Regulation of Mitochondrial Dynamics by Dynamin-Related Protein-1 in Acute Cardiorenal Syndrome

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ABSTRACT
Experimental evidence has clarified distant organ dysfunctions induced by AKI. Crosstalk between the kidney and heart, which has been recognized recently as cardiorenal syndrome, appears to have an important role in clinical settings, but the mechanisms by which AKI causes cardiac injury remain poorly understood. Both the kidney and heart are highly energy-demanding organs that are rich in mitochondria. Therefore, we investigated the role of mitochondrial dynamics in kidney–heart organ crosstalk. Renal ischemia reperfusion (IR) injury was induced by bilateral renal artery clamping for 30 min in 8-week-old male C57BL/6 mice. Electron microscopy showed a significant increase of mitochondrial fragmentation in the heart at 24 h. Cardiomyocyte apoptosis and cardiac dysfunction, evaluated by echocardiography, were observed at 72 h. Among the mitochondrial dynamics regulating molecules, dynamin-related protein 1 (Drp1), which regulates fission, and mitofusin 1, mitofusin 2, and optic atrophy 1, which regulate fusion, only Drp1 was increased in the mitochondrial fraction of the heart. A Drp1 inhibitor, mdivi-1, administered before IR decreased mitochondrial fragmentation and cardiomyocyte apoptosis significantly and improved cardiac dysfunction induced by renal IR. This study showed that renal IR injury induced fragmentation of mitochondria in a fission-dominant manner with Drp1 activation and subsequent cardiomyocyte apoptosis in the heart. Furthermore, cardiac dysfunction induced by renal IR was improved by Drp1 inhibition. These data suggest that mitochondrial fragmentation by fission machinery may be a new therapeutic target in cardiac dysfunction induced by AKI.


AKI has recently been recognized as an extremely severe complication in critically ill patients. Despite the progress of patient management in critical care, the mortality of AKI has not changed: it remains as 50%–70%.1,2 Of particular note is a recent large observational study using the Veterans Affairs database that demonstrated that the United States veterans with AKI alone have worse outcomes than those diagnosed with an myocardial infarction in the absence of AKI.3 Therefore, development of new AKI diagnosis and treatment is urgently necessary to improve critically ill patient outcomes.

Although AKI is associated with poor outcome of critically ill patients, it is assumed that renal dysfunction alone will not be sufficient to increase mortality. Remote organ effects caused by AKI might contribute to the poor outcome of AKI patients. Recently, organ crosstalk between the kidney and heart has been recognized as cardiorenal syndrome.4 Several different mechanisms by which AKI causes cardiac dysfunction by fission machinery may be a new therapeutic target in cardiac dysfunction induced by AKI.

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injury might include systemic immunologic reactions, sympathetic nervous and renin–angiotensin–aldosterone system activation, and increased oxidative stress.5,6 Clinically, other factors, such as excess fluid accumulation, hypertension, acidemia, and electrolyte disturbance, appear to worsen AKI-induced cardiac injury.7 However, distant organ effects of AKI on the heart (acute renocardiac syndrome4) have not been clarified sufficiently through basic research to date. Only a few animal studies have described pathologic changes, such as cellular apoptosis and capillary vascular congestion, in the heart after renal ischemia reperfusion (IR) or glycerol injection–induced rhabdomyolysis.8–10

Mitochondria are highly dynamic in response to the environment. Their morphologic changes, including fission and fusion, can be observed in several different types of cancer and in neurologic and cardiovascular diseases.11 Both fusion and fission are mediated by several guanosine triphosphatases (GTPases). Fusion of the outer membrane of mitochondria is regulated by mitofusin 1 (Mfn1) and mitofusin 2 (Mfn2),12,13 whereas the inner membrane fusion involves optic atrophy 1 (OPA1).14 Mitochondria fission is mainly regulated by dynamin-related protein 1 (Drp1), which is a cytosolic protein that will move to the outer mitochondrial membrane by activation. Reportedly, that inhibiting mitochondrial fission protects the heart and kidney from IR injury.15–17 Nevertheless, it remains uncertain whether mitochondrial fission is further involved in the distant organ effects of AKI on the heart. This study was undertaken to clarify the role of mitochondrial dynamics in AKI-induced cardiac injury, namely acute renocardiac syndrome, using a mouse renal IR model.

RESULTS

Acute Renocardiac Injury Demonstrated by Mitochondrial Fragmentation, Cellular Apoptosis, and Cardiac Dysfunction

Mouse renal IR was induced by 30 min of bilateral renal artery clamping in male C57BL/6 mice. Remarkable increase of BUN and severe pathologic injuries, including tubular epithelial cell necrosis, were observed 24 h later (Supplemental Figure 1). Pathologic analysis using electron microscopy revealed significantly increased mitochondrial fragmentation in cardiomyocytes in the renal IR group compared with the sham group (Figure 1). Increased cytochrome c release into the cytosol was observed in the renal IR group (Figure 2). At 24 and 72 h after renal IR, cardiomyocyte apoptosis evaluated by immunohistochemistry and Western blot analysis of activated caspase-3 was increased significantly in the renal IR group (Figure 3). Finally, renal IR injury caused marked depression of cardiac function at 72 h after the surgery, as indicated by reduced fractional shortening on echocardiography (Table 1).

Mitochondrial Dynamics Regulating Proteins and TNF-α in the Heart after Renal IR

We evaluated the mitochondrial dynamics regulating proteins of Mfn1, Mfn2, Opa1 (fusion), and Drp1 (fission) with whole heart tissue lysates and extracted mitochondrial fractions. Heart specimens were collected 24 h after renal IR injury. Among the examined proteins in the mitochondrial fraction, we found that only Drp1 increased significantly in the renal IR group compared with the sham group (Figure 4A). No difference was observed in

Figure 1. Mitochondrial fragmentation in the heart induced by renal IR injury. (A) Electron microscopy showed renal IR increased fragmented mitochondria in the cardiomyocytes after 24 h. Original magnification ×10,000. (B) The area of mitochondria was significantly less in the IR group mice than in the sham-operated mice (n=5 in each group). #P<0.05 versus sham.
fusion regulating protein amounts (Mfn1, Mfn2, and OPA1) in the mitochondrial fraction of the heart (Figure 4A). No mitochondrial dynamics regulating proteins in the whole tissue lysates differed between the sham and renal IR groups (Figure 4B).

TNF-α expression in the heart was examined using real-time PCR. In accordance with a previous report, we observed increased expression of TNF-α in the heart after renal IR (Supplemental Figure 2).

Drp1 Inhibitor Mitochondrial Division Inhibitor-1 Attenuated Acute Renocardiac Injury

To ascertain the role of Drp1 and mitochondrial fragmentation in the heart, a pharmacologic inhibitor of Drp1 called mitochondrial division inhibitor-1 (mdivi-1) was administered (50 mg/kg) to mice with renal IR. Although mdivi-1 treatment had not suppressed BUN or shown plasma creatinine elevation at 24 or 72 h after renal IR (Figure 5), mitochondrial fragmentation in the heart at 24 h was significantly more improved in the mdivi-1 group than in the vehicle group (Figure 6A). The mdivi-1 treatment suppressed Drp1 translocation to the mitochondria (Figure 6B) and also suppressed cytochrome c release into the cytosol (Figure 6C). Apoptosis of cardiomyocytes and depression of cardiac function examined by echocardiography at 72 h after renal IR were improved by mdivi-1 treatment (Figure 7). However, mdivi-1 treatment did not suppress expression of TNF-α in the heart (Supplemental Figure 2).

Delayed mdivi-1 treatment that was started at 6 h after renal IR did not suppress elevation of BUN or plasma creatinine (Figure 8), but it showed a protective effect on mitochondrial fragmentation with reduction of Drp1 translocation to the mitochondria in the heart (Figure 9). Cardiomyocyte apoptosis and cardiac function were also improved by delayed treatment using mdivi-1 (Figure 10).

This study also evaluated whether mdivi-1 attenuated renal injury related to mitochondrial fragmentation and renal cell apoptosis. In contrast with the protection by mdivi-1 treatment in the heart, no difference of mitochondrial fragmentation or activated caspase-3 level was apparent between mdivi-1–treated and untreated mice (Supplementary Figure 3).

DISCUSSION

Complication of heart and kidney dysfunction in critically ill patients has a substantial effect on patient outcomes. These two disorders worsen each other synergistically. Therefore, identifying the pathway of organ interaction between the heart and kidney is expected to be crucial for developing targeted therapies to improve the outcomes. This study demonstrated, for the first time ever reported to our knowledge, that the depression of cardiac function caused by AKI was associated with morphologic effect on the heart, as evidenced by mitochondrial fragmentation and apoptosis, and that Drp1 activation plays a crucial role in AKI-induced cardiac injury (acute renocardiac syndrome). It should be addressed that a Drp1 inhibitor mdivi-1 suppressed cardiac injury after renal IR, even when administered 6 h after renal ischemia insult.

The kidney and heart require large amounts of energy production. Therefore, they are rich in mitochondria. Consequently, it can be assumed that mitochondrial damage contributes to the pathogenesis of acute kidney and heart dysfunction. Recently, Brooks and colleagues reported a remarkable morphologic change of mitochondria in acute kidney injury models, including IR and cisplatin injection. Mitochondrial fragmentation was observed earlier than cytochrome c release and cellular apoptosis. Dominant-negative and small interfering RNA knockdown experiments demonstrated the role of Drp1 in mitochondrial fragmentation. Sharp and colleagues showed that Drp1 activation during cardiac IR caused left ventricle dysfunction, which was reversed by Drp1 inhibition. This study demonstrated that renal IR caused cardiac mitochondrial damage via Drp1 activation. This result might support the view that...
mitochondrial fission by Drp1 activation is a common pathway by which IR injury in one organ causes injury to another remote organ.

Mitochondria fragmentation describes abnormally small mitochondria and is reportedly observed in apoptotic cells. A crucial role for mitochondrial fragmentation in apoptosis has been reported. A molecular pathway in which increased mitochondrial fission induces cellular apoptosis by Drp1 activation has been suggested. Frank and colleagues reported that inhibition of Drp1 by overexpressing dominant-negative mutant in cultured cells prevented the release of cytochrome c and suppressed apoptosis. Germain and colleagues reported that Drp1-dependent cristae remodeling caused cytochrome c release from mitochondria. Recently, Ban-Ishihara reported that Drp1-dependent mitochondrial fission regulates cytochrome c release via mitochondrial DNA distribution and cristae reformation.

Presumably, mitochondrial fragmentation is determined as a balance between fission and fusion of mitochondria. Although accumulation of fission GTPase Drp1 on mitochondria during apoptosis has been reported by several studies, insufficient mitochondrial fusion might also contribute to cellular apoptosis. Three large GTPases in the dynamin family mediate mitochondrial fusion: Mfn1, Mfn2, and OPA1. These fusion GTPases are reportedly related to apoptosis. However, no change of their expressions was found in mitochondrial fractions or whole tissue lysates of the heart after renal IR in this study. Therefore, mitochondrial fragmentation appeared to be caused mainly by fission machinery in AKI-induced cardiac injury. These results were obtained using Western blot analysis with the mitochondria fraction extracted from heart tissue. Because electron microscopy of the heart tissue showed more fragmented mitochondria in the renal IR group than in the sham group, evaluation of mitochondrial dynamics regulation molecules of Drp1, Mfn1, Mfn2, and OPA1 was influenced by the different number of fragmented mitochondria between these two groups. However, we observed a difference of protein amounts between the renal IR and sham groups not in Mfn1, Mfn2, OPA1, but in Drp1 only. Moreover, we observed reduced mitochondrial fragmentation by Drp1 inhibition in the experiment of mdivi-1 treatment. Therefore, mitochondrial fragmentation

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Figure 3. Apoptosis of cardiomyocytes induced by renal IR injury. (A) Representative image of fluorescence immunohistochemistry for activated caspase-3 in the heart. Original magnification ×630. (B) Representative image of Western blot analysis for activated caspase-3 in whole tissue lysates. Original magnification ×400. (C) Histogram showing the relative density of bands compared with α-tubulin (n=4–6 in each group). #P<0.05 versus sham. *P<0.05 versus 24 h.

Table 1. Echocardiography after renal IR injury

<table>
<thead>
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<th>Parameter</th>
<th>Sham</th>
<th>IR 24 h</th>
<th>IR 72 h</th>
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</thead>
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<tr>
<td>Heart rate (beats/min)</td>
<td>608±18</td>
<td>544±44</td>
<td>580±27</td>
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<tr>
<td>LVDd (mm)</td>
<td>2.42±0.17</td>
<td>2.41±0.15</td>
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<td>LVDs (mm)</td>
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<td>1.15±0.12</td>
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<tr>
<td>IVSW (mm)</td>
<td>0.86±0.06</td>
<td>0.75±0.03</td>
<td>0.95±0.02</td>
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<tr>
<td>PW (mm)</td>
<td>1.08±0.12</td>
<td>0.98±0.04</td>
<td>1.17±0.13</td>
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<tr>
<td>FS (%)</td>
<td>62.3±1.1</td>
<td>66.4±3.5</td>
<td>50.8±3.4*</td>
</tr>
</tbody>
</table>

Values are means±SEM (n=7 in each group). LVDd, diastolic left ventricular dimension; LVDs, systolic left ventricular dimension; IVSW, intraventricular septum wall width; PW, posterior wall width; FS, fractional shortening. *P<0.05 versus sham.
observed in the heart of the renal IR group might be caused at least partly by a Drp1-dependent pathway.

Drp1 is an intensively investigated mitochondrial fission-regulating molecule. Approximately 97% of Drp1 is located in the cytoplasm under normative conditions. However, Drp1 will translocate to the outer membrane of mitochondria by activation and initiate the mitochondrial fission process. Several post-translational modifications, such as serine phosphorylation and ubiquitin E3 ligase, regulated Drp1 activity. An inhibitor of mitochondrial division has been identified using yeast screening of chemical libraries. This compound (mdivi-1) inhibits Drp1 assembly and GTPase activity. It also inhibits subsequent mitochondrial fission. Therefore, mdivi-1 is used widely for exploration of the role of mitochondrial fission in apoptosis. Protection by mdivi-1 administration against organ dysfunction, including that of the heart and kidney, has already been reported in mouse IR injury models. In accordance with these reports, we showed protection by mdivi-1 of cardiac injury caused by AKI, addressing the role of Drp1 in the pathophysiology of remote organ injury by AKI. Although pharmacokinetic properties, including the half-life of mdivi-1 in vivo, are not known, several in vivo studies have demonstrated protection by mdivi-1 in different organ IR injury models at 12–48 h after mdivi-1 injection. This study showed reduced Drp1 assembly to the mitochondrial fraction at 24 h and suppression of apoptosis in the heart at 72 h; however, it remains unclear whether mdivi-1 worked only at the initiation phase of renal ischemia-induced cardiac injury or prolonged effects of mdivi-1 suppress cardiac cell apoptosis.

Results of this study do not clarify why Drp1 in the cardiomyocytes was activated by renal IR injury. Several possible pathways can be considered. First, unknown humoral mediator accumulated in blood circulation attributable to decreased renal clearance might induce Drp1 activation. We recently demonstrated increased blood high-mobility group protein B1–induced lung injury via toll-like receptor 4 in another mouse AKI model of bilateral nephrectomy. Second, IR injury to one organ might cause systemic reaction, possibly mediated by inflammatory cytokines or cells. Andrés-Hernando and colleagues reported that proinflammatory cytokines IL-6, chemokine (C-X-C motif) ligand 1, IL-1β, and TNF-α were increased not only in the kidney, but in the spleen and liver in renal IR injury. Awad and colleagues observed a marked increase of neutrophil margination in the lungs in a mouse renal IR model. Patschan and colleagues demonstrated that endothelial progenitor cell homing to the spleen was induced by renal IR. We observed increased TNF-α expression in the heart after renal IR injury in accordance with a previous report. However, a Drp1 inhibitor, mdivi-1, did not reduce TNF-α expression in the heart; however, it reduced cardiomyocyte apoptosis. These results suggest that TNF-α does not play a crucial role in mitochondrial fragmentation and apoptosis in this model of cardiac injury after renal IR. Drp1 activation by TNF-α can be inhibited by mdivi-1 because this additional experiment cannot exclude the role of TNF-α–induced Drp1 activation. Further investigation must be conducted to ascertain the responsible pathways in cardiac injury caused by renal IR. Additionally, we did not clarify the mechanism by which cardiomyocyte apoptosis caused decreased cardiac function evaluated by functional shortening of the left ventricle.
detailed evaluation of cardiac function using ultrasound and other methods should be done in future studies.

Although mdivi-1 treatment attenuated cardiac injury after renal IR in this study, no significant renal protection by mdivi-1 was observed. A previous report described significant protection of mdivi-1 treatment in vivo on the same AKI model of mouse renal IR injury. However, a marked difference exists between these studies; the animal model in the previous study appears to be more severe because BUN and blood creatinine in the vehicle-treated mice were much higher in the previous study than those in this study (BUN 250 versus 150 mg/dl, creatinine 2.5 versus 1.2 mg/dl). Therefore, it is possible that severity of renal ischemic insult has some effect on the benefit from mdivi-1 treatment. In addition, the optimal dose and timing of mdivi-1 treatment for renal protection might be different from those for cardiac protection. These issues should be investigated further.

In conclusion, this study demonstrated that Drp1-mediated mitochondrial fragmentation and subsequent organ disorder was caused in the heart during renal IR injury. Although clinical situations in which AKI results in acute cardiac dysfunction are observed frequently and are defined recently as acute cardio-renal syndrome, the pathophysiology of this syndrome has not been well demonstrated. Therefore, results obtained from this study strongly suggest that mitochondria fission and apoptosis in the heart as a distant organ effect by AKI should be regarded seriously. They can be good therapeutic targets to improve critically ill patients.
**CONCISE METHODS**

**Animals and Surgical Protocol**
Eight-week-old male C57BL/6 mice were obtained from Japan SLC, Inc. (Hamamatsu, Japan). The mice were kept on a 12 h light/dark cycle with free access to diet and water. All experiments were conducted in accordance with the National Institutes of Health’s (NIH’s) Guide for the Care and Use of Laboratory Animals; all were approved by The University of Tokyo Institutional Review Board.

An IR model induced by 30 min of bilateral renal artery clamp was produced as described in a previous report. At 1 h before surgery, mdivi-1 (50 mg/kg dissolved in DMSO [Enzo Life Sciences Inc., Tokyo, Japan]) was injected intraperitoneally. An equal amount of DMSO was injected as vehicle. In another experiment, the same amount of mdivi-1 or only vehicle (DMSO) was given 6 h after surgery (delayed-treatment group). The mice were euthanized 24 and 72 h after surgery. Blood, kidney, and heart specimens were taken for analyses.

**Blood Chemistry**
BUN was measured using the urease indophenol method (Urea N B test; Wako Pure Chemical Industries Ltd., Osaka, Japan). Plasma creatinine measurements using HPLC were conducted as described previously.

**Electron Microscopy**
Hearts and kidneys of the animals were perfused with PBS with subsequent fixation in 0.1 M phosphate buffer, 4% paraformaldehyde, and 2.5% glutaraldehyde. The tissue block was examined at high magnification ($\times10,000$). To assess the mitochondrial fragmentation, digital images with scale bars were collected in electron microscopy. The areas of individual mitochondria were measured by tracing using NIH ImageJ software (http://rsbweb.nih.gov/ij/). For each heart, the respective areas of 100–150 interbrillar mitochondria were measured in 10 randomly selected electron micrographs of longitudinally arranged cardiomyocytes. For each kidney, mitochondrial fragmentation was evaluated using methods described in a previous report with minor modification. Briefly, the respective lengths of approximately 100 individual mitochondria in a single cell were measured to ascertain the percentage of cells that showed filamentous mitochondria <10% long (>2 $\mu$m). In all, 100 cells in four individual animals from each group (sham-operated, mdivi-1–treated, and untreated ischemic kidneys) were evaluated.

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**Figure 7.** Effects of Drp1 inhibitor mdivi-1 on the heart in mouse renal IR injury. (A) Apoptosis evaluated by Western blot analysis for activated caspase-3 was reduced by mdivi-1 treatment ($n=6$ per group). *P<0.05 versus sham. **P<0.05 versus IR 72 h. (B) Quantitative analysis of the fractional shortenings (FS) showed the protection of mdivi-1 ($n=4–6$ per group). *P<0.05 versus sham. **P<0.05 versus IR 72 h.

**Figure 8.** Effects of delayed mdivi-1 treatment on renal function in mouse renal IR injury. (A) BUN and (B) plasma creatinine (Cre) concentration of each group ($n=7$ per group).

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BUN was measured using the urease indophenol method (Urea N B test; Wako Pure Chemical Industries Ltd., Osaka, Japan). Plasma creatinine measurements using HPLC were conducted as described previously.
Immunohistochemistry for Activated Caspase-3

Frozen sections of 5 μm thickness were dried and fixed with ice-cold acetone. After washing the slides with PBS, we incubated them with anti-activated caspase-3 antibody (Cell Signaling Technology, Beverly, MA) and fluorescence conjugated secondary antibody (Invitrogen) for the primary antibody for 40 min. The sections were then examined visually using confocal microscopy (LSM 510 Meta NLO imaging system; Carl Zeiss).

Terminal Deoxyuridiine Triphosphate Nick End-Labeling Assay

Apoptotic cells in the heart were identified using terminal deoxyuridiine triphosphate nick end-labeling assay with a CardioTACS In Situ Apoptosis Detection Kit (R&D Systems) according to the manufacturer's instructions. The final supernatants were stocked as cytosol fraction. The presence of the voltage-dependent anion channel (VDAC) only in the mitochondrial fraction demonstrated by Western blot confirmed that the mitochondria fraction was obtained correctly.

Western Blot Analysis

Protein samples were extracted from the heart and kidney with radioimmunoprecipitation assay buffer containing a protease inhibitor cocktail, as described previously.41 After centrifugation of the samples, the supernatants were used as heart whole tissue lysates. The lysates were boiled in sample buffer containing 5% SDS with 20% 2-mercaptoethanol and were separated on a 10%–15% SDS-PAGE. After transferring proteins from the gel to a polyvinylidene difluoride membrane (Amersham Biosciences Corp., Uppsala, Sweden), Western blot analysis was performed using 1:1000 diluted anti-Drp1 (BD Biosciences, San Jose, CA), Mfn1 (Abnova Corp., Taipei, Taiwan), Mfn2 (Abnova Corp., Taipei, Taiwan), OPA1 (BD Transduction Laboratories), cytochrome c (BD Biosciences Pharmingen, NJ), VDAC (Abcam....
Inc., Cambridge, MA), and 1:4000 diluted α-tubulin (Sigma-Aldrich, St Louis, MO) antibodies, with incubation overnight at 4°C. Subsequently, the chemiluminescent signal labeled using ECL Plus (Amersham Biosciences Corp.) was detected using a CCD camera system (LAS-4000 mini; Fuji Photo Film Co., Tokyo, Japan). The membrane was then incubated at 50°C for 30 min in a stripping buffer (2% SDS, 100 mM 2-mercaptoethanol, and 12% 0.5 M Tris–HCl, pH 6.8) to remove all probes. The reprobing procedure was performed further with the antibody to α-tubulin. In mitochondrial fractions and whole heart tissue lysates, targeting proteins were normalized, respectively, to VDAC and α-tubulin.

**Real-Time PCR Assay for TNF-α Expression in the Heart**
The mRNA of TNF-α was examined using real-time quantitative PCR, as explained in earlier reports of the literature.34,41 Quantification of gene expressions was calculated relative to β-actin. Amplification data were analyzed using software (Prism Sequence Detection System version 2.1; Applied Biosystems, Foster City, CA).

**Echocardiography**
*In vivo* cardiac morphology was assessed in nonanesthetized mice using transthoracic echocardiography with an ultrasound machine (SONOS 4500; Philips Medical System, Santa Clara, CA). The M-mode left ventricular end-systolic and end-diastolic dimensions, intraventricular septum wall thickness, and posterior wall thickness were averaged from 3 to 5 beats. The left ventricular percentage of fractional shortening was calculated as described in earlier reports.42,43

**Statistical Analyses**
The results of the statistical analyses are expressed as means±SEM. Differences between groups were analyzed for statistical significance using t tests. Results for which P<0.05 were inferred as statistically significant. These calculations were conducted using software (JMP 9.0; SAS Institute Inc., Cary, NC).

**ACKNOWLEDGMENTS**
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**DISCLOSURES**
None

**REFERENCES**
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This article contains supplemental material online at http://jasn.asnjournals.org/lookup/suppl/doi:10.1681/ASN.2014080750/-/DCSupplemental.
Supplemental Figure 1. Renal dysfunction and pathological changes induced by renal ischemia reperfusion injury.
BUN (A) and plasma creatinine (Cre) (B) at 24 h after renal ischemia reperfusion (IR) injury was significantly increased compared with the sham-operated mice (n=7 in each group). # p < .05 versus sham. (C) Renal tubular necrosis caused by IR is shown in Periodic acid–Schiff (PAS) staining. Original magnification: ×400. Bar = 50 μm.
Supplemental Figure 2. TNF-α expression in the heart induced by renal ischemia reperfusion injury. Renal ischemia reperfusion increased cardiac TNF-α expression but mdivi-1 did not suppress this response (n=6 per group). # p < .05 versus sham. * p < .05 versus IR+vehicle 24 h.
Supplemental Figure 3. Effect of Drp1 inhibitor mdivi-1 on ischemic renal mitochondria and renal cell apoptosis.
Mitochondrial fragmentation in the kidney was observed 24 hours after renal ischemia reperfusion (A and B). Increased Drp1 expression in the mitochondrial fraction (C) and activated caspase-3 expression (D) were also observed. Treatment of mdivi-1 did not attenuate these renal injury. # p < .05 versus sham.

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Suppl. Fig. 3