Late Repair of Ischemic Mitral Regurgitation Does Not Prevent Left Ventricular Remodeling

Importance of Timing for Beneficial Repair

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Background—Ischemic mitral regurgitation (MR) is a frequent complication of myocardial infarction associated with left ventricular (LV) dilatation and dysfunction, which doubles mortality. At the molecular level, moderate ischemic MR is characterized by a biphasic response, with initial compensatory rise in prohypertrophic and antiapoptotic signals, followed by their exhaustion. We have shown that early MR repair 30 days after myocardial infarction is associated with LV reverse remodeling. It is not known whether MR repair performed after the exhaustion of compensatory mechanisms is also beneficial. We hypothesized that late repair will not result in LV reverse remodeling.

Methods and Results—Twelve sheep underwent distal left anterior descending coronary artery ligation to create apical myocardial infarction and implantation of an LV-to-left atrium shunt to create standardized moderate volume overload. At 90 days, animals were randomized to shunt closure (late repair) versus sham (no repair). LV remodeling was assessed by 3-dimensional echocardiography, dP/dt, preload-recruitable stroke work, and myocardial biopsies. At 90 days, animals had moderate volume overload, LV dilatation, and reduced ejection fraction (all P<0.01 versus baseline, P=NS between groups). Shunt closure at 90 days corrected the volume overload (regurgitant fraction 6±5% versus 27±16% for late repair versus sham, P<0.01) but was not associated with changes in LV volumes (end-diastolic volume 106±15 versus 110±22 mL; end-systolic volume 35±6 versus 36±6 mL) or increases in preload-recruitable stroke work (41±7 versus 39±13 mL mm Hg) or dP/dt (803±210 versus 732±194 mm Hg/s) at 135 days (all P=NS). Activated Akt, central in the hypertrophic process, and signal transducer and activator of transcription 3 (STAT3), a critical node in the hypertrophic stimulus by cytokines, were equally depressed in both groups.

Conclusions—Late correction of moderate volume overload after myocardial infarction did not improve LV volume or contractility. Upregulation of prohypertrophic intracellular pathways was not observed. This contrasts with previously reported study in which early repair (30 days) reversed LV remodeling. This suggests a window of opportunity to repair ischemic MR after which no beneficial effect on LV is observed, despite successful repair. (Circulation. 2013;128[suppl 1]:S248-S252.)

Key Words: heart disease, ischemic • mitral valve regurgitation • remodeling
with comparable infarction alone, with an initial rise in pro-
hyptertrophic and antiapoptotic signals (specifically, the Akt-
phosphatidylinositol 3'-kinase and gp130-janus kinase (JAK)/
signal transducer and activator of transcription 3 (STAT3)
pathways), followed by their exhaustion. In the same model,
early repair (after 1 month)\textsuperscript{10} of the MR-equivalent LV-to-LA
shunt prevents downregulation of these compensatory path-
ways and induces LV reverse remodeling manifested by nor-
malization of both functional and anatomic parameters, also
consistent with other ischemic MR models and therapeutic
approaches.\textsuperscript{10,19,20} However, it is not known whether the molec-
ular alterations and LV remodeling can be reversed by a late
intervention done when the prohypertrophic and antiapoptotic
signals are alreadydownregulated.

Based on this observed biphasic molecular response in isch-
emic MR, we specifically hypothesized that late MR repair
would not result in LV reverse remodeling. This was tested
using our previously described animal model of ischemic MR
combining an apical MI and an LV-to-LA shunt, with shunt
ligation at 3 months after MI to simulate MR repair.

**Methods**

**Ischemic MR Model**

A previously described ischemic MR model was created in 12 adult
Dorsett hybrid sheep (weight >45 kg).\textsuperscript{17,18} This model, combining an
apical infarction by distal left anterior descending coronary artery
ligation (not causing MR by itself) and an LV to LA shunt (8-cm
long, 8-mm diameter–reinforced Teflon graft with a cross-sectional
area of 50 mm\textsuperscript{2} implanted into the LV base and left atrial appendage
by color flow Doppler. Three-dimensional analysis was performed
using our previously described animal model of ischemic MR
combining an apical MI and an LV-to-LA shunt, with shunt
ligation at 3 months after MI to simulate MR repair.

**Echocardiography Analysis**

Standard 2-dimensional and full-volume 3-dimensional data sets
were obtained epicardially at each time point (baseline, 90 days, and
135 days) using high-frequency (SS, X3) probes and a Philips iE33
scanner (Andover, MA). Shunt patency or closure was confirmed
by color flow Doppler. Three-dimensional analysis was performed
blinded to group and time point using the custom software Omni4D
(MD Handschumacher) as previously described.\textsuperscript{18} LV end-diastolic
and end-systolic volumes (ESV) were measured and LV ejection frac-
tion computed. Regurgitant volume was calculated as total 3-dimen-
sional stroke volume (end-diastolic volume–ESV) minus forward
aortic stroke volume calculated by pulse wave Doppler and the con-
tinuity equation. Regurgitant volume and fraction, therefore, included
the regurgitant flow through the LV-to-LA shunt, but also accounted
for any additional functional MR that could have occurred secondary
to LV dilatation in the context of combined MI and volume overload.

**LV Pressure–Volume Analysis**

At baseline and day 135, a high-fidelity conductance catheter
(Scisense, Ontario, Canada) was inserted transapically to record LV
maximal \( \frac{dP}{dt} \) and pressure–volume relationship under different
preload conditions by simultaneously partially occluding the inferior
vena cava, with subsequent computation of preload-recruitable stroke
work (PLRSW),\textsuperscript{18,21} a preload independent measure of LV systolic
contraction.

**Tissue Samples and Molecular Analysis**

Myocardial needle biopsy was performed at baseline before model
creation, and both peri-infarct and remote myocardium were biopsied
at days 90 and 135. We measured levels of pAKT protein kinase B,
central in the hypertrophic process, and pSTAT3, a critical node in
the hypertrophic stimulus by cytokines. Both these, at their respective
levels (cytosol and membrane), are important crosstalks in prohy-
pertrophic signaling.\textsuperscript{18} Western blot analysis was performed on cell
lyses from biopsies at baseline, day 90, and day 135. pSTAT3 and
anti-phosphoAkt (Santa Cruz Biotechnology, Santa Cruz, CA) were
detected with peroxidase-conjugated anti-mouse IgG and chemilumi-
nescence, with α-actin as the housekeeping control. Integrated blot
pixel density was assessed using standard software (ImageJ; National
Institutes of Health, Bethesda, MD).

**Statistics**

Continuous variables are expressed as mean±SD. Differences within
and between groups at different time points were assessed with
repeated-measures ANOVA. Rank-based ANOVA was performed
when normality was excluded by the Shapiro–Wilk test. \( P<0.05 \) was
considered significant. Based on previous data with the same model,
we calculated that a sample size of 6 per group would provide
\( \geq 80\% \) power (\( \alpha \text{ level, 0.05} \)) to detect an effect comparable with early
MR repair between groups (25 mL/mm Hg in preload-recruitable
stroke work and 40 mL in ESV). Statistical analysis was performed
with Stata/IC 11.2 (StataCorp LP, TX). The authors had full access to
and take full responsibility for the integrity of data.

**Results**

**LV Volumes and Function**

A total of 16 sheep underwent the initial procedure of MI+MR.
Four sheep died suddenly within the first 48 hours, presumably
from malignant arrhythmia. A total of 12 sheep completed the
protocol successfully, without death occurring after random-
imization of MR repair versus sham procedure. As expected, the

![Figure 1. Schematic representation (left) and perioperative view (right) of the left ventricle-to-left atrial appendage shunt. MR indicates mitral regurgitation; and MI, myocardial infarction.](image)
initial procedure of MI+MR resulted in a progressive increase in LV end-diastolic volume and ESV (both $P<0.01$ versus baseline), a decrease in LV ejection fraction ($P<0.01$ versus baseline), and a moderate volume overload (regurgitant volume $15\pm4$ mL; regurgitation fraction $34\pm8\%$) at 90 days, with no significant difference between groups (Figure 3). Shunt ligation at 90 days in the late repair group successfully corrected the volume overload, whereas the no repair group had persistent regurgitation until euthanasia (regurgitant volume $2a\pm2$ versus $12\pm7$ mL for late repair versus no repair, $P=0.007$). However, this late correction did not prevent progression of LV remodeling at 135 days, and both end-diastolic volume ($106\pm15$ versus $110\pm22$ mL for late repair versus no repair, $P=0.72$) and ESV ($69\pm15$ mL versus $70\pm16$ mL for late repair versus no repair, $P=0.95$) were further increased at euthanasia with reduced LV ejection fraction ($35\pm6$ versus $36\pm6\%$ for late repair versus no repair, $P=0.70$), with no significant difference between groups (Figure 3). LV hemodynamics showed the same pattern: preload-recruitable stroke work was significantly and equally reduced at euthanasia in both groups (Figure 4; $41\pm7$ versus $39\pm13$ mL·mm Hg, $P=0.70$). Maximal $dP/dt$ also declined over time comparably in both groups at euthanasia ($803\pm210$ versus $732\pm194$ mm Hg/s, $P=0.56$).

**Molecular Analysis**

Western blot–integrated density of prohypertrophic markers pAKT and pSTAT3 at 3 time points is displayed in Figure 5. As expected, both groups had similarly decreased signals for these markers at 90 days. However, late repair did not result in recovery, and both groups were equally depressed at day 135 ($P=0.16$).

**Discussion**

In this study, we show that late correction of a moderate volume overload 3 months post-MI does not reverse or prevent further LV remodeling and dysfunction, with consistent outcomes at the functional and molecular levels. These results contrast with a previously published study, in which early (1 month) repair was associated with complete normalization of ventricular volume, hemodynamic alteration, and molecular alteration.

Despite its clear association with poorer prognosis, treatment of ischemic MR remains highly controversial. Both clinical and experimental studies yield conflicting results. Part of this controversy is attributable to the complex nature of LV remodeling associated with ischemic MR: the MI initially causes the MR, but both MI and MR can contribute to cellular and functional changes in the noninfarcted LV. The relative contribution of MI and MR has been difficult, if not impossible, to separate in clinical studies. Other confounders relate to MR surgical correction, often performed with simultaneous coronary revascularization and with the frustrating problem of recurrent MR, treating the volume overload often only partially or temporarily. This animal model of apical MI with LV-to-LA shunt can help resolve these difficulties. By isolating the effect of moderate volume overload on comparable MI size and location, it previously demonstrated the additional contribution of moderate MR to LV remodeling. The second advantage is the adequate and persistent correction of volume overload by shunt ligation, simulating a successful and minimally invasive MR repair. In this controlled environment, the current experimental study provides another possible explanation for discrepancies between clinical studies by suggesting that a successful surgical correction can produce variable results if not performed in a timely manner.

This variable response to correction at different time points needs to be related to the previously observed biphasic molecular response in the myocardium after MI and MR. Cardiac remodeling in ischemic MR is dynamic: increased apoptotic tone (caspase-3) has been shown in remote myocardium, partially counterbalanced by transiently increased

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**Figure 3.** Left ventricular (LV) volume and function in both groups overtime. **A**, LV end-diastolic volume (EDV). **B**, LV end-systolic volume (ESV). **C**, LV ejection fraction (LVEF). **D**, Regurgitant fraction. Despite successful shunt closure and marked reduction in volume overload at 90 days, no difference is seen at 135 days vs sheep without repair. LV volumes are equally increased as the LVEF decreases.
prohypertrophic and antiapoptotic pathways: the gp130-STAT3 pathway is related to cytokine-mediated hypertrophy, and protein kinase B (Akt) is a serine/threonine-specific protein kinase promoting growth factor–mediated cell survival and hypertrophy. Signaling of both pathways is increased after MI, but the presence of moderate volume overload is associated with subsequent downregulation paralleling the transition to heart failure.17,24 Early MR repair when performed while gp130 and Akt are still overexpressed can interrupt the vicious remodeling cycle and reverse heart failure. The lack of success of the same procedure after downregulation of these compensatory pathways indicates a point of no return for LV remodeling in ischemic MR and suggests a limited time window to correct the MR, with less or no beneficial effect if the intervention is not performed within this interval. The persistent negative remodeling observed after late MR repair suggests that these profound molecular alterations not only prevent reversal of heart failure but could also contribute to further LV remodeling even after the correction of the initial volume overload.

Clinical Correlation and Future Studies
Clinical criteria to correct MR surgically are generally based on MR severity, LV dimensions, and function. Although clinically important and useful, these parameters might not be sufficient to capture the whole spectrum of LV remodeling or completely predict outcome after surgery for ischemic MR. Although the current study suggests that earlier repair is better to prevent LV remodeling, determining the best timing for intervention in a clinical setting will remain a challenge because each patient presents variable MI size, MR severity, and myocardial substrate and, therefore, an individual time window. Although preoperative echocardiography assessment can help predict procedural success in terms of MR correction25 and that parameters, such as LV dimension, can be useful to assess the remodeling, it is unclear at this point how well imaging (other than LV dimensions and function) and biomarkers can improve the prediction of ventricular response after successful MR repair. Another question will be whether and how current and future medical therapies can prolong the time during which beneficial repair can be performed. Well-designed clinical and experimental studies are needed to answer these relevant questions properly.

Limitations
While helping to understand the effects and potential implications of volume overload correction on LV remodeling, direct extrapolation to patients with ischemic MR cannot be made at this point. This animal model was designed to separate the results of MI and volume overload on LV remodeling and to study the effects of successful MR repair, but does not fully represent the clinical scenario. However, these results highlight the dynamic nature of LV remodeling in ischemic MR and the importance of timing when considering surgical intervention. Our power was sufficient to rule out changes of the magnitude observed by early repair, but the relative small sample size could not rule out a smaller beneficial effect of late repair compared with no treatment. Although we previously used this model to show the beneficial effects of early repair,18 LV volume was measured differently in the current study (real-time 3-dimensional echocardiography versus...
sequential rotational acquisitions). This may possibly account for the larger variability in volume measurement observed in the previous study. Thus, although the differences in evolution after repair in the 2 studies are striking, direct comparison of LV volume between the studies cannot be made.

Conclusions
In this ischemic MR animal model, late MR repair was not beneficial to prevent additional LV remodeling, in contrast to earlier repair. This suggests a point of no return after which no or limited beneficial effect of MR repair can be achieved, despite adequate correction of volume overload. The timing of valve repair in ischemic MR needs to be assessed in future clinical studies because this could, in part, resolve controversies and discrepant findings in this area.

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Disclosures
None.

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