博士論文

論文題目

Cardiac mitochondria injury induced by renal ischemia:

Possible role of heart-kidney crosstalk in AKI

(虚血再灌流傷害腎が心臓のミトコンドリア障害へ

与える影響についての検討)

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Doctoral Thesis

Cardiac mitochondria injury induced by renal ischemia:

Possible role of heart-kidney crosstalk in AKI

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1. Abbreviations

IR	Ischemia reperfusion
AKI	Acute kidney injury
KC	Keratinocyte-derived chemoattaractant
G-CSF	Granulocyte-colony stimulating factor
IL6	Interleukin 6
ALI	Acute lung injury
GTPase	Guanosine triphosphatase
Mfn1	Mitofusin 1
Mfn2	Mitofusin 2
Drp1	Dynamin-related protein 1
DMSO	Dimethyl sulfoxide
EM	Electron microscopy
BUN	Blood urea nitrogen
VDAC	Voltage-dependent anion channel
RIPA	Radioimmunoprecipitation assay
SDS	Sodium dodecyl sulfate

PMSF	Phenylmethylsulfonyl fluoride
FS	Fractional shortening
LV	Left ventricle
LVDd	Left ventricular end-diastolic diameter
LVDs	Left ventricular end-systolic diameter
IVST	Intraventricular septum wall thickness
PWT	Posterior wall thickness
LVM	Left ventricular mass
Mdivi-1	Mitochondrial division inhibitor-1
HMGB1	High-mobility group protein box 1
RNA	Ribonucleic acid
CXCL1	Chemokine (C-X-C motif) ligand 1
TNF-α	Tumor necrosis factor-α

2. Abstract

Although the clinical overlap between kidney and heart dysfunction, which has recently been recognized as cardiorenal syndrome, appears to play an important role in clinical settings, the mechanisms by which AKI causes cardiac injury remains poorly understood. Because both the kidney and the heart are highly energy-demanding organs that are rich in mitochondria, we investigated the role of mitochondrial dynamics in kidney–heart organ interaction. Renal IR injury was induced by bilateral renal artery clamping for 30 min in eight-week old male C57BL/6 mice. Electron microscopy showed a significant increase of mitochondrial fragmentation in the heart at 24 h. Apoptosis in cardiac tissue and cardiac dysfunction evaluated by echocardiography were observed at 72 h. Among the mitochondrial dynamics regulating molecules of Drp1 (fission) and Mfn1, Mfn2, OPA1 (fusion), only Drp1 was increased in the mitochondrial fraction of the heart. A Drp1 inhibitor, mdivi-1, significantly decreased mitochondrial fragmentation and apoptosis in the heart, and improved cardiac dysfunction induced by renal IR. This study showed that renal IR injury induced mitochondria fragmentation in a fission-dominant manner with Drp1 activation and subsequent 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 can be a new therapeutic target in cardiac dysfunction induced by AKI.

3. Introduction

Acute kidney injury (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 been improved: it remains as 50–70%.[1,2] Of particular note is a recent large observational study using the Veterans Affairs (VA) database demonstrated that U.S. veterans with AKI alone have worse outcomes than those diagnosed with myocardial infarction (MI) 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 outcomes of critically ill patients, it is assumed that renal dysfunction alone is not sufficient to increase mortality. Remote organ effects caused by AKI might contribute to the poor outcomes of AKI patients. Recently, a pathophysiologic disorder of the heart and the kidneys whereby acute or chronic dysfunction of one organ may induce acute or chronic dysfunction of the other organ has been defined as cardiorenal syndrome.[4] Several different mechanisms by which AKI causes cardiac injury might include the systemic immunological reactions, the 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 syndrome[4]) have not been clarified sufficiently through basic research to date. Only a few animal studies described the pathological changes such as cellular apoptosis and capillary vascular congestion in the heart after renal ischemia reperfusion or glycerol injection-induced rhabdomyolysis.[8-10]

For other organs, recent investigations have identified some possible mechanisms by which AKI induces remote organ injury. Regarding a connection between the kidney and the brain, severe AKI resulted in increases of the pro-inflammatory chemokines such as keratinocyte-derived chemoattractant (KC) and granulocyte-colony stimulating factor (G-CSF) levels in the cerebral cortex.[11] The bilateral renal IR injury induced increase of vascular permeability, neutrophil infiltration, and interleukin-6 (IL-6) expression in the lung and resulted in acute lung injury (ALI).[12] AKI also induced hepatic oxidative stress and promoted inflammation, hepatocyte apoptosis, and tissue damage in the liver.[13]

Mitochondria are highly dynamic in response to the environment. Its morphological changes including fission and fusion can be observed in several different diseases of cancer, neurologic and cardiovascular diseases.[14] 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),[15,16] whereas the inner membrane fusion involves optic atrophy 1 (OPA1).[17] 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.[18-20] In brief, inhibiting mitochondria fragmentation reduced cytosolic calcium levels and prevented mitochondrial permeability transition pore opening and cell death both in the heart and the kidney. 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 ischemia reperfusion (IR) model.

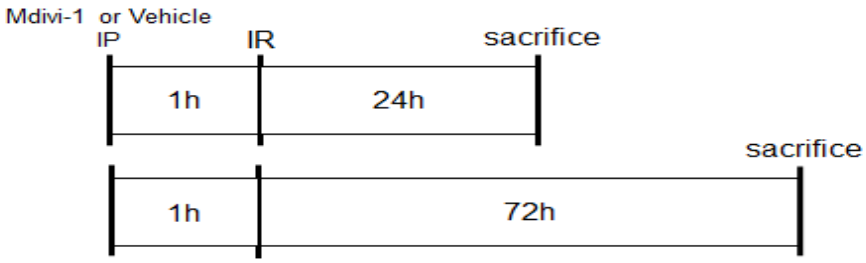
4. Experimental procedures

4-1. 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 NIH Guide for the Care and Use of Laboratory Animals (U.S. Department of Health and Human Services Public Health Services, National Institutes of Health, NIH publication No. 86-23, 1985) and were approved by The University of Tokyo Institutional Review Board.

An ischemia reperfusion (IR) model induced by 30 min bilateral renal artery clamp was produced as described in a previous report.[21] The animals were anesthetized and flank incisions were made to expose the renal pedicles for bilateral clamping to induce 30 minutes of renal ischemia. The clamps were then released for reperfusion with indicated times. Control animals were subjected to sham operation without renal pedicle clamping. Mdivi-1 (Enzo Life Sciences Inc., Tokyo, Japan) at the dosage of 50 mg/kg dissolved in dimethyl sulfoxide (DMSO) was injected intraperitoneally 1 h before surgery. The dose of mdivi-1 was equal to that in a previous report, in which mdivi-1 showed protection on renal IR.[18] An equal amount of DMSO was injected as vehicle. The mice were killed 24 h and 72 h after surgery. Blood, kidney, and heart specimens

were taken for analyses. Experimental protocol of the present study is described below.



4-2. Blood Chemistry

Blood urea nitrogen (BUN) was measured using the urease indophenol method (Urea N B test; Wako Pure Chemical Industries Ltd., Osaka, Japan).

4-3. Electron Microscopy

Hearts of the animals were perfused with PBS with subsequent fixation in 0.1M phosphate buffer, 4% paraformaldehyde, and 2.5% glutaraldehyde. The tissue block was examined at high magnification (×10,000). To ascertain the mitochondria fragmentation, digital images with scale bars were collected in EM. The areas of individual mitochondria were measured by tracing using NIH Image-J software (http://rsbweb.nih.gov/ij/). For each heart, the area of >100 to 150 interfibrillar

mitochondria were measured in 10 randomly selected electron micrographs of longitudinally arranged cardiomyocytes.

4-4. Immunohistochemistry for activated caspase-3

We used immunofluorescence double staining for detection of activated caspase-3 expression. 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 Inc., Beverly, MA, USA) and fluorescence conjugated secondary antibody (Invitrogen Corp.) for the primary antibody for 40 min. TOPRO-3 (Invitrogen Corp.) was incubated for nuclear staining with secondary antibody. The sections were then examined visually using confocal microscopy (LSM 510 Meta NLO imaging system; Carl Zeiss Inc.).

4-5. Mitochondria fraction isolation

The mitochondria fraction was isolated from heart tissue by differential centrifugation using a mitochondria isolation kit for tissue (Thermo Scientific, Rockford, USA) 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.

4-6. Western analysis

Samples obtained at 24 or 72 hours after the surgery were homogenized on ice in a radioimmunoprecipitation assay (RIPA) buffer [0.1% sodium dodecyl sulfate (SDS), 0.5% sodium deoxycholate, 1% Igepal CA-630 (Sigma Chemical Co.), 9.1 mmol/L dibasic sodium phosphate, 1.7 mmol/L monobasic sodium phosphate, and 150 mmol/L NaCl, pH 7.4]. Protease inhibitors such as phenylmethylsulfonyl fluoride (PMSF) (174 g/mL) (Sigma Chemical Co.), aprotinin (6 g/mL) (Sigma Chemical Co.), and leupeptin (10g/mL) (Sigma Chemical Co.) were added to the buffer. [22] 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 separated on a 10–15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. After transferring proteins from the gel to a polyvinylidene di-fluoride membrane (Amersham Biosciences Corp., Uppsala, Sweden), Western blot analysis was performed using 1:1000 diluted anti-Drp1 (BD Biosciences, San Jose, USA), Mfn1 (Abnova Corp., Taipei, Taiwan), Mfn2 (Abnova Corp., Taipei, Taiwan), OPA1 (BD Transduction

Laboratories, USA), cytochrome *c* (BD Biosciences Pharmingen, New Jersey, USA), VDAC (Abcam plc., Cambridge, USA) and α-tubulin (Sigma-Aldrich Corp., St. Louis, USA) antibodies and incubated overnight at 4°C. Subsequently, the chemiluminescent signal labeled using ECL plus (Amersham Biosciences Corp.) was detected using ultraviolet transillumination (Fujifilm Corp.). The membrane was then incubated in a stripping buffer (2% SDS, 100 mM 2-mercaptoethanol, and 12% 0.5 M Tris–HCl, pH 6.8) at 50°C for 30 minutes to remove all probes. The re-probing 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.

4-7. Echocardiography

In vivo cardiac morphology was assessed in non-anesthetized mice using transthoracic echocardiography with an ultrasound machine (SONOS 4500; Philips Medical System, Santa Clara, CA). The M-mode left ventricular (LV) end-systolic and end-diastolic dimensions were averaged from 3 to 5 beats. Fractional shortening (FS) is an index of systolic function that is obtained by measuring the LV end-diastolic diameter and LV end-systolic diameter, dividing the difference by the LV end-diastolic diameter.

Echocardiographic LV mass (in milligrams) of mice was calculated by use of an uncorrected cube assumption from a previously described formula:

$$\text{LV mass} = (\text{LVDd} + \text{IVST} + \text{PWT})^3 - (\text{LVDd})^3.$$ [23]

LVDd; left ventricular end-diastolic diameter, IVST; intraventricular septum wall thickness, PWT; posterior wall thickness.

4-8. Statistical analysis

The results of statistical analyses are expressed as means ± SEM. Differences between groups were analyzed for statistical significance using one-way analysis of variance followed by Tukey-Kramer test for multiple comparisons. Results for which $P < .05$ were inferred as statistically significant. These calculations were performed using software (JMP 8.0; SAS Institute Inc., Cary, NC, USA).

5. Results

5-1. Acute renocardiac injury demonstrated by mitochondrial fragmentation, cellular apoptosis, and cardiac dysfunction

Mouse renal ischemia reperfusion was induced by 30 min bilateral renal artery clamping in male C57BL/6 mice. Remarkable increase of blood urea nitrogen (BUN) (131.90 ± 6.61mg/dl) was observed compared with the sham group (13.58 ± 1.00mg/dl). We confirmed this mouse IR model sufficiently induced acute renal insults by measuring BUN (Fig. 1A). Severe pathological injuries including tubular epithelial cell necrosis, which could be seen in human severe AKI patients, were also observed 24 h later in IR group (Fig. 1B). Pathological analysis by electron microscopy revealed that significantly increased mitochondrial fragmentation in the cardiac tissue in the renal IR group compared with the sham group. Mitochondria area was larger in the IR group ($0.85 \pm 0.16 \mu m^2$) than the sham group ($0.45 \pm 0.12 \mu m^2$). The irregularity of cristae was also observed in the IR group (Fig. 2A, B). Condensed nucleus was also detected in the IR group, which was observed during apoptosis (Fig. 2C). Release of cytochrome c into the cytosol is known to stimulate apoptotic cascade. In this experiment, increased cytochrome *c* release into the cytosol was observed in the renal IR group (Fig. 3). At 24 and 72 h after renal IR, apoptosis in cardiac tissue evaluated by immunohistochemistry

and Western analysis of activated caspase-3 was increased significantly in the renal IR group. Blue staining indicated nucleus and green staining which was seen around the nucleus or interstitium indicated activated caspase-3. Both Western blotting and immunohistochemistry detected apoptosis more frequently at 72h after IR than 24h (Fig. 4). Finally, renal IR injury caused a marked depression of cardiac function at 72 h after the surgery, as indicated by reduced fractional shortening on echocardiography (Fig.5). There were no significant differences in LVDs and LVDd between the groups. Other parameters of echocardiography including heart rate, intraventricular septum wall thickness, posterior wall thickness and left ventricular mass were not significantly different between the groups (Table 1).

Figure 1

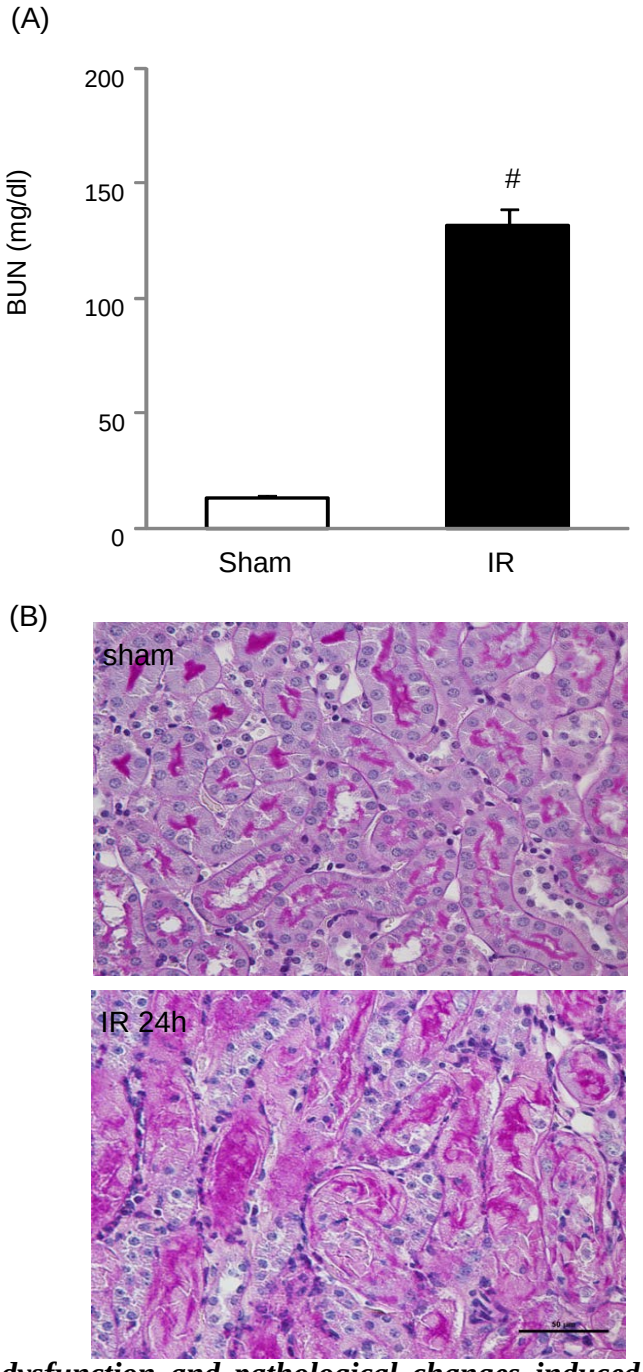


Figure 1. Renal dysfunction and pathological changes induced by renal ischemia reperfusion injury.

(A) BUN 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. (B) Renal tubular necrosis caused by IR is shown in Periodic acid–Schiff (PAS) staining. Original magnification: ×400. Bar = 50 μm.

Figure 2

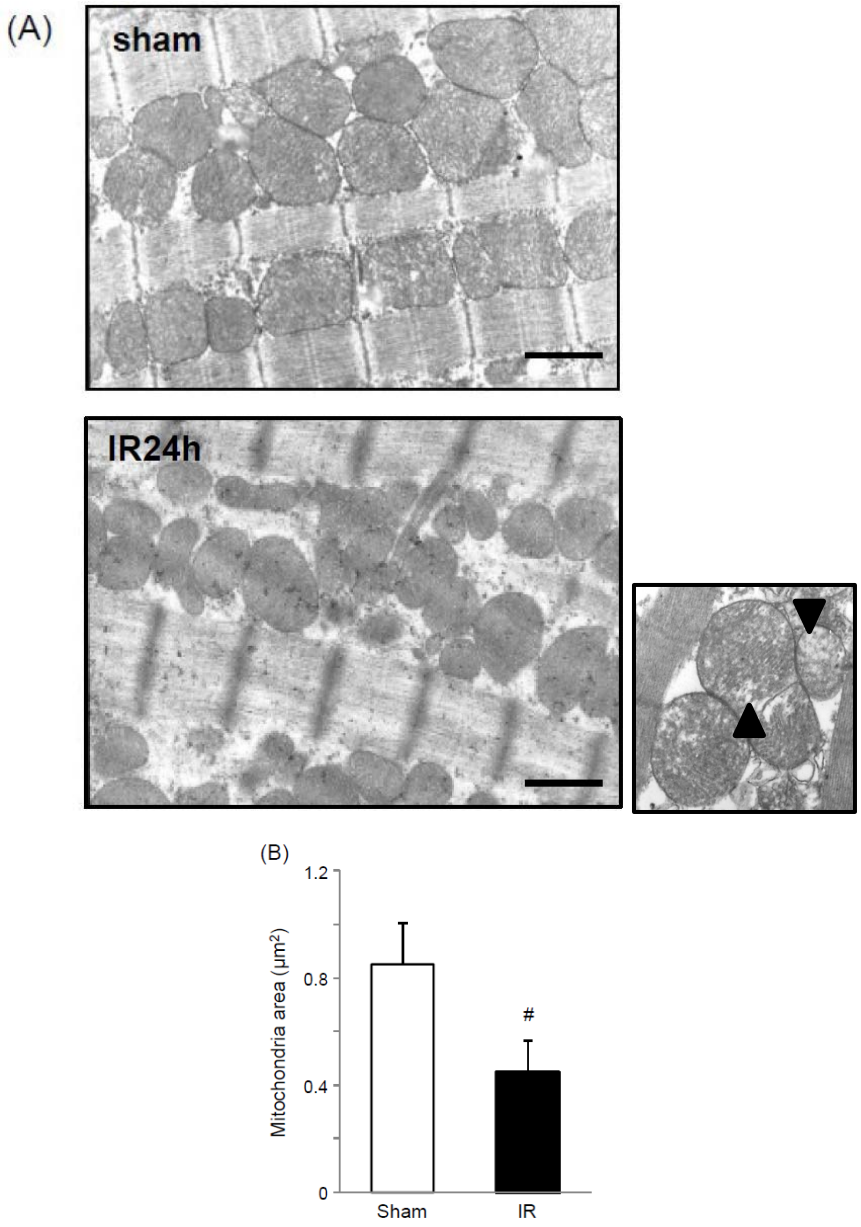


Figure 2. Mitochondrial fragmentation in the heart induced by renal ischemia reperfusion injury.

(A) Electron microscopy showed renal ischemia reperfusion (IR) increased fragmented mitochondria in the cardiac tissue after 24 h. (B) The mitochondria area was significantly reduced in the IR group compared with the sham-operated mice (*n*=5 in each group). Original magnification: ×10,000. Bar = 1μm. The irregularity of cristae was also observed in the IR group (arrowhead, Original magnification: ×25,000.) # $p < .05$ versus sham.

Figure 2

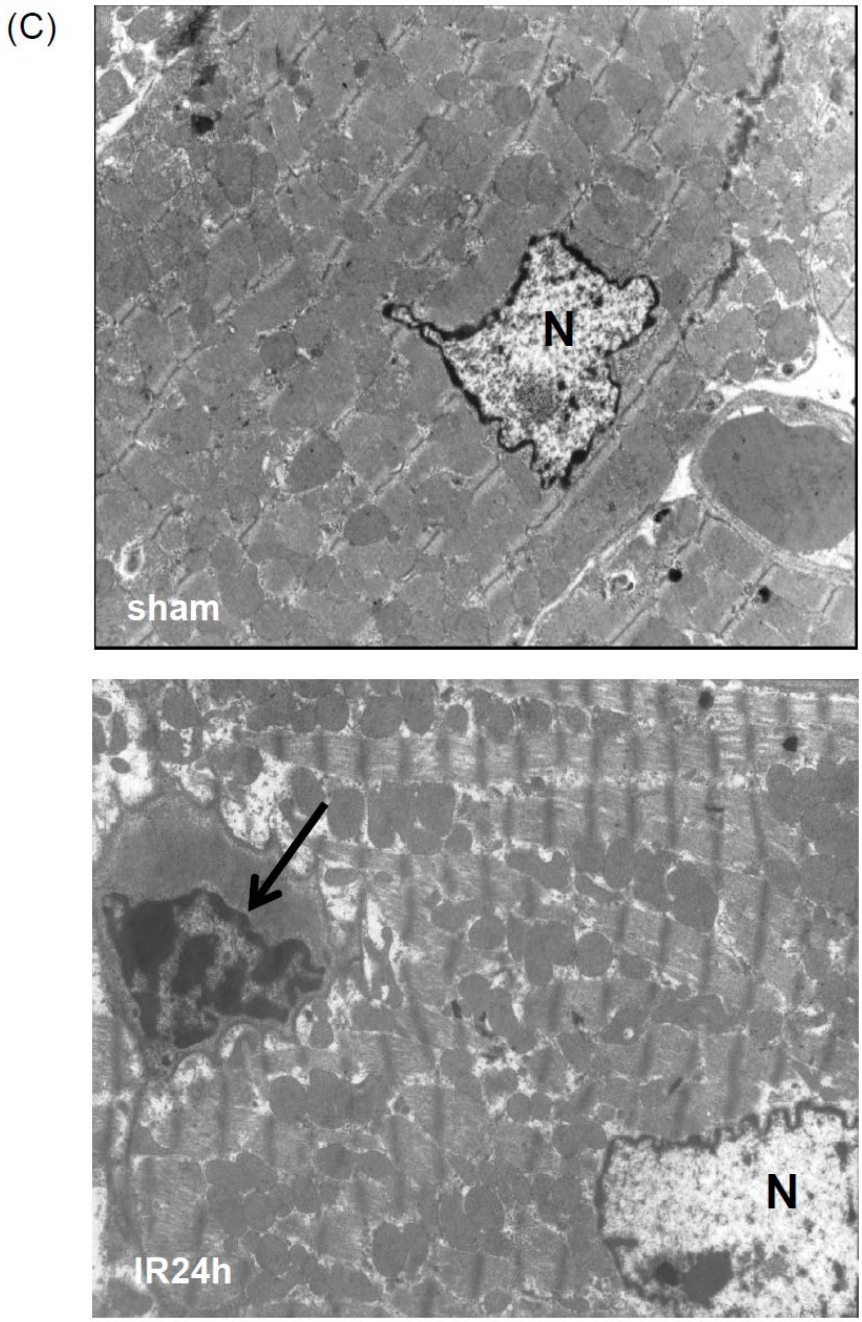


Figure 2. Mitochondrial fragmentation in the heart induced by renal ischemia reperfusion injury.

(C) Electron microscopy showed renal ischemia reperfusion (IR) increased fragmented mitochondria in the cardiac tissue after 24 h. N indicated normal nucleus, whereas condensed nucleus was detected in the heart after renal IR24h. Original magnification: ×5,000.

Figure 3

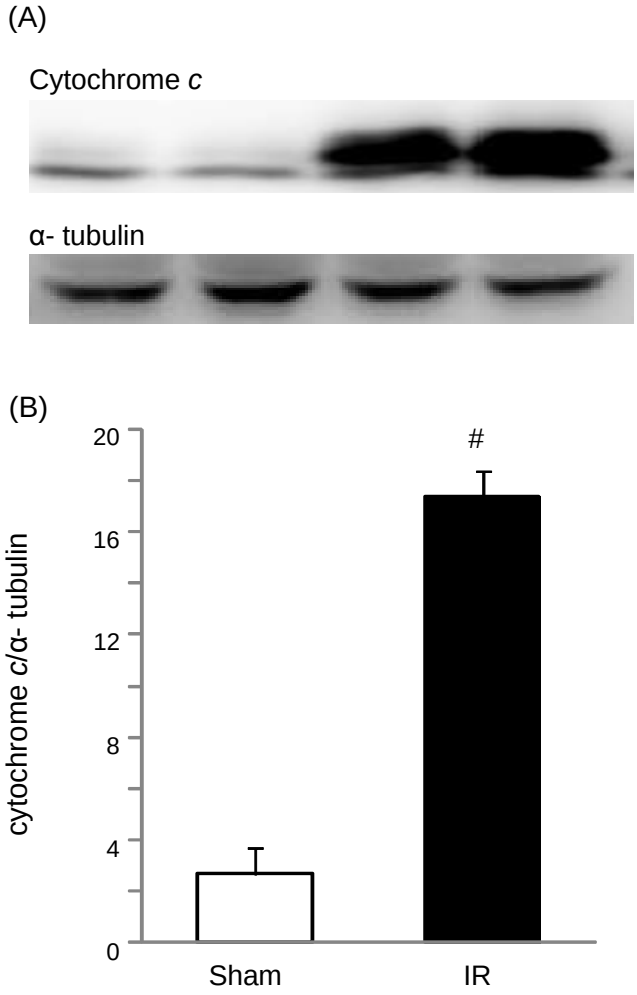


Figure 3. Cytochrome c release into the cytosol of cardiac tissue was increased by renal ischemia reperfusion injury.

(A) Representative image of Western blot analysis for cytochrome c in the cytosolic fractions. (B) Bar graph shows the relative density of bands compared with α-tubulin (*n*=6 in each group). # $p < .05$ versus sham. IR, ischemia perfusion.

Figure 4

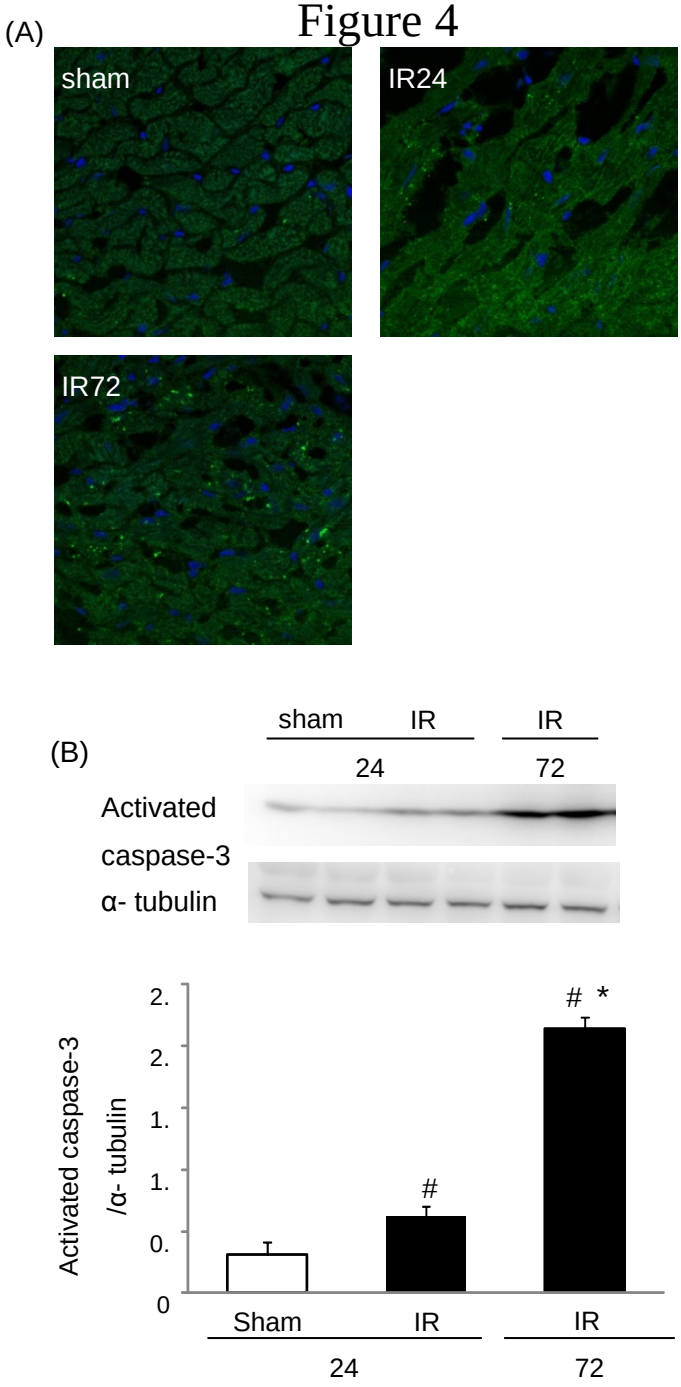


Figure 4. Apoptosis of cardiac tissue induced by renal ischemia reperfusion injury.
(A) Representative image of fluorescence immunohistochemistry for activated caspase-3 in the heart. (B) Representative image of Western blot analysis for activated caspase-3 in the whole tissue lysates. Bar graphs showing the relative density of bands compared with α-tubulin (n=4–6 in each group). # $p < .05$ versus sham. * $p < .05$ versus 24 h. IR, ischemia perfusion.

Figure 5

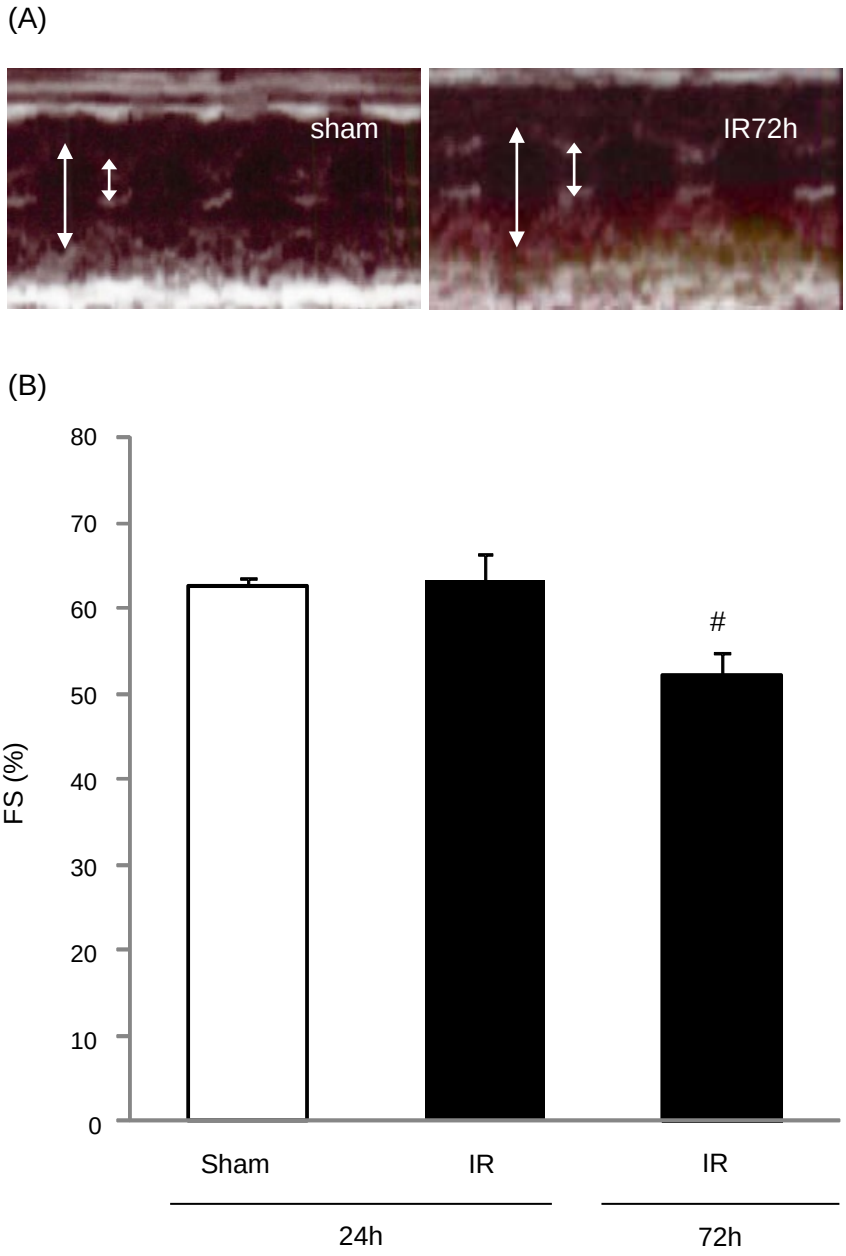


Figure 5. Echocardiography after renal ischemia reperfusion injury.

(A) Representative M modes from echocardiograms in a sham-operated mouse and a mouse exposed to renal ischemia reperfusion. (B) Results of quantitative analysis of the fractional shortenings (FS) from an echocardiogram (n=7 in each group). # $p < .05$ versus sham.

Table 1. Echocardiography after renal ischemia reperfusion injury

	Sham	IR 24h	IR 72h
Heart rate (/min)	608 ± 18	544 ± 44	580 ± 27
LVDd (mm)	2.42 ± 0.17	2.41 ± 0.15	2.33 ± 0.14
LVDs (mm)	0.91 ± 0.06	0.81 ± 0.09	1.15 ± 0.12
IVST (mm)	0.86 ± 0.06	0.75 ± 0.03	0.95 ± 0.02
PWT (mm)	1.08 ± 0.12	0.98 ± 0.04	1.17 ± 0.13
LVM (mg)	58.26 ± 7.00	51.72 ± 5.07	72.79 ± 8.70
FS (%)	62.3 ± 1.1	66.4 ± 3.5	50.8 ± 3.4 #

Values are means ± SE (*n*=5-7 in each group). # $p < .05$ versus sham. LVDd and LVDs ; left ventricular end-diastolic and end-systolic diameter, respectively, IVST, intraventricular septum wall thickness, PWT; posterior wall thickness, LVM; left ventricular mass, FS; fractional shortening

5-2. Mitochondrial dynamics regulating proteins in the heart after renal IR

We evaluated 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. All these mitochondrial dynamics regulating proteins in the whole tissue lysates did not differ between the sham and the renal IR groups (Fig. 6A). However, when we examined protein amounts in the mitochondrial fraction, we found only Drp1 was increased significantly in the renal IR group compared with the sham group. No difference was observed in fusion regulating protein amounts (Mfn1, Mfn2, and OPA1) in the mitochondrial fraction of the heart. Because it is assumed that mitochondrial fragmentation is determined by a balance between fission and fusion even in the normal condition, this result that increased Drp1 in the heart indicated Drp1 may promote mitochondrial fragmentation (Fig. 6B).

Figure 6

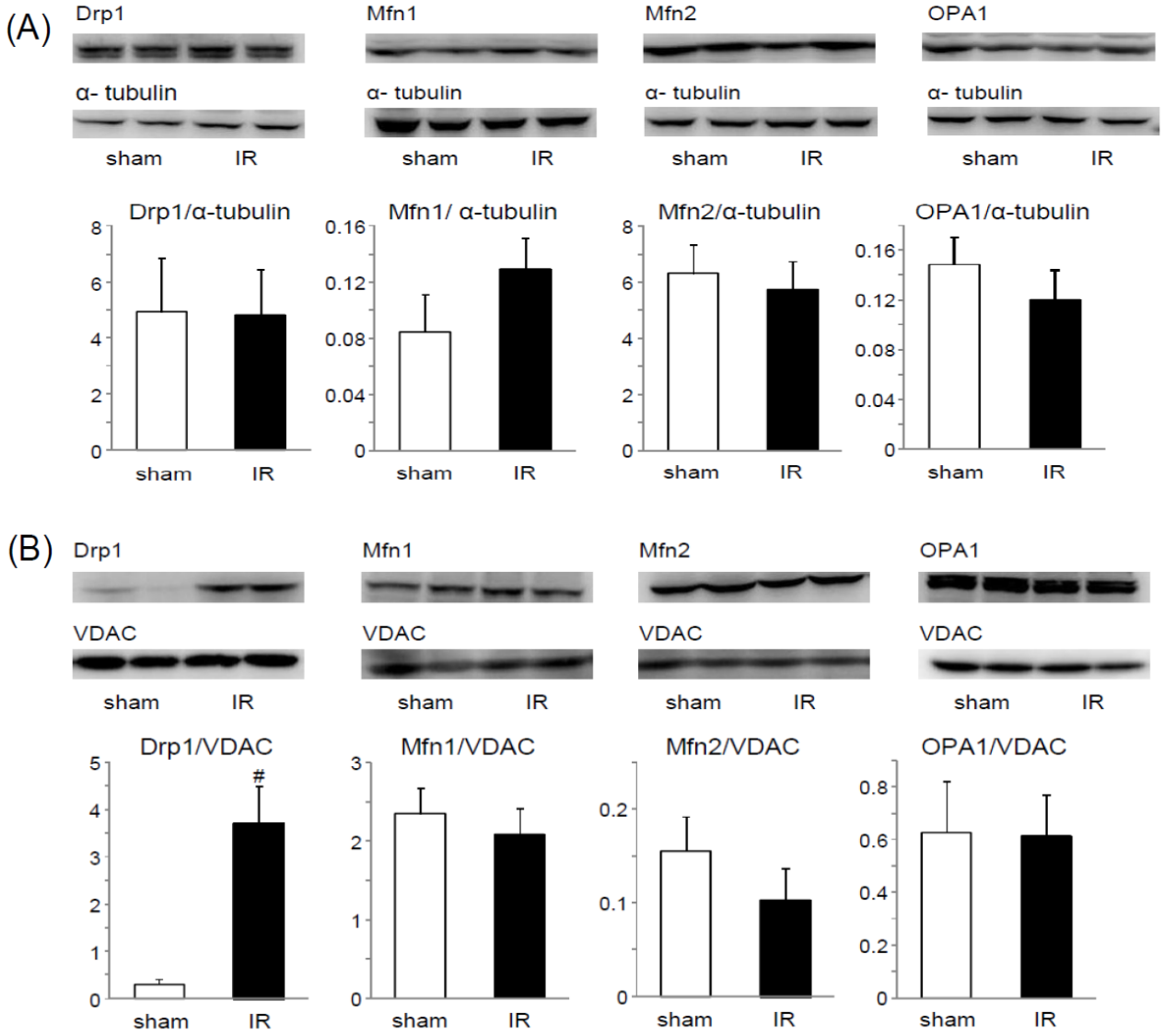


Figure 6. Mitochondrial dynamics regulating proteins in the heart 24 h after renal ischemia reperfusion injury.

(A) Amounts of each protein in whole tissue lysate were evaluated using Western blot analysis. Bar graphs shows the relative density of bands compared with α-tubulin (*n*=4–6 in each group). (B) Amounts of each protein in mitochondrial fraction were evaluated using Western blot analysis. Bar graphs indicates the relative density of bands compared with VDAC (*n*=4–6 in each group). # $p < .05$ versus sham. (A) Amounts of each protein in whole tissue lysate were evaluated using Western blot analysis. Bar graphs shows the relative density of bands compared with α-tubulin (*n*=4–6 in each group).

5-3. Drp1 inhibitor mdivi-1 attenuated acute renocardiac injury

To ascertain the role of Drp1 and mitochondrial fragmentation in the heart, a pharmacological inhibitor of Drp1 called mitochondrial division inhibitor-1 (mdivi-1), which inhibits Drp1 assembly by suppressing its GTPase activity, was administered at the dosage of 50 mg/kg to mice exposed to renal IR. Significantly elevated BUN was detected in both of at 24h (127.00 ± 7.44mg/dl) and 72h after the surgery in the vehicle-treated mice (152.89 ± 30.86mg/dl). Although mdivi-1 treatment did not suppress BUN elevation at 24 h after renal IR (142.54 ± 4.74mg/dl), the tendency of improvement of BUN at 72 h was detected in the mdivi-1 treated mice (86.79 ± 36.53mg/dl). However, it did not reach statistically significance (Fig. 7A). Mitochondrial fragmentation in the heart at 24 h was significantly more improved in the mdivi-1 group than in the vehicle group, which was confirmed by electron microscopy. Mitochondria area was significantly increased in the mdivi-1 treated IR group (0.88 ± 0.20 μm^2) than the vehicle group (0.51 ± 0.12 μm^2) (Fig. 7B). Mdivi-1 treatment suppressed Drp1 translocation to the mitochondria (Fig. 7C) and also suppressed cytochrome c release into the cytosol (Fig. 7D). Apoptosis in cardiac tissue and depression of cardiac function examined by echocardiography at 72 h after renal IR were improved by mdivi-1 treatment (Figs. 7E and 7F).

Figure 7

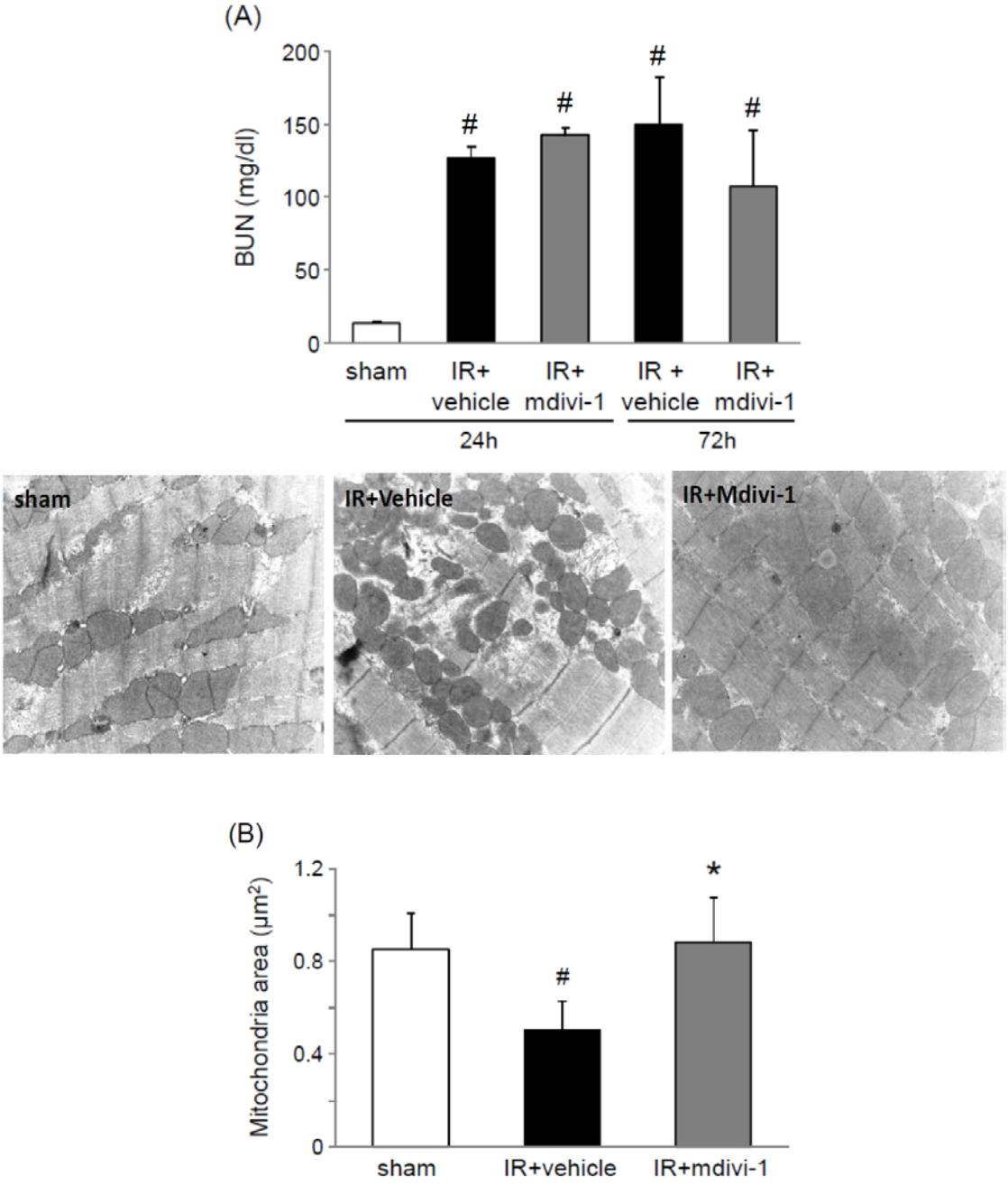


Figure 7. Effects of Drp1 inhibitor mdivi-1 on the heart in mouse renal ischemia reperfusion injury.

(A) BUN concentration of each group is shown (n=7 per group). (B) Mitochondrial fragmentation was reduced by mdivi-1 treatment (n=5 per group). # $p < .05$ versus sham. * $p < .05$ versus IR.

Figure 7

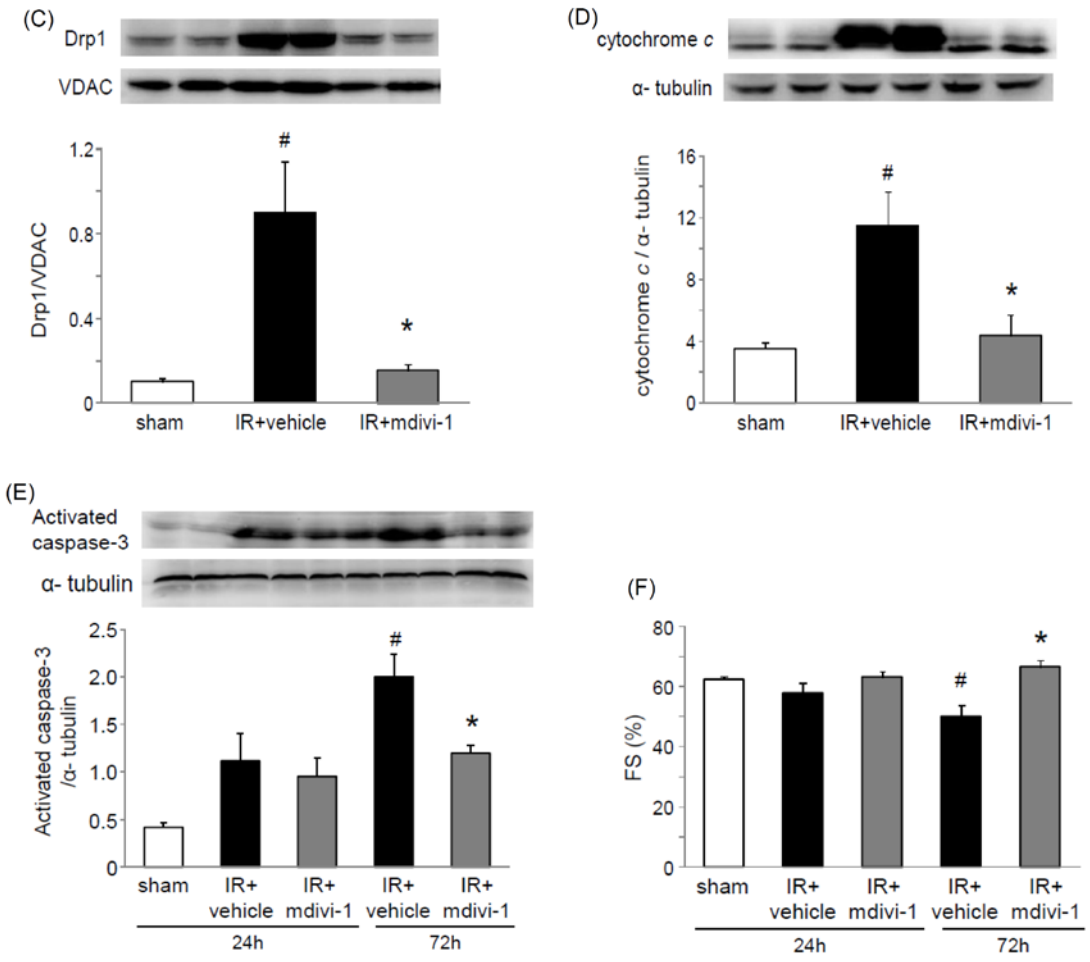


Figure 7. Effects of Drp1 inhibitor mdivi-1 on the heart in mouse renal ischemia reperfusion injury.

(C) Drp1 expression in the mitochondria fraction was reduced by mdivi-1 treatment (*n*=8–10 per group). # $p < .05$ versus sham. * $p < .05$ versus IR. (D) Cytochrome *c* release into the cytosolic fraction was suppressed by mdivi-1 treatment (*n*=6 per group). # $p < .05$ versus sham. * $p < .05$ versus IR. (E) Apoptosis evaluated by Western blot analysis for activated caspase-3 was reduced by mdivi-1 treatment (*n*=6 per group). # $p < .05$ versus sham. * $p < .05$ versus IR 72 h. (F) Quantitative analysis of the fractional shortenings (FS) showed the protection of mdivi-1 (*n*=4–6 per group). # $p < .05$ versus sham. * $p < .05$ versus IR 72 h.

6. Discussion

Recently, organ interactions between the kidney and other organs such as heart, lung, and brain have been investigated. A number of clinical studies showed treatment against acute renal dysfunction by using blood purification techniques cannot sufficiently improve the outcomes of critically ill patients complicated with AKI even though uremia determined by blood urea and creatinine levels were perfectly controlled. This suggests AKI induces and/or amplifies other organ injury by unknown mechanisms. To clarify these issues, basic research has been conducted mostly with bilateral renal ischemia reperfusion (IR) injury model, which causes both decreased renal function and ischemic organ injury in the kidney. [24] This model is clinically relevant given that a significant portion of in-hospital AKI is thought to be caused by ischemic insults such as decreased cardiac output, renal vascular constriction, and renal transplantation.[25] Another AKI model of bilateral nephrectomy is also used for investigation of organ interaction by AKI, however this model may represent the isolated impact of absent renal function. These two models of IR and bilateral nephrectomy can be used to compare the impacts of ischemic insults against the kidney and lack of kidney function alone. To achieve clinical relevance, we decided to use renal IR injury model because it is supposed to mimic clinical human AKI setting as described above.

Complication of heart and kidney dysfunction in critically ill patients has a substantial

impact on their outcomes. These two disorders worsen each other synergistically. Therefore, identifying the pathway of organ interaction between the heart and the kidney will be crucial for developing targeted therapies to improve the outcomes. This study demonstrated for the first time ever reported that the depression of cardiac function caused by AKI was associated with morphological change in the heart as seen by mitochondrial fragmentation and apoptosis and that Drp1 activation plays a crucial role in AKI-induced cardiac injury (acute renocardiac syndrome).

The kidney and the heart require large amounts of energy production. Therefore they are rich in mitochondria. Consequently, it can be assumed that mitochondrial damage contributes to the pathogeneses of acute kidney and heart dysfunction. Recently, Brooks and colleagues reported a remarkable morphological change of mitochondria in acute kidney injury models including ischemia reperfusion and cisplatin injection.[18] Mitochondrial fragmentation was observed earlier than cytochrome *c* release and cellular apoptosis. Dominant-negative or siRNA knockdown experiments demonstrated the role of Drp1 in mitochondrial fragmentation.[18] Sharp and colleagues showed that Drp1 activation during cardiac IR caused left ventricle dysfunction, which was reversed by Drp1 inhibition.[20] The present 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 another remote organ injury. However, possible mediators that induced Drp1 activation in the heart still remained uncertain. In addition, we did not investigate other distant organ effects by renal IR such as brain, lung or liver. Therefore, it is also unclear whether Drp1 activation induced by renal IR injury was heart-specific or not.

Mitochondria fragmentation describes abnormally small mitochondria and is reportedly observed in apoptotic cells.[26,27] A crucial role for mitochondrial fragmentation in apoptosis has been reported.[28] It is assumed that mitochondrial fragmentation is determined by a balance between fission and fusion of mitochondria. Although accumulation of a fission GTPase Drp1 on mitochondria during apoptosis has been reported by several studies,[29-32] insufficient mitochondrial fusion might also contribute cellular apoptosis. Three large GTPases in the dynamin family mediate mitochondrial fusion: Mfn1, Mfn2, and OPA1. These fusion GTPases are reportedly related to apoptosis.[33,34] However, we found no change of their expressions in mitochondrial fractions and 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.

Drp1 is an intensively investigated mitochondrial fission-regulating molecule.

Approximately 97% of Drp1 locates in the cytoplasm under normal 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.[35,36] An inhibitor of mitochondrial division has been identified using yeast screening of chemical libraries.[37] This compound (mdivi-1) inhibits Drp1 assembly and GTPase activity, and inhibits subsequent mitochondrial fission. Therefore, mdivi-1 is widely used for exploring 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 ischemia reperfusion injury models.[18-20] In accordance with these reports, we showed protection by mdivi-1 on 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 and the duration of efficacy of mdivi-1 in vivo are not known, several studies have demonstrated protection by mdivi-1 in organ ischemia reperfusion injury models at 10 minutes to 1 hour after mdivi-1 injection.[15-17]

This study does not clarify why Drp1 in the heart was activated by renal ischemia reperfusion 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 (HMGB1) induced lung injury via toll-like receptor 4 in another mouse AKI model of bilateral nephrectomy.[38] Second, ischemia reperfusion injury on one organ might cause systemic reaction possibly mediated by inflammatory cytokines or cells. Andrés-Hernando and colleagues reported proinflammatory cytokines IL-6, CXCL1, IL-1β, and TNF-α were increased not only in the kidney, but in the spleen and liver in renal ischemia reperfusion injury.[39] Awad and colleagues observed a marked increase of neutrophil margination in the lungs in mouse renal ischemia reperfusion model.[40] Patschan and colleagues demonstrated that endothelial progenitor cell homing to the spleen was induced by renal ischemia reperfusion.[41] Another limitation of this study was that it was uncertain whether observed fragmented mitochondria were all related to apoptosis induction in the heart. Because mitochondria fission and fusion frequently occur to keep the quality of mitochondria in organs under physiological condition, mitochondrial fission cannot always induce cellular apoptosis. Based on the observation of this study, we may be able to speculate that relatively enhanced fission pathway compared with fusion pathway resulted in increased mitochondrial fragmentation followed by apoptosis in heart. Further investigation must

be conducted to ascertain the responsible pathways in cardiac injury caused by renal ischemia reperfusion and the mechanism by which enhanced mitochondrial fission determines cellular fate towards apoptosis.

In conclusion, this study demonstrated for the first time that Drp1-mediated mitochondrial fragmentation and subsequent organ disorder was caused in the heart during renal ischemia reperfusion injury. Although clinical situations in which AKI results in acute cardiac dysfunction are frequently observed and recently defined as acute renocardiac syndrome,[4] the pathophysiology of this syndrome has not been well demonstrated by basic research. Therefore, result obtained by this study strongly suggests that mitochondria fission and apoptosis in the heart as a distant organ effect by AKI can be a good therapeutic target to improve critically ill patients. Moreover, Drp1 inhibition should be examined to prevent cardiac dysfunction related with AKI.

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