Short Communication

Coating with paclitaxel improves graft survival in a porcine model of haemodialysis graft stenosis

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Abstract

Background. Most commonly resulting from intimal hyperplasia at the venous anastomosis, stenosis leading to thrombosis is a major cause of failure of polytetrafluoroethylene (PTFE) dialysis grafts. We recently reported that coating haemodialysis grafts with paclitaxel could reduce neointimal hyperplasia. This study tested whether paclitaxel-coating could prolong graft survival in a porcine model.

Methods. PTFE grafts were double-coated with paclitaxel. Bilateral grafts were created between the carotid arteries and the external jugular veins, and we evaluated graft survival by weekly measurements of blood flow for 12 weeks.

Results. We successfully implanted four pairs of paclitaxel-coated grafts and four pairs of control grafts in eight Landrace pigs. One control pig had to be euthanized at 4 weeks after graft placement. The grafts in the other three controls and four paclitaxel pigs survived until harvesting of the grafts. All paclitaxel-coated grafts remained patent for 12 weeks without decrease of blood flow. Median blood flow was 702 ml/min at three weeks and 818 ml/min at 12 weeks after placement. In contrast, the four control grafts lost luminal patency at 5, 6, 6 and 8 weeks, respectively. In Kaplan–Meier analysis, paclitaxel-coated grafts showed better survival than uncoated grafts ($P=0.011$).

Conclusions. Double-coating with paclitaxel improved graft survival. Coated PTFE grafts may be effective for the prevention of graft failure in patients on haemodialysis.

Keywords: graft; haemodialysis; paclitaxel; polytetrafluoroethylene; vascular access

Introduction

The major complication associated with haemodialysis grafts is stenosis at the site of anastomosis and subsequent thrombosis. Several pharmacologic agents, such as warfarin, aspirin and clopidogrel, have been used to prevent graft thrombosis in haemodialysis patients [1,2]; however, these agents have been associated with an increased risk of bleeding, and thus their routine use for the prevention of graft thrombosis has not been recommended [1,2]. Another recent approach to improve long-term graft patency has focused on the early detection and correction of stenosis. Several studies have indicated that the frequency of graft thrombosis can be reduced with close monitoring of graft blood flow [3–5], but stenosis is still likely to recur. The annual incidence of graft thrombosis has been reported to be 30–65%, with a mean of 45% [6,7]. Therefore, novel strategies to prevent graft stenosis are needed to reduce the cost and morbidity associated with maintenance haemodialysis.

Paclitaxel is a potent anti-proliferative agent that inhibits the disassembly of cell microtubules [8]. Over the past several years, extensive studies have been done of the local delivery of paclitaxel to the vessel wall to prevent neointimal hyperplasia in intracoronary stents [9–11]. Consequently, the use of paclitaxel-eluting coronary stents has been accepted as a standard practice in the field of interventional cardiology to reduce in-stent restenosis and improve luminal patency [12].

Drug-eluting systems can be divided into three components: a platform, a carrier and the active drug.
Paclitaxel coating prolongs HD graft survival

In the case of drug-eluting coronary stents, bare metal plays the role of a platform and polymers are most commonly used as carriers [12]. The polymers allow sustained and predictable drug release, but carry the potential risk of inflammation and thrombosis [13]. The PTFE haemodialysis graft itself has a rough and diffusely pitted surface, which can hold the drug to be released for a while—and therefore does not require an additional carrier, unlike the bare metal coronary stents.

Our research, along with those of others, in animal models has shown that the sustained local delivery of anti-proliferative agents can inhibit neointimal hyperplasia at the anastomoses of haemodialysis grafts. However, the proven beneficial effects of this approach have been limited to the reduction of histological or angiographic luminal stenosis for short durations [14–17]. Therefore, we evaluated whether coating grafts with paclitaxel could prolong graft survival in a porcine model.

Materials and methods

Graft preparation

We used commercially available, reinforced, thin-walled, ringed polytetrafluoroethylene (PTFE) vascular grafts (IMPRA, BARD Inc.) 6 mm in diameter and 15 cm long. We tried two different techniques for coating grafts with paclitaxel (Genexol®, Samyang Genex Inc., Korea), a single-coating technique and a double-coating technique. Briefly, dry paclitaxel was dissolved in acetone (Fisher Scientific) to a concentration of 0.5 mg/ml for the single-coating technique and 0.3 mg/ml for the double-coating technique. The PTFE haemodialysis grafts were immersed vertically into the solution and incubated for 30 min at 37°C. All paclitaxel-coated grafts were kept under vacuum overnight to remove the solvent completely.

The amount of the paclitaxel loaded was 0.388 μg/mm² in the single-coating technique and 0.380 μg/mm² in the double-coating technique. The in vitro drug release profiles of the two types of paclitaxel-coated grafts, were obtained as reported in a previous study [15]. On the basis of the results of that in vitro release test, we selected the double-coated grafts for further animal study.

Animals and operation

In all, eight male Landrace pigs, weighing 50 ± 3 kg, were used in this study. They were maintained in standard animal care facilities at Samsung Biomedical Research Institute. All procedures and care were in conformity with the Guidelines for the Care and Use of Laboratory Animals, published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996).

All grafts were positioned between the common carotid artery and the external jugular vein, as described previously [15,18]. Briefly, intravenous heparin 5000 IU was administered before vessel manipulation. Next, the carotid artery was clamped, and an 8 mm arteriotomy was performed. An end-to-side anastomosis was created at a 45° angle. Venous anastomoses were created in a similar manner. Two or three weeks later, contralateral arteriovenous grafts were implanted in the same manner. When the pigs were eventually sacrificed at the end of the study, tissue was excised in the same manner as described in our previous study.

Study design

A total of 16 arteriovenous grafts were created bilaterally in the eight pigs. Each pig received either two double-coated paclitaxel grafts or two control (uncoated) ePTFE grafts. Graft patency was monitored weekly by palpation and Doppler sonography. Pigs were anesthetized each week; we then located the grafts by palpation and measured the blood flow through the graft, using a Doppler sonograph [SONOACE PICO, HL5-9ED SONOACE PICO PROBE (flat type, 40 mm field of view and 5–9 MHz frequency rate), Medison, Korea]. Volume flow was calculated automatically by the system using the following formula: volume flow (ml/min)=π × diameter (cm²)/4 × mean blood velocity (cm/s) × 60 (s/min). The average of three consecutive measurements was recorded in units of millilitres per minute. Graft failure was defined as an impalpable thrill and undetectable blood flow. At 12 weeks after the second graft placement, or when both grafts had failed, the pigs were euthanized, and both PTFE grafts were excised along with adjacent vessels.

Starting 7 days pre-operatively, all pigs received acetylsalicylic acid, 100 mg/day, until sacrifice. Clopidogrel, 75 mg, was added 1 day before surgery and was continued till sacrifice.

Statistical analyses

We used repeated-measures analysis of variance (ANOVA) to compare the in vitro drug release profiles of the two kinds of paclitaxel-coated grafts and the Mann–Whitney test to compare blood flow values between double-coated grafts and control grafts. The Kaplan–Meier method along with the log-rank test was used to estimate graft survival. A P-value of less than 0.05 was considered statistically significant. We used SPSS 11.0 for Windows for all statistical analyses.

Results

In vitro release profiles

We first compared the in vitro paclitaxel release profiles of the grafts coated by one of the two techniques (Figure 1). In both, drug release went through an initial burst phase within the first 48 h followed by a longer lasting and slower sustained release. Until the ninth week, the drug release rate of double-coated grafts was slower than for the single-coated grafts (P < 0.001) (Figure 1A). For the single-coated grafts, 53.6% of the total load of paclitaxel was released within the first 5 weeks, and then only a very small quantity of
paclitaxel could be detected between the 5th and 12th weeks by high performance liquid chromatography. In vitro, the double-coated grafts released 30% of their paclitaxel load within the first 5 weeks, and enough paclitaxel remained in the grafts to be released over the next 7 weeks (Figure 1B). On the basis of the results of that in vitro release test, we used the double-coated grafts for further animal study.

Blood flow measurement and graft survival

We successfully implanted eight paclitaxel-coated grafts and eight control grafts in eight Landrace pigs. One of the four control pigs had progressive neck swelling after the placement of the second graft, so it was euthanized 4 weeks later. Since the patency of both grafts in this pig was confirmed in the excised tissue, in survival analysis we considered each of the two grafts as cases censored without failure at 4 and 7 weeks after placement. In addition, and for the same reason, blood flow was not measured for these two control grafts. The other three control pigs and the four paclitaxel pigs survived until sacrifice and harvesting of the grafts.

Figure 2 shows the weekly measurements of blood flow in all grafts. In the majority of the subjects, neck swelling and erythema resolved by the second week; and thus, reliable measurements of blood flow were obtained from the third week onwards after placement. Graft flow remained unaffected until 12 weeks after placement in the eight paclitaxel-coated grafts (Figure 2). The median blood flow in them was 702 ml/min at 3 weeks after placement and 818 ml/min at 12 weeks after placement. In contrast, four out of six control grafts had reduction in blood flow, and they finally lost luminal patency at 5, 6, 6 and 8 weeks. Only two control grafts remained patent for 12 weeks. However, the blood flow of those two control grafts did show a decreasing trend over the study period (from 831 to 594 ml/min and from 548 to 493 ml/min), but this reduction was not statistically significant. Figure 3 shows a comparison of graft survival between the two groups. Paclitaxel-coated grafts had a better graft survival than the control grafts ($P = 0.011$).

Discussion

This study demonstrated that grafts double-coated with paclitaxel had prolonged graft survival in a porcine model of haemodialysis grafts stenosis when compared with uncoated grafts. Therefore, this approach may be effective for reducing the complications of haemodialysis vascular access in the clinical setting.

There have been several attempts to reduce the neointimal hyperplasia of haemodialysis grafts by delivering anti-proliferative agents locally. However, favourable results to date have been limited to the reduction of histologically or angiographically measured luminal stenosis [14–17]. In this study, we used, for the first time, a porcine model of haemodialysis graft to determine the effect of the local delivery of an anti-proliferative agent on graft survival—although
we did not investigate all the factors responsible for this neointimal hyperplasia, such as the angle between the graft and the vessels it joins or possible differences between the diameters of the graft and the vessels. We obtained favourable results, because of the remarkable efficacy of the paclitaxel-coated grafts and the longer duration of study than in previous trials. Our results provide a rationale for future clinical trials to confirm whether paclitaxel-coated grafts can prolong haemodialysis graft survival in humans.

The following two techniques for local drug delivery have been attempted in animal models of haemodialysis graft stenosis: including the percutaneous injection of polymer mixed with drugs and an intraluminal stent bearing anti-proliferative agents [16,17]. In the former of these approaches, an injection applies the anti-proliferative agent only to the exterior of the vessels—whereas stenosis originates in the intima of vessels. The latter technique might have the risk of further endothelial injury. Furthermore, both techniques entail an additional invasive procedure. In contrast, paclitaxel-coated grafts could be implanted in a single operation, which therefore would be expected to obviate additional damage to the endothelium. The paclitaxel-coated grafts could be obtained by simple dipping techniques, and then the arteriovenous grafts constructed as would be conventional grafts. Thus, using paclitaxel-coated grafts, as we did in this study, would have both economic and practical advantages over other techniques.

We compared the in vitro release profiles of single-coated and double-coated grafts in the present study, and double-coating resulted in longer and slower drug release than single-coating. Although single-coated grafts could also inhibit neointimal hyperplasia at the venous anastomosis site of grafts, as reported in our previous study, we used the double-coated grafts for comparison of graft survival in our present study, because we postulated that sustained retention of drug on grafts might be safe and efficient. As a result, double-coating with paclitaxel prolonged graft survival in our porcine model of haemodialysis graft stenosis when compared with uncoated grafts.

Several concerns remain. First, the lowest effective dose of paclitaxel has not yet been determined. The dosage employed in this study was approximately 1000-fold lower than the dose that has been used clinically [19]. Accordingly, the systemic plasma levels of paclitaxel related to an implanted arteriovenous graft are expected to be very low, and unlikely to cause adverse systemic effects. Second, it has not been evaluated whether the paclitaxel adherent to the external surface of a graft is safe for the overlying skin, especially when the graft is implanted in the upper extremity. Locally concentrated paclitaxel may cause inflammation in adjacent tissues, or it may prevent an inflammatory reaction to the foreign PTFE grafts. The third question is whether paclitaxel-coated grafts are able to tolerate repeated needle punctures over time. If paclitaxel coating could inhibit haemos-tasis after a needle puncture, we could only use paclitaxel coating at the ends of the graft. Fourth, 12 weeks of follow-up is rather short for forming an opinion about long-term clinical success; therefore in this respect, our results cannot but be considered preliminary. Nevertheless, median survival in a porcine model of graft stenosis is >8 weeks. Thus, we presumed 12 weeks to be sufficient for evaluating graft survival in a porcine model (and the cost of maintaining experimental pigs for more than 12 weeks would be much higher).

The present study demonstrated that coating grafts with paclitaxel prolongs graft survival. Therefore, the best strategy for preventing neointimal hyperplasia may be sustained local delivery of anti-proliferative agents, paclitaxel-coated grafts may be the instrument for achieving this.

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Conflict of interest statement. None declared.

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