

Hepatocyte Growth Factor Prevents Pulmonary Ischemia–Reperfusion Injury in Mice

Akiko Makiuchi, MD,^a Kazuhiro Yamaura, MD, PhD,^a Shinya Mizuno, PhD,^b Kunio Matsumoto, PhD,^b Toshikazu Nakamura, PhD,^b Jun Amano, MD, PhD,^a and Ken-ichi Ito, MD, PhD^a

Background: Ischemia-reperfusion (IR) injury after lung transplantation leads to significant morbidity and mortality in recipients, which remains the major obstacle in clinical lung transplantation. To reduce pulmonary graft dysfunction and improve prognosis after lung transplantation, prevention of IR-induced lung injury in the peri-operative period is required. In the present study, we investigated the effects of recombinant hepatocyte growth factor (HGF) on pulmonary IR injury using a murine model system.

Methods: To assess the protective effect of HGF against lung injury, mice with pulmonary IR were divided into two groups and injected with 500 µg/kg of human recombinant HGF or the same dose of saline alone as a control.

Results: After pulmonary IR injury, the lung injury score increased in a time-dependent manner up to 24 hours. A significant reduction of lung injury score was observed with the administration of exogenous HGF. Moreover, the ratio of apoptotic cells was significantly reduced in mice treated with HGF. Significantly increased expression of Bcl-xL was observed after IR in mice administered HGF as compared with saline-treated controls. In contrast, expression of Bax was reduced significantly in HGF-treated mice. Serum levels of endogenous murine HGF were increased significantly in HGF-treated mice.

Conclusions: Our findings indicate that administration of exogenous HGF ameliorates the pulmonary tissue injury induced by IR, which may provide an alternative for prevention of IR-induced lung injury in the peri-operative period in lung transplantation. *J Heart Lung Transplant* 2007;26:935–43. Copyright © 2007 by the International Society for Heart and Lung Transplantation.

Despite refinements in lung preservation and improvements in surgical techniques and peri-operative care, ischemia-reperfusion (IR)-induced lung injury remains the main cause of primary graft failure after lung transplantation.^{1–3} In addition to being a predictive factor of significant morbidity and mortality in the early post-operative period, severe IR injury has been reported to be an independent predictive factor for both development and progression of bronchiolitis obliterans syndrome (BOS), which is the most common cause of late death after lung transplantation.^{1,4} Previous studies have demonstrated that IR injury involves neu-

trophil infiltration into lung tissue that can damage the surrounding parenchyma, which can result in severe acute pulmonary dysfunction in the early post-operative period.^{5–7} On the other hand, IR injury after lung transplantation has been shown to be associated with the up-regulation of various cytokines, which contribute to the induction of an inflammatory response in lung allografts.^{8,9} To reduce pulmonary graft dysfunction and improve prognosis after lung transplantation, prevention of IR-induced lung injury in the peri-operative period is thought to be essential.

Hepatocyte growth factor (HGF), which was initially detected as a potent mitogen for mature hepatocytes,^{10–12} is a pleiotropic factor, exerting mitogenic, tubulogenic and anti-apoptotic activities over a wide spectrum of target cells with signaling via the HGF receptor c-Met.^{13–15} The pleiotropic properties of HGF render it specifically suited to promoting tissue repair and regeneration after injury.^{16–18} In some organs, HGF has been shown to protect the tissues from IR injury by reducing apoptosis.^{19–22} Recently, we demonstrated that administration of HGF can induce significant reduction of apoptosis in cardiomyocytes, and can then prolong survival in cardiac allografts in a murine model.²³ In addition, HGF was reported to be useful as an

From the ^aDepartment of Surgery (II), Shinshu University School of Medicine, Matsumoto, Japan; and ^bDivision of Biochemistry, Biomedical Research Center, Osaka University Graduate School of Medicine, Suita, Japan.

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Reprint requests: Ken-ichi Ito, MD, Department of Surgery (II), Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390-8621, Japan. Telephone: +81-263-37-2657. Fax: +81-263-37-2721. E-mail: kenito@hsp.md.shinshu-u.ac.jp

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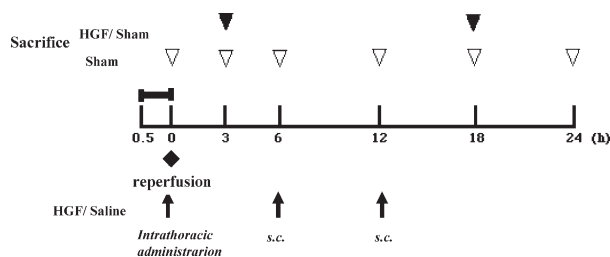


Figure 1. Schematic illustration of experimental design. Black arrows indicate the timing of administration of HGF or normal saline. White and black arrowheads indicate the time-points when mice in the sham-operated, saline-treated and HGF-treated groups were euthanized after de-clamping of the left hilum, respectively.

indicator for heart graft cell rejection.²⁴ HGF also acts as a potent multifunctional pulmotrophic factor in injured lung tissue^{25–29} and enhances tissue remodeling via improvement of blood perfusion.^{30–34} With regard to IR injury of the lung, endogenous HGF has been shown to be induced in the ischemia-reperfused lung and may play an important role in regeneration of the injured lung.²⁷ However, there have been no previous studies to examine the effects of exogenous HGF on pulmonary IR injury.

In the present study, to evaluate the preventive effect of HGF against pulmonary IR injury, we investigated the effects of human recombinant HGF on pulmonary IR injury using a murine model system and evaluated whether HGF plays a prominent role in prevention of lung tissue damage after pulmonary IR as an anti-apoptotic factor.

METHODS

Experimental Animals

Female ICR mice (Charles River Japan, Inc., Yokohama, Japan), weighing 30 to 35 g, were used in this study. The animals were kept under standard conditions and fed ordinary pellet diet and tap water *ad libitum*. 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*, prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1996).

Pulmonary IR Model

A left lateral thoracotomy was performed in the fifth intercostal space, and the left hilum was exposed and clamped for 30 minutes using a microvascular clip. After implementation of ischemia, the left lung was reperfused by de-clamping of the left hilum. The thoracic incision was closed in two layers and the mouse was extubated after restoration of spontaneous breath-

ing. The sham-operated mice underwent a simple left thoracotomy in which the left hilum was not clamped.

Recombinant HGF Treatment

Human recombinant HGF (hrHGF) was purified as described previously.³⁵ To assess the protective effect of HGF against lung injury, mice with pulmonary IR were divided randomly into two groups and injected with 500 µg/kg of hrHGF dissolved in 0.1 ml of saline or the same dose of saline alone as a control. The first administration was performed intra-thoracically just before de-clamping, and then subcutaneously at 6 hours and 12 hours after de-clamping of the left hilum. The mice were euthanized at 3 hours and 18 hours after de-clamping. Each experimental group consisted of five mice. The time courses of the three groups (sham-operated, HGF-treated and saline-treated groups) are shown in Figure 1.

Measurement of HGF in Plasma

Blood samples were obtained from mice at 3 hours and 18 hours after de-clamping of the left hilum in each group. Plasma HGF concentrations were measured using enzyme-linked immunosorbent assay (ELISA) kits for human HGF and rodent HGF (Institute of Immunology, Tokyo, Japan) according to the protocol supplied by the manufacturer. All the samples were assayed at least in duplicate.

Quantitative Evaluation of Lung Injury After Pulmonary IR

Lung tissues were fixed in 70% ethanol at 4°C for 12 hours, then embedded in paraffin. Each section was cut to a thickness of 4 µm and stained with hematoxylin-eosin. For quantitative histopathologic analysis of lung injury, a scoring system was adopted based on the following four criteria: (1) pulmonary edema; (2) in-

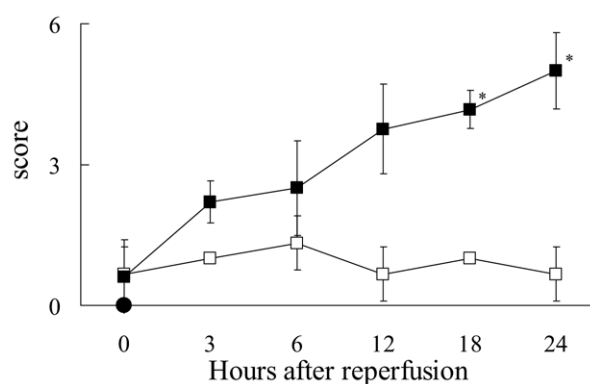


Figure 2. Time courses of changes in the injury scores of the lung tissues after IR injury in sham-operated (white squares) or IR (black squares) mice (black circle: naive). The left lungs of the mice were harvested at each time-point after reperfusion and were evaluated for the injury scores (* $p < 0.05$).

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