Postinfarction Gene Therapy Against Transforming Growth Factor-β Signal Modulates Infarct Tissue Dynamics and Attenuates Left Ventricular Remodeling and Heart Failure

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Background—Fibrosis and progressive failure are prominent pathophysiological features of hearts after myocardial infarction (MI). We examined the effects of inhibiting transforming growth factor-β (TGF-β) signaling on post-MI cardiac fibrosis and ventricular remodeling and function.

Methods and Results—MI was induced in mice by left coronary artery ligation. An adenovirus harboring soluble TGF-β type II receptor (Ad.CAG-sTβRII), a competitive inhibitor of TGF-β, was then injected into the hindlimb muscles on day 3 after MI (control, Ad.CAG-LacZ). Post-MI survival was significantly improved among sTβRII-treated mice (96% versus control at 71%), which also showed a significant attenuation of ventricular dilatation and improved function 4 weeks after MI. At the same time, histological analysis showed reduced fibrous tissue formation. Although MI size did not differ in the 2 groups, MI thickness was greater and circumference was smaller in the sTβRII-treated group; within the infarcted area, α-smooth muscle actin–positive cells were abundant, which might have contributed to infarct contraction. Apoptosis among myofibroblasts in granulation tissue during the subacute stage (10 days after MI) was less frequent in the sTβRII-treated group, and sTβRII directly inhibited Fas-induced apoptosis in cultured myofibroblasts. Finally, treatment of MI-bearing mice with sTβRII was ineffective if started during the chronic stage (4 weeks after MI).

Conclusions—Postinfarction gene therapy aimed at suppressing TGF-β signaling mitigates cardiac remodeling by affecting cardiac fibrosis and infarct tissue dynamics (apoptosis inhibition and infarct contraction). This suggests that such therapy may represent a new approach to the treatment of post-MI heart failure, applicable during the subacute stage. (Circulation. 2005;111:2430-2437.)

Key Words: heart failure • gene therapy • myocardial infarction • transforming growth factors

Myocardial infarction (MI) often leads to left ventricular (LV) remodeling, which is characterized by ventricular dilatation, diminished cardiac performance, and poor recovery of function.1 Thus, patients who escape death during the acute stage of a large MI are at high risk of developing heart failure during the chronic stage. Indeed, patients with postinfarction heart failure account for nearly half of the candidates for cardiac transplantation.2 The extent of the cardiomyocyte death during the acute stage of MI is a critical determinant of the subsequent ventricular remodeling and eventual heart failure, but the complex process of cardiac remodeling is not determined solely by that; hypertrophic responses occur in cardiomyocytes in the surviving portion of the ventricle, followed by ventricular dilatation due to architectural rearrangement of the cardiomyocytes and interstitial cells making up the myocardium.3–5 In that regard, myocardial fibrosis is one of the most characteristic structural changes in infarcted hearts and contributes to both systolic and diastolic dysfunction.6,7

Several lines of evidence point to the critical role played by transforming growth factor-β (TGF-β) during the progression of myocardial fibrosis: (1) TGF-β1 induces increases in both the production and secretion of collagen, increases the abundance of collagen type I and III mRNA in cultured rat cardiac fibroblasts, and stimulates the expression of extracellular matrix proteins in vivo6; (2) in vivo gene transfer of TGF-β1 can induce myocardial fibrosis6; (3) expression of TGF-β is markedly increased in both infarcted and noninfarcted areas...
of hearts after MI; and (4) TGF-β is associated with angiotensin II–mediated fibrosis, whereas inhibition of angiotensin II signaling mitigates post-MI cardiac remodeling and improves function. Collectively, these findings suggest strongly that TGF-β plays a critical role during the healing process after MI and thus affects cardiac remodeling and function during the chronic stage.

Soluble TGF-β type II receptor (sTβRII) inhibits the action of TGF-β, most likely by adsorbing TGF-β or by acting as a dominant negative receptor. In the present study we hypothesized that postinfarction treatment with sTβRII would mitigate chronic heart failure by affecting the LV remodeling process. We therefore constructed a recombinant adenoviral vector expressing the extracellular domain of the TGF-β type II receptor fused to human immunoglobulin Fc and started its transduction into mouse hindlimbs (systemic transfection) on the third day after MI, a time when therapy would not affect acute ischemic death of cardiomyocytes. We then examined the effects on LV structure and function during the chronic stage of MI and sought possible mechanisms responsible for our observations made both in vitro and in vivo.

Methods

Replication-Defective Recombinant Adenoviral Vectors

A replication-defective adenoviral vector, Ad-TβRIEX-Fc, which expresses the extracellular domain of the type II TGF-β receptor13 fused to the Fc portion of human IgG1 under the transcriptional control of cytomegalovirus immediate early enhancer and a modified chicken β-actin promoter, was constructed by in vitro ligation as previously described. Likewise, control Ad-LacZ was prepared as previously described.

Measurement of sTβRII in Plasma

Plasma concentrations of sTβRII after adenoviral transfection were measured in mice (n = 5) by detecting human IgG-Fc with the use of an enzyme-linked immunosorbent assay (Institute of Immunology).

Experimental Protocols

The study was approved by our institutional animal research committee. MI was induced in 10-week-old male C57BL/6J mice (Chubu Kagaku, Nagoya, Japan) by ligating the left coronary artery as previously described. Likewise, control Ad-LacZ was prepared as previously described.

Protocol 1 (Treatment at Subacute Stage)

MI was induced in 75 mice. Of those, 55 survived to the third day after MI and were entered into the study. They were then randomly assigned to sTβRII (n = 27) and LacZ (n = 28) treatment groups and were followed up for 4 weeks after MI. Fifteen sham-operated mice were subjected to either of the treatments (LacZ, n = 7; sTβRII, n = 8) and similarly assessed. In another experiment, on the third day after MI, 10 mice were divided into sTβRII and LacZ treatment groups (n = 5 each), and the survivors (n = 4 in the sTβRII group and n = 3 in the LacZ group) were euthanized on day 10 after MI.

Protocol 2 (In Vitro Experiment)

MI was induced in mice, and 10 days later cardiac myofibroblasts were obtained from the infarcted areas of the hearts according to the method previously described with modification. Briefly, the heart was resected, and the infarcted area was removed. The tissue was then minced and incubated with collagenase type II (Worthington) in Krebs-Ringer buffer for 30 minutes at 37°C. The dissociated cells were plated on 10-cm dishes for 1 hour and then rigorously washed with buffer. The attached remaining nonmyocytes were cultured in DMEM supplemented with 5% mouse serum, which was obtained from mice 7 days after transfection with Ad.CAG-TβRII or Ad.CMV-LacZ. The cells were used for experimentation during the second and third passages. More than 90% of the cells were found to be α-smooth muscle actin (SMA) positive. A mixture of agonistic anti-Fas antibody (1 µg/mL; Pharmingen) and actinomycin D (0.05 µg/mL; Sigma) was applied for 24 hours to induce apoptosis.

Protocol 3 (Treatment at Chronic Stage)

MI was induced in 33 mice that were subsequently observed for 4 weeks with no treatment. At that time, scarring was well established in the infarcts of the 25 surviving mice, and gene treatment with LacZ (n = 11) or sTβRII (n = 14) was started. These mice were then examined 4 weeks after an additional 4 weeks (8 weeks after MI). In another set of animals, we evaluated sTβRII in the 5-week-old infarcted area (1 week after viral injection) by Western blot using LacZ gene– and sTβRII gene–treated hearts (n = 3 each). This was to confirm accessibility of sTβRII into scar tissue.

Physiological Studies

Echocardiograms were recorded 4 weeks after MI with the use of an echocardiographic system (Aloka) equipped with a 7.5-MHz imaging transducer. The right carotid artery was then cannulated with a micromanometer-tipped catheter (SPR 407, Millar Instruments) that advanced into the left ventricle via the aorta for recording pressures and ±dP/dt.

Histological Analysis

After the physiological analyses, all surviving mice were euthanized, and their hearts were removed. The excised hearts were cut into 2 transverse slices; the basal specimens were fixed in 10% buffered formalin and embedded in paraffin, after which 4-µm-thick sections were stained with hematoxylin-eosin, Masson’s trichrome, and Sirius red F3BA (0.1% solution in saturated aqueous picric acid) (Aldrich). Quantitative assessments of cell size, cell population, and fibrotic area were performed on 20 randomly chosen high-power fields (HPF) in each section with the use of a LUXEX F multipurpose color image processor (Nireco). Quantitative assessments of cardiomyocyte size (as the transverse diameter), cell population, vessel population, and fibrotic area were performed on 20 randomly chosen HPF in each section with a LUXEX F multipurpose color image processor (Nireco). The number of cardiomyocytes evaluated was 198 ± 12 cells per heart. Vessels were identified as the lumens outlined by Flik-1–positive endothelial cells on the Flik-1–immuno-stained sections.

Immunohistochemical Analysis

Deparaffinized 4-µm-thick sections or cultured cells were incubated with primary antibody against α-SMA (Sigma), Flk-1 (Santa Cruz), or pan-leukocyte antigen (CD45, Pharmingen), after which they were immunostained with dianobenzidinhydrochloride or labeled with immunofluorescent Alexa Fluor 488 or 568 (Molecular Probes). Nuclei were stained with hematoxylin or Hoechst 33342. Immunohistochemical analysis was performed with the use of the in situ terminal deoxynucleotidyl transferase–mediated nick-end labeling (TUNEL) method with an ApopTag kit (Intergene) as previously described.

For double immunofluorescence, tissue sections or cells were stained first with the use of an FITC-conjugated ApopTag kit (Integen) and then with anti-α-SMA or anti–Flk-1 followed by labeling with Alexa Fluor 568.

Western Blotting

Proteins (100 µg) extracted from hearts in protocol 1 were subjected to 14% polyacrylamide gel electrophoresis and then transferred onto polyvinylidene difluoride membranes. The membranes were then probed with the primary antibody against matrix metalloproteinase-2 (MMP-2) (Daiichi Fine Chemical Co) or atrial natriuretic peptide (ANP) (Santa Cruz).
Infarct tissues were subjected to Western blotting for sTβRII by anti-human IgG antibody (DAKO). The blots were visualized by means of chemiluminescence (ELC, Amersham), and the signals were quantified by densitometry. β-Actin (analyzed with antibody from Sigma) was the loading control.

Statistical Analysis
Values are shown as mean±SEM. Survival was analyzed by the Kaplan-Meier method with the log-rank Cox-Mantel method. The significance of differences was evaluated with Student t tests. Values of P<0.05 were considered significant.

Results
Plasma Levels of Exogenous sTβRII
Among mice receiving sTβRII gene transfection, the plasma levels of exogenous sTβRII reached 23.7±4.3 and 49.0±5.4 μg/mL, respectively, 3 and 7 days after the injection (6 and 10 days after MI, respectively), a time when the infarcted area was composed of granulation tissue (Figure 1A). Levels declined steeply thereafter, and sTβRII was undetectable in the plasma 2 weeks after MI. No sTβRII was detected in the LacZ-treated mice at any time. Accessibility of sTβRII into scar tissue was confirmed by Western blotting (Figure 1B). All sham-operated mice survived until 4 weeks after surgery.

Effect of Anti–TGF-β Treatment at Subacute Stage (Protocol 1)
Four Weeks After MI
The survival rate was significantly higher among sTβRII-treated mice than among LacZ-treated control mice 4 weeks after MI (Figure 2A): 26 of 27 mice (96%) in the sTβRII-treated group survived versus 20 of 28 mice (71%) in the control group (P<0.05).

Echocardiography and cardiac catheterization performed 4 weeks after MI showed control mice to have severe LV remodeling with marked enlargement of the LV cavity and signs of reduced cardiac function compared with the sham-operated mice (Figure 2B): decreased LV percent fractional shortening and ±dP/dt and increased LV end-diastolic pressure. These parameters were all attenuated in sTβRII-treated mice (Figure 2B), indicating mitigation of postinfarct remodeling and improved cardiac function. In the sham-operated mice, there was no significant difference in cardiac function 4 weeks after surgery between the sTβRII gene– and LacZ gene–treated group, indicating a negligible effect of sTβRII treatment on cardiac function of sham-operated mice (data not shown).

There was no significant difference in heart weights (Lac Z, 166±9 mg versus sTβRII, 168±6 mg) or in ratios of heart weight to body weight (Lac Z, 6±0.2 mg/g versus sTβRII, 6±0.3 mg/g) between the groups. Although hearts from LacZ-treated mice showed marked LV dilatation with a thin
infarcted segment 4 weeks after MI, those from sTβRII-treated mice presented smaller LV cavities (Figure 3A1 and 3A2). Both the absolute area of the infarct and the percentage of the whole LV area taken up by the infarct were comparable between the LacZ- and sTβRII-treated mice (Figure 3B and 3C). On the other hand, the circumferential length of the infarcted segment was shorter and the infarct was thicker in the sTβRII-treated mice (Figure 3D and 3E).

By 4 weeks after MI, the infarcted areas of LacZ-treated mice had been replaced by fibrous scar tissue (Figure 4A1). The infarcts of sTβRII-treated mice, by contrast, contained not only collagen fibers but also numerous cells (Figure 4A2). The noncardiomyocyte population in the infarcted areas was significantly greater in the sTβRII-treated mice (Figure 4A3), as was the percent infarcted area taken up by extravascular α-SMA–positive cells (Figure 4B2). Asterisk in B2 indicates a bundle of α-SMA–positive cells. C, Sirius red–stained preparations of noninfarcted (C1 and C2) and infarcted (C4 and C5) areas in LacZ-treated (C1 and C4) and sTβRII-treated (C2 and C5) mice; graphs show percentage of noninfarcted (C3) and infarcted (C6) areas taken up by collagen fibers.

control (0.9±0.1 cells per HPF) and sTβRII-treated hearts (0.8±0.2 cells per HPF; P=NS). The amount of fibrosis assessed in Sirius red–stained sections was significantly reduced in the noninfarcted LV walls and in the infarct region of the sTβRII-treated mice (Figure 4C1 to 4C6). MMP-2 in hearts with 4-week-old MI was greater in hearts with MI compared with the sham-operated hearts, but it was not significantly affected by the sTβRII treatment (Figure 5A and 5B), suggesting a negligible association of the gelatinase activity with sTβRII-induced antifibrosis in the present experimental setting. In addition, the transverse diameters of cardiomyocytes in the noninfarcted areas were significantly greater in the LacZ-treated (17.7±0.3 μm) than in the sTβRII-treated (15.1±0.3 μm) mice (Figure 5C), suggesting that the compensatory cardiomyocyte hypertrophy was more developed in the control mice. Consistent with this finding, Western blot analysis revealed reduced ANP expression in the sTβRII-treated hearts (Figure 5A and 5B).
Ten Days After MI

By 10 days after MI, the infarcted areas were composed of granulation tissue, and TUNEL assays indicated that apoptosis was ongoing in both the LacZ- and sTβRII-treated groups. However, the incidence of TUNEL-positive cells was significantly smaller in the sTβRII-treated than in the LacZ-treated group (Figure 6A1 to 6A3). Moreover, double-immunofluorescence assays (TUNEL followed by anti–Flk-1 or anti–α-SMA antibody) revealed that within the sTβRII-treated group, the incidence of apoptosis was reduced among myofibroblasts/smooth muscle cells (Figure 6B1 and 6B2) but not among endothelial cells (LacZ, 3.5±0.5% versus sTβRII, 3.5±0.2%; P=NS), which suggests that sTβRII may specifically inhibit apoptosis among myofibroblasts. TUNEL-positive cardiomyocytes were extremely rare (<0.01%) in both groups.

Effect of sTβRII on Fas-Induced Apoptosis In Vitro (Protocol 2)

Myofibroblasts obtained from the infarcted areas of mouse hearts 10 days after MI were cultured in medium containing 5% serum collected from LacZ- or sTβRII-treated mice. When the cells were then subjected to Fas-induced apoptosis for 24 hours, the incidence of TUNEL-positive myofibroblasts was significantly lower among cells cultured with sTβRII-containing serum (16±2.9%) than among those cultured with normal serum (43±5.2%; P<0.05) (Figure 7). However, such an apoptosis-inhibitory effect by sTβRII-containing serum was completely canceled by an addition of TGF-β1 at the concentration of 1 μg/mL (Figure 7). These findings suggest that sTβRII exerts a direct antiapoptotic effect on cardiac myofibroblasts.

Effect of Anti–TGF-β Treatment at Chronic Stage (Protocol 3)

Using protocol 3, we determined the extent to which inhibiting apoptosis among granulation tissue cells is responsible for the beneficial effects on post-MI heart failure. For this purpose, the sTβRII gene therapy was started at a more chronic stage of MI, after the granulation tissue had already been replaced with scar tissue. The sTβRII (n=14) or LacZ (n=11) gene was delivered to mice 4 weeks after MI, and the mice were examined after an additional 4 weeks (8 weeks after MI). Accessibility of sTβRII into scar tissue was confirmed by Western blotting (Figure 1B). One of 14 sTβRII-treated mice and none of the 11 LacZ-treated mice died during the additional 4-week follow-up (P=NS). This time we found no difference in ventricular geometry or function between the sTβRII-treated and LacZ-treated groups (Table), clearly indicating that the preventive effect of sTβRII gene therapy on heart failure is attributable to its action on granulation tissue during the subacute stage of MI.

Discussion

The present study revealed that postinfarction sTβRII gene therapy, begun at the subacute stage of MI, alleviated adverse remodeling and improved function of the LV during the chronic stage. In addition, we provide novel insights into the
mechanism of the beneficial effect of the TGF-β signal inhibition.

Mechanisms of Beneficial Effects of sTβRII on Postinfarction Heart Failure

The mechanisms responsible for the beneficial effects of inhibiting TGF-β signaling on post-MI heart failure appear somewhat complicated, probably reflecting the multiple biological effects of TGF-β. TGF-β signaling acts as a strong inducer of extracellular matrix and as an immunomodulator of chemotaxis by fibroblasts and inflammatory cells.18–20 In the infarcted heart, TGF-β expression is regulated by locally generated angiotensin II via angiotensin II type 1 receptor binding, and angiotensin-converting enzyme inhibitors and angiotensin II type 1 receptor blockade attenuated postinfarction ventricular remodeling equally.11,12 However, the manner in which direct inhibition of TGF-β signaling in the infarcted heart affects the postinfarction process has not been well elucidated. In the present study inhibition of TGF-β signaling by exogenous sTβRII significantly reduced cardiac fibrosis, confirming the fibrogenetic effect of TGF-β on post-MI hearts. Because myocardial fibrosis contributes to both systolic and diastolic dysfunction, reducing it by inhibiting TGF-β signaling is one way in which to mitigate LV remodeling and heart failure. MMP-2 activity seemed to be not significantly associated with sTβRII-induced antifibrosis in the present experimental setting.

Perhaps the most notable finding of the present study is the effect of anti–TGF-β therapy on infarct geometry, ie, the shortening of the infarcted segment and the thickening of the infarcted wall, without a change in absolute infarcted area. Contraction of the infarcted tissue likely contributes to suppression of LV dilatation. Because wall stress is proportional to the cavity diameter and inversely proportional to the wall thickness (Laplace’s law)21 and because wall stress and adverse LV remodeling (dilatation) have a vicious relationship, accelerating one another, it is easy to surmise that such an alteration in the geometry of the infarct would markedly improve the hemodynamic state of the heart.

Figure 7. Effect of sTβRII-containing sera on Fas-induced apoptosis among cultured nonmyocytes obtained from the infarcted tissue of hearts 10 days after MI (protocol 2). Confocal micrographs (A) show TUNEL-positive/α-SMA–positive cultured cells. B, Percentage of apoptotic myofibroblasts.

<table>
<thead>
<tr>
<th></th>
<th>LacZ (n=11)</th>
<th>sTβRII (n=13)</th>
<th>P</th>
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<tr>
<td>LVED diameter, mm</td>
<td>6.0±0.1</td>
<td>6.3±0.2</td>
<td>0.11</td>
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<tr>
<td>% Fractional shortening</td>
<td>16.7±0.6</td>
<td>16.1±0.6</td>
<td>0.53</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>502±21</td>
<td>544±20</td>
<td>0.16</td>
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<tr>
<td>LVSP, mm Hg</td>
<td>85±3</td>
<td>80±3</td>
<td>0.21</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>9±1</td>
<td>9±1</td>
<td>0.84</td>
</tr>
<tr>
<td>+dP/dt, mm Hg/s</td>
<td>3847±101</td>
<td>3608±150</td>
<td>0.22</td>
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<tr>
<td>−dP/dt, mm Hg/s</td>
<td>3642±166</td>
<td>3337±144</td>
<td>0.18</td>
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<tr>
<td>MI area, ×10^3 μm^2</td>
<td>2.1±0.2</td>
<td>2.4±0.3</td>
<td>0.53</td>
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<tr>
<td>% MI area</td>
<td>21.5±3.2</td>
<td>24.2±3.0</td>
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<tr>
<td>MI segmental length, ×10^3 μm</td>
<td>6.1±0.9</td>
<td>6.4±0.7</td>
<td>0.78</td>
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<td>MI wall thickness, ×10^2 μm</td>
<td>2.4±0.4</td>
<td>2.0±0.2</td>
<td>0.43</td>
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<tr>
<td>% Fibrosis in non-MI area</td>
<td>17.7±1.8</td>
<td>18.1±1.9</td>
<td>0.88</td>
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<tr>
<td>% Fibrosis in MI area</td>
<td>50.6±3.6</td>
<td>51.5±3.9</td>
<td>0.56</td>
</tr>
<tr>
<td>Cell population in MI area, cells/10^5 μm^2</td>
<td>149±6</td>
<td>144±5</td>
<td>0.56</td>
</tr>
<tr>
<td>Area of α-SMA–positive cells, %</td>
<td>1.9±0.9</td>
<td>2.0±0.2</td>
<td>0.75</td>
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LVED indicates LV end-diastolic; LVSP, LV peak systolic pressure; and LVEDP, LV end-diastolic pressure.
Inhibition of TGF-β signaling also qualitatively altered the infarct tissue. We found an increased abundance of α-SMA–positive cells (myofibroblasts and smooth muscle cells) in the extravascular area of infarcts in stBRII-treated hearts. Those cells are well known to play an important role in wound contraction during the healing process,22 and to then disappear via apoptosis.23,24 Recently, we reported that blockade of myofibroblast apoptosis by the treatment with pan-caspase inhibitor or with soluble Fas, a competitive inhibitor of Fas, attenuates postinfarction ventricular remodeling and heart failure.25,26 We speculate that the preserved myofibroblasts may contribute structurally to the thickening of the infarct scar. In addition, although the property of contractile function of these myofibroblasts has not been elucidated, it is conceivable that contractile myofibroblasts that are running parallel with the infarct circumference may shrink the infarct into coronal directions and increase the infarct thickness.

It is thus notable that stBRII had a direct inhibitory effect on apoptosis among myofibroblasts in granulation tissue, both in vivo and in vitro. This is consistent with the report by Hagimoto et al,27 who showed that TGF-β1 sensitizes pulmonary epithelial cells to Fas-induced apoptosis. Conversely, TGF-β is known to promote transdifferentiation of fibroblasts into myofibroblasts,28 ie, inhibition of TGF-β signaling possibly results in reduction of myofibroblast population. Inhibition of TGF-β signaling thus appears to have reciprocal effects on myofibroblast population: its reduction through interferring with transdifferentiation from fibroblasts and its augmentation through blocking apoptotic death. In the present experimental setting, the gene product peaked during the granulation tissue phase (1-week-old infarct) when myofibroblasts were already abundant but their apoptosis was ongoing. In the 4-week-old infarct tissue, however, naturally occurring apoptosis was already complete in the control MI hearts. These findings may explain our data that the population of α-SMA–positive cells was balanced to gain in the post-MI scar tissue of the TGF-β signal–inhibited hearts. Taken together, these findings suggest that myofibroblasts escaping apoptosis may survive even during the chronic stage of MI, accumulate, form bundles, and contribute to infarct contraction. In addition, this mechanism appears critical for functional improvement, as transfection of the stBRII gene was ineffective if started during the chronic stage of MI, when most α-SMA–positive cells have already disappeared (see protocol 3 above).

Because in the present study stBRII gene therapy was started on the third day after MI, it is unlikely that it influenced cardiomyocyte apoptosis during the acute stage. It is also unlikely that this therapy affected cardiomyocyte survival by inhibiting apoptosis at the subacute or chronic stages. This is because, in contrast to an earlier report,4 we found that apoptosis was negligible among cardiomyocytes at any stage of MI.

**Time Window Within Which to Inhibit TGF-β Signaling**

TGF-β signaling is believed to have cardioprotective effect during ischemia/reperfusion, perhaps as a result of inhibition of tumor necrosis factor-α release, improvement of endothelium-dependent relaxation, prevention of reactive oxygen species generation, and/or inhibition of upregulation of matrix metalloproteinase-1.29,30 For these reasons, inhibition of TGF-β signaling during the acute stage of MI is considered harmful. In addition, our data indicate that late inhibition of TGF-β signaling (during the scar phase of MI) is without effect. It thus appears that there is a therapeutic time window that is critical for inhibition of TGF-β signaling to elicit the beneficial effects on post-MI heart failure.

**Limitations and Clinical Implications**

There is considerable evidence indicating that the TGF-β signal exerts a protective effect against atherosclerosis in mouse models by preventing lipid lesion formation.31–33 This potential limitation might have to be taken into account in application of the anti–TGF-β strategy.

Rapid recanalization of the occluded coronary artery is presently the best clinical approach to the treatment of acute MI; if performed in time, it enables salvage of the ischemic myocardial cells. Unfortunately, most patients miss the chance for coronary reperfusion therapy because to be effective it must be performed within a few hours after the onset of infarction.34 The present findings suggest that this novel therapeutic strategy may mitigate the chronic progressive heart failure seen in patients after large MIs. When initiated during the subacute stage, inhibition of TGF-β signaling may benefit patients who missed the chance for coronary reperfusion.

**Acknowledgments**

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