The Collagenase Total Occlusion-1 (CTO-1) Trial:

A Phase I, Dose Escalation, Safety Study

Running title: Strauss et al.; CTO-1 trial

Bradley H. Strauss, MD, PhD1; Azriel B. Osherov, MD1; Sam Radhakrishnan, MD1; GB John Mancini, MD2; Allison Manners, BSc3; John D. Sparkes, MSc1; Robert J. Chisholm, MD4

1Schulich Heart Center, Sunnybrook Health Sciences Center, University of Toronto, Toronto; 2University of British Columbia, Vancouver; 3Matrizyme Pharma Inc, Toronto; 4Terrence Donnelly Heart Center, St. Michael’s Hospital, University of Toronto, Toronto, Canada

Correspondence:

Bradley H Strauss, MD, PhD
Sunnybrook Health Sciences Center
2075 Bayview Avenue, D4-06
Toronto, Ontario, Canada
Phone: 416 480 6066
Fax: 416 480 6174
E-mail: Bradley.strauss@sunnybrook.ca

Journal Subject Codes: [7] Chronic ischemic heart disease; [23] Catheter-based coronary and valvular interventions: other; [27] Other Treatment; [98] Other Research
Abstract:

**Background** - Percutaneous interventions for chronic total occlusions (CTO) have low success rates, primarily due to failure of guidewire crossing. Collagen-rich matrix constitutes the main barrier to CTO crossing. In preclinical studies, local delivery of a bacterial collagenase formulation improved guidewire crossing. The Collagenase Total Occlusion-1 (CTO-1) Trial is a Phase 1, dose escalation trial to assess the safety and efficacy of collagenase therapy to facilitate guidewire crossing in coronary artery chronic occlusions.

**Methods and Results** - Twenty subjects, with ≥1 previous failure of CTO guidewire crossing, were enrolled at two sites. Subjects were treated in four distinct cohorts of five patients, with escalation of collagenase dose in each cohort from 300 to 1200 μg. Collagenase was locally delivered into the occlusions using either an over-the-wire balloon (OTW) system (n=8) or finecross microcatheter (n=12) for a period of 30 minutes. Subjects were brought back to the catheterization laboratory for guidewire crossing and angioplasty the following day. Guidewire crossing was successfully achieved in 15 subjects (75%). A soft tip guidewire (Whisper, Pilot-50, Fielder XT) was either the sole or predominant guidewire used in 75% of successful crossings. Non-STEMIs occurred in three patients due to sidebranch ischemia during stenting. Computed tomographic angiography at three months showed no late complications and patent stents in successfully treated CTO. Anginal improvement occurred with a reduction in CCS class from baseline to three months (2.5±0.6 versus 0.9±0.9, p<0.001).

**Conclusions** - Local delivery of collagenase into coronary CTO is feasible and safe with encouraging guidewire crossing results in previously failed cases. Larger clinical trials are required to determine efficacy.

**Clinical Trial Registration Information**: The CTO-1 trial has been registered on [www.clinicaltrials.gov](http://www.clinicaltrials.gov) (NCT01271335).

**Key words**: chronic total occlusion, collagenase, angioplasty, coronary artery disease, percutaneous coronary intervention
Introduction

Success rates of percutaneous coronary interventions (PCI) in chronic total occlusions (CTO) have been suboptimal in the range of 55-80%. Data from the NCDR (National Cardiovascular Data Registry) showed that PCI attempt rates for chronic occlusions in the United States remain low in the range of 12% and have not changed over the past 5 years, despite advances in techniques and technology. The main reason for failure is inability to cross the CTO with a guidewire. Preclinical model studies and human autopsy studies have shown that collagen is the predominant component in the occluded lesions, and acts as occlusive barrier at the proximal fibrous cap. Collagenase, a matrix metalloproteinase enzyme that selectively degrades type I collagen, has been shown to facilitate guidewire crossing in an animal model of CTO. The objectives of this study were to assess the feasibility, safety and efficacy of locally delivering collagenase into chronic occlusions in order to facilitate guidewire crossing in patients with at least one previously failed attempt to revascularize CTO.

Methods

The trial was designed as a prospective, two center, open-label, Phase I safety and tolerability, single ascending dose study. The trial was approved by Health Canada and the Institutional Research Ethics Boards (REB) at the two participating sites. All subjects provided written informed consent prior to participation in the trial. Study subjects were enrolled in four discrete dosing cohorts, with escalation of collagenase (referred to as MZ-004) dose with each cohort (300, 600, 900, and 1200 μg). Five subjects were enrolled in each dose cohort. All eligible subjects must have had at least one previous failed attempt of percutaneous revascularization of the CTO. Collagenase dose escalation was done on the
recommendation of an independent Data Safety Monitoring Board (DSMB) after angiographic
and clinical review of each trial subject within the previous dose cohort and in accordance with a
written and approved DSMB Charter. The trial subjects were screened for inclusion and
exclusion criteria prior to being eligible to be treated in a two-stage procedure. Once screened,
the trial subjects were brought into the hospital and the collagenase injection was performed on
Day 0. Subjects were monitored overnight in a holding bay and returned to the catheterization
laboratory on Day 1 for the PCI attempt. Subjects underwent 2D echo studies for assessment of
left ventricular (LV) function and pericardial effusions on Day 1 post collagenase injection prior
to the PCI procedure, and again on Day 2 prior to discharge. A follow-up computed
tomographic (CT) coronary angiogram was performed at 3 months to assess for any
pericardial/myocardial changes and the patency of the stents. In the 1st dose cohort, occlusion
duration (where known) was ≤2 years. However, for the 2nd to 4th dose cohorts, there was no
CTO age restriction; this protocol amendment was approved by each center’s REB and Health
Canada.

Patients eligible for inclusion included male or female subjects over 35 year old, with a
known chronic occlusion of duration more than six weeks, or absence of ischemic event in the
six weeks prior to enrolment, or in subjects with CTO of unknown duration, with a clinical
indication for revascularization, and at least one failed attempt to cross the occlusion and the
absence of pericardial effusion on 2D echocardiogram. Main exclusion criteria included
saphenous vein graft occlusion, ostial coronary occlusion, renal dysfunction (creatinine >2 x
ULN), pregnant women or recent (within 6 weeks) acute coronary syndrome.

The primary objective of the study was to evaluate the safety and tolerability of acute
intracoronary doses of collagenase (MZ-004). The primary outcome of the study was the
frequency, severity and relatedness of cardiac adverse events (AE’s) and serious adverse events (SAE’s) reported during the treatment and follow-up phases. An AE was defined as any untoward medical occurrence that a subject experienced while involved in the clinical investigative study (expected or unexpected). This may or may not have a causal relationship with this treatment. A SAE was defined as any untoward medical occurrence during the conduct of the study that resulted in death, was life-threatening, required in-patient hospitalization or prolongation of existing hospitalization or resulted in persistent or significant disability. The safety and tolerability of the treatment during the treatment period was assessed by serial cardiac enzymes at 6 and 18 hours following the procedures and the presence of pericardial effusions, and at three months when subjects underwent a CT coronary angiogram to assess for delayed vascular and myocardial effects. A pericardial effusion >1 cm or any echocardiographic signs of tamponade on Day 1 was a contraindication to performing the PCI revascularization procedure. The secondary endpoint was guidewire crossing success. Since this was the first experience of using collagenase in human coronary CTO cases, it was determined a priori that a success rate in any cohort of ≥30% (ie. at least 2 of the 5 patients in that specific cohort successfully crossed with a guidewire) would suggest the possibility of a beneficial clinical effect and would support further clinical testing of MZ-004 at that dose in future studies.

Angiographic images were quantitatively analyzed pre and post PCI using previously described and validated software at the Cardiovascular Imaging Research Core Laboratory, University of British Columbia, Vancouver, British Columbia (director, GBJM).12,13 The pre PCI angiogram was used to determine occlusion length and for qualitative assessments, including stump morphology (blunt or tapered), presence of bridging collaterals, and location of branches relative to the CTO.
Details of Collagenase

MZ-004 is a proprietary bacterial collagenase formulation manufactured in the United States for Matrizyme Pharma under GMP conditions. This form of collagenase, is a mixture of several isoforms of collagenase, primarily isoform IA and II. MZ-004 is produced from the fermentation of bacterium *Clostridium histolyticum* (*C. histolyticum*), which has been chromatographically purified (and therefore appropriate for clinical use) and shown to be active by two individual enzymatic activity assays, collagen digestion (CDU) and Furylacryloyl-Leucine-Glycyl-Propyl-Alanine (FALGPA) hydrolysis. The fermentation product also contains small amounts (≤1 unit / mg protein) of additional proteolytic enzymes, including neutral proteases and clostripain, which are controlled to approved specifications to produce a high activity formulation. MZ-004 has had the neutral proteases (caseinases) removed.

*C. histolyticum* collagenase was selected for use over mammalian and human collagenases because it is more efficient at breaking down different types of collagen in body. Human collagenase tends to cleave Type I collagen at a point called the ¾ position of the molecule, and is capable of cleaving all three strands in the helix at this one locus, leaving two segments, one of 71 kDa and the other of 24 kDa, both of which can be further digested by trypsin and other non-specific proteases. Bacterial collagenase derived from *C. histolyticum* has been well characterized and has been shown to be extremely effective at degrading all types of collagen. It is able to cleave collagen molecules predominantly at sequences that include GLY–PRO-X-GLY-PRO-Y, with cleavage occurring between the X-GLY component, where X and Y represent any amino acid. This sequence is very common in collagen molecules, allowing for scission into numerous small polypeptide chains. This allows for complete and rapid digestion of collagen without the aid of other proteases, making it very attractive for use in
therapeutic applications where collagen is to be degraded with exogenous delivery of collagenase.

MZ-004 (Lot # 059K7250, CDU 1148 units/mg solid, FALGPA 8.8 units/mg solid) was supplied in individual 2 ml Type I glass vials as a lyophilized / sterile powder that was stored at 80°C. On the day of dosing, 1 vial of lyophilized MZ-004 was removed from the freezer, and thawed at room temperature for 15-20 minutes. The entire vial of MZ-004 (2 mg) was diluted with sterile 0.9 % normal saline (USP). Subsequent dilutions with sterile 0.9 % normal saline were necessary to arrive at the specific dose cohort dose level. Once the correct dose had been achieved, the solution was filtered through a 0.2 μm disk filter into a sterile vial, and then drawn up into the individual subject’s syringe and labelled accordingly, ready for subject administration.

**Collagenase Administration**

Collagenase was locally injected into the occlusion through either a 2.0 mm diameter over-the-wire (OTW) angioplasty catheter (n=8 patients, Voyageur, Abbott Vascular) or finecross microcatheter (n=12 patients, Terumo). In the initial cases, the OTW angioplasty catheter was advanced under fluoroscopic guidance into the coronary artery immediately proximal to the CTO. The balloon was inflated to 4 atmospheres to prevent proximal run off of the infusate. The guidewire was removed, and the solution containing collagenase was administered over 15 minutes through the wire port, and then flushed with 0.3ml of saline over 5 minutes. Overall, the balloon was inflated for 30 minutes. During the second dosing cohort, it became apparent in some cases that the OTW catheter could not be advanced close enough to the occlusion due to tortuosity of the coronary artery. Thus the collagenase delivery protocol was changed to a finecross catheter that was positioned directly into the CTO over a guidewire that
was advanced a short distance (first 2-3 mm) into the occlusion. The collagenase formulation was slowly injected over 15 minutes and flushed with 0.3 ml saline over 5 minutes. The catheter was left in place for an additional 10 minutes. After balloon deflation or removal of finecross catheter, a final contrast injection was done to assess for dissections in the artery proximal to the occlusion or in the CTO itself.

The coronary percutaneous revascularization attempt was done 18-24 hours following administration of the collagenase, using standard techniques and equipment. Types of guidewires, fluoroscopy time, procedure times and contrast volumes were recorded.

For the collagenase injection procedure, it was not necessary to visualize the distal lumen by collaterals through dual coronary artery contrast angiograms since the injection was limited to the first 2-3 mm of the occlusion. However, during the attempt to cross the occlusion the following day, it was always necessary to properly visualize the distal lumen to ensure a safe guidewire crossing. This often necessitated dual coronary artery angiograms. Anticoagulation for all procedures was done with heparin alone with a target ACT of >250 for the collagenase injection procedure and >300 for the PCI procedure. An example of the stages of the procedure, including positioning of the finecross microcatheter for the CTO injection, is shown in Figure 1.

**Statistical analysis**

Results are reported as the 50th percentile (median) and as the 25th (Q1) and 75th (Q3) percentiles, except for ejection fraction, CCS angina class, CTO age, number and length of stents, which are reported as mean ± SD. No formal sample size justification for efficacy was performed since this was a dose-finding safety, Phase 1 study. Changes in CCS angina score were compared using a paired Student t-test that compared the average change in CCS score with no change. A p-value of <0.05 was considered statistically significant.
Results

Patient Details

Twenty patients with coronary CTO were consecutively enrolled from November 3rd, 2009, to January 5th, 2011. Baseline characteristics are shown in Table 1. All patients were male, median age was 63 (range 43-79), and 35% were diabetics. A previous myocardial infarction was known in 25% of cases and left ventricular ejection fraction was 55±10%. Three patients (15%) had Q waves on ECG in the territory of the CTO artery; one patient had viability on magnetic resonance imaging and the other two patients had hypokinesia in the CTO territory by echocardiography. Two patients had a previous CABG. By history, the median occlusion age was 12.5 months (Q1=10.5 months, Q3=48 months). The median time since the initial first documentation of the occlusion on coronary angiography was 6 months (Q1=4.5 months, Q3=12.5 months), with a range of 1 month to 60 months. The CTO was located in the left anterior descending artery (LAD) in 45%, the left circumflex artery in 30%, and the right coronary artery (RCA) in 25% of the cases (Table 2). The median occlusion length was 18 mm (Q1=15 mm, Q3=25 mm) and 1/3 of lesions were in excess of 20 mm length. Blunt entry was present in 55% of the cases.

Details of Previously Failed Attempts

The mean number of guidewires used per case in the initial failed attempt was 3.4±0.9 guidewires (range 2-5). Guidewire characteristics were grouped into three categories: 1. Polymer-jacketed tip guidewires (eg. Pilot, Whisper, Fielder XT), 2. Exposed coil, moderate stiffness guidewires (eg. Cross-it XT 100, 200; Vascular Progress 40,80; Miracle Bros 3, 4.5, 6) and 3. Exposed coil- stiff guidewires (eg. Progress 120T, 200T; Miracle Bros 12; Confianza Pro 9 and Pro 12). A guidewire from each of the three categories was used in nine patients (45% of
the study patients) and two categories in an additional ten patients (50%). A guidewire from only one category (Category 2) was used in one patient. The mean fluoroscopy time per failed case was 38±19 minutes. Fluoroscopy time exceeded 20 minutes in 90% and 30 minutes in 60%. The mean contrast use per case was 288±134 ml, and in 70% of cases, exceeded 200 ml of contrast.

**Clinical outcome and adverse events (Table 3)**

Collagenase was safely delivered to all 20 patients at the four dosing cohorts, ranging from 300 µg to 1200 µg. The intracoronary injection of collagenase was well tolerated without angiographic dissections, or abnormalities in cardiac enzymes, ECG changes or echocardiographic changes. The median fluoroscopy time and contrast use for the collagenase delivery procedure was 14.1 minutes (Q1=11 min, Q3=19 min) and 165 ml (Q1=120 ml, Q3=205 ml), respectively.

In all cases, the PCI attempt was made 18 – 24 hours post collagenase dose with no safety signals coming from any safety parameter evaluated. There were 5 adverse events in the study, including 3 non-ST segment elevation infarctions, 1 asymptomatic small pericardial effusion and 1 case of lung metastases in a previously undiagnosed colon cancer, which was an incidental finding on the 3 month coronary CT angiogram. Three (15%) patients, one each in the 300, 600, 900 µg dose cohorts, had CPK elevations post PCI (total CK [IU/L]/CK-MB mass [ng/ml]: 411/16, 493/54 and 330/80), all due to sidebranch occlusions at the proximal end of the occlusions. These non-ST segment elevation infarctions (NSTEMIs) had uncomplicated hospital courses. Serial echocardiographic studies identified only a small (<1 cm), asymptomatic pericardial effusion in one patient in the 3\textsuperscript{rd} dose cohort (900 µg) at one day after the PCI attempt.
There were no deaths or unexpected SAEs reported during the study. There were 3 SAEs, which included the 3 NSTEMIs since the event prolonged hospitalization (by 1 day in two patients and 2 days in 1 patient). The SAEs were deemed to be not related to the study drug as judged by the DSMB, but rather were expected due to the procedure. There did not appear to be any dose relationship with respect to AEs or severity of the events, with the highest dose cohort (1,200 μg) having no reported cardiovascular events in any of the five patients that were included.

All patients underwent percutaneous revascularization on Day 1 and guidewire crossing was successful in 15 cases (75%) with TIMI-3 flow. Stenting was successfully done in 14 cases on Day 1. In one case of a circumflex occlusion, the guidewire was successfully directed into a smaller obtuse marginal (OM) branch rather than the desired OM branch. This patient was brought back one month later and stented into the smaller OM since the guidewire still only entered this branch. In one case of a LAD CTO, the initial guidewire crossing was mistakenly deemed to have been intraluminal; a subintimal balloon dilation was done and the guidewire could not be repositioned into the true distal lumen. The occlusion was easily crossed (fluoroscopy crossing time <5 minutes) and stented when the patient was brought back at three months for a re-attempt. Thus the overall stenting success rate in the study was 80%.

CTO crossing was unsuccessful in four cases. One case was a LAD occlusion in which the OTW balloon catheter could not be adequately advanced up to the CTO, and the infusate was injected into a sidebranch. This prompted the change to a finecross microcatheter for injecting collagenase. The other three cases were in RCA occlusions: two were due to calcification (heavy in 1 case and a very localized but linear calcification in the other case) and one case in a very long (35 mm length), tortuous CTO.
In 75% of the successfully crossed occlusions, soft polymer-jacketed tip guidewires (Whisper, Pilot-50 and/or Fielder XT) either completely crossed the lesion (eight cases) or were advanced to the very distal end of the CTO and only required a stiff tip guidewire (Confianza Pro 9 or Pro 12) to re-enter a short distance into the ongoing lumen (four cases). In 25% of the cases, a stiff tip guidewire was the principal guidewire used for the CTO crossing.

The mean number and length of stents was 2.6±0.9 and 66.2±25.6 mm, respectively. The median fluoroscopy guidewire crossing time was 20.6 minutes (Q1=14 min, Q3=43.7 min). The median total fluoscopy time for the entire case was 59.5 minutes (Q1=42.7 min, Q3=63.0 min) and the median contrast use was 438 ml (Q1=328 ml, Q3=542 ml).

CT coronary angiograms were performed in 19 patients at three months. All stented arteries remained patent and there was no evidence of aneurysm or dissection in any arteries. No myocardial or pericardial effects were evident. One patient that could not undergo CT angiography, due to fast heart rate and contraindication to beta blockade, underwent repeat angiogram at six months, which showed patent coronary stents. Two patients had repeat revascularization within six months due to asymptomatic restenoses on coronary CT angiograms: proximal to the stents in one patient and distal to the stents in the second patient. Clinical improvement was evident in the overall patient population with a reduction from baseline CCS angina class 2.5±0.6 to 0.9±0.9 at 3 months (p<0.001).

Discussion

The presence of a CTO remains the most common reason for referral to bypass surgery. In the Bypass Angioplasty Revascularization Investigation (BARI) trial, the presence of chronic occlusion was the main reason that patients were deemed unsuitable for PTCA, and was present
in 68% of the 8,000 patients.\(^\text{17}\) The same preponderance was noted in the recently published Syntax registry,\(^\text{18}\) in which the surgical arm had a significantly higher prevalence of chronic occlusions compare to the percutaneous revascularization arm, 56.4% vs. 36.5%, respectively. The principal findings of the Phase 1, dose-escalation CTO-1 clinical study are that collagenase delivery into chronic occlusions is feasible, safe and associated with encouraging guidewire crossing success rates in symptomatic patients with previously failed attempts. The rationale of using collagenase in CTO is based on evidence from human pathology and experimental model studies indicating that collagen is the major component of the occlusions, particularly at the proximal fibrous cap.\(^\text{7-9}\) Collagenase has recently been shown to successfully treat Dupuytren’s contracture, another disease characterized by excessive collagen accumulation.\(^\text{19}\) Extensive preclinical studies in a rabbit femoral artery chronic occlusion model have previously shown that local delivery of collagenase significantly improved guidewire crossing.\(^\text{10,11}\)

It is generally acknowledged that success rates are lower in reattempt CTO cases than in first attempts. In the Japanese CTO [J-CTO registry], re-attempts were reported in 54 cases (10.2% of the overall study cases)\(^\text{20}\). The success rate for guidewire crossing was 72.2% and was significantly lower than first attempt cases. The actual lesion success rate (final diameter stenosis <50%) was 68.5%. In the European CTO (ERCTO) registry, Galassi et al reported a 76.1% success rate on reattempts of 498 CTO lesions\(^\text{21}\). These success rates in previously attempted CTO were achieved through innovations in technology and technique, including the retrograde approach. In the J-CTO registry, the retrograde approach was used in 25% of the overall cases, although specific data on reattempts is not available. In the ERCTO registry, the overall registry had 11.8% cases attempted by retrograde approach; however the retrograde approach was utilized in 24% of the reattempt cases (Dr. A. Galassi, personal communication). Although the
retrograde approach has been shown in one study to significantly improve CTO success rates in complex cases (58.9% versus 75.2%), this particular technique is currently limited to a small number of highly trained, experienced operators since it more complex than anterograde approach and also utilizes increased radiation dose and contrast use. The case selection between these two registries and our study cannot be reliably compared. However, the ability to successfully cross complex CTO lesions in an anterograde approach, particularly with soft tip guidewires, should be particularly appealing to interventional operators provided that larger, prospective trials show similar results.

The CTO-1 study patients had many high complexity lesional characteristics. By protocol design, all patients had one failed angioplasty attempt and one patient had undergone two unsuccessful attempts. The details of the failed, initial PCI attempt are consistent with a significant effort in almost all of the cases. Occlusion length (median 18 mm, 1/3 lesions >20 mm) and the high percentage of lesions with blunt entry (55%) and bridge collaterals (30%) also are markers for procedural difficulty. Despite these adverse features, the overall guidewire success rate in our study population was 75%. Moreover, guidewire crossing was accomplished with soft tip guidewires (tip loads 1.0-1.5 g) through the entire occlusion or up to the distal cap in 75% of the cases, consistent with significant softening of the hard, occlusive plaque with the collagenase. Even in cases where stiffer tip guidewires were required for traversing most of the CTO distance, the collagenase likely had an effect considering the previous failure in these lesions with any type of guidewire and/ or technique. Recent data in preclinical models has shown significant reduction of puncture forces in the proximal cap of CTO in collagenase treated arteries (unpublished data). The requirement of stiffer tip guidewires to cross the most distal part of the occlusion in some cases in this study suggests that the effect of the collagenase is
predominantly at the proximal fibrous cap and the body of the CTO, with lesser effects at the distal cap.

The technique of collagenase injection into the occlusion is a critical aspect of this procedure. Based on our experience in preclinical studies, the initial patients were injected through an OTW balloon catheter that was positioned either into the first part of the occlusion or just proximal to the lesion. Due to limitations in catheter flexibility in negotiating bends in the coronary artery anatomy before the occlusion, it was difficult in several cases to advance the OTW balloon as distally as desired, even when the guidewire was advanced into the first part of the occlusion. In one case (case #6), the collagenase was injected despite suboptimal OTW position and it was evident that the infusate was diverted down a side branch. This was the only case in which a guidewire could not be advanced any distance at all into the occlusion on the following day. Due to this technical limitation, we changed to the finecross microcatheter, which could be advanced in all cases into the first part of the CTO, regardless of the coronary anatomy. Although not encountered in this study, failure to properly advance the microcatheter into the initial part the occlusion should be considered a contraindication to collagenase use to avoid loss of the collagenase infusate into a side branch.

The microcatheter was routinely advanced approximately 2-3 mm into the occlusion to ensure that the injection would be inside the occlusion. The collagenase was injected slowly, essentially drop-by-drop, over 15 minutes to ensure no damage inside the CTO; the final angiogram in all cases confirmed the absence of any dissections or contrast retention after the collagenase injection. In some cases, there was tactile sensation of some resistance to the injection, but it could always be accomplished with gentle pressure on the injection syringe. The initial angiogram performed on the following day did not appear substantially different than the
post collagenase injection and no dissections were evident. However, the finecross very easily advanced deeper into the CTO in many of the cases on the following day, consistent with overnight plaque softening effects of the collagenase.

In the four failed cases, there was a significant contributing reason. The failed cases highlight the essential requirement for proper collagenase injection and for appropriate lesion selection; the main reasons for failure were excessive calcification, lesion length and tortuosity of the occluded segment. Based on this initial experience, we would recommend against using collagenase in heavily calcified vessels (collagenase does not act on calcium) and lesions >30 mm in length.

Percutaneous revascularization of chronic occlusions, even with collagenase, remains a complex procedure. The CTO arteries in our study were diffusely diseased before and after the occlusion, requiring treatment by a mean stent length of >60 mm. Previous CTO studies have similarly reported long mean stent lengths in the range of 45-56 mm. The overall fluoroscopy times (guidewire crossing and total procedure) and contrast use in this initial experience are still much longer than angioplasty procedures in non-occluded arteries. There is also the additional fluoroscopy time and contrast use associated with the collagenase delivery procedure. Nevertheless, the success rates in revascularizing this challenging group of symptomatic, previously failed cases is very promising, and additional clinical experience with collagenase should improve procedure and fluoroscopy times and contrast use.

Future studies are required to demonstrate the safety and efficacy in larger patient populations. There are several issues that could be addressed in future studies. The maximum dose used in this study was based on preclinical toxicity studies and was tolerated without significant side effects. It is unclear whether the use of higher collagen doses for more
recalcitrant occlusions is necessary or tolerated. Our initial experience suggests that the dose of 1200 μg seems to be very reasonable in terms of safety and efficacy. Specific delivery devices to optimize the depth of penetration of the collagenase into the CTO may be desirable. Some technical challenges still remain, especially crossing the distal part of the lesion where the “softening” effect of the collagenase was less evident. It is also possible that the collagenase could be combined with other crossing devices or techniques (e.g. retrograde) to improve crossing rates and shorten procedural times. The current treatment regimen requires a two-day procedure. Previous preclinical work has shown that a 72- hour waiting period between delivery and guidewire crossing is also effective, and in some cases, may be more clinically convenient. Whether the collagenase procedure can be shortened into a single day procedure remains to be determined; however, the time required for collagenase injection seems well suited to chronic occlusions diagnosed at the time of angiography when operators may have limited time to attempt the CTO given the prescheduled list of cases. The patient can then be brought back for revascularization at a more suitable time.

In summary, the CTO-1 clinical trial has shown that local delivery of collagenase into coronary CTO is feasible and safe, with encouraging guidewire crossing results in previously failed cases. Larger clinical trials are required to determine efficacy.

Acknowledgements: The authors would like to gratefully acknowledge the efforts and advice of the members of the DSMB, Dr. Merrill Knudtson (chair), Dr. Madhu Natarajan, Dr. Vladimir Dzavik and the study coordinators, Ms. Lyn Balleza and Ms. Elaine Hsu.

Funding Sources: This study was supported by the Canadian Institute of Health Research (Grant #MOP 93814)
Conflict of Interest Disclosures: Dr. Strauss holds intellectual property on the use of collagenase in chronic total occlusions, and is founder of Matrizyme Pharma Inc, a company that is commercializing collagenase for use in chronic total occlusions.

References:


**Table 1. Patient Characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ±SD)</td>
<td>63±11 years</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>20</td>
<td>100%</td>
</tr>
<tr>
<td>Hypertension</td>
<td>9</td>
<td>45%</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>7</td>
<td>35%</td>
</tr>
<tr>
<td>Previous myocardial infarction</td>
<td>5</td>
<td>25%</td>
</tr>
<tr>
<td>Previous CABG (%)</td>
<td>2</td>
<td>10%</td>
</tr>
<tr>
<td>Family history CAD (%)</td>
<td>9</td>
<td>45%</td>
</tr>
<tr>
<td>Former/current smoker (%)</td>
<td>12</td>
<td>60%</td>
</tr>
<tr>
<td>CCS Angina Class II/III</td>
<td>10/9</td>
<td>50/45%</td>
</tr>
<tr>
<td>LV EF (mean ±SD)</td>
<td>55%±10%</td>
<td></td>
</tr>
<tr>
<td>Q waves in the CTO territory</td>
<td>3</td>
<td>15%</td>
</tr>
<tr>
<td>Lesion Characteristics</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>------------------------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>LAD/LCx/RCA</td>
<td>45%/30%/25%</td>
<td></td>
</tr>
<tr>
<td>Median Occlusion age by history</td>
<td>12.5 mth (Q1=10.5 mth, Q3=48 mth)</td>
<td></td>
</tr>
<tr>
<td>0-3 months</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>4-6 months</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>7-12 months</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>1-2 yr</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>&gt;2 yr</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Median Lesion Length</td>
<td>18 mm (Q1=15 mm, Q3=25 mm)</td>
<td></td>
</tr>
<tr>
<td>Lesion Length&gt;20 mm</td>
<td>35%</td>
<td></td>
</tr>
<tr>
<td>Median Reference Diameter</td>
<td>2.93 mm (Q1=2.35 mm, Q3=3.26 mm)</td>
<td></td>
</tr>
<tr>
<td>Blunt Entry</td>
<td>55%</td>
<td></td>
</tr>
<tr>
<td>Side Branch at Occlusion</td>
<td>45%</td>
<td></td>
</tr>
</tbody>
</table>

Q1=25th percentile, Q3=75th percentile
### Table 3. PCI Procedural Data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Successful guidewire crossing</td>
<td>15 (75%)</td>
</tr>
<tr>
<td>Median Contrast volume</td>
<td>438 ml (Q1=328 ml, Q3=542 ml)</td>
</tr>
<tr>
<td>Median Fluoroscopy Time Wire Crossing:</td>
<td>20.6 minutes (Q1=14.0 min, Q3=43.7 min)</td>
</tr>
<tr>
<td>Total case</td>
<td>59.5 minutes (Q1=42.7 min, Q3=63.0 min)</td>
</tr>
<tr>
<td>Stent number (Average ±SD)</td>
<td>2.6±0.9</td>
</tr>
<tr>
<td>Stent length</td>
<td>66.2 ±25.6 mm</td>
</tr>
<tr>
<td>Stent diameter</td>
<td>2.95±0.35 mm</td>
</tr>
</tbody>
</table>

Q1=25th percentile, Q3=75th percentile
Figure Legends:

**Figure 1.** Left circumflex chronic total occlusion in a 58 year old man treated with 1200 micrograms collagenase. A. Pre procedural angiogram showing CTO (indicated by two white arrows). B. Positioning of the finecross microcatheter (dot in distal end of catheter indicated by white arrow) into first few mm of occlusion for the injection. C. Pre PCI angiogram the following day showing no deleterious effects of the collagenase injection. D. Post PCI angiogram after successful recanalization and placement of two Promus drug eluting stents with total stent length of 43 mm.
The Collagenase Total Occlusion-1 (CTO-1) Trial: A Phase I, Dose Escalation, Safety Study
Bradley H. Strauss, Azriel B. Osherov, Sam Radhakrishnan, GB John Mancini, Allison Manners,
John D. Sparkes and Robert J. Chisholm

Circulation, published online December 16, 2011:
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2011 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circ.ahajournals.org/content/early/2011/12/16/CIRCULATIONAHA.111.063198

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in
Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once
the online version of the published article for which permission is being requested is located, click Request
Permissions in the middle column of the Web page under Services. Further information about this process is
available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/