Blockade of NF-κB improves cardiac function and survival after myocardial infarction

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Kawano, Shunichi, Toru Kubota, Yoshiya Monden, Takaki Tsutsumi, Takahiro Inoue, Natsumi Kawamura, Hiroyuki Tsutsumi, and Kenji Sunagawa. Blockade of NF-κB improves cardiac function and survival after myocardial infarction. Am J Physiol Heart Circ Physiol 291: H1337–H1344, 2006. First published April 21, 2006; doi:10.1152/ajpheart.01175.2005—NF-κB is a key transcription factor that regulates inflammatory processes. In the present study, we tested the hypothesis that blockade of NF-κB ameliorates cardiac remodeling and failure after myocardial infarction (MI). Knockout mice with targeted disruption of the p50 subunit of NF-κB (KO) were used to block the activation of NF-κB. MI was induced by ligation of the left coronary artery in male KO and age-matched wild-type (WT) mice. NF-κB was activated in noninfarct as well as infarct myocardium in WT + MI mice, while the activity was completely abolished in KO mice. Blockade of NF-κB significantly reduced early ventricular rupture after MI and improved survival by ameliorating congestive heart failure. Echocardiographic and pressure measurements revealed that left ventricular fractional shortening and maximum rate of rise of left ventricular pressure were significantly increased and end-diastolic pressure was significantly decreased in KO + MI mice compared with WT + MI mice. Histological analysis demonstrated significant suppression of myocyte hypertrophy as well as interstitial fibrosis in the noninfarct myocardium of KO + MI mice. Blockade of NF-κB did not ameliorate expression of proinflammatory cytokines in infarct or noninfarct myocardium. In contrast, phosphorylation of c-Jun NH2-terminal kinase was almost completely abrogated in KO + MI mice. The present study demonstrates that targeted disruption of the p50 subunit of NF-κB reduces ventricular rupture as well as improves cardiac function and survival after MI. Blockade of NF-κB might be a new therapeutic strategy to attenuate cardiac remodeling and failure after MI.

Nuclear factor-κB (NF-κB) is a key transcription factor that regulates inflammatory processes (1). Recent studies have indicated that NF-κB may play important roles in cardiac hypertrophy and remodeling besides promoting inflammation. First, NF-κB has been shown to be activated in the failing human heart (5, 23), where expression of proinflammatory cytokines is exacerbated (10, 22). Second, in vitro studies have shown that activation of NF-κB is required for hypertrophic growth of cardiomyocytes in response to G protein-coupled receptor agonists, including phenylephrine, endothelin-1, and ANG II (7, 18). Third, recent in vivo studies have demonstrated that blockade of NF-κB ameliorates myocardial hypertrophy in response to aortic banding (12) and chronic infusion of ANG II (9). Finally, blockade of NF-κB improves cardiac function and survival without affecting myocardial inflammation in TNF-α-induced cardiomyopathy (8). Therefore, blockade of NF-κB may be a new therapeutic strategy for heart failure by attenuating myocardial hypertrophy and remodeling.

Myocardial infarction (MI) is a major cause of heart failure in most of the developed countries. NF-κB has been shown to be activated after myocardial ischemia. However, the role of NF-κB in MI remains controversial. Morishita et al. (15) reported that blockade of NF-κB reduced the extent of MI in a rat model of ischemia-reperfusion injury (15), suggesting that activation of NF-κB is cytotoxic in ischemia. The reduction of MI size by NF-κB blockade was also observed in a murine model of ischemia-reperfusion injury (2). In contrast, Misra et al. (14) reported that blockade of NF-κB increased infarct size in a murine model of permanent coronary ligation (14), suggesting that the activation of NF-κB might promote cell survival in MI. Furthermore, no study has investigated the long-term effects of NF-κB blockade on cardiac remodeling and failure late after MI. Therefore, the purpose of the present study was to investigate the role of NF-κB activation in early and late phases of MI using a mouse model of permanent coronary ligation. Mice with targeted disruption of the p50 subunit of NF-κB were used to confer chronic inhibition of NF-κB in vivo (19). The results demonstrated that blockade of NF-κB prevented ventricular rupture early after MI and improved survival by ameliorating cardiac dysfunction in the late phase, suggesting that blockade of NF-κB might be a new therapeutic strategy to attenuate ventricular rupture and remodeling after MI.

MATERIALS AND METHODS

Animal model. Mice with targeted disruption of the p50 subunit of NF-κB (19), backcrossed into the FVB background more than six generations (8, 9), were used to block the activation of NF-κB. These mice were born normally without any major defects. Homoknockout mice (KO) were compared with age- and gender-matched wild-type littermates (WT) in each analysis to minimize the effect of genetic background variation. Male mice at the age of 8–14 wk were used unless mentioned otherwise. We induced MI in both WT and KO mice by ligation of the left coronary artery at 2–3 mm from the tip of the left auricle under pentobarbital sodium anesthesia (50 mg/kg ip) as previously reported (20). Sham operation without coronary artery ligation was also performed in WT and KO mice. After the operation, mice were housed under climate-controlled conditions and were provided standard food and water ad libitum. During the study period of 12 wk.

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cages were inspected daily for animals that had died. All dead mice were examined for the presence of pleural effusion and cardiac rupture as well as MI. The cause of death in each mouse was classified as congestive heart failure when the presence of pleural effusion (serous fluid within the chest wall cavity) and increased lung weight were observed or ventricular rupture when the presence of a blood clot within the pericardial sac was found. This experiment was reviewed and approved by the Committee of the Ethics on Animal Experiment, Kyushu University Graduate School of Medical Sciences and carried out in compliance with the Guideline for Animal Experiment, Kyushu University and the Law (No. 105) and Notification (No. 6) of the Government. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85–23, revised 1996).

Electrophoretic mobility shift assay. Activation of NF-κB was evaluated by electrophoretic mobility shift assays (EMSA) according to the manufacturer’s instructions (Gel Shift Assay System E3300, Promega, Madison, WI). Nuclear protein was isolated from the myocardium as previously reported (8, 9). For supershift reactions, 1 μg of anti-p50 or -p65 antibody (sc-114X or sc-472X; Santa Cruz, Paso Robles, CA) was added after 20 min of binding reaction, with further incubation for 30 min on ice. Samples were resolved on a 5% acrylamide gel in 0.25% Tris-borate-EDTA buffer.

Echocardiographic and hemodynamic measurements. Four weeks after the operation, mice underwent physiological evaluation with echocardiography and left heart catheterization as previously reported (20). After anesthetization with pentobarbital sodium (30 mg/kg body wt ip, Abbott), a mouse was positioned supine. A 7.5-MHz transducer connected to a dedicated ultrasonographic system (SSD-5500 ALOKA) was applied to the left hemithorax. Two-dimensional targeted M-mode imaging was obtained from the short-axis view at the level of the greatest left ventricular (LV) dimension. After echocardiography, a 1.4-F micromanometer-tipped catheter (Millar Instruments) was inserted into the right carotid artery and then advanced into the LV for pressure measurement under additional anesthesia with 2.5% Avertin (3 μl/kg body wt ip, Aldrich Chemical).

Infarct size and myocardial histopathology. After hemodynamic study, the heart was excised and fixed in 4% paraformaldehyde for the evaluation of infarct size and histopathology. Infarct size was determined by methods described previously for rats (17) and also for mice (16, 20). Briefly, the LV was cut from apex to base into four transverse sections. Five-micrometer sections were sliced and stained with Masson’s trichrome. Infarct length was measured along the endocardial and epicardial surfaces from each of the LV sections, and the values from all specimens were summed. Total LV circumference was calculated as the sum of endocardial and epicardial segment lengths from all LV sections. Infarct size (in percent) was calculated as total infarct circumference divided by total LV circumference. Cross-sectional area of cardiomyocytes and collagen volume fraction of noninfarct myocardium were determined by quantitative morphometry of tissue sections as previously reported (20).

RNase protection assay. Multiprobe RNase protection assay (RPA) was performed according to the manufacturer’s protocol (RiboQuant, PharMingen) with 5 μg of total RNA (8, 9). A custom template set containing murine TNF-α; IL-1β; IL-6; transforming growth factor (TGF)-β1; regulated on activation, normal T cell expressed and secreted (RANTES); monocyte chemoattractant protein (MCP)-1; and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was hybridized to the RNA. After RNase digestion, protected probes were resolved on denaturing polyacrylamide gels and quantified by NIH image. The value of each hybridized probe was normalized to that of GAPDH included in each template set as an internal control.

Activity of MAPK. Western blotting analysis was performed by methods described previously (9). Briefly, the noninfarct LV was homogenized with a lysis buffer containing 25 mM Tris, pH 7.4, 150 mM NaCl, 5 mM EDTA, 1 mM NaVO4, 10 mM NaF, 1% (vol/vol) Triton X-100, and 1% (vol/vol) glycerol. Equal amounts of the heart homogenate (30 μg) were separated by SDS-PAGE on 10% (wt/vol) gels, transferred onto a nitrocellulose membrane (Trans-Blot Transfer Medium, Bio-Rad Lab), and blocked with 5% skimmed milk at room temperature for 60 min. The membranes were subjected to immunoblot analyses with anti-phospho-extracellular signal-regulated kinase (ERK) antibody (no. 9106; Cell Signaling Technology), anti-phospho-c-Jun NH2-terminal kinase (JNK) antibody (no. 9255; Cell Signaling Technology), or anti-phospho-p38 antibody (no. 9211; Cell Signaling Technology). Duplicate samples were subjected to immunoblot analyses with anti-ERK antibody (no. 9102; Cell Signaling Technology), anti-JNK1 antibody (sc-474; Santa Cruz Biotechnology), or anti-p38 antibody (no. 9212; Cell Signaling Technology). Immunodetection was accomplished with a horseradish anti-rabbit or anti-mouse secondary antibody (1:2,000 dilution; Amersham) and using an enhanced chemiluminescence kit (Amersham).

Evaluation of infarct size 24 h after coronary ligation. Evans blue dye (1%) was perfused into the aorta and coronary arteries, and tissue sections were weighed and then incubated with a 1.5% triphenyltetrazolium chloride solution at 37°C for 20 min. The infarct area (pale area), the area at risk (nonblue area), and the total LV area from each section were measured, multiplied by the weight of the section, and then totaled from all sections (20).

DNA ladder. Genomic DNA was isolated from the LV using a proteinase K method as previously described (20). To visualize the DNA ladder, fragmented DNA was amplified by ligation-mediated PCR (Maxim Biotech, South San Francisco, CA). Briefly, after overnight ligation with specially designed adapters, 25 ng of DNA in 50 μl of solution was amplified with 35 cycles of PCR and resolved on a 1.5% agarose/ethidium bromide gel.

Statistics. Results are presented as means ± SD. Survival analysis was performed by the Kaplan-Meier methods. ANOVA with Student-Newman-Keuls post hoc test or χ2 test was used for statistical comparison. Differences were considered significant at a value of P < 0.05.

RESULTS

Activation of NF-κB in infarct and noninfarct myocardium. EMSA was performed with nuclear protein isolated from infarct myocardium 24 h after MI and noninfarct myocardium 7 days after MI. Compared with WT + sham-operated mice, NF-κB was further activated in infarct (Fig. 1A) and noninfarct myocardium (Fig. 1B) of WT + MI mice. In contrast, activation of NF-κB was completely abolished in KO + sham-operated and KO + MI mice. Most of NF-κB band in infarct myocardium was supershifted with the anti-p50 antibody (Fig. 1C), suggesting that the majority of NF-κB was p50-p50 homodimers or p50-p65 heterodimers.

Improved survival after MI in NF-κB KO mice. Within 24 h after the operation, 44 of 119 WT + MI (37%) and 46 of 121 KO + MI mice (38%, P = 0.973) died of cardiogenic shock without ventricular rupture or bleeding. In contrast, none of 16 WT + sham-operated and 20 KO + sham-operated mice died after the operation. Survival analysis was performed up to 12 wk in these survived animals. Within 7 days after MI, 25 of 75 WT + MI (33%) and 19 of 75 KO + MI mice (25%, P = 0.370) died. Although the total mortality was not different statistically, the rate of ventricular rupture was significantly lower in KO + MI mice (11 of 75) than WT + MI mice (22 of 75, P < 0.05). As shown in Fig. 2, the survival rate up to 12 wk after MI was significantly higher in KO + MI mice (73.3%) than WT + MI mice (56.0%, P < 0.05). No ventricular rupture was observed after 7 days. All the autopsied mice exhibited marked cardiomegaly and pleural effusion, suggest-
ing that they died of congestive heart failure. These results suggest that blockade of NF-κB may prevent ventricular rupture early after MI and improve the survival with ameliorating congestive heart failure thereafter.

**Attenuated cardiac dysfunction in KO + MI mice.** Cardiac function was evaluated 4 wk after the operation by using echocardiography and left heart catheterization. The results are summarized in Table 1. Echocardiography revealed no significant differences in cardiac morphology and function between WT + sham-operated and KO + sham-operated mice. Although both WT + MI and KO + MI mice had significantly larger LV dimensions and significantly lower fractional shortening than WT + sham-operated mice, LV systolic dimension was significantly smaller and fractional shortening was significantly higher in KO + MI mice than in WT + MI mice. As in echocardiographic parameters, LV pressure parameters were not significantly different between WT + sham-operated and KO + sham-operated mice. LV systolic pressure, maximum rate of rise of LV pressure (+dP/dt max), and peak rate of LV pressure fall (−dP/dt min) were significantly lower and LV end-diastolic pressure was significantly higher in WT + MI mice than in WT + sham-operated mice. In contrast, there were no significant differences in LV systolic pressure, +dP/dt max, −dP/dt min, and end-diastolic pressure between KO + sham-operated and KO + MI mice. LV systolic pressure and +dP/dt max were significantly higher and LV end-diastolic pressure was significantly lower in KO + MI mice than WT + MI mice. These results suggest that LV dysfunction after MI was significantly ameliorated in KO mice.

Infarct size was evaluated after hemodynamic evaluation in each mouse. Because infarct size was not different between WT + MI and KO + MI mice (Table 1), the differences in cardiac function were not attributable to infarct size variation.

**Amelioration of myocyte hypertrophy and interstitial fibrosis in KO + MI mice.** Table 2 summarizes the heart and lung weights 4 wk after the operation. Compared with WT + sham-operated mice, there were significant increases in LV weight, atrial weight, and lung weight in WT + MI mice, consistent with the increased LV end-diastolic pressure after MI. No differences in RV weight, LV weight, atrial weight, and lung weight were observed between WT + sham-operated and KO + sham-operated mice. Compared with WT + MI mice, there were significant decreases in atrial weight and lung weight in KO + MI mice, in agreement with the attenuated elevation of LV end-diastolic pressure in KO + MI mice.

Cross-sectional area of cardiomyocytes and collagen volume fraction of noninfarct myocardium were evaluated with Masson-trichrome staining (Fig. 3A). As summarized in Fig. 3B,
the cross-sectional area of cardiomyocytes in noninfect myocardium was significantly increased in WT + MI mice. In contrast, the cross-sectional area was not increased statistically in KO + MI mice. As summarized in Fig. 3C, collagen volume fraction was significantly increased in WT + MI mice compared with WT + sham-operated mice and was smaller in KO + MI mice. These results indicated that myocyte hypertrophy and interstitial fibrosis in noninfect myocardium after MI were attenuated in KO mice.

**Myocardial expression of cytokines.** Expression of proinflammatory cytokines was assessed by multiprobe RPA (Fig. 4). Proinflammatory cytokines and chemokines, including RANTES, TNF-α, IL-1β, IL-6, TGF-β, and MCP-1, were upregulated in infect myocardium 24 h after MI in KO mice as well as in WT mice (Fig. 4, A and B). Although we had expected that the expression of proinflammatory cytokines and chemokines would be attenuated by NF-κB KO, there were no differences in the expression of IL-1β, IL-6, TGF-β, and MCP-1 between WT + MI and KO + MI mice. On the contrary, the expression of RANTES and TNF-α was enhanced in KO + MI mice.

As in the infect myocardium, expression of proinflammatory cytokines was evaluated in noninfect myocardium 4 wk after MI (Fig. 4, C and D). Compared with WT + sham-operated mice, expression of TNF-α and IL-6 was significantly increased in the noninfect myocardium of WT + MI mice. Blockade of NF-κB activation did not affect IL-6 but rather enhanced TNF-α expression in KO + MI mice. These results suggest that the induction of proinflammatory cytokines in infarct and noninfect myocardium was mediated by NF-κB-independent pathways.

**Phosphorylation of MAP kinases.** Activation of MAP kinases has been shown to play an important role in cardiac hypertrophy and remodeling. As shown in Fig. 5, both ERK and JNK, but not p38, were phosphorylated in noninfect myocardium 7 days after MI in WT mice. There were no significant differences in the protein levels of ERK, JNK, or p38 between WT and KO mice. However, NF-κB KO almost completely abolished the phosphorylation of JNK, although it did not affect that of ERK. The selective abrogation of JNK phosphorylation might play an important role in the attenuation of cardiac remodeling and dysfunction after MI in KO mice.

**Infarct size and apoptosis 24 h after MI.** Because the rate of ventricular rupture was significantly lower in KO + MI mice, another group of animals with WT + MI (n = 5) and KO + MI (n = 6) were evaluated at 24 h after MI to elucidate the underlying mechanisms. As summarized in Table 3, the infarct area in KO + MI mice was significantly lower than that in WT + MI mice, which may suggest less myocardial damage early after MI in KO mice. As indicated by the DNA ladder assay, although apoptosis was increased in the infect myocardium at 24 h after MI, the extent of apoptosis was not different between WT and KO mice (Fig. 6). Therefore, the difference in infarct size may not be attributed to apoptosis.

### Table 2. Heart and lung weights

<table>
<thead>
<tr>
<th></th>
<th>WT + Sham Operated</th>
<th>KO + Sham Operated</th>
<th>WT + MI</th>
<th>KO + MI</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW, g</td>
<td>6</td>
<td>6</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Heart weight/BW, mg/g</td>
<td>30.7±1.5</td>
<td>30.6±2.5</td>
<td>29.9±1.7</td>
<td>30.0±1.9</td>
</tr>
<tr>
<td>Left ventricular weight/BW, mg/g</td>
<td>4.31±0.71</td>
<td>4.28±0.49</td>
<td>5.71±0.75*</td>
<td>4.99±0.49†</td>
</tr>
<tr>
<td>Right ventricular weight/BW, mg/g</td>
<td>2.89±0.49</td>
<td>2.80±0.28</td>
<td>3.64±0.64*</td>
<td>3.23±0.42</td>
</tr>
<tr>
<td>Atrial weight/BW, mg/g</td>
<td>0.75±0.18</td>
<td>0.85±0.20</td>
<td>0.76±0.25</td>
<td>0.78±0.12</td>
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<tr>
<td>Lung weight/BW, mg/g</td>
<td>0.68±0.23</td>
<td>0.63±0.24</td>
<td>1.30±0.37*</td>
<td>0.97±0.28</td>
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Data are means ± SD; n indicates no. of animals studied; BW, body weight. *P < 0.05 vs. WT + sham operated; †P < 0.05 vs. WT + MI.
DISCUSSION

In the present study, we investigated the role of NF-κB in the pathogenesis of cardiac remodeling and heart failure after MI. We confirmed that NF-κB was activated in non-infarct as well as infarct myocardium in WT + MI mice. Targeted disruption of the p50 subunit of NF-κB abrogated the activation of NF-κB and ameliorated myocyte hypertrophy, interstitial fibrosis, and cardiac dysfunction in KO + MI mice, indicating that cardiac remodeling and heart failure after MI is mediated by the activation of NF-κB. In contrast, induction of proinflammatory cytokines and chemokines was not ameliorated but rather enhanced in KO + MI mice, suggesting that myocardial inflammation after MI might be mediated by NF-κB-independent pathways. Although the precise mechanisms by which blockade of NF-κB ameliorated cardiac dysfunction and heart failure after MI remain undetermined, selective abrogation of JNK phosphorylation in KO mice might play an important role. These results indicate that blockade of NF-κB might be a new therapeutic strategy to prevent ventricular remodeling and heart failure after MI.

The role of NF-κB activation in acute myocardial ischemia remains controversial and might be different between transient and sustained myocardial ischemia. Studies using a model of ischemia-reperfusion injury have shown that blockade of NF-κB reduces infarct size and protects myocytes from ischemic insult (2, 15). In contrast, a study using a model of permanent coronary ligation has reported that blockade of NF-κB promotes myocyte apoptosis and increases infarct size 24 h after MI (14). However, the long-term effects of NF-κB blockade after permanent coronary ligation have not been investigated. In the present study, we used a MI model of permanent coronary ligation to evaluate the long-term effects of NF-κB blockade on ventricular remodeling and heart failure. Because we ligated the coronary artery carefully to ensure that the area at risk was consistent, the infarct size evaluated 4 wk after MI was not different between WT + MI and KO + MI mice. However, the rate of ventricular rupture was significantly different between WT + MI and KO + MI mice. All the ventricular ruptures occurred within 7 days after MI, and the incidence was twice higher in WT + MI mice. Because ventricular rupture may reflect an imbalance between myocardial death and repair, the reduction of ventricular rupture in KO + MI mice suggests that blockade of NF-κB might have retarded myocardial cell death and/or enhanced tissue repair and scar formation to maintain integrity of the infarct myocardium. Taken together, these results suggest that the net effect of NF-κB blockade is not harmful but desirable even after permanent occlusion of coronary arteries.

We have previously reported that blockade of NF-κB ameliorates myocardial hypertrophy in response to chronic infusion of ANG II (9) and improves cardiac function and survival in TNF-α-induced cardiomyopathy (8). In the present study, we have demonstrated that blockade of NF-κB improves cardiac function and survival after MI with blockade of NF-κB.
amelioration of myocyte hypertrophy and interstitial fibrosis. Although the precise mechanisms by which NF-κB promotes myocyte hypertrophy remain undetermined, it is of interest that phosphorylation of JNK was abrogated in p50-knockout mice. Because the protein level of JNK is not affected in p50-knockout mice, expression or activation of upstream kinases may be modulated by NF-κB pathways. MAP kinase signaling pathways, including ERK, JNK, and p38, are supposed to play an important role in cardiac hypertrophy and remodeling because they are phosphorylated and activated by G protein-coupled receptor agonists such as phenylephrine, endothelin-1, and ANG II (3, 21). Especially, JNK is also termed the stress-activated protein kinase because it is additionally activated by cellular stresses such as reactive oxygen species and proinflammatory cytokines, including IL-1β and TNF-α (21). Substrates of JNK are transcription factors, including c-Jun, ATF2, and Elk1 (21). Inhibition of JNK has been shown to abrogate ventricular hypertrophy in vivo in response to pressure overload (4) or G_q overexpression (13). Therefore, the attenuated myocyte hypertrophy after MI in p50-knockout mice may be mediated by the abrogation of the JNK pathways.

Although activation of NF-κB has been shown to induce various proinflammatory cytokines and chemokines, including TNF-α (1), we have previously reported that expression of proinflammatory cytokines in response to chronic infusion of ANG II or TNF-α-induced cardiomyopathy was not abrogated by targeted disruption of the p50 subunit of NF-κB (8, 9). In the present study, expression of proinflammatory cytokines in infarct and noninfarct myocardium was not ameliorated but rather enhanced in KO MI mice. These results suggest that expression of proinflammatory cytokines in the failing heart may be mediated by NF-κB-independent pathways. Because blockade of NF-κB improved cardiac function with amelioration of myocyte hypertrophy and interstitial fibrosis in KO + MI mice, myocardial expression of proinflammatory cytokines may not be harmful in the progression of ventricular remodeling and heart failure after MI unless NF-κB is activated.

The paradoxical increase of TNF-α and RANTES in KO + MI mice might be explained by the difference in tran-
The WT MI mice showed an increase in wall thickness compared to the sham-operated group. Although the difference was not statistically significant, the wall thickness in MI mice was significantly greater than that in sham-operated mice, with a ratio of 260/201 = 1.29. Because area is a two-dimensional parameter, the square root of cross-sectional area should correlate with wall thickness. Indeed, the square root of 1.29 is 1.14, which is close to the ratio of wall thickness of 1.18. Because the resolution of echocardiography is limited, it might not be able to consistently differentiate differences in thickness of <20%.

In the present study, to achieve complete and chronic inhibition of NF-κB in vivo, we used gene-manipulated mice lacking the p50 subunit of NF-κB. These mice show no developmental abnormalities but exhibit multifocal defects in B-cell-mediated immune responses and nonspecific responses to infection. In these animals, B cells do not proliferate in response to bacterial lipopolysaccharide and are defective in basal and specific antibody production (19). Although we did not detect any adverse effects or premature death in the p50-knockout mice during the observation period, systemic inhibition of NF-κB may be deleterious in the long run. Therefore, it may be desirable to employ cardiac-specific inhibition of NF-κB to minimize immunological detrimental effects. Furthermore, the results observed in gene-manipulated mice may be different from the outcomes of pharmacological interventions because an inherent deficit of a specific gene may alter the expression and functions of other genes as compensation mechanisms. Therefore, further studies are required to draw a final conclusion that blockade of NF-κB is beneficial and applicable as a therapeutic strategy for patients with MI.

**Table 3. Area at risk and infarct area 24 h after coronary ligation**

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<thead>
<tr>
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<th>WT + MI</th>
<th>KO + MI</th>
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<tr>
<td>n</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Area at risk, %</td>
<td>39.7±2.98</td>
<td>37.9±2.68</td>
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<tr>
<td>Infarct area, %</td>
<td>20.6±2.80</td>
<td>14.9±2.21*</td>
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<tr>
<td>Infarct area/area at risk, %</td>
<td>52.1±5.90</td>
<td>39.4±6.96*</td>
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Data are means ± SD; n indicates no. of animals studied. *P < 0.05 vs. WT + MI.

**GRANTS**

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