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A perivascular system releasing sirolimus prevented intimal hyperplasia in a rabbit model in a medium-term study

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A B S T R A C T

The main complication of aortocoronary reconstruction with vein grafts is restenosis in the course of time. The aim was to assess the effect of a periadventitial polyester mesh releasing sirolimus on intimal hyperplasia of autologous grafts. We implanted *v. jugularis ext.* into *a. carotis communis* in rabbits. The vein graft was either intact, or was wrapped with a pure polyester mesh, or with a sirolimus-releasing mesh. Three and six weeks after surgery, the veins were subjected to standard histological staining and the thicknesses of the *tunica intima*, the media and the intima–media complex were measured. Wrapping the vein with a mesh releasing sirolimus or with a pure mesh decreased the thickness of the intima in comparison with a vein graft by 73 ± 11% or 73 ± 8% after 3 weeks, and by 73 ± 9% or 59 ± 12% after 6 weeks, respectively. Sirolimus-releasing meshes reduced the thickness of the media by 65 ± 9% and 20 ± 12% after 3 and 6 weeks. The thickness of the intima–media complex in grafts with sirolimus-releasing meshes decreased by 60 ± 6% and 30 ± 13% in comparison with pure PES meshes, after 3 and 6 weeks, respectively. A periadventitial polyester mesh releasing sirolimus has the potential to become an effective device in preventing vein graft restenosis.

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1. Introduction

Occlusion of the graft lumen is considered as the main complication in the follow-up of patients after aortocoronary vein bypass grafting. This occurs almost in 15% within the first year, and increases up to 50% of occluded veins after 10 years (Motwani and Topol, 1998). In patent vein grafts, 7% show signs of degeneration after 1 year and 77% after 10 years (Fitzgibbon et al., 1996).

Due to a mechanical mismatch after implantation, an autologous vein graft in arterial circulation is prone to remodelling. This is accompanied by excessive proliferation of vascular smooth muscle cells (VSMC), which can migrate into the intima and produce extracellular matrix. Graft remodelling thus results in intimal hyperplasia and graft stenosis. In clinical practice, autologous graft stenosis is usually dealt with (after percutaneous transluminal angioplasty) by inserting stents, often loaded with antiproliferative drugs, e.g. sirolimus-eluting stents (Cypher™, Cordis J&J, NJ), or paclitaxel-eluting stents, into the graft lumen (Colombo and Iakovou, 2004). However, the use of stents is limited by the relatively complicated process for inserting them, by increased mechanical strain on the vessel wall, and by local damage to the endothelium and VSMC leading to reactivation of VSMC growth and restenosis of the vessel. In addition, the stents can be released and can move inside the vessel (Jeremy et al., 2004). Similar problems are associated with drug-releasing polymeric films covering the luminal surface of a vessel, e.g. hydrogel films loaded with paclitaxel (Livnat et al., 2005).

From this point of view, an external drug delivering system, i.e. placed on the adventitial surface of the vascular graft, seems to be more advantageous. Periadventitial delivery of heparin from matrices placed adjacent to rat carotid arteries was successfully

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used in the case of heparin, which is well known to attenuate proliferation of VSMC (Edelman et al., 1990). Other antiproliferative drugs have also been found to inhibit neointimal hyperplasia of vein grafts in animal experimental models after local extraluminal application, for example suramin, C-type natriuretic peptide, cilostazol, and sirolimus (Schachner et al., 2004a,b; Hu et al., 1999; Fujinaga et al., 2004). Especially sirolimus (Rapamycin) is a drug well known for its potent antiproliferative action. In addition to its application in drug-eluting stents (Colombo and Iakovou, 2004), this macrocyclic lactone has also been used clinically for immunosuppressive therapy after organ transplantation (Roque et al., 2001). The mechanism of its antiproliferative effect is very complex (Yakupoglu and Kahan, 2003; Regar et al., 2001), involving mainly blocking the transition from the G1 phase to the S phase of the cell cycle by interacting with a specific target protein (mTOR, mammalian target of sirolimus) and inhibiting its activation. Our earlier study focused on the kinetics of the release of sirolimus from polyester meshes *in vitro* (Filova et al., 2011). In these experiments, the release of sirolimus from polyester meshes coated with a degradable copolymer loaded with sirolimus was detected for several weeks. The proliferation of VSMC in culture plates was inhibited if sirolimus-releasing meshes were used. For the present study, we assumed that the graft wall of the vessel would be thin enough to enable diffusion of sirolimus released from a periadventitially placed mesh into the tunica media, so that VSMC proliferation could be inhibited.

It has been suggested that thickening of the intima of the vein grafts increased in the distended regions, where the grafts were subjected to low flow velocity (Dobrin, 1995). Thus, we expected that in addition to the antiproliferative effects of sirolimus, our meshes would also reduce the risk of graft restenosis by minimizing distension of the graft. From this point of view, encouraging results were obtained in an *in vivo* study performed on sheep. Sheathing the implanted vein grafts with a pressure-resistant polyester (torlen/dacron) mesh significantly reduced intima thickening in these grafts compared to control untreated vein grafts within 12 weeks after implantation (Krejca et al., 2002). Taken together, the mesh placed around the graft should serve as a mechanical support for the graft wall, which increases the resistance of the graft against high pressure, decreases tangential stress and retards graft degeneration (Jeremy et al., 2004; Krejca et al., 2002).

We therefore developed a novel combined device composed of two synergistically effective components: a mechanically supportive polyester mesh, and an antiproliferative drug (sirolimus). Our work extended previous preliminary study about early intimal changes in the autologous vein grafts (Skalsky et al., 2011). We evaluated the dynamics of the vascular wall changes during a medium-term examination in vein grafts in rabbits, wrapped with a sirolimus-releasing polyester mesh. Sustained release of sirolimus within a period of several weeks suppressed VSMC proliferation for the time necessary for re-endothelialization of the graft. These effects reduced autologous graft remodelling and the need for subsequent treatment.

2. Materials and methods

2.1. Materials

Polyester mesh (CHS 50, PES mesh) was obtained from VUP Joint-Stock Co., Brno, CR. Purasorb PLC 7015, and a grade copolymer of L-lactide and ε-caprolactone (70/30 molar ratio, inherent viscosity midpoint of 1.5 dl/g) was purchased from PURAC biomaterials. Sirolimus (Rapamycin from Streptomyces hygroscopicus, Cat. No. R0395) was obtained from Sigma–Aldrich (Germany).

2.2. Mesh impregnation

Polyester mesh impregnation was described in a previous paper (Filova et al., 2011). Briefly, the mesh was coated with a solution containing 5.2 mg of sirolimus and 36.4 mg of purasorb in 1 ml of chlorobenzene–ethanol (1.75:1, v/v). It was dried, and then coated for a second time with the same solution and dried again. Finally, the impregnated mesh contained 0.14 mg of sirolimus per cm^2. The mesh was dried out in a vacuum oven for 3 weeks, and was then sterilized with ethylene oxide (sirolimus-releasing PES mesh).

2.3. Implantation procedures

The experiments on laboratory animals were approved by the Authorization No. 48/2009 issued by the Chief Hygienist of the Czech Republic, the Ministry of Health of the Czech Republic according to the law No. 246/1992 of the Collection and in compliance with further regulations, for the protection of animals against suffering, and in accordance with the Project of Experiments and the statement of the Ethical Committee. Male Giant Chinchilla rabbits (3.0–3.5 kg; $n = 65$; Table 1) were anaesthetized using an intramuscular injection of ketamine hydrochloride (30.0 mg/kg). Anaesthesia was maintained with isoflurane (2.5–3.0%), inhaled through a mask. Heparin (300 IU/kg) was given intravenously to the animals. The operative procedure was performed with an aseptic technique with operating glasses (magnification 2.5×). The right external jugular vein and the right common carotid artery were exposed through a vertical midline cervical incision. The vein bypass grafts were constructed using an anastomotic cuff technique (Jiang et al., 2004) (Fig. 1).

A segment of the external jugular vein approximately 2 cm in length was harvested for an autologous reversed-vein graft; the segments were also used as the graft_0 control group. The polymer cuffs were prepared from a 4F endovascular catheter (Terumo Medical Corp, Elkton, MD). The jugular vein ends were passed through a cuff, everted, and fixed using 8-0 monofilament polypropylene silk. The common carotid artery was clamped distally and proximally, and the lumen was then exposed using a small arteriotomy, and the cuffed, reversed vein ends were inserted. A second 8-0 polypropylene silk was used to ligature (i.e. secure) the artery around the cuff on both sides. The back wall of the carotid artery between the cuffs was excised to allow a vein graft extension (an untreated autologous graft). Finally, a pure PES mesh or a sirolimus-releasing PES mesh was put on around the vein graft and fixed by polypropylene 8/0 on the side (autologous graft wrapped with a PES mesh, an autologous graft wrapped with sirolimus-releasing PES mesh) (Jiang et al., 2004).

Groups of animals were euthanized 3 and 6 weeks after implantation. The specimens were equally divided in the middle of the vein graft (without the cuff segment) to obtain equal parts for histology and for immunohistochemistry.

2.4. Histology and immunohistochemistry

The venous grafts were divided into two parts. One part was fixed in 10% formalin and then it was embedded in paraffin. The second part was embedded in Sakura Finetek Tissue Tek© Cryomold holders and Sakura Finetek Tissue Tek© OCT Compound (both from Sakura Finetek, Tokyo, Japan). The samples were subsequently frozen in 2-methylbutane (Fluka Chemika, Buchs, Switzerland) cooled by liquid nitrogen, and then stored at −80 °C.

2.5. Histological analysis

The samples embedded in paraffin were cut into 3–4 μm sections and stained with haematoxylin-eosin, Van Gieson with

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