Effects of Medium Molecular Weight Heparinyl Phenylalanine on Type I Hypersensitivity

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Abstract. Background/Aim: We investigated the inhibitory action of medium molecular weight heparinyl phenylalanine (MHF) on type I hypersensitivity in comparison with medium molecular weight heparinyl arginine (MHR). Materials and Methods: MHF and MHR were synthesized from heparin (HE) to decrease the side-effect of HE based on its anticoagulant action and used in this study. Results: MHF demonstrated a significant inhibitory action on 48-h homologous passive cutaneous anaphylaxis in rats. Although MHF did not affect the death of mice injected with a lethal dose of histamine, it significantly prolonged the survival time of mice administered a lethal dose of compound 48/80. On the other hand, MHR did not inhibit type I hypersensitivity. Conclusion: The inhibitory action of MHF on the type I allergic reaction was due to a reduction or delay in histamine release from mast cells. MHF may be a potent anti-allergic agent.

In Japan, the number of patients with cedar pollen allergy increases every year (1), and the development of novel preventive or therapeutic medicines for type I hypersensitivity, including cedar pollen allergy, is expected.

Heparin (HE) was reported to inhibit the immediate cutaneous reaction and acute bronchoconstriction response induced by stimuli that produce immunological and non-immunological mast cell degranulation without attenuating the effects of histamine (2, 3). As HE is a well-known anticoagulant, bleeding may be a side-effect of HE use as an anti-allergic agent.

We previously synthesized twelve novel medium molecular weight heparinyl amino acid derivatives (MHADs) to reduce the anticoagulant activity of HE and investigated their beneficial pharmacological activity. Among them, medium molecular weight heparinyl phenylalanine (MHF), medium molecular weight heparinyl leucine (MHL), and medium molecular weight heparinyl tyrosine (MHY) functioned as indirect radical scavengers in vitro (4). MHF and MHL were superior to HE as safe radical scavengers in vivo. MHF, in particular, exhibited not only preventive effects, but also therapeutic effects on ischemic paw edema in mice (5). MHF and MHL also demonstrated radical scavenging ability by increasing the extracellular superoxide dismutase (EC-SOD) activity, and MHF was superior to HE and MHL (6). Furthermore, MHR prevented scorpion venom-induced acute pulmonary edema in rats (7).

In this report, we focused on MHF and MHR as anti-allergic agents according to our previous studies, and examined their inhibitory action on 48-h homologous passive cutaneous anaphylaxis (PCA) in rats, the death of mice due to a lethal dose of compound 48/80, and the death of mice due to a lethal dose of histamine.

Materials and Methods

Animals. Specific pathogen-free male Wistar rats (8 weeks old), female Wistar rats (5 weeks old), and male ICR mice (3.5 weeks old) were purchased from Charles River Laboratories Japan, Inc. (Yokohama, Japan), and used for the experiment after a one-week acclimation period. The animals were maintained at 23±2˚C (room temperature) and 50±5% relative humidity under an artificial 12-h light-dark cycle (7:00 on-19:00 off). Food and water were given ad libitum during the experimental period. All procedures followed the official regulations for the care and use of laboratory animals approved by the animal experimentation committee of Fuso Pharmaceutical Industries Ltd. (approval number: PDS9611).

Materials. MHF and MHR (mean molecular weight: 8,500-10,000) were synthesized at Fuso Pharmaceutical Industries Ltd. Research and Development Center (Osaka, Japan) (8), and bulk HE (Scientific Protein Laboratories, Waunakee, WI, USA) was used.
at a significance level of 0.05.

Samples among the groups differed. The differences were assessed by ANOVA followed by the Spjotvoll-Stotland test (corrected Tukey’s test) because the number of significance was evaluated. The survival time was measured.

Preparation of the anti-ovalbumin rat serum. The anti-ovalbumin (OVA) rat serum was prepared after modification of the method by Stotland LM and Share NN (9). Four days after the operation to remove the spleen from female rats, 0.25 ml of an equal mixture of saline containing 2.0 mg/ml of OVA and 2.0% aluminum hydroxide gel was subcutaneously injected into the limbs. In addition, dead Bordetella pertussis cells (2×10^10 cells/ml saline) were injected intraperitoneally. After five days, as an additional sensitization, 1.0 ml of an equal mixture of saline containing 2.0 mg/ml of OVA and 2.0% aluminum hydroxide gel was subcutaneously injected into the back of rats. Two weeks after the last sensitization, blood was collected from the abdominal aorta and serum was prepared. The antibody titer of this antiserum was 128.

Induction of 48-h homologous PCA in rats and treatment with MHR or MHF. One hundred μl/site of diluted anti-OVA rat serum to antibody titer 16 in saline was intradermally injected into the shaved back skin of rats. Forty-eight hours later, 10 mg/ml/rat of Evan’s blue saline solution containing 1 mg of OVA was injected into the tail vein of rats to induce an allergic reaction. Thirty min later, rats were euthanized by exsanguination and the amount of leaked dye in the back skin was measured using the method of Katayama et al. (10). MHR, MHF (0.3, 3.0, and 30.0 mg/ml/kg, respectively), or saline was injected into the tail vein of rats five min before inducing the allergic reaction.

Administration of MHR, MHF and compound 48/80 in mice. The experiments were carried out according to the method by Tasaka et al. (11). MHR, MHF (10 mg/10 ml/kg), or saline was injected into the tail vein of the mice. Five min later, compound 48/80 (3 mg/10 ml/kg) (Sigma-Aldrich Co., St. Louis, MO, USA) was injected into the tail vein, and the survival time was measured.

Results

Effects of MHR and MHF on the survival time of mice administered with compound 48/80. MHR did not significantly prolong survival time of mice, whereas MHF significantly prolonged the survival time of the compound 48/80-administered mice (p<0.01, Table II).

| Compounds | Dose (mg/kg) | Survival time (sec) |
|-----------|--------------|---------------------|
| Vehicle (Saline) | | 86.5±7.3 |
| MHR | 10.0 | 116.5±18.6 |
| MHF | 10.0 | 190.9±24.9** |

Each value represents the mean±S.E. for 12-13 animals. **p<0.01, compared with vehicle (Saline) (Spjotvoll and Stoline test).

| Compounds | Dose (mg/kg) | No. of survival | No. of deaths |
|-----------|--------------|----------------|--------------|
| Vehicle (Saline) | | 0 | 9 |
| MHR | 10.0 | 0 | 9 |
| MHF | 10.0 | 0 | 9 |

Preparation of the anti-ovalbumin rat serum. The anti-ovalbumin (OVA) rat serum was prepared after modification of the method by Stotland LM and Share NN (9). Four days after the operation to remove the spleen from female rats, 0.25 ml of an equal mixture of saline containing 2.0 mg/ml of OVA and 2.0% aluminum hydroxide gel was subcutaneously injected into the limbs. In addition, dead Bordetella pertussis cells (2×10^10 cells/ml saline) were injected intraperitoneally. After five days, as an additional sensitization, 1.0 ml of an equal mixture of saline containing 2.0 mg/ml of OVA and 2.0% aluminum hydroxide gel was subcutaneously injected into the back of rats. Two weeks after the last sensitization, blood was collected from the abdominal aorta and serum was prepared. The antibody titer of this antiserum was 128.

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Administration of MHR, MHF and compound 48/80 in mice. The experiments were carried out according to the method by Tasaka et al. (11). MHR, MHF (10 mg/10 ml/kg), or saline was injected into the tail vein of the mice. Five min later, compound 48/80 (3 mg/10 ml/kg) (Sigma-Aldrich Co., St. Louis, MO, USA) was injected into the tail vein, and the survival time was measured.

Administration of MHR, or MHF and histamine in mice. MHR, MHF (10 mg/10 ml/kg), or saline was injected into the tail vein of mice. Five min later, histamine (Sigma-Aldrich) (400 mg/10 ml/kg) was injected into the tail vein, and 3 h later, survival was evaluated.

Statistical analysis. Data are presented as the mean±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 differed. The differences were assessed at a significance level of 0.05.
Effects of MHR and MHF on histamine-induced death of mice. Mice administered with a lethal dose of histamine died in all groups (each group consisted of nine animals) within two min. Consequentially, both MHR and MHF exhibited no inhibitory effects on histamine (Table III).

Discussion

Homologous PCA in rats is considered to be caused by a mediator (mainly histamine) released from mast cells by the reaction of IgE antibody and the antigen (12). Although MHR did not inhibit the allergic reaction in the current study, MHF reduced it significantly. Accordingly, we examined the inhibitory effects of MHF on mediator release from mast cells and the antagonistic action of MHF on histamine H1 receptors in comparison with MHR.

First, compound 48/80 is a potent histamine liberator (13) and a non-immunologic stimulus for mast cells (14). The effects of MHR and MHF on survival time of mice administered with compound 48/80 were examined. As a result, MHF but not MHR significantly prolonged the survival time of mice after injection of compound 48/80. Furthermore, we investigated the actions of MHR and MHF on survival time of mice administered histamine. Both MHF and MHR had no effect on the survival time after injection of histamine. According to these results, we consider MHF to be able to reduce the release of histamine from mast cells, but MHF cannot antagonize the receptor of histamine. Thus, MHF may inhibit type I allergic reactions by reducing histamine release.

Tasaka et al. reported that compound 48/80 increased IP3 content and the resulting Ca2+ release from the intracellular Ca stores preceding histamine release from murine mast cells (15). On the other hand, anaphylactic histamine release from mast cells in allergic diseases is caused by the influx of a large amount of Ca2+ into cells (16). In other words, regardless of an allergic or non-allergic reaction, the increase in Ca2+ in mast cells triggers histamine release from mast cells.

In addition, Sugiyama demonstrated that degranulation due to an allergic or non-allergic reaction is inhibited by the membrane stabilizing action of mast cells (17). It can be speculated that MHF negatively influences Ca2+ kinetics, and reduces or delays the histamine release from mast cells by stabilizing the mast cell membrane. In conclusion, MHF may be a potent anti-allergic agent with few adverse effects.

Conflicts of Interest

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

References

1 Fukushima Y, Onuki A, Oya S and Ikezawa T: Relationship between cedar pollen count and cedar-specific IgE test. Jpn J Med Technol 64: 610-616, 2015.

2 Ahmed T, Abraham WM and D’Brot J: Effects of inhaled heparin on immunologic and nonimmunologic bronchoconstrictor responses in sheep. Am Rev Respir Dis 145: 566-570, 1992.

3 Lucio J, D’Brot J, Guo CB, Abraham WM, Lichtenstein LM, Kagey-Soboto A and Ahmed T: Immunologic mast cell-mediated responses and histamine release are attenuated by heparin. J Appl Physiol 73: 1093-1101, 1992.

4 Takeda S, Toda T and Nakamura K: Middle molecular weight heparinyl amino acid derivatives (MHADs) function as indirect radical scavengers in vitro. Pharmacol Pharm 7: 117-123, 2016.

5 Takeda S, Toda T and Nakamura K: Inhibitory effects of medium molecular weight heparinyl amino acid derivatives on ischemic paw edema in mice. In Vivo 30: 433-437, 2016.

6 Takeda S, Toda T and Nakamura K: Effects of medium molecular weight heparinyl phenylalanine on superoxide dismutase activity in mice. In Vivo 30: 841-844, 2016.

7 Takeda S, Toda T and Nakamura K: Effects of medium molecular weight heparinyl arginine on scorpion venom-induced pulmonary edema in rats. In Vivo 32: 537-539, 2018.

8 Araki H, Nishikawa H, Tanaka S, Nakamura K, Ohtani H, Nishimura S, Shimada C, Takeda S, Kawai K, Kitagawa C, Kuwahara M and Abe T: Japan Patent Kokai 1997-286803, 1997.

9 Stotland LM and Share NN: Pharmacological studies on active bronchial anaphylaxis in the rat. Can J Physiol Pharmacol 52: 1119-1125, 1974.

10 Katayama S, Shionoya H and Ohtake S: A new method for extraction of extravasated dye in the skin and the influence of fasting stress on passive cutaneous anaphylaxis in guinea pigs and rats. Microbiol Immunol 22: 89-101, 1978.

11 Tasaka K, Kamei C, Izushi K, Tsujimoto S and Yoshida T: Comparison of pharmacological properties of optical isomers and a racemic mixture of epinastine. Arzneimittel-Forschung 41: 219-223, 1991.

12 Azuma Y: Pharmacological studies on rat passive cutaneous anaphylaxis (PCA). Nihon Yakurigaku Zasshi 76: 685-696, 1980.

13 Paton WD: Compound 48/80: a potent histamine liberator. Br J Chemother 6: 499-508, 1951.

14 Barrett KE, Pluznik DH and Metcalfe DD: Histamine release and a non-immunologic stimulus for mast cells (14). The effects of MHR and MHF on survival time of mice administered histamine. Both MHF and MHR had no effect on the survival time after injection of histamine. According to these results, we consider MHF to be able to reduce the release of histamine from mast cells, but MHF cannot antagonize the receptor of histamine. Thus, MHF may inhibit type I allergic reactions by reducing histamine release.

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55