Does Implantation of Sonomicrometry Crystals Alter Regional Cardiac Muscle Function?

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Background: **Sonomicrometry is a gold standard in experimental studies on myocardial motion. However, limited information exists regarding mechanical and biochemical changes produced by sonomicrometry crystal (SC) insertion into the myocardial wall.**
Methods: **In 10 open-chest pigs, we implanted SCs into the inner half of apical anterior and midposterior regions. Longitudinal strains (systolic lengthening, end-systolic, peak shortening, and postsystolic shortening strains) and strain rate (SR) measurements (peak systolic ejection and early and late diastolic SRs) were obtained by Doppler SR echocardiography along with troponin I levels measured from peripheral blood before and after SC insertion.**
Results: **SR and strain parameters did not change significantly after SC implantation. Troponin I levels increased significantly from less than 0.010 to 0.129 ± 0.138 μg/L ($P < .005$) after SC implantation.**
Conclusions: **Our study demonstrates that despite biochemical evidence of myocardial injury, carefully implanted SCs do not alter systolic or diastolic regional myocardial function assessed by Doppler echocardiography.** (J Am Soc Echocardiogr 2007;20:1407-1412.)

Since the mid-1950s,[1,2] sonomicrometry crystal (SC) implantation has been widely used in experimental cardiovascular physiology studies. This technique continues to be the reference standard for measuring regional myocardial deformation.[3-6] Implantation of foreign objects into the myocardium could, however, lead to injury and alter regional myocardial function. Although only minimal damage to a myocardial wall as a result of SC implantation was found,[7] the effects of SC insertion on regional myocardial function have not been systematically evaluated.

Strain rate (SR) echocardiography has been established for evaluation of regional myocardial deformation and has been validated independently of sonomicrometry with nuclear magnetic resonance.[8] SR echocardiography has been broadly used to assess alterations of myocardial deformation caused by various factors such as ischemia, reperfusion, or metabolic inhibition.[6,9,10] In this study, we explore whether SC embedding causes measurable differences in SR echocardiography analysis of regional myocardial function. Troponin I served as a sensitive biomarker of myocardial injury.[11]

METHODS

The study was approved by our institutional animal care and use committee.

Animal Preparation

Twelve adolescent swine weighing 45 to 60 kg were studied under general anesthesia induced with an intramuscular injection of ketamine hydrochloride (20 mg/kg) and xylazine (2 mg/kg) and maintained with an intravenous infusion of ketamine hydrochloride (2 mg/kg/L), fentanyl (0.02 mg/kg/L), and etomidate (0.08 mg/kg/L). Each animal was intubated, mechanically ventilated (Servo Ventilator 900C, Siemens, Danvers, Mass), and normal ranges of blood gases maintained by periodic checks. After sternotomy, a pericardial cradle was constructed. Introducer sheaths (Terumo Medical Corp, Elkton, Md) were placed in common carotid arteries or femoral arter-

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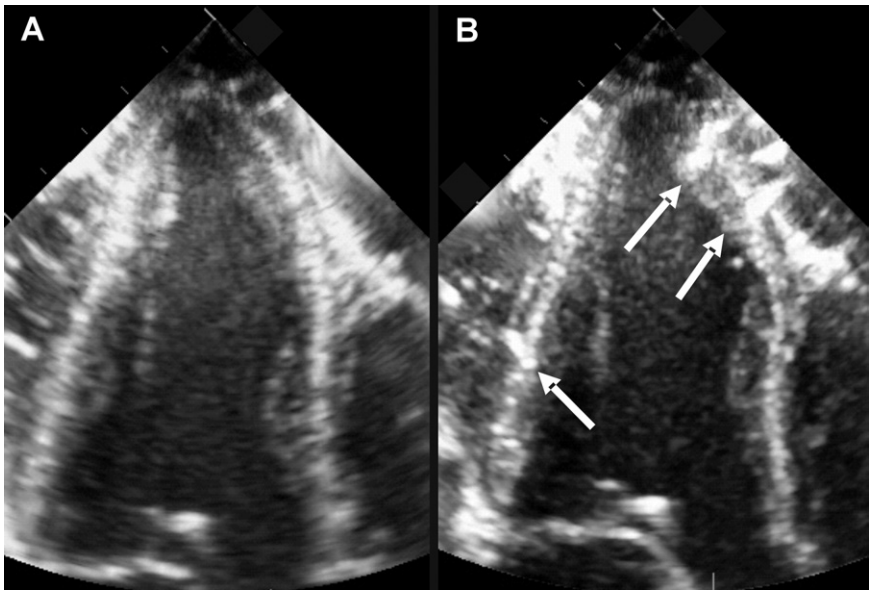

Figure 1 Gray-scale long-axis view of left ventricle before (**A**) and after (**B**) sonomicrometry crystal (SC) insertion. *Arrows*, Position of SCs in apical anterior and midposterior regions.

ies and both internal jugular veins for obtaining blood samples, administration of anesthesia and fluids, and insertion of catheters. Pressure catheters (Millar Instruments Inc, Houston, Tex) were placed into the left ventricle (LV) and ascending aorta. The animals were fully anticoagulated (activated clot times >240 seconds) with heparin after insertion of crystals. Electrocardiography and LV and aortic pressures were monitored continuously and recorded using the sonomicrometry computer system (Sonometrics Corp, London, Ontario, Canada) and software (CardioSoft, Sonometrics Corp.).

Testing and Control Regions

The apical anterior and midposterior walls served as testing regions for SC insertion, whereas the midlateral wall represented a control region with no SC implanted.

Hemodynamic Data Analysis

Peak LV pressure (LVP) during systole and end-diastolic pressure were measured and values of the peaks of positive and negative time derivative of pressure (dP/dt) and heart rate were calculated from LVP tracings.

Sonomicrometry

After careful puncturing of the epicardium and a superficial myocardial layer with a 12-gauge needle, a pair of spherical SCs (~2 mm in diameter) was inserted into the inner half of the myocardial apical anterior and midposterior wall segments (Figure 1). Within each pair, the crystals were placed approximately 10 to 15 mm apart and oriented along the LV long axis to measure predominantly longitudinal motion. Superficial vessels were avoided during SC insertion; no suture was required to maintain the crystals in their position. Mutual motion of SCs was recorded at a rate of 250 Hz and instantaneous (Lagrangian) strains calculated.[3] Longitudinal systolic lengthening strain (SL), end-systolic strain (ES), and peak shortening strain (PkS) were expressed as the percent deformation with respect to the preceding end-diastolic state. Postsystolic shortening strain (PSS) was calculated as the difference between ES and PkS magnitudes.[12] The midlateral segment served as an intact control region with no SCs embedded.

Two-dimensional and SR Echocardiography

An ultrasound system (Vivid 7, GE Healthcare, Milwaukee, Wis) equipped with a 3.5-MHz transducer was used for epicardial scans and data were archived digitally on a magneto-optical disk. A 2-dimensional gray-scale wide-sector scan was performed in apical long-axis projection to verify the position of SCs and check for intramural hematoma that could be caused by SC insertion. Narrow-sector apical long-axis and 4-chamber projections were used to obtain scans at 220 frames/s or higher for off-line analyses (EchoPAC, GE Healthcare) of longitudinal tissue Doppler velocities in the apical anterior, midlateral, and midposterior segments. SR measurements were obtained from the tissue velocity data, and strains were integrated from SRs over the period of an electrocardiographic R-R interval.[3] Measurement samples were carefully placed to match the position of crystal pairs, and data from 3 consecutive cardiac cycles were averaged to reduce the influence of noise. Peak ejection SRs and early (E) and late (A) diastolic SRs were measured in all 3 segments. The same strain parameters (ie, SL, ES, PkS, and PSS) were calculated as those measured by sonomicrometry (Figure 2).

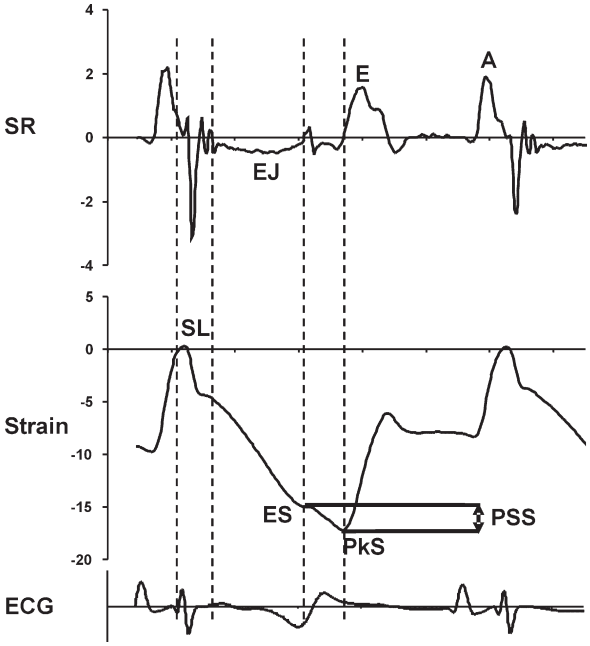

Figure 2 Strain rate (SR) and strain curves obtained from midposterior wall before sonomicrometry crystal insertion. *Top*, SR curve. *Vertical dashed lines*, Isovolumic contraction and relaxation phases of cardiac cycle. Peak ejection SR is denoted by peak ejection SR (*EJ*) reflects regional systolic function. Regional diastolic function was described by early relaxation peak SR (*E*) after mitral valve opening and by late relaxation SR caused by atrial contraction (*A*). *Bottom*, Strain curve derived from SR curve. Only minimal systolic lengthening (*SL*) is present at beginning of isovolumic contraction and isovolumic relaxation is characterized by postsystolic shortening (*PSS*). *ES*, End-systolic strain; *PkS*, peak shortening strain.

Timing of cardiac phases was based on the long-axis view of aortic and mitral valve opening and closure.

Biochemical Detection of Myocardial Injury

Troponin I levels (μg/L) were measured with electrochemiluminiscence immunoassay using the Elecsys 2010 analyzer (Roche Diagnostics, Indianapolis, Ind) from peripheral blood samples.[13]

Study Protocol

Before embedding any of the SCs, baseline recordings of electrocardiography, pressures, and 2-dimensional and SR echocardiography were performed and a first sample of blood for troponin I analysis was withdrawn. SCs were then implanted and full anticoagulation started. Approximately 90 to 120 minutes after SC insertion, all measurements were repeated along with sonomicrometry recordings and the second blood draw for troponin I analysis.

Statistical Analysis

Data are presented as mean ± SD of 3 measurements. A Wilcoxon signed rank test was performed for hemodynamic and echocardiographic data comparison before and after SC insertion. A sign test was used for comparison of troponin I levels before and after SC insertion. Linear regression analysis with a least squares method and Bland-Altman plot were used to compare data obtained by SR echocardiography and those measured by sonomicrometry.[14] Intraobserver and interobserver variabilities of SR and strain measurements were assessed in 5 randomly chosen animals in the apical anterior, midlateral, and midposterior segments before and after SC insertion, and presented as the difference between measurements expressed as a percentage of the mean. *P* values less than .05 were considered statistically significant.

Table 1 Hemodynamic measurements

	Baseline	After SC insertion
Peak LVP	102.7 ± 11.0	98.7 ± 13.8
+dP/dt	1496 ± 511	1307 ± 369
−dP/dt	−2046 ± 617	−1891 ± 531
ED LVP	7.5 ± 1.7	7.6 ± 1.7
HR	76.4 ± 16.9	72.0 ± 13.8

$+dP/dt$ and $-dP/dt$, Peak positive and negative, respectively, rate of pressure change; *ED*, end-diastolic; *HR*, heart rate; *LVP*, left ventricular pressure; *SC*, sonomicrometry crystal.There was no statistically significant difference in any measured hemodynamic parameter between baseline and after SC insertion.

RESULTS

Of the 12 animals used, one animal was excluded for pericarditis before baseline recordings and one animal for sustained arrhythmias after baseline recordings before insertion of SCs. Data for a complete protocol were obtained from 10 animals and careful SC embedding caused no apparent bleeding or any other complication.

LV Hemodynamic Data

There was no statistically significant difference of peak LVP, end-diastolic LVP, positive dP/dt, negative dP/dt, or heart rate before and after SC insertion (Table 1).

Two-dimensional Echocardiography

Two-dimensional echocardiography visualized SCs embedded within the myocardial wall (Figure 1) and did not show any excessive local myocardial backscatter suggestive of intramural bleeding after SC insertion.

Echocardiographic and Sonomicrometry Strain and SR Data

Tables 2 and 3 summarize the strain and SR magnitudes obtained by SR echocardiography and sonomicrometry. No changes were observed in patterns of strain or SR curves after implantation of crystals

Table 2 Strain parameters by echocardiography and sonomicrometry

Segment	Parameter	Baseline, %	After SC insertion. %
Apical anterior by echocardiography	SL	0.1 ± 0.2	0.1 ± 0.1
	ES	−11.5 ± 1.3	−11.1 ± 2.4
	PkS	−11.6 ± 1.2	−11.3 ± 2.3
	PSS	0.1 ± 0.3	0.2 ± 0.5
Midposterior by echocardiography	SL	0.2 ± 0.4	0.2 ± 0.4
	ES	−15.9 ± 1.5	−15.5 ± 1.2
	PkS	−16.6 ± 2.1	−16.0 ± 1.3
	PSS	0.7 ± 1.3	0.5 ± 0.8
Midlateral by echocardiography	SL	0.1 ± 0.2	0.1 ± 0.3
	ES	−18.7 ± 2.1	−18.3 ± 2.0
	PkS	−19.1 ± 2.4	−18.8 ± 2.4
	PSS	0.5 ± 0.8	0.5 ± 0.8
Apical anterior region by sonomicrometry	SL	N/A	0.2 ± 0.5
	ES	N/A	−12.7 ± 4.6
	PkS	N/A	−13.3 ± 4.6
	PSS	N/A	0.6 ± 1.0
Midposterior region by sonomicrometry	SL	N/A	0.1 ± 0.2
	ES	N/A	−17.9 ± 4.5
	PkS	N/A	−18.0 ± 5.5
	PSS	N/A	0.6 ± 0.9

ES, End-systolic strain; *N/A*, not applicable; *PkS*, peak shortening strain; *PSS*, postsystolic shortening strain; *SC*, sonomicrometry crystal; *SL*, peak systolic lengthening strain.
There were no statistically significant differences in all measured strain variables between baseline and after SC insertion.

Table 3 Strain parameters by echocardiography

Segment	Parameter	Baseline, s–1	After SC insertion, s–1
Apical anterior	EJ	−0.70 ± 0.12	−0.66 ± 0.17
	E	0.98 ± 0.25	1.07 ± 0.76
	A	0.81 ± 0.43	0.94 ± 0.41
Midposterior	EJ	−0.82 ± 0.21	−0.82 ± 0.23
	E	1.63 ± 0.85	1.44 ± 0.55
	A	1.89 ± 0.57	2.04 ± 0.73
Midlateral	EJ	−0.94 ± 0.22	−0.87 ± 0.23
	E	1.71 ± 0.78	1.94 ± 0.68
	A	2.51 ± 0.93	2.40 ± 0.66

A, Late relaxation strain rate; *E*, early relaxation strain rate; *EJ*, peak ejection strain rate; *SC*, sonomicrometry crystal.
There were no statistically significant differences in all measured strain rate variables between baseline and after SC insertion.

(Figure 3) in the testing and control regions. A small-magnitude systolic lengthening, found by SR echocardiography in 20% of anterior apical, 20% of midposterior, and 10% of midlateral segments at baseline, remained at the same rate of occurrence after insertion of the SCs. Postsystolic shortening of small magnitude was present in 30% of anterior apical, 40% of midposterior, and 30% of midlateral segments at baseline and in 20%, 40%, and 30% of the segments, respectively, after SC insertion. There were no statistical differences in strain contraction parameters (ES, PkS) or in systolic SR (peak ejection). Regional diastolic SRs E and A were not different after SC insertion from those measured at baseline. In the control midlateral segment, all strain and SR parameters remained unchanged as well. Intraobserver and interobserver variabilities were, respectively, 13.6% and 16.6% for SL, 11.4% and 12.7% for ES, 10.9% and 13.1% for PkS, 10.7% and 12.8% for PSS, 10.1% and 12.4% for peak ejection, 11.0% and 13.2% for E, and 15.5% and 17.5% for A. Linear regression analysis of SR echocardiography and sonomicrometry indicated high correlation (r = 0.91, $P < .0001$) and the Bland-Altman agreement analysis showed only a small bias (bias ± 2SD, −1.0 ± 7.5%).

Biochemical Detection of Myocardial Injury

At baseline, the troponin I levels were below 0.01 μg/L, but increased significantly to 0.129 ± 0.138 μg/L ($P < .005$) after SC insertion.

DISCUSSION

Although elevated troponin I levels indicated myocardial injury, this study demonstrates that careful embedding of SCs does not measurably alter systolic or diastolic regional myocardial function as assessed by Doppler SR echocardiography and does not cause or lead to an increase in the magnitude of systolic lengthening or postsystolic shortening.

Potential Mechanisms of Regional Myocardial Alteration Function Caused by SC Insertion

The initial epicardial puncture and the following SC insertion and tunneling can injure both myofibers and vessels. Some damage to myocyte integrity was, indeed, present and signaled by a significant increase of troponin I levels in peripheral blood. However, there was no alteration of strain or SR magnitudes or patterns in the implantation region. Although the areas with superficially positioned coronary arteries and their branches were avoided, SC insertion could injure coronary arteries or veins within the myocardial wall. However, there was not any remarkable bleeding from needle puncture before SC insertion or from a tunnel formed by SC insertion. Moreover, 2-dimensional echocardiography, which is suitable for identification of intramural hematoma,[15] did not reveal any noticeable intramural bleeding.

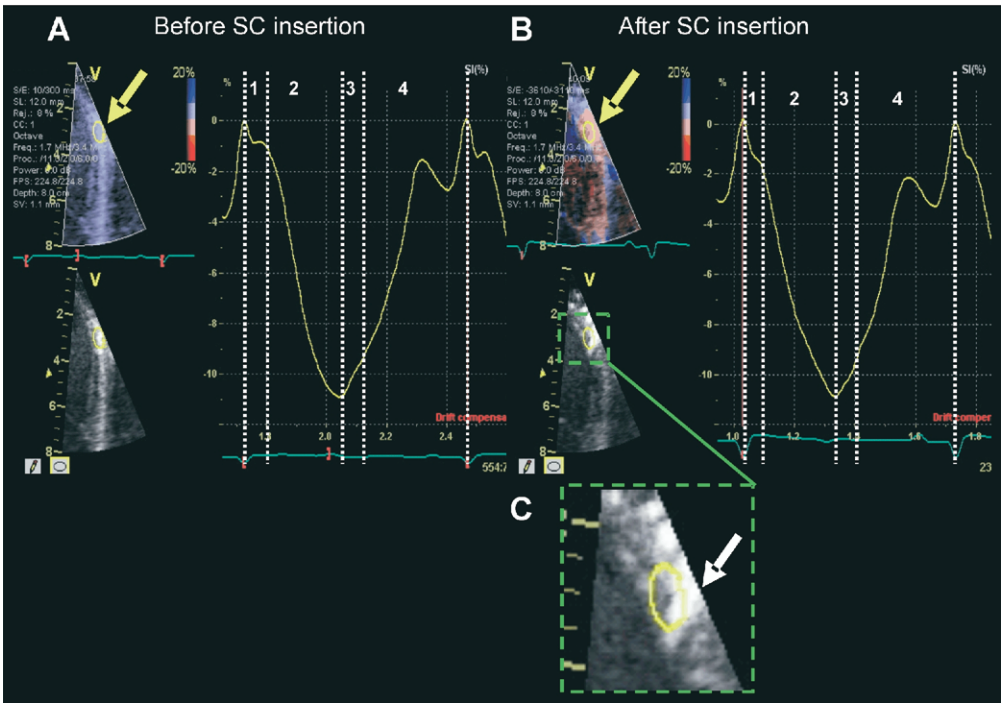

Figure 3 Examples of strain curves obtained before (**A**) and after (**B**) sonomicrometry crystal (SC) insertion into apical anterior region of left ventricle. *Yellow arrows* point to position of sample volume used for strain and strain analysis. *Dotted lines*, Phases of cardiac cycle: *1*, isovolumic contraction; *2*, ejection; *3*, isovolumic relaxation; *4*, diastolic filling phases. *Dashed box* (**C**) depicts magnified region of SC insertion; *white arrow*, inserted crystal; *yellow ovoid*, sample window for strain and strain rate analysis. Pattern of strain curve did not change after embedding of SC into myocardium. There was no systolic lengthening or postsystolic shortening present before and after implantation of SC. No change in pattern of strain curve was observed after SC embedding into inner half of apical anterior wall.

Strain and SR Analysis in Identification of Myocardial Functional Alteration

Strain and SR echocardiography are sensitive to changes in regional myocardial deformation induced by an ischemic injury[6,9] or just by selective inhibition of myocardial energy metabolism.[10] Regional dysfunction induced by these factors can include diminishing of strain and SR magnitudes, development of systolic lengthening, and shifting of myocardial contraction to the postsystolic period with generation of postsystolic shortening. However, SL and PSS of low magnitudes have been found to be physiologic phenomenon[16] that help the LV to reshape during isovolumic phases of cardiac cycle.[5,17] In this study, the presence of PSS was in 30% of evaluated segments, which corresponds with a previous report.[16] SL and PSS related to ischemic regional myocardial injury can be clearly distinguished from those occurring in normal myocardium based on magnitudes and timings of their peaks.[16] Even though injuries caused by SC insertion may not be remarkable or identified, it is conceivable that an incompressible foreign object, such as a SC with its connecting wire, could produce a mechanical obstacle to normal myocardial motion. However, we did not observe any increase in the magnitudes of SL or PSS after SC insertion; neither did we find alteration in any other parameter of regional mechanical function measured by strain and SR echocardiography.

Mechanical Myocardial Injury and Troponin Levels

An increased troponin level in peripheral blood is a sensitive biomarker of myocardial injury and necrosis, and this test is routinely used for diagnosis of acute coronary syndromes. However, troponin levels do not discriminate between ischemic and nonischemic (eg, traumatic) causes of myocardial injury. Increased levels of troponin I were reported in various conditions, even without apparent myocardial dysfunction.[18-20] Similarly, in this study, troponin I levels were elevated after insertion of SCs, but no measurable alterations in regional myocardial function were present.

Limitations

Considering 3-dimensional myocardial architecture, alterations of LV torsion and circumferential strains by SC insertion are possible and were not measured in our study. We also did not assess the long-term impact of SC insertion. This would require an experimental model with a surviving animal, but selective evaluation of the functional impact of SC insertion would be complicated because of the development of postoperative adhesions. We have not evaluated the extent of necrosis by the commonly used method of triphenyltetrazolium chloride staining, neither did we perform microscopic analyses of myocardial injury. Our study was focused on functional changes and the histologic description of limited necrosis was provided by others.[7]

Conclusion

Careful insertion of ultrasonic crystals into myocardium does not cause alterations in regional systolic or diastolic myocardial function measurable by SR and strain echocardiography, although the related microinjury can be detected with biochemical markers, such as elevated troponin I levels in peripheral blood.

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