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Remote Ischemic Pre-Conditioning Alleviates Contrast-Induced Acute Kidney Injury in Patients With Moderate Chronic Kidney Disease

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Background: Although remote ischemic preconditioning (RIPC) is shown to preserve kidney function in patients at high risk of contrast-induced acute kidney injury (CI-AKI), the effect in patients at low-moderate risk remains unknown. The preventive effects of RIPC in patients not at high risk of CI-AKI were examined, and biomarkers with anticipated roles in renal protection via RIPC investigated.

Methods and Results: Sixty patients who had moderate chronic kidney disease and who underwent angiography were randomly assigned to the control (n=30) or RIPC (intermittent arm ischemia, n=30) group. The baseline characteristics in the 2 groups did not differ significantly. CI-AKI was evaluated by measuring urinary liver-type fatty acid-binding protein (L-FABP). Biomarkers were measured before and 24 and 48 h after angiography. Twenty-four hours after angiography, the percent change in urinary L-FABP level in the RIPC group was significantly smaller than in the control group (41.3±15.6 vs. 159±34.1%, P=0.003). L-FABP-based CI-AKI developed in 8 control patients (26.9%) vs. only 2 patients in the RIPC group (7.7%), suggesting that RIPC prevents CI-AKI. Factors contributing to CI-AKI were analyzed. Neither high-sensitivity C-reactive protein nor pentraxine-3 level differed significantly between the 2 groups, while the percent change in asymmetrical dimethy larginine (ADMA) level and blood derivatives of reactive oxidative metabolite levels were significantly smaller in the RIPC group.

Conclusions: RIPC alleviates CI-AKI in patients at low-moderate risk. This effect might be mediated partly by decreasing oxidative stress and plasma ADMA level. (*Circ J* 2013; **77:** 3037–3044)

Key Words: Contrast-induced acute kidney injury; Coronary angiography; Liver-type fatty acid-binding protein; Remote ischemic preconditioning

The use of contrast medium for diagnostic and interventional cardiovascular procedures can cause contrast-induced acute kidney injury (CI-AKI). Risk factors for CI-AKI include chronic kidney disease (CKD), diabetes mellitus (DM), congestive heart failure, intravascular volume depletion, and use of a large volume of contrast medium.[1,2] Although the mechanism of CI-AKI is multifactorial, the consensus pathogenesis involves combined hypoxic and toxic renal tubular damage with renal endothelial dysfunction and decreased intrarenal microcirculation. Contrast medium-induced hypoxia of the renal medulla leads to the production of renal free radicals via post-ischemic oxidative stress.[3–5] Although CI-AKI is associated with prolonged hospitalization and adverse clinical outcome,[6] the established preventive approach to CI-AKI simply involves identifying patients at risk, minimizing the volume of contrast medium, and providing adequate i.v. volume expansion.[7–10] Therefore, the development of a novel therapeutic approach for CI-AKI is desirable.

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Although creatinine is widely used, it is a suboptimal marker of renal injury because it does not rapidly reflect the altered glomerular filtration rate (GFR) or degree of tubular injury.[11] The canonical creatinine-based CI-AKI has a predictable time course, during which creatinine level increases from 24 to 48 h after contrast medium exposure and peaks within 2–5 days. Moreover, renal function must decrease by more than half before an increase in serum creatinine is detected. The urinary excretion of liver-type fatty acid-binding protein (L-FABP) is a newly emerging biomarker that reflects tubulointerstitial damage, including ischemic and toxic insults, and increases rapidly and peaks within 24 h after use of contrast medium.[12] In addition, urinary L-FABP level is increased after contrast medium use in

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mild-to-moderate CKD, during which no patient exhibits an increase in creatinine.[1] Therefore, urinary L-FABP is a more useful marker than creatinine for the early detection of CI-AKI.[12–14]

In 2006, Cheung et al discovered remote ischemic preconditioning (RIPC), in which transient non-lethal ischemia and reperfusion applied to 1 organ protects another organ from a subsequent episode of lethal ischemia and reperfusion injury.[15–18] Several studies have shown that RIPC can preserve kidney function in patients undergoing elective endovascular or open surgical repair for an abdominal aortic aneurysm and elective coronary artery bypass graft surgery.[19–21] In addition, RIPC can prevent contrast-medium-induced nephropathy. Er et al showed that RIPC induced by intermittent upper-arm ischemia before an invasive coronary procedure, dramatically reduces the incidence of contrast medium-induced nephropathy (CIN)[22] in patients at high risk of CI-AKI according to the Mehran risk score.[23] The preventive effects of RIPC in patients at low-to-moderate risk of CI-AKI, however, remain elusive. In addition, the underlying mechanism responsible for the renal protective effect is unclear. We tested the hypothesis that RIPC attenuates CI-AKI in patients who are not at high risk. Moreover, we investigated biomarkers with anticipated roles in the renal protection resulting from RIPC.

Methods

Subjects

Eligible patients who had moderate CKD, who were not in cardiogenic shock and acute renal failure, and who were undergoing angiography were enrolled in this study. Sixty patients scheduled for elective angiographic procedures from February 2011 to October 2012 who had moderate CKD were enrolled in this study. CKD was defined using estimated GFR (eGFR), calculated using the equation of the modification of the diet in renal study, for Japanese individuals as recommended by the Japanese Society of Nephrology as: eGFR ($ml \cdot min^{-1} \cdot 1.73\,m^{-2}$) = $194 \times [\text{serum creatine}]^{-1.094} \times [\text{age}]^{-0.287} \times [0.739 \text{ if female}]$. Moderate CKD was defined as eGFR=30–60 $ml \cdot min^{-1} \cdot 1.73\,m^{-2}$. Patients were excluded if the contrast volume used was <40 ml or >300 ml after angiographic procedures. The study objectives and protocol were fully explained to the patients and written informed consent was obtained from all patients. The following 4 hospitals participated in this study: Department of Cardiovascular and Respiratory Medicine Akita University Hospital; Division of Cardiovascular Medicine, Honjo Daiich Hospital; Yuri Kumiai General Hospital; and Yamamoto Kumiai General Hospital. The numbers of enrolled patients were 43, 10, 3, and 4, respectively.

This study was approved by the regional ethics committee in accordance with the standards of the Declaration of Helsinki.

Study Protocol

This was designed as a prospective, randomized, non-blinded, multicenter study. Sealed envelopes were used to randomly assign treatment with only i.v. volume (control group); or i.v. volume combined with RIPC (RIPC group) at the time of scheduled coronary angiography (CAG) or percutaneous coronary intervention (PCI). Randomization was conducted at the Department of Cardiovascular and Respiratory Medicine, Akita University Hospital, in a single setting. All patients were given 0.9% isotonic saline i.v. at a rate of at least 1.0 ml/kg/h beginning at least 4 h before angiography, until 12 h after contrast exposure. All patients were allowed to drink if they were thirsty. RIPC consisted of intermittent upper arm ischemia involving 4 cycles of 5-min inflation of a blood pressure cuff to 200 mmHg and 5-min deflation. The RIPC protocol was applied 2 h before starting the procedure. CAG and PCI were performed using standard techniques. Interventional devices were selected by the operators. Serious complications did not occur in either group. The low-osmolality contrast medium iopamidol (350 mg iodine/ml; Iopamiron®, Bayer Pharmaceutical, Japan) was used. The amount of contrast medium used in each patient was recorded after the procedure. The primary endpoint was the incidence of L-FABP-based CI-AKI as stated in a previous report in which the disease-monitoring L-FABP cut-off level in patients with CKD was 17.4 μg/g Cr.[24] L-FABP-based CI-AKI was defined as L-FABP >17.4 μg/g Cr within 24 h after use of the contrast medium. If baseline L-FABP was >17.4 μg/g Cr, L-FABP-based CI-AKI was defined as an increase >25% from baseline. Secondary endpoints were the level of high-sensitivity C-reactive protein (hs-CRP), plasma pentraxin 3 (PTX3), the derivatives of reactive oxidative metabolites (D-ROM), and asymmetrical dimethylarginine (ADMA) at 24 h, and serum creatinine, eGFR and cystatin C at 48 h after contrast medium exposure. Incidence of creatinine-based CI-AKI was defined as an increase in serum creatinine >25% from baseline or an absolute increase ≥0.5 mg/dl within 48 h after use of contrast medium in accordance with previous reports.[2,22,23,25]

We are planning a study of a continuous response variable from independent controls and experimental subjects with 1 control per experimental subject. In a previous study,[1] the response within each subject group was normally distributed with standard deviation 39.4 μg/g Cr. If the true mean difference between experimental and control subjects is 30.2 μg/g Cr, then we need to study 28 experimental subjects and 28 control subjects to reject the null hypothesis that the population mean of the experimental and control groups is equal with a probability (power) of 0.8. The type I error probability associated with this test of the null hypothesis is 0.05. Therefore, we designed this study as an exploratory effort to assemble the data required to verify this, and determined the sample size considering the operability of this study.

Measurement of Biomarkers

Urinary L-FABP level was measured on specific ELISA, as described previously.[12,26,27] Previous studies have shown that urinary L-FABP level increases and peaks at 24 h after use of contrast medium.[1,12] Based on these results, we measured urinary L-FABP before RIPC, and at 24 h and 48 h after use of contrast medium. Additionally, we measured other biomarkers (hs-CRP, PTX3, D-ROM and ADMA) that may affect urinary L-FABP level before and 24 h after use of contrast medium. D-ROM was measured using a free radical and antioxidant potential determination device (Free Radical Analytical System 4; Health and Diagnostics, Grosseto, Italy).[28] The normal range of D-ROM is between 250 and 300 Carr units.[29] The test was linear up to 500 Carr units and had very good analytical performance, with intra-assay and inter-assay coefficients of variation <4%.[29,30] Serum ADMA level was measured on high-performance liquid chromatography.[31] In a previous study, serum ADMA level in the normal eGFR group was 0.37±0.07 nmol/ml.[32]

In contrast to urinary L-FABP level, the increase in serum creatinine level is detected at 48 h after use of contrast medium.[22,25] Contrast medium-induced elevation of cystatin C is found at 24 h and persists at least 48 h.[22,33] Therefore, we estimated serum creatinine, eGFR and cystatin C, at 48 h after use of contrast medium.

Statistical Analysis

Continuous variables are expressed as mean±SD or ±SE. Con-

Table 1. Baseline CKD Subject Characteristics

	All patients (n=60)	Control group (n=30)	RIPC group (n=30)	P-value
Age (years)	71.1±7.8	70.8±7.6	71.3±8.1	0.81
Male sex	43 (71.7)	23 (76.7)	20 (66.7)	0.41
BMI (kg/m²)	23.6±3.4	23.6±2.8	23.7±3.9	0.86
Underlying heart disease				
Ischemic heart disease	46 (76.7)	24 (80.0)	22 (73.3)	0.55
Peripheral artery disease	7 (13.5)	3 (11.5)	4 (15.4)	0.69
Macrovascular disease	5 (9.6)	3 (11.5)	2 (7.7)	0.65
Valvular heart disease	4 (7.7)	1 (3.8)	3 (11.5)	0.31
Pulmonary artery hypertension	2 (3.8)	1 (3.8)	1 (3.8)	1.00
Cardiomyopathy	5 (9.6)	3 (11.5)	2 (7.7)	0.65
Bradycardia	7 (13.5)	3 (11.5)	4 (15.4)	0.69
Blood pressure				
Systolic (mmHg)	120.5±16.8	119.3±15.4	120.5±18.2	0.78
Diastolic (mmHg)	68.6±12.4	69.0±11.8	68.30±13.3	0.83
Heart rate (beats/min)	69.8±9.90	70.8±10.2	69.1±9.73	0.50
Contrast medium usage (ml)	92.4±36.1	91.8±39.4	92.9±33.2	0.90
Total volume of infusion (ml)	1,402±160	1,399±156	1,405±166	0.89
Medication				
ACEIs/ARBs	41 (68.3)	22 (73.3)	19 (63.3)	0.50
β-blockers	26 (43.3)	12 (40.0)	14 (46.6)	0.63
Diuretics	20 (33.3)	11 (36.7)	9 (30.0)	0.60
Ca antagonists	33 (55.0)	18 (60.0)	15 (50.0)	0.49
Statins	45 (75.0)	23 (76.7)	22 (73.3)	0.83
Diabetes medicines	20 (33.3)	9 (30.0)	11 (36.7)	0.60
Sulfonylurea	10 (16.7)	6 (20.0)	4 (13.3)	0.50
Laboratory data				
eGFR (ml·min⁻¹·1.73 m⁻²)	48.2±7.9	48.9±6.0	47.4±9.4	0.47
Serum creatinine (mg/dl)	1.13±0.24	1.12±0.17	1.15±0.29	0.59
Cystatin C (mg/L)	1.43±0.46	1.38±0.28	1.47±0.59	0.44
Hemoglobin (g/dl)	12.5±1.9	12.3±1.7	12.7±2.0	0.47
Integer CI-AKI risk score				
Mean (Q1–3)	6 (5–9)	6 (4–8)	6 (4–9)	0.44
<5	23 (38.3)	12 (40.0)	11 (36.7)	0.79
6–10	31 (51.7)	17 (56.7)	14 (46.7)	0.45
11–15	6 (10.0)	1 (3.3)	5 (16.7)	0.09
>16	0 (0.0)	0 (0.0)	0 (0.0)	

Data given as mean±SD, n (%) or as specified.
ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CI-AKI, contrast-induced acute kidney injury; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; RIPC, remote ischemic pre-conditioning.

tinuous, normally distributed data were analyzed using Student's t-test; Mann-Whitney U-test was used for non-normally distributed data. Correlations were evaluated using Pearson's correlation coefficient. P<0.05 was considered to indicate statistical significance. All statistical analysis was performed using SPSS for Windows version 19.0 (SPSS, Chicago, IL, USA). Statistical analysis included all randomized subjects.

Results

Patient Characteristics

In total, 60 patients were included in this study and no patient was excluded after randomization. The baseline characteristics of the 60 enrolled CKD patients are summarized in Table 1. The 2 groups did not differ statistically in terms of age, gender, body mass index (BMI), underlying diseases, blood pressure, heart rate, contrast medium use, total volume of saline infusion, ejection fraction, medication, or laboratory data including eGFR, serum creatinine, cystatin C, and hemoglobin levels. In patients with DM, sulfonylurea possibly influences the effects of RIPC by inhibiting ATP-sensitive potassium channels, but there was no significant difference in sulfonylurea use between the 2 groups (control vs. RIPC: 20% vs. 13%, P=0.50; Table 1). The risk of developing CI-AKI was evaluated using the Mehran risk score.[23] All subjects were categorized as low-to-moderate risk and there was no significant difference in the mean risk score between 2 groups.

RIPC Reduces Prevalence of CI-AKI

Twenty-four hours after use of the contrast medium, urinary L-FABP level in the control group increased significantly from 7.2±5.4 to 14.0±8.8 μg/g Cr (P<0.001), whereas that in the RIPC

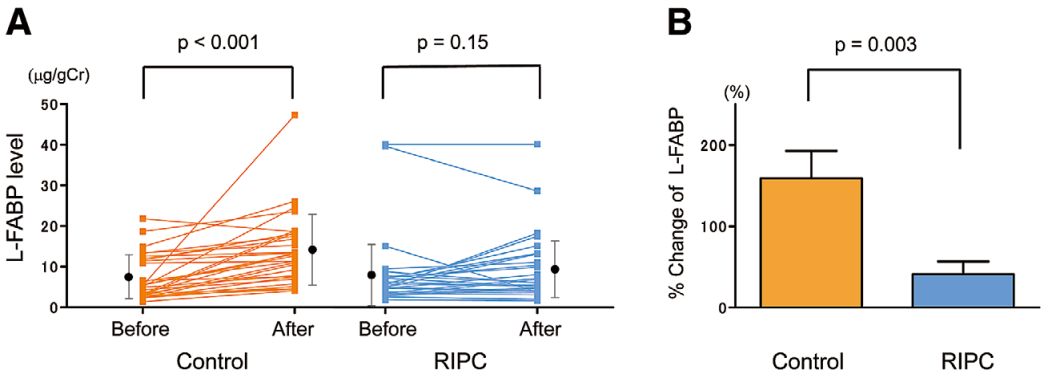

Figure 1. Urinary liver-type fatty acid-binding protein (L-FABP) level before and at 24 h after use of contrast medium in patients with or without remote ischemic preconditioning (RIPC). (**A**) Changes in individual data (mean, filled circle; standard deviation, vertical bars); (**B**) percent change (mean, columns; standard error, vertical bar).

Table 2. Change in Biomarkers After Use of Contrast Medium

	Control	RIPC	P-value
L-FABP (μg/g Cr)			
Before	7.2±5.4	8.0±9.1	0.7
24 h after	14.0±8.8	9.4±8.3	0.04
48 h after	13.4±5.7	8.3±6.5	0.21
(%) change of L-FABP			
24 h after	159±34.1	41.3±15.6	0.003
48 h after	62.4±26.8	40.0±17.9	0.48
Serum creatinine (mg/dl)			
Before	1.12±0.17	1.15±0.29	0.59
48 h after	1.19±0.30	1.16±0.25	0.7
eGFR (ml·min^{-1}·1.73 m^{-2})			
Before	48.9±6.0	47.4±9.4	0.44
48 h after	47.3±7.1	49.0±10.3	0.46
Cystatin C (mg/L)			
Before	1.38±0.28	1.47±0.59	0.44
48 h after	1.46±0.34	1.45±0.53	0.81

Data given as mean±SD (absolute change) or mean±SE (% change).
L-FABP, urinary liver-type fatty acid-binding protein. Other abbreviations as in Table 1.

Table 3. Prevalence of L-FABP-Based CI-AKI

	CI-AKI (–) n (%)	CI-AKI (+) n (%)
Control	22 (73.3)	8 (26.9)
RIPC	28 (93.3)	2 (7.7)

Abbreviations as in Tables 1,2.

group did not change significantly (8.0±9.1 vs. 9.4±8.3 μg/g Cr, P=0.15; Figure 1A). The percent change in the urinary L-FABP level at 24 h in the control group was significantly larger than in the RIPC group (159±34.1 vs. 41.3±15.6%, P=0.003; Figure 1B). The difference in the mean % change of L-FABP at 24 h was 118, and the 95% confidence interval (95% CI) was 42.7–193. There was no significant difference in %change of L-FABP level at 48 h between the control and RIPC group (62.4±26.8 vs. 40.0±17.9%, P=0.48; Table 2). When comparing changes in urinary L-FABP level at 24 h, the creatinine, eGFR, and cystatin C did not change significantly 48 h after the angiographic procedure in either group (Table 2).

L-FABP-based CI-AKI developed in 10 patients overall: 8 in the control group and 2 in the RIPC group. The difference in the prevalence of CI-AKI in the 2 groups was significant (26.9 vs. 7.7%, P=0.038; Table 3).

RIPC and Biomarkers

To explore the mechanism responsible for the renal protective effect of RIPC, we examined the inflammatory biomarkers hs-CRP and PTX3. As shown in Figure 2, the percent change in hs-CRP (143±47.5 vs. 104±36.7%, P=0.53) and PTX3 (5.1±6.2 vs. 8.4±6.2%, P=0.71; Figures 2A,B) did not differ significantly between the control and RIPC groups at 24 h after contrast medium.

The plasma level of D-ROM, an oxidative stress biomarker, was assayed before and 24 h after angiography. Figure 2C shows that plasma D-ROM level increased significantly (P=0.01)

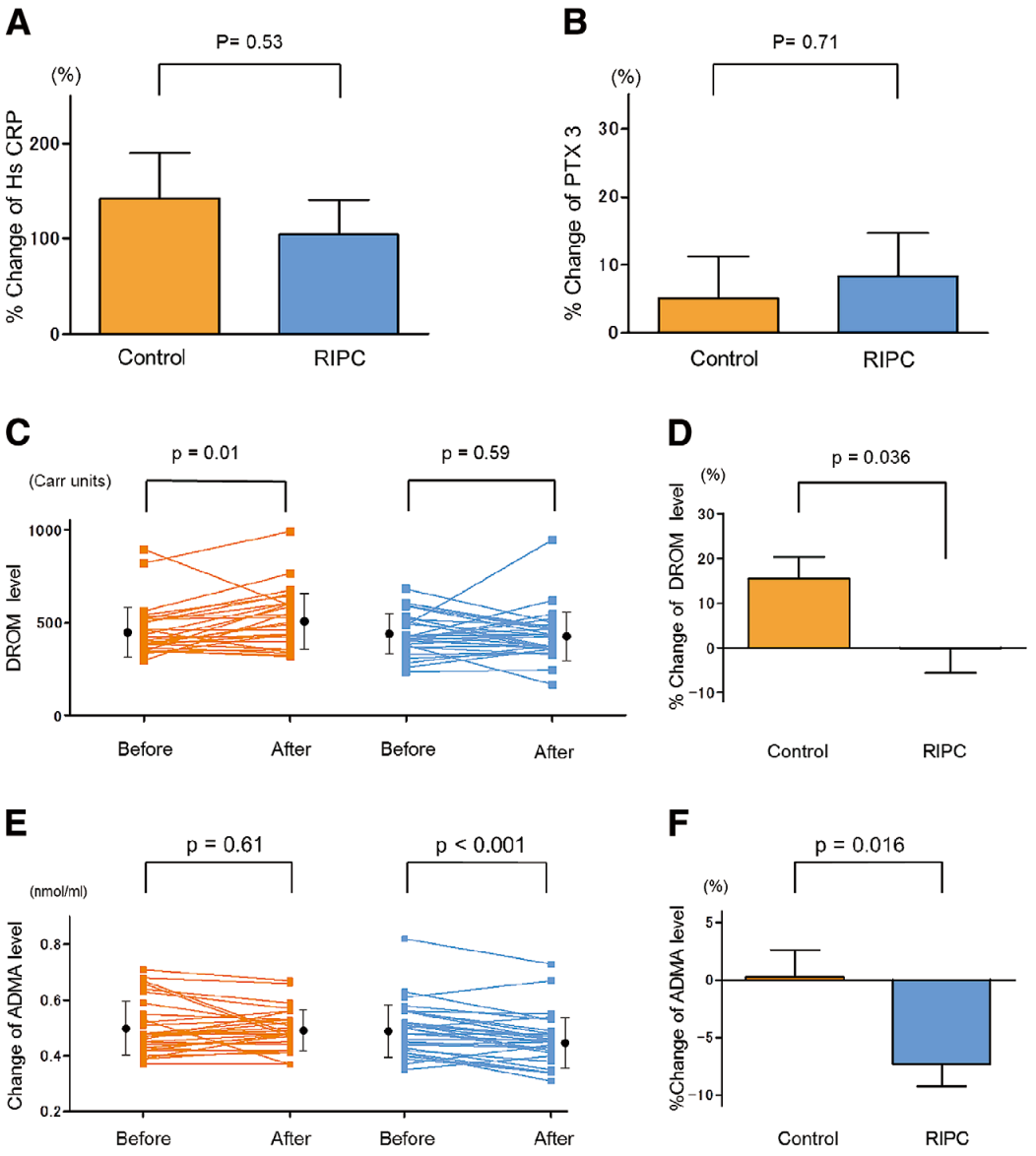

Figure 2. Changes in biomarkers before and at 24 h after use of contrast medium in patients with or without RIPC. (**A**,**B**) Percent changes in inflammation biomarkers. (**C**) Change and (**D**) % change in derivatives of reactive oxidative metabolite (D-ROM) level. (**E**) Change and (**F**) % change in derivatives of asymmetrical dimethylarginine (ADMA) level. (**A**,**B**,**D**,**F**) Data given as mean (bar) and standard errors (vertical bars). (**C**,**E**) Data given as mean (filled circle) and standard deviation (vertical bars). hs-CRP, high-sensitivity C-reactive protein; PTX-3, pentraxine-3; RIPC, remote ischemic preconditioning.

from 448±132 to 508±151 Carr units after use of contrast medium in the control group, while it did not change significantly in the RIPC group (441±109 vs. 427±132 Carr units, P=0.59). At 24 h, the percent change in plasma D-ROM level was larger in the control group than in the RIPC group (15.5±4.8 vs. −0.11±5.4%, P=0.036; Figure 2D).

To clarify the involvement of ADMA, an endogenous inhibitor of nitric oxide synthetase (NOS), in RIPC, plasma ADMA level was evaluated before and 24 h after angiography. In the control group, plasma ADMA level did not change significantly (0.5±0.1 to 0.49±0.07 nmol/ml; P=0.61) after use of contrast medium, whereas the level decreased significantly in the RIPC group from 0.50±0.09 to 0.46±0.09 nmol/ml (P<0.001; Figure 2E). At 24 h, the percent change in plasma ADMA level was significantly larger in the RIPC group than in the controls (−7.33±1.9 vs. 0.26±2.4%, P=0.016; Figure 2F).

Change in L-FABP and Change in D-ROM or ADMA

Figure 3 shows the correlations between change in D-ROM or ADMA and that in L-FABP. Significant correlations were found between D-ROM (r=0.43, P<0.001, Figure 3A) or ADMA (r=0.42, P=0.001, Figure 3B) and the change in L-FABP.

Discussion

Although numerous attempts have been made to prevent CI-

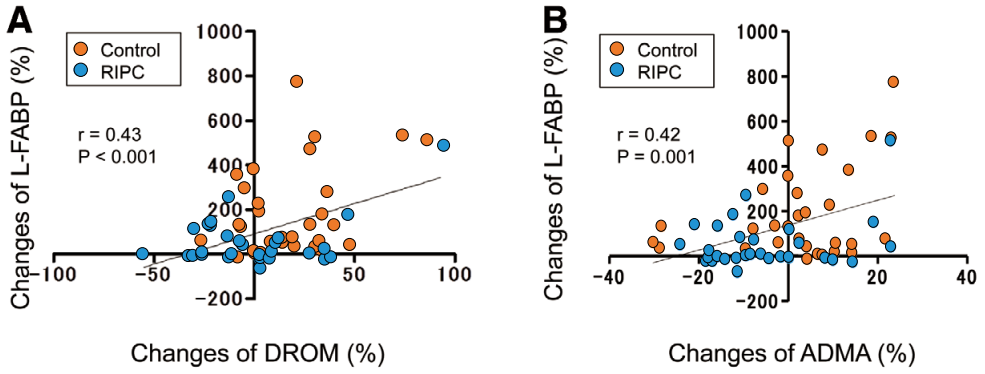

Figure 3. Correlations between % change in (**A**) D-ROM or (**B**) ADMA and %change in L-FABP at 24 h after use of contrast medium. ADMA, asymmetrical dimethylarginine; D-ROM, derivatives of reactive oxidative metabolites; L-FABP, liver-type fatty acid-binding protein; RIPC, remote ischemic preconditioning.

AKI, none is superior to adequate i.v. volume expansion.[7–10] In recent years, there has been renewed interest in RIPC. This study provides novel insights into the preventative effects of RIPC on CI-AKI. We found that (1) RIPC attenuated the prevalence of L-FABP-based CI-AKI after angiographic procedures in patients with low-to-moderate risk; (2) RIPC inhibited increases in urinary L-FABP, D-ROM, and ADMA level after exposure to contrast medium; and (3) the change in L-FABP level was correlated with the changes in the D-ROM and ADMA levels.

In this study, there was no significant difference in the serum creatinine level between the RIPC and control groups, resulting in the low incidence of canonical CIN, which is defined as an increase in serum creatinine >25% from baseline or an absolute increase ≥0.5 mg/dl within 48 h after use of contrast medium. There were several reasons for the low incidence of CIN in this study. First, the enrolled subjects were at low-to-moderate risk for developing CI-AKI compared with those in previous studies. The prevalence of CIN, however, is >20% even in low-moderate-risk patients.[23] We believe the prevention of CI-AKI is important even in patients with low-to-moderate risk. Second, the present study was conducted according to the protocol involving adequate i.v. volume expansion proposed by Stacul et al.[34] Third, the mean dose of contrast medium was lower in the present study than in previous studies (92 vs. 186–190 ml).[1,35] For reasons already stated, the incidence of creatinine-based CIN in the present study was lower. Nevertheless, urinary L-FABP level at 24 h was obviously increased after use of contrast in the control group.

L-FABP expression in the proximal tubules is acutely upregulated under tubular stress due to ischemia or the presence of toxins.[36] Accordingly, the urinary excretion of L-FABP might precede the occurrence of cell structure damage.[13,36] As Er et al described, serum creatinine is an inadequate marker of CI-AKI because of its low sensitivity, and cystatin C is more sensitive than serum creatinine in high-risk patients.[22] In the present study, however, we found that eGFR, creatinine, and cystatin C did not change significantly 48 h after the angiographic procedures. Only urinary L-FABP level was altered in response to contrast medium exposure. Similarly, Kato et al reported that urinary L-FABP level was increased significantly in the moderate CKD groups compared with before contrast medium, whereas other urinary biomarkers, serum creatinine, and cystatin C did not increase significantly from baseline.[1] Furthermore, Nakamura et al showed that urinary L-FABP sensitively and rapidly reflected renal injury compared with creatinine in patients after contrast medium use. This implies that urinary L-FABP is a more useful marker for the early detection of kidney injury than creatinine, even in low-risk CKD patients.[12–14,37] Conversely, creatinine level may mask latent CI-AKI in the clinical setting. Therefore, we evaluated the effects of RIPC on CI-AKI by measuring L-FABP level. Although the accurate L-FABP cut-off level for determining CI-AKI has not yet been decided, Kamijo et al reported the disease-monitoring L-FABP cut-off level in patients with CKD to be 17.4 μg/g Cr.[24] Accordingly, we used the same L-FABP cut-off to determine CI-AKI.

Although the precise mechanisms responsible for CI-AKI are not completely understood, renal ischemic injury and tubular epithelial cell toxicity have been proposed.[38] The infusion of contrast medium, with the attendant increases in osmotic load and viscosity, elicits hypoxia of the renal medulla and leads to renal free radical production via post-ischemic oxidative stress.[3] Post-ischemic free radical production might be the mechanism responsible for CI-AKI.[3] We measured serum D-ROM level as a marker of oxidative stress. After infusing contrast medium, D-ROM significantly increased in the controls compared with the RIPC groups (**Figure 2D**). Although the underlying mechanisms for the renal-protective effects of RIPC are not fully understood, Tapuria et al have shown that RIPC can reduce oxidative stress via the release of biochemical messengers.[39] Moreover, the cardioprotective effect of RIPC on reperfusion injury has been shown to be associated with activation of the phosphatidylinositol 3-kinase/Akt (PI3K-Akt) pathway.[40] The PI3K-Akt pathway reduces oxidative stress by activating nuclear factor-erythroid 2-related factor 2,[41] and induces expression of superoxide dismutase.[42] These antioxidative effects likely contribute to the prevention of the L-FABP-based CI-AKI.

In rat CI-AKI models, the inhibition of prostaglandin or NO synthesis decreases outer medullary blood flow and aggravates regional hypoxia.[43,44] Indeed, L-arginine improves the contrast-induced altered renal hemodynamics and renal dysfunction in hypercholesterolemic rats.[45] These results suggest that amelioration of the altered medullary nitrovasodilation is a possible target in preventing CI-AKI. As shown in **Figure 2F**, RIPC significantly reduced ADMA level after angiographic procedures compared to the controls. This implies that more

NO is available in the RIPC group than in the controls. ADMA is an endogenous competitive inhibitor of NOS, and reactive oxygen species are involved in the elevation of ADMA level.[46] Lin et al reported that application of an antioxidant reversed ADMA accumulation in human endothelial cells.[47] These observations support our idea that RIPC reduces oxidative stress and results in decrease in ADMA level. Although we could not estimate endothelial function in the present study directly, the improvements in peritubular capillary endothelial function might be involved in the renal protective effect of RIPC.

The pro-inflammatory action of contrast medium in human renal proximal tubular epithelial cells has been postulated.[48] The anti-inflammatory effect of RIPC, however, remains controversial. Konstantinov et al showed that RIPC suppressed the expression of pro-inflammatory genes in circulating leukocytes in humans.[49] Conversely, Iliodromitis et al reported that RIPC failed to reduce the increased circulating level of CRP after coronary angioplasty with stenting.[50] In the present study, there was no significant difference in the hs-CRP and plasma PTX3 levels between before and after use of contrast medium (**Figures 2A**,**B**). Further studies are needed to clarify this issue.

The present study has some limitations. First, the study design was non-blinded and the sample size was small. Second, we were unable to observe a sufficient number of events, such as CIN, mortality, and the need for dialysis as endpoints. A much larger study is required to confirm the beneficial effects of RIPC on CI-AKI. Third, we did not examine urinary neutrophil gelatinase-associated lipocalin (NGAL) level, which is also a useful marker of early CI-AKI. Bachorzewska-Gajewska et al reported that the rise in urinary NGAL and L-FABP levels has a similar time course after use of contrast medium.[51,52] Thus, both markers can be detected before a rise in serum creatinine level.[53] Furthermore, we did not examine creatinine clearance (CCr), which is also a useful marker of renal function. Examining changes in NGAL and CCr in this type of study is important. To confirm the effects of RIPC on CI-AKI, further studies using NGAL and CCr are needed. Fourth, because the RIPC procedure requires 40 min from start to finish, applying RIPC immediately before angiography was difficult in this study. Several studies in animal models and humans, however, have shown that the effects of RIPC can persist for at least 24 h.[39] Therefore, we applied RIPC approximately 2 h before CAG (average, 131±12 min). Further study is necessary to determine which RIPC protocol is best for preventing CI-AKI.

Conclusions

RIPC alleviates CI-AKI in patients at low-moderate risk. This beneficial effect might be mediated partly by decreasing oxidative stress and plasma ADMA level.

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