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Short Communication

In vivo feasibility study of ultrasound potentiated collagenase therapy of chronic total occlusions

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ABSTRACT

Arterial chronic total occlusions (CTOs) pose considerable challenges for percutaneous interventions, due primarily to the presence of stiff proximal fibrous caps (PFCs) which act as a barrier to the penetration of guide wires. A new approach under development for improving the success rate of guide wire crossing in CTOs is to employ collagenase to degrade the mechanical integrity of the PFCs. This has been shown to be feasible in preclinical work and in a Phase 1 clinical trial. In a recent study we demonstrated using *ex vivo* experimental CTO specimens that ultrasound-stimulated microbubbles (USMBs) could potentiate the effects of collagenase and result in increased mechanical degradation of the PFCs of CTOs. Here we report the results of the first *in vivo* study examining the feasibility of this approach, which demonstrates that the force required to puncture through the PFCs of CTOs is reduced with combined USMB + collagenase treatments relative to collagenase only treatments. This approach has the potential to further improve the efficacy of the emerging technique of collagenase facilitation of percutaneous interventions for CTO.

1. Introduction

Arterial chronic total occlusions (CTOs) of the coronary and peripheral vasculature are typically defined as vessels with blockages that result in absent antegrade blood flow for a period of >3 months [1]. The majority of CTOs begin as relatively soft thrombotic occlusions and with aging progressively undergo tissue reorganization and are increasingly invested with collagen. Eventually CTOs can develop complex morphologies that include features such as microvessels, calcifications, and the presence of proximal fibrous caps (PFCs) comprised of densely packed collagen [1–3]. Revascularization of coronary artery CTO by percutaneous coronary interventions (PCI) results in significant relief of angina symptoms and improvements in cardiac function [4–6], PCI procedures to restore flow in CTO cases are more complex than in stenosed but patent vessels, and generally involve first advancing a guide wire through the occlusion, then restoring the lumen with an angioplasty balloon, and subsequently introducing a stent to maintain vessel patency. Coronary CTO PCI success rates are at present limited to the 55–80% range [7–9]. The suboptimal success rates for attempted CTO PCI procedures are mainly due to inability to advance a guide wire across the mechanical barrier presented by the stiff collagen rich PFC [1,4,9,10]. Newer techniques and innovations to overcome this limitation is a high clinical priority.

We have previously proposed the use of collagenase to enzymatically degrade the mechanical integrity of PFCs and thereby reduce their resistance to guide wire penetration. This was initially demonstrated in preclinical CTO models in rabbit femoral arteries [11,12], where the approach to locally inject the collagenase through an over-the-wire balloon catheter into the space just proximal to the PFC for 30 min. After a waiting period (ranging from 24 h to 72 h) that was necessary to permit time for sufficient mechanical PFC degradation to occur, guide wire crossing rates were significantly improved. This approach was recently assessed in a first-in-man Phase 1 clinical trial, where it was found to be safe, feasible and with encouraging crossing success rates (75%) in a cohort of patients that previously had failed CTO attempts [13]. In this clinical study, there was a 1 day waiting period following collagenase exposures before guide wire crossing was attempted. Collagenase treatments therefore appear to hold significant potential to improve the success rates of CTO PCI

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procedures, though it is of interest to improve this approach and ideally have it confined to a single catheterization session.

With a view to further improving collagenase mediated PFC degradation, we have undertaken to investigate the potential of ultrasound-stimulated microbubbles (USMBs) to enhance the activity of collagenase. This work is inspired by previous research demonstrating the ability of USMBs to facilitate the degradation of vessel occluding thrombus, both with and without the use of lytic enzymes [14,15]. Recently, a feasibility study [16] was conducted using excised experimental CTOs situated in rabbit femoral arteries. The CTOs were subjected to one of four treatment scenarios: collagenase-only, USMB only, USMB exposures in the presence of collagenase, and USMB exposures followed by collagenase treatments. US exposures (1 MHz; 1.7 MPa peak negative pressure; 0.16% duty cycle) were delivered with a spherically focused transducer situated in the water bath, with bolus injections of Definity™ (Lantheus Medical Imaging, Billerca MA) released with a catheter near the CTO PFCs. The effectiveness of treatments was assessed with a recently reported puncture force test [17] that quantified the amount of force required for a probe to penetrate the PFC, and biochemical assays to indicate levels of relevant collagen degradation products present in the supranatent solution adjacent to the PFC. The USMB-only treatments did not produce a significant reduction in puncture force levels relative to control or collagenase-only groups. However, both the concurrent and sequential USMB + collagenase groups resulted in significant reductions in puncture force levels relative to the control and collagenase only groups. Similarly, these two groups exhibited enhancements of hydroxyproline levels. These results therefore suggest the feasibility of USMBs to potentiate and accelerate the effects of collagenase treatments of CTO PFCs. A primary limitation of this work was that it was conducted *ex vivo*, and while this permitted the collection of degradation products to confirm collagenase activity, it did not capture effects such as blood flow that could wash microbubbles (MBs) away, as well as potential physiologic factors that may influence the activity of collagenase.

In the present study we build upon this *ex vivo* work and perform the first *in vivo* test of this approach. Since USMB stand-alone treatments were shown not to have a measurable effect in the *ex vivo* experiments, we focus on the key groups relevant to determining if this technique might be clinically relevant: comparing collagenase-only with USMB + collagenase treatments.

2. Materials and methods

2.1. CTO model

CTOs were formed in 19 rabbit femoral arteries using the thrombin injection model [2,11], which produces CTOs with several relevant features present in the human disease, including the presence of microvessels, proximal fibrous caps, and collagen-rich cores. Experiments were performed on 12 week old CTOs and all *in vivo* work was approved by the Sunnybrook institutional animal care committee.

2.1.1. Ultrasound configuration

A custom apparatus was developed to couple the therapy beam to the PFC under ultrasound guidance. Therapeutic US (1 MHz) was delivered using an extracorporeally situated spherically focused transducer (11.2 mm 6 dB one-way beamwidth; 1.5″ diameter, 6″ focal length). As with the *ex vivo* study, we employed low duty cycle (0.001) 1 MHz ultrasound. Specifically, following each release of MBs (described below), the exposure sequence executed was comprised of a series of short bursts (fifty 100 μs pulses at 1 ms spacing) with peak negative pressures of 1.8 MPa, as measured with a 0.2 mm hydrophone (Onda Corp, CA) at the transducer focus. The transducer was mounted within a hollow Perspex® cylinder containing degassed water, which was bounded at its distal end by a transducer sleeve (CIV-flex, Civco, IA). The therapy transducer focus was co-registered to a location within the image plane of a Phillips L12-5 probe, which was mounted to the side of the therapy transducer (Fig. 1A). The ultrasound imaging was performed in either B-scan mode or low mechanical index (0.05) contrast imaging mode. This apparatus was attached to a universal joint (Manfrotto Model 488, Italy), which was in turn mounted to an x–y–z position stage (Velmex, NY) creating 6 degrees of freedom to facilitate vessel targeting (Fig. 1B).

2.2. Targeting and treatment sequence

During the targeting and treatment procedure, the animals were anesthetized with 1–2% isoflurane and placed in a supine position on a C-arm fluoroscopy table to permit access to the femoral artery region. The skin was resected to expose muscle tissue (typically 2–4 mm thick) overlying the CTO region. As a first step in targeting the therapy transducer focus to the PFC region, an over-the-wire (OTW) angioplasty balloon catheter (Long Cobra, SciMed) was introduced though the carotid artery and advanced under fluoroscopy guidance into the femoral artery with its tip situated ~10 mm from the CTO entrance (PFC). A wire-mesh frame, visible on both fluoroscopy and ultrasound imaging, was then positioned as a fiduciary marker at the muscle surface and sutured into place such that the PFC was located at its center (Fig. 2A). The therapy transducer focus was then positioned to be at the PFC using the imaging transducer, with the wire frame echoes employed as points of reference. Finally, the location of the therapy focus relative to the PFC was confirmed using ultrasound contrast imaging following injection of microbubbles through the catheter tip (example shown later), and any necessary positional adjustments were made at this time to ensure that the known therapy focus location within the ultrasound image was positioned at the PFC. Treatments then commenced immediately following the targeting procedure.

As noted, there were two experimental groups: collagenase only, and USMB + collagenase. Depending on the treatment group, a sequence of 10 bolus injections (~1 s injection time; injection intervals of 5 s) of either 0.1 ml of saline (collagenase-only group) or microbubble solution (USMB + collagenase group) were delivered via the central wire port of the OTW balloon catheter. For the combined treatment group, the MB solution was exposed to the therapeutic ultrasound sequence (fifty 0.1 ms pulses separated by 1 ms) immediately after each injection (Fig. 2B). Following the completion of the injection/exposure sequence, the animals underwent collagenase treatments. This process consisted of inflating the catheter balloon and then injecting 330 μL of the collagenase solution (see below) into the arterial lumen between the PFC and balloon. The balloon was left inflated for approximately 60 min and then removed. Following this, a stent was deployed immediately proximal to the CTO to facilitate vessel mounting in the puncture force tests [16,17]. After a 1 h waiting period, the animals were then sacrificed with euthanol and the CTO tissue was immediately harvested. Puncture force testing was conducted within approximately 1 h of tissue removal. Note that the sequential treatment approach is employed, as concurrent treatments would require injections to be made after balloon inflation to avoid collagenase washout, and this would cause pressurization and collapse of the bubbles prior to insonation. A group of $n = 3$ rabbits with CTOs were employed to establish the above targeting protocols prior to collecting data for the purposes of comparing therapeutic effects.