JACC Vol. 17, No. 5
April 1991:1213–22

Bleeding Time Prolongation and Bleeding During Infusion of Recombinant Tissue-Type Plasminogen Activator in Dogs: Potentiation By Aspirin and Reversal With Aprotinin

HARRY D. GARABEDIAN, SM,* HERMAN K. GOLD, MD, FACC,*
ROBERT C. LEINBACH, MD, FACC,* TRACY A. SVIZZERO,*
DIANNE M. FINKELSTEIN, PHD,* J. LUIS GUERRERO,* DÉSIRÉ COLLEN, MD, PHD*†

Boston, Massachusetts and Burlington, Vermont

Thrombolytic therapy is associated with a bleeding tendency that may be exacerbated by adjunctive antiplatelet agents. The effect of recombinant tissue-type plasminogen activator (rt-PA) alone or in combination with aspirin on serial measurements of template bleeding time, ex vivo platelet aggregation and coagulation factors and the frequency of bleeding was studied in dogs. During infusion of rt-PA (15, 30 or 60 μg/kg per min for 90 min), a dose-related increase in bleeding time was observed.

In a randomized blinded study of 25 dogs, the baseline bleeding time (mean ± SD) was 3.5 ± 1 min in control animals and 4 ± 2 min after oral aspirin (15 mg/kg body weight). Infusion of rt-PA (15 μg/kg per min for 90 min) prolonged the bleeding time to a maximum of 15 ± 12 min. In contrast, combined aspirin and rt-PA therapy produced an increase to >30 min during infusion, reverting to 13 ± 10 min within 2 h after cessation of infusion.

Recurrent continuous bleeding from incision sites occurred in one of six dogs given aspirin alone, two of seven given rt-PA alone and all six dogs given both aspirin and rt-PA ($p = 0.02$). Bleeding time >9 min correlated significantly with bleeding frequency ($p < 0.0001$), with a sensitivity of 100% and a specificity of 87%.

Intravenous bolus injection of aprotinin (20,000 kallikrein inhibitor units/kg body weight) in six dogs given both rt-PA and aspirin produced a decrease in bleeding time from >30 min to 9.5 ± 9 min and resulted in cessation of bleeding. Thus, bleeding and bleeding time prolongation in this canine model are potentiated by a marked interactive effect of rt-PA and aspirin that is rapidly reversible. Template bleeding times may provide a useful quantitative index for monitoring the bleeding tendency associated with thrombolytic therapy.

(J Am Coll Cardiol 1991;17:1213–22)

Thrombolytic therapy has become an established method for treating coronary artery thrombosis in patients with acute myocardial infarction (1). Intravenous infusion of recombinant tissue-type plasminogen activator (rt-PA) recanalizes occluded coronary arteries (2–4), preserves left ventricular function (5–7) and reduces mortality (7). Adjunctive therapy with aspirin has also been shown to further improve survival after administration of streptokinase (8).

The major side effect of thrombolytic therapy is bleeding, which on occasion may be life-threatening. Concern has focused on the frequency of intracranial bleeding, which although not observed in the initial studies with rt-PA (2–4), has occurred subsequently in as many as 1.6% of treated patients (6,9). More recently, administration of rt-PA at the currently recommended 100 mg dose has established an intracranial bleeding frequency of 0.5% (10) and 0.1% excess stroke rate relative to patients not treated with thrombolytic agents (7). Available comparative trials of streptokinase and rt-PA indicate that hemorrhagic complications do not correlate strictly with the extent of fibrinogen breakdown produced by thrombolytic therapy (3,4,11,12). Thus, although its pathogenesis is not established, bleeding associated with thrombolytic therapy seems to result not from disturbance of the hemostatic system, but from vascular injury (13) and possibly from interference of the thrombolytic agent with hemostatic plug formation.

On the basis of the latter hypothesis, we postulated that measurement of laboratory variables in vivo may provide a reliable marker for monitoring hemorrhagic risk during thrombolytic therapy. In a recent pilot study (14), significant prolongation of bleeding time that correlated with the occurrence of spontaneous bleeding was observed during infusion of rt-PA in patients with acute myocardial infarction.

The present investigation was designed to extend these observations by examining the effect of rt-PA administered

From the *Cardiac Division and the Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts and †Departments of Biochemistry and Medicine, University of Vermont College of Medicine, Burlington, Vermont. This study was supported by Grants HL-26215 (Ischemia Specialized Center of Research) and HL-35058 (Thrombosis Specialized Center of Research) from the National Heart, Lung, and Blood Institute, Bethesda, Maryland and by a sponsored research grant between Massachusetts General Hospital, Boston, Massachusetts and Genentech, Inc., South San Francisco, California.

Manuscript received July 30, 1990; revised manuscript received October 24, 1990, accepted November 9, 1990.

Address for reprints: Herman K. Gold, MD, Cardiac Unit, ACC 4, Suite 480, Massachusetts General Hospital, Fruit Street, Boston, Massachusetts 02114.

0735-1097/91/$3.50

under controlled experimental conditions alone or in combination with aspirin on measurements of hemostasis and the frequency of bleeding in dogs. The time course and extent of changes in template bleeding time, ex vivo platelet aggregation and coagulation factors were investigated. In a preliminary study, different doses of rt-PA alone and in combination with aspirin were administered. In the second phase, dogs were assigned randomly to treatment with fixed doses of rt-PA or aspirin, both or neither. Investigators performing these tests did not know which study drugs had been administered. Finally, the reversibility with intravenous aprotinin, a serine protease inhibitor, of bleeding time prolongation and bleeding after combined therapy with aspirin and rt-PA was tested.

Reagents. Recombinant human tissue-type plasminogen activator (rt-PA) (Genentech, Inc.) was provided in vials containing 50 mg of lyophilized material in predominantly single chain form (Activase). Adenosine 5'-diphosphate (ADP) and lyophilized aprotinin were purchased from Sigma Chemical Co., and epinephrine and arachidonic acid from BioData Corporation; all agents were reconstituted in distilled water. Aspirin and vitamin tablets were purchased from the hospital pharmacy.

Animal preparation and treatment. Adult mongrel dogs weighing 5 to 18 kg were anesthesized with sodium pentobarbital (30 mg/kg intravenously, followed by 10 mg bolus injections as needed), intubated and placed on a mechanical respirator. The femoral artery and vein were then cannulated, with local hemostasis controlled by careful vessel ligation. No heparin was administered.

Infusion of rt-PA or placebo was administered at doses adjusted to body weight (μg/kg per min for 90 min) into the femoral vein by means of a constant rate infusion pump (Harvard Apparatus). Aspirin or placebo tablets were given orally 2 to 4 h before induction of anesthesia at the indicated doses per kg body weight. Aspirin tablets (80 or 325 mg) were administered wholly or in part in a total dose closely approximating the indicated dose per kg body weight. Blood pressure was monitored from the femoral artery. These studies were performed in conformance to the Position of the American Heart Association on Research Animal Use adopted November 11, 1984.

Design of the studies. The experimental sequence for treatment and measurement of laboratory variables is illustrated in Figure 1.

Phase 1. In the preliminary dose-finding study, 26 dogs were allocated to treatment with saline solution (Group I) or to 15 (Group II), 30 (Group III) or 60 (Group IV) μg/kg per min of rt-PA for 90 min. Then, in six additional dogs, rt-PA infusion at 15 μg/kg per min for 90 min was administered in combination with oral aspirin doses of 10 (Group V), 15 (Group VI) or 40 or 100 (Group VII) mg/kg body weight.

Phase 2. The second phase was designed to document the effect of combined rt-PA and aspirin therapy on bleeding time and frequency of bleeding in a randomized prospective study design, with the investigators performing laboratory measurements and assessment of bleeding without knowledge of the study drugs administered. Twenty-five dogs were assigned to one of the following treatments groups: 1) rt-PA excipient infusion for 90 min and aspirin placebo (Group I); 2) 15 μg/kg per min of rt-PA for 90 min and aspirin placebo (Group II); 3) rt-PA excipient and 15 mg/kg of aspirin (Group III); or 4) 15 μg/kg per min of rt-PA for 90 min and 15 mg/kg of aspirin.

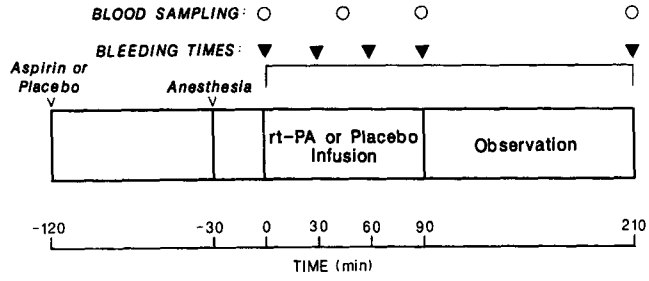

Figure 1. Schematic representation of the time course of intervention, study drug infusion and analyses. Oral aspirin or placebo administration preceded induction of anesthesia by approximately 2 to 4 h, after which femoral venous and arterial lines were placed and baseline bleeding time and blood sampling performed. Infusion of recombinant tissue-type plasminogen activator (rt-PA) or placebo was then done for the next 90 min, followed by a 2 h observation period and final bleeding time determination and blood sampling. O = Whole blood samples processed as described in Methods and used for ex vivo platelet aggregation and measurement of plasma fibrinogen, alpha$_2$-antiplasmin and rt-PA-related antigen levels and platelet counts. ▼ = intervals at which template bleeding time was measured.

Phase 3. In the third phase of this study, the efficacy of an antifibrinolytic agent in reversing bleeding time prolongation and bleeding was tested. Six additional dogs treated with 15 mg/kg of aspirin and 15 μg/kg per min of rt-PA for 90 min were given an intravenous bolus injection of 20,000 kallikrein inhibitor units (KIU)/kg body weight of reconstituted, lyophilized aprotinin 60 to 90 min after the start of rt-PA infusion.

Bleeding and template bleeding times. Template bleeding times were measured before (usually in duplicate) and 30, 60, 90 and 210 min after the start of infusion. A spring-activated surgical steel blade instrument (Surgicutt International, Technidyne Corporation) designed to produce a standardized incision (5 mm long by 1 mm deep) was applied to a shaved foreleg. Incisions were performed parallel to and below the antecubital crease. Flow of blood from the wound was blotted at 30 s intervals with filter paper disks (Whatman #5, Whatman International Ltd.), carefully avoiding direct contact with the wound. The duration of bleeding was quantitated by timing the interval (to the nearest 30 s) from incision to cessation of bleeding as demonstrated by no further staining of the filter paper. The range of normal values for bleeding time in anesthesized dogs established previously by our laboratory is from 1 to 9 min. Values >30 min were assigned a 30 min value for purposes of statistical analysis.

Bleeding documented after the start of infusion was defined as the occurrence of renewed spontaneous oozing from formerly coagulated bleeding time or femoral incision sites. Perturbation of incision wounds was carefully avoided. The occurrence of spontaneous bleeding after the start of infusion was closely monitored for the duration of each experiment.

Platelet aggregation. Arterial blood samples (10 ml) for ex vivo platelet aggregation studies were collected in 0.1 ml of 40% trisodium citrate containing 500 KIU of reconstituted, lyophilized aprotinin/ml of blood to preclude proteolysis in vitro. In previous experiments (unpublished observations), identical aggregation patterns were obtained in duplicate platelet-rich plasma samples with and without addition of reconstituted, lyophilized aprotinin. In contrast, use of commercially available aprotinin reconstituted in 0.9% benzyl alcohol suppressed the maximal percent change in transmittance (%ΔT) and caused platelet disaggregation in vitro. Samples were obtained before and 45, 90 and 210 min after the start of infusion. Platelet-rich plasma was prepared by centrifugation at 700 *g* for 3.5 min at 22°C and removal of the supernatant with a plastic pipette. The platelet-rich plasma was then stored in a capped polystyrene test tube at 22°C, and aggregation tests were performed within 3 h. Platelet-poor plasma was prepared from the remaining blood sample by centrifugation at 2,000 *g* for 8 min at 22°C and removal of the supernatant. Platelet counts were determined with an automated particle counter (Thrombocounter C, Coulter Electronics, Inc.) equipped with a 70 μm aperture. Platelet concentration in the platelet-rich plasma was first adjusted to 3×10^8/ml and then 0.4 ml aliquots of platelet-rich plasma were aggregated with ADP (7.5 to 20 μ*M* final concentration).

In the randomized blinded study, tests were also performed with arachidonic acid (0.9 μM final concentration) to document blocking of platelet aggregation due to aspirin inhibition of cyclooxygenase. Platelet-rich plasma was pretreated with epinephrine (0.5 μM final concentration) for 30 s at 37°C before addition of arachidonic acid. In the absence of aspirin, addition of arachidonic acid always produces aggregation of canine platelet-rich plasma if preceded by epinephrine potentiation, but aggregation does not always occur in response to arachidonic acid alone (15). Canine platelet-rich plasma does not aggregate with the addition of epinephrine alone. All aggregations were performed in a dual channel aggregometer (Chrono-log Corporation) at 37°C with constant stirring at 1,200 rpm. Quantitative analysis of aggregation was determined as the maximal percent change in transmittance (%ΔT) (measured from baseline to the lowest portion of the curve) after addition of agonist.

Hemostasis analysis. Arterial blood samples (4.5 ml) for measurement of fibrinogen were collected into 0.5 ml of trisodium citrate (0.11 M) and 200 KIU of aprotinin/ml of blood before and 45, 90 and 210 min after the start of infusion. Additional samples for measurement of alpha$_2$-antiplasmin were collected into sodium citrate in the absence of aprotinin before and 210 min after the start of infusion. To measure the concentration of rt-PA-related antigen in plasma, 2 ml blood samples were collected into 0.05 ml (15%) liquid ethylenediaminetetraacetate (K_3) (EDTA) at 15 min intervals during infusion and at 210 min after the start of infusion. Plasma was separated at 22°C by centrifugation at 2,300 *g* from blood kept on ice until the end of the experiment and then stored at −20°C until assayed. Samples were also collected into EDTA immediately before the start of infusion for measurement of serum salicylate (16) and before and 210 min after the start of infusion for measurement of platelet count.

Fibrinogen was measured by a modified version (17) of the coagulation rate assay of Clauss (18) and expressed as a percent of pretreatment values. An enzyme-linked immunosorbent assay based on monoclonal antibodies was used to measure the plasma concentration of rt-PA-related antigen (19). Alpha$_2$-antiplasmin was assayed using the chromogenic substrate S-2251 (KabiVitrum) and expressed in percent by comparison with a standard curve obtained by serial dilution of a normal plasma pool (20).

Statistical analysis. Results are expressed as mean values ± SD. Student's *t* test for paired or unpaired values was used to evaluate two-tailed levels of significance of differences within or between groups. A Wilcoxon test was used to evaluate differences in cases in which the data were not normally distributed. The significance of differences between groups was analyzed using the Fisher or Kruskal-Wallis exact test (21). The relation between bleeding time and rt-PA dose in the dose-response study was analyzed using the Jonckheere-Terpstra test (21). The correlation of bleeding with prolonged bleeding time or decreased plasma fibrinogen concentration was analyzed by stepwise logistic regression (BMDP Statistical Software, University of California–Los Angeles). Variables used in this model were bleeding time >9 and ≥15 min and fibrinogen concentration <1 g/liter at 90 min. Two-tailed p values <0.05 were considered statistically significant.

Results

Dose-Response Study

Template bleeding times. Table 1 summarizes the results of serial template bleeding times in the initial group of 26 dogs. In the control group (Group I), bleeding times were within the normal range and remained essentially unchanged from baseline values throughout the experiment. Proportional increases were observed in association with infusion of rt-PA at progressively higher doses (Groups II to IV) ($p = 0.003$). The increase in bleeding time to a maximum of 11 ± 10 min during infusion of 15 μg/kg per min of rt-PA for 90 min (Group II) was attributable mainly to a single 30 min value. All other bleeding times measured at this infusion rate remained ≤9 min (upper limit of normal). When the rt-PA

Table 1. Template Bleeding Times During and After Administration of Recombinant Tissue-Type Plasminogen Activator (rt-PA) and Aspirin: Results of the Dose-Response Study

Group	Protocol		No. of Dogs	Template Bleeding Time (min)				
	rt-PA (μg/kg per min)	Aspirin (mg/kg)		Pre	30 Min	60 Min	90 Min	210 Min
I	0	0	3	3.0 ± 2.0 (1.0–5.0)	2.0 ± 1.5 (1.0–3.0)	2.5 ± 1.0 (2.0–3.5)	3.0 ± 2.0 (1.0–5.0)	3.0 ± 2.0 (1.0–5.0)
II	15	0	6	3.5 ± 1.0 (2.0–5.0)	5.0 ± 1.5 (2.5–7.5)	11 ± 10 (5.0–30)	5.5 ± 1.5 (3.0–7.5)	5.5 ± 1.5 (4.0–8.0)
III	30	0	6	4.5 ± 2.5 (2.0–9.0)	10 ± 7.0 (3.5–20)	12 ± 9.0 (6.0–30)	14 ± 9.0 (7.0–30)	10 ± 9.0 (2.0–25)
IV	60	0	5	3.5 ± 3.0 (1.0–8.5)	6.5 ± 6.5 (3.0–18)	15 ± 10 (2.0–30)	22 ± 9.0 (9.0–30)	6.0 ± 5.0 (1.0–11)
V	15	10	2	8.0 ± 0.4 (7.5–8)	14 ± 4.0 (11–17)	21 ± 13 (12–30)	13 ± 9.0 (6–19)	5.5 ± 2.0 (4–7)
VI	15	15	2	6.0 ± 1.5 (5–7)	10 ± 1.5 (9–11)	30 (30)	30 (30)	15 ± 3.5 (12–17)
VII	15	40 or 100	2	5 (5)	29 ± 1.5 (28–30)	30 (30)	25 ± 8 (19–30)	19 (19)

Data are mean values ± SD and (range) at time points before and after the start of rt-PA infusion. Group I = dogs given saline solution; Groups II to IV = dogs treated with 15, 30 or 60 μg/kg per min, respectively, of rt-PA infusion for 90 min; Groups V to VII = dogs treated with rt-PA infusion at 15 μg/kg per min plus oral aspirin in doses of 10 (Group V), 15 (Group VI) or 40 or 100 (Group VII) mg/kg body weight. Pre = before rt-PA administration.

dose was doubled (Group III), more frequent prolongation of the bleeding time was observed, with values >9 min obtained in three dogs during infusion (maximum of >30 min in two), which normalized 2 h after the end of the infusion in two of these dogs. A fourfold rt-PA dosage increase (Group IV) produced a maximal bleeding time of 22 ± 9 min, with peak values >9 min (>30 min in one dog) observed in all dogs, reverting to <9 min within 2 h after the end of infusion in all but one dog.

In aspirin-free dogs (Groups I to IV), serum salicylate concentration measured 8 ± 18 μg/ml and the bleeding time before start of infusion averaged 3.5 ± 2 min. Four hours after ingestion of 10 (Group V) or 15 (Group VI) mg/kg of aspirin, salicylate levels increased to 50 ± 20 and 47 ± 3.5 μg/ml, respectively, and baseline bleeding times were modestly but significantly prolonged ($p < 0.01$ versus aspirin-free dogs). During infusion of 15 μg/kg per min of rt-PA for 90 min, marked prolongation of bleeding time to >30 min was obtained in three of these four aspirin-treated dogs. In the remaining dog given 10 mg/kg of aspirin, the maximal bleeding time during infusion was 12 min in association with the lowest salicylate level (36 μg/ml) in aspirin-treated dogs. At the end of infusion, the bleeding time in both dogs given 15 mg/kg of aspirin remained >30 min, whereas in the two dogs receiving 10 mg/kg of aspirin, these values measured 6 and 19 min. Within the next 2 h, the average bleeding time decreased by approximately 50% in both groups.

Serum salicylate levels were 97 and 200 μg/ml in two dogs given a higher dose of aspirin of 40 or 100 mg/kg (Group VII), respectively. During rt-PA infusion, the bleeding time prolonged to >30 min, remaining >9 min in both dogs at the end of the observation period.

The serum salicylate level measured after a dose of 10 mg/kg of aspirin demonstrated a large standard deviation and was lowest in the only nonresponsive (maximal bleeding time of 12 min with no observed bleeding) dog from the initial combined therapy groups. These findings suggested the possibility that blood levels of aspirin sufficient for complete cyclooxygenase inhibition may not be produced consistently with this dose in all dogs. Accordingly, a slightly higher aspirin dosage (15 mg/kg) was selected for use in the second and third phases of this study.

Bleeding and bleeding time prolongation. No bleeding was observed from femoral or bleeding time incision sites in any dog before the start of the infusion. There was no bleeding in control dogs (Group I). Of the nine dogs with a maximal bleeding time >9 min during administration of rt-PA alone, bleeding, which always persisted for >10 min, was observed in five (one in Group II and two in each of Groups III and IV). No bleeding was observed in the eight dogs with a bleeding time ≤9 min during rt-PA infusion alone. Bleeding that always persisted >10 min was observed in five of six dogs in the combined therapy groups (Groups V to VII), all with a maximal bleeding time >30 min. The one dog with no bleeding received 10 mg/kg of aspirin and demonstrated a peak bleeding time of 12 min.

Table 2. Platelet Aggregation and Hemostasis Variables After Administration of Recombinant Tissue-Type Plasminogen Activator (rt-PA) and Aspirin: Results of the Dose-Response Study

	Platelet Aggregation (%ΔT)			Plasma rt-PA (μg/ml)				Fibrinogen (g/liter)			$Alpha_2$-Antiplasmin (%)	
Group	Pre	90 Min	210 Min	30 Min	60 Min	90 Min	210 Min	Pre	90 Min	210 Min	Pre	210 Min
I	60 ± 21 (38–80)	70 ± 20 (50–79)	55 ± 5 (50–60)	—	—	—	—	1.8 ± 0.1	1.5 ± 0.1	1.6 ± 0.1	76 ± 7	71 ± 8
II	55 ± 18 (28–73)	64 ± 15 (35–75)	56 ± 35 (13–106)	0.6 ± 0.1	0.8 ± 0.2	0.9 ± 0.3	0.08 ± 0.03	2.2 ± 1.0	1.1 ± 0.5	1.2 ± 0.7	79 ± 13	40 ± 20
III	66 ± 9 (50–76)	56 ± 8 (46–68)	59 ± 18 (34–73)	1.1 ± 0.4	1.8 ± 0.6	1.6 ± 0.9	0.3 ± 0.3	1.7 ± 0.5	1.0 ± 0.5	1.2 ± 0.7	81 ± 10	25 ± 8
IV	58 ± 18 (38–75)	35 ± 28 (0–69)	34 ± 35 (5–81)	4.6 ± 1.5	5.4 ± 3.4	6.4 ± 5.6	0.4 ± 0.3	1.9 ± 0.6	0.3 ± 0.3	0.19 ± 0.12	80 ± 17	14 ± 6
V	99 ± 1 (98–100)	70 ± 42 (40–100)	70 ± 53 (33–106)	0.7 ± 0.4	1.0 ± 0.7	1.2 ± 0.8	0.06 ± 0.02	3.2 ± 0.5	1.1 ± 1.6	1.4 ± 2.0	80 ± 5	38 ± 25
VI	73 ± 5 (69–75)	75 ± 4 (73–78)	75 ± 9 (69–81)	0.7 ± 0.14	0.8 ± 0.07	0.8 ± 0.07	0.05 ± 0.01	3.3 ± 0.8	2.8 ± 1.3	2.7 ± 1.1	97 ± 19	62 ± 29
VII	55 ± 2 (53–56)	41 ± 39 (14–69)	48 ± 35 (23–73)	1.1 ± 0.4	1.4 ± 0.4	1.5 ± 0.4	0.09 ± 0.02	1.9 ± 0.2	0.6 ± 0.7	0.5 ± 0.7	74 ± 16	31 ± 8

Data are mean values ± SD and (range). %ΔT = maximal percent change in transmittance.

The frequency of bleeding was related significantly to prolongation of the bleeding time to >9 min ($p < 0.0001$), yielding a sensitivity and specificity of 100% and 69%, respectively. In dogs given rt-PA alone, bleeding was not related significantly to the dose of rt-PA ($p = 0.29$).

Platelet aggregation and hemostasis. Table 2 shows results of ex vivo platelet aggregation tests and hemostasis analysis. Substantial (40%) diminution of ADP-induced platelet aggregation from the baseline value was observed concomitantly with plasma fibrinogen depletion at the end of the highest rt-PA infusion (Group IV) and persisted for the next 2 h. During this interval, however, the bleeding time shortened from 22 ± 9 to 6 ± 5 min. In all other treatment groups, the extent of aggregation with ADP remained essentially unchanged from baseline values during the experiment. No discrete secondary wave of aggregation was observed in dogs that received aspirin.

Systemic fibrinolytic activation characterized by consumption of $alpha_2$-antiplasmin and fibrinogen breakdown was observed in all groups treated with rt-PA (Table 2). These changes and plasma levels of rt-PA were generally proportional to the rate of infusion. Maximal effects were again observed at an infusion rate of 60 μg/kg per min for 90 min, yielding plateau levels of rt-PA in plasma of >5 μg/ml and an intense systemic fibrinolytic state characterized by $alpha_2$-antiplasmin depletion to 14 ± 6% and fibrinogen degradation to 0.19 ± 0.12 g/liter. Administration of aspirin did not significantly change the effect of an rt-PA infusion at 15 μg/kg per min for 90 min on hemostatic variables. Platelet counts remained unchanged in all groups at mean values between 250,000 and 350,000/mm^3.

Randomized Blinded Study

To preclude the influence of technical variability and subjective analysis in performance of bleeding time measurements and classification of bleeding, a prospective blinded study in which 26 dogs were randomized to receive either aspirin (15 mg/kg), rt-PA (15 μg/kg per min for 90 min), both or neither was performed. One dog was excluded because of technical failure in performance of template bleeding times. The remaining 25 dogs form the study group.

Template bleeding times. Table 3 summarizes the results of serial template bleeding times in the four randomized groups. In control dogs (Group I) given rt-PA excipient infusion and aspirin placebo, bleeding time remained essentially unchanged from baseline values throughout the experiment and all values were within the normal range. Infusion of rt-PA alone (Group II) resulted in moderate prolongation of bleeding time to a maximum of 15 ± 12 min ($p = 0.01$). Of four dogs demonstrating values >9 min during infusion, the value was >30 min in two. Within the next 2 h, the bleeding time normalized to 8.0 ± 5.5 min, but values >9 min were still observed in three dogs. Aspirin administration (Group III) produced minor nonsignificant prolongation of baseline bleeding time in comparison with the control group, which remained unchanged during and after infusion of rt-PA placebo. Only a single value >9 min was observed in this

Table 3. Template Bleeding Times and Bleeding After Administration of Recombinant Tissue-Type Plasminogen Activator (rt-PA) and Aspirin: Results of the Randomized Study

Group	Protocol		No. of Dogs	Template Bleeding Time (min)				
	rt-PA (μg/kg per min)	Aspirin (mg/kg)		Pre	30 Min	60 Min	90 Min	210 Min
I	0	0	6	3.5 ± 1.0 (2.5–6.0)	4.5 ± 2.0 (3.5–7.5)	4.0 ± 1.5 (2.5–6.0)	3.5 ± 1.5 (1.5–5.5)	4.0 ± 2.5 (1.5–7.0)
II	15	0	7	4.5 ± 2.5 (2.5–8.0)	14 ± 12 (3.0–30)	15 ± 12 (3.0–30)	12 ± 11 (3.0–22)	8.0 ± 5.5 (3.0–18)
III	0	15	6	4.0 ± 2.0 (2.0–7.0)	5.0 ± 4.0 (1.0–12)	5.5 ± 3.5 (1.5–11)	4.0 ± 3.0 (2.0–9.0)	4.0 ± 2.5 (2.0–8.5)
IV	15	15	6	5.5 ± 2.0 (2.5–7.5)	29 ± 2.0 (25–30)	30 (30)	30 (30)	13 ± 10 (6–30)

Data are mean values ± SD and (range).

group. In the aspirin/rt-PA combination treatment group (Group IV), the bleeding time increased to >30 min in all dogs at 60 and 90 min after the start of infusion. Two hours after completion of rt-PA infusion, the bleeding time reverted to 13 ± 10 min, with values >9 min in only two dogs.

Bleeding and treatment. Bleeding was not observed from femoral or template bleeding time incision sites in any dog before the start of infusion. In placebo-treated dogs (Group I), no bleeding was documented. One dog in the aspirin group (Group III) demonstrated slight oozing throughout the experiment from the femoral catheterization site in association with a maximal bleeding time of 9 min. In the rt-PA group (Group II), bleeding was observed in two of seven dogs. In one dog, bleeding from the catheterization and preinfusion bleeding time incision sites started between 45 and 60 min after the start of rt-PA infusion and continued throughout the experiment. In this dog, the bleeding time was 22 min at the end of infusion and shortened only to 18 min at the end of the observation period (210 min) in association with a plasma fibrinogen level of 0.13 g/liter. In the remaining dog, oozing from the preinfusion bleeding time incision wound occurred during the infusion, but stopped within 30 min after the end of rt-PA therapy.

In the combined aspirin and rt-PA treatment group (Group IV), bleeding from the catheterization and preinfusion bleeding time wounds was observed in all dogs. It began within 15 min after the start of the infusion in three dogs, within 45 min in one dog and within 60 min in two dogs and persisted for the duration of infusion. Continued oozing throughout the experiment occurred in one dog in which the bleeding time remained >30 min and circulating fibrinogen decreased to an unmeasurable level. In all other dogs, bleeding stopped during the observation period after cessation of the rt-PA infusion.

The frequency of bleeding in the randomized study was not significantly increased in dogs given aspirin (Group III) ($p > 0.5$) or rt-PA (Group II) alone ($p = 0.46$) in comparison with the placebo group (Group I). In contrast, dogs treated with both aspirin and rt-PA demonstrated a significantly greater frequency of bleeding than those receiving rt-PA alone ($p = 0.02$). Although small but significant effects of aspirin or rt-PA alone on bleeding may have been missed as a result of the small sample size, these findings indicate that the interactive effect of aspirin and rt-PA was the most important determinant of bleeding.

Platelet aggregation and hemostasis (Table 4). No substantial variations in the extent of ADP-induced aggregation from baseline measurements were detected during the experiment in any treatment group. Epinephrine-potentiated, arachidonic acid-induced platelet aggregation performed immediately before the start of infusion was abolished in all dogs given aspirin (Groups III and IV), documenting cyclooxygenase inhibition. A discrete secondary phase of aggregation was also not detected in these dogs. Plasma levels of rt-PA, fibrinogen and alpha$_2$-antiplasmin were comparable with results obtained in the corresponding groups during the initial dose-response study. Platelet counts did not change significantly in any of the groups.

Correlation of bleeding time with bleeding frequency or treatment. Table 5 summarizes the observed relation between bleeding and the longest template bleeding time measured (at 60 or 90 min) after the start of the infusion. Bleeding, which occurred in 9 of 25 dogs, correlated significantly with prolongation of the bleeding time to >9 min ($p < 0.0001$), thereby predicting bleeding with a sensitivity of 100% (9 of 9 dogs) and a specificity of 87% (14 of 16 dogs).

No statistically significant relation was observed between prolongation of bleeding time and therapy in the aspirin and placebo groups. This relation was marginally significant in the rt-PA-treated dogs in comparison with placebo-treated dogs ($p = 0.04$). In contrast, the bleeding time demonstrated significant prolongation when rt-PA was combined with aspirin as compared with placebo treatment ($p < 0.0001$).

Table 4. Platelet Aggregation and Hemostasis Variables After Administration of Recombinant Tissue-Type Plasminogen Activator (rt-PA) and Aspirin: Results of the Randomized Study

	ADP Aggregation (%ΔT)			EPI-AA Aggregation (%ΔT)	Plasma rt-PA (μg/ml)				Fibrinogen (g/liter)			$Alpha_2$-Antiplasmin (%)		Serum Salicylate (μg/ml)
Group	Pre	90 Min	210 Min	Pre	30 Min	60 Min	90 Min	210 Min	Pre	90 Min	210 Min	Pre	210 Min	Pre
I	91 ± 18 (75–113)	85 ± 16 (61–106)	83 ± 16 (69–113)	83 ± 24 (63–113)	—	—	—	—	2.8 ± 1.4	2.6 ± 1.2	2.5 ± 1.3	73 ± 14	72 ± 12	19 ± 11
II	88 ± 16 (60–105)	85 ± 15 (75–113)	89 ± 16 (65–113)	89 ± 14 (69–106)	0.7 ± 0.2	0.8 ± 0.4	0.8 ± 0.4	0.06 ± 0.02	2.5 ± 1.0	1.6 ± 1.2	2.1 ± 1.6	67 ± 7	30 ± 11	19 ± 13
III	79 ± 13 (64–93)	83 ± 14 (70–109)	84 ± 18 (70–113)	0	—	—	—	—	3.1 ± 1.6	3.1 ± 1.6	3.1 ± 1.7	70 ± 9	65 ± 13	61 ± 9
IV	79 ± 11 (70–98)	63 ± 31 (10–94)	66 ± 25 (16–84)	0	0.8 ± 0.3	0.9 ± 0.4	0.9 ± 0.5	0.09 ± 0.06	3.0 ± 1.1	1.8 ± 1.0	1.9 ± 1.2	72 ± 8	38 ± 21	55 ± 12

Data are mean values ± SD and (range). ADP = adenosine 5'-diphosphate; EPI-AA = epinephrine and arachidonic acid; %ΔT = maximal percent change in transmittance.

Combined study groups. Corresponding data from the dose-response and randomized blinded studies were pooled and statistical analysis performed, yielding significance levels identical to results presented in Table 4. Prolongation of the bleeding time to >9 min predicted bleeding with a sensitivity of 100% (19 of 19 dogs) and a specificity of 78% (25 of 32 dogs).

Persistent bleeding after cessation of the rt-PA infusion in association with a prolonged bleeding time and plasma fibrinogen depletion was observed in two dogs in the randomized study. To determine the relation between decreased plasma fibrinogen concentration and bleeding time prolongation with the occurrence of bleeding, stepwise logistic regression of bleeding versus fibrinogen concentration at 90 min (<1 g/liter) and versus bleeding time (>9 and ≥15 min) was performed for all dogs. This analysis indicated that bleeding time was by far ($p < 0.0001$) the strongest predictor of bleeding. Although decreased fibrinogen level was also related to bleeding ($p < 0.02$), in our logistic regression model that included bleeding time, this measurement added no significant predictive value ($p = 0.78$). In a smaller sample size of dogs receiving rt-PA alone (Groups II, III and IV in the dose-finding study and Group II in the randomized study), bleeding time was also the strongest predictor of bleeding ($p = 0.01$). Fibrinogen values added no significant predictive value in a model that included bleeding time ($p = 0.28$).

Table 5. Correlation of Bleeding Time With Bleeding and Treatment in 25 Dogs in the Randomized Blinded Study

	Bleeding Time (min)				
	≤9	10–14	15–29	>30	p Value
Bleeding vs.	0	1	0	8	<0.0001
No bleeding	14	1	1	0	
Aspirin vs.	5	1	0	0	>0.1
Placebo	6	0	0	0	
rt-PA vs.	3	1	1	2	0.04
Placebo	6	0	0	0	
Aspirin + rt-PA vs.	0	0	0	6	<0.0001
Placebo	6	0	0	0	

rt-PA = recombinant tissue-type plasminogen activator.

Reversal of bleeding and bleeding time prolongation with aprotinin. Intravenous bolus administration of 20,000 KIU of aprotinin/kg body weight was tested in six additional dogs given 15 mg/kg of aspirin and 15 μg/kg per min of rt-PA for 90 min. In these dogs, epinephrine-potentiated, arachidonic acid-induced platelet aggregation was abolished. The maximal percent change in transmittance (%ΔT) of ADP-induced aggregation, which produced only a single wave, was 78 ± 9% before rt-PA infusion and 71 ± 11% and 70 ± 35% at 90 and 210 min, respectively. Plasma rt-PA levels increased to 1.0 ± 0.4 μg/ml at 30 min, plateaued at 1.1 ± 0.4 μg/ml at 60 min and declined to 0.1 ± 0.04 μg/ml at 210 min. Plasma fibrinogen concentration was 1.9 ± 0.5 g/liter before infusion of rt-PA, 1.1 ± 0.7 g/liter at 90 min and 1.3 ± 0.7 g/liter at 210 min. Levels of $alpha_2$-antiplasmin were 62 ± 7% before infusion, decreasing to 48 ± 12% at 210 min. Administration of aprotinin 60 to 90 min after the start of rt-PA infusion resulted in abrupt and marked shortening of the bleeding time from >30 min in all dogs to ≤9 min in five dogs measured 2 min after injection (Fig. 2). In addition, bleeding in five dogs stopped within 10 min after aprotinin injection. In the remaining dog, persistent oozing from incisions made after the start of infusion occurred in association with a bleeding time of 21 min at 90 min, which decreased to 9 min at the end of the experiment; the plasma fibrinogen concentration was unmeasurable in this dog.

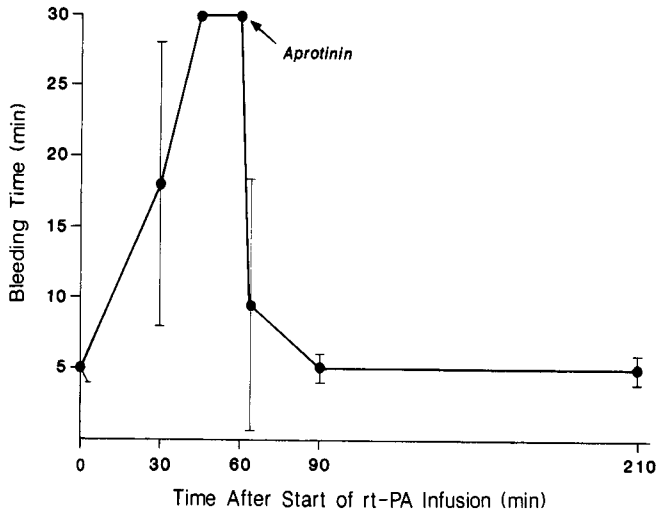

Figure 2. In six dogs treated with the combination of 15 mg/kg of aspirin and 15 μg/kg per min of recombinant tissue-type plasminogen activator (rt-PA) for 90 min, a bolus injection of 20,000 KIU of aprotinin/kg body weight was given after bleeding time prolonged to >30 min in all dogs. The abrupt and marked decrease in bleeding time observed after this treatment was accompanied by cessation of bleeding. Results are expressed as mean ± standard deviation obtained at times indicated after the start of rt-PA infusion.

Discussion

Rationale of the study. The main objective of this study was to examine the effect of recombinant tissue-type plasminogen activator (rt-PA) administered under controlled experimental conditions alone or in combination with aspirin on measurements of hemostasis and determine whether template bleeding time measurements correlate with the observed hemorrhagic tendency. Although a relation between bleeding time prolongation and recent aspirin ingestion with the frequency of spontaneous bleeding was observed previously (14) in a pilot study in patients given rt-PA, the preliminary nature and retrospective design of that study provided the rationale for the current prospective study.

The selection of dogs for these experiments was based on the extensive use of this species in preclinical studies of coronary arterial thrombolysis (22) and adjunctive therapy (23). As background for future investigations on the relative fibrinolytic potency and hemostatic effects of new agents or combinations for thrombolytic therapy, the necessity to define laboratory markers for monitoring a bleeding tendency was indicated. Such a model would obviously be valuable for evaluating the safety and efficacy of novel therapeutic regimens.

Relation of Therapy, Bleeding Time Prolongation and Bleeding

Effect of rt-PA. In the initial dose-finding phase, infusion of 15 μg/kg per min of rt-PA for 90 min (1.35 mg/kg total dose) caused only minimal prolongation of the mean bleeding time associated with bleeding in only one dog. In contrast, extensive systemic fibrinogen degradation and substantial diminution of platelet aggregation ex vivo were observed with a fourfold higher dose (60 μg/kg per min). At the end of this infusion, the average bleeding time had increased more than sixfold over baseline values, with bleeding observed in two dogs. However, substantial reversal of this prolongation occurred within 2 h after cessation of infusion unaccompanied by an increase in plasma fibrinogen levels or improvement in platelet aggregation. Thus, bleeding time prolongation could not be attributed to induction of a systemic lytic state or altered platelet aggregability alone.

Effect of aspirin and rt-PA. In contrast to the minor effects produced during separate administration, aspirin combined with rt-PA (15 μg/kg per min for 90 min) resulted in bleeding time values >30 min and recurrent continuous bleeding from previously coagulated wounds in five of six dogs. This prolongation was again reversed to a large extent 2 h after the end of infusion. These results demonstrated a marked interactive effect of aspirin and rt-PA on bleeding and bleeding time prolongation and a highly significant relation between the occurrence of bleeding and bleeding time >9 min.

Although bleeding time is generally recognized as the most basic overall test of platelet hemostatic function in vivo, its measurement and interpretation may be affected by problems of variability in part due to operator technique or intersubject differences in skin texture. Subjective analysis, particularly with respect to classification of bleeding and quantitation of bleeding time, may also have biased the initial phase of this study. Therefore, objective confirmation was obtained by a prospective randomized blinded study.

For this phase, a single fixed dose of aspirin (15 mg/kg) was selected as the minimal requirement for achieving consistent platelet cyclooxygenase inhibition, confirmed by epinephrine-potentiated, arachidonic acid-induced platelet aggregation. A similar minimal dose was also required to inhibit aggregation in a previous rabbit study (24). Although substantially larger than that recommended for patients given thrombolytic therapy, this dose of aspirin is nevertheless less than the intravenous regimen (20 or 35 mg/kg) used to abolish platelet aggregation in previous canine studies (25,26). These results suggest the need for higher doses of aspirin to obtain platelet cyclooxygenase inhibition in dogs.

Aspirin was then tested alone or in combination with an rt-PA dose (15 μg/kg per min for 90 min) at which only minor effects on bleeding frequency and prolongation of bleeding time were observed in the initial study group. This infusion rate produced plateau levels of rt-PA-related antigen in plasma approximately 50% of peak values observed in patients given rt-PA at the currently recommended 100 mg dose (27).

Effect of aspirin and rt-PA on bleeding and bleeding time prolongation. The results obtained also demonstrated the marked interactive effect of aspirin and rt-PA on bleeding and bleeding time prolongation. The frequency of bleeding

was significantly greater in dogs receiving combined therapy than in those receiving rt-PA alone. In addition, bleeding time values >9 min correlated with bleeding with a sensitivity of 100% (all 9 dogs with bleeding had a bleeding time >9 min) and a specificity of 87% (14 of 16 dogs without bleeding had a bleeding time <9 min). Stepwise logistic regression, using a model that included variables of fibrinogen concentration and bleeding time, excluded the possibility that plasma fibrinogen measurement was a more significantly predictive marker for bleeding. However, because of the unavailability of canine antiserum to measure fibrinogen degradation products, the relation between bleeding frequency and fibrinogen degradation product elevation produced during thrombolytic infusion, as demonstrated in a previous clinical trial (28), could not be tested specifically in the present study. Taken together, these observations suggest that bleeding time determination may provide a useful quantitative end point for the bleeding tendency associated with thrombolytic infusion and adjunctive antiplatelet therapy.

Presumptive Mechanism, Reversibility and Antifibrinolytic Therapy

The possibility that barbiturate anesthesia may have induced slight platelet dysfunction affecting control bleeding times or ADP-induced platelet aggregation ex vivo (29) cannot be discounted. However, the similar response of the template bleeding time to the combination of rt-PA and aspirin in dogs and patients with acute myocardial infarction suggests that its influence on platelet function in vivo is minimal.

Role of combined aspirin and rt-PA therapy. Similar increases in bleeding time were observed at 90 min in the initial dose-finding study with either 60 μg/kg per min of rt-PA alone or the combination of aspirin and 15 μg/kg per min of rt-PA, but prolongation occurred earlier and was more persistent in the latter group. Bleeding was also more frequent in the groups receiving combined administration of rt-PA and aspirin than in those receiving either agent alone, indicating that it may result primarily from an interactive effect of aspirin and rt-PA. Thus, it is likely that both the mechanism and extent of bleeding time prolongation are important in producing the bleeding tendency. These results also suggest that prolongation of the template bleeding time may be a more reliable marker of hemorrhagic tendency when agents affecting both the coagulation protein and platelet components of hemostasis are given in combination.

Role of interference with formation of the hemostatic plug. Although its origin remains unclear, shortening of the bleeding time observed after cessation of rt-PA infusion together with resolution of bleeding in six of nine randomized dogs argue against slowly reversible processes that might be related, for example, to degradation of plasma coagulation proteins or platelet membrane receptors. The transient nature of these phenomena suggests instead that bleeding may primarily be due to a reversible interference with formation of the hemostatic plug. This hypothesis is further supported by the immediate reversal of bleeding and bleeding time prolongation with aprotinin, a serine protease inhibitor with potent antiplasmin activity (30). Reversibility of bleeding time prolongation induced by rt-PA and aspirin with a bolus injection of recombinant plasminogen activator inhibitor has been recently reported in a rabbit model (31). Thus, interference of hemostatic plug formation by plasmin generated during the course of thrombolytic infusion is the most obvious explanation for the occurrence of bleeding events in this study. Instability of the hemostatic plug due to decreased interdigitation of platelets after aspirin administration (32) may have initially impaired hemostasis, producing a predisposition to renewed bleeding after the start of thrombolytic therapy.

Aprotinin as an antidote. Our findings indicate that administration of an antifibrinolytic agent may be an effective antidote to life-threatening bleeding associated with thrombolytic therapy. Indeed, aprotinin has been successfully used to reduce blood loss associated with cardiac surgery (33,34), even after recent aspirin ingestion (35). However, its safety and efficacy in patients receiving thrombolytic infusion require further investigation.

Effect on surgical wounds. In a previous study (23) in which rt-PA was combined with aspirin, prolongation of the bleeding time was also observed, but this did not appear to predispose to excessive hemorrhage from surgical wounds. In that study, however, at the earliest, aspirin and rt-PA were given 1 h after completion of surgery, which possibly permitted normal hemostatic plug formation in the surgical wounds.

Clinical Relevance and Future Studies

In the present study, we did not investigate whether the dose of rt-PA contributes to the bleeding tendency in dogs given aspirin. Such a contribution is, however, suggested by the results of the Thrombolysis in Myocardial Infarction (TIMI) II trial (10), which indicated that infusion of 150 mg of rt-PA was associated with a higher frequency of bleeding in patients given aspirin compared with the currently used 100 mg dose. This study was also not designed to investigate whether bleeding may be prevented by controlling the bleeding time, and this question can only be answered adequately in prospective trials with administration schemes that are modified on the basis of changes in the bleeding time.

Because of its clinical application, the effect of heparin, which was not used in this study, on the bleeding tendency observed with the combination of aspirin and rt-PA needs to be evaluated in future studies. However, the already profuse and continuous nature of the hemorrhagic tendency observed with combined therapy raises the potentially more relevant question of the efficacy of antifibrinolytic therapy in the setting of heparin anticoagulation. Finally, an appropriate follow-up study would be to examine the effect of

streptokinase administration on bleeding time prolongation and frequency of bleeding in the present model.

Whether the observed correlation between 1) bleeding time prolongation and bleeding, and 2) the interactive effect of aspirin and rt-PA on both the bleeding time and bleeding has significant clinical implications needs to be confirmed prospectively in large patient trials. This canine model may be particularly useful for investigation of alternative administration schemes of combined rt-PA/antiplatelet therapy not only in terms of thrombolytic potency, but also as related to bleeding tendency. In this context, the template bleeding time, which constitutes the first identified quantitative index for monitoring the bleeding tendency associated with thrombolytic therapy, may prove useful.

We thank Lawrence W. Gimple, MD for helpful discussions and advice during the course of this study.

References

1. Collen D, Stump DC, Gold HK. Thrombolytic therapy. Annu Rev Med 1988;39:405–23.
2. Collen D, Topol EJ, Tiefenbrunn AJ, et al. Coronary thrombolysis with recombinant human tissue-type plasminogen activator: a prospective, randomized, placebo-controlled trial. Circulation 1984;70:1012–7.
3. Chesebro JH, Knatterud G, Roberts R, et al. Thrombolysis in Myocardial Infarction (TIMI) trial, phase I: a comparison between intravenous tissue plasminogen activator and intravenous streptokinase. Circulation 1987; 76:142–54.
4. Verstraete M, Bernard R, Bory M, et al. Randomised trial of intravenous recombinant tissue-type plasminogen activator versus intravenous streptokinase in acute myocardial infarction. Lancet 1985;1:842–7.
5. Guerci AD, Gerstenblith G, Brinker JA, et al. A randomized trial of intravenous tissue plasminogen activator for acute myocardial infarction with subsequent randomization to elective coronary angioplasty. N Engl J Med 1987;317:1613–8.
6. Van de Werf F, Arnold AER, and the European Cooperative Study Group. Effect of intravenous tissue plasminogen activator on infarct-size, left ventricular function and survival in patients with acute myocardial infarction. Br Med J 1988;297:1374–9.
7. Wilcox RG, Olsson CG, Skene AM, von der Lippe G, Jensen G, Hampton JR. Trial of tissue plasminogen activator for mortality reduction in acute myocardial infarction. Lancet 1988;2:525–9.
8. ISIS-2 (Second International Study of Infarct Survival) Collaborative Group. Randomised trial of intravenous streptokinase, oral aspirin, both or neither among 17,189 cases of suspected acute myocardial infarction: ISIS-2. Lancet 1988;2:349–59.
9. Braunwald E, Knatterud GL, Passamani E, Robertson TL, Solomon R. Update from the Thrombolysis in Myocardial Infarction Trial. J Am Coll Cardiol 1987;10:970.
10. TIMI Study Group. Comparison of invasive and conservative strategies after treatment with intravenous tissue plasminogen activator in acute myocardial infarction. N Engl J Med 1989;320:618–27.
11. White HD, Rivers JT, Maslowski A, et al. Effect of intravenous streptokinase as compared with that of tissue plasminogen activator on left ventricular function after first myocardial infarction. N Engl J Med 1989;320:817–21.
12. Magnani B, for the PAIMS Investigators. Plasminogen Activator Italian Multicenter Study (PAIMS): comparison of intravenous recombinant single-chain human tissue-type plasminogen activator (rt-PA) with intravenous streptokinase in acute myocardial infarction. J Am Coll Cardiol 1989;13:19–26.
13. Marder VJ, Sherry S. Thrombolytic therapy: current status. N Engl J Med 1988;380:1512–20.
14. Gimple LW, Gold HK, Leinbach RC, et al. Correlation between template bleeding times and spontaneous bleeding during treatment of acute myocardial infarction with recombinant tissue-type plasminogen activator. Circulation 1989;80:581–8.
15. Johnson GJ, Leis LA, Rao GHR, White JG. Arachidonate-induced platelet aggregation in the dog. Thromb Res 1979;14:147–54.
16. Trinder P. Rapid determination of salicylate in biological fluids. Biochem J 1954;57:301–3.
17. Vermylen C, De Vreker RA, Verstraete M. A rapid enzymatic method for assay of fibrinogen: the fibrin polymerization time test (FPT). Clin Chim Acta 1963;8:418–24.
18. Clauss A. Gerinnungsphysiologische Schnellmethode zur Bestimmung des Fibrinogens. Acta Haematol 1957;17:237–46.
19. Holvoet P, Cleemput H, Collen D. Assay of human tissue-type plasminogen activator (t-PA) with an enzyme linked immunosorbent assay (ELISA) based on three murine monoclonal antibodies to tissue-type plasminogen activator. Thromb Haemost 1985;54:684–7.
20. Edy J, De Cock F, Collen D. Inhibition of plasmin by normal and antiplasmin depleted plasma. Thromb Res 1976;8:513–8.
21. Hollander M, Wolfe DA. Nonparametric Statistical Methods. New York: Wiley, 1973:120.
22. Bergman SR, Fox FAA, Ter-Pogossian MM, Sobel BE, Collen D. Clot-selective coronary thrombolysis with tissue-type plasminogen activator. Science 1983;220:1181–3.
23. Yasuda T, Gold HK, Fallon JT, et al. Monoclonal antibody against the platelet glycoprotein (GP) IIb/IIIa receptor prevents coronary artery reocclusion after reperfusion with recombinant tissue-type plasminogen activator. J Clin Invest 1988;81:1284–91.
24. Jang IK, Gold HK, Leinbach RC, Fallon JT, Collen D. In vivo thrombin inhibition enhances and sustains arterial recanalization with recombinant tissue-type plasminogen activator. Circ Res 1990;67:1552–61.
25. Folts JD, Crowell EB, Rowe GG. Platelet aggregation in partially obstructed vessels and its elimination with aspirin. Circulation 1976;54:365–70.
26. Fitzgerald DJ, Wright F, FitzGerald GA. Increased thromboxane biosynthesis during coronary thrombolysis. Circ Res 1989;65:83–94.
27. Lucore CL, Sobel BE. Interactions of tissue-type plasminogen activator and their pharmacologic implications. Circulation 1988;77:660–9.
28. Rao AK, Pratt C, Berke A, et al. Thrombolysis in Myocardial Infarction (TIMI) trial, phase I: hemorrhagic manifestations and changes in plasma fibrinogen and the fibrinolytic system in patients treated with recombinant tissue plasminogen activator and streptokinase. J Am Coll Cardiol 1988; 11:1–11.
29. O'Rourke ST, Folts JD, Albrecht RM. Inhibition of canine platelet aggregation by barbiturates. J Lab Clin Med 1986;108:206–12.
30. Fritz H, Wunderer G. Biochemistry and applications of aprotinin, the kallikrein inhibitor from bovine organs. Arzneimittel Forsch/Drug Res 1983;33:479–94.
31. Vaughn DE, De Clerck PJ, De Mol M, Collen D. Recombinant plasminogen activator inhibitor-1 reverses the bleeding tendency associated with the combined administration of tissue-type plasminogen activator and aspirin in rabbits. J Clin Invest 1989;84:586–91.
32. Wester J, Sixma JJ, Geuze JJ, van der Veen J. Morphology of the early hemostasis in human skin wounds. Lab Invest 1978;39:298–311.
33. Royston D, Bidstrup BP, Taylor KM, Sapsford RN. Effect of aprotinin on need for blood transfusion after open-heart surgery. Lancet 1987;2:1289–91.
34. Fraedrich G, Engler H, Kanz L, Schlosser V. High dose aprotinin regimen in open heart surgery: a prospective randomized double blind trial (abstr). Circulation 1989;80(suppl II):II-158.
35. Bidstrup BP, Royston D, McGuiness C, Sapsford RN. Aprotinin reduces bleeding in patients taking aspirin at the time of open heart surgery (abstr). Circulation 1989;80(suppl II):II-158.