Effect of Dextran and Enoxaparin on Early ePTFE Graft Thrombogenicity in Sheep

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Objectives: To evaluate the effect of low molecular weight heparin (LMWH), dextran 70 and their combination on platelet adhesion and fibrinogen uptake in ePTFE grafts in an experimental sheep model.

Design: Prospective open study.

Setting: Animal Laboratory of a University Hospital.

Materials: Early thrombogenicity of ePTFE grafts was studied after interposition in the two common carotid arteries of 40 adult sheep. The animals received one of four different treatment regimens in a double blind randomised way: enoxaparin and polygeline, saline and dextran 70, enoxaparin and dextran 70 or saline and polygeline (control). The substances were administered i.v. with a total dose of 75 antifactor-Xa U/kg for enoxaparin and 1.0 g/kg for dextran 70. Polygeline and saline were used as placebo substances in equivalent volumes. On one side (random allocation) the carotid blood flow was restricted to 25 ml/min, on the other side it was left unrestricted.

Chief outcome measures: The following variables were studied: 1) fibrinogen uptake; 2) platelet uptake; 3) early graft patency; 4) blood flow in patent grafts; 5) visible presence of graft thrombus; 6) thrombus weight.

Main results: The results verified the importance of adequate blood flow as only 30% of grafts with restricted blood flow in the control group were patent compared with 80% of those with unrestricted blood flow (p = 0.038). Dextran 70, enoxaparin and the combination of the two increased early graft patency (p < 0.05) and reduced thrombus weights (p < 0.05) in grafts with restricted blood flow. The relative number of grafts with thrombus free surface was increased in the unrestricted blood flow situation.

Conclusions: Dextran 70 and enoxaparin appeared to be equally effective in decreasing fibrinogen and platelet uptake in the grafts. Their combination was not significantly more effective although there was a favourable trend.

Key Words: Enoxaparin; Dextran; ePTFE graft; Low molecular weight heparin; Early graft patency; Platelet adhesion; Fibrinogen uptake.

Introduction

There are several reasons why the early- and long-term graft patency after arterial bypass reconstruction is limited. Technical errors, high peripheral resistance, low blood flow and the thrombogenicity of the graft material may lead to graft occlusion.¹ Blood abnormalities such as increased platelet adhesiveness² and decreased fibrinolytic activity in the postoperative period³ may also affect the outcome. Furthermore, patients with atherosclerotic disease frequently have an elevated hematocrit and increased blood viscosity.⁴ Probably the best arterial substitute available is the autologous vein.⁵,⁶ However, the vein is not always available and the use of prosthetic material, with higher thrombogenicity may lead to decreased patency.

Clinical data show that an increased postoperative platelet uptake in grafts is related to reduced 1-year patency⁷ making studies of pharmacological adjuvant therapy to increase graft survival of interest. Several clinical and experimental studies have focused on this problem. Various antiplatelet agents such as acetyl salicylic acid alone⁸ or combined with dipyridamole⁹,¹⁰ agents blocking the synthesis of thromboxane,¹¹ the thromboxane receptor¹² or both¹³ seem to diminish graft thrombogenicity. Dextran 40 also improves early graft patency in humans.¹⁴ Experimental studies have also shown favourable effects of dextran 40, increasing early graft patency¹⁵ and
reducing leukocyte and platelet adhesion. Similarly, unfractionated heparin (UH) has been used in vascular reconstructive surgery since the 40s intraoperatively and postoperatively and even bound to the prosthetic surface.

After the development of low molecular weight heparin (LMWH) the use of UH has diminished in venous thromboprophylaxis. In arterial reconstructive surgery LMWH is inadequately documented with only few studies published. Experimental data indicate that LMWH may augmentate the antithrombotic effects of dextran when used in venous thromboprophylaxis without affecting haemostasis.

The aim of this study was to evaluate the effect of LMWH, dextran 70 and their combination on platelet adhesion and fibrinogen uptake in ePTFE grafts in an experimental sheep model.

Material and Methods

The study was approved by the Ethics Committee for Laboratory Animal Research, Lund University, Sweden, and conducted according to the rules of European Convention for Laboratory Animal Care.

Animals

Forty apparently healthy adult Swedish farm sheep of both sexes were studied. The median body weight was 27 kg (range 22–34 kg). The sheep were fed a standard diet and given water ad libitum. A leg vein cannula (Venflon®, BOC Ohmeda, Sweden) was used for injection of anaesthetics and test substances. The animals were anaesthetised with sodium pentobarbital (Pentothal® Sodium 25 mg/ml) and intubated. Respiration was maintained with a respirator (Cervo 900, Siemens Elema, Sweden) with a mixture of oxygen (50 to 65%) and nitrous oxide (50 to 35%), at a volume of 5.0 to 7.51/min and monitored with frequent blood gas analyses. The blood pressure was monitored continuously. Anaesthesia was maintained with a continuous infusion of sodium pentothal 2.5% (0.15 to 0.3 ml/min). Adequate hydration was maintained with a continuous infusion of saline (80 ml/h).

Radionuclide labelling

Sixty ml of blood from a jugular vein was used to prepare a platelet rich plasma suspension. The platelet fraction was examined by microscopy for contamination and for the amount of cells available for radionuclide labelling. The platelets were labelled with 12.5 MBq indium oxinate with a half-life of 67.4 hours (DRN 4908 Indium oxinate, Mallinkrodt Medical, The Netherlands). The platelet fraction was then checked in a gamma counter for labelling efficiency (Fig. 1). The fibrinogen used in the investigation was a sheep fibrinogen delivered in a pre-labelled suspension according to McFarlane. The amount used in each sheep was 1.0 ml labelled with 2 MBq iodine (Amersham International, U.K.).

Experimental model

The sheep were positioned in a supine position and through a 30 cm ventral midline neck incision both carotids were exposed. Small tributaries were ligated and divided. The carotids were wrapped in a thin plastic film to assure moisture and placed in separate lead collimators, one on each side. Four sodium iodine, gamma radiation detectors (Model 0.91 × M.510/.75 BLP-X, Bicron, U.S.A.) were placed in the collimators, two detectors on each side. The detectors were connected to a multi-channel analyser set up (EG & G Ortec, Nuclear Systems, U.S.A.) and a personal computer (Victor 486) for detection of signals that were stored simultaneously in the computer. The sheep were covered with a lead apron to minimise scattered radiation interference. After injection of the

Fig. 1. Platelet harvest (platelets × 10⁹)-left, and platelet labelling efficiency (%)-right, in the groups studied (mean ± S.E.M.). No statistical difference was found between the four groups.
$^{111}$Indium labelled platelets and the $^{125}$Iodine labelled fibrinogen, baseline isotope activity was measured during five 2 min intervals. This activity was used as baseline for the uptake measurements in the ePTFE grafts in the individual sheep. After the baseline isotope uptake measurements, the carotids were clamped and partially resected. The vessels were flushed with isotonic saline to prevent thrombosis but heparin was not used. An 11.5 cm thin-walled ePTFE graft, 6 mm in diameter (Gore-Tex™, Gore & Associates, Inc., U.S.A.) was interposed in each carotid vessel with an oblique end to end anastomosis with a running suture of CV 7.0, Gore-Tex™ (Gore & Associates, Inc., U.S.A.). The flow was re-established on both sides simultaneously and free vessel/graft flow was allowed for 5 min to ensure haemostasis in the anastomosis. The vessels were again wrapped in a thin plastic film and placed in the lead collimators, one on each side. To increase the thrombogenicity of the graft and to mimic a poor run-off situation the flow was restricted to 25 ml/min (+20%) in one carotid (randomly selected) with a specially made flow-restriction clamp, on the native artery distal to the distal anastomosis. The contralateral carotid had an unrestricted blood flow. The blood flow was monitored simultaneously in both carotids with a transit time ultrasound flow meter (TC 101, Transonic Inc., U.S.A.) and registered in a Macintosh™ computer with CHART/4 - Mac Lab™ software (Analog Digital Instrument, U.S.A.) for later analysis. The ultrasound probes were placed on the native arteries proximal to the proximal anastomosis. The four sodium iodine, gamma radiation detectors were placed in the collimators and the radionuclide uptake measured in the ePTFE graft 1.5 cm from the anastomosis. The uptake was measured during 3h 20 rain (200 min) in repeated 2 min intervals. The occurrence of graft occlusion was registered with the transit time ultrasound flowmeter. After the observation time the grafts were removed and opened lengthways and inspected for the presence of visible thrombus. Any thrombus was removed from the graft in a standard manner by scraping the graft. The excess fluid was removed with absorption paper and the thrombus weighed.

Substances

The following substances were used: Enoxaparin (Klexane®, Rhône-Poulenc Rorer, France). Mean molecular weight 4500 Da. Declared specific activity 100 antifactor-Xa U/mg. The total injected dose was 73 antifactor-Xa U/kg body weight i.v., with a starting dose of 55 antifactor-Xa U/kg repeated with one third of the starting dose (18 antifactor-Xa U/kg) after 2 h. Dextran 70 (Macrodex® with sodium chloride, Pharmacia, Sweden) with a mean molecular weight of 70 000 Da. The total dose was 1.0 g/kg body weight (16.5 ml/kg) by i.v. route during 30 min. Prior to the dextran infusion 20 ml of dextran 1 (Promiten®, Pharmacia, Sweden) was given intravenously to check for possible anaphylactic reactions. Polygeline (Hae-macel®, Behring, Germany) was used as a placebo substance for dextran for volume expansion. It has a molecular weight of 5000-15 000 Da with no antithrombotic or fibrinolytic effects and a negligible effect on platelet function. The infused polygeline dose was 16.5 ml/kg body weight. Saline (0.9% NaCl) was used as placebo substance for enoxaparin and dextran 1.

Experimental design

In a blind randomised fashion the animals received one of four treatment regimes. 1) Saline with polygeline (control group), 2) Enoxaparin with polygeline, 3) Enoxaparin with dextran, 4) Saline with dextran. Ten animals were included in each treatment group. The test solutions were prepared separately for individual animals in coded vials and the investigator was unaware of the given treatment. The polygeline and dextran was infused during the insertion of the grafts. The enoxaparin or respective placebo substance (saline) were injected during the haemostatic pause when the flow was re-established after the insertion of the grafts. The doses of the test substances were based on recommendations by the manufacturer for venous thromboprophylaxis in clinical practice for high risk situations. Due to the short half-life of LMWH after i.v. injection in sheep a further dose, one third of the original dose, was given after 2 h. The following variables were studied: 1) fibrinogen uptake, 2) platelet uptake, 3) early graft patency, 4) blood flow in patent grafts, 5) visible presence of graft thrombus 6) thrombus weight.

Statistical analysis

The values are expressed as mean ± S.E.M. unless otherwise stated. The radionuclide uptake was analysed and related to the baseline radionuclide uptake. The resulting curves show the relative change in uptake when compared with the baseline measurements. The results were evaluated with Mann-Whitney U test. Fischer’s exact test was used to evaluate
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Fig. 2. Early graft patency (%). *p<0.05 when compared with respective control group. Patency was significantly lower in the control group with restricted blood flow when compared with the group with unrestricted blood flow (p = 0.038).

Fig. 3. Mean thrombus weights (g ± S.E.M.). *p < 0.05 compared with the control group. The mean thrombus weights were lower in the control group with unrestricted blood flow when compared with restricted blood flow (p = 0.04).

Results

No adverse effects or bleeding complications were observed during the study. All animals survived the observation period and were sacrificed at the end of the experiment.

Early graft patency

The three treatment regimens significantly increased the early graft patency in the restricted flow system (Fig. 2). Seven grafts occluded in the control group with restricted blood flow (median 61 min, range 30–100). One graft with restricted blood flow occluded in the enoxaparin treated group and one in the dextran treated group at 135 and 190 min respectively. Two grafts with unrestricted blood flow occluded in the control group after 60 and 150 min. One graft occluded in the enoxaparin treated group and one in the dextran treated group after 100 and 105 min respectively with unrestricted blood flow. No occlusion occurred in the enoxaparin/dextran treated group with restricted or unrestricted blood flow. No technical errors that may have resulted in graft occlusion were observed in the occluded grafts. The graft with unrestricted blood flow that occluded after 150 min had lower flow than the other grafts in the control group, during the whole observation period. The reason for this is unknown but the animal also had a lower arterial pressure than the other animals in the group. A wide variation in blood flow was observed in the vessels with unrestricted blood flow (43–395 ml/min). Generally the flow in the patent grafts tended to increase successively during the observation period but no statistical differences were observed between or within the groups.

Thrombus

All grafts in the control groups had some thrombus. No thrombus-free surface was seen in the control group with restricted flow. Generally the mural thrombi were more pronounced in the anastomotic regions with their thickest part distally. The thrombi in the grafts with unrestricted blood flow were generally "whitish" and thin but more compact than the more "reddish" thrombi in the grafts with restricted blood flow, irrespective of given treatment. The groups treated respectively with enoxaparin, dextran or their combination had thrombus-free surface in 50, 50 and 60% of grafts with unrestricted blood flow (p < 0.05 when compared with the control group). Two grafts with restricted blood flow in the group treated with...
enoxaparin and dextran and one treated with enoxaparin had no visible mural thrombus at all.

The mean thrombus weights were significantly lower in the treatment groups with restricted blood flow than in the control group (Fig. 3); enoxaparin $p=0.045$, dextran $p=0.04$ and enoxaparin/dextran $p=0.002$. No similar reduction was seen in the treatment groups with unrestricted blood flow.

**Radionuclide activity**

In grafts with restricted blood flow, all three treatment groups were similar in fibrinogen and platelet uptake without significant difference between them (Figs 4 and 5). Although there was a numerically higher uptake in the control group this was only significant in the first 44 min for both fibrinogen and platelets except for fibrinogen in the proximal part of the grafts. The uptake has only been calculated in patent grafts. The control group therefore “faded out” as the grafts occluded during the observation period.

In grafts with unrestricted blood flow, the treatment regimens significantly reduced the fibrinogen uptake for the first 88 min in the proximal part of the grafts and in the entire distal part, except in some sporadic measurements, when compared with the control group (Figs 6 and 7). The platelet uptake was similarly reduced in the treatment groups for up to
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Fig. 6. Relative fibrinogen uptake in the proximal part of grafts with unrestricted blood flow based on baseline uptake. Each column represents average uptake during 22 minutes interval (mean ± S.E.M.). *p < 0.05 compared with controls.

132 min in the distal part of the grafts but not in the proximal part (Figs 8 and 9).

Discussion

Graft performance in clinical practice depends on several factors which can be classified according to Virchow's triad: the surface of the prosthesis; the blood flow; and the characteristics of the circulating blood. Low blood flow, secondary to poor in- or outflow, appears to be one of the most important factors associated with reduced graft patency. However, the flow situation is difficult to improve except when based on pure technical error or by increasing the graft flow by distal AV-fistula. Autogenous venous graft material is still superior to prosthetic material in long term patency. In cases where an autologous vein cannot be harvested, prosthetic material is the only material available for arterial reconstruction. Several experimental and clinical studies have suggested that ePTFE grafts are superior to Dacron in terms of patency and platelet adhesion. However, this appears to be true only in the immediate intra- and postoperative period as after 6 months there was...
no difference\(^4\) and with time, all grafts, including autologous veins, biological as well as prosthetic substitutes, develop thrombogenic surface alterations. Low flow, high intra- and postoperative thrombogenicity and possible thrombogenic blood factors make adjuvant pharmacological treatment to decrease the risk of graft thrombosis attractive.

In the present study we investigated the effect of dextran 70 and enoxaparin on ePTFE platelet adhesion, fibrinogen uptake and graft patency. The two substances have different mechanisms of action. The antithrombotic effect of enoxaparin is partially mediated through inhibition of factor Xa and to a lesser extent of factor IIa.\(^{43}\) It also affects platelets, endothelium and fibrinolysis.\(^{44}\) Dextran 70 is volume expanding, to an extent comparable to albumin and gelatin, and flow promoting.\(^{45,46}\) It coats the blood corpuscles and endothelium with a dextran film,\(^{47}\) changes the structure of fibrin\(^{48}\) and thrombi\(^{49}\) and enhances lysability of the thrombus.\(^{50}\) Furthermore, dextran decreases platelet adhesiveness to foreign surfaces with peak effect 2 to 6 h after infusion.\(^{51}\) Von Willebrand factor (factor VIII related antigen) is decreased in the presence of dextran but other coagulation factors only to an extent which can be explained by the haemodilution.\(^{50,52,53}\) Polygeline,
that has no thromboprophylactic properties and does not affect coagulation or haemostasis. The initial volume expansion effect is similar to that of dextran 70 but of shorter duration. However, 3 to 4 h after infusion similar volume expansion is achieved in animals. The purpose of using polye- line in the control group was to correct for the volume expansion effects of dextran 70. After 20, 90 and 180 min there was no significant difference in blood flow between the groups.

The experimental model used in the present study simulates situations with different expected patency rates: one highly thrombogenic low flow system and another less thrombogenic high flow system. The results verify the importance of adequate blood flow. The grafts with restricted blood flow in the control group were patent in 30% compared with 80% of grafts with unrestricted flow (p = 0.038). The tested substances did not influence the patency or thrombus weights when there was unrestricted blood flow (although numerical differences were observed). However, the grafts were significantly more often thrombus-free than in the control group. The substances appeared to affect mainly the distal part of the graft where the deposition of platelets and fibrinogen was highest.

In restricted blood flow, the treatments influenced thrombus weight as much as patency but not thrombus-free surface. The graft loss due to early occlusion makes statistical analysis with a limited number of patent grafts in the control group less valid after 88 min regarding platelet- and fibrinogen uptake but the values were numerically higher when compared with the control groups. In conclusion in the present study dextran 70 and enoxaparin appear equally effective in decreasing early thrombogenicity. Their combination is not significantly more effective although there was a favourable trend.

Acknowledgements

This study was supported by grants from Stig and Ragna Gorhons Foundation, Sweden and the Swedish Medical Research Council (00759). Thanks to Rhône-Poulenc Rorer, Sweden for kindly providing the ePTFE test substance (Klexane®) and Gore Inc., Sweden for providing ePTFE grafts and suture material (Gore Tex®). Thanks to Mrs. Gertie Jönsson for her skilful technical assistance in performing this investigation.

References


Accepted 18 August 1994