Thrombosis and Haemostasis – © F. K. Schattauer Verlagsgesellschaft mbH (Stuttgart) 68 (3) 306–309 (1992)

Effect of DDAVP on Endotoxin-Induced Intravascular Coagulation in Rabbits

M. J. Paloma, J. A. Páramo, and E. Rocha

From the Hematology Service, University Clinic, Faculty of Medicine, University of Navarra, Pamplona, Spain

Summary

We have evaluated the effect of 1-Deamino-8D-arginine vasopressin (DDAVP) on an experimental model of intravascular coagulation (DIC) induced in rabbits by injection of 20 μg kg^{-1} h^{-1} during 6 h of *E. coli* lipopolysaccharide. DDAVP significantly ameliorated the platelet drop and fibrinogen decrease ($p < 0.01$) induced by endotoxin in control animals. A significant reduction in factor XII consumption ($p < 0.01$) and a decrease in the generation of endotoxin induced PAI-1 activity in rabbits circulation was also observed ($p < 0.005$). Moreover, fibrin deposition in kidneys of rabbits receiving DDAVP was significantly reduced as compared to control animals. Finally, the mortality rate in the control group was significantly higher than in DDAVP-treated rabbits ($p < 0.01$). The hemostatic changes induced by DDAVP correlated with lower fibrin deposition and reduction in mortality rates.

Introduction

Disseminated intravascular coagulation (DIC) is a common effect of the presence of endotoxins, released from the bacterial wall, within the circulation (1). Although the reactions implicated in the initiation of DIC are not completely understood, endotoxin may trigger blood coagulation by causing endothelial damage and/or by stimulating the generation of procoagulant activity from different cell types affecting both the intrinsic and extrinsic pathways (2–4). Endotoxin has been shown to activate factor XII and a direct evidence that exposure to tissue factor after endotoxin is an important mechanism of intravascular coagulation has also been reported (2, 5).

On the other hand, an impairment of the fibrinolytic system may contribute to DIC by retarding the clearance of fibrin from circulation, which is to a great extent due to an increase in the blood levels of type 1 plasminogen activator inhibitor (PAI-1), as experimentally demonstrated in rabbits infused with endotoxin (6).

This inhibitor plays a pivotal role in the control of fibrinolysis. Elevated PAI-1 levels appear to be a significant factor for clot formation when a thrombogenic stimulus such as endotoxin is administered. That is indicated by the fact that high PAI-1 has been found in different clinical conditions related to thrombosis as well as in human sepsis (7–10). In addition, recent in vivo data have also shown a correlation between the endotoxin-induced generation of PAI-1 and the presence of fibrin deposition in tissues. Finally, the control of PAI-1 generation may represent an useful approach in endotoxin-induced DIC (11).

1-Deamino-8D-arginine vasopressin (DDAVP) is a synthetic vasopressin analogue which displays a variety of biological effects on hemostasis, such as the release of von Willebrand factor (vWF) and tissue plasminogen activator (t-PA) (12). It also shortens the bleeding time (13) and favors platelet adhesion to the subendothelium (14), which is probably a consequence of the release of high molecular weight multimers of vWF (15, 16). Its exact mechanism of action is, however, not completely understood. It could be mediated by a receptor present in the central nervous system or via release of catecholamines (17).

On the basis of the possible actions of DDAVP on different coagulation and fibrinolysis parameters, and its effect in experimental animals (18, 19), we investigated whether this vasopressin analogue would be able to counteract the changes in blood coagulation and fibrinolysis, especially the PAI-1 levels, as well as to reduce mortality in rabbits infused with endotoxin.

Material and Methods

Experimental Studies

Male New Zealand rabbits weighing 2.5–3 kg were anesthetized by intravenous infusion of 10 mg/kg body weight of Nembutal (Abbot Lab) via a marginal ear vein, where a catheter was placed to administer treatment. DIC was induced in 20 rabbits by intravenous infusion, via the contralateral marginal ear vein, of 20 μg kg^{-1} h^{-1} of endotoxin (lipopolysaccharide from *E. coli* 0128:B8, Sigma Chemical Co., St. Louis, MO) during 6 h. Blood samples were taken via a catheter inserted into a femoral vein.

Surviving rabbits were sacrificed 18 h after the end of endotoxin infusion, by intravenous injection of 60 mg/kg body weight of Nembutal, for histological examination of kidney sections.

Treatment Schedule

Treatment was started simultaneously with endotoxin infusion. Twenty rabbits were used as controls and were infused with 20 ml saline solution during 20 min and 20 rabbits received DDAVP (Minirin, Ferring Lab. Sweden) at a dose of 0.4 μg/kg in 20 ml of normal saline during 20 min. Additional saline solution (10 ml/h) was then administered in all rabbits to complete 6 h.

Five rabbits not receiving endotoxin were given DDAVP in a similar way to the treatment groups, to study the possible effect of this drug on different hemostasis parameters.

Laboratory Methods

Blood samples were taken before endotoxin, at 2 and at 6 h of infusion. A sample for platelet count was processed immediately. Platelet-poor plasma, obtained by centrifugation for 10 min at 15,000 × *g*, was stored in aliquots in capped polypropylene Eppendorf micro test tubes at −70 °C until used. Fibrinogen was determined by the Clauss method. Factor XII was assayed by a one stage method using factor XII deficient plasma (Organon Teknika, USA) as substrate. t-PA activity was determined by spectrophotometric assay (20). Briefly, diluted euglobulin fraction was mixed in a microtiter plate to a final volume of 200 μl with 0.02 M Tris.HCl, pH 7.5, 0.1% Tween 80, 0.3 mmol/l S-2251 (Kabi Diagnostica, Sweden), 0.13 μmol/l human plasminogen (Biopool, Sweden) and 0.13 mg/ml CNBr fibrinogen fragments (Kabi Diagnostica, Sweden). The plate was incubated at 37° C and the change in absorbance at 405 nm was measured. Results were expressed in mU/ml. t-PA inhibitor capacity of rabbit plasma (refer to as PAI-1 activity) was measured by an amidolytic assay as previously described (11). PAI-1 activity was expressed in units of t-PA inhibited per milliliter.

Correspondence to: Dr. E. Rocha, Hematology Service, University Clinic, Avda Pio XII s/n, Pamplona, Spain

The endotoxin concentration was determined by using a limulus lysate chromogenic peptide substrate (Coatest Endotoxin, Kabi Diagnostica, Sweden). Results were expressed in EU/ml.

Histological Examination

Sections of kidneys were fixed in formalin, stained with hematoxilin-eosin and examined for the presence of fibrin microthrombi by a pathologist who was unaware of the experimental protocol for the individual animals. Tissue sections were scored on a scale from 0 to 4 as previously described (11).

Data Analysis

The results of experiments are expressed as mean + SEM. Statistical analysis for groups comparison was performed with the Mann-Whitney test. For differences in mortality between control and treated groups the Fisher's exact test was used.

Results

Effect of DDAVP on Endotoxin-induced Changes in Coagulation and Fibrinolysis

Table 1 and Fig. 1 show the plasma levels of the different coagulation and fibrinolysis parameters in the control group and in the DDAVP group. The endotoxin concentration in both groups is also shown in Table 1. No differences in any of these parameters were observed between groups in the baseline samples.

Infusion of endotoxin into rabbits caused a significant decrease of platelets, fibrinogen and factor XII at 2 and 6 h of infusion ($p < 0.0001$). There was also a significant reduction of t-PA specially 6 h after endotoxin ($p < 0.05$). PAI-1 levels showed, however, a marked increase 2 h after endotoxin infusion ($p < 0.0007$) to maximum values at 6 h ($p < 0.0001$).

Infusion of DDAVP significantly ameliorated the platelet drop at 2 ($p < 0.01$) and 6 h ($p < 0.005$) as well as the fibrinogen decrease at 6 h of endotoxin ($p < 0.005$) as compared with control animals.

However, the most striking changes in components of coagulation and fibrinolysis were on factor XII and PAI-1 levels (Fig. 1). Whereas in control animals factor XII activity decreased gradually at 2 and 6 h of endotoxin, in the DDAVP infused animals this activity was normal and thus statistical significant differences between groups were found in that period of time ($p < 0.01$). DDAVP infusion was also able to reduce the high PAI-1 levels observed in control animals at 2 and 6 h of endotoxin. The difference in the rate of increase of PAI-1 activity between controls and DDAVP treated rabbits was highly significant ($p < 0.005$). No differences in t-PA levels were observed in the treated group as compared to the control.

In the group of rabbits only receiving DDAVP (without endotoxin), no changes in platelets, fibrinogen, t-PA and PAI-1 were observed (not shown). However, we found a slight increase of factor XII at 2 h (102.14 ± 5.80%) with respect to the basal value (91.20 ± 6.41%).

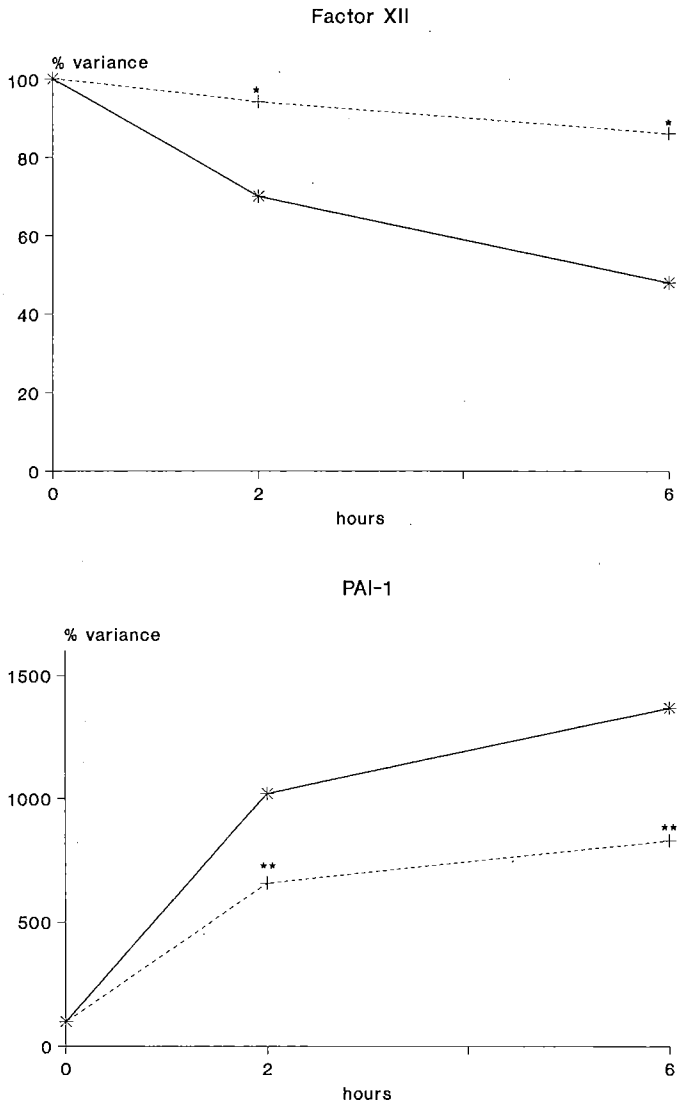

Fig. 1 Levels of factor XII and PAI-1 after endotoxin in controls (———) and DDAVP-treated (– – –) rabbits. Values are expressed as the mean percent of the initial value before the injection of endotoxin. * $p < 0.01$ and ** $p < 0.005$ as compared to the control

Effect of DDAVP on Fibrin Deposits in Kidneys

Intense deposits of fibrin within glomerular capillaries were observed in the majority of control rabbits. However, as shown in

Table 1 Hemostatic parameters and endotoxin concentrations before and after endotoxin infusion in rabbits. Results are expressed as mean ± SEM

	Control ($n = 20$)			DDAVP ($n = 20$)		
Parameter	Basal	2 h	6 h	Basal	2 h	6 h
Platelet ($\times 10^9$/l)	517.59 ± 10.88	272.94 ± 35.22	150.14 ± 10.48	485.84 ± 26.36	336.54 ± 32.00*	229.39 ± 20.81**
Fibrinogen (mg/dl)	267.49 ± 15.43	193.99 ± 11.76	146.84 ± 10.41	250.24 ± 12.96	175.49 ± 9.24	180.24 ± 10.60**
Factor XII (%)	93.19 ± 9.56	74.54 ± 4.99	51.44 ± 4.41	89.19 ± 3.66	80.54 ± 2.55*	73.24 ± 2.52*
t-PA (mU/ml)	53.89 ± 2.50	44.24 ± 3.77	43.39 ± 3.51	59.04 ± 3.20	44.26 ± 3.14	38.79 ± 4.16
PAI-1 (U/ml)	4.33 ± 0.28	39.10 ± 1.88	53.83 ± 1.23	4.15 ± 0.33	22.87 ± 1.64**	29.65 ± 3.12**
Endotoxin (EU/ml)	0.30 ± 0.10	19.80 ± 1.21	24.62 ± 1.60	0.25 ± 0.08	17.50 ± 1.30	30.20 ± 3.18

* $p < 0.01$ and ** $p < 0.005$ with respect to control.

Fig. 2 the score of fibrin deposition in kidneys was significantly reduced in rabbits infused with DDAVP ($p = 0.0015$).

Effect of DDAVP on Mortality

In the control group 14 out of 20 rabbits (70%) died shortly after the endotoxin infusion whereas only 7 out of 20 (35%) treated with DDAVP died within 18 h from the start of the experiment when they were sacrificed for histological studies. Mortality rate in the control group was significantly higher than in the treated group ($p < 0.01$).

An interesting finding was that the increase in plasma PAI-1 activity (Fig. 3) was significantly higher at 2 and 6 h ($p < 0.01$) in animals that died following endotoxin infusion than in survivors.

Discussion

This study shows that intravenous infusion of DDAVP in rabbits is able to significantly reduce not only the endotoxin-induced hemostatic changes but also the fibrin deposition in kidneys. Interestingly, DDAVP also diminished mortality associated with endotoxin in our experimental model.

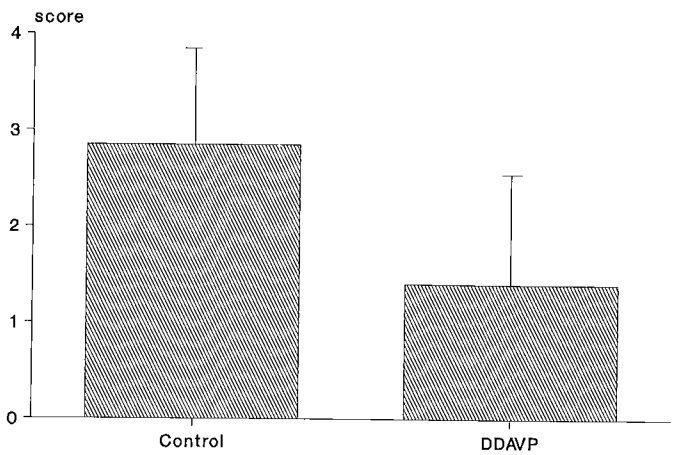

Fig. 2 Score of fibrin deposition in kidneys induced by endotoxin in control and DDAVP-treated rabbits, showing a significant reduction in the DDAVP group ($p = 0.0015$)

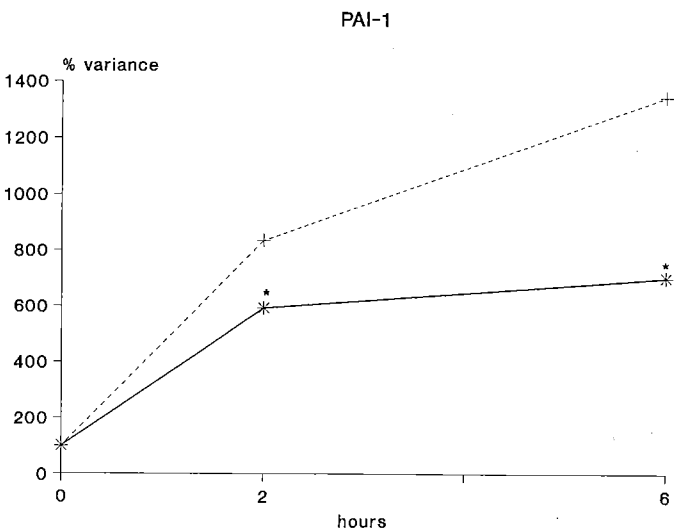

Fig. 3 PAI-1 levels as related to mortality in endotoxin-treated rabbits. The increase in plasma PAI-1 activity in animals that died ($n = 21$) following endotoxin (–––) was significantly higher than in survivors ($n = 19$) (———). * $p < 0.01$

DIC was induced in rabbits as observed by the decline in platelets and coagulation factors and the intense presence of fibrin deposits in kidneys. Moreover, high PAI-1 levels were observed in all animals at 2 and 6 h of endotoxin. These results are in agreement with previous reports which have emphasized the correlation between high PAI-1 levels and fibrin deposits in endotoxin-induced DIC (6, 11). Endotoxin, by increasing the PAI-1 levels, promoted fibrin deposition in ancrod-treated rabbits as recently demonstrated by Krishnamurti et al. (19), who suggested that PAI-1 would prevent endogenous fibrinolysis that occurs after a thrombogenic stimulus.

It has also been shown in healthy subjects that the intravenous injection of endotoxin or tumor necrosis factor induces a rapid activation and subsequent inhibition of the fibrinolytic system, mainly related to high PAI-1 activity (21, 22).

In this study we report that the infusion of DDAVP induces important changes in several hemostatic factors and reduces the generation of PAI-1 activity in the plasma of endotoxin-treated animals. This effect resulted in a reduction in fibrin deposition in kidneys and 50% lower mortality rate. The action of DDAVP is not related to a possible interaction of this drug with endotoxin, since the circulating endotoxin levels in the two different animal groups were similar.

DDAVP has been shown to be useful in human clinical conditions characterized by a prolonged bleeding time such as uremia, liver cirrhosis and extracorporeal circulation (23–27), but its effect on DIC had not been previously reported.

The changes in coagulation parameters show that DDAVP partially prevented the intravascular coagulation as assessed by the inhibition of the consumption of platelets, fibrinogen and factor XII. The most striking difference was on factor XII activity, which was significantly increased at 2 and 6 h as compared with control animals only receiving endotoxin. We believe that this previously unknown effect of DDAVP is very important by the central role of factor XII in the generation of vasoactive kinins and the development of shock (2). Our results seem to confirm very recent data on the fibrinolytic response upon DDAVP in factor XII deficient patients (28). The infusion of 0.4 µg/kg was able to induce a 10–20% increase in factor XII activity in the mild deficient group, suggesting that the factor XII dependent fibrinolysis can be stimulated by the infusion of DDAVP. We have also found a slight increase of factor XII in rabbits only treated with DDAVP. However, other unknown effects of this drug on intrinsic coagulation or contact systems cannot be ruled out (29).

DDAVP was also able to reduce the high PAI-1 levels observed in endotoxin-treated rabbits. Since this drug is known to increase the t-PA levels in humans it might be speculated that the reduction of inhibitor activity in rabbits might be due to an increase in plasminogen activator. However, there is controversy as to the effect of DDAVP on t-PA release in rabbits; whereas some authors have found a slight increase of t-PA in normal and afibrinogenemic rabbits (18, 19), others have not observed such an effect (25, 30). Our results also suggest no effect of DDAVP on t-PA release in this model, although a possible effect cannot be ruled out since the samples were taken 2 h after DDAVP infusion and an early release might have occurred. Nevertheless, we did not observe changes in t-PA levels in rabbits simultaneously treated with endotoxin and DDAVP in relation to those only receiving endotoxin. Therefore, the reason for the reduction of endotoxin-induced PAI-1 generation in rabbits treated with DDAVP is still unclear.

Rabbits injected with endotoxin developed glomerular microclots that could be partially prevented by DDAVP. The striking

difference in microscopic appearance between kidney sections from control and treated rabbits leaves no doubts as to the beneficial effect of the drug on the amount of fibrin deposition.

Finally, DDAVP significantly reduced mortality as compared with rabbits only infused with endotoxin. Since PAI-1 activity was significantly higher in animals that died following endotoxin than in survivors it could be speculated that the control of intravascular coagulation via a reduction of PAI-1 levels in the DDAVP-treated rabbits could have a beneficial effect. We had previouly demonstrated that a combination of heparin plus antithrombin III prevented the endotoxin-induced generation of PAI-1 activity in rabbits which correlated with the reduced presence of fibrin deposits in kidneys and with a lower mortality rate (11).

DDAVP only partially corrected the endotoxin-induced DIC but it had a beneficial effect in terms of mortality, suggesting that something other than intravascular coagulation may be responsible for the death of animals. It is known that intravascular coagulation and shock are independent manifestations of endotoxemia. DDAVP would prevent the generation of vasoactive kinins and the development of shock via the inhibition of the consumption of factor XII.

In conclusion, this study demonstrates that the treatment with DDAVP in rabbits simultaneously infused with endotoxin reduces the increase of PAI-1 and the consumption of factor XII. These changes correlated with lower fibrin deposition and reduction in mortality rates. If these findings apply to humans our results would suggest that the survival of patients with septicemia may be improved by the administration of DDAVP.

Acknowledgements

This work was supported by grant PB85-0036 from the DGICYT of the Ministerio de Educación y Ciencia, Spain.

REFERENCES

1. Morrison DC, Ulevitch RJ. The effects of bacterial endotoxins on host mediation systems. A review. Am J Pathol 1978; 93: 526–617.
2. Morrison DC, Cochrane CG. Direct evidence for Hageman factor (factor XII) activation by bacterial lipopolysaccharides (endotoxins). J Exp Med 1974; 140: 797–811.
3. Osterud B, Bjorklid E. Human factor VII associated with endotoxin stimulated monocytes in whole blood. Biochem Biophys Res Commun 1982; 108: 620–6.
4. Warr TA, Rao LVM, Rapaport SI. Disseminated intravascular coagulation in rabbits induced by administration of endotoxin or tissue factor: Effect of anti-tissue factor antibodies and measurement of plasma extrinsic pathway inhibitor activity. Blood 1990; 75: 1481–9.
5. Muller-Berghaus G, Schneberger R. Hageman factor activation in the generalized Shwartz reaction induced by endotoxin. Br J Haematol 1971; 21: 513–27.
6. Colucci M, Páramo JA, Collen D. Generation in plasma of a fast acting inhibitor of plasminogen activator in response to endotoxin stimulation. J Clin Invest 1985; 75: 818–24.
7. Páramo JA, Colucci M, Collen D, Van de Werf F. Plasminogen activator inhibitor in the blood of patients with coronary artery disease. Br Med J 1985; 291: 573–4.
8. Páramo JA, Fernández FJ, Rocha E. Plasminogen activator inhibitor activity in bacterial infection. Thromb Haemostas 1988; 59: 451–4.
9. Rocha E, Páramo JA, Fernández FJ, Cuesta B, Hernández M, Paloma MJ, Rifón J. Clotting activation and impairment of fibrinolysis in malignancy. Thromb Res 1989; 54: 699–707.
10. Páramo JA, Pérez JL, Serrano M, Rocha E. Types 1 and 2 plasminogen activator inhibitor and tumor necrosis factor alpha in patients with sepsis. Thromb Haemostas 1990; 64: 3–6.
11. Gómez C, Páramo JA, Colucci M, Rocha E. Effect of heparin and/or antithrombin III on the generation of endotoxin-induced plasminogen activator inhibitor. Thromb Haemostas 1989; 62: 694–8.
12. Richardson DW, Robinson AG. Desmopressin. Ann Intern Med 1985; 103: 228–39.
13. Kobrinsky NL, Israel ED, Gerrard JM, et al. Shortening of the bleeding time by 1-deamino-8-D-arginine vasopressin in various bleeding disorders. Lancet 1984; 1: 1145–8.
14. Escolar G, Cases A, Montcagudo J, et al. Uremic plasma after infusion of desmopressin (DDAVP) improves the interaction of normal platelets with vessel subendothelium. J Lab Clin Med 1989; 114: 36–42.
15. Ruggeri ZM, Manucci PM, Lombardi R, Federici AB, Zimmerman TS. Multimeric composition of factor VIII/von Willebrand factor following administration of DDAVP: implications for pathophysiology and therapy of von Willebrand's diseases subtypes. Blood 1982; 59: 1272–8.
16. Vicente V, Alberca I, López Borrasca A. Response of factor VIII/von Willebrand factor to intranasal DDAVP in healthy subjects and mild haemophiliacs (with observations in patients with combined deficiency of factors V and VIII). Thromb Haemostas 1982; 48: 91–3.
17. Grant MB, Guay C, Tottenberg R. Desmopressin stimulates parallel norepinephrine and tissue plasminogen activator release in normal subjects and patients with diabetes mellitus. Thromb Haemostas 1988; 59: 269–72.
18. Hasset MA, Krishnamurti C, Barr CF, Alving BM. The rabbit as a model for studies of fibrinolysis. Thromb Res 1986; 43: 313–8.
19. Krishnamurti C, Barr CF, Hasset MA, Young GD, Alving BM. Plasminogen activator inhibitor: a regulator of ancrod-induced fibrin deposition in rabbits. Blood 1987; 69: 798–803.
20. Verheijen JH, Mullaart E, Chang GTG, Kluft C, Winjgaards GA. A simple, sensitive spectrophotometric assay for extrinsic (tissue-type) plasminogen activator applicable to measurements in plasma. Thromb Haemostas 1982; 48: 266–9.
21. Suffredini AF, Harpel PC, Parrillo JE. Promotion and subsequent inhibition of plasminogen activation after administration of intravenous endotoxin to normal subjects. N Engl J Med 1989; 320: 1165–72.
22. Van der Poll T, Levi M, Buller HR, et al. Fibrinolytic response to tumor necrosis factor in healthy subjects. J Exp Med 1991; 174: 729–32.
23. Mannucci PM, Remuzzi G, Pusineri F, et al. Deamino-8-D-arginine vasopressin shortens the bleeding time in uremia. N Engl J Med 1983; 308: 8–12.
24. Mannucci PM, Vicente V, Vianello L, et al. Controlled trial of desmopressin (DDAVP) in liver cirrhosis and other conditions associated with prolonged bleeding time. Blood 1986; 67: 1148–53.
25. Mannucci PM. Desmopressin: a nontransfusional form of treatment for congenital and acquired bleeding disorders. Blood 1988; 72: 1449–55.
26. Salzman EW, Weinstein MJ, Weintraub RM, et al. Treatment with desmopressin acetate to reduce blood loss after cardiac surgery. N Engl J Med 1986; 314: 1402–6.
27. Rocha E, Llorens R, Páramo JA, Arcas R, Cuesta B, Martin Trenor A. Does desmopressin acetate reduce blood loss after surgery in patients on cardiopulomary bypass? Circulation 1988; 77: 1319–23.
28. Levi M, Biemond B, Brandjes D, Hack CE, ten Cate JW. Fibrinolytic response upon DDAVP in factor XII deficient patients. Br J Haematol 1990; 76 (Suppl 1): 11 (Abstr).
29. Vicente V, Alberca I, Calles I, López Borrasca A. Effect of DDAVP on contact phase of blood coagulation. Br J Haematol 1984; 56: 679–80.
30. Smith D, Gilbert M, Owen WG. Tissue plasminogen activator release in vivo in response to vasoactive agents. Blood 1985; 66: 835–9.

Received September 18, 1991 Accepted after revision April 9, 1992