sobel have shown that heparin does not interfere with the binding of t-PA to thrombi at therapeutic concentrations.²⁴ Each of these observations has potential clinical implications. The enhancement of t-PA activity by heparin could optimize thrombolysis with t-PA, while a heparin-induced reduction of the ability of t-PA to be stimulated by fibrin could nullify the advantage of t-PA over the first generation thrombolytic agent.

In the clinical setting, heparin is administered during thrombolysis for several reasons, including limitation of thrombus formation during thrombolysis, prevention of acute reocclusion, as well as before administration of thrombolytic agents as part of treatment for unstable angina or during cardiac catheterization and coronary angiography. We have assessed the ability of heparin to enhance the thrombolytic effect of rt-PA and to prevent fibrin accretion onto the thrombi during thrombolysis with rt-PA in a rabbit animal model. We conclude that heparin is not effective in enhancing the thrombolytic effect of rt-PA nor in preventing the extension of residual thrombi during rt-PA treatment. Our experimental findings are in keeping with the results obtained by Topol et al showing that the addition of intravenous heparin to rt-PA in myocardial infarction patients did not significantly affect early coronary patency rates.¹⁵ It should be noted that we selected "therapeutic" doses of heparin, doses producing 0.3 to 0.6 antifactor Xa levels, able to inhibit thrombus growth in absence of rt-PA.²⁶ This ability was confirmed in our control experiments (Fig 2). The addition of heparin did not result in an increased systemic fibrinolytic activation and fibrinogen breakdown. This result suggests that the heparin-induced inhibition of the stimulation of rt-PA by fibrin observed *in vitro* at physiologic concentrations does not occur *in vivo* at the higher concentrations achieved during pharmacologic thrombolysis.

A major problem in the use of rt-PA as well as other thrombolytic agents has been rethrombosis. Thus, the results

of the experiments on thrombus growth inhibition deserve further comments. We observed that fibrin still accumulates on the thrombi during effective thrombolysis induced by rt-PA. The fact that lysing thrombi can still accumulate fibrin is not a new observation,²⁷ but the phenomenon was never so clearly quantified before. One possible explanation is that thrombogenic material is exposed by the dissolution of the thrombus. Alternatively, it has been hypothesized that thrombolytic therapy is associated with thrombin generation and this contributes to the rethrombosis.^{28,29} Thrombin produced in association with lytic therapy is generated on thrombus surface where it is protected from fluid-phase inhibitors.²⁸ Indeed, thrombin bound to thrombus is less susceptible to inhibition by natural inhibitors and standard heparin than circulating thrombin.²⁸ Weitz et al have recently found that in a purified system rt-PA can cleave fibropeptide A and B from fibrinogen, exerting a sort of thrombinlike activity.³⁰ Should this effect occur on the surface of the thrombus, it could explain the active absorption of new fibrin on the thrombi during t-PA treatment. In our experiments, heparin was clearly ineffective in preventing thrombus growth during rt-PA treatment. On the other hand, heparin was effective when infused alone or after rt-PA was cleared from the circulation, suggesting that the efficacy of heparin is compromised by rt-PA. It has been recently shown that fibrin monomers II protect thrombin from inactivation by heparin-antithrombin III.³¹ Thus, it is tempting to speculate that fibrin monomers II might be produced on the thrombus surface by thrombin or the thrombinlike activity of rt-PA,³⁰ and this could compromise the antithrombin activity of heparin during rt-PA treatment.

Our *in vitro* experiments confirmed that the lysis of clots produced by rt-PA enhances fibrin accretion on the clot. r-Hirudin and PPACK, two inhibitors of thrombin, are able to inhibit fibrin accretion on the clots induced by rt-PA while heparin is not. PPACK, which in addition to thrombin also inhibits t-PA, inhibits both rt-PA-induced thrombolysis and

Table 2. In Vitro Thrombolysis Induced by Saline, r-Hirudin, PPACK, and Heparin Incubated With and Without rt-PA

In Vitro Thrombolysis (%)			
Saline	3.6% \pm 1.2%	t-PA + Saline	25.1% \pm 4.9%
Heparin	4.1% \pm 1.9%	t-PA + Heparin	27.0% \pm 4.6%
PPACK	2.8% \pm 3.1%	t-PA + PPACK	6.1% \pm 3.1%
r-Hirudin	3.9% \pm 2.1%	t-PA + Hirudin	26.1% \pm 5.1%

Table 3. In Vitro Inhibition of Fibrin Accretion on the Clots Induced by Saline, r-Hirudin, PPACK, and Heparin Incubated With and Without rt-PA

In Vitro Thrombus Growth Inhibition (μ g)			
Saline	161 \pm 29	t-PA + Saline	296 \pm 34
Heparin	58 \pm 6	t-PA + Heparin	246 \pm 39
PPACK	37 \pm 4	t-PA + PPACK	43 \pm 5
r-Hirudin	39 \pm 4	t-PA + Hirudin	48 \pm 6

fibrin accretion on the clots. These observations are consistent with a potential role of thrombin generated during rt-PA-induced lysis in the thrombus extension after thrombolysis. The limited ability of heparin to inhibit thrombin on the thrombus surface and the surrounding plasma could explain its failure in inhibiting the accretion of new fibrin on the clot. r-Hirudin is more effective than heparin in inhibiting fibrin accretion on the clots. This difference could be explained by its greater ability to neutralize thrombin both in solution and bound fibrin during t-PA-induced thrombolysis as recently observed by Mirshahi et al.³² Heparin was more effective than saline in preventing thrombus growth after therapy with rt-PA was stopped. Our experimental findings are in keeping with the results of two recently published clinical trials in which the effects of adding intravenous heparin to rt-PA in myocardial infarction patients was assessed by evaluating coronary patency rates at a mean of 18³³ and 55 hours³⁴ after beginning of treatment. The addition of intravenous heparin to rt-PA was associated with an improved patency rate when compared with aspirin³³ or no

adjunctive therapy.³⁴ Our experimental findings are in apparent conflict with the results of the GISSI 2 study, which showed that adding subcutaneous heparin to either rt-PA or streptokinase did not improve survival rates.³⁵ Whether the difference between the patency studies and the GISSI 2 study, in which heparin therapy was started 12 hours after the thrombolytic agents, is due to the different heparin dosage schedule or to the irrelevance of patency in determining mortality remains to be established.

In conclusion, in our experiments heparin was unable to enhance the thrombolytic activity of rt-PA and prevent thrombus growth during rt-PA treatment. Heparin was more effective than saline in preventing thrombus growth when administered after rt-PA treatment.

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