Myocardial infarction impairs renal function, induces renal interstitial fibrosis, and increases renal KIM-1 expression: implications for cardiorenal syndrome

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Lekawanvijit S, Kompa AR, Zhang Y, Wang BH, Kelly DJ, Krum H. Myocardial infarction impairs renal function, induces renal interstitial fibrosis, and increases renal KIM-1 expression: implications for cardiorenal syndrome. Am J Physiol Heart Circ Physiol 302:H1884–H1893, 2012. First published February 24, 2012; doi:10.1152/ajpheart.00967.2011.—Progressive decline in renal function coexists with myocardial infarction (MI); however, little is known about its pathophysiology. This study aimed to systematically identify post-MI renal changes (functional, histological, and molecular) over time in a rat MI model and examine potential mechanisms that may underlie these changes. Rats were randomized into three groups: nonoperated, sham, and MI. Cardiac and renal function was assessed before death at 1, 4, 8, 12, and 16 wk with tissues collected for histological, protein, and gene studies. Tail-cuff blood pressure was lower in MI than sham and nonoperated animals only at 1 wk (P < 0.05). Systolic function was reduced (P < 0.0001) while heart/body weight and left ventricle/body weight were significantly greater in MI animals at all time points. Glomerular filtration rate decreased following MI at 1 and 4 wk (P < 0.05) but not at 8 and 12 wk and then deteriorated further at 16 wk (P = 0.052). Increased IL-6 gene and transforming growth factor (TGF)-β protein expression as well as macrophage infiltration in kidney cortex was detected at 1 wk (P < 0.05). Renal cortical interstitial fibrosis was significantly greater in MI animals from 4 wk, while TGF-β bioactivity (phospho-Smad2) was upregulated at all time points. The degree of fibrosis increased and was maximal at 16 wk. In addition, kidney injury molecule-1-positive staining in the tubules was more prominent in MI animals, maximal at 1 wk. In conclusion, renal impairment occurs early post-MI and is associated with hemodynamic and structural changes in the kidney possibly via activation of the Smad2 signaling pathway.

ACCELERATED PROGRESSIVE DECLINE in renal function is a frequent accompaniment of myocardial infarction (MI) (20), manifesting the so-called cardiorenal syndrome. Approximately 20% of hospitalized patients with acute MI have renal impairment (41), and one-quarter (24.23%) of these patients died during hospitalization, approximately four times higher than those without renal impairment (15).

The mechanisms underlying the renal dysfunction that follows MI are poorly understood. After MI, an inflammatory response rapidly appears at the infarct site. The inflammatory cells are a source of various cytokines, such as IL-6, TNF-α, IL-1β, and transforming growth factor (TGF)-β (11, 39), which are involved in the initial stages of postischemic wound healing and eventually lead to fibrosis/scar formation. The post-MI inflammatory response is also likely to be systemic, as evidenced by increased circulating levels of cytokines/inflammatory markers in both human and experimental models (3, 17, 27).

Renal interstitial fibrosis is a common sequelae of progressive kidney disease and leads to disturbance of renal function irrespective of the nature of the initial injury (8). Several cytokines and growth factors activated as part of the inflammatory process, in particular TGF-β, appear to be major contributors to renal fibrosis (9). Given the above, it is likely that systemic inflammatory responses involved in post-MI cardiac repair may be implicated in both cardiac and renal fibrosis contributing to progressive dysfunction of both organs.

Neurohormonal activation and hemodynamic disturbance may also have detrimental effects on post-MI renal pathology. Neurohormonal activation, mainly the renin-angiotensin-aldosterone and sympathetic nervous systems, has been demonstrated in both humans and animals post-acute MI (6, 10, 35, 42) and is associated with cardiac remodeling, which can be abrogated by neurohormonal blockade (10). Similar to the inflammatory response, activation of neurohormonal systems can be systemic. Hemodynamic disturbances are not uncommon in acute MI (54). Dysfunction of infarcted, stunned, and/or hibernating myocardium as well as MI-induced arrhythmias may affect cardiac pump function leading to congestive heart failure, cardiogenic shock and/or systemic hypotension that can lead to hypoperfusion of end organs including kidney.

Given the multiplicity of heart-kidney interactions, as outlined above, the aim of the present study was to systematically identify post-MI renal changes (functional, histological, and molecular) over time (1, 4, 8, 12, and 16 wk) in a rat MI model and to examine in detail the potential mechanisms that may underlie the changes observed.

METHODS

Study Design

Male Sprague-Dawley rats [220–250 g body weight (BW), 6–8 wk old] were randomized into three groups: nonoperated, sham, and MI. MI was induced by left anterior descending ligation (50). Nonoperated animals were included in this study to discount the effect of anesthesia from surgery. Prospectively, each subgroup was further randomized over a five-point time course: 1, 4, 8, 12, and 16 wk (Fig. 1). The investigation conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (PHS Approved Animal Welfare Assurance No. A5587-01). All animal usage was also approved by St. Vincent’s Hospital’s Animal Ethics Committee (AEC) in accordance with National Health and Medical Research Council’s Guide for the Care and Use of Laboratory Animals (AEC No. 034/08).

At the endpoint for each time point, systolic blood pressure (BP) was measured in conscious rats using the tail-cuff method before
Male Sprague-Dawley rat (non-operated, sham, MI)

1 wk
4 wks
8 wks
12 wks
16 wks

Endpoint
- Tail-cuff blood pressure
- Urine collection for 24-hour albumin
- Glomerular filtration rate (GFR): 99mTc-DTPA method
- Echocardiogram and Millar catheterization
- Tissue harvest (kidney and heart)

Fig. 1. Experimental design. MI, myocardial infarction; GFR, glomerular filtration rate; 99mTc-DTPA, 99technetium-diethylene triamine penta-acetic acid.

being placed in a metabolic cage for a 24-h period with free access to food and water. The glomerular filtration rate (GFR) was then performed, and animals were allowed to rest. On the following day, cardiac function was assessed by echocardiography and Millar catheterization, respectively, before tissue harvest.

Cardiac Function Assessment—Echocardiography and Millar Catheterization

Before death, echocardiography was performed in lightly anesthetized animals (40 mg/kg ketamine and 5 mg/kg ip xylazine) using a Vivid 7 (GE Vingmed, Horten, Norway) echocardiography machine with a 10-MHz phased array probe to measure ejection fraction and fractional shortening. The procedure was performed as per published standard protocol and as routinely used in our laboratory (43).

Animals were anesthetized with pentobarbital (30 mg/kg ip) and intubated for cardiac catheterization procedures, as previously described (26). Briefly, animals were ventilated and a 2-F miniaturized combined catheter/micromanometer (model SPR838; Millar Instruments, Houston, TX) was inserted into the right common carotid artery to obtain aortic BP and then advanced into the left ventricle to obtain left ventricular pressure-volume loops. Pressure-volume loops were recorded at steady state and during transient preload reduction, achieved by occlusion of the inferior vena cava and portal vein with the ventilator turned off and animal apnoeic. The following validated parameters were assessed using Millar conductance data acquisition and analysis software PVAN 3.2: left ventricular end-systolic pressure, left ventricular end-diastolic pressure, maximal and minimal change in pressure over time (dP/dmax and −dP/dmin), tau (t Logistic), and the slope of the preload recruitable stroke work relationship.

GFR

One day before death, GFR was performed to measure kidney function. Briefly, animals were intravenously injected with a radioactive isotope, 99technetium-diethylene triamine penta-acetic acid (99mTc-DTPA), which is excreted solely by the glomerulus (25). The DTPA is prepared at a rate of 37 MBq/ml (1 mCi/ml), and 0.26 ml of this solution was injected into each rat. Animals were bled 43 min after injection, and their plasma radioactivity was measured to evaluate the rate of DTPA excretion; this was compared with the counts of the standard reference prepared at the time of injection (24). The calculated GFR was corrected for BW recorded before the procedure and reported as GFR per kilogram.

Twenty-Four Hour Urine Albumin

Urine samples obtained from metabolic caging 2 days before death were stored at −20°C for urine albumin measurement. A double antibody radioimmunoassay was used, as previously described (22).

Histological Study

Hearts and kidneys were removed from the animal after Millar catheterization, weighed, fixed in neutral buffered formalin, and then processed for histopathology and immunohistochemistry.

Infarct size. Cross sections of left ventricle of all MI animals were stained with picrosirius red and scanned (Aperio; Aperio Technologies, Vista, CA) for infarct size analysis. Infarct size was reported in animals with transmural infarction as the proportion of the mean endocardial and epicardial circumference occupied by the infarct (16).

Renal pathology and immunohistochemistry. Kidney sections were stained with picrosirius red for matrix deposition. Focal interstitial fibrosis/scarring was defined as an increase in matrix deposition in interstitial spaces that is distinguishable from the surrounding area. Perivascular areas were excluded. Tissue expression of macrophages, phosphorylated Smad2, and kidney injury molecule-1 (KIM-1) was assessed immunohistochemically (33), using mouse anti-C668 (AbD Serotec, Raleigh, NC; 1:300 dilution), rabbit anti-phospho-Smad2 (Cell Signaling Technology, Boston, MA; 1:400 dilution), and goat anti-KIM-1 (R&D Systems, Minneapolis, MN; 1:200 dilution) antibodies, respectively. Numbers of macrophages (CD68 immunoreactive cells) and KIM-1-positive tubules were counted from whole kidney sections for analysis. The immunoperoxidase technique was quantified by positive diaminobenzidine staining.

Quantitation of matrix deposition and phospho-Smad2 expression.

Renal cortical tissue (30 mg) was homogenized with 1 ml of modified RIPA buffer in the presence of protease and phosphatase inhibitors. Equal amounts of protein (30 μg) were separated by 10% SDS-PAGE and electrophoretically transferred to nitrocellulose membranes (Amersham Biosciences). Western blot analysis was performed as per manufacturer’s protocol with specific antibodies [TGF-β, phospho-p44/42, p44/42, phospho-p38, p38, phospho-SAPK/INK, SAPK/INK, phospho-NF-κB, phosphorylated, NF-κB p65 antibodies (Cell Signaling Technology, Beverly, MA); neutrophil gelatinase-associated lipocalin (NGAL) antibody (Santa Cruz, Santa Cruz, CA); and anti-p65 antibody (NeoMarkers, Fremont, CA)] and then visualized by enhanced chemiluminescent reagents (Thermo Scientific, Rockford, IL). Band intensity was analyzed using program ImageJ software (National Center for Biotechnology Information). Pan-actin and total-proteins were used as endogenous controls to correct for nonphosphorylated proteins and corresponding phosphorylated proteins, respectively.

Quantitative mRNA Expression in Renal Cortical Tissue

Total RNA was extracted from 30 mg renal cortical tissue using Qiagen RNeasy kit (Qiagen, Hilden, Germany). cDNA was reverse transcribed, and triplicate cDNA aliquots were amplified using sequence-specific primers (Geneworks, Adelaide, SA, Australia) with
SYBR Green detection (Applied Biosystems) using an ABI prism 7900HT sequence detection system (Applied Biosystems). Real-time PCR was used to quantify profibrotic (TGF-β1 and connective tissue growth factor), inflammatory cytokine (TNF-α and IL-6), and lipocalin2/NGAL gene expression. The primer pairs were designed using Primer Express 2.0 software (Applied Biosystems) based on published sequences (http://www.ncbi.nlm.nih.gov). 18S rRNA was used as an endogenous control in all experiments to correct for the expression of each gene.

Statistical Analysis

Data are presented as means ± SE. One-way ANOVA with Bonferroni’s multiple comparison test or Kruskal-Wallis test with Dunn’s multiple comparison test were used for comparisons among all groups for parametric and nonparametric data, respectively. For comparisons between two groups, unpaired Student t-test was used for parametric data and Mann Whitney test for nonparametric data. All statistical analyses were performed using GraphPad Prism 5. A two-tailed P value of <0.05 was considered statistically significant.

RESULTS

The final total number of animals used in this study was 105 (29 nonoperated, 29 sham, and 47 MI animals). The number of animals in each group at each time point is shown in Supplemental Table S1. Overall mortality rate after MI surgery was 29.54%.

There was no difference in infarct size across five time points (means of 40.55–46.30%; Supplemental Table S1).

The heart weight/BW and left ventricular weight/BW were significantly greater in MI compared with sham and nonoperated animals at all time points. One-week MI animals had a significantly greater lung/BW than sham. There was no difference in heart weight/BW, left ventricular weight/BW, and

Fig. 2. Systolic blood pressure (BP) decreases at 1-wk post-MI. MI animals at 1 wk show a significant lower systolic tail-cuff BP compared with nonoperated and sham animals (P < 0.05; top). Non-op, nonoperated. There is no difference between groups at the other time points.
lung/BW between nonoperated and sham animals. The kidney/BW was not different among groups at all time points (Supplemental Table S1).

Cardiac Function and Hemodynamic Assessment

**Echocardiographic study.** MI animals had significantly lower ejection fraction and fractional shortening than sham and nonoperated animals at all time points (Supplemental Table S1).

**Systolic tail-cuff BP.** At 1 wk, MI animals had significantly lower tail-cuff BP compared with sham and nonoperated animals (nonoperated = 129.0 ± 3.3, sham = 124.7 ± 1.4, and MI = 115.8 ± 2.7 mmHg; \( P < 0.05 \); Fig. 2). There was no difference in systolic BP among groups at the other time points.

**Millar catheterization.** MI animals had a significantly lower systolic BP compared with sham and nonoperated animals at 1 wk; however, BP was not different at the other time points (Supplemental Table S1). No difference in diastolic BP and heart rate was found at all time points between animal groups.

Systolic as well as diastolic dysfunction was found in MI animals at all time points, as assessed by decreased \( \frac{dP}{dt_{max}} \) and preload recruitable stroke work and decreased \( -\frac{dP}{dt_{min}} \) and increased Tau logistic, respectively (Supplemental Table S1). Left ventricular end-systolic pressure was significantly decreased in 1 wk MI compared with sham animals, while left ventricular end-diastolic pressure was significantly increased in MI animals from 4 wk onwards.

Renal Function Assessment

**Endpoint GFR.** GFR was significantly decreased in MI compared with sham animals at 1 and 4 wk \( (P < 0.05) \) and was borderline reduced at 16 wk \( (P = 0.052; \) Supplemental Table S1). Smad2-activated renal interstitial fibrosis, and kidney injury molecule-1 (KIM-1) expression.

A: decline in GFR is observed at 1 and 4 wk following MI. Recovery in GFR is seen after 4 wk post-MI before a sudden deterioration at 16 wk. B: increase in 24-h urine albumin is shown over time in post-MI animals. MI animals have greater levels of albumin at 16-wk compared with the 1-wk time point \( (P < 0.05) \). C: increased renal cortical interstitial fibrosis is first observed in MI animals from 4 wk post-MI and is greatest at 16 wk \( (P < 0.05 \) vs. 1- and 4-wk MI animals). D: significant activation of phospho-Smad2 is persistently demonstrated in MI animals at all time points. E: KIM-1 expression on renal tissue is significantly increased in MI animals at all time points and maximal at 1 wk. There is an increasing trend of KIM-1 expression in MI animals from the 4-wk time point onwards. Compared with 4 wk, MI animals at 1, 12, and 16 wk show a significantly greater degree of renal KIM-1 expression \( (P < 0.01) \).
24-h urine albumin. There was no statistically significant difference in albuminuria amongst the groups; however, a directional trend towards an increase in urine albumin excretion in MI compared with sham and nonoperated animals was observed from 8 wk onwards. When considering only MI animals, 24-h albuminuria increased over time and was significantly greater at 16 wk compared with 1 wk (Fig. 3B).

Renal Tissue Studies

Renal interstitial matrix deposition (fibrosis). Focal tubulointerstitial scarring with inflammatory cell infiltration was observed in 3 MI animals at 16 wk (3/8) and 1 MI animal at 8 wk (1/12; Fig. 4, representative images). The lesions were mainly located between the cortex and the corticomedullary region. Nonoperated, sham, and the remaining MI animals showed no morphological change.

In nonscarring areas, MI animals showed substantial peritubular interstitial fibrosis in the renal cortex compared with a lesser degree in nonoperated and sham animals (Fig. 5, top). Quantitation of renal cortical interstitial fibrosis significantly increased in MI animals at all time points compared with sham and nonoperated animals. The degree of fibrosis was greatest at 16 wk post-MI (Fig. 3C).

Renal macrophage infiltration. At the 1-wk time point, MI animals had significantly a greater number of CD68-immunoreactive cells in renal cortex than nonoperated and sham animals (Fig. 6A). Most of these cells were located in interstitial spaces. There was no significant increase at the other time points.

IL-6 mRNA expression was significantly upregulated in MI animals at 1 wk post-MI (Fig. 6B) but not at the other time points. There was no difference in TNF-α mRNA expression between groups at all time points.

Smad-dependent TGF-β signaling pathway. Renal TGF-β1 gene expression in MI animals showed an increased trend at 4, 8, and 12 wk that was statistically significant at the 8-wk time point (P = 0.02 vs. sham). There was no difference in connective tissue growth factor mRNA expression between groups at all time points.

TGF-β protein expression by Western blot analysis was significantly upregulated in MI animals only at 1 wk (Fig. 7). Phospho-NF-κB p65 (P = 0.06 vs. sham), phospho-SAPK/JNK (P = 0.17 vs. sham), and phospho-p38 (P = 0.24 vs. sham) but not phospho-p44/42 expression showed a nonsignificant increase in the kidney of MI compared with sham animals at 1 wk (data not shown).

MI animals showed an increase in phospho-Smad2 immunostaining in the kidney at all time points (Fig. 5, bottom). The proportional area of phospho-Smad2-positive staining was significantly greater in MI animals at all time points compared with sham and nonoperated animals (P < 0.01) with an upward trend at 16 wk post-MI that was not statistically significant compared with the earlier time points (Fig. 3D).

Kidney Injury Biomarkers

KIM-1. MI animals had significantly more KIM-1-positive tubules than the other two groups at all time points (representative images shown in Fig. 8) except at 8 wk (P = 0.068). Interestingly, KIM-1 expression demonstrated a peak at 1 wk post-MI with a significant reduction at 4 wk before increasing again at 8–16 wk (Fig. 3E).

NGAL. There was no increase in NGAL protein (1, 4, and 16 wk) and mRNA expression at all time points in kidney (data not shown).

DISCUSSION

Renal dysfunction is an independent risk factor for adverse cardiovascular events and death in post-MI patients (2, 23). The degree of impaired renal function is associated with increased short- and long-term mortality in this setting (14). Even mild renal dysfunction, which may be transient during the MI admission, has been found to independently affect long-term (10 year) survival (41). However, assessment of the pathophysiologial changes in the kidney following an acute MI, as well as mechanisms underlying these changes, has not been extensively studied.

The present study demonstrated time-course renal functional, structural, and molecular changes following acute MI. Renal dysfunction occurred early at 1 wk and was associated with increased TGF-β and phospho-Smad2 protein expression as well as increased IL-6 mRNA expression in the kidney. By 4 wk, MI animals had developed renal interstitial fibrosis with persistent phospho-Smad2 activation.

Acute kidney injury found in MI animals at 1 wk may be attributable to hemodynamic alterations. After a large, acute MI, systolic dysfunction is common. With an average infarct size of 46.3% in 1-wk MI animals, a significant decrease in ejection fraction and fractional shortening, and a significant increase in lung/BW, it is likely that animals had acute decompen sated heart failure associated with associated hypotension. As a result, renal blood flow may have been affected, contributing to an early decline in GFR.

Upregulated IL-6 and TGF-β expression with an increase in macrophage infiltration observed in the kidney at 1 wk may be contributory to the subsequent renal fibrosis. A reduction in renal blood flow may lead to some degree of ischemic injury, which can trigger local inflammatory processes. Macrophages,
typically found to be increased in the kidney in progressive renal disease, are the major source of fibrogenic cytokines including IL-6 and TGF-β (38). Furthermore, in the post-MI setting the infarcted heart can be a source of systemic cytokines (3, 17, 27) that could accelerate the local renal inflammatory and fibrosis processes. Higher plasma IL-6 and C-reactive protein levels have been demonstrated in postacute-MI patients with impaired renal function than those without (37). The Smad-dependent TGF-β signaling pathway is likely to be a key contributor to fibrogenesis in both heart and kidney in the post-MI setting. Activation of this pathway, well known to be involved in post-MI cardiac fibrosis (11), has been demonstrated in the kidney in the present study. The time course of this pathway activation appears to be related; cardiac TGF-β overexpression has been reported as early as days 2–3 post-MI (30, 49), while an increase in renal TGF-β protein expression was observed at 1 wk post-MI in the present study.

At 4 wk, systolic BP returned to normal levels while GFR recovery occurred at the 8-wk time point. Adaptive mechanisms such as neurohormonal activation and renal compensatory reaction, e.g., tubuloglomerular feedback, which occur when renal perfusion pressure is reduced (40), may be contributory to the maintenance of glomerular filtration to preserve renal function. Recovery of worsening renal function has also been observed in patients following acute MI. Approximately 40% of acute MI patients with mild to severe degree of acute kidney injury have transient, reversible renal dysfunction. Crucially, this is still associated with a poor 3-yr mortality rate (15).

While hemodynamic derangement is likely to cause early renal dysfunction at 1 wk as previously discussed, persistent activation of the renal fibrosis pathway may underlie subsequent worsening renal function at 16 wk as excessive matrix deposition has effects on physiological function. In the present

Fig. 5. MI increases fibrosis and Smad2 in the kidney. **Top**: MI animals show a greater degree of renal interstitial fibrosis, pink color surrounding renal tubulem. Picrosirius red staining, magnification = 200, in representative images from 16-wk animals is shown. **Middle and bottom**: immunohistochemical staining of phospho-Smad2 demonstrates more positive nuclear staining in renal tubular epithelial cells in MI animals at all time points than nonoperated and sham animals. Magnification = 200.
study, increased renal interstitial fibrosis was identified in MI animals from 4 wk and tended to increase over time with constant activation of phospho-Smad2 in kidney. Smad2 is a specific downstream mediator of TGF-β (34). It is likely that GFR could worsen further beyond the period of 16 wk in part due to this continuous fibrogenesis. As TGF-β protein expression was found upregulated in MI animals at the 1-wk time point and an increase in renal TGF-β1 mRNA expression was seen at 8 wk when fibrosis started to appear, this suggests that the Smad-dependent TGF-β pathway activation may occur in a biphasic pattern. TGF-β is known to be involved in fibroblast proliferation and extracellular matrix deposition, but it also plays a crucial role in suppression of inflammation and usually appears during the transition phase from inflammation to fibrosis (12). After inflammation has subsided, peak levels of TGF-β may be decreased but still continuously exerts its profibrotic effect on post-MI kidney.

Other intracellular signaling mediators such as mitogen-activated protein kinases (MAPKs) and NF-κB, which have been reported to be involved in both renal (18, 29) and cardiac fibrosis (13, 45) and are downstream of the TGF-β pathway (7), were also investigated in the present study. With the use of Western blot analysis, phospho-NF-κB, phospho-SAPK/JNK, and phospho-p38 but not phospho-p44/42 showed a trend towards increased expression in the kidney compared with sham animals at 1 wk. It is possible that NF-κB and JNK and p38 MAPKs may also be involved in post-MI renal fibrosis. Further mechanistic work to elucidate the renal profibrotic changes observed post-MI in the present study is still required.

To our knowledge, there has been only one abstract report describing renal interstitial fibrosis with a mild reduction in GFR at 3 wk post-MI in a rat model (32). In that study, MI animals developed systolic dysfunction without heart failure and there was no difference in BP between MI and sham animals. MI animals showed greater fibrosis in the renal cortex and medulla with significant renal gene dysregulation related to cell proliferation, metabolic processes, and cell communication by microarray analysis. The mechanisms underlying these changes, however, were not described.

Considering that hypoxic/ischemic injury due to systemic hypotension might play a crucial role in post-MI renal changes, a model of renal ischemia may also be used to compare with the present study. Temporary bilateral renal arterial occlusion [ischemic/reperfusion (I/R) model] causes transient renal impairment that is reversible 2 wk after I/R induction (4). Renal function tended to gradually worsen again over time together with more profound morphological abnormalities and fibrosis. Interesting, TGF-β1 expression was increased in a biphasic pattern. This renal I/R injury study (4) also demonstrated a progressive increase in 24-h urine albumin from the earliest time points measured (creatinine clearance independent) similar to the present study (GFR independent). Although the timing is not exactly the same due to the different cause and magnitude of ischemia, the pattern of changes and progression appears similar in the I/R and post-MI models.

Neurohormonal activation may play a crucial role in post-MI fibrosis-associated renal impairment. Activation of neurohormonal processes occurs rapidly following acute MI, likely related, at least in part, by hemodynamic derangements; in patients with overt heart failure and/or large infarct size, this activation is commonly sustained (46). With regard to subsequent fibrosis, a close link between neurohormonal activation and the TGF-β system has been demonstrated. Angiotensin II and other components of the renin-angiotensin-aldosterone system can stimulate TGF-β activity in both heart (28) and kidney (53). Aldosterone is closely associated with perivascular inflammation, a process frequently preceding fibrosis (31),

Fig. 6. Renal macrophage infiltration and interleukin-6 (IL-6) mRNA expression increases at 1-wk MI. A: significant increase in renal macrophage infiltration is demonstrated in 1-wk MI animals (P < 0.01 vs. sham). B: quantitative real-time PCR shows increased renal IL-6 mRNA expression in MI animals at 1 wk (P < 0.05 vs. sham) but no difference between groups at the other time points.

Fig. 7. Increased renal transforming growth factor (TGF)-β protein expression is observed at 1-wk post-MI. Western blot analysis shows upregulated renal TGF-β protein expression in MI animals compared with sham animals at 1 wk post-MI (*P < 0.05).
and angiotensin II is involved in direct phosphorylation of renal Smads, independent of TGF-β (53). Furthermore, sympathetic and endothelin systems are associated with TGF-β mediated fibrosis (1, 5). Finally, blockade of the above systems has demonstrated not only retardation of progression of chronic kidney disease but also inhibition of renal inflammation, TGF-β1 expression, and fibrosis (47, 55).

Although the present study did not measure circulating neurohormonal levels, based on the above considerations it is likely that activation of the above key neurohormonal systems post-MI is likely contributory to the findings observed in the kidney. Experimental blockade of key neurohormonal systems may therefore help clarify the contribution of these mechanisms to the post-MI renal pathology observed in the present study.

Effective clinical tools are needed for the early detection of renal injury. Blood urea nitrogen and serum creatinine levels are most popularly used in clinic to measure renal function, but they are not sensitive or do not change rapidly enough to detect early impairment. Serum creatinine indicates damage when substantial renal mass has been lost (44). Due to its functional reserve capacity, minor effects on renal function are difficult to detect (48). Estimated GFR, which is creatinine derived, seems to be a better option, but it is also affected by many factors such as age, gender, and ethnicity. In this regard, substances such as enzymes and intracellular proteins produced/released from injured cells are likely to be more sensitive and precisely predictive. Given the clinical relevance of renal injury and impairment, there have of late been considerable efforts to identify early markers of kidney injury. KIM-1 and NGAL seem to be most promising of the recently described biomarkers (21, 44). This may relate to their specific induction at the target site of injury (19, 36). Moreover, KIM-1 expression in renal tissue has been reported to be correlated with renal damage and worsening renal function in various human renal diseases (51). The present study showed that only KIM-1 gene expression was significantly increased in post-MI kidney while NGAL was unchanged. KIM-1 expression also had a biphasic pattern but in the opposite direction to GFR, except at the 4-wk time point. Importantly, KIM-1 expression was dramatically upregulated at 1 wk while GFR decreased. Since NGAL expression is rapidly induced after injury (as early as 2 h) and remains elevated for only a short period (52), the earliest post-MI time point of 1 wk, used in this study, may have been too late to detect substantive changes in NGAL expression.

In conclusion, the present study has systematically demonstrated the nature, time course, and mechanisms underlying renal injury in the week following MI. Early kidney injury is likely contributed by hemodynamic alterations. The later phase is associated with interstitial fibrosis, which appears to be mediated via a Smad-dependent TGF-β signaling pathway. These two phases are sequential and overlapping; once kidney injury is triggered, the process does not dissipate over time despite transient renal dysfunction. KIM-1 may be a potentially useful kidney injury biomarker for early detection and monitoring of disease progression. Further mechanistic work, involving neurohormonal inhibitors and other relevant pharmacological agents might help clarify the pathophysiology of post-MI renal changes observed in the present study, as well as potentially being of therapeutic benefit in this clinical setting.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: S.L., A.R.K., D.J.K., and H.K. conception and design of research; S.L. and A.R.K. performed experiments; S.L. and A.R.K. analyzed...
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POST-MI RENAL PATHOPHYSIOLOGICAL CHANGES


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