PECULIAR MECHANISMS OF GRAFT RECOVERY THROUGH ANTI-INFLAMMATORY RESPONSES AFTER RAT LUNG TRANSPLANTATION FROM DONATION AFTER CARDIAC DEATH

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Abstract

Background: Although lung transplantation from donation after cardiac death (DCD), especially uncontrolled DCD, is limited by warm ischemic periods, the molecular mechanism of warm ischemia-reperfusion-injury (IRI) has not been well elucidated. The purpose of this study was to clarify the particular longitudinal mechanisms of molecular factors involved in warm IRI.

Methods: Cold ischemic-time (CIT)-group lungs were retrieved and subjected to 3-h of cold preservation, whereas warm ischemic-time (WIT)-group lungs were retrieved after 3-h of warm ischemia. Orthotopic rat lung transplantation was performed and the grafts were reperfused for 1 or 4-h. The graft functions, gene expression, and activation of inflammatory molecules in the grafts were analyzed. Exhaled-carbon-monoxide-concentration (ExCO-C) was measured during reperfusion.

Results: Only the WIT-group showed obvious primary graft dysfunction at 1-h reperfusion, but the graft function was recovered during 4-h reperfusion. Most of pro-inflammatory cytokines and stress-induced molecules showed different expression and activation patterns between CIT and WIT groups. In the WIT-group, the expressions of anti-inflammatory molecules, IL-10 and HO-1, were significantly increased at 1-h reperfusion compared to the CIT-group, and these high levels were maintained through 4-h reperfusion. Furthermore, ExCO-C levels in the

WIT-group increased immediately after reperfusion compared to the CIT-group.

Conclusions: This study indicates that warm IRI may involve a different mechanism than cold IRI and anti-inflammatory pathways may play important roles in the graft recovery after lung transplantation from uncontrolled DCD.

1. Introduction

Lung transplantation has become an accepted clinical option for patients with end-stage pulmonary diseases (1). However, the shortage of brain-dead donors remains a serious problem and the annual death rate for patients on lung transplant waiting lists varies from 13 to 30% (1, 2). Using lungs from donation after cardiac death (DCD) might be a useful method to increase the donor pool, and the clinical application of uncontrolled DCD has recently been put to practical use (3-5).

Ischemia-reperfusion injury (IRI) in lung transplantation leads to severe primary graft dysfunction (PGD), which unfortunately progresses to early morbidity and mortality in 10–20% of recipients (6). Lungs from uncontrolled DCD are inevitably subjected to warm ischemia, and the warm ischemic tolerance time is limited compared to cold ischemia (7-10). Therefore, IRI after warm ischemia is the most obstructive issue for progress with tentative transplantation of lungs from uncontrolled DCD. However, the graft functions of DCD have only recently been discussed, and the mechanism of warm IRI has not been well studied.

2. Objective

The aim of this study was to demonstrate specific longitudinal changes in primary graft function and inflammatory molecule expression levels induced by warm IRI. To clarify the characteristics of warm IRI, it was necessary to explore the differences between cold and warm IRI using the orthotopic vascularized lung transplant model.

3. Materials and Methods

3.1. Lung transplantation

Male Sprague-Dawley rats (Charles River, Yokohama, Japan) weighing 250–300 g were used for the experiments. The orthotopic single left lung transplantation was performed as previously reported using the cuff technique (11, 12). The donor rat was anesthetized by inhalation of diethyl ether following intraperitoneal injection of pentobarbital sodium (60 mg/kg). After tracheotomy, the rat was ventilated with a mixture of halothane and oxygen to maintain anesthesia, heparinized by the intrahepatical injection of 600 IU/body heparin through a small laparotomy, and divided into 1 of 2 groups (Figure 1). In the cold ischemic-time (CIT) group, a median laparosternotomy was performed and the donor lung was retrieved after flushing with low-potassium dextran glucose (LPDG) solution. The graft underwent cold (4°C) preservation for 3-h. In the warm ischemic-time (WIT) group, the donor lung underwent 3-h of warm

ischemia. In brief, the donor was exsanguinated by cutting the inferior vena cava through a small laparotomy and immediately put to death. The tracheal tube was disconnected and the cadaver was stored in a chamber at room temperature (28°C). After 3-h warm ischemic period, a median laparosternotomy was performed and the lung was retrieved after flushing with LPDG solution. In both CIT and WIT groups, the total ischemic-time including the time of transplantation was 4-h. The recipient was anesthetized and a left thoracotomy was performed. The pulmonary vessels and bronchus were anastomosed with standard cuff technique. After reperfusion, the recipient was kept anesthetized with a mixture of 0.5% halothane and oxygen under ventilation and sacrificed at 1-h (-1hRe) or 4-h (-4hRe) after reperfusion (n = 5 each). In the sham group, the rats underwent only anesthesia and thoracotomy (n = 5).

3.2. Blood gas analysis

The right hilum was clamped for 5-min after changing the tidal volume and respiratory rate ventilator settings to 0.6 ml/100g and 100 breaths/min, respectively. The arterial blood sample was taken from the ascending aorta to analyze the blood gas on a FiO_2 1.0. Finally, the graft lung was removed and separated into 3 blocks (upper, middle and lower) and stored at -80°C.

3.3. Histology and neutrophil infiltrations

Middle part of the horizontal section of donor lung was fixed in 10% formalin and stained with

Haematoxylin-Eosin (H&E). Under high power magnification (HPF; X200), five fields from central to peripheral parts on one slice were accurately sampling with equal probability of each graft lung and neutrophils were identified and counted for two slices in a blinded fashion by a pathologist. The average numbers of neutrophils/HPF were used for SEM.

3.4. Western blot analysis

The lower specimen was homogenized on ice using an ultrasonic processor in 1 X lysis buffer (RIPA buffer; Cell Signaling, Beverly, MA) containing a cocktail of protease inhibitors (Complete Mini; Roche, Indianapolis, IN). Equal amounts of each protein sample (50 µg) were loaded on sodium dodecyl sulfate (SDS)-polyacrylamide gels and electroblotted to a polyvinylidene difluoride (PVDF) transfer membrane. The membranes were incubated overnight at 4°C using a primary antibody against phospho-p38 mitogen-activated protein kinase (MAPK) (Bio Vision, Mountain View, CA). The site of primary antibody binding was visualized with horseradish peroxidase-conjugated rabbit antibody (Santa Cruz Biotechnology, Santa Cruz, CA) followed by the detection step and exposure to film. The same membrane was rinsed and re-blotted with a primary antibody against total–p38 (Delta Biolabs, Gilroy, CA) and the same detection and exposure to film processes were repeated. The image was analyzed by densitometry (Image J software version 1.2; Media Cybernetics, Silver Spring, MD) to assess

the relative level of p38 activation in the different groups by calculating the ratio of phospho-p38/total p38.

3.5. Real-time RT-PCR technique

The upper specimen was homogenized and RNA was isolated using TRIZOL Reagent (Invitrogen, Carlsbad, CA) following the manufacture's protocol. DNA was removed using the DNA-free kit (TURBO DNA-free; Ambion, Austin, TX) following the manufacturer's protocol. Total RNA (2 μg) was used for reverse transcription (RT) using the SuperScriptTM III-RT Kit (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. The resulting cDNA sample was diluted 1:20 and 1 μl was used for real-time RT-PCR.

Real-time RT-PCR was performed using LightCycler technology (Roche Diagnostics, Mannheim, Germany) to quantify the expression of the following mRNAs: tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, IL-10, monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein-2 (MIP-2), plasminogen activator inhibitor-1 (PAI-1), intercellular adhesion molecule-1 (ICAM-1), cyclooxygenase-2 (COX-2), heme oxygenase-1 (HO-1), and β-actin. The forward and reverse primers and Taq probes were designed using Probe Finder software (https://www.roche-applied-science.com/sis/rtpcr/upl/index.jsp) from Roche Diagnostics. Each primer was designed to span 2 different exons. Real-time RT-PCR was

performed using the LightCycler TaqMan Master from Roche Diagnostics with each primer pair and taq probe following the manufacturer's protocol.

The second derivative maximum method was used to automatically estimate the crossing points for individual samples. The relative amount of target gene was calculated on the basis of the cross-point analysis (LightCycler software version 3.5; Roche Diagnostics, Mannheim, Germany) on an external standard curve created by blotting the log number of copies by using serial dilutions of 1 control sample. The relative data for experimental genes were normalized as a ratio to β-actin concentration and shown compared to the mean data of the sham group.

3.6. Exhaled Carbon-monoxide (CO) measurement

Exhaled-carbon-monoxide-concentration (ExCO-C) was analyzed using a CO analyzer (CarbolyzerTM mBA-2000; Taiyo Instruments Inc., Osaka, Japan). Another series of single left lung transplantation followed by 4-h of reperfusion were performed as described above in each group (n = 5 each). A sampling adaptor was side attached to the ventilator circuit for collecting the exhaled air, and the ExCO-C was measured during the 4-h reperfusion at 13 time points: 0, 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, and 240 min. The mean ExCO-C for 1 min was calculated by the special software package (CarbolyzerTM Data BOX; Taiyo Instruments Inc.) at each time point. The excretion change level of ExCO-C at each time point was calculated

from the ExCO-C level using the starting point as a base level.

3.7. Statistical analysis

All values are presented as mean ± standard deviation (SD). The one-way repeated-measures analysis of variance (ANOVA) followed by Tukey-Kramer test for multiple comparisons were used to compare the levels of phospho-p38, mRNA levels, and ExCO-C between groups. Probability values less than 0.05 were regarded as statistically significant for all tests. All analyses were performed using Stat View 5.0 Program for Windows (SAS Institute Inc., Cary, NC).

3.8. Animal Care

The Animal Care Committee at Okayama University approved the protocol for this study. All rats received humane care in compliance with The Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals (NIH Publication No.86-23, 1996).

4. Results

4.1. Pulmonary Graft Function

The PaO_2 level of the WIT-1hRe group was significantly lower than those of the CIT-1hRe and sham groups ($p < 0.01$), although the PaO_2 of the CIT-1hRe was the same as that of the sham group (Figure 2 A). However, the PaO_2 of the WIT-4hRe group was significantly higher than that of the WIT-1hRe group ($p < 0.01$), indicating that the pulmonary function of the graft subjected to 3-h of warm ischemia recovered during the 4-h reperfusion period. The recovery of pulmonary function was clear and the WIT-4hRe group showed comparable oxygenation relative to the sham and CIT-4hRe groups. Histological examination showed alveolar oedema only in WIT-1hRe group (Figure 2 B). All groups showed significant neutrophil infiltrations compared to sham group ($p < 0.01$). A significant increase of neutrophils was observed in WIT-1hRe group, whereas they were significantly decreased in WIT-4hRe group ($p < 0.01$) (Figure 2 C).

4.2. Pro-inflammatory cytokines and stress-induced molecules

The expression levels of all pro-inflammatory molecules were significantly increased in the WIT-1hRe group than the sham group ($p < 0.01$) (Figure 3), and the expression levels of IL-1β, IL-6, MCP-1, ICAM-1, and COX2 were significantly higher in the WIT-1hRe group compared to the CIT-1hRe group ($p < 0.01$). However, these significant differences were no

longer observed at 4-h reperfusion between CIT and WIT groups with the exception of IL-6. TNF-α and MIP-2 expression levels were not significantly different between the CIT and WIT groups at 1-h or 4-h reperfusion, although MIP-2 expression levels were significantly decreased after 4-h reperfusion in both groups ($p < 0.01$). The expression of PAI-1, comparable between the CIT-1hRe and WIT-1hRe groups, decreased in the CIT-4hRe group and was significantly lower than that of the WIT-4hRe group ($p < 0.01$).

4.3. Anti-inflammatory cytokine and molecules

IL-10 gene expression was significantly increased in the WIT-1hRe group than the sham group, and this high expression level was maintained at 4-h reperfusion (Figure 4A). In the CIT-1hRe group, the IL-10 expression levels were significantly lower than those in the WIT-1hRe group ($p < 0.01$) and were up-regulated only at 4-h reperfusion. The gene expression patterns of HO-1 and IL-10 were the same (Figure 4A). P38 was activated only at 4-h reperfusion in the CIT group, whereas the WIT group showed significant activation of p38 at 1-h reperfusion (Figure 4B), and this activation was maintained until 4-h reperfusion. P38 activation showed the same pattern as the mRNA expression levels of IL-10 and HO-1.

4.4. Exhaled CO measurement

Changes in ExCO-C during the 4-h reperfusion are indicated in Figure 4C. In the CIT group, the ExCO-C started to increase approximately 120-min after reperfusion and slightly increased until 240-min after reperfusion. On the other hand, the ExCO-C in the WIT group was rapidly elevated after reperfusion, and these levels were significantly higher than CIT group from 60 to 105-min after reperfusion. The ExCO-C gradually increased in the WIT group from 105-min until 240-min after reperfusion and was also significantly different from the CIT group from 180 to 240-min (60, 75, 90, 105, 180, 210, and 240 min: $p < 0.05$).

5. Discussion

IRI after lung transplantation is thought to be a biphasic process (13, 14). The initial phase of injury occurs within the first 30 minutes of reperfusion, and mainly depends on donor characteristics of endothelial cells and alveolar macrophages(15). Endothelial cell is known to contribute to PGD by its inductions of powerful vasoconstrictors and vascular hyper permeability factors, like endothelin-1 and vascular endothelial growth factor(16). Moreover, activated resident alveolar macrophages in donor lung are also known to mediate early phase of IRI by its productions of several proinflammatory cytokines and chemokines(15, 17). These mediators result in the recruitment and activation of

recipient neutrophils to the transplanted lung, eliciting a subsequent late phase of injury that generally occurs 4 to 6 hours after reperfusion. Activated neutrophils produce a number of proteolytic enzymes and reactive oxygen species (ROS) that produce end-organ damage in the allograft. Most of rodent models of lung cold IRI demonstrated that the late phase of injury show worse graft function than the initial phase of injury (18, 19). In this study, DCD lungs were subjected to warm ischemia in the closed cadaver thoracic cavity similar to a clinical situation of uncontrolled DCD (Maastricht classification Category I NHBD) and transplanted orthotopically. In contrast to cold IRI models, the pulmonary graft function and neutrophil infiltrations of uncontrolled DCD group improved after 4-h reperfusion in the recipient body. Recent clinical reports also indicated that DCD lung grafts could recover from the first PGD within 6-h after reperfusion (20, 21). Furthermore, our group showed the recovery potential of DCD lung grafts while reperfusion and it started within 1-h after reperfusion by using swine model as well (22). These findings suggest that uncontrolled DCD lung may possess good recovery potential and warm IRI may involve a different mechanism than cold IRI.

We analyzed the differences of longitudinal changes in gene expression levels of inflammatory cytokines and molecules between cold and warm IRI. In cold IRI, proinflammatory cytokines such as TNF-α, IL-1β, IL-6, and COX2 and chemokines such as MIP-2 and MCP-1 (23, 24) have been identified as molecular factors that regulate IRI in lung transplants (25-28). In

addition, PAI-1 and ICAM-1 are proinflammatory molecules (29, 30) that reportedly lead to IRI by fibrin deposit and cell adhesion in rat lung transplantation and mouse IRI models (25-27). In the present study, all proinflammatory mediators were upregulated by warm IRI, although IL-1β, COX2, and ICAM-1 were not increased by cold IRI as previously reported (15, 31). Significant differences in the gene expression levels of IL-1β, IL-6, MCP-1, COX2, and ICAM-1 were observed at 1-h reperfusion between cold and warm IRI, and these differences were correlated with blood gas oxygenation. However, the expressions of most of these genes in the warm IRI group had decreased to comparable levels of the cold IRI group during 4-h after reperfusion. The reduction of these proinflammatory cytokines after warm IRI was consistent with improvement of pulmonary function. On the other hand, TNF-α and MIP-2 were also upregulated by cold IRI, but the expression levels were comparable to warm IRI at both 1 h and 4 h after reperfusion. Thus, proinflammatory cytokines showed different and various expression patterns between cold and warm IRI, suggesting that both the degree of injury and the mechanism may be different in cold and warm IRI.

Anti-inflammatory molecules were also examined as we considered them to play an important role in the recovery process of DCD lungs, because they have been shown to reduce IRI and their inhibition promotes lung injury (32-36). IL-10 is a pleiotropic cytokine that mainly functions to limit and terminate inflammatory responses, and exogenous IL-10 has been shown

to prevent lung IRI in several studies (35-38). HO-1 is also induced by a variety of oxidant/inflammation stresses, plays an important role in the cytoprotective response (32, 33), and reduces IRI by producing CO, biliverdin, and bilirubin (34, 39-43). In the current study, the differences between cold and warm IRI in the expressions and activations of anti-inflammatory pathways were more obvious than those of pro-inflammatory molecules. IL-10 and HO-1 displayed different expression patterns in the grafts that underwent warm IRI compared to cold IRI. After cold IRI, the expression levels of IL-10 and HO-1 gradually increased over the reperfusion period. On the other hand, IL-10 and HO-1 expression levels were significantly higher at 1-h reperfusion after warm ischemia when the graft showed severe PGD. IL-10 and HO-1 were still highly expressed 4-h after warm IRI, while the graft recovered from IRI. These results suggest that these anti-inflammatory mediators are expressed early after warm IRI and may play a pivotal role in graft recovery. Several reports have demonstrated that IL-10 induces the expression of HO-1 via p38 activation and the anti-inflammatory molecules are thought to be further amplified by their own mutual positive-feedback circuit (44, 45). We observed a similar association between activated p38 and IL-10 and HO-1 expression in this study, and this indicated that the anti-inflammatory signaling pathway from IL-10 to HO-1 through p38 activation was rapidly induced in the graft after warm IRI to heal the severe injury associated with ischemic conditions.

We measured ExCO-C to further study the anti-inflammatory system after warm IRI. CO, a product of heme breakdown mediated by HO-1, diffuses out of cells, enters the bloodstream to form carboxyhemoglobin, and is transported to the lungs for excretion (46). Previous studies reported that ExCO-C was increased in inflammatory airway diseases, systemic inflammatory diseases like sepsis and critical illness, and in IRI of living donor liver transplantation (47, 48). Recently, Vos et al. demonstrated the correlation between ExCO-C and airway neutrophilia after lung transplantation, and it could be used as a noninvasive marker for airway inflammation (49). On the other hand, a low exogenous dose of CO has been shown to reduce IRI in the lung and other solid organ transplantation models (28, 42, 50-52), and even in cerebral (53) and myocardial (54) infarction models. Consistent with HO-1 expression, ExCO-C was rapidly elevated after warm IRI following reperfusion but not after cold IRI. This finding suggests that CO may have a protective effect on the graft and reduce IRI after warm IRI and also supports the importance of the anti-inflammatory process in graft recovery.

Warnecke et al. reviewed the differences between cold and warm IRI and commented that there are no major differences on lung functions (55). However, the current study demonstrated that differences may exist in the processes and changes associated with cold and warm IRI. This study also suggested that anti-inflammatory molecules such as IL-10, HO-1, and CO are rapidly induced after warm IRI and may have potential to recover the graft against warm IRI. In case of

lung retrieval, ex-vivo treatment, and medication for the recipient after lung transplantation form uncontrolled DCD, specific strategy by utilizing these molecules may be effective for the reduction of IRI. However, further studies are required to substantiate these findings by suppression or overexpression of inflammatory and anti-inflammatory molecules using inhibitors or knockout mice lung transplantation models.

Although this study may have shown the differences after reperfusion, the differences during cold and warm ischemia have to be considered as well. During ischemia, macrophage and endothelial cells have been shown to induce cytokines and ROS(15, 17), but cytokines have been shown little up-regulations while both cold and warm ischemic period(56, 57). The current study also showed rapid cytokine up-regulation only after reperfusion with little expression while ischemic period in preliminary experiments (data not shown). Metabolism and cell death during ischemia may also contribute to the differences between cold and warm IRI. While hypothermia decreases metabolic rate, warm ischemia leads a sharp decrease of adenosine triphosphate (ATP) and then generates ROS(15, 17, 58). Apoptosis is not present during ischemia but after reperfusion, and does not correlate with pulmonary function(59). However, 2-h of warm ischemia has been shown to cause 36% of cell necrosis and necrotic cells release intracellular proteins just after reperfusion(60). The rapid inflammatory change after warm ischemia in our

study may have been caused by these proteins released from necrotic cells right after reperfusion. However, it is difficult to reveal the mechanisms during ischemia to cause the differences between cold and warm ischemia from our results, and further experiments targeting metabolic rate and cell death may be necessary to clarify the mechanisms.

In summary, this novel report delineated the differences between cold and warm ischemia-reperfusion at the molecular level by using the orthotopic rat lung transplantation model. Furthermore, anti-inflammatory molecules may play important roles in the graft recovery after warm IR. These findings have possibilities to lead to novel therapies against IRI after lung transplantation from uncontrolled DCD to increase the potential donor pool.

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Figure legends

Fig. 1

Schematic overview of the rat lung transplant experiment. All donor rats were heparinized and followed by sudden cardiac arrest by the protocol described in the text. All donor lungs were flushed with LPDG solution before the harvest. The donor lung for cold ischemia (CIT) was preserved at 4°C after lung retrieval and the lung of uncontrolled DCD (WIT) was kept in a cadaver at 28°C for 3 h before lung retrieval. In both groups, orthotopic vascularized single left lung transplantation was performed and the graft was reperfused after 4 h of total ischemic time. The grafts were assessed at 1 and 4 h after reperfusion (n = 5 in each group).

Fig. 2

Pulmonary graft functions after cold and warm ischemia-reperfusion. (A) Arterial oxygenation (PaO_2) was determined with isolated lung graft circulation and ventilation on a FiO_2 1.0 (n = 5 in each group). Data are expressed as means ± SD. PaO_2 levels of the WIT group were significantly worse than the sham and CIT groups after 1h reperfusion (*$p < 0.05$; vs. sham, ‡$p < 0.05$; vs. CIT at the same reperfusion time). The PaO_2 level of the WIT group at 4-h reperfusion was markedly improved compared to 1-h reperfusion (†$p < 0.05$; 1 h vs. 4 h after

reperfusion in each group), and there were no significant differences when compared to the sham and CIT group after 4 h of reperfusion.

(B) Representative histologic analysis (n = 5) of lung grafts from sham operations (a), 1-h and 4-h reperfusion after cold ischemia (b, c) and 1-h and 4-h reperfusion after warm ischemia (d, e). Lung grafts were stained with hematoxylin-eosin. (X200 magnification)

(C) The total neutrophil counts per one HPF are shown as a mean ± SD (*$p < 0.05$). Neutrophils were significantly infiltrated into graft lung after ischemia-reperfusion (*$p < 0.05$; vs. Sham). The intragraft neutrophil numbers after 4-h reperfusion were markedly decreased compared to 1-h reperfusion after warm ischemia ($^{\dagger}p < 0.05$).

Figure 3

Intragraft mRNA expression levels of proinflammatory cytokines and stress-induced molecules after ischemia-reperfusion. The expression level of mRNA was quantified using real-time RT-PCR and is shown relative to the β-actin housekeeping gene mRNA level. The relative mRNA level of each group (n = 5) was compared to the sham group (n = 5), and the fold data are expressed as means ± SD. (*$p < 0.05$; vs. sham, ‡$p < 0.05$; CIT vs. WIT at the same reperfusion time, †$p < 0.05$; 1 h vs. 4 h after reperfusion in each group).

Figure 4

Longitudinal changes of anti-inflammatory factors induced by cold and warm ischemia-reperfusion injury. Data are expressed as means ± SD. (A) mRNA expression levels of IL-10 and HO-1 were quantified using real-time RT-PCR and are shown relative to the β-actin housekeeping gene mRNA expression level. The relative mRNA fold level of each group (n = 5) is expressed compared to the sham group (n = 5). (‡$p < 0.05$; CIT vs. WIT at the same reperfusion time, †$p < 0.05$; 1 h vs. 4 h after reperfusion in each group). (B) Levels of phosphorylated-p38 (phospho-p38) in isolated lung grafts (n = 5) at 1 and 4 h of reperfusion after cold and warm ischemia were evaluated by western blot analysis. A representative image of 5 independent experiments is shown. Band intensity was quantified and expressed as the ratio of phospho-p38 to total-p38. (‡$p < 0.05$; CIT vs. WIT at the same reperfusion time, †$p < 0.05$; 1 h vs. 4 h after reperfusion in each group). (C) Changes in exhaled carbon monoxide concentrations (ExCO-C) during reperfusion after rat lung transplant. The ExCO-C of the recipient rat transplanted with the DCD lung gradually increased throughout the reperfusion period, but the ExCO-C from the recipient rat transplanted under CIT conditions showed an increased level from 120 min after the reperfusion time point. Significant differences between

CIT and WIT were identified at 60, 75, 90, 105, 210, and 240 min after reperfusion (§$p < 0.05$; CIT vs. WIT at the same reperfusion time)

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Figure 1

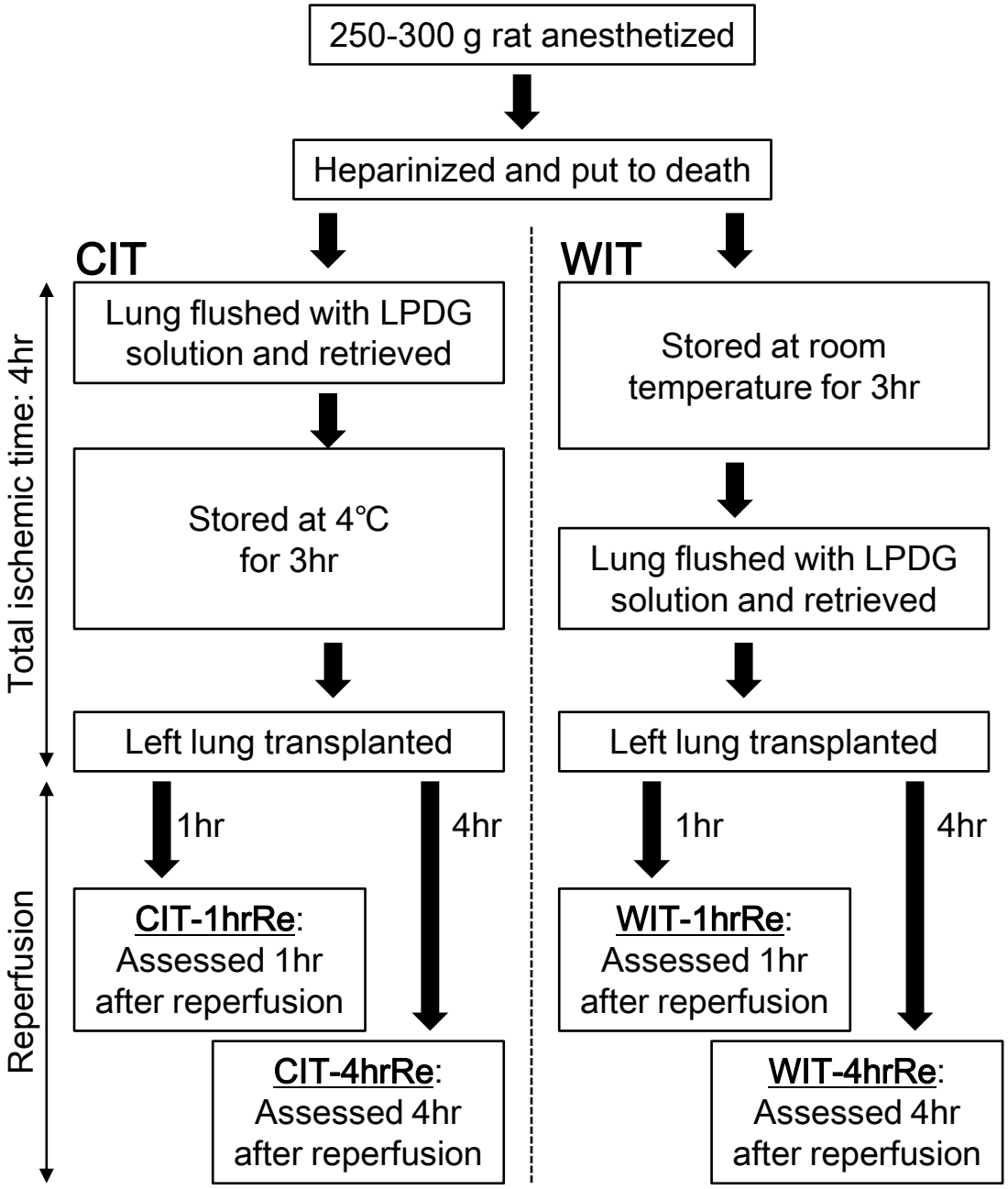
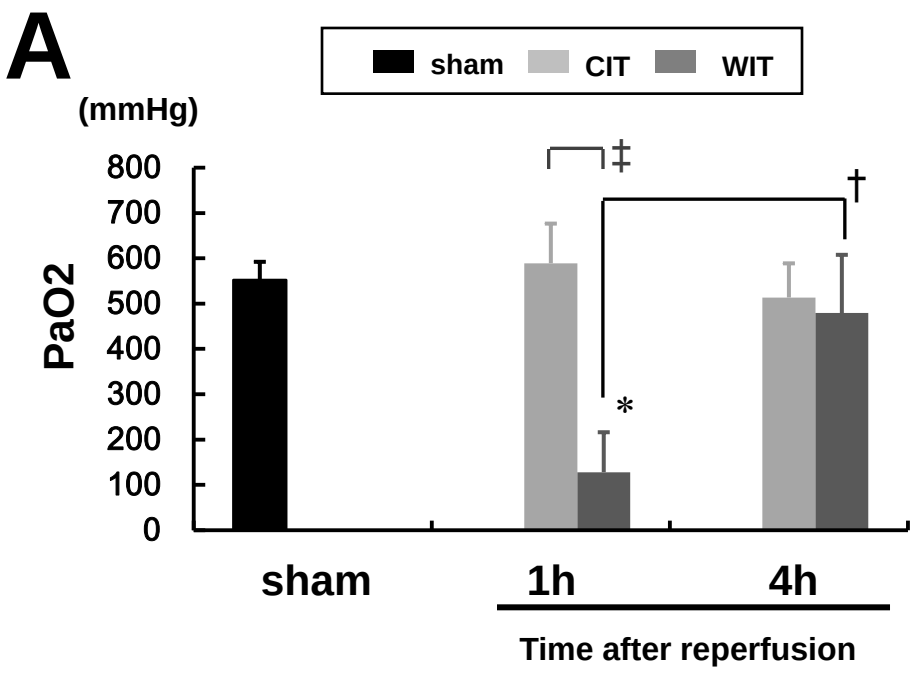
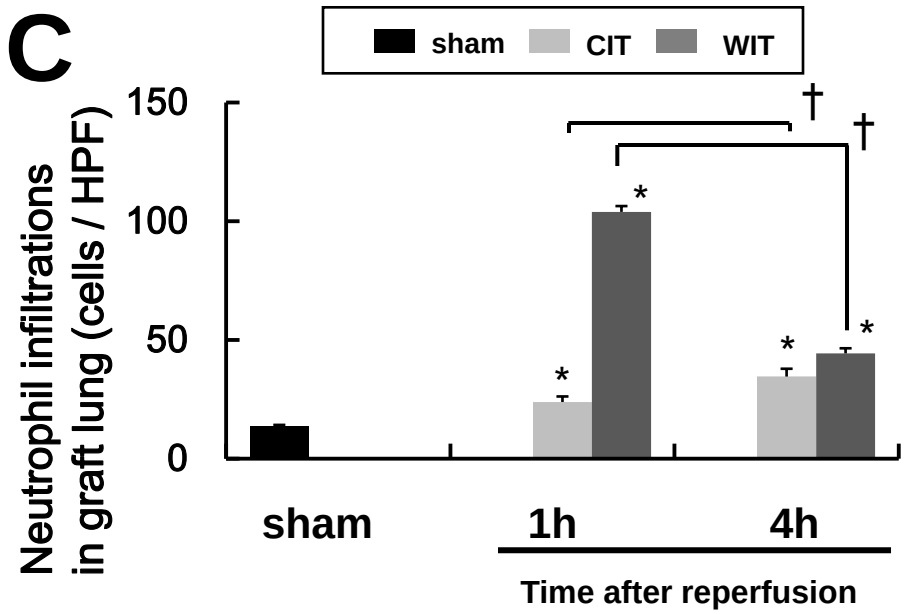

250-300 g rat anesthetized
Heparinized and put to death
CIT
WIT
Lung flushed with LPDG solution and retrieved
Stored at room temperature for 3hr
Stored at 4℃ for 3hr
Lung flushed with LPDG solution and retrieved
Left lung transplanted
Left lung transplanted
Total ischemic time: 4hr
1hr
4hr
1hr
4hr
Reperfusion
CIT-1hrRe: Assessed 1hr after reperfusion
WIT-1hrRe: Assessed 1hr after reperfusion
CIT-4hrRe: Assessed 4hr after reperfusion
WIT-4hrRe: Assessed 4hr after reperfusion

Figure 2


A
sham
CIT
WIT
(mmHg)
PaO2
800
700
600
500
400
300
200
100
0
‡
†
*
sham
1h
4h
Time after reperfusion


C
sham
CIT
WIT
Neutrophil infiltrations
in graft lung (cells / HPF)
150
100
50
0
†
†
*
*
*
*
*
sham
1h
4h
Time after reperfusion

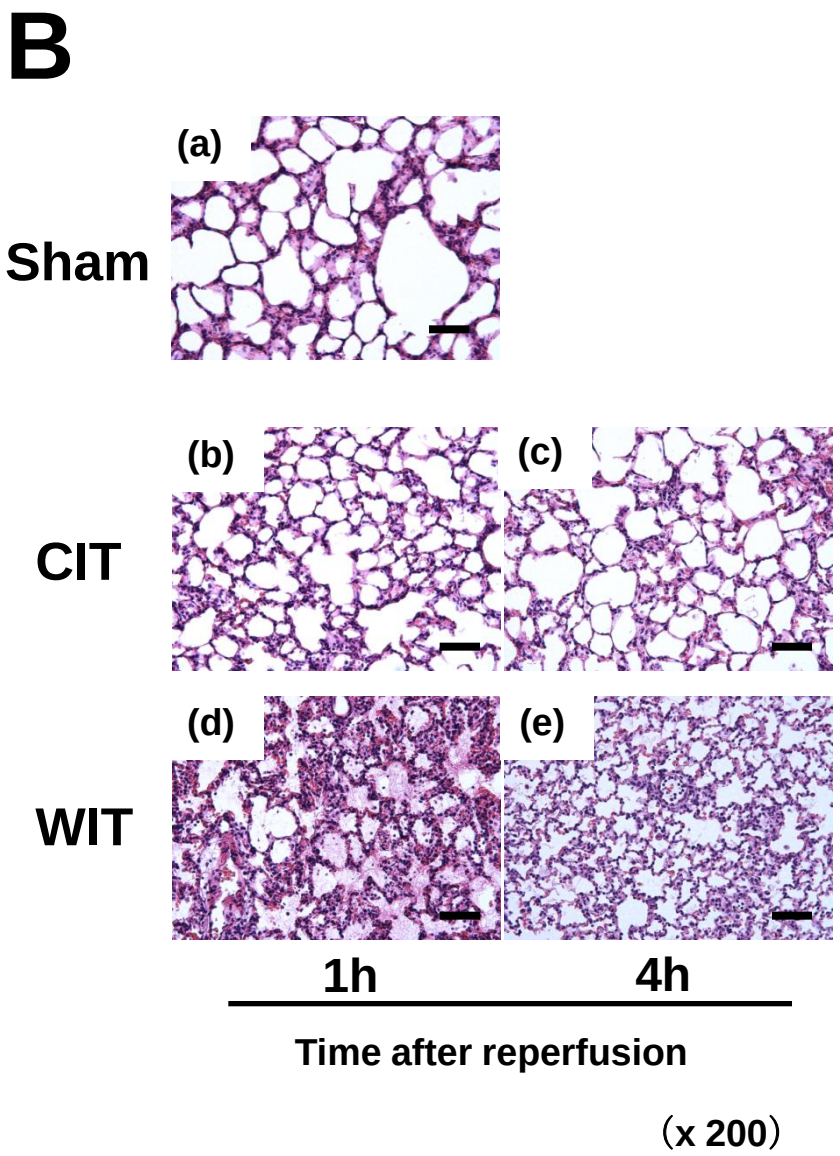

B
Sham
CIT
WIT
(a)
(b)
(c)
(d)
(e)
1h
4h
Time after reperfusion
(x 200)

Figure 3

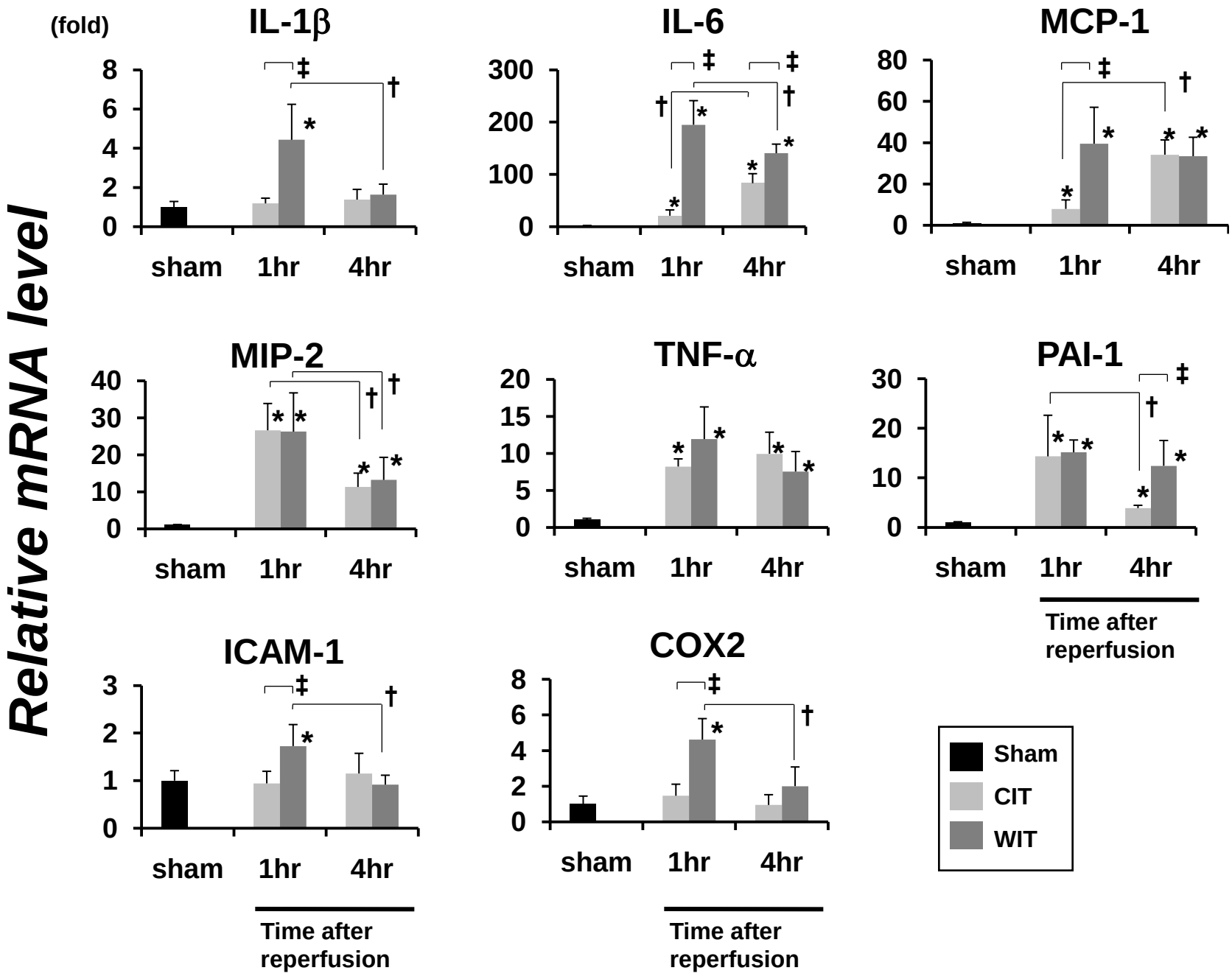
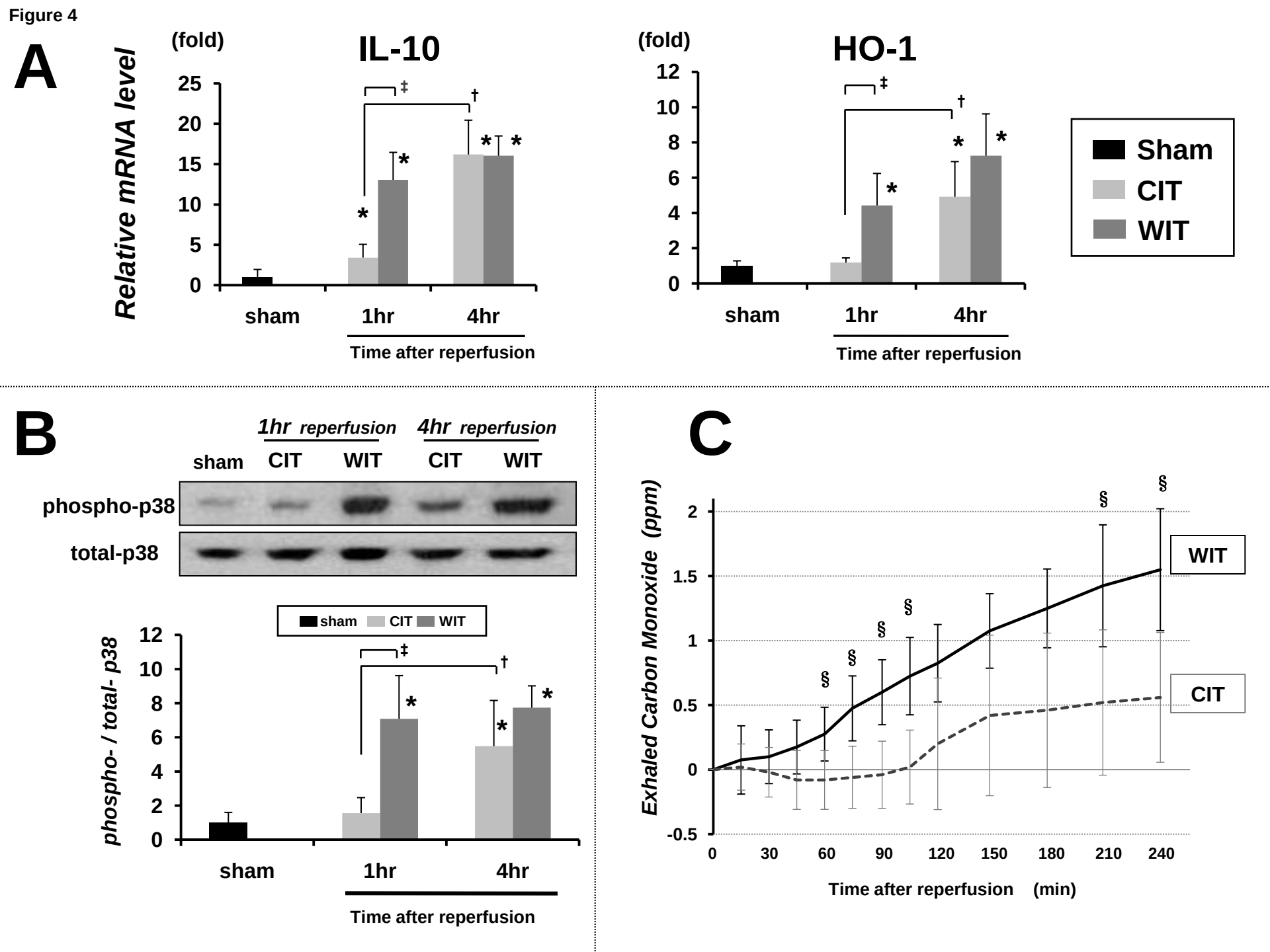

Relative mRNA level
(fold)
IL-1β
IL-6
MCP-1
MIP-2
TNF-α
PAI-1
ICAM-1
COX2
sham
1hr
4hr
Time after reperfusion
Sham
CIT
WIT

Figure 4


A
IL-10
(fold)
Relative mRNA level
25
20
15
10
5
0
sham
1hr
4hr
Time after reperfusion
HO-1
(fold)
12
10
8
6
4
2
0
Sham
CIT
WIT
B
1hr reperfusion
4hr reperfusion
sham
CIT
WIT
CIT
WIT
phospho-p38
total-p38
phospho- / total- p38
C
Exhaled Carbon Monoxide (ppm)
2
1.5
1
0.5
0
-0.5
0
30
60
90
120
150
180
210
240
Time after reperfusion (min)
WIT
CIT