Targeted inhibition of activin receptor-like kinase 5 signaling attenuates cardiac dysfunction following myocardial infarction

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CARDIOVASCULAR DISEASE is the leading cause of mortality in the developed world. It is estimated that around 30% of the total health care expenditure on cardiovascular disease in the United States in 2009 would be due to coronary heart disease (21a). Despite current advances in the prevention and management of coronary heart disease and acute myocardial infarction (MI), heart failure post-MI remains a significant clinical problem with substantial morbidity, hospitalization rates, and mortality (33). Novel therapies are urgently required to improve the long-term prognosis and to reduce the morbidity of patients following MI.

Post-MI remodeling describes a sequence of events leading to infarct expansion, thinning of the myocardium at the site of infarct, compensatory eccentric hypertrophy of the viable myo-cardium, and progressive dilatation of the left ventricle (LV) leading to heart failure and ultimately death (33). The deposition of fibrillar type I and type III collagen, which are the principal structural components of the interstitium in the peri-infarct and noninfarct zone (NIZ) of MI hearts undergoing remodeling, is a key contributor to both systolic and diastolic dysfunction (14). In addition, myocyte hypertrophy that occurs post-MI is a marker for poor prognosis in advanced heart failure (7). Thus both pathological fibrosis and hypertrophy remain key characteristic findings of cardiac remodeling.

A pivotal mediator in the pathogenesis of cardiac remodeling is the profibrotic cytokine transforming growth factor-β (TGF-β) (12, 19). Upon binding of TGF-β to the constitutively active type II receptor, type I receptor, which is also termed activin receptor-like kinase 5 (ALK5), is recruited and activated. ALK5 then phosphorylates and activates Smad2/3, which forms a functional transcription complex with Smad4 to initiate transcription responses (12). ALK5 activity is an integral component in the TGF-β-mediated regulation of the extracellular matrix (ECM) (19) and indirectly the hypertrophic effects of TGF-β signaling in cardiomyocytes (28).

Previous work by our group has focused on the functional and structural effects of TGF-β inhibition using various approaches in experimental diabetic nephropathy and diabetic cardiomyopathy (16, 22, 23). However, toxicity has prevented these strategies from moving to human clinical trials. More recently, 4-[4-[3-(pyridin-2-yl)-1H-pyrazol-4-yl]pyridin-2-yl]-N-(tetrahydro-2H-pyran-4-yl)benzamide (GW788388), an orally active ALK5 inhibitor, has shown promise in the attenuation of TGF-β activity and has been reported to reduce the expression of collagen IA1 in a rat model of puromycin aminonucleoside-induced renal fibrosis (10) and to attenuate diabetic nephropathy in db/db mice (25).

In this study we thus aimed to further explore the effects of the novel TGF-β receptor inhibitor, GW788388, in a rat model of MI and to examine whether the prevention of LV remodeling by blocking TGF-β signaling would attenuate cardiac dysfunction post-MI.

METHODS

Animal model and tissue preparation. MI was induced in 30 male Sprague-Dawley rats, aged 8–10 wk, by ligation of the left anterior descending coronary artery as previously described (1). Briefly, the animals were anesthetized with a gaseous mixture of 95% oxygen and 5% isoflurane and were intubated and connected to a ventilator from which they received 3% isoflurane-97% oxygen to maintain anesthesia during the course of surgery. The animals were also given subcutaneous buprenorphine (0.03 mg/kg) for analgesia. Another 15 sham-operated animals underwent thoracotomy and incision of pericardial sac but not left anterior descending coronary artery ligation.

Heart weight-to-body weight ratio and left ventricular hypertrophy. Heart weight-to-body weight ratio and left ventricular hypertrophy were measured by weighting the heart and left ventricle at the end of the treatment period. The heart weight-to-body weight ratio was calculated as heart weight divided by body weight at the end of the treatment period. The left ventricular hypertrophy was calculated as the ratio of left ventricular weight to body weight at the end of the treatment period. The heart weight-to-body weight ratio and left ventricular hypertrophy were calculated as the mean ± SEM for each group.
Table 1. Baseline characteristics of sham-operated and MI rats treated with or without ALK5 inhibitor at 35 days

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Sham + GW</th>
<th>MI</th>
<th>MI + GW</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>9</td>
<td>6</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>BW, g</td>
<td>410 ± 11</td>
<td>435 ± 7</td>
<td>402 ± 8</td>
<td>433 ± 17</td>
</tr>
<tr>
<td>LV/BW, mg/g</td>
<td>2.07 ± 0.06</td>
<td>2.07 ± 0.04</td>
<td>2.26 ± 0.04*</td>
<td>2.15 ± 0.1</td>
</tr>
<tr>
<td>Lung/BW, mg/C</td>
<td>3.29 ± 0.1</td>
<td>3.41 ± 0.2</td>
<td>4.19 ± 0.1*</td>
<td>3.09 ± 0.1†</td>
</tr>
</tbody>
</table>

Values are means ± SE; N, rats/group. ALK5, activin receptor-like kinase 5; MI, myocardial infarction; GW, GW788388; LV/BW and Lung/BW, ratios of left ventricle (LV) and lung weight to total body weight (BW), respectively. *P < 0.05 vs. sham; †P < 0.05 vs. MI.

Five MI animals did not survive the surgery and were excluded from the study.

One week postsurgery, sham-operated (N = 6) and infarcted animals (N = 10) were randomized to treatment with the ALK5 inhibitor GW788388 (GSK) at a dosage of 50 mg·kg⁻¹·day⁻¹ by gavage, which has been shown to significantly attenuate collagen overexpression in a rodent model of dimethylacetamide-induced liver disease (10). Untreated rats, that is, sham-operated (N = 9) and MI animals (N = 15), were gavaged with vehicle (1% carboxymethyl cellulose solution). Four animals with <25% infarct size as determined postmortem by histology were excluded from further analyses.

Animals were housed and maintained at a constant room temperature (21 ± 1°C) and 12-h:12-h light-dark cycle and were fed standard chow and given water ad libitum. Four weeks later, the animals were euthanized with Lethabarb (pentobarbitone; 60 mg/kg) by intra-peritoneal injection. LV was separated, blotted once, and weighed. The LV was then sectioned immediately into three parts where the top was fixed in optimum cutting temperature compound, the middle was fixed in neutral-buffered formalin, and the bottom was snapped frozen (−80°C).

Experimental procedures adhered to guidelines of the National Health and Medical Research Council of Australia’s Code for the Care and Use of Animals for Scientific Purpose and conformed by the National Institutes of Health (NIH Publication NO. 85-23, Revised 1996) and were approved by the Bioethics Committee of St. Vincent’s Hospital (Melbourne, Australia).
antibody (Dako). Furthermore, the infiltration of macrophages was examined using the mouse monoclonal rat macrophage marker (CD68/ED1, Serotec, Raleigh, NC).

For immunohistochemistry, tissue sections were dewaxed and washed in distilled water before an antigen retrieval step was performed to unmask antigens. The sections were then incubated with 3% hydrogen peroxide (Sigma-Aldrich) for 15 min to quench any endogenous peroxidase activity. The tissue sections were incubated with 1:10 normal goat serum or normal swine serum for 30 min before being incubated overnight at 4°C with the primary antibody. The following day, the slides were washed thoroughly with PBS and treated with biotinylated goat anti-mouse or anti-rabbit antibody (Dako) for 1 hr rabbit anti-goat (1:200)-biotinylated IgG (Dako) for 1 h, followed by avidin-biotin peroxidase complex for another hour (Vector). The localization of the peroxidase conjugates was achieved using 3,3'-diaminobenzidine tetrahydrochloride (Dako) as a chromagen.

Western blot analyses of heart tissue. The content of Smad2/3 was assessed by using specific mouse anti-Smad2/3 antibody (BD Biosciences, San Jose, CA), and activated (phosphorylated) Smad2 was examined using rabbit antibody that detects endogenous levels of Smad2 only when dually phosphorylated at Ser465 and Ser467 (Cell Signaling, Beverly, CA).

Western blot analysis was performed as previously described (1). In brief, small pieces of frozen heart tissue were homogenized in ice-cold lysis buffer containing 50 mM Tris (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 0.5% Triton X-100 containing 1 mM Na3VO4, 50 mM NaF, 25 mM β-glycerophosphate, 10 mM Na

Table 2. Echocardiographic characteristics of sham-operated and MI rats treated with or without ALK5 inhibitor at 35 days

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Sham + GW</th>
<th>MI</th>
<th>MI + GW</th>
</tr>
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<tbody>
<tr>
<td>HR, beats/min</td>
<td>259 ± 36</td>
<td>285 ± 14</td>
<td>234 ± 15</td>
<td>259 ± 10</td>
</tr>
<tr>
<td>LVIDd, cm</td>
<td>0.832 ± 0.02</td>
<td>0.850 ± 0.04</td>
<td>0.965 ± 0.03*</td>
<td>0.879 ± 0.08†</td>
</tr>
<tr>
<td>LVIDs, cm</td>
<td>0.516 ± 0.03</td>
<td>0.524 ± 0.04</td>
<td>0.779 ± 0.04*</td>
<td>0.675 ± 0.11*†</td>
</tr>
<tr>
<td>FS, %</td>
<td>38.12 ± 2.0</td>
<td>38.62 ± 1.9</td>
<td>19.51 ± 2.2*</td>
<td>25.48 ± 6.4*†</td>
</tr>
<tr>
<td>EF (M-mode), %</td>
<td>73.17 ± 2.4</td>
<td>73.95 ± 2.5</td>
<td>43.67 ± 4.1*</td>
<td>51.78 ± 10.3*†</td>
</tr>
<tr>
<td>FAC, %</td>
<td>56.55 ± 2.9</td>
<td>56.66 ± 3.6</td>
<td>34.73 ± 2.2*</td>
<td>45.79 ± 6.1*†</td>
</tr>
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</table>

Values are means ± SE. HR, heart rate; LVIDd and LVIDs, left ventricular internal diameters in diastole and systole, respectively; FS, fractional shortening; EF, ejection fraction; FAC, fractional area change. *P < 0.05 vs. sham; †P < 0.05 vs. MI.

Fig. 2. Representative images of transforming growth factor-β (TGF-β) immunostaining (brown) in the noninfarct zone (NIZ) of the hearts of sham-operated (A), sham-operated treated with GW788388 (B), MI (C), and MI treated with GW788388 (D) rats. There was a significant increase of TGF-β immunostaining in the MI animals, and this increase was not affected by ALK5 inhibition. Quantitation of TGF-β immunostaining in the NIZ is shown (E). Values are expressed as means ± SE. *P < 0.01 vs. sham. Original magnification, ×200.
pyrophosphate, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 1 mM PMSF, and 1 mM DTT. After 30 min incubation on ice, the lysates were centrifuged to remove cell debris. Protein concentration was determined using Bradford reagent (Bio-Rad, Hercules, CA) and standardized with a known amount of BSA.

Protein (100 μg) was resolved on a 10% SDS-polyacrylamide gel by denaturing discontinuous gel electrophoresis according to the Laemmli method and transferred to a polyvinylidene difluoride membrane (Roche, Mannheim, Germany). Nonspecific binding sites were blocked for 1 h [5% nonfat milk and Tris-buffered saline, and 0.1% Tween 20 (TBST)], after which the membranes were exposed to primary antibodies diluted in 5% nonfat milk and TBST overnight at 4°C. The membranes were then washed three times with TBST and incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. The membranes were washed three times and the proteins were detected by enhanced chemiluminescence system (Roche). The membranes were washed with TBST and incubated with blocking solution (5% nonfat milk in TBST) again before being immunobotted with pan-actin, which served as the loading control. The bands corresponding to Smad2/3 (58 kDa), phospho-Smad2 (60 kDa), and pan-actin (42 kDa) were quantitated using gel documentation (Bio-Rad) and quantitated by densitometry using Quantity One Software (Bio-Rad). At least six samples were analyzed from each group in four separate gels.

**Infarct size.** Picrosirius red-stained slides were examined under light microscopy, digitized, and then analyzed using image analysis (AIS, Analytical Imaging Station Version 6.0, Ontario, Canada). Infarct size was assessed morphologically and calculated as the ratio of scar average circumferences of the endocardium and the epicardium to LV average circumferences of the endocardium and the epicardium, as previously described (1).

**Quantification of matrix deposition and TGF-β, phospho-Smad2, and α-SMA expression.** All analyses of the changes in cardiac structure were assessed in a masked protocol using 6 to 10 tissue sections from each experimental group. The accumulation of matrix and the extent of immunostaining of phospho-Smad2, TGF-β, α-SMA, collagen I, and collagen III were quantified using computer-assisted image analysis software. In brief, 10 random nonoverlapping fields from each stained section around the NIZ were captured and digitized using an AxioImager.A1 microscope (Carl Zeiss AxioVision) at ×200 magnification. The digital images were then loaded onto a Pentium D Dell computer. The areas of red on picrosirius red-stained sections (for matrix) or brown on immunostained sections (for phospho-Smad2, TGF-β,
α-SMA, collagen I, and collagen III) were highlighted using a selective color tool for their color ranges, and the proportional area of the tissue with their respective ranges of color was then quantified. The calculation of the proportional area stained red or brown was then determined using image analysis software AIS (Analytic Imaging Station version 6.0, Imaging Research).

**Measurement of cardiomyocyte hypertrophy.** To assess the morphology of the myocytes, a tissue section from each LV was stained with hematoxylin and eosin stain. The measurement of mean myocyte cross-sectional area (CSA) was performed as previously described (15). In brief, circular-shaped cardiomyocytes were selected and images were taken at ×200 magnification. Up to 20 fields per LV were assessed, and only myofibers with intact cellular membranes from fields were assessed and the circumferences of carefully selected 40 to 50 cells per LV were traced and digitized to calculate mean CSA using image analysis software AIS (Analytic Imaging Station version 6.0, Imaging Research).

**Quantification for ED1/CD68-positive cells.** The macrophage number was estimated by manually counting the CD68/ED1-positive cells under a standard light microscope under ×400 magnification in 10 randomized nonoverlapping fields in the NIZ.

**Statistical analyses.** Data are expressed as means ± SE. Statistical significance was determined by ANOVA with a Fisher post hoc comparison. All analyses were performed using StatView II and graphics package (Abacus Concepts, Berkeley, CA). P < 0.05 was considered as statistically significant unless otherwise stated.

**RESULTS**

**Animal characteristic.** There was no difference in body weight observed across all experimental groups. However, MI rats had a significant increase in LV weight-to-body weight ratio, which was not effected by ALK5 inhibition (Table 1). MI rats also had a significant increase in the lung weight-to-body weight ratio, and this was significantly attenuated by the treatment with the ALK5 inhibitor (Table 1).

**Echocardiography.** After 5 wk postsurgery, MI rats showed a significant increase in LVIDd and LVIDs. MI rats were also associated with significant systolic dysfunction reported as a decrease in FS, ejection fraction, and FAC. ALK5 inhibition was associated with a significant improvement in LVIDd, LVIDs, FS, ejection fraction, and FAC (Fig. 1 and Table 2).

**Infarct size.** Myocardial infarct size was similar in the untreated and treated MI groups (33 ± 2.5 and 34 ± 2.1%, respectively).

**TGF-β protein and activity.** Immunohistochemistry analysis revealed that the protein expression of TGF-β was significantly increased in MI rats compared with sham-operated rats. ALK5 inhibition had no effect on the total TGF-β (P = 0.101, Fig. 2). However, immunostaining of phospho-Smad2, which served as a marker for the biological activity of TGF-β, was signifi-

![Fig. 4. Photomicrographs of α-smooth muscle actin (α-SMA) immunostaining (brown) in sham-operated (A), sham-operated treated with GW788388 (B), MI (C), and MI treated with GW788388 (D) rats. There was an increase of the accumulation of α-SMA positively stained cells in the interstitium in the MI rats (indicated by arrows), and ALK5 inhibition was associated with a significant reduction in the presence of these cells. Quantitation of α-SMA immunostaining in the NIZ is shown (E). Values are expressed as means ± SE. *P < 0.001 vs. sham; †P < 0.01 vs. MI. Original magnification, ×400.](http://ajpheart.physiology.org/issue/298/5/AHP/JH1/
significantly increased in rats following MI, and ALK5 inhibition significantly attenuated the levels of phospho-Smad2 in MI rats \((P < 0.01, \text{Fig. 3C})\).

Consistent with immunohistochemistry results, Western blot analysis also demonstrated a significant increase in phospho-Smad2 in MI animals, together with an increase in total Smad2/3 protein expression. Both phospho-Smad2 and total Smad2/3 were reduced by ALK5 inhibition. Furthermore, there was a significant increase in the ratio of phospho-Smad2 to total Smad2/3, and this was also attenuated by the ALK5 inhibitor \((P < 0.05, \text{Fig. 3})\).

\(\alpha\)-SMA expression. Positively stained vascular smooth muscle cells were excluded from the quantitation. We found that there were very few myofibroblasts observed around the myocardium in the sham-operated animals (Fig. 4). However, there was a fourfold increase of \(\alpha\)-SMA-positive cells in the NIZ \((P < 0.001)\) in MI animals. This increase of \(\alpha\)-SMA was significantly attenuated by ALK5 inhibitor \((P < 0.01)\) (Fig. 4).

Myocardial matrix accumulation. On picrosirius red-stained sections, ECM deposition in the NIZ was significantly higher in the MI animals compared with sham-operated animals \((P < 0.001, \text{Fig. 5})\). Treatment with the ALK5 inhibitor significantly attenuated the deposition of matrix throughout the NIZ to levels similar to those seen in sham-operated animals \((P < 0.001, \text{Fig. 5})\).

Immunostaining for fibrillar collagen type I and III demonstrated significant increased collagen in the NIZ of MI rats compared with sham-operated animals (Fig. 6). ALK5 inhibition significantly attenuated the deposition of collagen I \((P < 0.05)\) in the NIZ (Fig. 6).

Cardiomyocyte hypertrophy. MI animals demonstrated a marked increase in cardiomyocyte CSA compared with sham-operated rats \((P < 0.01)\). ALK5 inhibition was associated with a significant attenuation of cardiomyocyte hypertrophy in MI rats \((P < 0.05, \text{Fig. 7})\).

Macrophage infiltration. In sham-operated rats, very few macrophages were detected in the myocardium. However, MI animals were associated with a dramatic increase in the number of macrophages in the NIZ, particularly within the interstitial regions \((P < 0.01)\). ALK5 inhibition had no effects on macrophage infiltration \((P = 0.574, \text{Fig. 8})\).

DISCUSSION

In the present study, the inhibition of TGF-\(\beta\) signaling with targeted ALK5 inhibition significantly attenuated the accumulation of collagen in the NIZ and reduced cardiomyocyte hypertrophy, leading to an improvement in systolic function in a rat model of MI.

The role of TGF-\(\beta\) as a critical mediator of cardiac fibrosis has instigated anti-TGF-\(\beta\) therapies aimed to improve the
prognosis of patients at risk of developing heart failure (9, 17). The present study took advantage of the recently developed compound GW788388, a potent, highly selective and orally active ALK5 inhibitor with an IC$_{50}$ of 0.018 ± 0.08 µM (10). More recently, it was found that GW788388 also inhibits TGF-β type II receptor and blocks TGF-β-induced Smad2 phosphorylation and Smad2/3 nuclear transduction (25). We have previously shown that increased TGF-β mRNA or protein
in pathological conditions such as diabetic cardiomyopathy may not necessarily reflect parallel changes in TGF-β activity (22). Thus, in addition to examining TGF-β protein, we look at one of its specific intracellular actions, the phosphorylation of its immediate downstream signaling molecule, Smad2. In the present study, GW788388 significantly attenuated the activity of TGF-β without affecting the protein expression of TGF-β.

TGF-β is expressed in the rat heart in both cardiac myocytes and fibroblasts (21). It has also been reported that TGF-β receptors are localized to both the rat cardiac myocytes (6) and the cardiac fibroblasts (20). Thus TGF-β may act in an autocrine and/or paracrine fashion to promote fibrosis. In the present study, immunostaining of TGF-β was mainly localized to the interstitium, enabling secreted TGF-β to act on both myocytes and fibroblasts, activating Smad proteins, thus leading to the activation of collagen transcription.

Enhanced TGF-β activity post-MI was associated with the development of fibrosis in the NIZ and myocyte hypertrophy. An increased deposition of ECM proteins (fibrosis) in the infarct region occurs in an attempt to maintain the structural integrity of the heart; however, fibrosis in the border or remote region contributes to the increased myocardial stiffness and dysfunction that follows MI (26). While collagen composes only 5% of the heart weight, it has an intrinsic stiffness 10,000–50,000 times greater than that of the relaxed myocardium (21), hence the increase in collagen I and III in the MI rats increases the stiffness of the heart and contributes to adverse remodeling.

Immediately after MI, irreversible cell necrosis can occur within minutes. Adult myocytes are terminally differentiated cells and as such are not able to divide, and thus they undergo hypertrophy in response to an increased wall stress (33). Human studies demonstrate that fibrosis and myocyte hypertrophy are key determinants of the magnitude of cardiac dysfunction in the development of heart failure with TGF-β as a critical mediator (11). Indeed, the inhibition of TGF-β signaling significantly attenuated the accumulation of collagen at the NIZ and also prevented myocyte hypertrophy while preserving systolic functions.

To date, the inhibition of TGF-β signaling in animal models of ischemic heart failure has provided controversial results. It

Fig. 7. Representative hematoxylin and eosin-stained sections showing marked increase in the cardiomyocyte cross-sectional area (CSA) in MI (C) compared with sham-operated (A) rats. Treatment with the ALK5 inhibitor, GW788388, was associated with a reduction in the CSA in MI animals (D). B: representative image of sham-operated animals treated with GW788388. Quantitation of cardiomyocyte CSA is shown (E). Values are expressed as means ± SE. *P < 0.01 vs. sham; †P < 0.05 vs. MI. Original magnification, ×400.
is thought that in the early phase of infarct healing, TGF-β may be important in the resolution of the inflammatory response by deactivating macrophages and by suppressing endothelial cell chemokine and cytokine synthesis (24). Thus the inhibition of TGF-β signaling 1 wk before MI (8) or within 24 h post-MI (13) increased mortality and exacerbated early cardiac dysfunction. Moreover, late inhibition of TGF-β after prominent fibrosis has been formed in the infarcted heart has no effect (24). Furthermore, anti-TGF-β therapy started 24 h to a few days post-MI significantly mitigated post-MI remodeling in mice (5, 13, 24). Thus, for a mediator as pleiotropic as TGF-β, there appears to be a narrow therapeutic time window that is critical for the inhibition of TGF-β signaling to elicit beneficial effects on post-MI ventricular remodeling. Our group has previously demonstrated that treatment with antifibrotic drugs 1 wk post-MI prevented potential impaired healing in the infarct zone and attenuated late ventricular remodeling and cardiac dysfunction (1). Thus we chose to start TGF-β inhibition therapy 1 wk after MI surgery in the current model.

Myofibroblasts are identified and characterized as interstitial nonvascular α-SMA-positive cells, which are not resident cells in normal myocardium but are extremely abundant at sites of fibrosis in the infarcted heart (27). These cells are differentiated from fibroblasts and/or pericytes, although signals responsible for such transformation are yet to be elucidated. However, local angiotensin II generation and TGF-β are believed to play an important role in this process (27, 30). Myofibroblasts are eliminated by apoptosis when the fibrotic process associated with tissue repair is complete (3, 4). However, in the infarcted heart, it was found that α-SMA-positive myofibroblasts can persist up to 17 years in human myocardial scars and are not found in normal hearts (31). In this study, we found that the expression of α-SMA was markedly increased in the NIZ in MI rats, whereas in sham-operated animals, α-SMA was mostly confined to the smooth muscle cells of blood vessels. By blocking of TGF-β signaling, the expression of α-SMA was dramatically reduced in the NIZ in MI rats, suggesting that TGF-β induced the activation of fibroblasts to myofibroblasts in ischemic heart injury.

Fibroblasts have been proposed to be the predominant cellular mediators of fibrosis in many organs, including the heart (29). Recently, Zeisberg et al. (34) demonstrated that cardiac fibrosis is associated with the emergence of adult fibroblasts with an endothelial cell origin through TGF-β-induced endothelial-mesenchymal transition (EndMT), which is a form of epithelial-mesenchymal transition that occurs during the embryonic development of the heart (34). It has been proposed that TGF-β not only activates endothelial cells to produce

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**Fig. 8.** Photomicrographs of ED1 immunostaining (brown) in sham-operated (A), sham-operated treated with GW788388 (B), MI (C), and MI treated with GW788388 (D) rats. There was minimal evidence of ED1 positively (ED1 pos)-stained cells in the interstitium of sham-operated animals, whereas MI animals were associated with a marked increase in the accumulation of positively stained interstitial cells (indicated by arrows), and this was not affected by ALK5 inhibition. Quantitation of ED1 positively stained cells in the NIZ is shown (E). Values are expressed as means ± SE. *P < 0.01 vs. sham. Original magnification, ×400.
fibrogenic mediators but also induces EndMT through the Smad pathway, thus contributing to the accumulation of cardiac fibroblasts in the fibrotic heart. Based on the observations of a reduction in α-SMA-positive cells in the treated animals, the inhibition of TGF-β signaling may also prevent the occurrence of EndMT in the infarcted heart, thus reducing the recruitment of activated fibroblasts.

Another important component of MI response is inflammation, which has been proposed to be a prerequisite for wound healing and scar formation. Macrophage is an important inflammatory cell type that modulates the inflammatory response post-MI (18). While an initial activation of macrophages is essential to facilitate wound healing, a prolonged activation can lead to the overproduction of chemokines, cytokines, and growth factors, such as TGF-β, promoting adverse cardiac remodeling following MI (18). We examined the presence of macrophages in this study and found that inhibition with the ALK5 inhibitor had no effect on macrophage accumulation in the infarcted heart.

In conclusion, treatment with a TGF-β type I receptor inhibitor, GW788388, led to a significant reduction of TGF-β activity, which attenuated systolic dysfunction and LV remodeling following experimental MI in rats.

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GRANTS

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DISCLOSURES

None.

REFERENCES


