Effects of Medium Molecular Weight Heparinyl Arginine on Scorpion Venom-induced Pulmonary Edema in Rats

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Abstract. Background/Aim: The aim of this study was to examine the inhibitory action of medium molecular weight heparinyl amino acid derivatives (MHADs) on scorpion venom (SV)-induced acute pulmonary edema. Materials and Methods: SV was injected into the tail vein of rats after MHAD pre-treatment. An hour later, lungs were removed from each experimental animal, followed by measurement of the lung/body index (LBI) and Na⁺/K⁺ ratio of the pulmonary tissue as indices for acute pulmonary edema. Results: Medium molecular weight heparinyl arginine (MHR)-administered rats exhibited significantly lower LBI and Na⁺/K⁺ ratios compared to control rats. Although the mechanism of inhibitory action of MHR on pulmonary edema is unclear, MHR inhibited the vascular permeability increase by SV because both LBI and Na⁺/K⁺ ratio of the pulmonary tissue remained at almost normal values. Conclusion: MHR may prevent scorpion venom-induced acute pulmonary edema and thus makes a good candidate for clinical use.

One well-known anticoagulant, heparin (HE), produces many pharmacological effects. However, there is a limit to its clinical use due to common serious side effects like bleeding and bruising. In recent years, low molecular weight HE (LMWH) has been developed. LMWH has a reduced bleeding tendency compared with HE, but has similar pharmacological effects. We have synthesized and studied a novel medium molecular weight HE (MHE) and its amino acid derivatives (MHADs), in which eleven kinds of amino acids were adducted to MHE to develop safe and effective heparin derivatives. We found that some MHADs had a function as indirect radical scavengers (1-3). In this paper, we examined the inhibitory action of MHADs on scorpion venom (SV)-induced acute pulmonary edema.

Materials and Methods

Animals. Specific pathogen-free male Wistar rats (6 weeks old) were purchased from Charles River Laboratories Japan, Inc. (Yokohama, Japan), and used for the experiment after a one-week acclimation. The rats were maintained at 23±2°C (room temperature) and 50±5% relative humidity under an artificial 12-hour light-dark cycle (7:00 on-19:00 off). Food and water were given ad libitum during the experimental period. All procedures followed the office regulations for the care and use of laboratory animals approved by the animal experimentation committee of Fuso Pharmaceutical Industries Ltd (approval number: PDS9405).

Materials. Medium molecular weight heparinyl histidine (MHH), medium molecular weight heparinyl methionine (MHM), medium molecular weight heparinyl arginine (MHR), and medium molecular weight heparinyl tyrosine (MHY) were synthesized from HE (Scientific Protein Laboratories, Waunakee, WI, USA) at the Research and Development Center of Fuso Pharmaceutical Industries LTD (Osaka, Japan), and the mean molecular weight of each was 8,500-10,000 (4). Amino acids were purchased from Wako (Osaka, Japan).

Measurement of lung/body index (LBI) and Na⁺/K⁺ ratio of the pulmonary tissue. The SV-induced pulmonary edema rats were prepared after modification of the method by Freire-Maia L and de Matos IM (5). SV (Tityus serrulatus venom) (Sigma-Aldrich Co., St. Louis, MO, USA) was injected into the tail vein of rats 30 minutes after MHAD (5,000 μg/kg) or saline pre-treatment. An hour later, rats were decapitated and lungs were removed after bloodletting, and the wet weight of lungs was measured. LBI was calculated by the method of Freire-Maia L and de Matos IM (5) from the wet weight of the lungs.

LBI: lung wet weight (g) ×100/body weight (g)

The Na⁺/K⁺ ratio of the pulmonary tissue was determined by flame photometry using the flame photometer MF-303 (JASCO Corporation, Tokyo, Japan).
In the present study, we examined the inhibitory effects of four kinds of MHADs on acute pulmonary edema. It is reported that acute pulmonary edema may occur by an increase in permeability of the alveolar capillary barrier in the case of acute lung injury (6-8), or by increase in pulmonary microvascular hydrostatic pressure in the case of cardiogenic pulmonary edema (9). We used SV derived from Tityus serrulatus to induce the acute pulmonary edema in rats in this study. In Brazil, medically remarkable scorpions belong to the genus Tityus and the most severe poisoning is caused by Tityus serrulatus (10). Miyamoto JG et al. demonstrated that intravenously injected Tityus serrulatus venom (200 μg/kg) induced Evans blue extravasation in 30 min in airways and hemorrhage in 60 min in the lungs. Furthermore, interleukin-1β and interleukin-6 were detected at 60 min in lung homogenates using male Wistar rats (11). Comellas AP et al. examined the effects of Tityus serrulatus venom on the ability of the lung to clear fluid and on alveolar epithelial Na, K-ATPase. As a result, lung edema clearance decreased by up to approximately 60% in male Sprague-Dawley rats injected intraperitoneally with the venom (2 mg/kg), and Na, K-ATPase α1- and β1-subunit protein abundance and activity decreased by 50% at the basolateral membranes of alveolar epithelial type II cells incubated with scorpion venom (10 μg/ml) for 60 min as compared with those of controls (12).

In this study, we selected the LBI and Na⁺/K⁺ ratio as the indices to evaluate the extent of acute lung edema in rats and their results support the validity of our experimental protocol. Freire-Maia L and de Matos IM indicated that HE prevented acute pulmonary edema induced by Tityus serrulatus scorpion venom in rats and their results suggested the inhibitory action of HE to be related to a decrease in the vascular permeability in the lung (5). However, it is known that HE causes bleeding as its representative adverse effect. On the other hand, MHR demonstrated a significant inhibitory action against acute pulmonary edema. Furthermore, the bleeding tendency with MHR was reduced to one-eighth of that of HE according to the activated partial thromboplastin time (APTT) (1).

In conclusion, MHR may prevent scorpion venom-induced acute pulmonary edema via the decrease in vascular permeability and alveolar epithelial Na, K-ATPase with a low risk of hemorrhage.

### Table I. Changes in LBI and Na⁺/K⁺ ratio of the pulmonary tissue in rats after injection of scorpion venom.

| Reagent | Dose (μg/kg) | LBI | Na⁺/K⁺ ratio |
|---------|--------------|-----|--------------|
| Normal (Saline) | 0.48±0.02 | 0.84±0.02 |
| Venom    | 0.44±0.01 | 0.70±0.01 |
| 250      | 0.73±0.05* | 1.73±0.14* |
| 500      | 0.88±0.04* | 2.03±0.17* |

Each value represents the mean±S.E. for 4-7 animals. *p<0.05 compared with controls (Spjotvoll and Stoline test).

**Statistical analysis.** Data are represented as the means±standard error (S.E.), and significance was evaluated by ANOVA followed by the Spjotvoll and Stoline test (corrected Tukey’s test), because the number of samples among the groups was different. The differences were assessed at a significance level of 0.05.

### Results

**Investigation of suitable doses of SV for establishment of acute pulmonary edema in rats.** When SV (250 or 500 μg/kg) was injected into the tail vein of rats, piloerection, dyspnea, exophthalmos, lacrimation with blood, spasm, sialorrhea, and squatting were observed. Remarkable pulmonary edema was observed on autopsy. The LBI and Na⁺/K⁺ ratio of the pulmonary tissue in rats administered 250 or 500 μg/kg of SV increased significantly (Table I).

In the group administered 500 μg/kg of SV, death was seen before the end of observations. Accordingly, we decided to administer 250 μg/kg of SV to rats in subsequent experiments.

**Inhibitory effects of MHADs on the SV-induced acute pulmonary edema.** Rats administered with MHR and MHY (5,000 μg/kg) exhibited significantly lower LBI values compared with the control rats (p<0.01). Furthermore, the MHR administered group had a significantly lower Na⁺/K⁺ ratio in the pulmonary tissue compared with the controls (p<0.05). On the other hand, MHH and MHM did not demonstrate any inhibitory effects on SV-induced acute pulmonary edema (Table II).

### Discussion

In the present study, we examined the inhibitory effects of four kinds of MHADs on acute pulmonary edema. It is reported that acute pulmonary edema may occur by an increase in permeability of the alveolar capillary barrier in the case of acute lung injury (6-8), or by increase in pulmonary microvascular hydrostatic pressure in the case of cardiogenic pulmonary edema (9). We used SV derived from Tityus serrulatus.
Conflicts of Interest

The Authors declare no conflict of interest associated with this manuscript.

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