Collagenase Plaque Digestion for Facilitating Guide Wire Crossing in Chronic Total Occlusions

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Background—Chronic total occlusions (CTOs) are associated with significant angina, impaired left ventricular function, and worse long-term outcomes. Percutaneous coronary interventions in CTO are unsuccessful in up to 50% of cases, primarily because of inability to cross the lesion with a guide wire. Collagen is the predominant component of the atherosclerotic plaque. The objective of this study was to determine the efficacy and toxicity of local delivery of a collagen-degrading enzyme to facilitate guide wire crossing in CTO.

Methods and Results—Type IA collagenase (100 or 450 μg) or placebo was locally administered to 45 CTOs in a rabbit femoral artery model. Mean occlusion duration was 16±5 weeks. Attempts to cross the CTO (mean length, 28±9 mm) with conventional guide wires were assessed at 72 hours after treatment. An additional 3 arteries per group were assessed for collagenase effects at 24 hours after treatment. Successful guide wire crossings were significantly higher in collagenase-treated arteries (13 of 21, 62%) than in placebo-treated arteries (7 of 24, 29%) (P=0.028). No adverse effects on arterial structure were observed in collagenase-treated arteries. At 24 hours, collagenase-treated arteries demonstrated increased collagenase protein, gelatinase activity, and collagen fragments.

Conclusions—Local delivery of collagenase can safely facilitate guide wire crossing of CTO. This novel approach could lead to higher percutaneous coronary intervention success rates in CTO. (Circulation. 2003;108:1259-1262.)

Key Words: angioplasty • occlusion • collagen

Percutaneous coronary interventions (PCIs) remain of limited application in the treatment of chronic total occlusions (CTOs), with success rates in the range of 50% to 70%. The main reason for failure is inability to cross the CTO with a guide wire because of the occlusive fibrotic plaque. Collagen is the major structural component of these atherosclerotic plaques. Collagenase, a matrix metalloproteinase (MMP), is the initial mediator of interstitial collagen degradation. The objectives of this study were to assess the efficacy and toxicity of locally delivered collagenase in facilitating guide wire crossing in CTO.

Methods

The Occlusion Model

Approval for experiments was obtained from St Michael’s Hospital Animal Care Committee. Male New Zealand white rabbits, weighing 3.0 to 3.5 kg, were used (Charles River Canada, St Constant, Quebec). Bovine thrombin solution (100 IU, Thrombostat, Parke-Davis) was injected into an isolated femoral artery segment. Ligatures were maintained up to 60 minutes to ensure a persistent occlusion. Arterial patency was assessed angiographically at a mean of 16±4 weeks (range, 10 to 25). In the first 2 rabbits, CTOs were examined pathologically to confirm the histologic features. The remaining CTOs were treated with collagenase or placebo as outlined below.

Collagenase Administration

An over-the-wire angioplasty catheter (3.0 mm diameter) was advanced under fluoroscopic guidance into the iliac artery. The occlusion length was measured using the known balloon length as a scaling device. An attempt was made to cross the CTO with 2 conventional 0.014-inch angioplasty guide wires (Wizdom, Cordis and Choice PT [Boston Scientific]). If wire crossing was unsuccessful and no angiographic dissection was evident, the CTO was entered into the study. The catheter was advanced immediately proximal to the CTO. The balloon was inflated to 4 atmospheres to prevent proximal run off. The guide wire was removed, and 1.5 mL solution containing either type IA collagenase (n=31 arteries, total dose 100 or 450 μg) or placebo (n=24 arteries) was administered through the wire port. Type IA collagenase (Sigma) is a bacterial collagenase formulation obtained from clostridium histolyticum. Clostridial endopeptidases, unlike mammalian collagenase, cleave collagen at multiple sites, resulting in many small peptides. The balloon was inflated for up to 60 minutes. In the first 10 CTOs, the operator (L.G.) gave open-label collagenase. Guide wire crossings were done within 1 hour of collagenase administration, and all attempts were failures. Based on this experience, a waiting period of 72 hours after administration was instituted. In the randomized, placebo-controlled trial, 45 CTOs were treated with either placebo (n=24) or collagen...
nase (n=21). Operator blinding was maintained by other colleagues preparing solutions, which were then drawn up by blinded technicians. The first phase (collagenase 100 µg versus placebo, n=14) and second phase (collagenase 450 µg versus placebo, n=31) were done by different operators (L.G. and B.H.S.). Guide wire attempts were continued until the following outcome: successful crossing, large dissection, or failure despite attempts for 25 minutes. Successful crossing was identified angiographically by free movement of the guide wire tip in the distal artery. No attempt at angioplasty was done to ensure that the arterial architecture was intact for analysis. After euthanasia, femoral arteries were removed and processed for histology (Movat and H&E stains). At least 3 cross-sections were examined per occluded segment. Internal elastic lamina (IEL) integrity was quantified by calculating the percent of disrupted IEL (disrupted IEL circumference/overall IEL circumference×100) using perimetry analysis by Image I software (National Institutes of Health). An additional 6 CTO arteries (3 collagenase [450 µg] and 3 placebo) were removed at 24 hours after drug administration. No guide wire attempts were made to assess collagenase effects without the confounding effects of guide wires. These arteries were assessed histologically and for the presence of gelatinase activity, interstitial collagenase (MMP-1) protein, and collagen fragments.

**Gelatinase Zymography**

Arterial extracts were electrophoresed on a 10% SDS-polyacrylamide gel containing 0.1% gelatin, as previously described. Gels were stained with 0.5% Coomassie brilliant blue. Areas of clearing indicated gelatinase activity.

**Western Blot Analysis for Interstitial Collagenase (MMP-1) and Collagen Fragments**

Arterial extracts were fractionated on 4% to 12% tris-glycine gels under nonreducing or reducing conditions for MMP-1 and collagen fragments, respectively, followed by electrophoretic electrophoresing onto nitrocellulose membranes. Anti-MMP-1 monoclonal (dilution 1:100, Calbiochem, San Diego, Calif) was used as primary antibody and detected with anti-mouse IgG-HRP (Sigma, St Louis, Mo) as a secondary antibody. For collagen fragments, a rabbit polyclonal primary antibody (COL2 ¼ C, HDM Diagnostics & Imaging Inc, Toronto, Ontario; dilution 1:1000) directed against cleaved human type II collagen was used. Anti-rabbit IgG-HRP (Santa Cruz Biotechnology, Santa Cruz, Calif) was used as a secondary antibody. A chemiluminescence detection system (ECL Plus, Amersham, Piscataway, NJ) was used followed by autoradiography.

**Statistics**

χ² analysis was used to assess differences between collagenase-treated and placebo-treated arteries. P<0.05 was considered statistically significant.

**Results**

**Characteristics of CTO**

Occlusion lengths were similar between collagenase-treated arteries (28.5±8.6 mm) and placebo-treated arteries (27.9±8.7 mm). There was minimal to absent fibrin remnants in the CTO (Figure 1). The CTO contained mature fibrous tissue, multiple small intraluminal vascular channels, along with occasional extracellular lipid deposits, pigment-filled macrophages, and lymphocytes. A common feature, even in nonintervened placebo-treated arteries, was disruption of the IEL at several sites with intervening fibrous tissue, suggesting chronic changes unrelated to either enzyme treatments or wire attempts.

**Angiographic Results at 72 Hours**

There was a significant increase in successful guide wire crossings in collagenase-treated compared with placebo-treated arteries (13 of 21 versus 7 of 24, P=0.028) (Table, Figure 2). There was no difference in dissection rates between collagenase-treated and placebo-treated arteries (Table).

**Histology After Crossing Attempts**

Blood-filled vascular channels and some plaque disruption were evident where the guide wire traversed the occlusion (Figure 3). The number of arteries without IEL disruption was nonsignificantly higher in the collagenase-treated compared with placebo-treated groups (40% versus 26%, P=0.40). There were no differences in percent disrupted IEL between collagenase-treated (15±17% [range, 0% to 52%]) and placebo-treated (20±24% [range, 0% to 74%], P=0.28) arteries. Minor subcutaneous bruising was present only in the 450-µg collagenase-treated group.

**Collagenase Effects at 24 Hours**

Gelatin zymography showed increased 92- and 82-kDa lytic bands, reflecting both proenzyme and activated forms of MMP-9, only in the collagenase-treated arteries (Figure 4A).

<table>
<thead>
<tr>
<th>Angiographic Success and Complication Rates in the Randomized Study</th>
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<tr>
<td><strong>Low-Dose Group (100 µg)</strong></td>
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<tr>
<td>Collagenase (n=14)</td>
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<tr>
<td><strong>Collagenase</strong></td>
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<tr>
<td>Successful crossing, %</td>
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<td>Dissection rate, %</td>
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*P=0.028 vs placebo; †P=NS vs placebo.

![Figure 1](http://circ.ahajournals.org/doi/fig/10.1161/CIRCULATIONAHA.103.134057)

Figure 1. CTO at 12 weeks. Lumen (L) is occluded by fibrotic intimal lesion. Small vascular channels are evident (B, arrow in C). M indicates media; A, adventitia. A and B, Movat, ×10; C, Movat, ×20; D, H&E, ×20.
Interstitial collagenase and collagen fragments were also increased in collagenase-treated arteries (Figures 4B and 4C).

There was extensive degradation of the occlusive plaque in 2 of the 3 arteries treated with 450 μg collagenase, which was not present in placebo-treated arteries (Figure 5). Overall, the percent IEL disruption in arteries after the 24-hour waiting period was 22% (12% in the collagenase group), which was not significantly different than the arteries that had undergone wiring attempts.

Discussion

This study demonstrates a novel approach to improve PCI rates in CTO. The administration of collagenase followed by a 72-hour waiting time resulted in a significant improvement (2-fold) in successful guide wire crossings. The collagenase-treated arteries did not demonstrate any increase in the number or severity of dissections, medial layer damage, or IEL integrity compared with placebo-treated arteries.

An important observation is the requirement for a waiting period after enzyme administration. We arbitrarily set a 72-hour waiting period to provide sufficient time for plaque degradation. However, even a 24-hour waiting period showed definite plaque degradation along with increased collagen fragments and collagenase activity. Absolute success rates were higher for both placebo and collagenase (450 μg) in the second phase, probably because of increased experience with the CTO model or different operators. Nevertheless, the relative success rates in favor of collagenase-treated arteries were similar in both phases, suggesting the lower dose may be as effective (and possibly safer) than the higher dose and would be the appropriate dose for initial clinical trials.

Figure 2. A through C, Successful attempt at 72 hours after collagenase. D through F, Unsuccessful attempt at 72 hours after placebo. Bl indicates contrast in bladder. CTO is evident between the 2 arrows (A and D). Guide wire (arrow) has successfully crossed the CTO (B). After guide wire attempt, there is no dissection. In E, guide wire (arrow) will not advance because of dissection (E). F, Contrast extravasation attributable to guide wire dissection (D).

Figure 4. A, Gelatin zymogram from a collagenase-treated artery (lane 1) and placebo-treated artery (lane 2). There was 92-kDa gelatinase (MMP-9) activity only in collagenase-treated arteries. Lytic bands were present at 92 and 82 kDa, reflecting both proenzyme and activated forms of MMP-9 in collagenase-treated arteries. B, Western blot analysis for interstitial collagenase (MMP-1). All lanes showed a ~93-kDa band, indicating interstitial collagenase. This band was markedly increased in the collagenase-treated arteries (lanes 1 and 2) compared with placebo treatment (lanes 3 and 4). Lane 5, Collagenase formulation (20 μg), positive control. C, Collagen fragments were increased in collagenase-treated (lanes 1 and 2) compared with placebo-treated (lanes 3 and 4) arteries.
There are several possible reasons to explain preferential degradation of the matrix in the occlusive plaque while sparing the outer layers of the vessel wall. First, newly formed collagen is the most vulnerable to the effects of MMPs.6 The organization of occlusive thrombus into a fibrointimal plaque is a dynamic process involving new collagen synthesis with variable cross-linkage. This fibrous tissue is most susceptible to collagenase degradation. Second, the occluding plaques are quite vascular, with a very abundant supply of small vasa-vasorum and intra-arterial arteries. This is evident in human pathological specimens2,7 and in our model. This microvascular network could provide diffusion of the enzymes along the length of the occluded segment.

Study Limitations

Although this animal CTO model shares many similarities to the human pathology, the extensive IEL disruption and medial thinning in human atherosclerosis may predispose to more serious vascular damage (such as perforation) with collagenase therapy. Longer-term studies are required to assess for late aneurysm formation or medial thinning. The efficacy of collagenase in specific subtypes of CTOs, such as heavy calcification, absent microchannels, or extremely old occlusions, also merits additional study. Importantly, however, Srivatsa et al2 have described some degree of neovascularization in >80% of plaques from human CTO. Arterial dissections would be a contraindication to collagenase therapy, because deeper arterial walls would be directly exposed. Bacterial-derived enzymes such as collagenase may be immunogenic, similar to streptokinase.

Summary

Local delivery of collagenase can degrade intimal plaque collagen in CTO and facilitate guide wire crossing without additional damage to the deep layers of the arterial wall. Additional development could lead to substantially higher PCI success rates in chronic CTO. Plans are underway for a clinical feasibility study.

Acknowledgments

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References

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