Effects of Intracoronary Collagenase Injection in a Porcine Model: A Safety Dose-Finding Study

Azriel B Osherov1,4, Sang Yup Lim1,3, Jagdish Butany2, Beiping Qiang1, Ronen Jaffe1,5, Ilana Erlich1 and Bradley H Strauss1,2*

1Schulich Heart Centre, Sunnybrook Health Sciences Centre, Canada
2University Health Network, University of Toronto, Toronto, Canada
3Korea University Ansan Hospital, Ansan, Korea
4Barzilai Medical Centre, Ben Gurion University, Ashkelon, Israel
5Lady Davis Carmel Medical Centre, Technion-Israel Institute of Technology, Haifa, Israel

Abstract

Objective: Chronic Total Occlusions (CTO) remains a challenge for Percutaneous Coronary Intervention (PCI). Initial studies of bacterial collagenase injections into experimental arterial CTOs and human coronary CTOs have indicated very promising effects to facilitate guide wire crossing in previously failed cases. The aim of the current study was to assess the vascular and cardiac effects of intracoronary collagenase injections in a porcine model.

Methods: Intracoronary injections of collagenase were done in the Left Anterior Descending (LAD) artery of 21 Yorkshire pigs through an over-the-wire balloon catheter. Two groups were studied: 24 hours after injection (n=14) and 30 days after injection (n=6). During balloon inflation, 8 ml of a phosphate buffered solution (PBS) containing either collagenase (range 50-3200 µg) or PBS alone (control) was injected. After sacrifice, hearts were photographed immediately and then fixed in 10% buffered formalin. Myocardial cross-sections were stained with H&E and Movat.

Results: One pig died acutely due to arterial dissection during balloon inflation. In collagenase-treated pigs, localized epicardial and myocardial haemorrhages were evident at 24 hr in the LAD distribution at doses more than 800 µg. The extent of haemorrhages was related to the dose and was generally mild at doses less than 1600 µg and moderate at doses more than 1600 µg. At 30 d, no evidence of haemorrhages was present. Mild myocardial fibrosis, likely due to ischemia, was present in both placebo and treatment groups. Balloon damaged LAD arteries showed mild intimal hyperplasia but no specific effects related to collagenase.

Conclusions: A single intracoronary injection of a bacterial collagenase formulation into normal coronary arteries caused dose-related, localized epicardial and myocardial haemorrhages, which were well tolerated without later sequelae.

Keywords: Coronary artery disease; Coronary angiography; Chronic disease; Occlusions; Angioplasty; Collagenases; Bacterial protein.

Background

Chronic Total Occlusions (CTO) are a common finding in patients undergoing diagnostic coronary angiograms with a prevalence of nearly 20% [1,2]. Success rates of Percutaneous Coronary Interventions (PCI) in CTO are approximately 70% outside of expert registries, mainly due to an inability to cross the CTO with a guide wire [3]. Collagen is the predominant matrix component in CTO, particularly at the proximal fibrous cap and serves as a major barrier to guide wire crossing [4,5]. Local injection of a bacterial-derived collagenase, a matrix metalloproteinase enzyme that selectively degrades type I collagen, has been shown to facilitate guide wire crossing in an animal model of CTO [6-9]. Recently we have reported a 75% success rate of guide wire crossing following intracoronary injection of bacterial collagenase into CTO that had previously failed attempts at revascularization [10]. The objectives of this study were to assess the cardiac and vascular effects of a range of collagenase doses administered into normal coronary arteries in a porcine model to provide safety information.

Methods

Animal model

This study was approved by the Animal Care Committee of St. Michael’s Hospital and adhered to the guidelines. The study was performed in normal Left Anterior Descending Coronary (LAD) arteries in 21 Yorkshire pigs, weighing 19-31 kg. Vascular access with a 6F sheath was obtained through a right carotid artery cut down. Coronary angiograms were performed using a 6F JLS guiding catheter. Pigs were injected with unfractionated heparin at a dose of 100 units/kg. A 0.014” angioplasty guide wire was then advanced to a distal location in the LAD artery. Depending on the arterial diameter, 2.0 mm diameter (n=8) or 2.5 mm diameter (n=13) over-the-wire angioplasty balloon catheters were advanced to mid LAD after the 2nd diagonal branch. The balloons were inflated at 15-22 atmospheres and vessel occlusion was confirmed by intracoronary contrast injection. The guide wire was removed from the guide wire port of the angioplasty catheter. While the balloon was inflated, an injection of 8 ml phosphate buffered solution containing either collagenase (50, 100, 250, 400, 600, 800, 1200, 1600, 3200 micrograms) or PBS alone (control) was injected through the guide wire port over 20 seconds. The collagenase formulation was manufactured by Sigma Aldrich from fermentation products of C. histolyticum (Catalogue # C2399 Type IA and II Lot - 046K8613 Sigma Aldrich, St. Louis, Mo). The balloon inflation was

*Corresponding author: Bradley H Strauss, Reichmann Chair in Cardiovascular Sciences, Schulich Heart Centre, Sunnybrook Health Sciences Centre, 2075 Bayview Avenue, D4-06, Toronto, Ontario, M4N 3M5 Canada, Tel: 4164806068; Fax: 4164806174; E-mail: bradley.strauss@sunnybrook.ca

Received May 21, 2014; Accepted June 24, 2014; Published June 30, 2014


Copyright: © 2014 Osherov AB, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
maintained for 8 minutes. After balloon deflation, the angioplasty balloon was removed and a coronary angiogram was performed. Animals were sacrificed at either 24 hr (n=14) or 30 d (n=6). Hearts were photographed immediately after removal and then placed in 10% buffered formalin for fixation. At least 4 cross-sections were taken from the mid ventricular level to the apex, and prepared with two stains: haematoxylin and eosin, and movat. The microscopic slides were examined by two investigators (JB, BHS).

Results

In all cases, coronary ischemia was present during the inflation, as evidenced by ST elevation on the monitor and occasional ventricular arrhythmias. One pig in the control group died during the procedure following the balloon inflation due to a ruptured balloon. Coronary angiograms after withdrawal of the over-the wire balloon catheters showed patent arteries beyond the balloon inflated segment in all pigs. After the procedure, the pig was returned to the cage. All remaining animals, including all collagenase treated pigs, survived until the scheduled time of sacrifice.

Early effects (at 24 hours)

**Coronary artery:** Angioplasty effects at the local site of balloon inflation, including intimal tears and perivascular inflammation were evident in both placebo-treated and at all doses in collagenase-treated arteries. There was no evidence of arterial damage at more distal arterial sites beyond the site of balloon inflation.

**Cardiac effects:** In placebo-treated and lower dose collagenase (up to 600 μg), no gross effects were evident (Figure 1 and Panel A,B). At higher doses (800-3600 μg), collagenase treated hearts had evidence of epicardial haemorrhages, located predominantly over the right ventricle and the apex of the left ventricle (Figure 1 and Panels C-E). Microscopically, mild focal epicardial haemorrhages was evident at 600-1200 μg and moderate myocardial haemorrhages over 1600 μg (Figure 2 and Panels A-E). A moderate myocardial haemorrhage was evident in one heart treated with 1600 μg (Figure 2 and Panels D,E). Pathological evidence of early ischemic change and mild cellular infiltrates in the myocardium were identified in both placebo- and collagenase-treated pigs.

Effects at 1 month

**Artery effects:** The coronary arteries were patent in all cases, although there was evidence of focal intimal thickening at the local site of balloon inflation in all of cases (Figure 3 and Panels A,B). There were no differences according to the treatment. In 1 case of collagenase treatment (600 μg), there was a marked perivascular inflammatory response in the LAD, with eosinophils, macrophages and multi-nucleated giant cell infiltration as well as an intense intimal hyperplastic response (Figure 3, Panel C). The inflammation extended outside of the vessel. The SMC fibers were disrupted in the media. This “hypersensitivity reaction” was only evident at the branch point of the LAD, suggesting it may have been related to a localized perforation of the vessel that subsequently had healed.

**Cardiac effects:** There was no evidence of haemorrhage in any of the hearts (Figure 4, Panels A-E). The coronary arteries were patent in all cases, although there was evidence of intimal thickening at the balloon inflated site in all of the cases, including placebo treated cases (Figure 5, Panels A, B). A medial tear with disruption of internal elastic lamina at the balloon inflated site were seen in one pig (Figure 5, Panel B). Focal myocardial fibrosis, a typical finding of healing infarcts, was present in all of the pigs with no differences attributable to treatment with collagenase or dose (Figure 5, Panels C, D).

Discussion

Local treatment with collagenase has been previously used in several clinical entities that have prominent collagen accumulation, including Dupuytren’s contractures and Peyronie’s disease [11-13]. The main adverse reaction reported in both of these conditions has been injection-site haemorrhage in 37% and ecchymosis in 25%. Of interest, direct injection of high-dose collagenase into the caudate nucleus of rats has been used to induce intracranial haemorrhages in a rat model [14]. In this model, bleeding originates from thin walled vessels, and has been attributed to effects of collagenase on degrading basement membrane collagen (particularly type IV collagen) and the
the artery within the myocardium. This allowed us to see the “worst case scenario” that could potentially occur in a situation whereby the collagenase would inadvertently be injected into a side branch rather than into the occluded artery. This study showed two types of effects: 1) balloon effects, including local vascular injury and ischemia related myocardial changes and 2) collagenase effects.

**Balloon induced local vascular injury and ischemia**

The angioplasty balloon was inflated for 8 minutes and in all cases, there were definite changes of ischemia on the ECG, including ST segment elevation and ventricular arrhythmias. Longer periods of ischemia were avoided to ensure survival of the animal. However, even after removing balloon, decreased flow was evident in many pigs, likely due to ongoing arterial spasm and possibly thrombus formation. Only 1 pig died during the procedure and that was due to balloon rupture in a control artery.

All balloon treated arteries demonstrated effects of acute and chronic arterial wall injury, according to the time of sacrifice. At 24 hours, the damaged arterial wall had inflammatory cellular infiltrate, loss of medial smooth muscle cells and occasional medial dissections. At 30 days there was a typical neointimal injury response at the site of injury. In 1 case of low dose collagenase, there was an aggressive perivascular inflammatory response that may have been related to a localized, healed perforation. Otherwise, there were no differences in the extent of these changes according to treatment group or collagenase dose.

In addition, a common finding in all animals, irrespective of treatment, was the presence of inflammatory cell infiltrates in epicardium and myocardium, usually very focal and perivascular. This appeared to be related to acute angioplasty effects or in some cases, likely just normally present in this strain of pigs. There was also evidence of ischemic damage at 24 hours (acute infarct/ischemia) or at 30 days (myocardial fibrosis). These histologic changes were usually subendocardial and focal, presumably due to the limited ischemic time, and occurred in both controls and collagenase treated animals. There resulting increased permeability [15]. These effects occurred as early as 30 min after the injection [14].

The presence of epicardial and myocardial haemorrhages during the first 24 hours following collagenase injection in our study was not attributable to changes in the larger epicardial artery where the injection was performed (see below). It is most likely that the haemorrhages originated from more distal branches of the arteries, probably at the thinner-walled arteriole level due to increased permeability, as noted in the cerebral artery rat model described above. In previous pre-clinical studies in a rabbit femoral artery chronic total occlusion model, we also observed dose-related subcutaneous haemorrhages in the perivascular tissue at both 24 hours and 72 hours post collagenase injection [6,7]. Similar to the findings of the current study, the media and adventitia at the site of the collagenase injection (and distally throughout the entire occluded arterial segment) remained intact. The haemorrhage was presumed to originate from permeability changes in distal small arterioles that were not amenable to pathologic study.

In the current study, we injected a range of collagenase doses into normal coronary arteries to assess the local arterial and cardiac effects. In contrast to our experimental rabbit femoral artery CTO model where collagenase could be trapped within the CTO, we injected collagenase into normal coronary arteries in swine without occlusions so that all of the delivered collagenase should reach the most distal branches of

---

**Figure 3:** Coronary artery treated with 600 μg collagenase at 30 d post injection. There was a prominent intimal thickening on the balloonered treated site of the coronary artery (indicated by black arrows in panel A and B) and a marked inflammatory response adjacent to coronary artery. Box in panel A indicates eosinophil’s, macrophages and giant cell infiltration, which is magnified in panel C. Black arrow in panel C indicate the multi nucleated giant cell infiltration.

**Figure 4:** A-H&E stain (<5 magnification); B-Movat stain (<5 magnification), C-H&E stain (<10 magnification). Gross pathology after 30 d of intracoronary collagenase injection. There was no evidence of haemorrhages in any of the hearts. Black arrow in panels indicates region of myocardial fibrosis. A= Placebo, B= 600 μg, C=800 μg, D=1600 μg, E=3200 μg.

**Figure 5:** Pathology of coronary artery and myocardium at 30 d following placebo treatment. Focal intimal thickening at the balloon treated region of the artery was evident (A and B). Black arrows in panel A and B indicate the intimal thickening. White arrows indicate a tear in the media and internal elastic lamina (Panel B). Myocardial fibrosis (indicated by black arrow), a typical finding of healing infarcts was present regardless of collagenase or placebo treatment (C, D). A=H&E stain, B=Movat stain, C=H&E stain, D=Movat stain.
were no differences in the magnitude of these changes between control and collagenase treatments.

**Collagenase-specific effects**

Collagenase treated animal’s demonstrated dose-related epicardial and myocardial haemorrhages.

All animals were pretreated with unfractionated heparin due to coronary artery instrumentation and to mimic the usual clinical angioplasty protocol. No or only mild, focal epicardial haemorrhages was present up to 600 µg, with mild haemorrhages at 800-1200 µg and moderate sized at 1600-3200 µg. Epicardial haemorrhages was predominantly right ventricular. Even at higher doses (1200-3200 µg) where epicardial haemorrhages were common findings, microscopic evidence of myocardial haemorrhages was mild and focal except in one 1600 µg treated animal.

The origin of these haemorrhagic changes did not appear to be from the epicardial arteries. The media and adventitia layers remained intact at all doses of collagenase at both time points. The bleeding likely is originating from distal small arterioles or capillaries that are thin walled with loose endothelial junctions that would be most susceptible to collagenase. It was reassuring that these haemorrhages had no short- or long-term sequelae, even at the highest dose. These effects were self-limiting and no evidence of either cardiac haemorrhages or aneurysm formation was present in any of the animals followed for 30 days.

Based on the findings of this study, we initiated a first-in-man human clinical trial using intracoronary collagenase that was successfully completed [10]. The maximum dose used in our first in-man study was 1200 µg and was tolerated without significant complications. Only 1 case had a small pericardial effusion, even at the highest dose. This study showed encouraging efficacy results with a 75% success rates in revascularizing coronary CTO that had previously failed attempts.

There were some differences between the experimental approach used in this swine coronary artery model and the clinical approach as follows: First, the experimental approaches for the rabbit CTO models used a collagenase injection through the wire port of an inflated over-the-wire balloon that was positioned adjacent to the CTO [-6-8]. In this study, we did not have a CTO created in porcine coronary artery. We still delivered the collagenase through the wire port of an over-the-wire balloon catheter that was inflated in the coronary artery. In the clinical trials, we started with this same approach of an over-the-wire balloon positioned immediately proximal to the CTO (n=8 patients) [10]. However, in 1 case, we could not position the over-the-wire balloon close enough to the CTO and inadvertently injected the collagenase into a side branch. Therefore, we adjusted the technique by switching to a micro catheter (Finecross), which was advanced approximately 2 mm into the chronic total occlusion. The guidewire was then removed and we injected the collagenase solution through the Finecross micro catheter in the subsequent patients enrolled in the study. Second, the infusion volume was different between the pre-clinical studies and the clinical study. In the current pig study, we injected collagenase diluted with 7-9 ml of Phosphate Buffered Saline (PBS). In the preclinical rabbit CTO studies, we injected collagenase diluted with 0.5-1.5 ml of PBS. In the human study, the collagenase was diluted into 0.9-1.2 ml normal saline, followed by 0.3 ml normal saline to ensure no collagenase was retained inside the catheter. Finally, there were differences in the balloon inflation time between the studies. In the current experimental CTO model using porcine coronary artery, we could not inflate the balloon more than 8 minutes due to acute ischemia since the artery was not previously occluded with a CTO and did not have a collateral blood supply. In the clinical study, during the initial phase when we used an over-the-wire balloon catheter, we inflated the balloon for 15-20 minutes during the collagenase injection and the balloon inflation was maintained for an additional 10 minutes after the injection. However there were no ischemic problems in the clinical cases since the CTO artery had collateral blood supply, which protected against ischemia during the balloon inflation.

Several issues could be addressed in future studies. It is unclear whether the use of higher collagenase doses, particularly for long occlusions, is necessary or tolerated. It is also possible that the collagenase could be combined with other devices or techniques to improve success rates of guide wire crossing and to shorten procedural times. Whether increasing the dose would enable the collagenase delivery and PCI attempt to be combined into a same day procedure remains to be determined. The effects of collagenase treatment on stent thrombosis or late restenosis also merits further study in a larger number of patients.

**Conclusion**

Single administration of intracoronary collagenase doses up to 1200 µg (in conjunction with unfractionated heparin) appears to be well tolerated acutely and chronically. At 24 hours, the only significant effects attributable to collagenase were focal, mild epicardial haemorrhages and in some cases myocardial haemorrhage, without clinical sequelae. At higher doses (up to 3200 µg), epicardial haemorrhages were increased but still only moderate and well tolerated.

**References**


