Electrical Homogenization of Ventricular Scar by Application of Collagenase
A Novel Strategy for Arrhythmia Therapy

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Background—Radiofrequency ablation for ventricular tachycardia is an established therapy. Use of chemical agents for scar homogenization represents an alternative approach. The purpose of this study was to characterize the efficacy of collagenase (CLG) application on epicardial ventricular scar homogenization.

Methods and Results—Myocardial infarcts were created in Yorkshire pigs (n=6) by intracoronary microsphere injection. After 46.6±4.3 days, CLG type 2, type 4, and purified CLG were applied in vitro (n=1) to myocardial tissue blocks containing normal myocardium, border zone, and dense scar. Histopathologic studies were performed to identify the optimal CLG subtype. In vivo high-density electroanatomic mapping of the epicardium was also performed, and border zone and dense scar surface area and late potentials were quantified before and after CLG-4 application (n=5). Of the CLG subtypes tested in vitro, CLG-4 provided the best scar modification and least damage to normal myocardium. During in vivo testing, CLG-4 application decreased border zone area (21.3±14.3 to 17.1±11.1 mm², P=0.043) and increased dense scar area (9.1±10.3 to 22.0±20.6 mm², P=0.043). The total scar area before and after CLG application was 30.4±23.4 and 39.2±29.5 mm², respectively (P=0.08). Late potentials were reduced by CLG-4 application (28.8±21.8 to 13.8±13.1, P=0.043). During CLG-4 application (50.0±15.5 minutes), systolic blood pressure and heart rate were not significantly changed (68.0±7.7 versus 61.8±5.3 mmHg, P=0.08; 77.4±7.3 versus 78.8±6.0 beats per minute, P=0.50, respectively).

Conclusions—Epicardial ventricular scar homogenization by CLG-4 application is feasible and effective. This represents the first report on bioenzymatic ablation of arrhythmogenic tissue as an alternative strategy for lesion formation. (Circ Arrhythm Electrophysiol. 2013;6:776-783.)

Key Words: bioenzymatic ablation ☻ collagenase ☻ radiofrequency ☻ scar homogenization ☻ ventricular tachycardia
myocardial scars. Optimal CLG subtype and concentration were determined by in vitro experiments and applied topically in vivo to epicardial scar regions.

Methods

Myocardial Infarct Induction

Myocardial infarcts were created in 6 female Yorkshire pigs (30–35 kg). Following a 12-hour fasting period, the animals were sedated with intramuscular injection of 1.4 mg/kg Telazol and were intubated. Ventilation was achieved with an endotracheal tube connected to a ventilator (Summit Medical, Bend, OR). General anesthesia was maintained with inhaled 1.5% to 2.5% isoflurane. Analgesia was maintained with buprenorphine (0.3 mg) intravenously hourly. Femoral arterial and venous access were obtained, and lidocaine (2.0 mg/kg), esmolol (1.0 mg/kg), and unfractionated heparin (10000 units) were given intravenously. Under fluoroscopic guidance, myocardial infarctions were created in the left circumflex (n=1), right coronary artery (n=2), and left anterior descending coronary arteries (n=3). An Amplatzer-type guide catheter was placed in the left main coronary artery or the right coronary artery. A 0.018-mm guide wire (HT BMW Universal, Abbott vascular, IL) was inserted into the left coronary artery or the right coronary artery. A 2.5 to 3.5 mm angioplasty balloon catheter (Fox ss, Abbott vascular) was advanced over a guide wire and inflated in the mid-left circumflex/left anterior descending or in the mid-right coronary artery. Thirty seconds after balloon inflation, a 10-mL suspension of sterile saline containing 3 to 5 mL polystyrene microspheres (Polybead 90.0 µm; Polysciences, PA) was injected through the central lumen of the balloon catheter. ECG and arterial pressure were monitored continually during infarction and recovery. Acute infarction was confirmed by ST segment elevation in the ECG leads. Five minutes after microsphere injection, the balloon catheter was removed. Animals were exsanguinated and observed with continuous ECG monitoring until able to ambulate without assistance. Animals were observed without additional antiarrhythmic drugs administered until terminal CLG application experiments.

In Vitro Testing of CLG Subtypes

Three types of clostridial CLGs (type 2 CLG [CLG-2], type 4 CLG [CLG-4], and purified CLG [CLSPA]; Worthington Biochemical Corporation, NJ) were evaluated to identify the optimal CLG subtype and concentration for scar homogenization.26–29 CLG-2 and CLG-4 were each tested at 6 concentrations (0.025%, 0.05%, 0.1%, 0.15%, 0.2%, and 0.4%), whereas CLSPA was tested at 6 dilutions (25, 50, 100, 150, 200, and 400 U/mL). The pieces of myocardium were obtained from a left circumflex infarct pig. The infarct was grossly evident on inspection and was cut out into 20 equal pieces. The pieces of myocardium were subsequently histologically confirmed to contain scar, BZ, and normal myocardium. A chemical buffer solution (HEPES [4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid]; Sigma-Aldrich, MO) was used to dissolve each CLG subtype. The CLG solutions were adjusted to pH 7.4 to 7.5 for experimentation. Pieces of tissue soaked into HEPES medium served as controls. The solutions containing tissue were incubated at 37°C for 24 hours and then into 70% ethanol after rinsing in dH2O. The sections were then placed in 10% buffered formalin for 24 to 48 hours. Ventilation was achieved with an endotracheal tube connected to a ventilator (Summit Medical, Bend, OR). General anesthesia was maintained with inhaled 1.5% to 2.5% isoflurane. Analgesia was maintained with buprenorphine (0.3 mg) intravenously hourly. Femoral arterial and venous access were obtained, and lidocaine (2.0 mg/kg), esmolol (1.0 mg/kg), and unfractionated heparin (10000 units) were given intravenously. Under fluoroscopic guidance, myocardial infarctions were created in the left circumflex (n=1), right coronary artery (n=2), and left anterior descending coronary arteries (n=3). An Amplatzer-type guide catheter was placed in the left main coronary artery or the right coronary artery. A 0.018-mm guide wire (HT BMW Universal, Abbott vascular, IL) was inserted into the left coronary artery or the right coronary artery. A 2.5 to 3.5 mm angioplasty balloon catheter (Fox ss, Abbott vascular) was advanced over a guide wire and inflated in the mid-left circumflex/left anterior descending or in the mid-right coronary artery. Thirty seconds after balloon inflation, a 10-mL suspension of sterile saline containing 3 to 5 mL polystyrene microspheres (Polybead 90.0 µm; Polysciences, PA) was injected through the central lumen of the balloon catheter. ECG and arterial pressure were monitored continually during infarction and recovery. Acute infarction was confirmed by ST segment elevation in the ECG leads. Five minutes after microsphere injection, the balloon catheter was removed. Animals were exsanguinated and observed with continuous ECG monitoring until able to ambulate without assistance. Animals were observed without additional antiarrhythmic drugs administered until terminal CLG application experiments.

EAM

Electroanatomic bipolar voltage mapping of epicardium was performed during sinus rhythm on the animals subjected to CLG application. The animals were heparinized during mapping and CLG application (3000 U unfractionated heparin intravenously every hour). The NavX patch system (EnSite; St Jude Medical) was used in the animal with closed chest topical approach, whereas an EnSite array catheter system (EnSite; St Jude Medical) was used in the animals with open chest approach. EnSite array was chosen to avoid the impact of air on impedance as assessed by NavX patch system. Data from both NavX patch and EnSite array systems were analyzed using NavX Velocity software (St Jude Medical). A duodecapolar catheter (Livewire, 2-2-2 mm spacing; St Jude Medical) and/or a 4-mm tip mapping catheter (SafireBLU; St Jude Medical) was used for epicardial mapping. During epicardial mapping, ≥500 points were collected for each pre- and post-CLG map.

Histopathologic Analysis

After euthanization, hearts were immediately explanted and rinsed thoroughly with cold saline (4°C). Subsequently, cold 10% buffered formalin was flushed down the coronary arteries repeatedly. These samples were then placed in 10% buffered formalin for 24 to 48 hours and then into 70% ethanol after rinsing in dH2O. The sections were embedded in paraffin and cut in 5-µm-thick sections and then stained with hematoxylin and eosin and trichrome elastic van Gieson stain. Slides were digitally scanned for measurement of lesion depth (Aperio XT; Aperio Technologies, Vista, CA).

Statistical Analysis

The exact permutation version of the nonparametric Wilcoxon signed-rank test was used to compute P values for paired comparisons of hemodynamic data, total scar, DS, and BZ areas before and after CLG application.

The exact nonparametric Mann–Whitney U test was used for unpaired comparisons of digestion depth. Data are displayed as dot plots. A P value <0.05 was considered significant. Analyses were performed with the use of SPSS (version 19.0) statistical software (SPSS, Chicago, IL).

We verified catheter stability and reproducibility of local electrograms (including LPs) by sampling repeatedly at each location at different time intervals. Two observers analyzed the morphology and timing of these potentials. In cases in which a measurement or electrogram was subject to interpretation, a consensus between the 2 observers was reached.
Table 1. Procedural Details of In Vitro Experiments

<table>
<thead>
<tr>
<th>Collagenase Subtype</th>
<th>Maximum Concentration</th>
<th>Application Time</th>
<th>Digestion Depth (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>n/a</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>CLSPA</td>
<td>400 (U/mL)</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>CLG-2</td>
<td>0.4 (%)</td>
<td>24</td>
<td>665.9±58.3</td>
</tr>
<tr>
<td>CLG-4</td>
<td>0.4 (%)</td>
<td>24</td>
<td>951.0±39.6*</td>
</tr>
</tbody>
</table>

Values for digestion depth are listed as mean±SEM. CLG indicates collagenase; and CLSPA, purified collagenase.
*P<0.05 when compared with CLG-2.

**Results**

**Determination of Optimal CLG Subtype**

In vitro scar digestion was performed from adjacent blocks of myocardial tissue containing scar, BZ, and normal-appearing myocardium. The procedural details are shown in Table 1. Three CLG subtypes were tested as previously described, CLG-2, CLG-4, and CLSPA. Concentrations tested were 0.025%, 0.05%, 0.1%, 0.15%, 0.2%, and 0.4% for CLG-2 and CLG-4, and 25, 50, 100, 150, 200, and 400 U/mL for CLSPA. Figure 1 shows histological results from digestion experiments. Figure 1A shows control tissue block soaked in HEPES solution, whereas Figure 1E and 1I demonstrates high-power views of the scar and normal-appearing myocardium, respectively, from Figure 1A. As shown in Figure 1B, 1F, and 1J, CLSPA (even at the highest concentration of 400 U/mL) showed minimal digestion of the scar. However, the effects of CLG-2 (Figure 1C, 1G, and 1K) and CLG-4 (Figure 1D, 1H, and 1L) can be appreciated easily at doses of 0.4% for both CLG-2 and CLG-4. Below this dose, minimal digestion of scar or myocardium occurred (data not shown). The effect on scar digestion appeared greater in CLG-4 specimen than CLG-2, whereas the damage to surviving myocardium was stronger in specimen of CLG-2. The maximum depth of digestion of surviving myocardium was 665.9±58.3 µm for CLG-4, whereas that for CLG-2 was 951±39.6 µm (P=0.01; Figure 1C and 1D; blue scale markers).

**Impact of CLG on Scar and BZ Surface Area**

In total, 4 animals underwent open chest surgical approach (Figure 2A and 2B), whereas 1 animal underwent a closed chest surgical approach using an ablation catheter with a piece of cellulose sponge fastened onto it (0.5×1.0 cm; Figure 2C). This allowed the catheter to be directly visible within the EAM system and selectively placed over the BZ region (Figure 2D). CLG-4 was applied on the RV epicardium for 30 minutes and LV epicardium for 60 minutes. In subject 5, CLG-4 was applied to 3 different sites for 30 minutes each (total 90 minutes) because of smaller sponge size. Mean CLG-4 application time was 60.0±9.5 minutes across all animals, after which voltage mapping of the region was immediately repeated. The procedural details are shown in Table 2.

Hemodynamics were recorded continuously throughout the experiment. Systolic blood pressure and heart rate were not significantly changed before and after CLG application (68.0±7.7 versus 61.8±5.3 mmHg, P=0.08; 77.4±7.3 versus 78.8±6.0 beats per minute, P=0.50, respectively).

Total low-voltage and DS surface areas were measured in the NavX velocity system. Regions identified as BZs were targeted for digestion with CLG-4. The total scar area before and after application of CLG-4 was 30.4±23.4 and 39.2±29.5 mm², respectively (P=0.08, n=5). Topical application of CLG-4 significantly reduced BZ surface area (21.3±14.3 to 17.1±11.1 mm²; P=0.043, n=5; Figure 3A). The reduction in BZ surface area was associated with an increase in DS surface area (9.1±10.3 to 22.0±20.6 mm²; P=0.043, n=5), indicating that CLG-4 application converted BZ areas to DS areas (Figure 3B). After CLG-4 delivery, the percentage of BZ was significantly reduced, whereas percent DS was significantly increased (78.0±7.5% to 53.0±9.7%, and 22.0±7.5% to 47.0±9.7%; P=0.043, n=5; Figure 3C).

High-density mapping was performed in all animals, and LPs were quantified from all points collected. Consistent with previous studies,11 the majority of LPs were distributed in the BZ. CLG-4 application significantly reduced the number of LPs (28.8±21.8 to 13.8±13.1; P=0.043, n=5; Figure 4). There was no significant difference in the total collected
points pre- and post-CLG-4 application (704.5±463.0 versus 753.7±438.0; \(P=0.14\)).

Histopathologic analyses were performed after CLG-4 application in all animals. Figure 5A and 5C shows representative images of scar tissue after CLG-4 application in subjects 1 and 5, respectively. Focal debris and inflammatory changes were observed at CLG-4 application sites, whereas surviving myocardium distant from CLG-4 application sites remained intact. Extracellular matrix at CLG-4 application sites was degraded and appeared loosened (Figure 5B and 5D).

**Discussion**

**Major Findings**

The major findings of the present study are (1) CLG application to the BZs results in chemical homogenization of myocardial scars, and (2) LPs are eradicated after the CLG application. The current study represents the first evaluation of bioenzymatic electrical scar homogenization.

**Mechanism and Characteristics of Lesion Formation**

The cardiac extracellular matrix is surrounded by myocardium and comprises several subtypes of collagens in normal hearts.\(^3\) Production of collagens induced by myofibroblasts after myocardial infarction has been described in animal models and in humans. The BZ of scars, regions where myocardial scars are adjacent to surviving myocardium, represents regions of slow conduction and sources of VT. In this study, these regions were targeted for chemical ablation by CLG. The mechanism of lesion formation by CLG is the chemical interruption of tissue architecture

<table>
<thead>
<tr>
<th>Table 2. Procedural Details of Collagenase Application Strategies</th>
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<td>Subject</td>
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</table>

Average±SEM 46.6±4.3 46.7±3.3 … … … … 60.0±9.5 1.63±0.15

Epi indicates epicardium; LAD, left anterior descending artery; LV, left ventricle; RCA, right coronary artery; and RV, right ventricle.
given its inherent activity not only on collagens, but also its actions as a protease, an aminopeptidase, and a tryptase. This disruption involves both the normal and fibrotic components of the electrophysiologically defined BZ, rendering the region electrically silent. This causes not only mechanical, but also electrical disconnection of surviving myocardium. These plural actions result in focal ablation of the cellular and extracellular matrix components of the targeted regions.

Ablation lesions created by radiofrequency energy delivery result in tissue coagulation necrosis, infiltration of inflammatory cells, and hemorrhage in central region, which ultimately form well-demarcated lesions. These lesions also result in obliteration of cellular and extracellular matrix components in normal myocardium and scar/BZ regions. Similarly, features of these ablation lesions are also observed in lesions created with high-intensity focused ultrasound, microwave, and laser technology. Biological interruption of electrical conduction was reported by Bunch et al., where lesions were formed by fibroblast injection to modify atrioventricular node function. That lesion was characterized by scar formation with collagen fibers. Other groups have performed delivery of biochemical solutions to myocardial scars by coronary artery injection or direct intramural injection. Our study shows a novel method of epicardial scar digestion in the beating heart, involving catheters and devices already widely used clinically. Bioenzymatic ablation by CLG represents an alternate mechanism of lesion

Figure 3. Change in low-voltage area after collagenase application: A, Bipolar voltage map before and after collagenase type 4 (CLG-4) application. Dense scar lesion with voltage <0.5 mV was delineated in gray areas. Yellow arrows indicate CLG-4 application site. B, Quantification of scar area in the animals with CLG-4 selectively applied (n=5). C, Quantification of border zone and dense scar distribution in scar area.

Figure 4. Collagenase application eliminates late potentials (LPs): A, Bipolar voltage map and isolated LP distribution before and after collagenase type 4 (CLG-4) application. Red arrows indicate LPs in the scar area, and red arrowheads indicate actual LPs on the electrogram. Blue arrows indicate LP eliminated points. B, Quantification of total collected points from electroanatomic maps before and after CLG-4 application. C, Quantification of the number of LPs before and after CLG-4 application. The white arrows denote a LP that remained after CLG-4 application.
formation, which results in focal tissue destruction in regions exposed to the agent, similar to the aforementioned mechanical strategies. Topical application by open chest approach yielded focal delivery of CLG and targeted digestion of the BZ regions only. We further showed feasibility of epicardial catheter–based delivery of CLG with focused effects on the BZ and on LPs.

Electrical Homogenization and Elimination of LPs
The present study demonstrates electrical homogenization of myocardial scars, eliminating heterogeneous low-voltage regions and converting them into electrically silent DS. In addition, LPs, which predominantly exist in regions denoted as BZs, were significantly reduced after CLG application. Furthermore, CLG application had no effects on LPs outside of the site of application. This is important as it indicates that the effects of CLG do not diffuse away and affect unintended sites. Although arrhythmia inducibility was not tested, significant reduction in LPs suggests reduced tissue arrhythmogenicity. Clinical studies have shown that targeting and eradicating LPs is an effective strategy for catheter-based treatment of VT.31,35 In the present study, CLG application was performed for 50±15.5 minutes at a concentration of 0.8% for CLG-4. This application time can be dramatically shortened by increasing the concentration of CLG, making it more compatible for clinical use. A higher concentration of CLG can be applied at sites of interest and the effects terminated by α2-macroglobulin or other safe antagonists after a short period of time.

Study Limitations
This study demonstrates the feasibility of CLG in homogenizing myocardial scars and reducing LPs, as a proof of concept. A number of important considerations should be taken. First, topical CLG application is more applicable for epicardial scars, although a percutaneous catheter providing a stable seal with the endocardium, excluding freely flowing blood, could allow modulation of endocardial scars. Since we did not perform endocardial voltage mapping, the effect of epicardial CLG application on the endocardium remains unknown. Hemodynamic measurements showed that CLG application did not cause hemodynamic compromise, although ventricular wall motion was not evaluated by echocardiography. Lastly, this study evaluated the effect of CLG application only in the acute phase, and the efficacy of lesion maintenance intermediate to long term remains unclear.

Conclusions
These data represent the first reported experimental evidence of the feasibility of bioenzymatic ablation of ventricular tissue. Further studies are warranted to investigate the safety and efficacy of this approach.

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Disclosures
The Regents of the University of California, Los Angeles, have intellectual property developed by the author (Dr Shivkumar) that relate to data reported in this paper. The other authors report no conflicts.
References


Catheter-based radiofrequency or cryogenic ablation is widely used for the treatment of ventricular and atrial arrhythmias. Occlusion, or infusion of alcohol into coronary artery branches, and alternative sources of energy, such as high-intensity focused ultrasound, ionizing radiation, and microwaves, are under investigation. Use of bioenzymes is a novel strategy for myocardial ablation. Our study tested this novel method of homogenization of myocardial substrate. Bioenzymatic tissue ablation using collagenases was studied. In vitro analyses were performed to identify the optimal collagenase subtype, whereas in vivo studies were performed to characterize the tissue effects on the epicardium of beating hearts. Electroanatomic mapping was performed before and after tissue ablation with collagenase type 4 in a porcine chronic infarct model. This demonstrated homogenization of heterogeneous scar border zones and elimination of late potentials in the areas that were bioenzymatically ablated. The clinical implications of this study are: (1) bioenzymatic ablation of select myocardial substrates is feasible; (2) this can be achieved in a timely period, similar to other methods of myocardial ablation currently in use; (3) the concentration of enzyme used and the degree of tissue ablation can be controlled; and (iv) there was no acute adverse effect of this therapeutic approach. This approach highlights the feasibility of myocardial tissue modulation for arrhythmia therapy and may also facilitate delivery of biologically active cellular or molecular therapies.
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