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Calcified plaque modification alters local drug delivery in the treatment of peripheral atherosclerosis

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ABSTRACT

Background: Calcific atherosclerosis is a major challenge to intraluminal drug delivery in peripheral artery disease (PAD).
Objectives: We evaluated the effects of orbital atherectomy on intraluminal paclitaxel delivery to human peripheral arteries with substantial calcified plaque.
Methods: Diagnostic angiography and 3-D rotational imaging of five fresh human lower limbs revealed calcification in all main arteries. The proximal or distal segment of each artery was treated using an orbital atherectomy system (OAS) under simulated blood flow and fluoroscopy. Explanted arterial segments underwent either histomorphometric assessment of effect or tracking of ^{14}C-labeled or fluorescent–labeled paclitaxel. Radiolabeled drug quantified bulk delivery and fluorescent label established penetration of drug over finer spatial domain in serial microscopic sections. Results were interpreted using a mathematical model of binding-diffusion mediated arterial drug distribution.
Results: Lesion composition affected paclitaxel absorption and distribution in cadaveric human peripheral arteries. Pretreatment imaging calcium scores in control femoropopliteal arterial segments correlated with a log-linear decline in the bulk absorption rate-constant of ^{14}C-labeled, declining 5.5-fold per calcified quadrant ($p = 0.05$, $n = 7$). Compared to controls, OAS-treated femoropopliteal segments exhibited 180 μm thinner intima ($p < 0.001$), 45% less plaque calcification, and 2 log orders higher paclitaxel bulk absorption rate-constants. Correspondingly, fluorescent paclitaxel penetrated deeper in OAS-treated femoropopliteal segments compared to controls, due to a 70% increase in diffusivity ($p < 0.001$).
Conclusions: These data illustrate that calcified plaque limited intravascular drug delivery, and controlled OAS treatment of calcific plaques resulted in greater drug permeability and improved adjunct drug delivery to diseased arteries.

1. Introduction

Considerable progress has been made in interventional treatments of peripheral artery disease (PAD), yet key challenges remain that are largely attributed to lesion morphology and calcific burden [1]. While percutaneous transluminal angioplasty relies on plaque fracture and vessel wall stretching [2], calcium alters morphology and compliance of the arterial wall reducing the effect of both angioplasty and stent deployment. Moreover, calcification increases the occurrence of flow limiting dissections and acute vessel recoil [3], and limits stent efficacy by increasing the risk of incomplete stent expansion, malapposition, and fractures [4]. Paclitaxel coated balloons (PCB) reduce restenosis in the superficial femoral and popliteal arteries but have not been proved to be effective in below-the-knee arteries [5,6]. Fanelli et al. [7] observed that late lumen loss and primary patency 12 months following PCB treatment correlated negatively with the degree of circumferential

Abbreviations: OAS, orbital atherectomy system; IVUS, intravascular ultrasound; PAD, peripheral artery disease; PCB, paclitaxel coated balloon; SEM, scanning electron microscopy; SFA, superficial femoral artery

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calcification as assessed by computerized tomographic angiography, and hypothesized that vessel wall calcification impedes paclitaxel absorption. These and other investigators have suggested that vessel preparation by atherectomy might remove barriers to intravascular drug absorption and distribution in the artery wall [8]. Arterial plaque can be removed by a range of intravascular atherectomy devices [9] that excise it using cutting blades (directional atherectomy) or sand it down via high speed concentric rotation of a diamond-encrusted elliptical burr (rotational atherectomy) or an eccentric crown that orbits along a spiral path and frictionally pulverizes hard surfaces into harmless microparticles (orbital atherectomy).

Data on adjunctive directional atherectomy and PCB from a single-center non-randomized study [10] looked promising, and additional studies are underway. However, clinical experience with adjunctive rotational atherectomy and coronary drug eluting stents has been mixed [11–14] and it remains unclear whether atherectomy did not enhance drug delivery or whether such benefits existed but were counterbalanced by restenotic response to the atherectomy related injuries [12]. Preclinical experiments are better suited for examining the barrier effect of plaque on drug absorption and distribution with or without atherectomy. Unfortunately, induction of mineralization in animal models has proven difficult and a recent study in atheromatous arteries in the pig model exhibited comparable paclitaxel deposition 30 days post PCB treatment in OAS-pretreated vs native femoral arteries [15].

The current study was designed to directly quantify the intravascular absorption and distribution of paclitaxel in calcific human peripheral arteries at baseline and after OAS treatment. This design allowed the quantification of the barrier properties of calcific intimal plaque on paclitaxel absorption and its modulation by arterial plaque modification in femoropoliteal arteries. The study was not designed to address the impact of medial calcification on drug absorption. Limited observations on drug delivery in calcified tibial arteries are reported to help with future study designs.

2. Methods

2.1. Experimental overview

Five fresh human lower limbs were obtained from a certified research institution and maintained on ice for further evaluation. 3-D rotational imaging (GE Healthcare Innova® 2100 Cath Lab, Milwaukee, Wisconsin, USA) was performed on all limbs to identify the distribution and severity of calcification in the lower extremity arteries. Intra-arterial sheaths were inserted proximally and distally to simulate human arterial blood flow with saline perfusion. Under fluoroscopic guidance, a guiding catheter was advanced through the sheath into designated peripheral arteries and angiographic images were obtained to identify lesion location for OA. The superficial femoral arteries (SFA), popliteal and tibial arteries were divided through angiographic measurements into approximately equal segment lengths, and the proximal or distal segment of each artery was treated using the DIAMONDBACK 360® OAS (Cardiovascular Systems, Inc., Saint Paul, MN) with Solid Crowns (1.25–2.0 mm), delivered as 3 consecutive OAS runs, at 60,000 rpm, 90,000 rpm and 120,000 rpm in 60–140 mm long lesions (91.7 ± 6.7 mm).

After OAS treatment, arteries were harvested and divided into 2–8 cm long tubular segments. Each segment was annotated relative to the location of the OAS treatment site, and assigned for histology, scanning electron microscopy (SEM), or drug incubation with ^{14}C-labeled paclitaxel for drug uptake analysis or fluorescent labeled paclitaxel for drug distribution analysis as described below (Fig. 1). Segments assigned for drug incubation were retrospectively scored for circumferential calcium burden based on 3-D images or angiography. Following Fanelli et al. [7], a score of 1–4 was assigned based on the number of quadrants exhibiting substantial calcification.

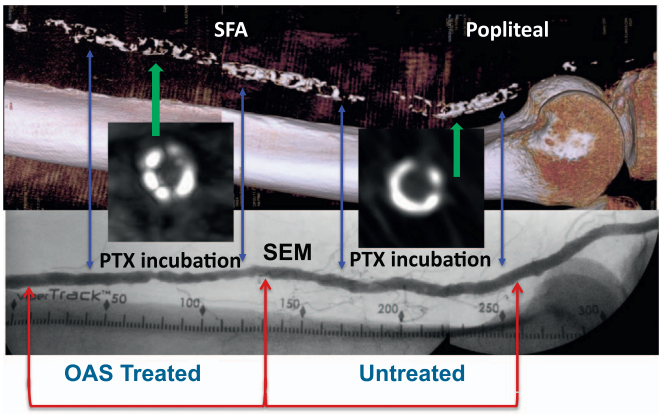

Fig. 1. Experimental paradigm. Representative angiographic image (bottom) of the femoropopliteal artery illustrating treatment location and vessel segmentation. Segments assigned for fluorescent or radiolabeled drug infusion are demarcated by arrows (blue) and their corresponding calcium burden depicted in reconstructed 3-D images (top). Inserts depict cross section views along green demarcation arrows. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.2. Drug incubation

Freshly excised arterial segments (3–8 cm long) originating from OAS-treated or untreated sites were trimmed to reduce edge effects, ligated with suture at one end (as were any side branches) and lumenally infused with a buffered solution (PBS/4% BSA) of radiolabeled (^{14}C, 10 μM) or fluorescent (Oregon Green 488, 10 μM) paclitaxel. Fully infused arterial segments were ligated in the open end and incubated in 50 mL PBS/4% BSA (37 °C) in a shaking water bath. After 1 h, incubated segments were rinsed with PBS to remove residual unbound drug, and processed for scintillation counting or fluorescent microscopy.

2.3. Analysis of radiolabeled drug uptake

After drug infusion, the sutured ends of each vessel were cut away, and the artery flushed with PBS to remove residual, unbound ^{14}C paclitaxel (Fig. 2A). Wash and incubation solution volumes were recorded for each artery and analyzed for isotope activity. The sutured ends and remaining segment (~ 1.5–2.5 cm) of each artery were weighed and incubated in excess Solvable® (Perkin Elmer) at 37 °C in a shaking water bath. Digested samples were analyzed for 1–2 min for isotope activity in a liquid scintillation counter. Scintillation counts were converted to drug weight and divided by tissue weight to obtain the bulk tissue content for each artery. Drug tissue content (M_{tissue}) was normalized to the (leakage corrected) infused drug dose (M_{dose}), and contrasted with the diffusion-binding model predicted uptake kinetics from a finite volume (Fig. 2B):

$$M_{tissue}(t)/M_{dose} = 1 - e^{k_u absorb\, t}\,\mathrm{erfc}(\sqrt{k_{absorb}\,t}), \quad k_{absorb}\,t < 1 \tag{1}$$

where

$$k_{absorb} \equiv \frac{D_{eff}\,(k_p f_u)^2}{(V_{lumen}/A_{mural})^2} \tag{2}$$

is a bulk absorption rate-constant, D_{eff} is the effective diffusion coefficient of drug in the penetrated tissue layer, k_p is the equilibrium partition coefficient of the drug in the tissue, f_u is the fraction of free drug in the infusate, V_{lumen} is the volume of infusate and A_{mural} is the mural surface area of the tissue, estimated by assuming a cylindrical lumen. The derivation and numerical validation of Eqs. (1–2) is detailed in the Supplemental methods (Figs. S1–S4). Evaluation of the right hand side of Eq. (1) at t = 1 h provided an estimate of the bulk absorption rate-constant k_{absorb}. For an inhomogeneous tissue, supplemental numerical