DEXTRAN'S ANTITHROMBOTIC PROPERTIES IN SMALL ARTERIES ARE NOT ALTERED BY LOW-MOLECULAR-WEIGHT HEPARIN OR THE FIBRINOLYTIC INHIBITOR TRANEXAMIC ACID: AN EXPERIMENTAL STUDY

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In two separate blind, randomized studies, 48 rabbits were divided into five groups. Three treated groups received dextran 70 (0.51 g/kg) as a single injection, dextran 70 combined with low molecular-weight heparin (LMWH) (560 IU/kg as anti-FXa and 140 IU/kg as APTT in 3 hr), and dextran 70 plus tranexamic acid (14 mg/kg) following severe arterial trauma (arteriotomy/intimectomy). Two control groups received the same amount of saline. The bleeding times from the arteriotomy were recorded, and patency rates and the weight of thrombotic materials were registered 2 hr after reperfusion of the trauma region. The bleeding times in three treated groups, all including dextran, were significantly prolonged compared to the control groups ($P < 0.05$ or 0.01). The patency rates of treated groups, which were 100% (18/18 vessels patent) in the dextran-treated group, 90% (18/20 vessels patent) in the dextran + LMWH group and 95% 19/20 vessels patent) in dextran + tranexamic acid group, were significantly higher than those of their control groups (55–67% patent vessels) ($p < 0.05$ or 0.01). The mean weights of thrombotic materials were significantly reduced in the treated groups compared to the corresponding control groups ($p < 0.01$). In conclusion, dextran 70 in an ordinary dose exerted such a profound antithrombotic effect (100% patency) in small traumatized arteries that the addition of a high dose of LMWH could not further improve patency rates or decrease thrombotic materials but did not prolong vessel bleeding times compared to the single dextran treatment. The addition to dextran 70 treatment of a clinical dose of the antifibrinolytic agent tranexamic acid did not disturb the good antithrombotic effect, suggesting that fibrinolysis enhancement is a less important characteristic of the dextran antithrombotic effect in small traumatized arteries.

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Dextran has been extensively used both as a plasma expander and an antithrombotic agent.[1] It was found that dextran could reduce the incidence of postoperative deep vein thrombosis,[2,3] the risk for fatal pulmonary emboli,[4] and occlusion rates of arterial grafts.[5] In some of our previous studies, dextran was demonstrated to improve significantly the vascular patency without disturbing platelet aggregation following anastomosis or intimectomy in experimental microsurgery.[6–8] These studies proved the beneficial use of dextran in microvascular surgery. In 1975, Aberg et al.[9] reported increased fragility and lysability of thrombi following dextran treatment in an ex vivo study. In addition, dextran enhances fibrinolysis and appears to protect plasmin from other inhibitory effects.[10–12] The relative importance of this proposed dextran-induced enhancement of fibrinolysis in preventing thrombus formation in small arteries is not known but is of great interest both clinically and experimentally. This is the rationale for testing, as in this study, a combination of dextran and the fibrinolysis inhibitor tranexamic acid in a common clinical antihemorrhagic dose. Low-molecular-weight heparin (LMWH; Fragmin; Kabi Pharmacia, AB Stockholm, Sweden), a new antithrombotic agent, has been widely accepted in clinical practice[13] due to its advantages compared to conventional heparin, such as better bioavailability, longer biological half-life after subcutaneous injection,[14] and fewer bleeding complications.[14,15] In our previous studies, LMWH in a high dose was shown to exert a profound antithrombotic effect in experimental microvascular surgery. This effect was as good as that of conventional heparin in veins (patency rates of traumatized vessels were increased from 0% to 40% following both LMWH and heparin treatment) but lower in arteries (vascular patencies were 80% in the LMWH treated group and 100% in heparin treated groups following intimectomy).[16,17] The results were based on large groups (30–33 vessels/group). A further study in this series has also demonstrated that a combination of dextran 70 and LMWH treatment can significantly enhance the venous patency following severe trauma (intimectomy) without causing prolonged vessel bleeding times.[18] It is not known if this combination therapy can also improve the thromboprophylactic effect of these agents in small arteries or causes bleed

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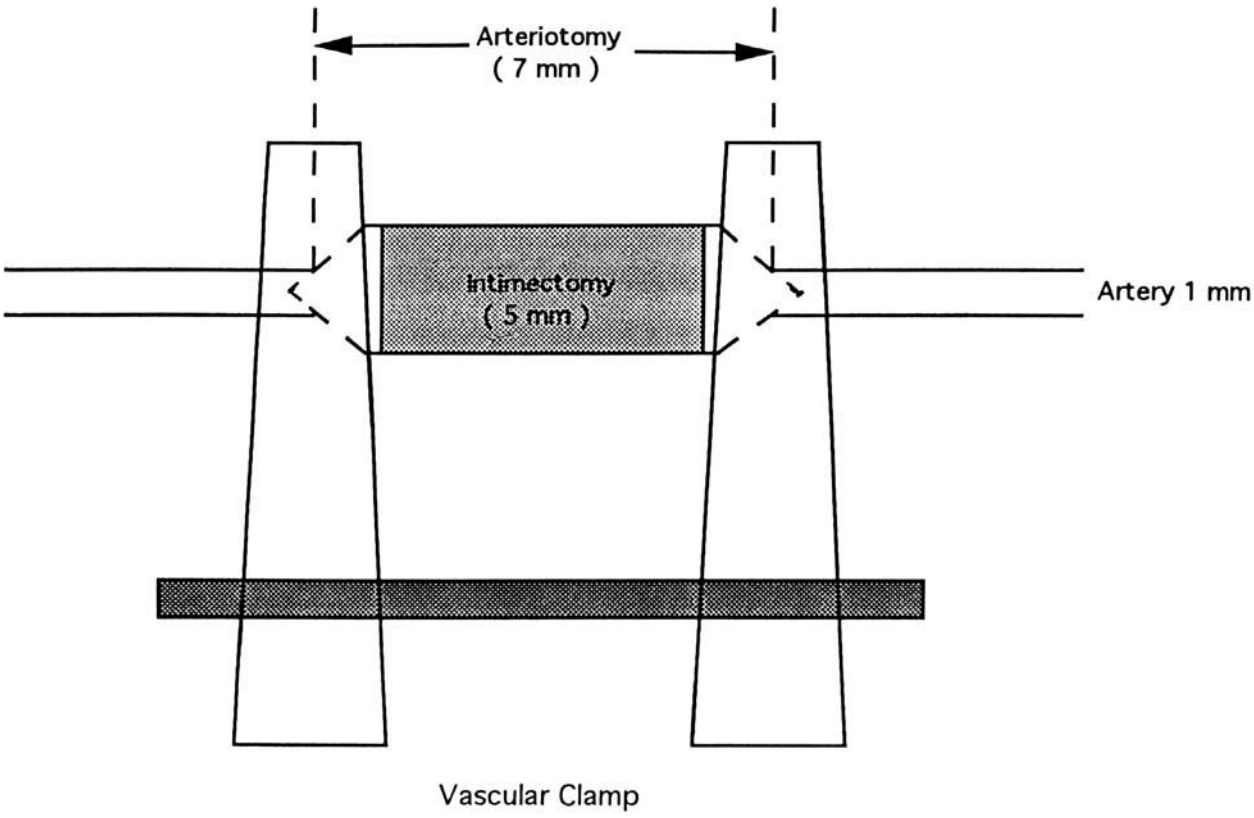

Figure 1. The prepared artery is placed in a double clamp, and a 7 mm longitudinal arteriotomy is performed. A deep intimectomy entering the medial layer is then performed using a special blade.

ing complications, but this would be of great interest in clinical practice.

MATERIALS AND METHODS

Male rabbits, weighing 2.5–3.8 kg, were kept on a standard pellet diet and given water ad libitum for at least 7 days prior to experiments and were kept according to the national guide for the care and use of laboratory animals.

Surgical Procedure

The rabbits were anaesthetized by intravenous injection of sodium pentobarbital (Mebumal; ACO Läkemedel, Solna, Sweden), and anaesthesia was maintained by intermittent administration of the same drug. Lidocaine (Xylocaine; 10 mg/ml; AB Astra Läkemedel, Sweden) was used as local anaesthesia in skin incisions of ears and groin area. The body temperature of rabbits was maintained at 39.6 ± 0.2°C using a heating pad. An incision was made in the groin area and a catheter, for infusion and the drawing of blood samples, was put into the aorta via the right femoral artery. The ears of rabbits were mounted on plastic moulds, and an S-shaped skin incision was performed along the central artery of both ears. The central arteries were dissected carefully, and blood flow was stopped by applying a double vascular clamp. Arteriotomy (7 mm) and intimectomy (5 mm) were performed (Fig. 1). The arteriotomy was closed with a running 10/0 monofilament nylon suture. All procedures, including arteriotomy/intimectomy, were performed by the same surgeon, using a microscope and normal microsurgical instruments. The blood flow was reestablished by removing the double vascular clamps.

Groups

The study consisted of two separate blind randomized studies. In the first part, 18 rabbits were divided into two groups, nine rabbits in each. The treated group received 8.5 ml/kg (0.51 g/kg) of dextran 70 (Macrodex; 60 mg/ml; Kabi Pharmacia AB, Stockholm, Sweden) 2 hr before removal of

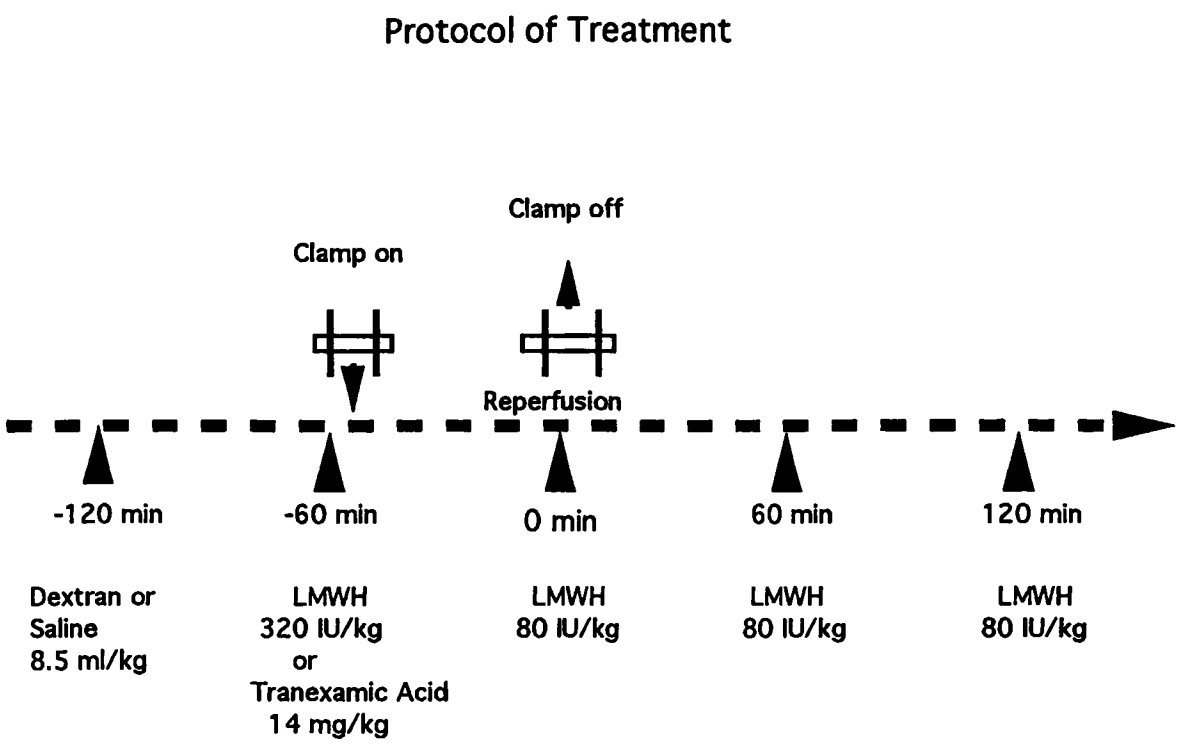

Figure 2. Times and doses of drugs administered.

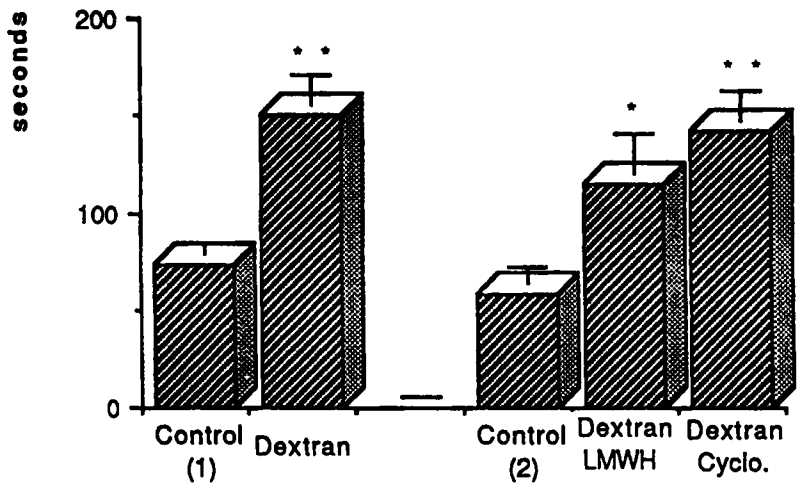

Figure 3. The addition of LMWH to dextran-treated animals did not increase vessel bleeding times.

the vessel clamp; the control group was treated the same way but with equal volumes of saline. In the second part of the study, 30 rabbits were divided into three groups, ten rabbits in each. Two treated groups received 8.5 ml/kg of dextran 70 2 hr before removal of the vessel clamps. One of these groups received LMWH 320 IU/kg as anti-FXa and 80 IU/kg as APTT 1 hr later, and then one-quarter doses were repeated each hour (totally 560 IU as anti-FXa and 140 IU as APTT was administered; Fig. 2). The other treated group received 14 mg/kg of tranexamic acid (Cyklokapron; 100 mg/ml; Kabi Pharmacia AB, Stockholm, Sweden) 1 hr after infusion of dextran resulting in tranexamic plasma combinations of ~ 30 microgram/ml after 5 minutes and 6–8, 3, and 2 microgram/ml after 1, 2, and 3 hours respectively. The control group was treated according to the protocol with equal volumes of saline.

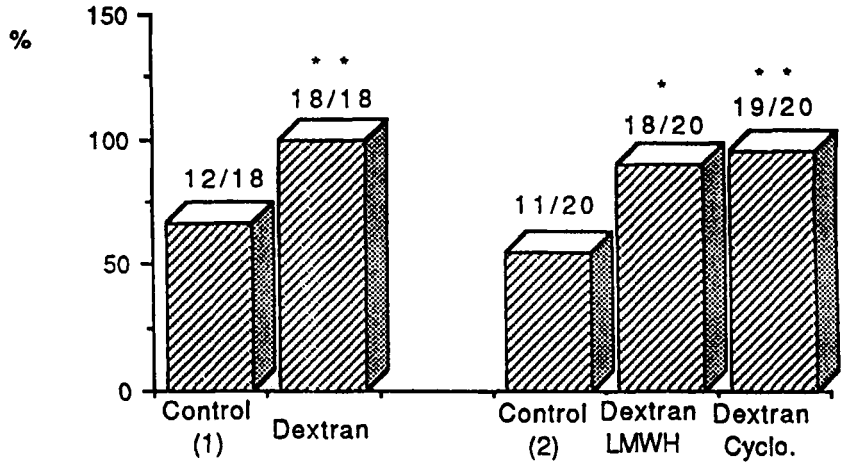

Figure 4. Dextran treatment in all groups lead to increased patency rates, despite the addition of tranexamic acid (Cyclo).

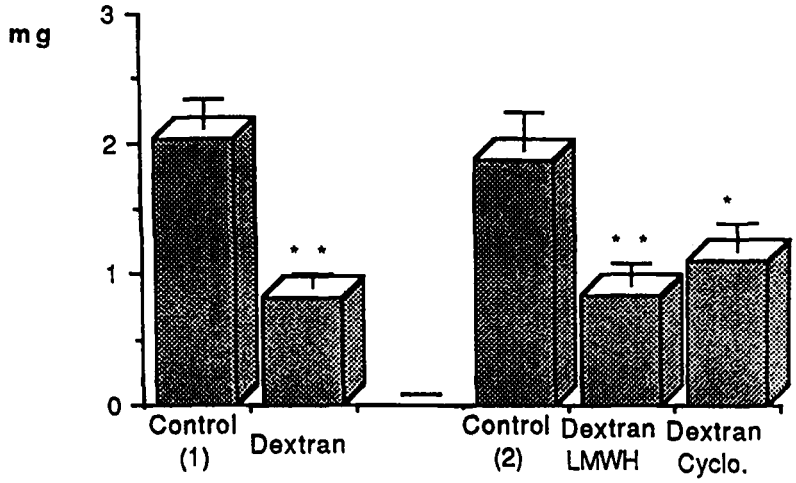

Figure 5. The increased patency rates in Figure 4 are reflected in decreased thrombotic materials without obvious effects of either an added high dose of LMWH or a clinical dose of tranexamic acid (Cyclo).

Bleeding Time

The time between the release of vessel clamps and complete cessation of bleeding from the arteriotomy was noted with a stopwatch in seconds as the total bleeding time.

Patency

Vessel patency was determined 2 hr after reestablishment of blood flow using an empty/refill test and was recorded as either good (rapid refilling) or no patency (no refilling). A segment of the artery distal to the arteriotomy was emptied of blood using two pairs of microforceps. Blood flow was restored by opening the proximal pair and patency was assessed.

Thrombotic Material

After finishing the patency test, segments of the arteries containing the traumatised regions were excised and opened longitudinally. The vessel with and without thrombotic material was weighed. The difference was registered as the weight of the intraluminal thrombotic material.

Haematocrit Determination

In the first part of the study, the haematocrit was measured according to routine procedures in both the dextran-treated and the control groups. These values were based on blood samples drawn just prior to infusion of dextran or

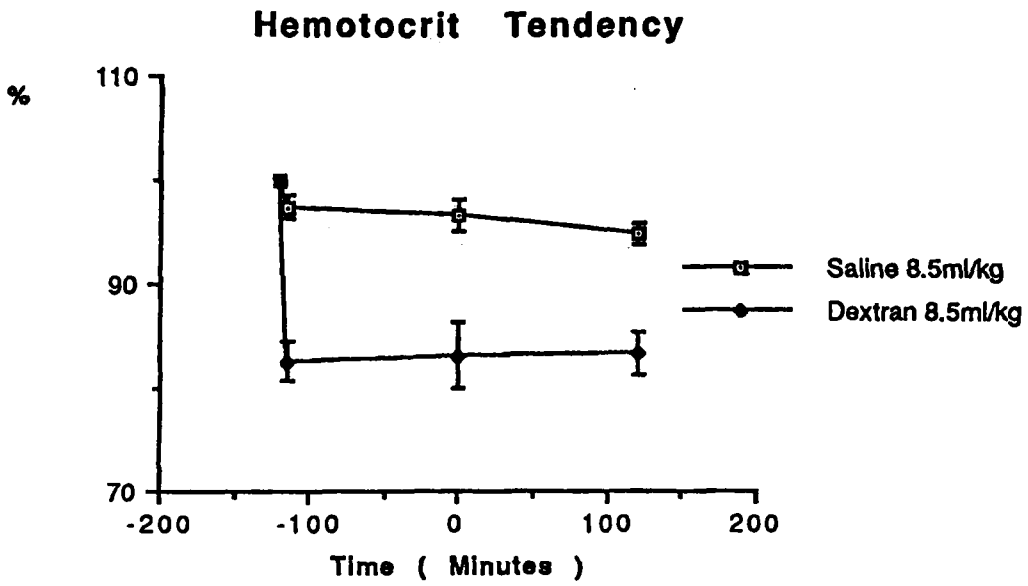

Figure 6. Dextran infusion immediately resulted in a significant hemodilution that persisted for at least 4 hr.

saline and at intervals of 5 min, 2 hr, and 4 hr after the infusion.

Fibrinolytic Activity

Plasma samples were collected for measurement of fibrinolytic activity 5 min prior to administration of solution and at intervals of 5 min, 2 hr, and 4 hr after the end of infusion. The plasma samples were kept in a refrigerator at −70°C until they were assayed. Thirty microlitres of plasma was directly applied to fibrin plates prepared according to Nilsson et al. with modification.[19] The plates were incubated for 12 hr at 37°C. The sizes of the lytic zones were expressed as the maximum chord length times the width of the maximum chord next to it.

Statistical Comparisons

Fisher's exact test was used to compare the difference of patency rates. Student's t test was used in comparisons of bleeding times, thrombotic materials, haematocrit, and fibrinolytic activity values.

RESULTS

Bleeding Time

In the first part of the study, the bleeding times were 73 ± 6 sec in the control group and 150 ± 15 sec in the dextran-treated group. The difference between the two groups is very significant ($p < 0.001$).

In the second part of the study, the bleeding times were 57 ± 8 sec in the control group, 115 ± 21 sec in the dextran + LMWH group, and 142 ± 16 sec in the dextran + tranexamic acid group. The differences were significant between treated groups and the control group ($p < 0.05$) but were not significant between the two treated groups (Fig. 3).

Patency Rates

The patency rates were 67% (12/18 vessels patent) in the control group and 100% (18/18 vessels patent) in the dextran group in the first part of the study. The difference between the two groups was statistically significant ($p < 0.01$). In the second part of the study, the patency rates were 55% (11/20 vessels patent) in the control group, 90% (18/20 vessels patent) in the dextran + LMWH group, and 95% (19/20 vessels patent) in the dextran + tranexamic acid group. The patency rates of treated groups were statistically significantly higher than that of the control group ($p < 0.05$ and $p < 0.01$, respectively; Fig. 4).

Thrombotic Material

In the first part of the study, the thrombotic materials of the dextran-treated group were 0.822 ± 0.095 mg and significantly less than in the control group, 2.028 ± 0.23 mg ($p < 0.01$). In the second part of the study, the thrombotic materials were 0.855 ± 0.163 mg in the dextran + LMWH group, 1.10 ± 0.213 mg in the dextran + tranexamic acid group, and 1.865 ± 0.275 mg in the control study. There were statistically significant differences between the treated groups and the control group ($p < 0.01$), but there was no difference between the two treated groups (Fig. 5).

Haematocrit Values

The haematocrit values following infusion of dextran was decreased by ~ 15–20%, representing statistically sig-

nificantly lower values than that of the control group receiving saline infusions ($p < 0.05$; Fig. 6).

Fibrinolytic Activities

Fibrinolysis, as measured with this method, was not increased in any of the groups.

DISCUSSION

The trauma model (arteriotomy/intimectomy) closely resembles clinical vascular injuries found in avulsion tramata.[18] No foreign materials, except ordinary microsutures, or blood flow restrictions were used. The purpose of the first part of the study was to investigate if dextran 70 in a moderate dose of 8.5 mg/kg (0.51 g/kg), reflected in a limited haemodilution (haematocrit values decreased by 15–20%), increase microarterial patency rates following a severe trauma (arteriotomy/intimectomy). This dextran 70 dose administered also 2 hr before reperfusion of small traumatized veins (venotomy/intimectomy), was previously shown to enhance significantly venous patency rates by addition to LMWH, administered as in the second part of this study, without causing bleeding complications.[19] As was stated above, our previous studies have shown that the same LMWH treatment as was used in this study, but as a single agent treatment, resulted in significantly increased patency rates and significantly decreased thrombotic materials in small arteries using the same severe trauma model (arteriotomy/intimectomy).[16]

Dextran treatment in this moderate dose was very efficient in preventing thrombosis during the most crucial 2 hr following reperfusion of the arteriotomy/intimectomy area and resulted in 100% patency. This being the case, the addition of LMWH could not further increase patency rates as seen in the second part of the study (Fig. 4). The thrombotic materials (Fig. 5) do not reveal any additional effect of LMWH, even though this dose was high, but a most important observation was that the combination therapy did not increase arterial vessel bleeding. In the previous venous study,[20] LMWH added to dextran treatment significantly reduced the thrombotic materials to very low levels (one-sixth that of the dextran group) without increasing vessel bleeding, perhaps reflecting that the high anti-FXa activity of LMWH (560 IU as anti-FXa but only 140 IU as APTT units was administered in this study) is an effective antithrombotic characteristic in veins but in small arteries a high antithrombin activity (APTT activity), as with standard heparin, is most important. The same administration of LMWH in the previous venous study[20] resulted in a steady state of ~ 2 IU/ml anti-FXa in plasma. APTT times were also increased, but, since the same dose of APTT units administered as standard heparin (140 IU) did not increase patency rates and rarely affected thrombotic materials, a high anti-FXa activity seems sufficient to prevent thrombosis in small veins. The addition of LMWH had no positive effects in small arteries in this experimental model and no negative effects, since the vessel bleeding times were unaffected and increased tissue bleeding was not observed. This is important in that this combination therapy was so efficient in preventing venous thrombosis and is therefore indicated when venous problems can be expected, this being a more common problem than arterial thrombosis according to most authors.[21,22]

In the second part of the study, one group was treated with dextran 70 combined with tranexamic acid, which inhibits fibrinolysis.[23] The reason is that in Scandinavia this antifibrinolytic agent is commonly used to prevent diffuse intra- or postoperative tissue bleeding occurring both with and without dextran treatment.[24] Dextran treatment increases tissue bleeding to some extent and has been shown to enhance fibrinolysis,[25] which could not be verified in our study with the method used. It is not known if the dextran-induced increase of fibrinolysis is one of the important factors in dextran's good antithrombotic qualities in our experimental microvascular procedures. The question therefore was whether a commonly used dose of the fibrinolysis inhibitor tranexamic acid would prevent partially or totally the antithrombotic effect of dextran in small arteries as seen in the first part of the study. The result shows that the antithrombotic effects of dextran in small arteries was not disturbed by the addition of tranexamic acid as seen in patency rates (Fig. 4) and thrombotic materials (Fig. 5). Furthermore, the vessel bleeding times, which are quite different from other bleeding tests, were not affected. It therefore seems safe to add tranexamic acid in this clinical dose 14 mg/kg body weight 1 hr before vessel perfusion and still retain the antithrombotic effect of dextran in small arteries. However, we do not know if this is valid also for small veins and higher concentrations of tranexamic acid. Separate tests must be performed to study that. Recently, commonly used end-to-end anastomoses were performed in both arteries and veins in animals treated with tranexamic acid, in the same way as in this study, without any effect on patency rates or thrombotic materials compared to untreated control groups (unpublished data).

In conclusion, dextran 70 in an ordinary dose exerted such a profound antithrombotic effect (100% patency) in small arteries that the addition of a high LMWH dose could not further improve patency rates or decrease thrombotic materials but did not prolong vessel bleeding times. The addition to dextran 70 treatment of a clinical dose of the antifibrinolytic agent tranexamic acid did not disturb the good antithrombotic effect, suggesting that fibrinolysis enhancement is a less important characteristic of the dextran antithrombotic effect in small traumatized arteries. Based on these and previous studies, dextran seems a good choice in preventing thrombosis in small arteries and the addition of LMWH in preventing small vein thrombosis during the most crucial 2 hr after reperfusion of the traumatized vessels.

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