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Perivascular sustained release of atorvastatin from a hydrogel-microparticle delivery system decreases intimal hyperplasia

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ABSTRACT

Intimal hyperplasia (IH) is the major cause of grafted vessel occlusion and occurs frequently after bypass intervention. No pharmaceutical formulation is currently available to prevent this pathology. Local perivascular delivery of an appropriate active compound released in a time-dependent manner (from day one up to 4 weeks) is necessary for an efficient single-administration preventive therapy. To this aim, we propose the combination of gel and microparticles delivery system containing atorvastatin (ATV). The incorporation of ATV in a cross-linked hyaluronic acid gel, provided *in vitro* a fast release over 3 days, while ATV-loaded poly-lactic-*co*-glycolic acid (PLGA) microparticles dispersed in the gel gave a sustained release over 4 weeks. *In vivo*, ATV formulations were applied perivascularly in mice undergoing carotid artery ligation. IH was significantly reduced (-68%) in presence of ATV incorporated in hyaluronic acid gel and encapsulated in microparticles compared to control. No significant IH alteration in IH was observed when ATV was incorporated only in the gel (fast release) or only in the microparticles (slow release) demonstrating that a biphasic release of ATV is essential to interfere with the development of IH.

ATV was detected in adjacent tissues 28 days after the intervention, showing the sustained presence of the drug *in vivo*. After four weeks ATV was not detected in remote tissues, except at a very low concentration (0.044 ng/mg) in the liver, suggesting a very low risk of systemic toxicity of locally delivered ATV. Additionally, the *ex vivo* data showed that ATV in solution permeates through isolated human saphenous veins and thus is a good candidate for perivascular delivery.

Our data demonstrate that a local biphasic ATV release on the mice ligated carotid efficiently prevents the development of IH without apparent toxicity.

1. Introduction

Despite the improved results of endovascular treatment (balloons, stents), open surgical revascularization remains the gold standard for the treatment of vascular occlusive diseases. It is performed in over one million patients per year worldwide [1]. Autologous saphenous vein graft is the conduit of choice for peripheral bypasses. However, graft failure may occur within up to five years following the intervention, leading to a redo surgery, amputation and increased mortality rate [2]. Indeed, the combination of hemodynamic factors, such as turbulent blood flow due to a mismatch between graft and artery mechanical properties as well as surgical manipulation, result in a progressive lumen occlusion due to cell proliferation (restenosis) [3]. This clinical occurrence is mainly driven by intimal hyperplasia (IH), which is the thickening of the inner layer of the vessel due to vascular smooth muscle cell (VSMCs) migration and proliferation as well as high extracellular matrix secretion (Fig. 1 upper panel) [4–6]. Even if aspirin and statins are prescribed, in the vast majority of patients, for secondary prevention of IH, the restenosis rate after open surgical bypass revascularization remains high [1,7–9].

A preventive therapy applied locally during the intervention, as a single administration, ensuring adequate dose of drug delivered on site can be a promising approach [10,11]. The application of the drug

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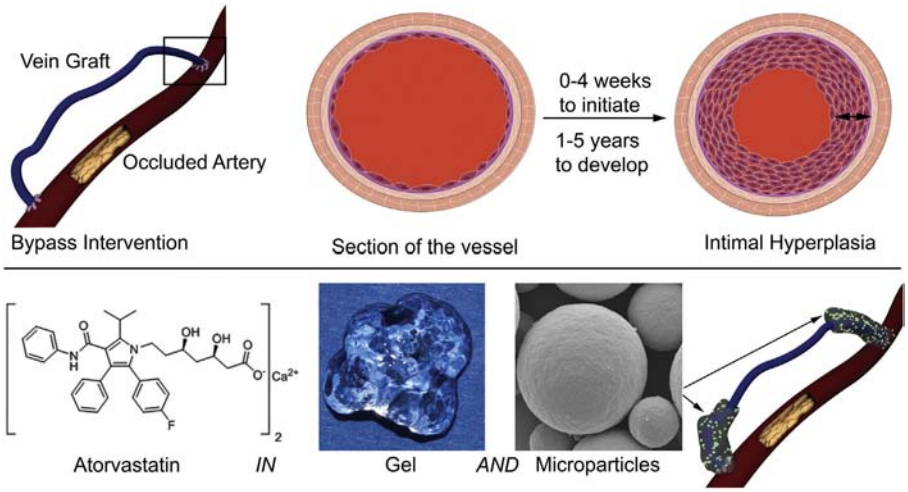

Fig. 1. Representation of the vein graft bypass surgery with the development of the intimal hyperplasia responsible for graft failure (upper panel) and the atorvastatin (ATV) delivery system proposed as a preventive treatment to preclude intimal hyperplasia development (lower panel).

around the vessel (perivascular) is easily achieved during surgery as the vessel is exposed and accessible. Several strategies have been developed for the perivascular administration of drugs aiming at preventing graft failure. For instance, sheaths [12], wraps [13], meshes [14], membranes [15], cuffs [16], particles [17,18] and gels [19–24] have been explored. To our knowledge, none of these technologies are used in clinical practice due to a lack of efficacy [25]. The limitations of these approaches include difficulty in handling and applying the formulations, as well as the need for repeated administrations due to a non-optimal drug release profile and the fast degradation of the polymers [21,26].

A cross-linked hyaluronic acid (clHA) hydrogel was selected as the vehicle for the local delivery. The selection of clHA was initially motivated by essential mechanical properties that would ensure ease of handling and conformable covering of the graft. Additionally, clHA is enzymatically degraded by hyaluronidases, avoiding the need for a second surgery to remove the implant [27]. Nevertheless, its cross-linked nature slows the degradation. When a drug is loaded in a gel, a fast diffusion-driven release in <3 days is observed [25]. However, ideally, a sustained release for at least 4 weeks is required to control IH progression [1]. Therefore a biphasic system is proposed, consisting of a clHA gel phase to provide a fast release of the compound and ensure the position of the formulation around the vessel and drug loaded-polylactic-*co*-glycolic acid (PLGA) microparticles to ensure the slow release of the drug (Fig. 1 lower panel).

Appropriate selection of the drug is essential to limit the inhibition of the vessel's re-endothelialization, which could lead to thrombus formation [25]. Inspired by the success of endovascular drug eluting stents, the recent literature on perivascular administration focuses on the delivery of antiproliferative compounds (paclitaxel, sunitinib, sirolimus) [12,13,17,18,23,28,29]. However, these compounds tend to inhibit the re-endothelialization of the vessel [18,30]. For this reason, we selected a non-antiproliferative pharmacological approach, atorvastatin (ATV). Although statins are traditionally thought to exert their beneficial effects on saphenous vein graft patency by reducing the blood's lipid content, it was also demonstrated that they inhibit IH in arterialized vein grafts by limiting macrophage infiltration and VSMC migration and proliferation [24,31–34].

We previously showed that when ATV is released from the clHA gel, it maintains its activity and inhibits cell proliferation and migration of hVSMC *in vitro* [35]. The objectives of the present study are: a) to prepare formulations with various *in vitro* release profiles; b) to demonstrate *in vivo* the efficacy of the formulations; and c) to explore whether ATV is able to permeate human vein grafts *ex vivo* from the perivascular side to the lumen.

2. Materials and methods

2.1. Materials

Poly(D,L-lactic-*co*-glycolic acid) (PLGA; Resomer® RG503 molecular weight 42.000 Da Boehringer Ingelheim; Ingelheim; Germany), atorvastatin calcium (Chemos GmbH; Regenstauf; Germany), atorvastastin-d5 sodium salt, (TRC, Canada), PVA or polyvinyl alcohol (Mowiol® 4-88; Kuraray Europe; Hattersheim am Main; Germany), chloroform (Chromasolv® plus for HPLC, ≥99.9%, 0.5–1.0% ethanol as stabilizer, Sigma-Aldrich Chemie GmbH, Steinheim, Germany), Toluidine Blue O (Merck, Germany) and Poloxamer 407 (Pluronic® F127, BASF, Germany) were used as received. Cross linked hyaluronic acid hydrogel (Fortelis extra®) was a generous gift from Anteis, Meyrin, Switzerland. All other chemicals were of analytical grade. Nitex® 03 with a 100-μm mesh opening was purchased from Sefar, Heiden, Switzerland. MF-Millipore GSWP02500 filters (0.22 μm) were purchased from Millipore Merck, Darmstadt, Germany. Vertical Franz's diffusion cells with an internal diameter of 20 mm were manufactured on demand by Glass Technology, Meyrin, Switzerland.

2.2. Microparticle preparation

Microparticles were prepared by an oil-in-water (o/w) solvent emulsion-evaporation process. We dissolved 375 mg of PLGA and 37.5 mg of ATV in 7 g of chloroform before emulsification in a PVA 2% aqueous solution at 1500 rpm for 20 min using a paddle stirrer (Eurostar digital, IKA-Werke, Staufen, Germany). The emulsion was added to 50 mL of water, and chloroform was evaporated at room temperature overnight at a stirring rate of 500 rpm. Unloaded microparticles were prepared similarly without ATV. The particles were washed and concentrated by successive steps of centrifugation/re-suspension before freeze-drying (Alpha 2-4 LSC, Christ, Kuhner, Switzerland). ATV-loaded microparticles are referred to as Matv and unloaded microparticles as M.

2.3. Microparticle size analysis

Freeze dried particles were suspended in water, and their size distribution was measured by laser diffraction on a Mastersizer S Long Bench equipped with a small volume sample dispersion unit and a dispersion unit controller set at 1100 rpm (Malvern Instruments Ltd, Worcestershire, UK). The analysis was performed using the Fraunhofer