Effect of the Free Radical Scavenger MCI-186 on Pulmonary Ischemia–Reperfusion Injury in Dogs

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Background: Free radical scavengers and superoxide dismutase have been found to protect against cerebral ischemic damage, and it was suggested that oxygen free radicals contribute to ischemia–reperfusion injury induced by cerebral ischemic damage. MCI-186 (3-methyl-1-phenyl-2-pyrazolin-5-one) is a potent scavenger and inhibitor of hydroxyl radicals and protective agent of peroxidative injury. The purpose of this study was to evaluate the effects of MCI-186 on pulmonary ischemia–reperfusion injury in a simulated transplanted lung model.

Methods: Fourteen dogs were divided into two groups ($n = 7$ each). In the MCI group, MCI-186 was continuously administered at 3 mg/kg/hour intravenously (IV) from 30 minutes before reperfusion until 30 minutes after reperfusion (total administration time 1 hour). Vehicle was administered in the control group. Warm ischemia was induced for 3 hours by clamping the left pulmonary artery and veins. Simultaneously, the left stem bronchus was bisected and then anastomosed before reperfusion. The right pulmonary artery was ligated 15 minutes after reperfusion, and the right stem bronchus was then bisected.

Results: The respiratory gas exchange, hemodynamic changes, wet-to-dry weight ratio (WDR) and malondialdehyde (MDA) concentration in the tissue were significantly improved ($p < 0.05$) in the MCI group. The histologic damage was more severe in the control group and polymorphonuclear neutrophil (PMN) infiltration was reduced in the MCI group.

Conclusion: MCI-186 has a protective effect on pulmonary ischemia–reperfusion injury through the inhibition of lipid peroxidation. J Heart Lung Transplant 2006;25:965–71. Copyright © 2006 by the International Society for Heart and Lung Transplantation.

Protecting against ischemia–reperfusion (I/R) injury is important for improving the results of organ transplantation. Several reports have suggested that pulmonary edema after lung transplantation is related to I/R injury.[1–4]

Reactive oxygen species (ROS) are known to be produced in excess following reperfusion, negatively affecting lipids, DNA and protein, and inducing cellular dysfunction or cell death.[5] ROS are strictly controlled within a normal range by free radical elimination systems, such as superoxide dismutase (SOD), catalase and glutathione peroxidase, which have evolved to maintain low steady-state levels of intracellular O_2 and H_2O_2 under normoxic conditions.[6] However, in cases of I/R injury, such as ischemic heart disease, cerebral infarction, thrombosis or transplantation, excessive free radicals are rapidly produced immediately after the reperfusion phase.

According to McCord,[7] the depletion of cellular ATP during ischemia results in an elevated concentration of AMP, which is catabolized to adenosine, inosine and then to hypoxanthine. The reduction of ATP in the tissues accelerates the permeability changes of the cell membranes. At the time of reperfusion, xanthine dehydrogenase is changed into xanthine oxidase by activated protease.[8] Consequently, a burst of superoxide radicals is produced with hypoxanthine and xanthine oxidase. In another pathway of superoxide production, activated neutrophils release free radicals through membrane-bound NADPH oxidase during reperfusion.[9] This is thought to be the most common pathway for I/R injury. Furthermore, free radicals, such as the hydroxyl radical (·OH), are generated from arachidonic acid released from the cell membrane during ischemia. These radicals are formed during the conversion of

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hydroperoxyeicosatetraenoic acid (HPETE) to hydroxyeicosatetraenoic acid (HETE) in the arachidonic acid cascade, and it is speculated that any type of HPETE can release ROS, which are probably OH radicals, during the conversion to HETE.[10,11]

The free radicals generated by these pathways cause severe damage to local tissues and systemic organs[7,12] through lipid peroxidation on the cell membranes, and cause increases in the affinity between neutrophils and endothelial cells through the adhesion molecules expressed on the surface of the activated endothelium.[13] The microcirculation is injured under this condition, and pulmonary edema is promoted. Free radical scavengers, SOD, catalase[14] and lazaroid,[15,16] have been found to be effective at preventing tissue damage from I/R injuries. Free radicals and lipid peroxide play a key role in cerebral damage during an early ischemic phase.[17–19]

The free radical scavenger MCI-186 (3-methyl-1-phenyl-2-pyrazolin-5-one) has been tested in I/R animal studies and was found to be beneficial in cerebral,[20–22] myocardial[23] and kidney[24,25] models. MCI-186 was also recently evaluated in liver models and found to be beneficial.[26–28] However, there are no reports on pulmonary I/R injury. Therefore, we evaluated the effects of MCI-186 on I/R injury in the canine simulated transplanted lung model.

MATERIALS AND METHODS

Animals and Operative Procedure

For this study, 14 dogs, weighing 13 to 16 kg, were divided into two groups ($n = 7$ each). In the MCI group, 3 mg/kg/hour intravenously (IV) of MCI-186 was continuously administered from 30 minutes before reperfusion until 30 minutes after reperfusion (total administration time 1 hour). It has been reported that the serum concentration of MCI-186 rapidly increased during the first 30 minutes and then reached a plateau until the infusion was stopped.[29,30] In the control group, vehicle was administered in the same manner. The animals were anesthetized with pentobarbital sodium (15 mg/kg) and pancuronium bromide (0.2 mg/kg) after administration of ketamine hydrochloride (2 mg/kg) by intramuscular injection. The animals were then intubated and mechanically ventilated at a tidal volume of 20 ml/kg and a rate of 12 breaths/min. Positive end-expiratory pressure (PEEP) was maintained at 5.0 cm H_2O, and the inspired o_2 fraction (Fio_2) was 1.0. Anesthesia was maintained by inhalation of 1% to 2% isoflurane. Muscular relaxation was obtained with additional pancuronium bromide (0.1 mg/kg). An arterial line was inserted into the left carotid artery to monitor the blood pressure and blood gases, and another catheter was inserted into the left atrium through an appendage to monitor the atrial pressure. A Swan–Ganz catheter was inserted into the left jugular vein to monitor the left pulmonary arterial pressure (L-PAP) and was used as a venous infusion line.

After a left thoracotomy at the fifth intercostal space, we performed hilar stripping of the left lung, the right stem bronchus and the right pulmonary artery. The nerves, bronchial arteries and lymphatics were completely transected. After administration of sodium heparin (100 U/kg), a 5-minute clamping test was performed by occluding the right pulmonary artery and the right stem bronchus with vascular clamps. Warm ischemia was then induced for 3 hours by clamping the left pulmonary artery and veins. Simultaneously, the left stem bronchus was bisected and then anastomosed before reperfusion. The right pulmonary artery was ligated 15 minutes after reperfusion, and the right stem bronchus was then bisected. Simultaneously, the tidal volume decreased to 10 ml/kg, and the respiratory rate increased to 20 breaths/min. Without ligating the right pulmonary artery after reperfusion, most blood would flow into the right pulmonary artery because pulmonary vascular resistance increases remarkably after reperfusion. Because we sought to evaluate the gas-exchange capacity and hemodynamics of the injured left lung only after warm I/R, we ligated the right pulmonary artery and bronchus after reperfusion. To avoid the sudden change in hemodynamics just after reperfusion, we ligated the right pulmonary artery at 15 minutes after reperfusion. In addition, we evaluated the function of the left lung only before ischemia; therefore, we isolated the right lung and performed the 5-minute clamping test. For the end-point of this study, blood samples and lung specimens were taken after 4 hours of reperfusion.

All animals 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*, published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996). This study was performed with the approval of the Animal Care and Experimental Committee, Gunma University, Showa Campus, Japan.

MCI-186

MCI-186 (Mitsubishi Pharma Corp., Osaka, Japan) was developed as a free radical scavenger for the amelioration of symptoms associated with acute stroke. The molecular weight of MCI-186 is 174.20, and its chemical structure is 3-methyl-1-phenyl-2-pyrazolin-5-one.

Measurement of Blood Gases

Arterial blood samples were taken to analyze blood gases before ischemia during the 5-minute clamping test and at 30, 60, 120, 180 and 240 minutes after