Myocardial Infarction Promoted the Contrast-Induced Nephropathy by Renal Injury

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Abstract

Background: The morbidity of myocardial infarction is keeping rise in this decade. Because of high safety and operability, percutaneous coronary intervention (PCI) has been used to conquer this disease for more than 20 years. An important complication of PCI is contrast induced nephropathy (CIN), which raises our attention. Previously, we started a study to explore the correlation between acute kidney injury and myonecrosis after scheduled percutaneous coronary intervention. Our study showed that the rate of CI-AKI in patients with post-procedural myocardial injury and undergoing elective PCI was higher than that in patients free of injury.

Methods: In this study, forty male rats were randomly divided into four groups: control group (n=8), CM group (n=12), AMI group (n=8) and AMI+CM group (n=12), then velocity of renal artery blood flow (VRABF), computer tomography (CT), serum creatinine (Scr), reactive oxidative species (ROS), periodic acid-Schiff (PAS) and TUNEL were used to estimate the injury of kidney. We analyzed 327 non-ST-segment elevation acute coronary syndrome subjects undertaking elective PCI. Serum levels of creatinine (SCr) and the eGFR before coronary angiography, and 24 – 72 h after contrast administration were recorded to assess the renal function.

Results: The data showed that VRABF was lower in AMI+CM group than CM group from 0 minute to 24h and CT number in cortex was higher in AMI+CM group than CM group at 4-hour. As well as the level of Scr in AMI+CM group displayed a significantly increase at 24-hour compared with CM group. The histopathologic scores and percentage of tubular cell apoptosis
were higher in AMI+CM group at 24-hour. In 327 patients, we found that CI-AKI occurred more often in subjects with post-procedural myonecrosis (PMN) than in those without PMN (20.8% versus 5.8%, respectively, P=0.001).

Conclusion: Compared to the elective patient, the injury of CIN exhibited a higher severity in AMI patient.

**Keywords:** Contrast induced nephropath, acute myocardial infarction.

**Introduction**

Contrast-induced nephropath (CIN), has been reported to be the third most common cause of hospital acquired renal failure. It is defined as the impairment of renal function and is measured as either a 25% increase in serum creatinine (SCr) from baseline or 0.5 mg/dL (44.2 µmol/L) increase in absolute value, within 48-72 hours of intravenous contrast administration. It was reported that the incidence of CIN in the general population has been calculated to be 2%. However, in the high-risk patients, who involved in chronic renal impairment, diabetes mellitus, congestive heart failure, and older age, the incidence has been calculated to be 20% to 30%[1-5]. AS the high fatal and emergency of AMI, PCI is considered as the best operation for patient. Whereas, there are sufficient case-reports shown that the incidence of CIN in AMI patient is much higher than elective patient[6, 7]. The previous studies partially revealed the reasons of high incidence of CIN was related to the injury of kidney. Based on the previous findings and other investigators’ observations, the implicated mechanisms of CIN include the changes of the renal circulation leading to ischemia and hypoxia, direct nephrotoxicity and oxidative stress, and the special condition
of the patients with AMI[8-11]. AMI is a process of the haemodynamic change, which can increase reactive oxygen species leading to oxidative stress. One clinical trial demonstrated that the risk of CIN between STEMI (ST-elevation myocardial infarction) and the NSTEMI (non-ST elevation myocardial infarction) have not significant differences[12], however, the pathophysiology process of CIN in AMI patients is still not clear. Therefore, the objective of this study was to investigate the mechanism of AMI induced higher incidence of CIN in vivo.

The animal experiments illustrated this process from biochemistry and Immunohistochemistry.

**Material and methods**

1. **Animal and experimental design**

40 male SD rats (20–24 months old and weighing 200-300g) were purchased from Shanghai Slaccas Animal Co. Animals were housed in the Animal Services Unit with a 12-hour light–dark cycle, with access to standard rodent chow and tap water. Rats were acclimatized for 7 days before randomization and then divided into four groups in the beginning of the study: (1) control group(n=8) (2)CM(n=12) (3)AMI(n=8) (4)AMI+CM (n=12). An anterior thoracotomy was performed to open the pericardium in all groups. The muscle layer and skin were closed separately without special treatment in the control and CM groups after 5 min. In the AMI group and AMI+CM group, the heart was explored and a 5-0 silk suture was tightened around the proximal left anterior descending coronary artery. After 45 minutes, CM iopromide (2g I/kg) were administrated through caudal vein for CM and AMI +CM groups while the equal normal saline
(ns) was for control and AMI groups. Before injection, all groups were examined the left ventricular ejection fraction (LVEF), and four of CM and AMI+CM groups were scanned by CT machine (16 slices Emotion somatom, Siemens, Germany) at baseline, 4h and 12h after the CM injected. At 0 min 2 min 8 min 15 min 4-hour 24-hour after injection, the VRBF using the Doppler ultrasound (Vevo2100 VisualSonics) were detected in the groups sacrificed at 24h. The blood was collected through inferior vena cava and right kidney was harvested for four rats of each group at 4h under anesthetized with pentobarbital sodium (45mg/kg). 24h after injection other four rats of each group was treated in the same way. The blood was centrifuged at 3000r/min for 12 minutes and the serum stored at -40°C. The right kidney was departed to two parts, one part was fixed in 10% buffered formalin, and the other was stored in -80°C.

2. Subjects and biomarkers of renal function

We retrospectively enrolled 327 subjects in the study, who undergone elective PCI for the treatment of stable angina pectoris (SAP) or unstable angina pectoris (UAP) or of non-ST-segment elevation myocardial infarction (NSTEMI). The clinical characteristics of the subjects in two groups (PMN and non-PMN), we measured SCr and eGFR levels during hospitalization that occurred before and closest to the time of coronary angiography, and again at 24 - 72 h post-dose.

3. Contrast agents

non-ionic iodinated CM was used: iopromide (Ultravist, Bayer Schering Pharma AG, Germany, 370mg iodine/ml) non-ionic monomeric, lower-osmolar iodinated CM (770mOsm/kg of water at 37°C)
4. Renal function assessment, ET-1 and Ang II measurement

Renal function was assessed by measuring serum creatinine by a colorimetric method based on the Jaffe reaction. Serum endothelin-1 and Ang II concentrations were measured with a commercially available radioimmunoassay kit (Phoenix Pharmaceuticals, Mountain View, CA, USA and Shanghai Yanyu Shangmao Company, Shanghai, China) according to the manufacturer's instruction.

5. Kidney ROS examination

One part of kidney stored at -80°C was homogenated to measure activity ROS level with a commercially an oxidation-sensitive fluorescent probe (DCFH-DA) according to the manufacturer's instructions (GENMED SCIENTIFICS INS USA). The DCF fluorescence intensity in the tissues was detected using Synergy HT fluorescence microplate reader (BIO-TEK, USA) at an excitation wavelength of 490 nm and an emission wavelength of 520 nm.

6. Renal histopathological examination

The one part of the right kidney was fixed in 10% buffered formalin, and embedded in paraffin. The kidney samples were cut to the sections of four micron thickness and stained by periodic acid-Schiff (PAS). Changes of acute renal injury were scored semiquantitatively. A minimum of 100 cortical tubular profiles from at least 10 different regions were assessed with 40 times magnification. The histopathological changes were evaluated and the total scores per 100 tubular profiles were derived no histopathological changes (score 0), tubular cytoplasm vacuolization (score 1), tubular epithelial cell flattening (score 1), brush border loss (score 1), interstitial edema (score 1), tubular cells necrosis (score 2), and tubular lumen obstruction...
7. Assessment of apoptosis

To detect the apoptotic cells, in situ terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling (TUNEL), staining was performed in paraffin embedded sections using a commercially available In Situ Cell Death Detection Kit (Roche Diagnostics, Germany) according to the manufacturer’s instruction. To visualize the total number of cells in the field, nuclei were counterstained with DAPI (blue fluorescence) (Roche Diagnostics, Germany). Fluorescent staining was visualized and digital images were taken on an imaging system with the appropriate argon beam lasers. Five high-power (40×) fields in each section were randomly selected, and the percentage of apoptotic cells over all the glomerular cells or tubular cells in each section was counted.

8. Gene expression analysis,

Each 4h group and 24h groups RNA sample was isolated by Trizol Reagents (Invitrogen, USA), and first-strand cDNA was synthesized by reverse transcriptase kit (Invitrogen, USA) according to manufacturers’ instruction. Primer for NF-ΚB p53 mRNA were 5'-CCAAAGACCCACCTCACC-3 (forward) and 5'-CGCATTCAAGTC ATAGTCCC-3 (reverse), and primer for Caspase-3 was 5'-GCTGATGTCTGGAG-3 (forward) and 5'-TCTTCTGAGTGGCAGTG AT-3 (reverse). Primer for GAPDH was 5'-CTGGACTGCGGTATTGAG -3 (forward) and 5'-GGGTGCCTGTAGAAGTGAC-3 (reverse). Real-time PCR was performed using SYBR green (eppendorf realplex instrument,). Data for each transcript were normalized to GAPDH as internal controls using the 2-ΔΔCt method.
9. Statistical analysis

All measurements are expressed as means±S.D. Data were analyzed using Student’s t-test or one-way ANOVA with Bonferroni correction for multiple comparisons between groups. A value of P < 0.05 was considered statistically significant.

Results

1. Changes of the renal function, serum ET-1, AngII and renal ROS.

As shown in FIG.1A, the level of ET-1 was higher in the CM, AMI+CM, and AMI groups than in control groups at 4h (p<0.05), and the ET-1 level of CM and AMI+CM groups was still high in 24h, and had no difference between the 4h and 24h in AMI+CM group (p>0.05). The level ET-1 of the AMI+CM group was higher than that of CM group at 4h (p<0.05), and there was no difference between the of CM group and AMI group in 4h (p>0.05) (FIG.1A). The Ang-II level increased in CM, AMI+CM and AMI groups in 4h. The AngII of AMI+CM group at 4h was highest in all groups (P<0.05). The level of AMI+CM went down in 24h. There was no difference in CM group and AMI group at 4h (FIG.1B). There was no significant difference in serum creatinine at 4h groups (p>0.05). After 24h, all groups of creatinine increased except the control group. In the 24h groups, the creatinine level of AMI+CM were highest in all groups (p<0.05) (FIG. 1C). ROS in kidney increased in CM, AMI+CM and AMI groups compared with the control group at 4h (p<0.05) and still increased at 24h. There was no significant differences in CM, AMI+CM and AMI groups at 4h (P>0.05). The ROS level in AMI+CM at 24h was the highest in all groups (P<0.05). The level of CM had no significant difference with the level of AMI group at 24-hour (P>0.05) (FIG. 1D). In the 327 subjects undergoing scheduled PCI and who had been...
diagnosed with myocardial, pre-procedure Scr levels were \((87.5 \pm 34.2)\) mmol/L in subjects with PMN and \((77.7 \pm 18.8)\) mmol/L in subjects without PMN \((P=0.12)\). While post-procedure Scr levels were \((94.2 \pm 43.6)\) mmol/L in subjects with PMN and \((80.7 \pm 18.9)\) mmol/L in subjects without PMN \((P=0.015)\). The pre-procedure eGFR level was \((85.0 \pm 25.1)\) ml/min in subjects with PMN and \((91.8 \pm 20.2)\) ml/min in subjects without PMN \((P=0.032)\). The post-procedure eGFR level was \((79.0 \pm 24.2)\) ml/min in subjects with PMN and \((87.8 \pm 19.3)\) ml/min in subjects without PMN \((P=0.004)\) (Table.1).

2. Changes of the velocity of renal artery blood flow

The VRABF in the AMI+CM group was lower than CM group from 0 min to 24h \((P<0.05)\). During the initial 2 min of CM infusion, the VRABF in CM group increased from 1042.7±92.6 mm/s at 0 min to 1290±107.80 mm/s at 2 min, and the AMI+CM group increased from 792.80±36.19 mm/s at 0 min to 1043.39±75.58 mm/s at 2 min. Afterwards, the VRABF decreased at 8min to basline and had no significantly changes from 8 min to 24-hour (FIG. 2A). From our study, the LVEF of AMI and AMI+CM groups decreased significantly compared with the CM group and control group before injection of CM \((p<0.05)\) (FIG. 2B). The ventricular wall motion was abnormal in AMI and AMI+CM groups detected by echocardiography (FIG. 2C).

3. The retention of CM in the kidney

In general, a higher attenuation in the renal cortex were observed after administration of the CM (FIG. 3A). Four hours after injection of the CM in the AMI+CM group and CM group, we observed an X-ray attenuation in the renal cortex of 124.85±15.37 and 65.06±7.13 HU, respectively (baseline values were 46.48±2.9 HU and 45.36±2.68 HU, respectively). Twelve
hours later after injection of the CM, no significant difference to the baseline were observed in the AMI+CM and CM groups (FIG. 3B).

4. Apoptosis in renal tubular cell

The renal tubular cells were stained by TUNEL to determine the renal tubular apoptosis. The TUNEL staining showed that there were significant apoptosis in CM group and AMI+CM group compared with control group at 4h and 24h. The apoptosis in AMI+CM group at 24h is more significant than other groups (FIG. 4A). The percentage of apoptotic tubular cells in AMI+CM 4h group was higher than that in AMI 4h and CM 4h (P<0.05), and still continued to exist at 24h. The percentage of apoptosis at 4-hour groups was lower than 24-hour groups except to control group (P<0.05). The percentage of apoptosis in AMI was lower than CM group. (FIG. 4B).

5. histomorphological analysis of renal injury

In the light microscopic examination, the proximal and distal tubules were normal, and there were no vacuolization, degeneration or necrotic differences in control group. There were significantly vacuolization, brush border loss and tubular cell necrosis in the AMI+CM 24h than other groups. There was more tubular lumen obstruction in AMI+CM group than other groups(FIG. 4C). The scores of AMI+CM at 4h were higher than the CM and AMI at 4h. The scores went higher at 24h in the CM and AMI+CM group (p<0.05), and the highest scores was the AMI+CM at 24h among all groups. The scores of AMI at 4h and 24h were both lower than that of CM at 4h and 24h (p<0.05) (FIG. 4D).

We evaluated the transcript level for the genes that encoded NF-κb and caspase-3 by real time polymerase chain reaction was related with apoptosis and kidney injury. The real-time PCR determination showed that the NF-κb expression of the CM, AMI+CM groups increased compared with control group at 4h (p<0.05) and was still high at 24h. The expression of AMI group increased at 24h but was lower than AMI+CM group (p<0.05) and the expression of the AMI+CM was higher than that of CM group at 24h (p<0.05) (FIG. 5A). The caspase-3 expression of CM, AMI+CM and AMI groups were increased significantly compared with the control group at 4h (p<0.05). The CM and AMI+CM groups also continued to go higher at 24h than that in 4h (p<0.05) while the AMI group decreased at 24h. Caspase-3 expression of the AMI+CM groups was higher than that of CM group at 4h and 24h (FIG. 5B).

Discussion

The present study demonstrates that acute myocardial ischemia negatively influences the process of CIN in vivo. To compare with AMI rat and normal rat both underwent CM, the renal injury caused by CM in AMI rat is more serious than the normal rat. This presented as increase in serum creatinine, apoptosis and scores of histopathology.

Many experiments proved that the solvent of CM could cause the haemodynamic alterations in kidney. The low blood flow and increased vascular resistance in cordial and medullary were partially related to the high viscosity, but the CM did not produce long term effects on the systemic or total renal blood flow[14-16]. Because the function of sodium reabsorption and about 80% of total renal oxygen consumption depend on the out medullary region, it is most vulnerable under CM exposure[17]. In our study, Doppler ultrasound was used to evaluate the
cardiac function and blood flow, the results demonstrated that LVEF in AMI group and AMI+CM group is significantly lower than the CM and control group. Interestingly, the data of VRBF in 24 hours has shown more decrease in AMI+CM group, which indicated that CM had no more effect on the renal artery blood flow in AMI+CM group than in CM group. By CM injection, severe renal injury was induced by lower perfusion and hypoxia that attributed to the low LVEF and unstable hemodynamic.

It was well known that endothelial cell in the microcirculation of the kidney could release vasoconstrictors (Endothelin/adenosine) and vasodilators (nitric oxide/prostaglandin)[18, 19]. Another investigation reported that CM injection caused the decrease of nitric oxide (NO) and PGE2 release in the cortex and medulla [20-22]. The same as our study, we found the Endothelin (ET-1) of AMI+CM groups are the highest in all groups, and started to increase at 4h and kept high level until 24h. Meanwhile, AngII as the other important CIN pathogenic factor was correspondence with ET-1 in all groups. According to the CT number analysis, AMI+CM group was higher than CM group at 4h after injection, which indicated renal artery and microcirculation disturbance in AMI+CM kidney by higher CM attenuation and lower blood flow.

Previous researches observed that increased tubular cytoplasm vacuolization and tubular lumen obstruct led to the injury of kidney, which attributed to the influences of CM and tubular protein. The present study also found the same result in histomorphology. The negatively changes in AMI+CM group illustrated that cardial ischemia also influenced the CM metabolism and injury kidney by prolonged CM retention as well as instability of renal microcirculation. In this situation, GFR and other toxic metabolites not only hurt the kidney but also made a circulation disorder. Therefore, the high mortality rate of rat AMI models by 2ml CM injection
indicated the relationship between circulation disorder and CIN.

The cell apoptosis always caused by Inflammatory and ROS, and plays a critical role in mediating tissue injury. In our study, Realtime-PCR was utilized to evaluate the RNA level of NF-kB, caspase-3 and ROS in all groups, which represented the effect of Inflammatory, apoptosis and oxidative damage in kidney. We found that the level of ROS and the expression of caspase-3 and NF-kb were also higher in the AMI+CM groups than other groups (P<0.05). Superoxide (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), and hydroxyl radical (OH$^-$) are the most common ROS, and oxidative stress augments the production of them in the mitochondria. O$_2$ rapidly scavenges nitric oxide (NO) and then blunt NO activity in the renal microvasculature. As a potent vasodilator, the decrease of NO can incurs hypoperfusion in kidney, and then ROS generates. AMI is a highly dynamic event with increasing production of ROS. The imbalance between ROS production and antioxidant defenses leads to oxidative stress, which can cause the oxidation and damage of macromolecules, membranes, proteins, and DNA[10]. CM also can make ROS increase in the out medulla given the decline of the medullary blood flow and oxygenation[23-25], and the administration of antioxidant SOD and ascorbic acid can prevent or attenuate the increase of MDA[24, 26], and in clinical trials, the antioxidant NAC or other has been proved to attenuate the decline of GFR[27]. In our study, we found the ROS of the AMI+CM group was the highest in all groups, and it increased at 4h and was higher at 24h. In AMI group, it was also higher than control groups, and had no significant differences with the CM group, this demonstrated that AMI contributed to the injury of kidney, it is conceivable that the generation of the ROS in AMI groups before the injection of CM alters medullary oxygen balance and enhances the ROS induced pathways activation.
The injection of CM can cause the renal tubular cell apoptosis[9], because of the hypoxic damage[28] and the direct influence on these cells[29], and in our study, the higher attenuation in AMI+CM group indicated the time of renal tubule cells contact with CM were prolonged which induced the percentage of apoptosis higher than CM group. Many experiments had shown that the ROS was associated with the apoptosis, the CM induced an increase in ROS production, then promoted the activation of the stress kinases JNK1/2 and p38, finally improved the caspase-3 activation[30]. AS we known, caspase-3 is one of important gene regulated apoptosis, and many studies has demonstrate that caspase-3 increased after injection of CM[13, 30-32]. In this study, we also found the caspase-3 increased at 4-hour and was still high at 24-hour after injection of CM. TUNEL data illustrated that AMI+CM group caused the severest apoptosis.

NF-kB is an important nuclear transcript factor. It has two side effects, on one hand NF-kB is related to the pro-apoptosis[33, 34]. On the other hand, NF-κB is critically involved in the processes of oxidative stress, some studies demonstrated that oxidative stress activates NF-Kb in cells and is involved in the contrast induced renal injury[35], this was similar with the previous study. We found the NF-Kb increased in AMI+CM group and CM group at 4h, and still went up at 24h in AMI+CM group. It was also higher in AMI group which associated with the oxidative stress as well. It indicated that NF-Kb was also important in renal injury after injection of CM when AMI occurred. Although many biomarkers such as ET-1, Ang II, caspase-3 of AMI group increased similar with CM group, but were more serious than those in CM group and control group. These results implied that myocardial infarction and systemic microcirculation disorder caused by coronary ligation influenced cardiac function while limited the CM
metabolism in kidney by renal injury. Myocardial ischemia limited cardiac function and caused blood redistribution, which finally resulted in the kidney circulation limitation. These two conditions promoted the incidence of CIN in myocardial infarction patients.

Figure legends

FIG.1 Effects of the CM on the renal function, plasma biochemical markers and the reactive oxygen species of kidney. Bar charts represent the changes of the serum endothelin-1(A), Angiotensin II(B), creatinine (C) and ROS in kidney(D). All data are presented as means±S.D (n=4). *P<0.05 VS 4h, #P<0.05 VS CM and AMI groups.

FIG.2 (A) The response of renal artery blood flow to CM of AMI+CM and CM groups, from 0min to 24h. (B) The LEVF of the four groups before injection. (C) The representative echocardiography of ventricular wall motion of each group. (n=4)

FIG.3 CM retention in AMI+CM and CM groups. (A) Representative CT scans of the kidney at baseline, 4-hour, 12-hour of AMI+CM and CM group. (B) Attenuation (HU) in the cortex in the kidney of rats injected with 2gl/kg of Iopromide 370 at 4hours and 12hours. All data are presented as means±S.D (n=4). *P<0.05 VS 4h, #P<0.05 VS CM and AMI groups.

FIG.4 (A) apoptosis in the renal tubules tissues in each group (TUNEL staining, green fluorescence). (B) The percentage of apoptosis in the renal tubules of each group. (C) Representative histomorphological kidney changes in each group. (D) The scores of tubular injury in the each group. All data are presented as means±S.D (n=4). *P<0.05 VS 4h, #P<0.05 VS AMI and CM groups.

FIG. 5 Expression of biomarkers for renal injury. (A) Expression of NF-κb and (B) caspase-3 4-hour and 24-hour after injected with CM. All data are presented as means±S.D (n=4). *P<0.05 VS 4h, #P<0.05 VS CM and AMI groups.
Table 1 Biomarkers of renal function. Values are given as means ± S.D, median (interquartile range), or number of patients with MA or CI-AKI/total patient number (percent).

Author Contributions: Zhijian Yang and Bo Chen conceived and designed the experiments; Zhiwen Tao, Ningtian Zhou performed the experiments; Zhiwen Tao and Fan Huang analyzed the data; Zhiwen Tao, Ningtian Zhou and Fan Huang wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

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Fig. 1

(A)

(B) Echocardiography before injection

(C)

Fig. 2
Fig 5

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