PGE₁, dexamethasone, U-74389G, or Bt₂-cAMP as an additive to promote protection by UW solution in I/R injury

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DESPITE INTENSIVE STUDIES, none of the clinically applied lung preservation solutions permits reliable preservation of human lung allografts for longer than 6 h (6, 21). The susceptibility of lung tissue to ischemia-reperfusion (I/R) injury has made preservation of lungs more difficult than of other organs (15, 22). Theoretically, a reduction in I/R injury will increase preservation time and improve the early lung function. Therefore, we used a reduction of the I/R injury in lung as a criterion to evaluate the preservation solutions.

The University of Wisconsin solution (UW) was developed by Wahlberg and colleagues in 1986 and has made a major impact on the preservation of solid organs for transplantation (45). Experimental and clinical applications of UW expanded to pancreas, kidney, liver, and even the heart. Considering the experience of the Pittsburgh lung transplant group, UW is at least equivalent to the modified Euro-Collins solution used in lung transplantation (14). However, the optimal solution for the preservation of lung is still undefined. Although UW works as a preservation solution for lung, its attenuation effect on I/R injury has not been explored. To improve UW as a lung preservation solution, we attempted to enhance its attenuation effects by adding some protective agents. A modified UW solution containing various protective agents was tested for lung injury, and we found that certain modifications protected the rat lung from I/R injury.

Substantial literature shows that some of the commonly used protective agents such as prostaglandin E₁ (PGE₁), dexamethasone (Dex), dibutylryl-adenosine 3',5'-cyclic monophosphate (Bt₂-cAMP), and U-74389G (an experimental drug of the lazaroid class) have been postulated to exert a positive effect on the reduction of acute lung injury (5, 13, 18, 27, 28, 36). In this study, we hypothesized that modifications of UW solution by adding protective agents will enhance its protective effects from the acute I/R lung injury. A well-established I/R rat model (39, 46) was adapted to investigate the protective effect of UW and of the modified solutions of UW on I/R injury. From the results obtained, we demonstrate that UW can attenuate I/R injury and that the modified UW solutions can further enhance the protective effect of UW against I/R lung injury.

METHODS

Preparation of isolated and perfused rat lungs. The procedures to prepare isolated-perfused lung in situ in the chest were previously described (39, 46). Male Sprague-Dawley rats (250–350 g body wt) were anesthetized with pentobarbital sodium (20–25 mg ip). A tracheotomy was performed and permitted ventilation with a Harvard rodent ventilator (model 683) at 55 breaths/min, a tidal volume of 2.5 ml, and positive end-expiratory pressure of 2 cmH₂O. The inspired gas mixture was 5% CO₂-95% air. After median sternotomy was performed, heparin (1 unit/g body wt) was injected into the right ventricle. Blood was drawn from the right ventricle and discarded. A cannula was placed in the pulmonary artery through a puncture into the right ventricle, and tight ligature
was placed around the main trunk of the pulmonary artery. A large catheter was inserted into the left atrium through the left ventricle and mitral valve, fixed by ligature at the apex of the heart, and was used to divert pulmonary venous outflow into a reservoir. A third ligature was placed above the atrioventricular junction to prevent perfusate flow into the ventricles. The lungs were perfused by using a peristaltic pump (Minipuls 2; Gilson Medical Electronic, Middleton, WI) with various perfusates at a constant flow of 0.03 ml·g body wt·min⁻¹. The initial 75 ml of lactate Ringer solution perfusate, which contained residual blood cells and plasma, were discarded and not recirculated. An additional 25 ml of various perfusates were used for recirculation. Perfusion fluid was maintained at 25°C. Pulmonary arterial (Ppa) and pulmonary venous (Ppv) pressures were continuously monitored with pressure transducers (Statham P23 ID) from a sidearm of the inflow and outflow cannulas and recorded on a polygraph recorder (Gould Instruments, Cleveland, OH). The Ppv was set at 2.5 mmHg by adjusting the height of the venous reservoir. Zone 3 condition (arterial > venous > alveolar pressures) was maintained throughout all experiments. The isolated perfused lung remained in situ, and the weight of the rat was monitored on an electrical balance and recorded on an oscillograph after digital-to-analog conversion. The changes in body weight were measured as a result of changes in lung weight, according to the method of Wang et al. (46). In this study, the isolated lung preparation was selected based on the following three criteria: 1) no leakage observed at the sites of cannula insertion, 2) no evidence of edema in lung examined by observation, and 3) an isogravimetric state.

Perfusates. Several perfusates were used. 1) Physiological salt solution (PSS) contained 4% bovine serum albumin (Sigma Chemical, St. Louis, MO) and (in mM) 119 NaCl, 4.7 KCl, 1.17 MgSO₄, 22.6 NaHCO₃, 1.18 KH₂PO₄, 3.2 CaCl₂, and 5.5 glucose. 2) UW (DuPont-Merck Pharmaceuticals, Wilmington, DE) was composed of (in g/l) 50 pentastarch, 35.83 lactobionic acid, 3.4 potassium phosphate monobasic, 1.23 raffinose pentahydrate, 1.34 magnesium sulfate heptahydrate, 1.34 adenosine, 0.136 allopurinol, and 0.922 glutathione. Osmolarity was at 320 mosmol, including sodium concentration of 29 meq/l and that of potassium of 125 meq/l; pH was 7.4 (Table 1). 3) Four kinds of modified UW solutions were prepared as follows: UW+U-74389G (0.04 g/ml, a potent inhibitor of lipid peroxidation, kindly provided by Upjohn); UW+Dex (Sigma Chemical; 0.04 mg/ml); UW+B₂-cAMP (Sigma Chemical; 1 mg/l); and UW+PGE₁ (Sigma Chemical; 20 µg/l).

Determination of pulmonary capillary pressure (Ppc). The Ppc was estimated by using the double occlusion method (16). Arterial inflow and venous outflow lines were occluded simultaneously, and the equilibrium Ppa and Ppv were measured. This equilibration pressure is well correlated with isogravimetric measurements of Ppc and also reflects the prevailing capillary pressure when the lung is not isogravimetric.

Calculation of pulmonary vascular resistance. The pulmonary arterial (Ra) and venous resistances (Rv) were calculated from the following equations: Ra = (Ppa – Ppv)/Q˙; and Rv = (Ppc – Ppv)/Q˙, respectively, where Q˙ is flow.

Measurement of microvascular permeability. Pulmonary capillary filtration coefficient (Kfc) was used as an index of microvascular permeability to water. The Kfc was measured by using the method described previously (9). Briefly, after an isogravimetric period, Ppv was rapidly elevated to 6–8 cmH₂O for 15 min. The increase in lung weight was recorded, and a characteristic rapid weight gain (vascular filling) was followed by a slower rate of weight gain. The rate of weight change of (AWt/μt) during the 6- to 14-min interval was analyzed by using linear regression of the log₁₀-transformed rates of weight changes per minute. The initial rate of weight gain was calculated by using extrapolation of AWt/μt to time 0. Kfc was calculated by dividing ΔWt/Δt at time 0 by the changes in Ppc that occurred after venous outflow pressure was increased, normalized using the baseline wet lung weight, and expressed as milliliters per minute per centimeter H₂O per 100 g of lung tissue.

Measurement of protein concentration in lung lavage fluid. All experiments were terminated after 60 min of closed extracorporeal perfusion, and the lungs were removed and wet weights were measured. The lungs were lavaged twice with saline (2.5 ml/lavage). Lavage samples were centrifuged at 1,500 g in at room temperature for 10 min. The concentration of protein was determined as previously described (8).

Tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) assay. After experimentation, 2 ml of perfusate in the reservoir were drawn into two tubings and Ppv stored at −70°C until the assay was performed. Based on the enzyme-linked immunosorbent assay (ELISA), these collected perfusate samples and standards were determined by mouse TNF-α or IL-1β kit (Genzyme, Cambridge, MA), respectively. All samples were tested in duplicate. The assay was done as follows. First, a 96-well microtiter plate (precoated with monoclonal anti-TNF-α or IL-1β) was used to capture TNF-α or IL-1β in standards and test samples. After the plate was washed to remove unbound material, a peroxidase-conjugated polyclonal anti-TNF-α or IL-1β (horseradish peroxidase conjugate), which binds to captured TNF-α or IL-1β, was added. The plate was washed again. Then a substrate solution was added, which initiated a peroxidase-catalyzed color change that was subsequently stopped by acidification. The absorbance measured on an ELISA reader (Microplate Reader 450; Bio-Rad, CA) at 450 nm was proportional to the concentration of TNF-α or IL-1β present in the standards or samples. A standard curve was obtained by plotting the concentrations of TNF-α or IL-1β standards vs. their resulting absorbance. The TNF-α or IL-1β concentrations in experimental samples were then determined using the standard curve.

Experimental protocols. The studies were divided into seven groups. 1) PSS (no I/R injury) as negative control that showed no I/R injury (n = 6); 2) PSS as positive control displaying I/R injury (n = 5); 3) UW (n = 7); 4) UW+U-
Kfc. There was a significant increase in microvascular permeability due to I/R as shown by rise in Kfc (0.84 ± 0.19) at 60 min of reperfusion in the PSS control group. In comparison with the PSS group, UW and modified UW produced a significant reduction in microvascular permeability at 60 min after reperfusion, as shown by a lower Kfc (UW: 0.52 ± 0.12; UW + U-74389G: 0.32 ± 0.05; UW + Dex: 0.32 ± 0.05; UW + Bt2-cAMP: 0.35 ± 0.09; UW + PGE1: 0.34 ± 0.05; P < 0.05). A larger reduction of Kfc was found in modified UW groups than in the UW group (P < 0.05). However, no significant difference in Kfc was seen among the different modified UW groups (Table 2).

Hemodynamics. Before ischemia, both UW and modified UW groups (groups 4, 5, 6, and 7) had significantly higher baseline values of Ppa, Ppv, isogravimetric Ppc, Ra, and Rv than those of the PSS group. After I/R challenge, significant increases in Ppa, Ppv, isogravimetric Ppc, Ra, and Rv were found in the PSS group. In contrast, there was a significant decrease of Ppa and Ppv in the UW and modified UW groups (Table 3).

Protein content in the lavage fluid. In the PSS group, protein in the lavage fluid was markedly increased to 428 ± 222.7 mg/100 ml after 60 min of reperfusion. In UW perfusate group, the protein concentration was 50.5 ± 18.4 mg/100 ml, a significant eightfold decrease in protein concentration of the PSS group. Among the modified UW groups, the UW + U-74389G group showed a significantly lower protein concentration (30.4 ± 13.4 mg/100 ml) in the bronchoalveolar lavage (BAL) fluid. All others have a significantly lower concentration than PSS groups (Table 2).

Lung weight gains (LWG). In the PSS group, LWG was 8.41 ± 4.0 g at 60 min postreperfusion. After I/R challenge in the UW group, LWG was only 2.2 ± 1.00 g (P < 0.05) (Fig. 1). Among the modified UW groups, the LWG was as follows: UW + Dex: 0.54 ± 0.51 g; UW + U-74389G: 0.44 ± 0.58 g; UW + Bt2-cAMP: 0.75 ± 1.77 g; and UW + PGE1: 0.72 ± 0.75 g. These results revealed that the modified UW groups had a greater reduction of LWG than the UW perfusate group (Fig. 1).

Changes in TNF-α and IL-1β. TNF-α levels of each group after I/R are illustrated in Fig. 2. There was less TNF-α in the UW group than in the PSS group (70 vs. 278 pg/ml). Further reduction of TNF-α levels was noted in all modified UW groups: UW + U-74389G group, 30 pg/ml; UW + Dex group, 30 pg/ml; UW + PGE1 group, 41 pg/ml; and UW + Bt2-cAMP group, 43 pg/ml. The reduction of TNF-α levels in UW + U-74389G and UW + Dex groups was significant in comparison to the UW group (P < 0.05). There was a linear relationship between TNF-α and LWG (r = 0.9, P < 0.0001) as well as between TNF-α and Kfc (r = 0.8, P < 0.0001) (Figs. 3 and 4). In the test of IL-1β, no significant differences in concentration were noted in all groups (Table 4).

Histological findings. In the PSS group, profound inflammatory cells infiltrate into the perivascular regions and alveoli (Fig. 5A). The lung tissues obtained from the UW group contained many fewer inflammatory cells in the interstitium and alveoli (Fig. 5B). Lung tissues that appeared normal were seen in the modified UW groups (Fig. 5, C and D).

DISCUSSION

In this study, the UW perfusate showed a significant decrease in Ppa, Kfc, LWG, TNF-α, and protein content in the lavage fluid. Inflammatory cell infiltration in alveoli was decreased. Therefore, the UW perfusate solution has a protective effect on I/R injury. In addition, a further decrease of LWG, Kfc, and inflammatory cell infiltration was observed in all groups of modified UW. We conclude that PGE1, Dex, U-74389G, or Bt2-cAMP added to UW solution gave better protection from I/R injury than did UW alone. In the UW + U-74389G group, BAL contained lower protein concentration. Using the reduction in I/R injury as an impor-

Table 2. Kfc values and protein concentrations of lavage fluid

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Kfc Baseline</th>
<th>Kfc After I/R</th>
<th>Protein in Lavage Fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: PSS (no I/R)</td>
<td>6</td>
<td>0.35 ± 0.05</td>
<td>0.37 ± 0.04</td>
<td>Not detected</td>
</tr>
<tr>
<td>2: PSS</td>
<td>6</td>
<td>0.42 ± 0.03</td>
<td>0.84 ± 0.19</td>
<td>428.0 ± 222.7</td>
</tr>
<tr>
<td>3: UW</td>
<td>7</td>
<td>0.41 ± 0.06</td>
<td>0.52 ± 0.12</td>
<td>50.5 ± 14.98</td>
</tr>
<tr>
<td>4: UW + U-74389G</td>
<td>5</td>
<td>0.27 ± 0.05</td>
<td>0.32 ± 0.05</td>
<td>34.0 ± 13.44</td>
</tr>
<tr>
<td>5: UW + Dex</td>
<td>5</td>
<td>0.29 ± 0.05</td>
<td>0.32 ± 0.05</td>
<td>37.4 ± 12.61</td>
</tr>
<tr>
<td>6: UW + Bt2-cAMP</td>
<td>5</td>
<td>0.29 ± 0.05</td>
<td>0.35 ± 0.09</td>
<td>32.5 ± 7.14</td>
</tr>
<tr>
<td>7: UW + PGE1</td>
<td>5</td>
<td>0.27 ± 0.05</td>
<td>0.34 ± 0.05</td>
<td>42.3 ± 20.67</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of rats. Capillary filtration coefficient (Kfc) is in ml min⁻¹ cmH₂O⁻¹ 100 g lung wt⁻¹; unit of protein concentration is mg/100 ml. PSS, physiological salt solution; I/R, ischemia-reperfusion; UW, University of Wisconsin solution; Dex, dexamethasone; Bt2-cAMP, dibutyryl adenosine 3',5'-cyclic monophosphate; PGE1, prostaglandin E1. *P < 0.05 compared with PSS; †P < 0.05 compared with baseline; ‡P < 0.05 compared with group 2; §P < 0.05 compared with UW group.
tant criterion of lung preservation, we found that U-74389 UW is the best solution.

At initial experimentation, the rat lungs were flushed with 75 ml of lactate Ringer solution under zone 3 conditions and $1 \times 10^5$ leukocytes/ml found in perfusate. Hence, the initial concentrations of leukocytes in all closed-perfusion circuits were the same; therefore, the variations in levels of I/R injury present in different groups of perfusate were not due to initial concentrations of cells. A marked leukocyte infiltration into the interstitial and perivascular regions in the PSS group was indicating that isolated lungs contained leukocytes and that these leukocytes played a role in I/R lung injury. Compared with changes observed in leukocyte infiltration in various groups of perfusates, we found that in the UW and modified UW groups many fewer circulating leukocytes were recruited into the lung.

![Fig. 1. Changes of lung weight gains (LWG). In physiological salt solution (PSS) group, LWG was markedly increased during reperfusion. After ischemia-reperfusion (I/R) challenge, LWG in University of Wisconsin solution (UW) group was significantly less than that in PSS group (P < 0.05). Modified UW groups showed further reduction in LWG (P < 0.05). PGE1, prostaglandin E1; Dex, dexamethasone; Bt2-cAMP, dibutyryl adenosine 3',5'-cyclic monophosphate; PSSC, PSS control.](#)

![Fig. 2. Tumor necrosis factor-α (TNF-α) concentration after I/R challenge in various groups. There is less TNF-α in UW group than in PSS group. Further reduction of TNF-α levels was noted in modified UW groups (UW+U-74389G group, UW+Dex group, UW+PGE1 group, and UW+Bt2-cAMP group). Reduction of TNF-α release in UW+U-74389G and UW+Dex was significant compared with UW solution (P < 0.05).](#)

### Table 3. Hemodynamics

<table>
<thead>
<tr>
<th>Group</th>
<th>Ppa (mmHg)</th>
<th>Ppv (mmHg)</th>
<th>Ppci (mmHg)</th>
<th>Ra (cmHg·min·ml⁻¹)</th>
<th>Rv (cmHg·min·ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before ischemia (baseline)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1: PSS (no I/R)</td>
<td>17.70 ± 0.97</td>
<td>2.80 ± 0.27</td>
<td>9.43 ± 0.24</td>
<td>0.98 ± 0.08</td>
<td>0.77 ± 0.06</td>
</tr>
<tr>
<td>2: PSS</td>
<td>18.70 ± 4.10</td>
<td>4.40 ± 1.78</td>
<td>10.69 ± 2.69</td>
<td>0.94 ± 0.18</td>
<td>0.74 ± 0.14</td>
</tr>
<tr>
<td>3: UW</td>
<td>28.30 ± 3.01*</td>
<td>2.10 ± 0.55</td>
<td>13.63 ± 1.38*</td>
<td>1.72 ± 0.20*</td>
<td>1.36 ± 0.16*</td>
</tr>
<tr>
<td>4: UW + U-74389G</td>
<td>27.50 ± 3.20*</td>
<td>1.40 ± 0.65</td>
<td>12.88 ± 1.49*</td>
<td>2.32 ± 1.35*</td>
<td>1.35 ± 0.17*</td>
</tr>
<tr>
<td>5: UW + Dex</td>
<td>27.30 ± 2.66*</td>
<td>2.30 ± 0.57</td>
<td>12.79 ± 0.48*</td>
<td>1.65 ± 0.20*</td>
<td>1.30 ± 0.16*</td>
</tr>
<tr>
<td>6: UW + Bt2-cAMP</td>
<td>28.50 ± 0.50</td>
<td>2.30 ± 0.45</td>
<td>13.63 ± 0.42*</td>
<td>1.72 ± 0.03*</td>
<td>1.36 ± 0.02*</td>
</tr>
<tr>
<td>7: UW + PGE1</td>
<td>29.80 ± 2.25*</td>
<td>2.40 ± 0.42</td>
<td>14.46 ± 0.81*</td>
<td>1.80 ± 0.17*</td>
<td>1.42 ± 0.13*</td>
</tr>
<tr>
<td>After I/R</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1: PSS (no I/R)</td>
<td>18.60 ± 1.64</td>
<td>1.80 ± 0.57</td>
<td>10.22 ± 0.81</td>
<td>1.04 ± 0.09</td>
<td>0.82 ± 0.07</td>
</tr>
<tr>
<td>2: PSS</td>
<td>27.00 ± 6.47†</td>
<td>4.30 ± 1.79</td>
<td>14.29 ± 3.25†</td>
<td>1.50 ± 0.41†</td>
<td>1.13 ± 0.38†</td>
</tr>
<tr>
<td>3: UW</td>
<td>26.10 ± 2.90†</td>
<td>2.20 ± 0.57</td>
<td>12.72 ± 1.45†</td>
<td>1.62 ± 0.26†</td>
<td>1.20 ± 0.12†</td>
</tr>
<tr>
<td>4: UW + U-74389G</td>
<td>24.00 ± 1.70†</td>
<td>1.50 ± 0.87</td>
<td>11.04 ± 0.82†</td>
<td>1.49 ± 0.09†</td>
<td>1.16 ± 0.07†</td>
</tr>
<tr>
<td>5: UW + Dex</td>
<td>23.80 ± 2.41‡</td>
<td>2.10 ± 0.74</td>
<td>11.47 ± 0.39‡</td>
<td>1.43 ± 0.21‡</td>
<td>1.12 ± 0.16†</td>
</tr>
<tr>
<td>6: UW + Bt2-cAMP</td>
<td>24.50 ± 1.00‡</td>
<td>2.20 ± 1.10</td>
<td>12.01 ± 1.10‡</td>
<td>1.54 ± 0.08‡</td>
<td>1.15 ± 0.04‡</td>
</tr>
<tr>
<td>7: UW + PGE1</td>
<td>25.90 ± 2.46‡</td>
<td>2.40 ± 1.19</td>
<td>12.74 ± 1.34‡</td>
<td>1.55 ± 0.17‡</td>
<td>1.21 ± 0.14‡</td>
</tr>
</tbody>
</table>

Values are means ± SD; Ppa and Ppv (mmHg), pulmonary arterial and venous pressure, respectively; Ppci (mmHg), isogravimetric capillary pressure; Ra and Rv (cmHg·min·ml⁻¹), arterial and venous resistance, respectively. *P < 0.05 compared with PSS (no I/R); †P < 0.05; ‡P < 0.001 compared with baseline.
This was due to reduction in leukocyte adherence to the capillary and migration to interstitial tissues and alveoli; therefore, less microvascular injury was seen (35).

Higher baseline values of Ppa were found in both the standard and modified UW solutions than in the PSS group. A high potassium concentration (125 meq/l in UW vs. 4.7 meq/l in PSS groups) in the UW may be responsible for the increased peripheral vascular resistance and Ppa during pulmonary arterial flush. It is well known that a high extracellular potassium concentration depolarizes smooth muscle cell membranes and causes smooth muscle contraction (32). After I/R, a significant increase in pulmonary vascular pressure and resistance was found, but not in Ppv, in the PSS. This was reportedly due to leukocyte aggregation and adherence to the capillary (35) and to released vasoactive substance. We suggest in this report that a significant decrease of pulmonary pressure and resistance observed in UW and modified UW groups resulted from a reduction of adherence and recruiting leukocytes to lung tissue. The pathological findings were also indicating less leukocyte migration.

The baseline values of Kfc in modified UW groups were lower than those in UW, but no significant difference of baseline Ppa existed in UW and modified UW. It suggests that protective agents added to UW decreased Kfc by altering permeability, not by changing surface area of pulmonary capillary, which resulted from vasoactive effects.

The exact mechanism of UW attenuation of I/R injury to the lung remains unclear. Definitely, the UW solution contains protective substances that prevent I/R injury. Other investigators (3, 40) have suggested that lactobionate and raffinose in UW are osmotically active nonmetabolic impermeants and that they suppress cellular swelling. Glutathione and allopurinol prevent and reduce cytotoxic injury from oxygen free radicals (3). In addition, adenosine provides the substrate for cell to regenerate ATP during reperfusion after cold storage (40). Previous studies have shown that the increased microvascular permeability associated with I/R can be reversed by cAMP (36). The mechanism by which Bt2-cAMP exerts beneficial effects in animal models of I/R lung injury and decreases microvascular permeability is not clearly known. A possible explanation is that the cytoskeleton altered the endothelial cells in vessels that produced tighter intercellular junctions (23, 38). Goodman et al. (12) have suggested an activated ionic transport from air space to interstitium, which contributes to the beneficial effect of Bt2-cAMP by removing edema fluid from the air space. In this study, we demonstrated that Bt2-cAMP modified the TNF-α release in UW perfusate and suggested that the mechanism of I/R attenuation is due to a multiple actions of Bt2-cAMP, including the inhibition of TNF-α production.

With the exception of the vasodilating effect (26, 30), PGE1 has a bronchodilating effect (29), inhibits aggregation of platelets and leukocytes (30, 46), suppresses
TNF-α production (23), has immunosuppressive effects (20, 23, 42), and has a variety of “cytoprotective” effects (10, 11, 34). However, the exact action that is responsible for amelioration of I/R injury is not clearly known.

Recently, a novel series of 21-aminosteroids (lazaroids), which are potent inhibitors of iron-dependent lipid peroxidation, has been developed (4). These agents have proven very effective in protecting tissues from ischemic damage. Our results, similar to others (2, 5, 16), showed that U-74389G enhanced UW preservation solution to protect against I/R injury. In this study, we demonstrated that U-74389G enhanced the UW by inhibiting TNF-α production and suggest that U-74389G in preservation solution was not only preventing lipid peroxidation but also suppressing TNF-α-mediated inflammation. TNF-α has been expressed in acute lung injury, reminiscent of acute respiratory distress syndrome in many animal models (41, 43). TNF-α was shown to induce the expression of both intercellular cell adhesion molecule-1 and endothelial leukocyte adhesion molecule-1 (26, 49) and to mediate polymorphonuclear neutrophil attachment to endothelial cells. Adherence of polymorphonuclear neutrophil to endothelial cells results in release of neutrophil-derived oxygen metabolites, which leads to vascular and tissue injury (1, 17, 19). The elevation of TNF-α during reperfusion after ischemia was accompanied by severe lung I/R injury in the PSS group. Similar results were also demonstrated by others in I/R injury of liver (7) and lung (33). Palace et al. (33) have suggested that neutrophil sequestration resulting in lung injury after reperfusion is dependent on generation of TNF-α. In contrast, studies of Serrick et al. (37) showed no elevation of TNF-α in a lung autograft animal model. The role of TNF-α in I/R injury is clear. This study demonstrated that the UW solution produced less TNF-α than the control group; the TNF-α levels revealed a significant correlation to the changes of LWG and Ktc, suggesting that TNF-α production was associated with the severity of lung injury.

In conclusion, the animal model used to measure I/R lung injury in our studies is simple, reliable, and inexpensive (39, 46). This method provides an effective way to screen solutions that would be best to use in lung transplantation.

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