N-acetylcysteine to ameliorate acute renal injury in a rat cardiopulmonary bypass model

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Supplemental material is available online.

Objective: Oxidative stress and systemic inflammation response contribute to acute renal injury post cardiac surgery. We hypothesized that administration of the antioxidant *N*-acetylcysteine would be beneficial to renal function after cardiopulmonary bypass in a rat model.

Methods: Male Sprague–Dawley rats were divided into four groups (each $n = 6$): sham group, cardiopulmonary bypass group, and two *N*-acetylcysteine–treated cardiopulmonary bypass groups (bolus doses of 200 and 500 mg/kg in cardiopulmonary bypass prime). Blood samples were collected at the beginning of cardiopulmonary bypass, at the cessation of cardiopulmonary bypass, and at 2 and 12 postoperative hours. The kidneys were harvested at 12 postoperative hours.

Results: Serum creatinine and cystatin C continuously increased in all cardiopulmonary bypass groups ($P < .05$ within groups). Tubular dilatation, tubular necrosis, and vacuole formation were found in epithelial cells in histomorphologic studies of the cardiopulmonary bypass groups, but *N*-acetylcysteine significantly reversed these effects ($P < .05$ between groups). Compared with the sham group, the reduced glutathione hormone content and the superoxide dismutase and catalase activities decreased in the cardiopulmonary bypass groups ($P < .01$). *N*-acetylcysteine–treated groups had higher levels of these antioxidants than the untreated bypass group ($P < .05$). Renal malondialdehyde, tumor necrosis factor α , and nuclear factor κ B were notably increased in all cardiopulmonary bypass groups relative to the sham group ($P < .01$), and *N*-acetylcysteine attenuated these changes dose dependently.

Conclusion: Administration of the antioxidant *N*-acetylcysteine preserved renal function after cardiopulmonary bypass dose dependently. Furthermore, oxidative stress and systemic inflammation were significantly reduced in the treated animals.

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Acute renal dysfunction affects about 5% to 31% of patients who undergo cardiac surgery with cardiopulmonary bypass (CPB), and it is associated with mortality.¹ Potential reasons for renal dysfunction include cardiovascular compromise, prolonged CPB exposure, increased catecholamine level, non-pulsatile flow, hypothermia, renal hypoperfusion, and the induction of inflammatory mediators.² These factors may collectively contribute to renal hypoxic-ischemic and systemic inflammatory responses. These insults result in generous formation of reactive oxygen species and depletion of endogenous antioxidants.

A number of possible strategies aimed at alleviating the development of renal dysfunction have been evaluated. Although commonly used approaches to prevent acute renal failure have included adequate hydration, mannitol, renal doses of dopamine, and loop diuretics, examination of the evidence does not support the continued use of all these regimens.³ Recently, several meta-analyses and randomized, controlled trials have demonstrated that *N*-acetylcysteine (NAC) attenuates contrast-associated declines in renal function, but the conclusion is still inconsis-

Abbreviations and Acronyms

CPB	= cardiopulmonary bypass
GSH	= reduced glutathione
MDA	= malondialdehyde
NAC	= <i>N</i> -acetylcysteine
NF- κ B	= nuclear factor κ B
SOD	= superoxide dismutase
TNF α	= tumor necrosis factor α

tent.^{4,5} NAC also attenuates kidney ischemia-reperfusion injury.⁶ These protective effects may arise because NAC is precursor of glutathione and scavenger of reactive oxygen species. NAC has also been used to ameliorate inflammatory response in lipopolysaccharide-induced acute lung injury in rats and in an acute pancreatitis experimental model.^{7,8}

Because NAC can ameliorate ischemia-reperfusion injury and inflammatory response, which are the main pathophysiologic changes seen with CPB, it should be protective in patients undergoing CPB.^{2,9-15} Several studies have shown its myocardial protective effect in bypass surgery, but research concerning its renal protective effect is limited, and the results seem controversial.^{2,11} There are several possible reasons why a treatment effect was not observed, with two being most important. First, serum creatinine is not a prompt marker of acute renal injury, and these studies lacked morphologic studies of renal tissue because of the clinical limitations. Second, the dose of NAC, although well studied for prevention of contrast-induced nephropathy, may be inadequate to counteract the hypoxic-ischemic insults to the renal tubular epithelial cells induced by CPB.² In this study we therefore aimed to determine the biochemical and morphologic renal effects of NAC at different doses in a rat model of CPB and to investigate the potential mechanism of this agent.

Materials and Methods**Animals and Groups**

Male Sprague-Dawley rats (450-550 g) were used for the experiment. All animals received humane care in compliance with "The Principles of Laboratory Animal Care" formulated by the National Society of Medical Research and with the "Guide for the Care and Use of Laboratory Animals" (<http://www.nap.edu/catalog/5140.html>). The following experimental protocol was approved by local ethical committee.

Rats were randomly assigned to one of four groups ($n = 6$ for each group): sham, CPB only (control), CPB plus low-dose (200 mg/kg) NAC (low NAC), and CPB plus high-dose (500 mg/kg) NAC (high NAC). NAC or placebo was added into the prime of CPB.

Surgical Procedure

The rat model of CPB was built according to Dong and colleagues,¹⁶ and we performed some modifications that made our model very similar to the one used by Modine and associates.¹⁷ Rats were anesthetized with intraperitoneal administration of butylone (60 mg/kg) at the beginning; additional pentobarbital was added to ensure an adequate depth of anesthesia, and the incisions were infiltrated with 2% lidocaine intermittently. The right femoral artery was cannulated with a 24-gauge polytetrafluoroethylene heparinized catheter to monitor arterial pressure and to collect blood samples. After administration of heparin (250 U/kg), a 16-gauge catheter, modified to a multiside-orifices cannula in the forepart, was inserted into the right jugular vein and advanced to the right atrium. A 22-gauge catheter was cannulated to the tail artery to serve as the arterial infusion line. Any rats that died during the institution of CPB were excluded.

The mini-CPB circuit comprised a venous reservoir, a specially designed membrane oxygenator, a roller pump, and sterile polyvinyl chloride tubing with an internal diameter of 3 mm for the venous and arterial lines (30 cm long; [Figure E1](#)). The roller pump was equipped with a silicone tube 15 cm in length with an internal diameter of 5 mm. The membrane oxygenator was specially designed with a surface area for gas exchange of 0.05 m² (Micro-1; Kewei Medical Instrument Inc, Dongguan, China), with its total assembly dynamic priming volume approximating 2 mL. Body central temperature was monitored with a rectal probe and kept at 36.5°C to 38.3°C by a heat lamp placed around the animal and the CPB equipment. We primed the CPB circuit with 12 mL of a solution of heparin (250 U/kg), hetastarch, and NAC or placebo. Before the initiation of extracorporeal circulation, the CPB set was examined carefully to avoid liquid and air leaks. The blood was drained from the right atrium through the jugular vein catheter to a 5-mL sterile open reservoir by gravity and siphon. A roller pump (BT00-300M; Lange Co, Baoding, China) was used to drive the blood through silicone arterial inflow tubing and then return it to the tail artery.

At the initiation of perfusion, the flow rate was gradually increased to 100 mL/(kg · min) and maintained for 60 minutes; it was then turned down step by step to maintain hemodynamic stability. When the rat was weaned from CPB, the tail artery catheter was removed, and the right jugular vein catheter was drawn back to superior vena. The remaining priming solution was infused gradually when the main arterial pressure was less than 60 mm Hg. After 1 hour of intensive postoperative care, the right jugular vein catheter and the femoral artery catheter were decannulated. Then the neck, tail, and groin incisions were sutured. Throughout the experiment, the mean arterial pressure was maintained about 60 to 80 mm Hg. The rats were given water and food 6 hours after the operation, and they were monitored for 12 postoperative hours.

Specimen Collection

Mean arterial pressures were recorded during the experiment. Blood samples (1 mL) were obtained from the femoral artery immediately after heparinization, at the end of CPB, and at 2 and 12 hours after operation. These were used for blood gas analyses and later determinations of serum creatinine, cystatin C, and tumor necrosis factor α (TNF- α). The urinary output in the first 2 hours

was also collected. At 12 hours after the operation, the kidneys were harvested for microscopic examination and biochemical analysis.

Biochemical Analysis of Renal Function

Serum creatinine was measured with a kinetic Jaffe method modified to reduce the effect of noncreatinine Jaffe-reacting chromogens. Serum cystatin C was measured by particle-enhanced nephelometric immunoassay (Beijing Strong Biotechnologies Inc, Beijing, China). Both were assayed on a chemistry analyzer (model 7600-020; Hitachi, Ltd, Tokyo, Japan).

Assays of Oxidative Stress Markers

The homogenate samples were prepared as described elsewhere.⁶ Protein measurements were analyzed according to the method of Lowry and colleagues.¹⁸ Total (copper-zinc and manganese) superoxide dismutase (SOD) activity was determined by the method of Sun and coworkers¹⁹; SOD activities were expressed as unit per milligram of protein. Catalase activity was determined according to the method of Aebi²⁰ and expressed as katal* per gram of protein. Tissue reduced glutathione (GSH) levels were measured by the method of Ellman²¹; values were expressed as nanomoles per milligram of protein. The level of malondialdehyde (MDA) in tissue homogenate was determined according to the method of Uchiyama and Mihara²² and expressed as nanomoles per gram of wet tissue.

Determination of Inflammation Markers

TNF- α concentrations in serum and renal tissues were quantified with enzyme-linked immunosorbent assay kits specific for the rat cytokines according to manufacturer instructions (Tepnel Lifecodes Corp, Stamford, Conn, for TNF- α). Values were expressed as picograms per milliliter for serum and picograms per milligram of protein for tissue samples.

The nuclear extracts were prepared as described elsewhere.²³ Electrophoretic mobility shift assay was performed with a commercial kit (Gel Shift Assay System; Promega Corporation, Madison, Wis). Nuclear factor κ B (NF- κ B) consensus oligonucleotide (AGT-TGA-GGG-GAC-TTT-CCC-AGG) was labeled with [γ -32P] adenosine triphosphate (Free Biotech, Beijing, China) with T4 polynucleotide kinase. Equal amounts of nuclear extract (60 μ g) were added to 9 μ L of gel shift binding buffer (10-mmol/L tris(hydroxymethyl)aminomethane hydrochloride, pH 7.5, 50-mmol/L sodium chloride, 0.5-mmol/L ethylenediaminetetraacetic acid, 1-mmol/L magnesium chloride, 0.5-mmol/L deoxythymidine triphosphate, 4% glycerol, 0.05-mg/mL polynucleotide deoxyinosine deoxycytosine) for 15 minutes at room temperature. The mixture was incubated for 30 minutes with 1 μ L of the phosphorus 32-labeled oligonucleotide probe. A 1- μ L portion of loading buffer was added, and the sample was electrophoresed in a 4% polyacrylamide gel at 390 V for 1 hour. The dried gel was exposed to x-ray film (Fuji Hyperfilm; Fuji Photo Film Co, Ltd, Tokyo, Japan) at μ 70°C. The intensity of the NF- κ B complex was quantified by densitometry.

Light Microscopy

For microscopic evaluation, formalin-fixed kidney samples were embedded in paraffin, and 4- μ m sections were prepared. The

sections were then stained with hematoxylin and eosin and scored according to a previously described semiquantitative scale designed to evaluate the degree of renal damage (tubular cell necrosis, cytoplasmic vacuole formation, hemorrhage, and tubular dilatation⁶) by an investigator (HS) who was blinded to the grouping.

Statistical Analysis

All values were expressed as mean $\pm$ SD. Data were analyzed with a commercially available statistical software package (SPSS for Windows version 13.0; SPSS Inc, Chicago, Ill). Either 1- or 2-way analysis of variance was used for comparisons between the groups where appropriate, and we examined the time courses of each measured parameter with repeated measures analysis of variance. Post hoc comparisons were performed with the Tukey test or Dunnett T3 test.

Results

Perioperative Physiologic Data

All the rats survived the CPB process, and their physiologic data before and after operation are summarized in Table 1. The preoperative data were similar for all the four groups and did not change postoperatively in the sham group. In all groups with CPB, mean arterial pressure decreased significantly from baseline to about 70 mm Hg ($P < .001$); Pao₂ and Paco₂ were both in our acceptable range; pH levels declined, indicating a tendency toward metabolic acidosis; and hematocrit significantly decreased from the hemodilution of CPB. Urinary volume increased in all CPB groups in the first 2 hours from the beginning of operation, and high-dose NAC significantly increased urinary output ($P = .018$). No rats died during 12 postoperative hours.

Time Courses of Renal Markers

The changes of the renal markers, containing serum creatinine and cystatin C, are depicted in Figure 1. Serum creatinine (Figure 1, A) was stable in the sham group throughout the experiment, whereas it continued increasing and was significant higher than the baseline in all three CPB groups ($P < .001$, $P < .001$, and $P = .002$, for control, low NAC, and high NAC, respectively). Compared with the sham group, serum creatinine increased significantly in the control and low-NAC groups ($P = .001$ and 0.005, respectively). NAC treatment significantly reduced the CPB-induced serum elevation relative to the control group ($P = .02$ and $P < .001$ for low NAC and high NAC, respectively); however, no significant difference was found between the two NAC treatment groups ($P = .348$).

Serum cystatin C showed a similar pattern to that of creatinine (Figure 1, B). Serum cystatin C rose gradually in the sham group after the operation, but it did not approach statistical significance; in contrast, cystatin C significantly increased postoperatively in the control group ($P < .001$), the low-NAC group ($P = .001$), and the high-NAC group ($P = .002$) relative to baseline. Cystatin C increased remarkably in all CPB groups ($P <$

*One katal represents the enzyme activity able to cleave 1 mol substrate per second.

TABLE 1. Physiologic data of the rats during experiments

	Mean arterial pressure (mm Hg)	pH	Pao ₂ (mm Hg)	Paco ₂ (mm Hg)	Hematocrit	Urine volume first 2 h
Sham						
Preoperative	108.5 ± 8.6	7.39 ± 0.02	162 ± 17.5	39 ± 2.4	46 ± 3.2	1.9 ± 0.6
Postoperative	106.5 ± 5.2	7.39 ± 0.01	164.7 ± 14.2	39.5 ± 1.5	44.5 ± 2.1	
CPB (control)						
Preoperative	112 ± 6.9	7.40 ± 0.01	160.5 ± 13.9	38.3 ± 2.6	46.5 ± 2.4	2.25 ± 0.7
Postoperative	76 ± 4.9*†	7.36 ± 0.01*†	351.7 ± 33.6*†	40.8 ± 3.1	30.8 ± 1.7	
CPB plus low-dose NAC						
Preoperative	105.5 ± 11	7.38 ± 0.01	156.8 ± 10.8	41.2 ± 3.7	44.8 ± 2.8	3.2 ± 1.1
Postoperative	75.2 ± 5.5*†	7.36 ± 0.01*†	351.33 ± 27.5*†	39.3 ± 2.2	28.8 ± 1.2	
CPB plus high-dose NAC						
Preoperative	108.3 ± 7.9	7.39 ± 0.02	160.5 ± 11.9	39.3 ± 3.0	46.0 ± 2.8	4.3 ± 1.5†‡
Postoperative	70.7 ± 5.6*†	7.36 ± 0.02*†	365.7 ± 30.2*†	38.0 ± 2.8	30.5 ± 1.9	

Values are means ± SD, n = 6. CPB, Cardiopulmonary bypass; NAC, N-acetylcysteine. * $P < .01$ for difference from baseline period. † $P < .01$ for difference from sham group. ‡ $P < .01$ for difference from control group.

.001, $P = .002$, and $P = .081$ for control, low NAC, and high NAC, respectively, vs sham). Relative to the control group, the administration of high-dose NAC significantly inhibited the elevation of cystatin C ($P = .03$); however, there were no significant differences between the two NAC treatment groups ($P = .310$).

Light Microscopic Examination Results

Histologic damage ranged from normal (sham group) to mild (NAC groups) and severe (CPB-only control group). Histologic changes, including tubular dilatation, tubular necrosis, and vacuole formation, were clearly observed in the kidneys of all CPB groups (Figure 2, B-D). Cortical tubular dilatation and vacuole formation were observed to some extent in all groups except the sham group (Figure 2, A). The control pathologic damage score after CPB-induced injury (3.3 ± 0.5) was significantly reduced in the high-dose NAC group (2.0 ± 0.6 , $P = .01$), whereas no difference was found between the control and low-dose NAC groups (2.7 ± 0.6 , $P = .157$). In the high-dose NAC group, generalized hemorrhage could be observed (Figure 2, D).

Effects of NAC on Oxidative Stress Markers

The effects of NAC on renal MDA and GSH contents and on SOD and catalase activities are shown in Table 2. The level of MDA significantly increased in control and low-NAC groups ($P < .001$ and $P = .013$, respectively) relative to the sham group, and treatment with high-dose NAC significantly reduced the CPB-enhanced MDA level ($P = .001$) relative to the control group. In the cases of the control and low-NAC groups, there were significant decreases ($P < .001$ and $P = .004$, respectively) in GSH content relative to the sham group. High-dose NAC treatment protected against CPB-induced reduction in GSH levels, as evidenced

by a significant increase in GSH content relative to the control ($P < .001$) and low-NAC ($P = .006$) groups. The activities of SOD and catalase were decreased significantly in the control group relative to the sham group ($P < .001$). When compared with the control group, however, low-dose NAC supplementation significantly increased the SOD activity ($P = .003$), whereas high-dose NAC treatment increased the activities of both SOD and catalase significantly ($P < .001$).

Effects of NAC on Serum TNF- α

The time course of serum TNF- α concentration is depicted in Figure 3. The levels of TNF- α increased remarkably postoperatively. The difference with time (immediately after heparinization, at the end of CPB, at 2 and 12 hours after operation) within groups was statistically significant ($P < .01$), and strongly significant differences were found in each comparison of two groups ($P < .01$).

Effects of NAC on Renal TNF- α and NF- κ B

TNF- α levels in the kidney tissues significantly increased in all CPB groups relative to the sham group (1.39 ± 0.31 pg/mg protein). Relative to the control group (5.71 ± 1.33 pg/mg protein), NAC significantly depressed TNF- α elevation (4.27 ± 0.57 pg/mg protein, $P < .01$), and high-dose NAC showed more apparent effect (3.09 ± 0.40 pg/mg protein, $P = .013$). The tendency of optical densities of renal NF- κ B was similar with that of renal TNF- α . Relative to the sham group (1.18 ± 0.06 times background), CPB caused significant increased expression of NF-kappa B (2.42 ± 0.19 , $P < .001$), whereas NAC treatment ameliorated this effect (1.89 ± 0.13 and 1.62 ± 0.10 , $P = .002$ and $P < .001$ for low NAC and high NAC, respectively, vs control), and a more significant effect was found in the high-NAC group ($P = .015$ vs low NAC).

Figure 1. Perioperative time courses of serum creatinine (A) and cystatin C (B). *NAC*, *N*-acetylcysteine; *T0*, before cardiopulmonary bypass; *T1*, at end of cardiopulmonary bypass; *T2*, 2 hours after cardiopulmonary bypass; *T3*, 12 hours after cardiopulmonary bypass. Asterisk indicates $P < .05$ versus baseline; crosshatch indicates $P < .05$ versus sham group; ampersand indicates $P < .05$ versus control group.

Discussion

Our data show that NAC prevents CPB-induced acute renal injury. Oxidative stress and systemic inflammation response were significantly lower in rats receiving NAC than in those

Figure 2. Photomicrographs of renal cortical tubules (hematoxylin-eosin, $\times 600$) from each group. Tubules appear normal in sham group (A), whereas they appear dilated and lined by flattened epithelial cells (some representing necrosis) in control group (B). In low-*N*-acetylcysteine group (C) and high-*N*-acetylcysteine group (D), tubules are dilated slightly, and cytoplasm of some tubular epithelial cells are filled with fine vacuoles.

receiving placebo, and a higher dose of NAC displayed better protective effects.

There have been many studies focusing on the renal protective effects of NAC. Most, however, have been based on renal ischemia-reperfusion models or toxic drug-induced kidney injury or acute inflammation models. Recently, there have been a few clinical trials concentrating on the effect of NAC in patients undergoing CPB, and their results are encouraging. Perioperative use of NAC showed cardiac and lung protective effects,^{10,12,14,15,24} but its renal effect in patients undergoing CPB seems inconsistent. Fish and associates¹¹ reported renal protection by NAC in patients undergoing coronary artery bypass grafting, but another randomized, controlled trial concluded that perioperative administration of NAC did not prevent postoperative renal dysfunction in high-risk patients undergoing coronary artery bypass grafting.² Although such clinical trials give us the most direct evaluation of NAC treatment, they cannot provide us with morphologic data on renal tissues because of ethics restrictions, and there are some uncontrolled influencing factors. Additionally, the dose of NAC used in clinical trials seems a little conservative.

As far as we knew, until now there has been only one study focusing on the effects of NAC in an animal CPB model.¹³ The authors used dogs in their experiment, and they concluded that NAC reduces lung reperfusion injury after deep hypothermia and total circulatory arrest. Nowadays, most researchers use large animals to establish a CPB

TABLE 2. Effects of *N*-acetylcysteine on malondialdehyde, reduced glutathione, superoxide dismutase, and catalase

Group	Malondialdehyde (nmol/g wet tissue)	Reduced glutathione (nmol/mg protein)	Superoxide dismutase (U/mg protein)	Catalase (katal/g protein)
Sham	1.55 ± 0.21	11.39 ± 0.98	95.18 ± 8.14	8.04 ± 0.60
Control	2.36 ± 0.23*	6.09 ± 1.90*	38.52 ± 2.81*	6.22 ± 0.65*
Low <i>N</i> -acetylcysteine	2.03 ± 0.30†	7.80 ± 1.74*	53.48 ± 3.82*‡	6.91 ± 0.39†
High <i>N</i> -acetylcysteine	1.74 ± 0.21‡	11.22 ± 1.56‡§	87.22 ± 8.25‡§	8.01 ± 0.51‡²

Values are mean ± SD, n = 6. **P* < .01 for difference from sham group. †*P* < .05 for difference from sham group. ‡*P* < .01 for difference from control group. §*P* < .05 for difference from low-*N*-acetylcysteine group. ²*P* < .05 for difference from low-*N*-acetylcysteine group.

model, because their anatomy and physiology are more similar to human beings. Large animal models, however, are increasingly expensive, require sophisticated surgical expertise, and require much more time and labor, whereas a rat model is more convenient and cost-effective.^{16,17}

We used a model designed by our colleagues in a previous study¹⁶; however, we made some modifications. First, we used the tail artery for our arterial line, instead of the right carotid artery. In our preliminary experiment, we found that when we used the tail artery, the arterial pressure was almost nonpulsatile during the extracorporeal circulation period. This was closer to the clinical situation. Second, we reduced the total prime volume to approximately 12 mL, and we did not add allogeneic blood to the prime. The hematocrit was in our ideal range and did not result in overhemodilution. Third, we kept the rats spontaneously breathing instead of using tracheal intubation. Our experiment achieved acceptable blood gas analysis results, which suggests that the membrane oxygenation and oxygen inhalation together were enough to satisfy the requirements of the rats. Finally, several drug doses in our experiment were

changed because of the results of our preliminary experiment.

There are a number of available renal function tests. Serum creatinine has been the most widely used marker in the last 40 years; however, its limitations are well known. It often overestimates glomerular filtration rate, it misses some subclinical kidney dysfunction, and its change falls behind glomerular filtration rate. Cystatin C, another glomerular filtration rate marker, has been well studied in the last two decades. The available data have indicated that serum cystatin C is superior to serum creatinine in various selected cases.²⁵ Recent research indicates that cystatin C is a reliable renal marker for patients undergoing on-pump coronary artery bypass grafting,²⁶ and it was proved in another study to be an earlier marker than serum creatinine to reflect acute renal dysfunction.²⁷ Additionally, a recent study showed that NAC only had an effect on creatinine levels in radiocontrast agent-induced nephropathy, without any effect on cystatin C levels.²⁸ We therefore used both creatinine and cystatin C as our renal function markers.

The time courses of these two markers were similar, consistent with the typical changes observed in clinical settings. Both injury markers, as well as the histologic findings, were attenuated dose dependently by the administration of NAC. These protective effects occurred mainly because of the relatively large doses we used in our experiment. In most previous studies, the widely used dose was 100 mg/kg into the prime, followed by infusion at 20 mg/(kg · h) or less.^{2,10-15} Because NAC is inexpensive, safe, and well tolerated, we used higher dose in this study and found a renal protective effect. We also used a dose of 1000 mg/kg in our preliminary experiment, but this caused hemodynamic instability.

A dose-dependent diuretic effect of NAC was also seen in this study. This is probably because of the high osmotic pressure and vasodilatation effect of the 20% NAC solution we used; it may also be one of the manifestations of protected renal function. Diuretics, such as mannitol and furosemide, can also significantly increase urinary volume and reduce tubular obstruction; however, recent studies concerning their renal protective effects have had inconsistent results.³ Mannitol prevented acute renal failure only in

Figure 3. Effect of *N*-acetylcysteine (NAC) on serum TNF- α level. T0, Before cardiopulmonary bypass; T1, at end of cardiopulmonary bypass; T2, 2 hours after cardiopulmonary bypass; T3, 12 hours after cardiopulmonary bypass. Serum TNF- α was elevated significantly at end of cardiopulmonary bypass and continued to increase. Significant differences were found within and between groups.

rabdomyolysis and kidney transplant surgery, whereas loop diuretics were associated with a higher risk of delayed recovery of renal function.²⁹ Furthermore, in our study NAC decreased serum creatinine and cystatin C and improved renal histologic condition rather than merely increasing urinary volume.

Our results demonstrate that oxidative stress induced reduction of antioxidants and elevation of MDA in renal tissue. These data are in good agreement with the work of other researchers.⁶ Our biochemical results demonstrated that antioxidant therapy with NAC prevented lipid peroxidation and caused increased activity of SOD and catalase in renal tissue after CPB, as well as a significant elevation of GSH level.

Because of its principal role in initiating the cascade of activation of other cytokines in the inflammatory response, TNF- α is regarded as the most important proinflammatory cytokine. NF- κ B is considered one of the most important transcription factors modulating its gene expression. NAC treatment significantly inhibited the CPB-induced inflammation response and reduced the tissue expression of NF- κ B, which confirms other research.^{23,30}

Apart from its antioxidative and anti-inflammatory characteristics, NAC may protect renal function through other mechanisms. Lessio and associates³¹ found that NAC blunts the reduction of inducible nitric oxide synthase expression and nitric oxide synthesis caused by cyclosporine (INN ciclosporin) in rat renal artery vascular smooth muscle culture cells.³¹ Nitric oxide-independent vasodilation with NAC has also been reported.⁴

Despite our encouraging findings, there were several limitations of this experiment. We only gave a bolus dose of NAC into the prime and only observed the renal function for 12 hours after the operation. Further long-term studies should be performed to investigate the protective effects of NAC in CPB models.

Conclusions

Our experiment demonstrated acute renal damage in a rat model of CPB, which was shown in both biochemical markers and morphologic examinations. NAC dose dependently ameliorated the CPB-induced kidney injury, probably through its antioxidative and anti-inflammatory properties. Further clinical trials with higher doses of NAC should be performed in patients undergoing CPB to evaluate its renal effects.

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Figure E1. Schematic of experimental model. *a.*, Artery; *v.*, vein; *ID*, inner diameter.