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Anti-edematous effects of epinastine, cetirizine and its enantiomers in λ-carrageenan-induced edema in rat hind paw

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Received March 7, 2024, accepted April 15, 2024

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Pharmazie 79: 98-100 (2024) *doi: 10.1691/ph.2024.4518*

Urticaria is induced by the histamine released from mast cells which develops wheals (edema) as a visual feature. In clinical practice, second-generation histamine H_1-receptor blockers are routinely used as the first-line symptomatic treatment for urticaria. Nevertheless, not much research has directly examined the second-generation histamine H_1-receptor blockers' ability to reduce edema. In this study, we directly evaluated the anti-edematous activities of three second-generation histamine H_1-receptor blockers available in the market (epinastine hydrochloride, cetirizine hydrochloride, and levocetirizine hydrochloride) using a λ-carrageenan-induced footpad edema model. One hour before the induction of edema with 1% λ-carrageenan injection, all second-generation histamine H_1-receptor blockers (5, 10, 50 and 100 mg/kg) were subcutaneously administered to rats. At 0.5 and 3 hours after λ-carrageenan administration, the edema volume was evaluated using a Plethysmometer. Epinastine hydrochloride significantly suppressed the edema growth in a dose-dependent manner. Cetirizine hydrochloride showed a slight anti-edematous effect, while levocetirizine significantly inhibited the development of edema in a dose-dependent manner. On the other hand, dextrocetirizine did not prevent edema from growing. In summary, second-generation histamine H_1-receptor blockers, at least those examined in this study, may be able to reduce the clinical symptoms of urticaria associated with edema. Levocetirizine hydrochloride is also anticipated to have stronger anti-edematous effects than cetirizine hydrochloride because levocetirizine is responsible for cetirizine's anti-edematous activity.

1. Introduction

Urticaria is defined as a condition that develops wheals, angioedema, or both, with some visual and sensational features such as edema (swelling), erythema, itching, burning, and tingling (Zuberbier et al. 2022). For more than half a century, histamine H_1-receptor blockers have been used for the treatment of urticaria because histamine released from mast cells is one of the key mediators for the development of urticaria. As the first-generation histamine H_1-receptor blockers have potent serious adverse effects, such as anti-cholinergic and sedative effects, and central nervous system depressant action, the international guideline for urticaria stated by EAACI/GA²LEN/EuroGuiDerm/APAAACI recommends second-generation histamine H_1-receptor blockers as the first-line symptomatic treatment for urticaria (Zuberbier et al. 2022). However, the anti-edematous effects of second-generation histamine H_1-receptor blockers have not been thoroughly studied.

Subcutaneous administration of λ-carrageenan acutely forms edema in and around the access site resulting from eliciting several inflammatory factors. Thus, a λ-carrageenan-induced footpad edema model has been leveraged for the evaluation of anti-inflammatory activities of active agents by contrasting edema volume before and after treatment for more than half-century (Alnusaire et al. 2023; Duarah et al. 2023; Ullah et al. 2023). In addition to anti-inflammatory activities, some studies evaluated the anti-edematous activities of first-generation H_1-receptor blockers in urticaria using a λ-carrageenan-induced footpad edema model (Higashino et al. 1992; Lloret and Moreno 1994). This is because the underlying biphasic edema formation mechanism, especially first-phase response, of the edema model is similar to that of urticaria: histamine and serotonin are implicated in the first-phase edema growth (about 0.5-1 hour after the λ-carrageenan administration) and prostaglandins, leukotrienes, and bradykinin are subsequently implicated in the second-phase edema growth (about 3 hours after the λ-carrageenan administration) (Morris 2003).

Based on these radical backgrounds, we assessed the anti-edematous activities of three second-generation histamine H_1-receptor blockers available on the market (epinastine hydrochloride, cetirizine hydrochloride, and levocetirizine hydrochloride; Fig. 1) in a λ-carrageenan-induced footpad edema model rat. Additionally, the anti-edematous activity of levocetirizine hydrochloride was contrasted with dextrocetirizine hydrochloride (Fig. 1), an enantiomer of levocetirizine hydrochloride, to reveal the structure-activity relationship in enantiomers.

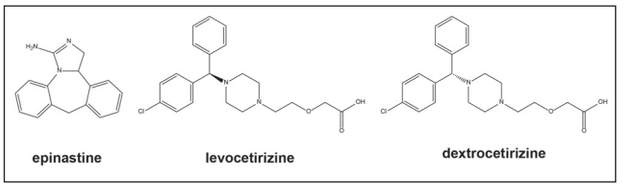

Fig. 1: Chemical structure of epinastine, levocetirizine, and dextrocetirizine. Cetirizine is a racemate mixture of levocetirizine and dextrocetirizine.

2. Investigations, results and discussion

2.1. Validation of λ-carrageenan-induced footpad edema model

First-generation histamine H_1-receptor blockers, diphenhydramine hydrochloride and *d*-chlorpheniramine maleate, were found to have anti-edematous actions in rats with footpad edema model generated by carrageenan (Higashino et al. 1992; Lloret and Moreno, 1994). Thus, we first evaluated the anti-edematous effects of diphenhydramine hydrochloride and *d*-chlorpheniramine maleate to confirm the validity of λ-carrageenan-induced footpad edema model rats used in this study. Similar to earlier reports (Higashino et al. 1992; Lloret and Moreno 1994), diphenhydramine hydrochloride and *d*-chlorpheniramine maleate suppressed the edema growth at 0.5 and 3 hours after λ-carrageenan administration (Fig. 2 and 3). Considering that histamine is linked to the onset of first-phase edema, which happens approximately 0.5–1 hour following the dose of λ-carrageenan (Morris, 2003), it makes sense to note the anti-edematous effects from the earlier period of time. Thus, we decided to evaluate the anti-edematous effects of second-generation histamine H_1-receptor blockers using this model. Furthermore, both first-generation histamine H_1-receptor blockers exhibited dose-dependent inhibition of edema growth after the λ-carrageenan administration.

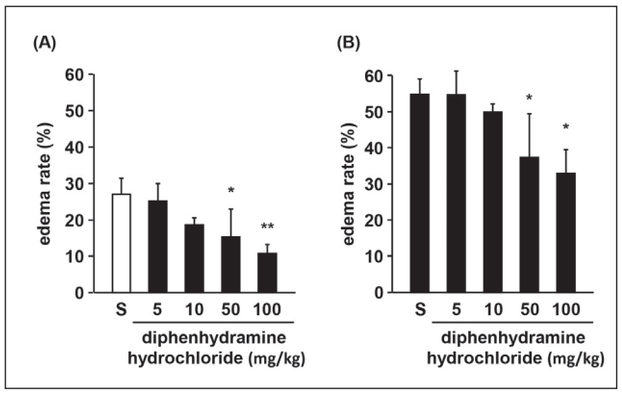

Fig. 2: The anti-inflammatory effects of diphenhydramine hydrochloride at (A) 0.5 and (B) 3 hours after λ-carrageenan administration. S: saline. Mean ± standard deviation (n=4). * p<0.05, ** p<0.01 vs saline

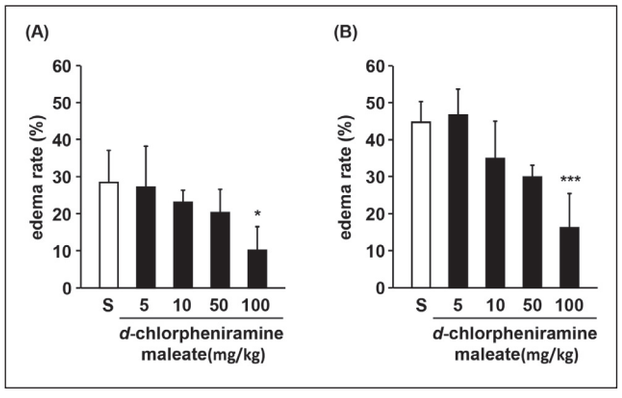

Fig. 3: The anti-inflammatory effects of *d*-chlorpheniramine maleate at (A) 0.5 and (B) 3 hours after λ-carrageenan administration. S: saline. Mean ± standard deviation (n=4-8). * p<0.05, ** p<0.01 vs saline

2.2. Anti-edematous effects of epinastine hydrochloride

Borazan et al. (2009) reported that epinastine hydrochloride suppressed eyelid swelling in patients of seasonal allergic conjunctivitis compared to a placebo, leading us to hypothesize that epinastine hydrochloride suppresses edema growth induced by λ-carrageenan. As expected, significant suppression was observed at epinastine hydrochloride doses of 50 and 100 mg/kg, 0.5 and 3 hours after λ-carrageenan administration (Fig. 4). Notably, the lower dose (5 mg/kg) of epinastine hydrochloride significantly inhibited the edema growth at 0.5 hours, but not at 3 hours, after λ-carrageenan administration. Such a distinct time-dependent inhibitory effect would be due to histamine involved with the edema growth at first-phase, but not second-phase, of edema formation by λ-carrageenan. These findings suggest that epinastine hydrochloride has anti-edematous effects and is expected to suppress the exacerbation of wheals in urticaria.

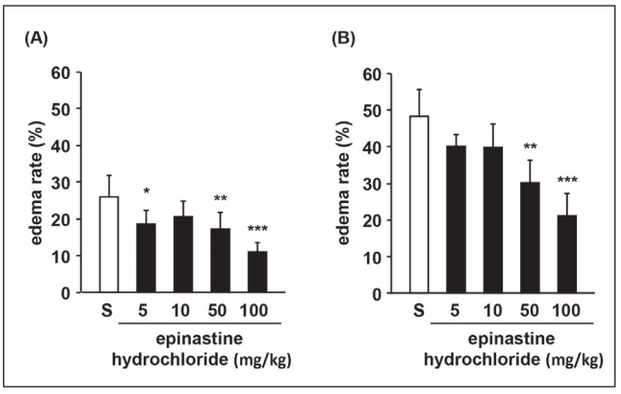

Fig. 4: The anti-inflammatory effects of epinastine hydrochloride at (A) 0.5 and (B) 3 hours after λ-carrageenan administration. S: saline. Mean ± standard deviation (n=4-12). * p<0.05, ** p<0.01, ** p<0.01 vs saline

2.3. Anti-edematous effects of cetirizine hydrochloride and its enantiomers

A double-blind multicenter study showed that long-term cetirizine intake alleviated the severity of edema in patients with chronic idiopathic urticaria (LaRosa et al. 2001). However, no significant anti-edematous effects of cetirizine hydrochloride were observed at 0.5 hours after λ-carrageenan administration (Fig. 5A). Three hours after λ-carrageenan was administered, there was a tendency of dose-dependent inhibitory effects, but it was much less pronounced than with epinastine hydrochloride. (Fig. 5B).

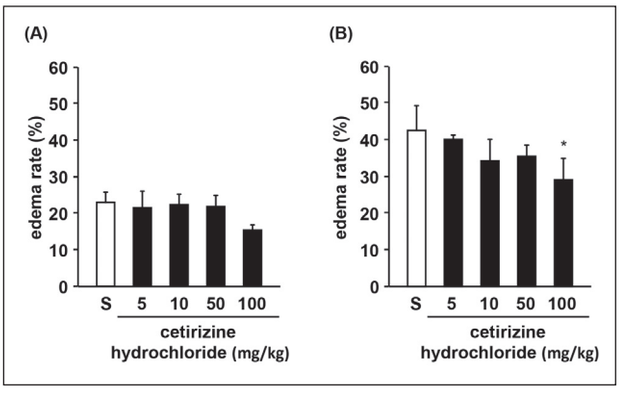

Fig. 5: The anti-inflammatory effects of cetirizine hydrochloride at (A) 0.5 and (B) 3 hours after λ-carrageenan administration. S: saline. Mean ± standard deviation (n=4). * p<0.05 vs saline

Cetirizine hydrochloride is a racemate mixture of levocetirizine (*R* enantiomer) and dextrocetirizine (*S* enantiomer) (Fig. 1). Prior *in vitro* research employing human airway epithelial cells showed that at the same dose, levocetirizine had higher anti-inflammatory activity than cetirizine (Shih et al. 2008). In addition, clinical studies in healthy subjects showed that the anti-histaminic activity of 10 mg cetirizine was comparable to 5 mg levocetirizine (Wang et al. 2001). These reports imply that the entire pharmacological activity of cetirizine is attributed to levocetirizine. Thus, we further evaluated the anti-edematous effect of levocetirizine hydrochloride. As shown in Fig. 6A and B, the anti-edematous effect of levocetirizine hydrochlo-

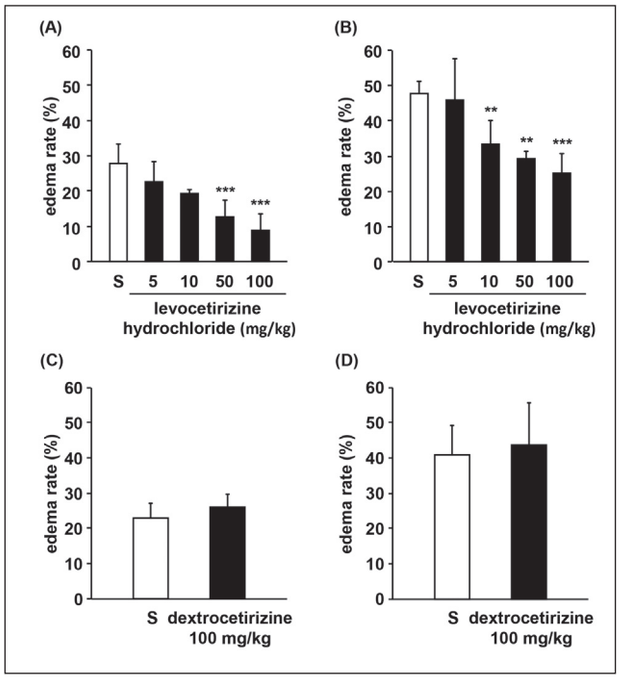

Fig. 6: The anti-inflammatory effects of levocetirizine and dextrocetirizine hydrochloride at (A, C) 0.5 and (B, D) 3 hours after λ-carrageenan administration. S: saline. Mean±standard deviation (n=4-8). ** p<0.01, *** p<0.001 vs saline

ride was stronger than that of cetirizine hydrochloride. On the other hand, a high dose (100 mg/kg) of dextrocetirizine hydrochloride did not exhibit an anti-edematous effect (Fig. 6C, D). Previously, the binding affinity of levocetirizine to the histamine H_1-receptor was found to be 30-fold higher than that of dextrocetirizine (Gillard et al. 2002). The affinity difference between levocetirizine and dextrocetirizine is attributed to the much higher dissociation rate from the histamine H_1-receptor in dextrocetirizine (0.12 min^{-1}) than in levocetirizine (0.005 min^{-1}). Since the histamine H_1-receptor blockers activity was exhibited by competitive inhibition of the histamine H_1-receptor, the high binding affinity of levocetirizine would be associated with stronger anti-edematous effect compared to the dextrocetirizine and cetirizine. Furthermore, as far as we are aware, no research has been done that compares the anti-edematous effects of cetirizine hydrochloride and its enantiomers to those caused by λ-carrageenan-induced edema. Levocetirizine hydrochloride is therefore anticipated to be more effective than cetirizine hydrochloride in reducing the formation of wheals in urticaria.

2.4. Conclusion

This study unequivocally demonstrated that the three second-generation histamine H_1-receptor blockers levocetirizine hydrochloride, cetirizine hydrochloride, and epinastine hydrochloride had some degree of anti-edematous activity. However, because the inactive enantiomer (dextrocetirizine) contaminated the cetirizine hydrochloride, the latter had only a minimal anti-edematous effect. These findings bolster the effectiveness of second-generation histamine H_1-receptor blockers in reducing urticaria patients' clinical symptoms associated with edema. Nevertheless, due to the lack of clinical and fundamental research determining the level of pharmacological activity of second-generation histamine H_1-receptor blockers, it is still unknown which of these drugs is most suited for treating urticaria. Which second-generation histamine H_1-receptor blockers to prioritise in urticaria may be determined by further research comparing anti-edematous effects in a λ-carrageenan-induced rat hind paw model under the same conditions.

4. Experimental

Anti-edematous effects of histamine H_1-receptor blockers were monitored in a λ-carrageenan-induced rat hind paw model like in our previous studies with minor modifications (Isobe et al. 2023; Ito et al. 2023; Taguchi et al. 2024). In short, the diphenhydramine hydrochloride (Sigma-Aldrich Japan), *d*-chlorpheniramine maleate (Sigma-Aldrich Japan), epinastine hydrochloride (Tokyo Chemical Industry CO., LTD.), cetirizine hydrochloride (Tokyo Chemical Industry CO., LTD.), levocetirizine hydrochloride (LKT Laboratories, Inc.), and dextrocetirizine hydrochloride (Tokyo Chemical Industry CO., LTD.) and λ-carrageenan (Nacalai Tesque Inc) were dissolved in saline. Either saline or histamine H_1-receptor blockers solution (0.5 mL/kg) was subcutaneously injected into the back of male Wistar rats (5-week-old, Sankyo Labo Service Corporation). One hour after each sample administration, edema was induced in the footpad of the rat's left posterior limb by subcutaneous injection of λ-carrageenan solution (1%, 0.1 mL/rat). Immediately before (0 hours) or 0.5 and 3 hours after λ-carrageenan injection, the volume of the left posterior limb ranging from toe to the first joint was measured using a plethysmometer (UNICOM Co., Ltd.). The edema rate (%) was calculated by dividing incremental foot volume 0.5 and 3 hours after λ-carrageenan injection by the foot volume of baseline (0 hours). The obtained data was statistically analyzed by Tukey test using SPSS® 22 for Windows software. Differences were considered statistically significant when the p-value was <0.05. The animal experiments were performed according to ARRIVE guidelines. All animal experiments were approved by the Animal Care and Use Committee of Keio University (11023-(0)).

Acknowledgments: This work was supported by a research grant from Keio University.

Disclosure: Kazuaki Matsumoto received grant support funding from Shionogi & Co., Ltd., Sumitomo Pharma Co., Ltd., and Meiji Seika Pharma Co., Ltd.

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