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Local perivascular delivery of anti-restenotic agents from a drug-eluting poly(ε-caprolactone) stent cuff

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Abstract

The introduction of drug-eluting stents (DES) to prevent in-stent restenosis is one of the major advances in interventional cardiology. Currently many types of DES are under evaluation for effectiveness and safety, a time-consuming and difficult procedure in humans. An animal model that allows rapid evaluation of the present and upcoming therapeutic approaches to prevent in-stent restenosis is most valuable and still lacking.

Here, a perivascular cuff to induce restenosis was constructed of a poly(ε-caprolactone) (PCL) formulation suitable for the controlled delivery of drugs. Placing the PCL cuff around the femoral artery, in vivo, resulted in reproducible restenosis-like lesions containing predominantly smooth muscle-actin positive cells. Loading the cuff with the anti-restenotic compounds paclitaxel and rapamycin resulted, in vitro, in a sustained and dose-dependent release for at least 3 weeks. Paclitaxel- and rapamycin-eluting PCL cuffs placed around the femoral artery of mice in vivo significantly reduced intimal thickening by $76 \pm 2\%$ and $75 \pm 6\%$, respectively, at 21 days. Perivascular sustained release of both anti-restenotic agents is restricted to the cuffed vessel segment with no systemic adverse effects or effect on cuffed contralateral femoral arteries.

Drug-eluting PCL cuffs provide an easy and rapid tool to evaluate anti-restenotic agents to be used in combination with the DES strategies.

Keywords: Controlled drug release; Local delivery; Drug-eluting stents; Poly(ε-caprolactone); Restenosis; Animal model

1. Introduction

Percutaneous transluminal coronary angioplasty (PTCA) was introduced in the late 1970s as a method to restore coronary blood flow in atherosclerotic coronary arteries in patients with (symptomatic) stenoses. Since then it has become widely accepted as an effective and safe treatment modality for single and multivessel coronary atherosclerosis disease. However, a major drawback to PTCA has been the occurrence of restenosis of the treated vessels, resulting in renewed symptoms and the need for repeated intervention in up to 50% of patients [1]. The introduction of intracoronary bare metal stents reduced the restenosis rate within 6 months, however a smaller portion of the patients (20–30%) still suffered of so called in-stent restenosis [2,3]. Recently, drug-eluting stents (DES)

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loaded with the anti-proliferative compounds paclitaxel and rapamycin were introduced very successfully in interventional cardiology. The restenosis rate dropped from 20–30% to 1–3% at 1 year [4,5]. Many new anti-proliferative, anti-inflammatory, anti-migratory or pro-healing compounds to be loaded onto stents are currently under evaluation. These DES are supposed to inhibit inflammation and neointimal growth and subsequently in-stent restenosis. However, little is known concerning the potential adverse effects of these anti-restenotic agents on vessel wall integrity and (re)healing, atherosclerotic lesions formation, progression, and plaque stability [see Ref. [6] for detailed review]. An animal model that allows rapid evaluation of the present and upcoming therapeutic approaches to prevent in-stent restenosis is most valuable and still lacking.

One well-defined mouse model of restenosis consists on the placement of a non-constrictive perivascular polyethylene cuff around the mouse femoral artery, which results in a reproducible and concentric intimal thickening within 2–3 weeks, mainly consisting of rapid induction of smooth muscle cells proliferation [7–9].

Drug-loaded polymer formulations, as the ones present in the majority of the DES coatings, are a rational technique to deliver compounds locally for a prolonged period of time to the vessel wall to inhibit intimal hyperplasia. Local application of drugs for anti-restenotic compounds evaluation is also possible using gelatin or pluronic (F-127) gels. A substantial disadvantage of these methods is that they are water-based, which restricts the half-life of the delivery system. Using a cuff made of a polymer suitable for eluting anti-restenotic compounds instead of the polyethylene cuff would be an important step towards an useful animal model for preclinical evaluation of new DES strategies in mice. Poly(ε-caprolactone) (PCL) is a biocompatible and biodegradable polymer belonging to the aliphatic polyester family [10,11]. Extensive in vitro and in vivo biocompatibility and efficacy studies have been performed, resulting in U.S. Food and Drug Administration (FDA) approval of a number of medical and drug delivery devices composed of PCL [12–15]. PCL has a relatively long biodegradation time and is therefore suitable for drug-eluting purposes [16,17]. In addition, PCL formulations have also been investigated as a stent eluting coating for paclitaxel in a rabbit model of restenosis [18] and in the Boston Scientific DES program (TAXUS^{TM}) [19]. Polymeric formulations consisting of PCL blended with poly(ethylene glycol) (PEG) have been developed in the past for local delivery of anti-oncogenic drugs [20–22]. The relatively hydrophilic PEG dissolves into the aqueous medium and open channels within the PCL matrix through which water can penetrate and drugs can be sustainly diffused out.

In the present study the non-constrictive perivascular cuff to induce restenosis was constructed of a blended polymeric formulation of PCL and PEG suitable for controlled drug delivery. The novel drug-eluting PCL cuff described here simultaneously induces reproducible intimal hyperplasia and allows local delivery of anti-proliferative compounds to the vessel wall. This new approach gives the possibility to evaluate the effects of the tested compounds on neointima formation, vessel wall integrity, and potential side effects. We show that, in vitro, paclitaxel and rapamycin-eluting PCL cuffs give a sustained release of the drug for at least 3 weeks. Consequently, this sustained release resulted in a substantially reduced neointima formation for both anti-restenotic agents tested, in vivo, with no systemic adverse effects or effect on cuffed contralateral femoral arteries.

2. Materials and methods

2.1. Materials

Poly(ethylene glycol) 300 (PEG; $H(OCH_2CH_2)_nOH$; MW 285–315) was obtained from J.T. Baker (Philipsburg, USA). Poly(ε-caprolactone) (PCL; $[-O(CH_2)_5CO-]_n$; MW 10,000–20,000) was purchased from Polysciences Inc. (Warrington, USA). Paclitaxel was kindly provided by Bristol-Myers Squibb Company (New Jersey, USA) and rapamycin was obtained from LC Laboratories (Woburn, USA). Phosphate-buffered saline (PBS) pH 7.4 was obtained from B. Braun (Melsungen, Germany) and *n*-octanol ($C_8H_{17}OH$; >99.0%) was supplied by Merck (Darmstadt, Germany).

2.2. Preparation of drug-eluting PCL cuffs

The PCL-based drug delivery cuffs were manufactured as previously described [20,23]. In brief, paclitaxel or rapamycin were first blended with PEG before this blend was mixed with molten PCL at 70 °C. The PCL:PEG ratio was 4:1 (w/w). Drug-loaded polymer cuffs were made from the different blended molten drug–polymer mixtures and designed to fit around the femoral artery of mice. Drug-eluting PCL cuffs had the shape of a longitudinal cut cylinder with an internal diameter of 0.5 mm, an external diameter of 1 mm, a length of 2 mm, and a weight of approximately 5 mg.

2.3. In vitro release profiles of paclitaxel and rapamycin

PCL cuffs were loaded with 0.5%, 1%, 2.5%, and 5% (w/w) paclitaxel ($n = 5$) or rapamycin ($n = 5$) and in vitro release profiles for both drugs were performed as previously described [22]. Cuffs of each composition were placed in 20 ml glass scintillation vials and cooled to 4 °C. Five milliliters of iced-cold PBS pH 7.4