Introduction

Acute lung injury (ALI), which is called as acute respiratory distress syndrome (ARDS) in its most severe clinical manifestation, affects around 150,000 patients per year in the U.S, with recent mortality rates being over 30 percent\(^1,2\). At present, there is no effective treatment for ALI except the low tidal volume ventilation, which is rather a way of reducing artificially induced ventilation–associated lung injury (VLI) than an active, specific way of treatment. ALI is characterized by neutrophil accumulation in the lungs, interstitial edema, disruption of epithelial integrity, and leakage of protein into the alveolar space\(^3-6\). Infection, associated with endotoxemia, and blood loss are frequent predisposing factors to the development of ALI. In experimental settings, endotoxemia or hemorrhage produces ALI\(^7\). Neutrophils, which play a central role in the ALI, produce proinflammatory mediators, including...
cytokines such as tumor necrosis factor α (TNF-α) and macrophage inflammatory peptide-2 (MIP-2) and demonstrate increased activation of transcriptional regulatory factors including cyclic adenosine monophosphate (cAMP) response element binding protein (CREB) and nuclear factor kappa B (NF-κB)\(^8\)-\(^10\).

Heparin presents a dazzling array of properties. In lung disease, it is used as an anticoagulant for thromboembolism, but its polyanionic nature confers a wide variety of other actions not related to anticoagulation\(^11\). Heparin is a potent antiinflammatory agent that inhibits neutrophil-derived elastase\(^12\), complement activation\(^13\), tumor necrosis factor-induced lung edema\(^14\), L- and P-selectins\(^15\), leukocyte rolling\(^16\) and neutrophil-induced injury of pulmonary alveolar epithelium\(^17\). However, therapeutic potential of heparin as an antiinflammatory treatment in lung injury is limited by its inherent anticoagulant activity. Attachment of heparin to antithrombin III and some other proteins is critically dependent on binding energies conferred by specific saccharide sequences or charged side groups, and anticoagulant activity can be removed from heparin by partial chemical desulfation\(^18\),\(^19\). However, removal of sulfates may have variable effects on other heparin-related activities, which appear to be related to simple charge neutralization of cationic proteins by the anionic polysaccharide\(^11\),\(^14\). This change often results in the loss of important pharmacological activities. Lyophilization of porcine mucosal heparin under extreme alkaline conditions (pH>13) produces a nonanticoagulant heparin (NCH) remarkable for the selective loss of only 2-O and 3-O sulfates, leaving 6-O and N-sulfates intact. Selectively O-desulfated heparin retains potent activity as an inhibitor of neutrophil protease, elastase and cathepsin G\(^18\). It also inhibited translocation of the NF-κB from the cytoplasm to the nucleus in human endothelial cells and attenuated myocardial reperfusion injury\(^20\). It is plausible that NCH could show therapeutic effects on inflammatory diseases of lung. However, as far as authors have searched, there has been no report which evaluated the therapeutic effects of NCH on animal models of the acute lung injury.

In the present study, we evaluated the therapeutic effects of selectively O-desulfated heparin on the mouse model of acute lung injury developed by endotoxemia or hemorrhage.

**Materials and Methods**

**Mice.** Male BALB/c mice, 8–12 week of age, were purchased from Harlan Sprague Dawley (Indianapolis, IN). The mice were kept on a 12:12-hour, light–dark cycle with free access to food and water. At least five mice were used for each experimental group. All experiments were conducted in accordance with institutional review board-approved protocols.

**Materials.** Nonanticoagulant heparin (2-O,3-O desulfated porcine intestinal heparin, NCH) was kindly donated by Dr. Thomas Kennedy (University of North Carolina, Chapel Hill, NC, USA). *Escherichia coli* 0111:B4 endotoxin (LPS) was purchased from Sigma (St. Louis, MO). Isofluorane was obtained from Abbott Laboratories (Chicago, IL, USA). Bicinchoninic acid (BCA) protein assay reagent was purchased from Pierce (Rockford, IL). All other reagents were purchased from Sigma unless otherwise noted in the text.

**Model for Hemorrhage.** Either PBS (200 μl) or NCH (6.25 mg/ml, 200 μl, 50 mg/kg) was injected into the tail vein. Just after the injection of either solution, hemorrhage model was induced into the mouse. In brief, mice were anesthetized with inhaled isofluorane. Cardiac puncture was used to