Renal ischemia/reperfusion against nephrectomy for induction of acute lung injury in rats

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ABSTRACT

Purpose: Acute kidney injury (AKI) induces acute lung injury (ALI) through releasing injurious mediators or impairing clearance of systemic factors. To determine the links between AKI and ALI, pulmonary and blood variables were evaluated following induction of AKI via different experimental models of bilateral renal ischemia/reperfusion (BIR: renal ischemia with uremia), unilateral renal ischemia/reperfusion (UIR: renal ischemia without uremia), bilateral nephrectomy (BNX: uremia without renal ischemia), and unilateral nephrectomy (UNX: without uremia and renal ischemia).

Methods: Ninety male Sprague–Dawley rats were divided into six groups. Animals had 1-h bilateral or 2-h unilateral renal ischemia followed by 24-h reperfusion in the BIR and UIR groups, respectively, and 24-h period following bilateral or unilateral nephrectomy in the BNX and UNX groups, respectively. There were also sham and control groups with and without sham-operation, respectively.

Results: Plasma malondialdehyde and nitric oxide were elevated by BIR more than UIR, but not changed by UNX and BNX. UIR slightly increased plasma creatinine, whereas BIR and BNX largely increased plasma creatinine, urea, K+ and osmolality and decreased arterial HCO3−/CO2, pH, and CO2. UNX and UIR did not affect lung, but BIR and BNX induced ALI with equal capillary leak and macrophages infiltration. However, there were more prominent lung edema and vascular congestion following BNX and more severe neutrophils infiltration and PaO2/FiO2 reduction following BIR.

Conclusion: Acutely accumulated systemic mediators following renal failure in the absence of kidneys vary from those due to combined renal failure with ischemic-reperfused kidneys and consequently they induce ALI with distinct characteristics.

Introduction

Patients with acute kidney injury (AKI) have high mortality rate in spite of substantial progresses in renal replacement therapies.1,2 Although AKI is a leading cause of death, the situation becomes more complicated with the incidence of distant organ injury, especially in the lung.1,3 Ischemia/reperfusion (I/R) is one of the common causes of AKI.4 Renal ischemia/reperfusion injury (IRI) may affect lung via not only releasing injurious mediators but also accumulating some plasma factors normally cleared by the kidney and establishing systemic uremia.3,5

To study the links between AKI and acute lung injury (ALI), two separate groups of investigators developed AKI via different experimental models of renal ischemia without uremia (unilateral renal ischemia/reperfusion; UIR), renal ischemia with uremia (bilateral renal ischemia/reperfusion; BIR), and uremia without renal ischemia (bilateral nephrectomy, BNX).6,7 The UIR model in the studies of both groups did not affect plasma urea, creatinine, and cytokines as well as lung. However, Rabb’s group observed induction of pulmonary injury in mice by BIR, but not BNX, and suggested that distant effects of renal IRI on lung can be conducted by released inflammatory molecules and cells from injured kidneys into circulation, but not due to absent renal clearance of systemic factors such as uremic toxins.8–10 In contrast, Faubel’s group found similar lung histological damage following both BNX and BIR in mice,7,11–14 and concluded that the absence of kidney function independent of renal tissue injury results in accumulation of systemic mediators to induce ALI.
In addition, there are contradictory findings in studies performed by other investigators\textsuperscript{15–19} that induced ALI by BNX or BIR and, totally, some opposite mechanisms have been suggested for conduction of injury from kidney to lung.

It is notable to mention that renal dysfunction was evaluated only by measurement of plasma urea and creatinine concentrations in all the above studies, and changes in other plasma variables were not determined that most likely have influences on lung response to AKI. On the other hand, we recently demonstrated that a 2-h unilateral renal ischemia and 24-h reperfusion had distant effect on the contralateral non-ischemic kidney to dampen its compensatory hyperperfusion and hyperfiltration, while the remnant kidney in the unilateral nephrectomy (UNX) model exhibited complete compensatory overfunction.\textsuperscript{20} Therefore, this investigation was designed to study the links between AKI and ALI through evaluating pulmonary structural and functional parameters along with measuring plasma levels of reactive oxygen species (ROS), nitric oxide (NO), creatinine, urea, Na\textsuperscript{+}, K\textsuperscript{+}, and osmolality as well as arterial pH, HCO\textsubscript{3}\textsuperscript{−}, O\textsubscript{2} and CO\textsubscript{2} tension, and blood pressure following induction of AKI by BIR, BNX, UIR, or UNX.

**Materials and methods**

**Experimental animals and groups**

This study was performed on 90 male Sprague–Dawley rats weighing 290–320 g, which were kept in cages at a temperature-controlled room (23 ± 1 \degree C) with 12-h artificial light and dark cycle. There were six groups of rats (n = 15), which were subjected to bilateral nephrectomy (BNX group), unilateral nephrectomy (UNX group), bilateral renal ischemia/reperfusion (BIR group), unilateral renal ischemia/reperfusion (UIR group) or sham-operation (sham group), as well as a group of rats without sham surgery (control group). All procedures conformed to the guidelines for the care and handling of animals prepared by the ethics committee of Shiraz University of Medical Sciences and in accordance with the international conventions on animal experimentation.

**Induction of renal ischemia/reperfusion and nephrectomy**

Each rat was anesthetized by intraperitoneal (I.P.) injection of pentobarbital sodium (60 mg/kg; Sigma, Poole, Dorset, UK), and then heparin (100 units) was I.P. injected to prevent intravascular blood clotting. A 1 mL blood sample was taken from the tail, centrifuged, and its plasma was aliquoted for measuring basal levels of its variables before exerting the related challenge in each group of rats as their pre-values. One part of plasma used for an immediate measurement of Na\textsuperscript{+}, K\textsuperscript{+} and osmolality, a second part was kept refrigerated for later assessment of creatinine and urea, and a third part was frozen in liquid nitrogen and preserved at −70 \degree C for later determination of malondialdehyde (MDA) and NO-metabolites.

After a midline electrosurgical laparotomy, there was removal of the right kidney in the UNX group or both kidneys in the BNX group. In rats subjected to renal I/R, clamping of the right renal pedicle for 2 h in the UIR group or both pedicles for 1 h in the BIR group was done and then blood reflow was assured by returning of the kidney color to normal. In the sham group, blood...
sampling and all surgical procedure were performed but renal pedicles were only manipulated. The abdominal incision of rats was sutured and they were allowed to recover from the anesthesia prior being returned to individual cages with access to food and water ad libitum.

**Experimental protocol**

Rats were weighed and re-anesthetized by I.P. injection of pentobarbital sodium at 23 h after the end of nephrectomy, renal ischemia, or sham-operation, while the rats of the control group were weighed and immediately anesthetized. Each rat was located supine on a heated surgical table and the probe of thermistor was placed in the rectum to maintain body temperature at 37 ± 1°C. A tracheotomy was performed and supplemental oxygen was blown across the end of the tube. Then, a cannula was placed into the right femoral artery and connected to a pressure transducer. After elapsing 15 min of equilibration, arterial pressure and heart rate were continuously recorded for 30 min using a PowerLab/8SP data acquisition system (AD Instruments, Bella Vista, NSW, Australia). Then, an arterial blood sample (1 mL) was taken into a cooled heparinized syringe, of which 0.2 mL was quickly analyzed for acid-base status and gases. The remainder of blood was centrifuged and part of the plasma was used for immediate measurement of electrolytes and osmolality, and the remainder of it was aliquoted and preserved as described before for later assaying of creatinine, urea, MDA, and NO-metabolites. Thereafter, the tracheal tube was aspirated and connected to a ventilator (Model 683; Harvard Apparatus Inc., Holliston, MA) for respiring room air at a rate of 60 breaths/min with a tidal volume of 1 mL per 100 g of body weight. After 10 min of mechanical ventilation, an arterial blood sample was taken to measure its O2 tension ($P_aO_2$) and by dividing it by the inspired fraction of oxygen ($F_iO_2$) in room air (21%), the ratio of $P_aO_2$ to $F_iO_2$ ($P_aO_2/F_iO_2$) was calculated. Meanwhile, the pressure of airways was measured during expiration by a pressure transducer connected to the PowerLab, and airway resistance was calculated by dividing airway pressure to the specified and constant air flow. Then, each group was subdivided into three different subgroups of $n = 5$ for (A) studying pulmonary performance at ex vivo condition in an isolated-perfused lung model, (B) obtaining bronchoalveolar lavage fluid (BALF), and also determining lung wet-to-dry weight ratio, and (C) removing kidneys and lungs for histopathological examination.

**The isolated perfused lung model**

In the subgroup A, heparin (150 units per 100 g body weight) was I.P. injected into anesthetized rat and then it received a normoxic–normocapnic gas (15.5%O2 and 5.5% CO2 balanced with N2) by the ventilator. As previously described, the chest was opened and pulmonary artery and left atrium were cannulated. The Krebs–Henseleit solution was used as the perfusate with the composition of 120.0 mM NaCl, 1.1 mM K2HPO4, 1.3 mM MgCl2, 4.3 mM KCl, 2.4 mM CaCl2, 13.3 mM glucose, and 1 g dextran (MW: 70,000; Sigma-Aldrich Chemie GmbH, Riedstrasse, Steinheim, Germany) per 100 mL. The lung was perfused with cooled (4°C) air bubble-free Krebs–Henseleit solution through the pulmonary artery cannula connected to a volumetric infusion pump (Model SM 2100; Jong Sang Techno Co. Ltd, Korea) with a pulsatile flow rate of 2 mL/min. The isolated-perfused lung was placed in a temperature equilibrated housing chamber and freely suspended from a force transducer. After rinsing the lung with perfusate to washout the blood, the perfusion circuit was closed with a total circuit volume of 40 mL. Meanwhile, the flow rate was slowly increased from 2 to 10 mL/min and the entire system (double glass reservoirs, tubing, and housing chamber) was heated from 4 to 40°C. Concomitantly, the left atrial pressure was set to 2–3 cmH2O by adjusting the height of venous part of the system to have zone 3 in the lung. A positive end expiratory pressure (PEEP) of 2 cmH2O was chosen to prevent regional alveolar collapse. The measurements of pressures in the left atrium, pulmonary artery, and airway by pressure transducers as well as lung weight by the force transducer were continuously performed and recorded through their connections to the PowerLab. For direct evaluation of pulmonary capillary permeability, capillary filtration coefficient ($K_c$) was determined gravimetrically from the slope of the lung weight-gain curve induced by sequential increasing venous pressure up to 10 mmHg for 8 min (hydrostatic challenge), and calculated as described by Segger et al.

**Bronchoalveolar lavage and lung wet-to-dry weight ratio**

In the subgroup B, BALF was obtained by slowly instilling 3 mL of warmed (~37°C) phosphate-buffered saline (PBS) with pH = 7.4 into lung through the tracheal tube. After 30 s, the fluid was withdrawn by gentle suction,
and the process was repeated twice. BALF (50 µL) was stained with 50 µL of Turk’s solution, and the total number of leukocytes was counted by means of a hemacytometer. The remainder of BALF was centrifuged at 1500 rpm for 10 min at 4 °C, and the supernatant was removed. The BALF supernatant together with a plasma sample, which had been obtained from the rat before taking BALF, were stored at −20 °C.20,26 Protein concentrations of the samples were measured by the Bradford method and presented as the ratio of BALF to plasma protein concentrations \( \times 1000 \) \( \left( \frac{[\text{Pr}]_{\text{BALF}}}{[\text{Pr}]_{\text{P}}} \times 1000 \right) \).

After taking BALF, the thorax was opened through a median sternotomy and all lobes of the lung were removed, cleaned from blood residues by PBS and weighed as wet weight. Then, they were placed in an oven with 90 °C for 72 h and their weight was taken as dry weight.16 The lung weight wet-to-dry ratio (wet/dry) was calculated as an index for pulmonary edema.

### Renal and pulmonary histopathological examinations

In the subgroup C, the abdomen and thorax of the rat were opened through the midline. Renal pedicles were tied off and then kidneys were removed, descapsulated, longitudinally sectioned, and preserved in the buffered 10% formaldehyde.27,28 Through the decapsulated, longitudinally sectioned, and preserved kidney, the abdomen and thorax of the rat were opened through the midline. Renal pedicles were tied off and then kidneys were removed, descapsulated, longitudinally sectioned, and preserved in the buffered 10% formaldehyde.27,28

The preserved kidneys and lungs were embedded in paraffin, and 5-µm sections were obtained by a microtome. Sections were subjected to routine staining with hematoxylin and eosin (H&E). In a blinded fashion, a pathologist colleague examined each section in at least 10 randomly selected non-overlapping fields under light microscope. The renal histopathology was quantified by one-way analysis of variance (ANOVA) followed by Duncan’s post-hoc test, and then the least significant difference (LSD) test for determining the exact level of \( p \) values. The histopathological scores were analyzed by the non-parametric Kruskal–Wallis multiple comparison test, and then a non-parametric Mann–Whitney test was used to determine statistical differences between groups. All the data analyses were performed using SPSS 11.5 software (SPSS Inc., Chicago, IL) and level of significance was set to \( p < 0.05 \).

### Results

#### Sham and control groups

The post-values of plasma creatinine, urea and electrolytes (Table 1), \( \text{NO}_2^-/\text{NO}_3^- \) and MDA (Figure 1) at 24 h
Renal histopathology

One hour of bilateral ischemia and 24-h reperfusion caused considerable cellular necrosis and exfoliation of cells in the proximal tubule (PT) with the average grades of 1.36 ± 0.07 at the cortex (Figure 6(A4)) and (B4)) and 4.02 ± 0.11 at the outer stripe of outer medulla (Figure 6(C4)) as well as in the thick ascending limb (TAL) with the average grades of 1.0 ± 0.0 at the cortex, 3.96 ± 0.13 at the outer stripe of outer medulla, and 1.0 ± 0.0 at the inner stripe of outer medulla. There were also low levels of tubular damages in the distal tubules as well as cortical and outer medullary collecting ducts. In addition, BIR resulted in enlargement of Bowman’s space with the average grade of 3.82 ± 0.16 (Figure 6(A3)), and vascular congestion and intratubular proteinaceous casts were intensively formed with the average grades of 4.65 ± 0.08 and 2.92 ± 0.47, respectively, in the inner stripe of outer medulla and around 1.0 in the cortex. In the post-ischemic kidney of the UIR group, whereas the average grade of TAL injury in the inner stripe of outer medulla was 1.47 ± 0.20. Also, distal tubules and collecting ducts in the cortex had considerable cellular injury with the average grades of 2.27 ± 0.17 and 1.98 ± 0.04, respectively, while injury of collecting ducts continued in the outer and inner stripe of outer medulla and the inner medulla with the grades of 1.64 ± 0.17, 1.75 ± 0.05, and 1.06 ± 0.04, respectively. In addition, enlargement of Bowman’s space had the average grade of 3.82 ± 0.16 (Figure 6(A3)), and vascular congestion and intratubular proteinaceous casts were intensively formed with the average grades of 4.65 ± 0.08 and 2.92 ± 0.47, respectively, in the inner stripe of outer medulla, and 1.36 ± 0.16 and 2.93 ± 0.15, respectively, in the inner medulla, as well as 1–1.5 in the outer stripe and cortex (Figures 6(B3–D3)). In contrast, the non-ischemic kidney of the UIR group (Figure 6(A2–D2)) and the remnant kidney of the UNX group did not show any sign of tubular injury, while their compensatory over function and increased blood flow were associated with medullary capillary hyperemia.

Plasma variables

Table 1 shows that the levels of plasma creatinine, urea, Na⁺, K⁺, and osmolality before performing renal nephrectomy and renal ischemia/reperfusion. The levels of plasma variables before and after nephrectomy and renal ischemia/reperfusion.

| Groups | Plasma creatinine (mg/dL) | Plasma urea (mg/dL) | Plasma sodium (μmol/mL) | Plasma potassium (μmol/mL) | Plasma osmolality (mosm/kgH₂O) |
|--------|--------------------------|---------------------|-------------------------|---------------------------|-------------------------------|
| Control | 0.33 ± 0.03              | 18.8 ± 0.6          | 144.4 ± 0.6             | 3.86 ± 0.07                | 301.2 ± 2.6                   |
| Sham   | 0.33 ± 0.03              | 18.6 ± 0.8          | 145.3 ± 0.6             | 3.79 ± 0.08                | 304.0 ± 1.4                   |
| Pre-values | 0.35 ± 0.03         | 17.7 ± 0.8          | 145.6 ± 0.6             | 3.81 ± 0.09                | 302.3 ± 2.4                   |
| Post-values | 0.32 ± 0.04             | 18.6 ± 0.8          | 145.3 ± 0.5             | 3.97 ± 0.16                | 303.7 ± 1.9                   |
| UIR    | 0.62 ± 0.04              | 21.3 ± 1.5          | 145.9 ± 0.6             | 3.94 ± 0.08                | 301.7 ± 2.6                   |
| Pre-values | 0.33 ± 0.04             | 18.7 ± 1.1          | 145.3 ± 0.5             | 3.97 ± 0.16                | 303.7 ± 1.9                   |
| Post-values | 0.33 ± 0.04             | 18.7 ± 1.1          | 145.3 ± 0.5             | 3.97 ± 0.16                | 303.7 ± 1.9                   |
| UNX    | 0.36 ± 0.04              | 18.5 ± 1.1          | 145.8 ± 0.5             | 3.61 ± 0.08                | 303.7 ± 2.7                   |
| Pre-values | 0.36 ± 0.03              | 18.5 ± 1.1          | 145.8 ± 0.5             | 3.61 ± 0.08                | 303.7 ± 2.7                   |
| Post-values | 0.36 ± 0.03              | 18.5 ± 1.1          | 145.8 ± 0.5             | 3.61 ± 0.08                | 303.7 ± 2.7                   |
| BIR    | 0.35 ± 0.03              | 20.1 ± 1.0          | 144.4 ± 0.6             | 4.08 ± 0.10                | 306.3 ± 2.9                   |
| Pre-values | 0.23 ± 0.01              | 142.0 ± 5.2         | 144.1 ± 0.6             | 5.49 ± 0.13                | 355.5 ± 2.9                   |
| Post-values | 0.23 ± 0.01              | 142.0 ± 5.2         | 144.1 ± 0.6             | 5.49 ± 0.13                | 355.5 ± 2.9                   |
| BNX    | 0.30 ± 0.02              | 18.3 ± 0.6          | 144.9 ± 0.5             | 4.00 ± 0.19                | 306.3 ± 1.1                   |
| Pre-values | 0.37 ± 0.13              | 139.9 ± 5.4         | 142.1 ± 0.6             | 5.32 ± 0.22                | 337.3 ± 3.4                   |
| Post-values | 0.37 ± 0.13              | 139.9 ± 5.4         | 142.1 ± 0.6             | 5.32 ± 0.22                | 337.3 ± 3.4                   |

The values are expressed as means ± SEM before (pre-values) and 24 h after (post-values) sham-operation (sham group), a 2-h unilateral or a 1-h bilateral renal ischemia (UIR and BIR groups, respectively), and unilateral or bilateral nephrectomy (UNX and BNX groups, respectively) in rats. There was no sham surgery in the control group. n = 15 in each group.

* p < 0.05, versus sham group at each period.

* c p < 0.05, UIR group versus BIR group or UNX group versus BNX group at each period.

* a p < 0.05, UIR group versus BIR group or UNX group versus BNX group at each period.

* b p < 0.05, versus sham group at each period.
ischemia, nephrectomy or sham-operation (pre-values) were not different among all groups. UNX did not change the levels of any plasma variables after 24 h (post-values), while UIR only elevated slightly post-values of plasma creatinine and urea from their pre-values \((p < 0.001 \text{ and } p < 0.05, \text{ respectively})\). Also, the post-value of plasma creatinine was higher in the UIR group than the sham and UNX groups (both \(p < 0.05\)). Both BIR and BNX resulted in severe rises of plasma creatinine, urea, \(K^+\), and osmolality after 24 h from their pre-values (all \(p < 0.001\)), and also their post-values in the sham group (all \(p < 0.001\)). Moreover, all of these plasma variables were higher in the BIR group than UIR group and the BNX group than UNX group (all \(p < 0.001\)). Post-value of plasma Na\(^+\) in the BNX group was decreased with respect to its pre-value \((p < 0.001)\) as well as the post-values of the BIR \((p < 0.05)\), UNX and sham \((p < 0.001)\) groups.

**Arterial acid–base and gases, mean arterial pressure, and heart rate**

Table 2 shows that the levels of \(P_\text{a}O_2\), \(P_\text{a}CO_2\), \(pH_a\) and \([\text{HCO}_3^-]_a\) as well as arterial pressure and heart rate after 24 h of unilateral ischemia, unilateral nephrectomy, and sham-operation were not different between the three groups. However, there were large falls in all arterial blood variables of the BIR and BNX group with respect to those of the sham, UIR, and UNX groups (all \(p < 0.001\)). The levels of \(pH_a\), \(P_\text{a}CO_2\), and \([\text{HCO}_3^-]_a\) were lower but \(P_\text{a}O_2\) was higher in the BNX group than the BIR group \((p < 0.05 \text{–} 0.01)\). In addition, mean arterial pressure and heart rate were decreased in the BIR (both \(p < 0.05\)) and BNX (both \(p < 0.001\)) groups with respect to the sham group. While, mean arterial pressure in the BNX group was much lower than that of the BIR and UNX groups (both \(p < 0.001\)). Heart rate was also lower in the BNX group than the BIR (both \(p < 0.01\)) and UNX \((p < 0.001)\) groups, as well as in the BIR group than the UIR group \((p < 0.05)\). The amounts of body weight loss after 24 h of performing the related challenge in all groups were similar.

**Plasma MDA and NO-metabolites**

The pre-values of plasma \(\text{NO}_2^-/\text{NO}_3^-\) (Figure 1(A)) were equal in all groups. At 24 h after the end of each...
challenge, the post-values of plasma NO\(_2^-\)/NO\(_3^-\) were not changed in the UNX and BNX group but elevated in the UIR and BIR groups from their pre-values and also the post-values of the sham, UNX and BNX groups (all \(p < 0.001\)). In addition, plasma NO\(_2^-\)/NO\(_3^-\) was raised after 24-h reperfusion in the BIR group more than the UIR group (\(p < 0.01\)).

Figure 2(B) shows that the pre-value of plasma MDA in the sham group was equal to those of the other groups. At 24 h after nephrectomy, plasma MDA was not changed in the UNX group but slightly increased in the BNX group (\(p < 0.01\)) with respect to their pre-values, however post-values of plasma MDA in both groups were not statistically different from that of the sham group. UIR and BIR resulted in large rises of plasma MDA after 24 h of reperfusion from the pre-values in the UIR and BIR groups and also the post-values of the sham, UNX, and BNX groups (\(p < 0.01\)–0.001). In addition, post-value of plasma MDA was higher in the BIR group than the UIR group (\(p < 0.01\)).

Table 2. The levels of arterial blood variables, heart rate, and weight loss after nephrectomy and renal ischemia/reperfusion.

| Groups   | pH\(_a\) | [HCO\(_3^-\)]\(_a\) (umol/mL) | P\(_{\text{CO}_2}\) (mmHg) | P\(_{\text{O}_2}\) (mmHg) | MAP (mmHg) | HR (beats/min) | Body weight loss (g) |
|----------|----------|-------------------------------|---------------------------|-------------------------|------------|----------------|---------------------|
| Control  | 7.359 ± 0.006 | 24.4 ± 0.5                   | 39.9 ± 0.3                | 92.8 ± 1.7              | 107.7 ± 3.1 | 391.4 ± 7.2    | −12.4 ± 1.2          |
| Sham     | 7.369 ± 0.008 | 24.1 ± 0.3                   | 38.9 ± 0.4                | 93.1 ± 3.2              | 106.8 ± 2.5 | 387.7 ± 8.7    | −12.6 ± 1.5          |
| UIR      | 7.363 ± 0.005\(^c\) | 24.3 ± 0.6\(^c\)         | 38.7 ± 0.6\(^c\)         | 90.4 ± 1.5\(^c\)       | 103.1 ± 2.4 | 385.6 ± 11.4\(^c\) | −12.6 ± 1.5          |
| UNX      | 7.385 ± 0.005\(^b,d\) | 24.8 ± 0.2\(^d\)        | 39.3 ± 0.3\(^d\)         | 92.0 ± 2.9\(^d\)       | 104.5 ± 2.9 | 400.1 ± 8.2\(^d\) | −12.7 ± 1.2          |
| BIR      | 7.238 ± 0.011\(^b,d\) | 13.1 ± 0.4\(^d\)         | 29.7 ± 2.6\(^d\)         | 66.9 ± 2.6\(^d\)       | 356.7 ± 9.6\(^d\) | 10.7 ± 0.9       | −10.7 ± 0.9          |
| BNX      | 7.193 ± 0.014\(^b\) | 11.5 ± 0.5\(^b\)         | 27.8 ± 0.5\(^b\)         | 75.9 ± 1.4\(^b\)       | 312.2 ± 9.8\(^b\) | 12.4 ± 1.2       | −12.7 ± 1.2          |

Values are means ± SEM for arterial pH (pH\(_a\)), bicarbonate concentration ([HCO\(_3^-\)]\(_a\)), carbon dioxide tension (P\(_{\text{CO}_2}\)) and oxygen tension (P\(_{\text{O}_2}\)), as well as mean arterial pressure (MAP), heart rate (HR), and body weight loss in rats at 24 h after sham-operation ( sham group), a 2-h unilateral or a 1-h bilateral renal ischemia (UIR and BIR groups, respectively) and unilateral or bilateral nephrectomy (UNX and BNX groups, respectively). There was no sham surgery in the control group. \(^n = 15\) in each group.

\(^a\)Values are means ± SEM for arterial pH (pH\(_a\)), bicarbonate concentration ([HCO\(_3^-\)]\(_a\)), carbon dioxide tension (P\(_{\text{CO}_2}\)) and oxygen tension (P\(_{\text{O}_2}\)), as well as mean arterial pressure (MAP), heart rate (HR), and body weight loss in rats at 24 h after sham-operation ( sham group), a 2-h unilateral or a 1-h bilateral renal ischemia (UIR and BIR groups, respectively) and unilateral or bilateral nephrectomy (UNX and BNX groups, respectively). There was no sham surgery in the control group. \(^n = 15\) in each group.

\(^b\)p < 0.05, versus sham group.

\(^c\)p < 0.05, UIR group versus BIR group or UNX group versus BNX group.

\(^d\)p < 0.05, UIR group versus UNX group or BIR group versus BNX group.

Figure 3. Changes in capillary permeability and water content of lungs after nephrectomy and renal ischemia/reperfusion. The values are expressed as means ± SEM for (A) capillary filtration coefficient (K\(_{\text{fc}}\)) in the isolated-perfused lungs, (B) ratio of bronchoalveolar lavage fluid (BALF) to plasma protein concentrations ×1000 ([Pr\(_{\text{BALF}}\)/Pr\(_{\text{Plasma}}\) × 1000), and (C) lung weight wet-to-dry ratio (wet/dry) at 24 h after sham-operation ( sham group), a 2-h unilateral or a 1-h bilateral renal ischemia (UIR and BIR groups, respectively) as well as unilateral or bilateral nephrectomy (UNX and BNX groups, respectively). There was no sham surgery in the control group. \(^n = 5\) in each group. \(*p < 0.05, **p < 0.01, ***p < 0.001, versus Sham group. \(^p < 0.05, §§p < 0.01, §§§p < 0.001, UIR group versus BIR group or UNX group versus BNX group.\)
Figure 4. Changes in arterial blood oxygenation and airway resistance after nephrectomy and renal ischemia/reperfusion. The values are expressed as means ± SEM for (A) ratio of arterial oxygen tension to inspired fraction of oxygen (P_{O2}/FiO2) and (B) airway resistance (AR) at 24 h after sham-operation (sham group), a 2-h unilateral or a 1-h bilateral renal ischemia (UIR and BIR groups, respectively) as well as unilateral or bilateral nephrectomy (UNX and BNX groups, respectively). There was no sham surgery in the control group. n = 15 in each group. *p < 0.05, ***p < 0.001, versus Sham group. §§§p < 0.001, UIR group versus BIR group or UNX group versus BNX group.

Figure 5. Lung tissue damages after nephrectomy and renal ischemia/reperfusion. Representative light microphotographs of the lungs in rats at 24 h after sham-operation (sham group), a 2-h unilateral or a 1-h bilateral renal ischemia (UIR and BIR groups, respectively) and unilateral or bilateral nephrectomy (UNX and BNX groups, respectively). There was no sham surgery in the control group, n = 5 in each group. (A1–A6) Hematoxylin and eosin (H&E) staining, magnification ×400, with indications of white arrows to increased interstitial thickness, black arrowheads to vascular congestion, and white arrowheads to alveolar hemorrhage. (B1–B6) H&E staining, magnification ×1000, showing increased infiltration of macrophage and neutrophils in the UIR, BIR, and BNX groups.
The lungs of the UNX (Figure 5(A3)) and UIR (Figure 5(A4)) groups had normal appearance similar to those of the control (Figure 5(A1)) and sham (Figure 5(A2)) groups. However, lung injuries were clearly observed in the BIR group (Figure 5(A5)) and BNX group (Figure 5(A6)) as alveolar hemorrhage (grades of 1.24 ± 0.12 and 1.61 ± 0.17, respectively), increased interstitial thickness (grades of 1.14 ± 0.14 and 1.92 ± 0.20, respectively), and vascular congestion (grades of 1.67 ± 0.19 and 2.59 ± 0.11, respectively). Figures 5(B1–B6) also show that the lung interstitial numbers of neutrophils and macrophages in the BIR and BNX groups as well as neutrophils in the UIR group were increased with respect to those of the control, sham, and UNX groups. Moreover, the lung total histopathological score was slightly higher in the BNX group than the BIR group (9.55 ± 0.69 versus 7.65 ± 0.70, p < 0.05).

Pulmonary leukocytes infiltration

Figure 2(A) indicates that there were considerable numbers of resident macrophages in the lung interstitium of the sham group, which were not changed in the UIR and UNX groups. However, the numbers of pulmonary interstitial macrophages equally increased in the BIR and BNX groups and became higher than those of the sham, UIR, and UNX groups (all p < 0.001).

The number of neutrophils in pulmonary interstitium (Figure 2(B)) of the sham group was very few and not changed in the UNX group. However, the numbers of pulmonary leukocytes infiltration

Figure 6. Renal tissue damages after nephrectomy and renal ischemia/reperfusion. Representative light microphotographs of the renal (A1–A4) cortex (B1–B4) juxtamedullary cortex, (C1–C4) outer medulla, and (D1–D4) inner medulla of rats at 24 h after sham-operation (sham group), a 2-h unilateral renal ischemia (UIR group) and a 1-h bilateral renal ischemia (BIR group), n = 5 in each group. Hematoxylin and eosin (H&E) staining, magnification ×400, with indications of black arrows to Bowman’s space, white arrows to tubular damage, black arrowheads to vascular congestion, and white arrowheads to intratubular proteinaceous cast.
lung interstitial neutrophils were elevated moderately in the UIR and BNX groups (both $p < 0.05$) and severely in the BIR group ($p < 0.001$) with respect to the sham group. Moreover, pulmonary interstitial neutrophils counts were higher in the BIR group than UIR group ($p < 0.001$) and the BNX group than UNX group ($p < 0.05$).

The total leukocytes numbers in BALF (Figure 2(C)) of the BIR and BNX groups, but not UIR and UNX groups, were largely increased compared to that of the sham group (both $p < 0.001$). In addition, cell number of BALF was higher in the BIR group than the BNX ($p < 0.01$) and UIR groups ($p < 0.001$), as well as the BNX group than the UNX group ($p < 0.001$).

**Pulmonary capillary permeability and edema**

The measured $K_{fc}$ in the isolated-perfused lung model (Figure 3(A)), as a direct indicator of pulmonary capillary permeability, in the UIR and UNX groups were not statistically different from that of the sham group. However, $K_{fc}$ was markedly and equally elevated in the isolated-perfused lungs of the BIR and BNX groups compared to the sham group (both $p < 0.01$). Also, pulmonary capillary permeability was higher in the BIR group than UIR group ($p < 0.05$) and the BNX group than UNX group ($p < 0.01$).

Figure 3(B) shows that UIR or UNX did not affect the protein concentration of alveolar fluid but BIR and BNX after 24 h resulted in similar rises of $[Pr]_{BALF}/[Pr]_P \times 1000$ in the BIR and BNX groups with respect to the sham, UNX, and UIR groups (all $p < 0.001$).

The lung wet/dry weight (Figure 3(C)) was not changed in the UIR and UNX groups but increased in the BIR and BNX groups ($p < 0.05$ and $p < 0.01$, respectively) compared to that of the sham group, however it was lower in the BIR group than the BNX group ($p < 0.05$). Moreover, it was larger in the BIR group than UIR group ($p < 0.05$) and the BNX group than UNX group ($p < 0.001$).

**Arterial blood oxygenation and airway resistance**

Figure 4(A) presents that $P_{aO_2}$/FiO$_2$ was dropped at 24 h after nephrectomy or renal ischemia to very low levels in the BNX and BIR groups (both $p < 0.001$) and slightly in the UIR group ($p < 0.05$) with respect to that of the sham group at the equivalent period. In addition, the BIR group had lower $P_{aO_2}$/FiO$_2$ than the BNX group ($p < 0.001$), and also it was much lower in the BIR group than UIR group and the BNX group than UNX group (both $p < 0.001$).

The airway resistance (Figure 4(B)) was not changed in the UIR and UNX groups but decreased in the BIR and BNX groups ($p < 0.001$) compared to that of the sham group. Moreover, it was moderately higher in the BNX group than the BIR group ($p < 0.05$), but largely lower in the BIR group than UIR group and the BNX group than UNX group (both $p < 0.001$).

**Discussion**

I/R induces damages in both vascular and tubular systems of the kidney. The vascular endothelial cell injury is associated with the attachment of leukocytes, red blood cells, and platelets that result in vascular congestion. Furthermore, an imbalance between production of vasoconstrictors and vasodilators increases renal arterial resistance, which with vascular congestion keep renal blood flow low during reperfusion period. The reduction of renal blood flow is more prominent in the medulla, where most microvessels are congested and create no-reflow areas. The renal I/R-induced tubular damages are either lethal, as apoptosis and necrosis, or sub-lethal. Disintegrations in cytoskeleton, intracellular attachment proteins, and integrins of the tubular epithelium lead to loss of cell polarity, shedding of brush borders, and detachment of viable cells. The sloughed cells, remnants of shed brush border, and other cellular debris as well as proteins form intratubular proteinaceous casts that obstruct tubular lumen and result in elevation of Bowman’s space pressure and its enlargement. The most severe epithelial injuries occur in the PT and TAL that have high active transport and especially at the outer medulla that a hypoxic state exists even in the normal condition. All of these histological damages were observed in the kidneys of rats subjected to 1-h bilateral renal ischemia followed by 24-h reperfusion. Additionally, there were slight tubular damages in the distal tubules and cortical and outer medullary collecting ducts, which were not seen in our previous studies with 30-min bilateral renal ischemia. Increasing the duration of renal ischemia to 2 h in the UIR group augmented all types of tissue damages in the post-ischemic kidney after 24 h of reperfusion, and even epithelial cells in the PT and TAL at the cortex and the outer stripe of outer medulla were completely destroyed and the whole length of distal tubules and collecting duct systems had considerable cellular injuries. However, the non-ischemic kidney of the UIR group and the remnant kidney of the UNX group had normal appearance with medullary capillary hyperemia due to their compensatory increased blood flow. In accordance, it was shown in patients subjected to partial nephrectomy for tumor resection that the longer warm
renal ischemic time (≥ 25 min) caused the greater irreversible damage throughout the operated kidney, while functional adaptation occurred in the normal contralateral kidney. The results of this study provide evidence that UNX and UIR do not affect lung, whereas BNX and BIR induce ALI but with distinct characteristics. Other studies have also shown that there are some differences between factors and mechanisms involved in the induction of ALI following BIR and BNX. Rabb’s group observed pulmonary injury in mice following BIR, but not BNX. Thereafter, this group showed that BIR of 60 min, but not 30 min, induced lung injury moderately after 6 h and severely after 36 h. However, Faubel’s group demonstrated that both BNX and BIR with only 22-min duration induced similar histological damages in lungs of mice after 24 h. Moreover, they found that BIR and BNX elevated multiple serum cytokines but with different profiles. In this study, the increases in pulmonary interstitial thickness and lung wet/dry weight, indicating pulmonary edema formation, were somehow more severe in the BNX than the BIR group. The impairment in alveolar fluid balance induced by BIR or BNX was shown to be associated with the down regulation of pulmonary epithelial Na+/K+ ATPase, Na+-channel, and aquaporin-5. Since the extracellular acidosis has an inhibitory effect on Na+/K+ ATPase activity, the lower pH₃ in the BNX group than the BIR group may imply that BNX probably has a stronger acidosis-induced disturbance of lung fluid balance than the BIR. It is also apparent from the levels of plasma Na⁺ in the BNX and BIR groups that the main reason for their increased plasma osmolality is highly elevated urea in both groups. Urea is a non-effective osmole and plasma Na⁺ was decreased by BNX, but not by BIR. Hence, the lowered plasma Na⁺ in the BNX group might cause more water entering into the lung cells during edema formation and also narrowing the lumens of pulmonary capillaries to lead to higher vascular congestion than the BIR group.

It has been proposed that the systemic release of injurious factors following IRI in an organ can lead to activation of pulmonary capillary endothelial cells with the resultant increases in their permeability and expression of adhesion molecules. The adherent neutrophils and macrophages subsequently transmigrate and enter the pulmonary interstitial space and along with the activated resident macrophages diffuse damage capillary endothelium and alveolar epithelium to induce ALI. The disruption of pulmonary microvascular barrier has been regarded as a central event in development of ALI induced by AKI. Hassoun et al. found that the BIR-induced pulmonary capillary leak was associated with the raised number of lung apoptotic cells, which in vivo immunofluorescence staining showed their colocalization predominantly with the endothelial cells and also few type-II epithelial cells in the injured lung. BNX and BIR caused equal increase in permeability of pulmonary capillaries in this study, as evidenced by their similarly raised levels of Kₑ in the isolated-perfused lungs and proteins in BALF. The pulmonary capillary leak was associated with the increased numbers of interstitial and alveolar neutrophils and macrophages in both BNX and BIR groups. Neutrophils and macrophages most likely play a main role in the pathogenesis of ALI by releasing proteases, ROS and cytokines. Importantly, pre-treating mice with a neutrophil elastase inhibitor (ONO-5046) attenuated the AKI-induced disruption in pulmonary microvascular barrier.

AKI-induced disruption of the capillary endothelium, leukocytes trafficking and tissue damages in the lung totally impair the exchange of O₂ between air and blood to result in acute onset of intensive hypoxemia and decrement of PₐO₂/FiO₂ to <300 in the patients with ALI. BIR caused less increases in lung wet/dry weight, interstitial thickness, and vascular congestion than BNX, but it led to a more reduction in PₐO₂/FiO₂ that was associated with the much larger number of neutrophils infiltration into lung of the BIR group with respect to the BNX group. Interestingly, the 2-h unilateral renal ischemia and 24-h reperfusion that did not induce any visible morphological damage in the lung could slightly decrease PₐO₂/FiO₂ in conjunction with the rise of lung interstitial neutrophils at the equal numbers of the BNX group. In addition, plasma levels of MDA and nitrite/nitrate were elevated in the UIR and BIR groups, but did not change in the BNX group. We recently showed that a 2-h unilateral renal ischemia followed by 24-h reperfusion had distant effects on the contralateral non-ischemic kidney, partly mediated by ROS and NO derived from inducible nitric oxide synthase (iNOS), to dampen its compensatory over function. In addition, co-treatment with α-tocopherol, as a potent antioxidant, and aminoguanidine, as an iNOS inhibitor, prevented the rises in plasma MDA and nitrite/nitrate and the fall in PₐO₂/FiO₂ following UIR. Hence, there can be the possibility that the elevated plasma ROS and NO in the UIR group may affect lung to promote moderate interstitial infiltration of neutrophils that slightly reduces PₐO₂/FiO₂. Of course, the increased infiltration of neutrophils into the lung interstitium of the UIR group was not accompanied with their migration into alveolar space as well as elevated interstitial and alveolar macrophages and, therefore, was not able to induce any pulmonary histological
damage. On the other hand, the increased urea, creatinine, K⁺ and H⁺ in the BNX group led to pulmonary infiltration of neutrophils at the equal numbers but macrophages at much larger numbers with respect to the UIR group, which together decreased largely P_{a}O₂/F_{i}O₂. When these systemic mediators combined with the highly increased plasma ROS and NO in the BIR group potentiated neutrophils infiltration into the lung that along with the infiltrated macrophages resulted in more reduction of P_{a}O₂/F_{i}O₂ than the BNX group.

The developed hypoxemia by BIR and BNX led to a compensatory response of decreased airway resistance. While, the more hypoxemia caused the more reduction of airway resistance in the BIR group compared to the BNX group. In addition, BNX- and BIR-induced metabolic acidosis caused compensatory respiratory hyperventilation to reduce P_{a}CO₂, however compensation was not full and the levels of pHa were still lower than normal in both BNX and BIR groups. Of course, the lower [HCO₃] and pHa in the BNX group than the BIR group resulted in more intensive hyperventilatory response with more reduction in P_{a}CO₂. Moreover, mean arterial pressure and heart rate were largely decreased after 24 h following BNX, which could be mainly due to the absence of renin–angiotensin system. Also, BIR moderately reduced arterial pressure and heart rate probably through its distant effect on heart as well as increased systemic NO vasodilatory action. In addition, decreases in pHa and P_{a}O₂ might contribute to fall of arterial pressure in both groups.

There were some limitations in this study. First, we had to anesthetize rats twice in two successive days; however, the inclusion of the control group showed that the first anesthesia and sham surgery did not affect any of considered parameters in this study. Second, since it was not possible to evaluate all pulmonary parameters in one rat, each group was subdivided into three subgroups. Third, this investigation was performed on rat models of AKI and, therefore, its data cannot be directly inferred to humans or clinical reality.

In summary, a 2-h unilateral renal ischemia and 24-h reperfusion resulted in rises of plasma ROS and NO, which probably had some effects on pulmonary endothelial reactivity processes to make them more susceptible to respond to factors promoting interstitial infiltration of neutrophils at a moderate level that only led to slightly reduced P_{a}O₂/F_{i}O₂, without causing any morphological changes in the lung. BNX-induced systemic accumulation of creatinine, urea, K⁺, and H⁺ could stimulate transmigration of neutrophils and macrophages into the interstitium and alveoli of lung, and when these accumulated systemic mediators accompanied with the highly elevated plasma ROS and NO due to the 1-h bilateral renal ischemia and 24-h reperfusion led to more pulmonary neutrophils trafficking and reductions of P_{a}O₂/F_{i}O₂ and airway resistance with respect to those caused by BNX. BIR and BNX induced ALI with equal capillary leak, but edema and vascular congestion were more prominently formed by BNX in association with the stronger metabolic acidosis, higher increase in plasma creatinine, and decreased plasma Na⁺. Totally, it can be concluded that there are some differences in acute accumulation of systemic mediators following renal failure in the absence of kidneys compared to those due to combined renal failure with ischemic-reperfused kidneys, and consequently they have different deleterious effects on the lung and induce ALI with distinct characteristics.

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