Thrombolytic Therapy Causes an Increase in Vascular Permeability That Is Reversed by 1-Deamino-8-ᴅ-Vasopressin

M. Audrey Rudd, PhD; Michael T. Johnstone, MD; LeRoy E. Rabbani, MD; Dorinda George, BA; J. Anthony Ware, MD; and Joseph Loscalzo, MD, PhD

Background. To examine the effect of plasminogen activator therapy on vascular permeability, we used a modified rabbit mesenteric model of extravascular tissue accumulation of radiolabeled albumin.

Methods and Results. Albumin deposition was measured after saline, tissue-type plasminogen activator (t-PA; 0.86 mg/kg for 1 hour followed by 0.29 mg/kg for 2 hours), or t-PA plus 1-deamino-8-ᴅ-arginine vasopressin (DDAVP; 0.6 mg/kg/hr for 30 minutes) infusion in animals with or without aspirin (ASA; 15-mg/kg bolus) pretreatment. In animals not given ASA, t-PA caused a 240% increase in tissue [¹²⁵I]albumin accumulation over time (p<0.001). DDAVP prevented the rise in albumin accumulation normally seen with t-PA alone (p<0.05) in animals not given ASA. In animals pretreated with ASA, t-PA similarly caused an increase in tissue albumin accumulation, but this was significantly attenuated from that of animals not given ASA (p<0.03). Interestingly, DDAVP failed to block the response to t-PA in the animals given ASA. Because increases in vascular permeability correlated with increases in bleeding time (r=0.37, p<0.03), these data suggest that the effect of plasmin generation on vascular permeability may contribute to the bleeding tendency seen with thrombolytic therapy. The ability of DDAVP to reverse the bleeding tendency and bleeding time may be due in part to its reversal of the increased vascular permeability induced by the administration of plasminogen activators.

Conclusions. These data show that plasminogen activation causes an increase in vascular permeability that is inhibited by DDAVP; ASA blunts this action of t-PA and prevents the DDAVP blockade of the increase in permeability induced by t-PA in this rabbit model. (Circulation 1991;84:2568–2573)

Although thrombolytic agents benefit patients with acute myocardial infarction, these agents can also cause spontaneous bleeding, which can be a life-threatening side effect. The mechanism underlying the bleeding tendency produced by thrombolytic therapy is not clearly understood.

We recently reported that the bleeding tendency observed after streptokinase administration did not correlate with any of the recognized determinants of bleeding such as platelet function, hematocrit, fibrinogen levels, and von Willebrand factor concentration or multimer distribution but instead correlated with bleeding time. We therefore concluded from these observations that one possible parameter that could account for the bleeding diathesis of thrombolytic therapy is a compromise in vessel integrity. The present study was designed to examine this possibility using vascular permeability to albumin as an index of vascular integrity. We used a rabbit model of mesenteric extravascular tissue accumulation of radiolabeled albumin to address this question. We further hypothesize that if the vessel integrity alterations are intimately involved in the bleeding incidence and bleeding time reflects these changes, then 1-deamino-8-ᴅ-
arginine vasopressin (DDAVP) might also affect or influence integrity. This appears to be a viable hypothesis since it has been shown by us as well as others\textsuperscript{25} that DDAVP can reverse bleeding time increases associated with various hemostatic disorders.

**Methods**

**Animal Preparation**

Female New Zealand White rabbits (3–4 kg) were initially anesthetized with ketamine (30–50 mg/kg i.m.) and subsequently given sodium pentobarbital (5–10 mg/kg i.v.) to maintain the level of anesthesia. After induction of anesthesia, the animals were surgically prepared for bilateral femoral artery and vein cannulation. Venous lines were used for drug infusions, and one of the arterial lines was used for blood pressure monitoring and blood sampling. The second femoral artery was used as access for the placement of a PE-50 cannula into the superior mesenteric artery (SMA). An abdominal incision was performed, and the large intestine was placed on saline-soaked gauze toward the right side of the animal’s abdominal cavity. The intestine was then covered with parafilm to prevent excessive insensible water loss. Lateral displacement of the large intestine made the SMA readily accessible for isolation. The cannula was filled with Evans blue dye so that its position could be easily discerned and then placed approximately 4 mm into the SMA. This cannula was used for injection of radioabeled albumin.

The study was divided into two series of experimental preparations containing three groups of animals each. Series 1 involved animals that were not pretreated with aspirin (ASA). After a 1-hour stabilization period, animals were infused with saline (n=5; 1.1 ml/hr for 3 hours), tissue-type plasminogen activator (t-PA) alone (n=5; 0.86 mg/kg/hr for 1 hour followed by 0.29 mg/kg/hr for 2 hours), or t-PA plus DDAVP (n=5; 0.3 \( \mu \)g/kg DDAVP for 30 minutes beginning at the end of the first hour of t-PA infusion). The animals in series 2 were pretreated with ASA (15-mg/kg i.v. bolus) 1 hour before beginning the various infusions (ASA plus saline, n=5; ASA plus t-PA, n=8; ASA plus t-PA plus DDAVP, n=5). This 1-hour incubation corresponded to the 1-hour stabilization period in series 1. Saline, t-PA alone, or t-PA plus DDAVP were given as in series 1.

**Tissue Sampling**

Radiolabeled albumin prepared as described below (bovine serum albumin fraction V, Sigma Chemical Co., St. Louis, Mo.) was injected at the beginning of the second hour of infusion of t-PA or saline. After injection of \([^{125}\text{I}]\)albumin into the SMA cannula, segments of small intestine were removed for counting radioactivity. All visible blood vessels surrounding each tissue sample were ligated before tissue excision. The isolated tissue segment was washed with saline to remove all blood and excrement. Each segment was swabbed dry with gauze and weighed before counting. Tissue samples were taken at 5, 15, 30, 60, and 120 minutes after injection of labeled albumin. All values were normalized to the 5-minute sampling period for each experiment and measured in counts per minute/gram of tissue.

**Bleeding Times**

Bleeding times were measured using previously described methods.\textsuperscript{1,4} Briefly, a shaved rabbit ear was placed into a 37°C water bath for 5 minutes to warm the ear. A full-thickness incision was made with a 15 stainless-steel surgical blade (Brad-Parker Becton Dickinson and Co., Lincoln Park, N.J.), with care taken to avoid any macroscopically visible vessels. The ear was returned to the saline bath and observed until all blood flow ceased. We also measured mesenteric bleeding times as described by Niewiarowski and colleagues.\textsuperscript{2} Microscopically visible veins on small bowel segments were cut and monitored for time to cessation of flow using gauze to collect the blood. Care was taken to avoid physical contact of the actual incision site during the bleeding time measurements.

**Protocol**

After surgery, the animals were allowed to recovery for 15–30 minutes before baseline blood samples were collected for plasmin activity determination (see Figure 1). Basal bleeding times were also measured. ASA was then given to the designated animals, and the animals were monitored for 1 hour before beginning the t-PA or saline infusion. In some animals, another bleeding time and blood sampling were obtained at the end of this incubation period. Saline or t-PA infusion was then begun, and a third bleeding time and blood sampling were performed at the end of the first hour of the t-PA infusion. It was at this point that radiolabeled albumin was injected into the
SMA cannula; tissue sampling was begun 5 minutes later (see above). Bleeding time and blood sampling were again performed at the second and third hours of t-PA or saline infusion.

**Assays**

Plasma plasmin activity was measured using a chromogenic substrate method as described previously. Plasma (50 μl) was incubated at 37°C with 0.8 mM H-d-valyl-L-leucyl-L-lysine-p-nitroanilide dihydrochloride (S2251) (Kabi Vitrum, Stockholm, Sweden) in 24-well microtiter plates. Optical density was read at 410 nm on a MiniReader II (Spectrophotometer, Dynatech Laboratory, Inc., Chantilly, Va.) at various time points during the incubation. Activity was calculated as change in absorbance per hour.

**Drugs and Reagents**

t-PA was obtained from Genentech, Inc., South San Francisco, Calif. DDAVP was kindly provided by Armour Pharmaceutical, Kankakee, Ill., as Stimate. Na[125I] was obtained from New England Nuclear, Boston, Mass. Bovine albumin (fraction V) was purchased from Sigma Chemical. All other agents were reagent grade or better.

**Radioiodination**

Radioiodination of albumin was performed using a modified version of the Iodo-bead method as previously described. Briefly, 0.5 μCi of Na[125I] was added to 1 ml of a 0.3 mg/ml albumin solution containing two Iodo-beads. After a 30-minute incubation, the reaction solution was passed over a Sephadex G-25 column, and fractions were collected and counted in a gamma counter. The first peak containing the [125I]albumin was assayed for specific activity, which averaged 2.175±642 cpm/ng (mean±SEM).

**Statistical Analysis**

All data within groups were analyzed by one-way analysis of variance (ANOVA) with a Newman-Keuls multiple comparison test. Data between groups were analyzed by two-way ANOVA. Values are given as mean±SEM. Probability values of less than 0.05 were considered statistically significant.

**Results**

In animals without ASA pretreatment (Figure 2A), t-PA caused a significant increase in the accumulation of [125I]albumin in extravascular mesenteric tissue as soon as 30 minutes, and this accumulation progressed during the 60- and 120-minute sampling times. These time periods corresponded to 1½, 2, and 3 hours, respectively, after the start of t-PA infusion. Tissue radioactivity increased to 171±17%, 212±36%, and 240±25% (p<0.05 versus baseline) at 30, 60, and 120 minutes, respectively. Saline control animals showed no significant change in permeability to albumin over time. To determine whether the change in tissue counts was the result of changes in blood volume within the particular tissue segments, we infused saline into the SMA to flush the tissue of intravascular blood. This was done immediately after the 120-minute sampling period, and tissue adjacent to the presaline washout sample was taken for comparison. We found no significant difference in tissue counts before or after the saline washout (232±48% versus 213±40%, n=3).

We also examined the effect of DDAVP on the vascular permeability changes seen after t-PA infusion. DDAVP, which was begun when the radiolabeled albumin bolus was injected, completely prevented the rise in tissue counts, with radioactivity accumulation being only 97±16%, 106±19%, and 110±22% of baseline at 30, 60, and 120 minutes, respectively.

Figure 2B shows the vascular permeability responses to saline, t-PA, or t-PA plus DDAVP in animals pretreated with ASA. t-PA caused an increase in tissue accumulation of albumin similar to that in animals not treated with ASA, increasing to 124±11%, 132±9%, and 158±9% (p<0.05 versus
baseline) at 30, 60, and 120 minutes, respectively. However, this response was significantly attenuated compared with animals not receiving ASA but given t-PA (two-way ANOVA; \( p < 0.03 \)). Saline (control) did not cause a change in tissue albumin content. Interestingly, when DDAVP was given to ASA-treated animals, it failed to prevent the rise in permeability induced by t-PA seen previously in animals not treated with ASA. The increase in tissue accumulation in the presence of DDAVP tended to be greater than that with t-PA alone (two-way ANOVA, \( p = 0.14 \)). The response to DDAVP alone was similar to that of saline control (data not shown, \( p = \text{NS} \)).

Bleeding time correlated significantly with permeability as shown in a plot of bleeding time 2 hours after the start of t-PA or saline infusion compared with the corresponding extravascular tissue content of \([^{125}]\)albumin at the same time point (Figure 3, \( r = 0.37, p = 0.025 \)). When permeability of control, t-PA, and t-PA plus ASA groups were plotted as a function of plasmin activity, we found an even stronger correlation (Figure 4, \( r = 0.69, p = 0.001 \)). The smaller, but significant \( r \) value for bleeding time may simply reflect the fact that there are other factors that contribute to bleeding in addition to vessel integrity.

### Discussion

Our results show that thrombolytic treatment causes an increase in vascular permeability as determined by extravascular radiolabeled albumin accumulation in mesenteric tissue. Adjunctive treatment with ASA did not prevent but significantly attenuated the permeability response to t-PA. DDAVP prevented the increase in tissue albumin accumulation after t-PA administration in animals not given ASA; however, DDAVP failed to do so in animals treated with ASA. We also demonstrate that permeability significantly correlated with bleeding time prolongation as well as with plasma plasmin activity.

One of the major side effects of thrombolytic therapy is spontaneous bleeding from nonsurgical as well as surgical sites. We have recently reported that bleeding noted in animals treated with a plasminogen activator correlated with bleeding time prolongation. This association was independent of any change in platelet function, decrease in hematocrit or fibrinogen levels, or alterations in von Willebrand factor antigen or multimer distribution. Because we could not detect any significant contribution to bleeding risk by any of these major determinants of bleeding, we hypothesized that alterations in vascular integrity may explain our observations. Using vascular permeability to albumin as an index of vascular integrity, we examined the effect of thrombolysis on albumin deposition in extravascular intestinal tissue.

It is beyond the scope of the present study to describe the specific means by which thrombolytic agents lead to alterations in vessel wall integrity. We can only speculate at this point as to possible mechanisms by which t-PA and/or plasmin may act to alter permeability. Several investigators have shown that plasmin can lead to the generation of kinins and complement activation, both of which can directly or indirectly alter endothelial cell and vascular permeability. In addition, certain proteolytic fragments of fibrinogen, such as Bj61-42, have also been shown to increase vascular permeability.

Another possibility involves the stimulation of platelets by plasmin, which then release agents that are capable of affecting endothelial permeability. For example, after infusion of thrombolytic agents, thromboxane \( A_2 \) levels rise. When endothelial cells are treated with this prostanoid, their microfilaments become disassembled, reducing junctional membrane apposition and increasing permeability. Other substances, such as histamine, an inflammatory agent, can also cause microfilament disassembly. By contrast, serotonin and norepinephrine cause stabilization of microfilaments and prevent extravasation of red blood cells and petechiae formation in experimental thrombocytopenia.

Other cell types in the vasculature can release cytokines upon stimulation that can alter vascular permeability. Interleukin (monocytic) and leukotriene (lymphocytic) cytokines can each produce an increase in albumin leakage into tissue. Other
leukocytic factors such as tumor necrosis factor, platelet-activating factor,\textsuperscript{21} and interferons can have similar effects on vascular permeability.\textsuperscript{18,20,22}

In addition, plasmin generation may lead to a functional state of thrombocytopenia. We have shown that infusion of thrombolytic agents\textsuperscript{22} produces a biphasic effect on platelet function—a early stimulatory phase and a later inhibitory phase. Adjunctive therapy with an antiplatelet agent prevents the early increase in aggregation response while prolonging the later decrease in aggregation response. The inhibitory phase may lead to events similar to those seen in thrombocytopenia that result in a net increase in vascular permeability. As stated earlier, infusion of serotonin reverses the increase in permeability and prevents petechiae formation\textsuperscript{15,17} in experimental thrombocytopenia. Platelet inhibition or dysfunction induced by plasmin may limit serotonin release from dense granule stores.

Last, plasmin may act directly on the basement membrane or subendothelial proteins\textsuperscript{23} to degrade essential component molecules required for anchoring endothelial cells to the matrix, thus leading to detachment of these cells at focal sites along the endothelial barrier. Becker and colleagues\textsuperscript{24} reported endothelial damage after streptokinase as well as urokinase infusion in rats; this action may also be the direct result of cytotoxicity of plasmin.

The mechanism by which DDAVP can reverse bleeding time\textsuperscript{1} and prevent bleeding complications in various clinical settings\textsuperscript{25} is not completely understood. DDAVP causes the release of von Willebrand factor from endothelial cells, a polymeric glycoprotein necessary for platelet agglutination or adhesion to endothelial surfaces.\textsuperscript{26} In the present study, DDAVP caused only a small decrease in bleeding time (495±232 seconds, t-PA, n=4; 393±175 seconds, t-PA plus DDAVP, n=4). However, in our earlier study with streptokinase, we found that DDAVP reversed bleeding times only if it exceeded 400–500 seconds. Because bleeding times in the present study with t-PA approximated this value, we were not able to detect dramatic changes in response to DDAVP. Our study with streptokinase revealed that although DDAVP reversed bleeding time in animals given streptokinase, its administration did not result in any significant increase in von Willebrand antigen or redistribution of von Willebrand multimer to the more hemostatic, larger-molecular-weight forms. One can therefore conclude as an alternative possibility that DDAVP acts on the endothelium to release a cytoprotective agent or agents that preserve or maintain an intact cellular permeability barrier. That this is a viable possibility is borne out by the facts that 1) prostaglandin I\textsubscript{2}, a possible cytoprotective prostanooid that prevents microfilament disassembly engendered by thromboxane A\textsubscript{2} release,\textsuperscript{17,27} is stimulated by DDAVP\textsuperscript{28} and inhibited by plasmin,\textsuperscript{29} and 2) ASA, a cyclooxygenase inhibitor, prevents the effects of DDAVP on reversing increases in vascular permeability in the present study.

Interestingly, the effect of ASA on the permeability response may be twofold. First, ASA attenuates the response to t-PA, perhaps by inhibiting thromboxane A\textsubscript{2} release. As discussed above, thromboxane A\textsubscript{2} can increase endothelial permeability.\textsuperscript{11} However, ASA also prevents the production of prostaglandin I\textsubscript{2}, a cytoprotective prostanooid that reduces endothelial leaking.\textsuperscript{17} If DDAVP stabilizes endothelial integrity by stimulating prostaglandin I\textsubscript{2} release, then ASA could also attenuate this action of DDAVP by inhibiting prostaglandin I\textsubscript{2} synthesis. Vascular permeability continues to increase with t-PA plus DDAVP in the presence of ASA, although to a reduced extent because of a decrease in thromboxane A\textsubscript{2} production caused by ASA. In addition, when cyclooxygenase is inhibited, leukotriene production is increased because of shunting of arachidonate to the lipoxygenase pathway. Leukotrienes may adversely modulate endothelial cell permeability, as does thromboxane A\textsubscript{2}.\textsuperscript{19} More detailed pharmacological studies will be needed to determine the specific mechanism by which DDAVP and plasmin manifest their actions on endothelial permeability.

Limitations

We recognize that these experiments do not prove that the accumulation of albumin in the perivascular or extravascular space occurs solely through a direct increase in endothelial permeability. There are other factors, such as intravascular and tissue pressures, lymph flow, and numbers of perfused capillaries, that may also be important in our observations. To address the issue of blood volume or perfused capillary density, we perfused saline through the artery immediately after the last tissue sampling and removed an adjacent tissue segment. We found no significant difference in the tissue counts before and after saline washout, suggesting that a change in blood volume is an unlikely explanation for our results. We did not extensively address all of the other potential factors in these experiments, but we did measure lymph flow in several animals treated with t-PA and found it to be increased. However, more experiments are needed to determine adequately the role of each of these parameters in our study.

We recently performed in vitro studies that suggest that plasmin generation can directly alter endothelial monolayer permeability.\textsuperscript{30} In a tissue culture system, we have shown that radioactive albumin passage across a monolayer of endothelial cells is directly correlated with plasmin activity. This suggests that our results may be the consequence of a direct increase in vascular permeability.

Summary

We have demonstrated that therapeutic doses of t-PA lead to an increase in vascular permeability and that this increase in permeability is positively correlated with bleeding time and plasmin activity. These observations suggest that the bleeding diathesis associated with thrombolytic therapy may be
due in part to a compromise of vessel wall integrity. This effect of thrombolytic therapy and its reversal by DDAVP provide new and potentially useful information for the optimal management of patients receiving thrombolytic agents.

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References


**Key Words**: tissue-type plasminogen activator • plasmin • vascular permeability • vasopressin • bleeding time
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