Effects of Olopatadine Hydrochloride on the Cutaneous Vascular Hyperpermeability and the Scratching Behavior Induced by Poly-L-Arginine in Rats

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ABSTRACT—Intradermal injections of poly-L-arginine induce cutaneous vascular hyperpermeability and scratching behavior in rats. Recently, we elucidated that the plasma extravasation involved both histamine and substance P, while the scratching behavior involved substance P, but not histamine. This study examined the effects of olopatadine hydrochloride (olopatadine), an antiallergic drug with histamine H₁-antagonistic action, on the poly-L-arginine-induced responses. Olopatadine (1 mg/kg, p.o.) significantly inhibited both the plasma extravasation and the scratching behavior, suggesting that its inhibitory effects are mediated by the suppression of neuropeptidergic action as well as histaminic action. Olopatadine seems to be a novel-type drug for the treatment of dermatitis.

Keywords: Cutaneous vascular hyperpermeability, Olopatadine hydrochloride, Scratching behavior

The polycations, including eosinophil granule major basic protein, are suggested to be involved in the pathogenesis of pruritic allergic dermatitis (1, 2). Previous studies demonstrated that polycations elicited vascular hyperpermeability in humans (3, 4) and animals (5). We recently observed that the cationic polypeptide poly-L-arginine induced not only cutaneous vascular hyperpermeability but also scratching behavior in rats (6). Moreover, we have elucidated that both histamine derived from mast cells and substance P are involved in the cutaneous plasma extravasation, while substance P, but not histamine, is involved in the scratching behavior. The poly-L-arginine-induced response in rats is assumed to partly mimic the pruritic allergic dermatitis in humans, although various other mediators including cytokines, in addition to polycations, are involved in the chronic phase of dermatitis.

Olopatadine hydrochloride (olopatadine) ((Z)-11-[3-dimethylamino)propylidene]-6,11-dihydrodibenz[b,e]-oxepin-2-acetic acid monohydrochloride, CAS 140462-76-6, KW-4679) is a novel antiallergic drug developed for the treatment of allergic diseases. Olopatadine has a potent histamine H₁-receptor antagonistic activity (7). Moreover, a previous study has shown that olopatadine inhibits the electrical field stimulation-induced tachykininergic contraction but not the substance P- or neurokinin A-induced contraction in the isolated guinea pig main bronchus, suggesting that this drug inhibits the release of transmitters from sensory nerves (8). The clinical studies showed that olopatadine exhibited prominent antipruritic effects in patients with allergic dermatitis (9). In the present study, we determined the effects of olopatadine on the cutaneous vascular hyperpermeability and scratching behavior induced by poly-L-arginine in rats in order to investigate the mechanism for the efficacy of this drug in the treatment of pruritic allergic dermatitis.

All animals were purchased from Japan SLC (Shizuoka). For the measurement of vascular permeability, 10-week-old male Wistar rats weighing 221 to 258 g were used. For the observation of scratching behavior, 5-week-old male Wistar rats weighing 96 to 113 g were used. The animals were acclimatized in an animal room maintained at a room temperature of 19 – 25°C and a relative humidity of 30 – 70% with a 12-h light-dark cycle (illuminated between 07:00 and 19:00 h). Food and water were freely available. This animal experiment was approved by the Animal Ethical Committee of Kyowa Hakko Kogyo Co., Ltd. (Shizuoka).

Poly-L-arginine hydrochloride (poly-L-arginine, MW 11,800 or 12,100) (Sigma Chemical Co., St. Louis, MO, USA) was dissolved in saline. Olopatadine (synthesized at Kyowa Hakko Kogyo Co., Ltd.) was dissolved in distilled water.

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The back skin of the rat was clipped, and poly-L-arginine (
50 μg/site) was injected intradermally at a volume of
50 μL/site (6). Immediately after the injection, the rat
received 0.5 mL of 1% Evans blue (Wako Pure Chemical
Industries, Osaka) intravenously. The rat was sacrificed
with CO2 gas 30 min after the elicitation of the cutaneous
reactions, and the skin at the reaction site was excised. The
dye content in the skin specimen was measured according
to a modification of the method described by Katayama
et al. (10). The specimen was dissolved in 1 mL of 1 mol/L
KOH solution. Nine milliliters of a mixture of 0.6 mol/L
H3PO4 solution and acetone (5:13) was added, and the
sample was centrifuged at 1200 × g for 10 min. The amount
of dye extracted in the supernatant was measured colori-
metrically at 620 nm (U-2001; Hitachi, Tokyo). The leaked
dye amount was calculated by subtracting the dye content
of untreated site from that of the intradermally injected site.

Scratching behavior was observed according to a modifi-
cation of the method described by Andoh et al. (11). The
rostral part of the back skin of the rat was clipped, and
the rat was put into an acrylic cage composed of 4 cells
(the size of each cell: 200 × 145 × 170 mm) and acclimated
for about 30 min. Poly-L-arginine (200 μg/site) was intra-
dermally injected into the rostral part of the back (around
the interscapular level) at a volume of 50 μL/site (6).
Immediately thereafter, the rat was put back to the same
cell, and the behavior was recorded using an 8-mm video
camera (CCD-TRV95 NTSC; Sony, Tokyo) for 30 min.
The scratching around the injected site with the hind-paws
was counted. The rat generally scratched several times in
about one second and a series of the scratchings was
counted as one incidence.

Olopatadine or distilled water was orally administered
at a volume of 10 mL/kg 1 h before the intradermal injec-
tion of poly-L-arginine.

The data were expressed as means ± standard error of
the mean (S.E.M). Analysis of statistical significance was
performed with the SAS system for Windows ver. 6.12
(SAS Institute, Cary, NC, USA). In the experiment of vas-
cular permeability, either the Student’s t-test or the Aspin-
Welch test was used for comparisons between the two
groups, after the variances of the data were evaluated
with the F-test. Multiple comparisons were made first by
the 1-way analysis of variance (ANOVA), followed by the
Dunnett test when appropriate. In the experiments of
scratching behavior, multiple comparisons were made first
by the Kruskal-Wallis test, followed by the Steel test
when appropriate. Differences were considered significant
if P values were <0.05.

An intradermal injection of poly-L-arginine (50 μg/site)
into the dorsal skin significantly increased cutaneous vas-
cular permeability (Fig. 1). Olopatadine at 0.03, 0.1 and
1 mg/kg (p.o.) significantly inhibited the cutaneous plasma
evacuation by 69.2%, 72.8% and 77.5%, respectively
(Fig. 1).

An intradermal injection of poly-L-arginine (200 μg/
site) into the rostral part of the back skin produced scratch-
ing behavior (Fig. 2). Olopatadine at 1 mg/kg (p.o.) signifi-
cantly inhibited the scratching behavior by 68.2% (Fig. 2).

Our recent study (6) demonstrated that the antihistamine

![Fig. 1. Effects of olopatadine (OL, 0.01 – 1 mg/kg) on the vascular permeability increase induced by poly-L-arginine in the rat skin. Distilled water or olopatadine was orally administered 1 h before the intradermal (i.d.) injection of saline or 50 μg/site of poly-L-arginine. Each value represents the mean ± S.E.M. from 8 animals. ***P<0.001, compared with the value in the saline i.d. group (open column).]({})

![Fig. 2. Effects of olopatadine (OL, 0.1 or 1 mg/kg) on the scratching behavior induced by poly-L-arginine. Distilled water or olopata-
dine was orally administered 1 h before the intradermal injection of 200 μg/site of poly-L-arginine. The scratching around the injected
site with the hind-paws was counted for 30 min after the intradermal
injection. Each value represents the mean ± S.E.M. from 8 animals.
*P<0.05, compared with the value in the vehicle-treated group.](https://example.com/fig2.png)
chlorpheniramine (10 mg/kg, p.o.) and the neurokinin-1 receptor antagonist LY303870 (10 mg/kg, i.v.) significantly inhibited the poly-L-arginine-induced cutaneous vascular hyperpermeability, suggesting that histamine and substance P are involved in the vascular hyperpermeability. On the other hand, LY303870 (10 mg/kg, i.v.) almost completely, but chlorpheniramine (10 and 30 mg/kg, p.o.) only minimally, inhibited the scratching following poly-L-arginine, suggesting that substance P but not histamine is involved in the scratching behavior (6). In the present study, olopatadine (p.o.) was found to significantly suppress both the cutaneous vascular hyperpermeability and the scratching behavior induced by poly-L-arginine. Olopatadine is a histamine H1-receptor antagonist (7), and this drug also inhibits the tachykininergic contraction in the isolated guinea pig main bronchus (8). These findings suggest that olopatadine inhibited the cutaneous vascular hyperpermeability by both its antihistaminic action and its inhibitory action on tachykinin release, whereas this drug inhibited the scratching behavior mainly by its inhibitory action on tachykinin release. Further studies, however, are required to investigate the more detailed mechanism for the inhibition by olopatadine, since other mediators such as nitric oxide (12) and bradykinin (13) are suggested to be involved in the poly-L-arginine-induced responses.

In this study, olopatadine inhibited the cutaneous plasma extravasation and the scratching behavior at 0.03 mg/kg or higher and at 1 mg/kg, respectively. There are two possible explanations for the mechanism for this dose discrepancy as follows. First, the difference in poly-L-arginine doses may have caused the difference in the effective doses between the plasma extravasation and the scratching behavior. In this study, we employed 50 and 200 μg/site of poly-L-arginine to evaluate the effects on the plasma leakage and the scratching behavior, respectively. Thereby, the relatively high dose of olopatadine may have been needed to suppress the scratching behavior, which was induced by the higher dose of poly-L-arginine. The second possibility is that the difference in the mediators involved in the cutaneous plasma leakage and the scratching behavior was responsible for that in the effective doses of olopatadine. It is possible that the antihistaminic action emerged at the lower doses of olopatadine than that inhibiting tachykinin release, and thus this played a role in its inhibitory effect on the plasma leakage, which is partly mediated by histamine. Alternatively, the synergistic inhibition by olopatadine of histaminergic and tachykinergic action may have resulted in its more potent effect on the plasma leakage than on the scratching behavior.

There is increasing evidence that neuropeptides are involved in the pathogenesis of allergic dermatitis. Ostlere et al. (14) showed that substance P immunoreactivity was observed in the papillary dermis of atopic dermatitis patients. Moreover, repeated topical treatment of the skin with capsaicin, leading to the degeneration of unmyelinated neupeptidergic nerves, is reported to significantly reduce pruritus in the patients with urticaria or atopic dermatitis (15). The inhibition by olopatadine of neuropeptidergic action seems to involve the clinical efficacy of this drug, especially on allergic pruritus. In fact, olopatadine has been shown to prominently ameliorate pruritus in the patients with allergic dermatitis (9).

Taken together, olopatadine was demonstrated to inhibit both the cutaneous plasma extravasation and the scratching behavior induced by poly-L-arginine. The suppression of tachykinin release, in addition to the antagonism of histamine H1 receptors, is likely to be involved in the inhibitory effect of olopatadine on the poly-L-arginine-induced responses. Olopatadine may be a novel-type of antiallergic drug, with an inhibitory action against tachykinin release, for the treatment of pruritic allergic dermatitis.

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