

Pharmacological Studies on the Novel Antiallergic Drug HQL-79: I. Antiallergic and Antiasthmatic Effects in Various Experimental Models

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ABSTRACT—The effects of oral administration of 4-benzhydryloxy-1-{3-(1*H*-tetrazol-5-yl)-propyl}piperidine (HQL-79), a newly synthesized antiallergic drug, in various experimental allergic and asthmatic models were investigated. HQL-79 markedly inhibited immediate hypersensitivity reactions such as passive cutaneous anaphylaxis in rats, antigen-induced bronchoconstriction and nasal vascular permeability in actively sensitized guinea pigs, like epinastine and ketotifen did. Airway eosinophilia in repeatedly antigen-exposed guinea pigs was suppressed by chronic administration of HQL-79 for 2 weeks. In another experiment, the antigen-induced late asthmatic response (LAR) in metyrapone-treated guinea pigs was also ameliorated by chronic treatment with HQL-79. Moreover, HQL-79 partially inhibited the toluene diisocyanate-induced delayed-type hypersensitivity (DTH) reaction in mice when administered chronically during the immunization period. The corticosteroid dexamethasone inhibited the airway inflammatory responses in guinea pigs and the DTH in mice. These results indicate that HQL-79 has potent inhibitory effects on the immediate hypersensitivity reactions, and when administered chronically, it also inhibits airway eosinophilia, LAR and DTH, similarly to corticosteroids.

Keywords: Antiallergic drug, Late asthmatic response, Airway inflammation, Delayed-type hypersensitivity, HQL-79

Antigen-induced allergic responses are closely implicated in the pathogenesis of allergic inflammatory diseases such as bronchial asthma, allergic rhinitis and dermatitis. In bronchial asthma, various chemical mediators released from antigen-stimulated mast cells or basophils elicit immediate hypersensitivity reactions such as bronchial contraction and airway plasma extravasation (1). Following the immediately occurring events, late-phase inflammatory responses such as airway edema, infiltration of inflammatory leukocytes and airway hyperresponsiveness are observed (2–4). Because asthmatic symptoms include the immediate hypersensitivity reactions and the late-phase inflammatory responses, a variety of medicines such as bronchodilators, corticosteroids and various kinds of antiallergics are used in combination for the treatment of asthma. It is important, therefore, for the development of new antiallergic and antiasthmatic drugs

to investigate the pharmacological effects not only on allergic immediate hypersensitivity reactions but also on the late-phase airway inflammatory responses.

In our laboratory, various series of compounds have been synthesized and screened for development as anti-allergic and antiasthmatic drugs, and some tetrazol derivatives have been found to have potent antiallergic effects in rats and guinea pigs. Among these compounds, HQL-79 [4-benzhydryloxy-1-{3-(1*H*-tetrazol-5-yl)-propyl}piperidine] has relatively strong antiallergic activities with few side-effects on the central nervous system in mice (5).

In the present study, antiallergic and antiasthmatic effects of HQL-79 were evaluated using various experimental models. Effects of HQL-79 on immediate hypersensitivity reactions such as passive cutaneous anaphylaxis (PCA) in rats, and antigen-induced bronchoconstriction and nasal vascular permeability in actively sensitized guinea pigs were examined, and compared to those of epinastine and ketotifen, which have been already established as potent antiallergic drugs with histamine-H₁

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blocking activities (6, 7). Effects of HQL-79 on airway inflammatory responses such as airway leukocyte-infiltration and late-phase asthmatic response (LAR) in repeatedly antigen-exposed guinea pigs, and on the delayed-type hypersensitivity (DTH) reaction in mice were also examined and compared to those of a corticosteroid, dexamethasone. The results show that HQL-79 is a potent, orally active antiallergic drug with anti-inflammatory activities similar to those of corticosteroids, which are often used for asthma therapy.

MATERIALS AND METHODS

Animals

Male Wistar rats, male ICR mice and male Hartley guinea pigs were purchased from Japan SLC, Inc. (Hamamatsu). Male Brown Norway rats were purchased from Seac Yoshitomi (Chikujou-gun, Fukuoka).

Drugs

HQL-79 was synthesized, and epinastine hydrochloride (epinastine) was extracted from Alesion[®] (Boehringer Ingelheim Japan, Kawanishi) at High Quality-Life Research Laboratories, Sumitomo Metal Industries, Ltd. (Souraku-gun, Kyoto). Ketotifen fumarate (ketotifen) was purchased from Sigma (St. Louis, MO, USA). Dexamethasone sodium phosphate solution (Decadron[®] phosphate injection; Banyu Pharmaceuticals, Tokyo) was used as dexamethasone. The chemical structure of HQL-79 is shown in Fig. 1. These drugs were suspended or dissolved in 0.5% methylcellulose for oral administration.

PCA in rats

2,4-Dinitrophenylated-ascaris extract (DNP-As; LSL Co., Ltd., Tokyo) was used as the antigen. Rat anti-DNP-As serum was obtained from male Brown Norway rats immunized according to the methods of Tada and Okumura (8). The anti-DNP IgE titer of the serum estimated by 48-hr homologous PCA was 1:2000.

Male Wistar rats (8-week-old, 175–190 g) were sensitized intradermally on their shaved backs with 0.1 ml of

200-fold diluted antiserum containing anti-DNP IgE. Forty-eight hours later, the rats were challenged by i.v. injection of 1 ml of saline containing 300 μ g of DNP-As and 5 mg of Evans blue (Sigma). The animals were sacrificed 30 min after antigen challenge, and the skin samples were removed. Evans blue was extracted and determined according to Katayama et al. (9). Test drugs were given orally 1 hr before antigen challenge. The inhibitory effects of test drugs were expressed as the percent reduction in dye leakage compared with that determined in vehicle-treated animals.

Antigen-induced bronchoconstriction and nasal vascular permeability in actively sensitized guinea pigs

Ovalbumin (OA, Sigma) was used as the antigen. Male guinea pigs (5-week-old, 300–330 g) were actively sensitized by exposure to aerosolized 1% OA (in saline) for 10 min and i.p. injection of a mixture of 10 μ g OA and 20 mg Al(OH)₃; 7 days later, they were then boosted by i.p. injection of 10 μ g OA alone. Experiments to measure antigen-induced bronchoconstriction and nasal vascular permeability were carried out 14 days after the sensitization.

Measurement of bronchoconstriction was based on the overflow principle of Konzett and Rössler (10). The animals were anesthetized by i.p. injection of 1.5 g/kg urethane (Aldrich Chemicals, Milwaukee, WI, USA), and the trachea was cannulated. The animals were ventilated by a rodent respirator (Ugo Basile, Comerio, Italy) at 60 strokes/min at a fixed volume of air under a constant pressure (10 cmH₂O). Spontaneous respiratory movement was stopped by i.v. administration of 5 mg/kg gallamine triethiodide (Sigma). Ventilation overflow from a side arm of the tracheal cannula was recorded with a Ugo Basile bronchospasm transducer as an index of change in airway resistance. The bronchoconstriction was induced by OA (0.3 mg/kg, i.v.). Test drugs were given orally 2 hr before antigen challenge. The inhibitory effects of test drugs were expressed as the percent reduction in ventilation overflow volume compared with that determined in vehicle-treated animals.

Measurement of nasal vascular permeability was performed according to the method of Kojima et al. (11). The animals were anesthetized and the trachea was cannulated for spontaneous respiration. Physiological saline and antigen was perfused through polyethylene tubing inserted into the nasal cavity at a rate of 0.2 ml/min with a perfusion pump (KD Scientific Inc., Boston, MA, USA). Saline was perfused for 10 min following the operation, then 1% Evans blue saline solution was injected intravenously (0.25 ml/100 g). Ten minutes after the Evans blue injection, 1% OA saline solution was perfused for 10 min, followed by perfusion of saline for a further

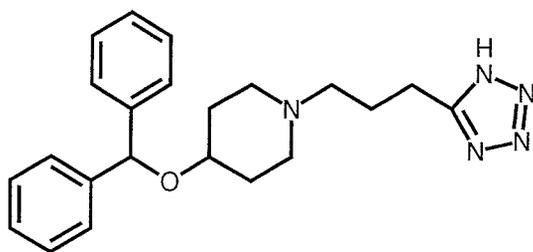


Fig. 1. Chemical structure of 4-benzhydryloxy-1-(3-(1H-tetrazol-5-yl)-propyl)piperidine (HQL-79).

20 min. The effluent was collected for 30 min after the antigen perfusion and then filtrated through a cellulose acetate filter (0.20 μm ; Advantec Toyo, Tokyo). The amount of Evans blue in the filtrate was determined at 620 nm. Test drugs were given orally 2 hr before antigen challenge. The inhibitory effects of test drugs were expressed as the percent reduction in dye leakage compared with that determined in vehicle-treated animals.

Airway leukocyte-infiltration in repeatedly antigen-exposed guinea pigs

Male guinea pigs were actively sensitized as described above (day 1). Thereafter, the animals were exposed to aerosolized 1% OA for 10 min on 3 occasions separated by 7 days (on days 8, 15 and 22), following pretreatment with i.p. injection of 10 mg/kg mepyramine (Research Biochemicals International, Natick, MA, USA) 30 min before the antigen exposure. Bronchoalveolar lavage (BAL) was performed 24 hr after the final antigen exposure. Animals were killed with an overdose (100 mg/kg, i.p.) of pentobarbital sodium (Nembutal[®]; Dinabot, Osaka). The trachea was cannulated and the lungs were lavaged by five 5-ml aliquots of phosphate-buffered saline (PBS) prewarmed at 37°C. The fluid recovered from each animal was pooled in a plastic tube and then centrifuged at 200 $\times g$ for 10 min at 4°C.

The cell pellet was resuspended in 1 ml of PBS. The total leukocyte number was counted with an automated hematology analyzer (Coulter[®] JT; Coulter Electronics, Inc., Hialeah, FL, USA). Differential leukocyte counts were undertaken on Wright-Giemsa-stained slides. A minimum of 200 cells were counted using standard morphologic criteria to classify the cells into mononuclear cells, eosinophils and neutrophils. Test drugs were administered orally 14 times from days 8 to 22 (HQL-79 and epinastine): 1 hr before and 6 hr after the antigen exposure on days 8, 15 and 22; and once a day on days 9, 10, 13, 14, 16, 17, 20 and 21. In the other experiment, test drugs were administered 4 times from days 20 to 22 (dexamethasone and HQL-79): 1 hr before and 6 hr after the antigen exposure on day 22; and once a day on days 20 and 21.

Antigen-induced LAR in metyrapone-treated guinea pigs

We have established another asthmatic model, since the incidence of LAR occurrence in the guinea pig model used for the BAL studies was not high enough for pharmacological studies on LAR.

Male guinea pigs were actively sensitized as described above (day 1). Thereafter, the animals were exposed to aerosolized 1% OA for 10 min on 3 occasions separated by 7 days (days 10, 17 and 24), following pretreatment with i.p. injection of 1 mg/kg mepyramine. In addition,

the animals were pretreated with metyrapone (2-methyl-1,2-di-pyridyl-1-propanone, Sigma), which inhibits 11 β -hydroxylase in glucocorticoid biosynthesis (12), before each antigen-exposure; metyrapone (10 mg/kg) was injected intraperitoneally 24 hr and 30 min before the first and the second antigen challenge (days 10 and 17), and intravenously administered 24 hr and 30 min before the final challenge (day 24).

Respiratory resistance (R_{rs}) was measured 15 min, 2, 4, 6 and 8 hr after the final antigen challenge (day 24), as described below. R_{rs} was expressed as the percentage of the pre-challenge (baseline) value measured 24 hr before the antigen challenge. HQL-79 was administered orally 13 times from days 10 to 24: 1 hr before and 6 hr after the antigen exposure on days 10 and 17; 1 hr before the final antigen exposure on day 24; and once a day on days 11, 12, 15, 16, 18, 19, 22 and 23. Dexamethasone was administered 3 times from days 22 to 24: 1 hr before the antigen exposure on day 24; and once a day on days 22 and 23.

Measurement of R_{rs} of conscious guinea pigs was performed by the forced oscillation technique according to the principles described by Mead (13), with a two-chambered, whole body plethysmograph connected to a noninvasive pulmonary mechanics analyzer (Model MBP-6000; Nihon Kohden-Kyoto/Biotex, Kyoto). In brief, a conscious guinea pig was placed in the two-chambered box with its neck fixed at the partition using a rubber collar. A sealing gel was applied around the neck to avoid air-movement between the body chamber and the head chamber. A 30-Hz sine wave oscillation was applied to the body surface by a loudspeaker (4 inches in diameter) driven by a sine wave generator and a power amplifier. Respiratory flow through the head chamber and the box pressures were measured with a differential pressure transducer (Model BDP-5; Nihon Kohden-Kyoto/Biotex). The 30-Hz component of the respiratory flow and the box pressure were extracted by a digital filter, and R_{rs} was calculated by the following formula: $R_{rs} = \Delta\text{pressure} / \Delta\text{flow}$ (cmH₂O/ml/sec).

Toluene diisocyanate (TDI)-induced DTH in mice

Male mice (7-week-old, 30–32 g) were sensitized by application of 0.1 ml of 1% toluene 2,4-diisocyanate (TDI; Wako Pure Chemicals, Osaka) in ethyl acetate onto their shaved abdomen. Seven days later, the animal was challenged with 20 μl of 1% TDI on the right ear. The DTH reaction was estimated by the vascular permeability in the challenged ear according to Tominaga et al. (14). At 15 hr after the challenge, 0.1 ml of 1% Evans blue saline solution was injected intravenously, and 5 hr later, the animals were exsanguinated and the ears were removed. Evans blue in the ears was extracted and determined as described above. Dye leakage by the

reaction was calculated by subtracting the amount of dye in the control left ear from that of the antigen-challenged right ear. Test drugs were given orally 1 hr before antigen challenge. In the case of chronic treatment, test drugs were given once a day during the 7 day-immunization period (7 times): on days 1 and 7, the drugs were given 1 hr before the antigen application. The inhibitory effects of test drugs were expressed as the percent reduction in dye leakage compared with that determined in vehicle-treated animals.

Statistics

The results obtained were expressed as the mean \pm S.E.M. The data were analyzed by one-way analysis of variance (ANOVA) or the Kruskal-Wallis test. To determine the significance of differences among the groups, Dunnett's or Scheffe's multiple comparison tests were used. The 50% inhibition dose was estimated by median effect analysis (15).

RESULTS

Effect of HQL-79 on PCA in rats

Orally administered HQL-79 dose-dependently inhibited the 48-hr PCA reaction in rats, and a significant effect was observed at doses of 0.1–1 mg/kg (Fig. 2). Epinastine and ketotifen significantly inhibited the PCA reaction at doses of 3–10 mg/kg (Fig. 2). HQL-79 showed substantially higher potency in inhibiting the PCA

reaction in rats than the reference drugs. The doses of HQL-79, epinastine and ketotifen required to inhibit the reaction by 50% (ID_{50} values) were estimated to be 0.16, 2.7 and 2.8 mg/kg, respectively.

Effects of HQL-79 on antigen-induced bronchoconstriction and nasal vascular permeability in actively sensitized guinea pigs

HQL-79 and ketotifen significantly inhibited antigen-induced bronchoconstriction in actively sensitized guinea pigs at doses greater than 0.03 mg/kg (Fig. 3). On the other hand, the significant effect of epinastine was observed at doses of 1–3 mg/kg (Fig. 3). The ID_{50} values of HQL-79, epinastine and ketotifen were estimated to be 0.025, 0.51 and 0.058 mg/kg, respectively.

HQL-79 and ketotifen significantly inhibited antigen-induced nasal vascular permeability in actively sensitized guinea pigs at doses of 0.01–1 mg/kg (Fig. 4). On the other hand, the significant effect of epinastine was observed at doses of 1–10 mg/kg (Fig. 4). The ID_{50} values of HQL-79, epinastine and ketotifen were estimated to be 0.0049, 0.16 and 0.0048 mg/kg, respectively.

The inhibitory effects of HQL-79 on the immediate hypersensitivity reactions such as antigen-induced bronchoconstriction and nasal vascular permeability in actively sensitized guinea pigs were comparable to those of ketotifen and stronger than those of epinastine, in terms of the minimum effective doses.

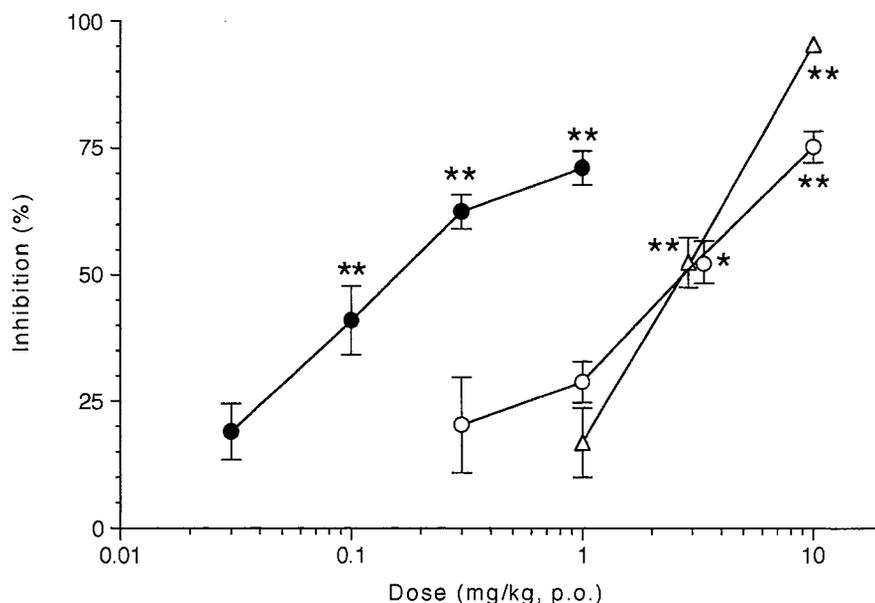


Fig. 2. Effects of HQL-79, epinastine and ketotifen on 48-hr homologous PCA reaction in rats. Test drugs were given orally 1 hr before antigen challenge. Each point represents the mean \pm S.E.M. of 8–12 rats. * $P < 0.05$, ** $P < 0.01$ vs vehicle-treated group (Scheffe's test). ●: HQL-79, △: Epinastine, ○: Ketotifen.

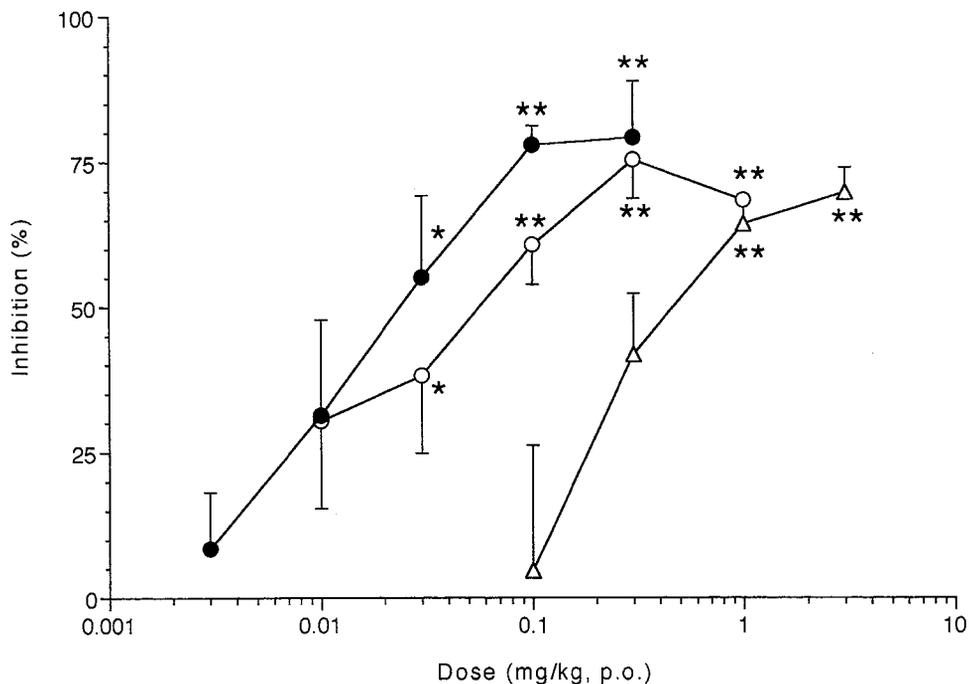


Fig. 3. Effects of HQL-79, epinastine and ketotifen on antigen-induced bronchoconstriction in actively sensitized guinea pigs. Test drugs were given orally 2 hr before antigen challenge. Each point represents the mean \pm S.E.M. of 6-8 guinea pigs. * $P < 0.05$, ** $P < 0.01$ vs vehicle-treated group (Scheffe's test). ●: HQL-79, △: Epinastine, ○: Ketotifen.

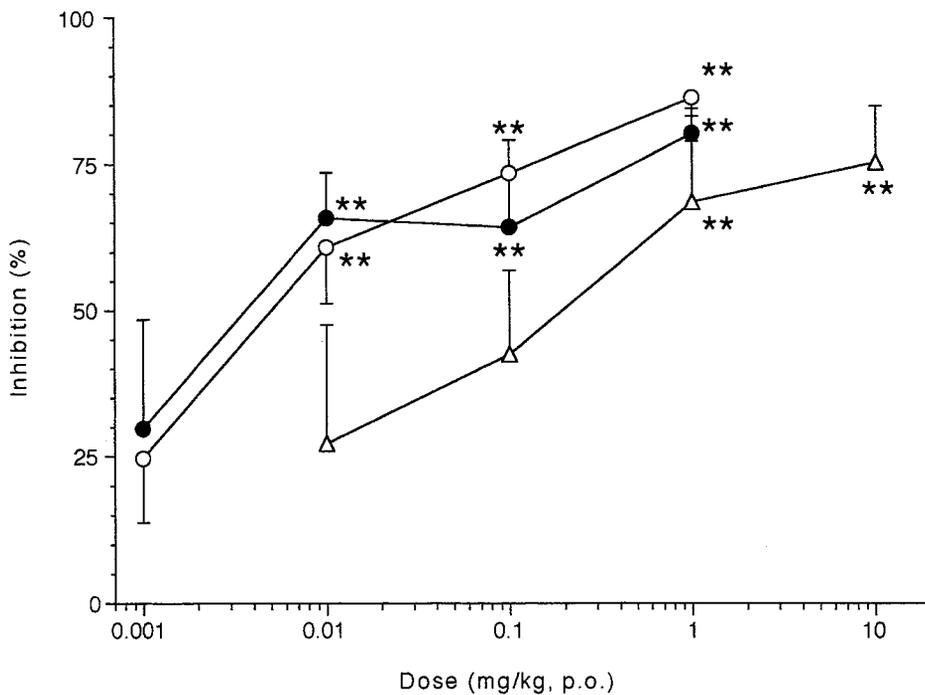


Fig. 4. Effects of HQL-79, epinastine and ketotifen on antigen-induced nasal vascular permeability in actively sensitized guinea pigs. Test drugs were given orally 2 hr before antigen challenge. Each point represents the mean \pm S.E.M. of 11 or 12 guinea pigs. ** $P < 0.01$ vs vehicle-treated group (Scheffe's test). ●: HQL-79, △: Epinastine, ○: Ketotifen.

Effects of HQL-79 on airway leukocyte-infiltration in repeatedly antigen-exposed guinea pigs

Table 1 shows the effects of the chronic administration (10 or 30 mg/kg, 14 times for 2 weeks) of HQL-79 and epinastine on airway leukocyte-infiltration in guinea pigs repeatedly exposed to antigen. Chronic administration of HQL-79 significantly reduced the eosinophil counts in the bronchoalveolar lavage fluid (BALF), and the effect was stronger than that of epinastine. In other experiments, dexamethasone and HQL-79 were administered 4 times from days 20 to 22. Dexamethasone (3, 10 mg/kg) significantly reduced the numbers of total leukocytes and eosinophils. On the other hand, the 3-day treatment with HQL-79 (10, 30 mg/kg) did not suppress the leukocyte infiltration into the guinea pig airway (Table 2).

Effect of HQL-79 on antigen-induced LAR in metyrapone-treated guinea pigs

In the metyrapone-treated guinea pigs, the challenge of

aerosolized antigen provoked a biphasic increase in R_{rs} . As shown in Fig. 5A, R_{rs} of the control-group animals measured at 15 min and at 2, 4, 6 and 8 hr after the final antigen challenge were $164.2 \pm 8.7\%$ and $129.1 \pm 4.3\%$, $147.0 \pm 5.2\%$, $139.7 \pm 4.3\%$ and $138.8 \pm 6.6\%$ of the pre-challenge value (0.509 ± 0.012 cmH₂O/ml/sec), respectively. The chronic administration of HQL-79 (10 or 30 mg/kg, 13 times for 2 weeks) clearly reduced both the immediate- and late-phase increase in R_{rs} (Fig. 5A). As shown in Fig. 5B, R_{rs} of the control-group animals measured at 15 min and 2, 4, 6 and 8 hr after the final antigen challenge were $150.5 \pm 6.3\%$ and $114.9 \pm 5.6\%$, $137.1 \pm 4.2\%$, $127.8 \pm 2.9\%$ and $113.0 \pm 2.6\%$ of the pre-challenge value (0.537 ± 0.013 cmH₂O/ml/sec), respectively. Dexamethasone (3 mg/kg \times 3) did not inhibit the immediate rise in R_{rs} , but significantly inhibited the late-phase response.

Table 1. Effects of the chronic administration of HQL-79 and epinastine on the leukocyte counts in BALF from repeatedly antigen exposed guinea pigs

Drugs	Dose (mg/kg)	n	Total leukocytes	Mononuclear cells (Cell counts, $\times 10^6$ cells)	Eosinophils	Neutrophils
Vehicle	—	6	56.0 \pm 7.8	19.0 \pm 4.2	22.5 \pm 2.5	14.5 \pm 3.7
HQL-79	10	6	39.4 \pm 10.7	13.8 \pm 2.8	12.0 \pm 3.5*	13.6 \pm 5.3
	30	6	30.7 \pm 2.7	14.7 \pm 1.5	5.4 \pm 2.9**	10.6 \pm 2.0
Epinastine	10	7	41.6 \pm 13.5	12.3 \pm 2.7	17.4 \pm 5.3	11.9 \pm 6.3
	30	7	37.1 \pm 9.4	14.6 \pm 2.7	13.0 \pm 3.9	9.4 \pm 4.5

Actively sensitized guinea pigs were exposed to aerosolized antigen on days 8, 15 and 22. HQL-79 and epinastine were given orally 14 times from days 8 to 22. Each value represents the mean \pm S.E.M. of 6 or 7 guinea pigs. *P < 0.05, **P < 0.01 vs vehicle-treated group (Dunnett's test).

Table 2. Effects of the 3-day treatment with dexamethasone and HQL-79 on the leukocyte counts in BALF from repeatedly antigen-exposed guinea pigs

Drugs	Dose (mg/kg)	n	Total leukocytes	Mononuclear cells (Cell counts, $\times 10^6$ cells)	Eosinophils	Neutrophils
Vehicle	—	6	48.6 \pm 8.2	17.7 \pm 2.7	17.7 \pm 3.8	13.1 \pm 3.7
Dexamethasone	3	6	23.5 \pm 6.9*	9.6 \pm 2.8	6.1 \pm 1.3*	7.8 \pm 3.3
	10	6	21.3 \pm 5.8*	10.6 \pm 2.4	7.0 \pm 2.0*	3.7 \pm 2.0
Vehicle	—	7	48.9 \pm 10.5	18.1 \pm 2.9	11.2 \pm 4.0	19.5 \pm 4.3
HQL-79	10	7	37.6 \pm 7.3	16.3 \pm 1.7	8.5 \pm 2.8	12.8 \pm 3.7
	30	7	37.5 \pm 6.0	15.6 \pm 1.8	10.9 \pm 1.6	11.1 \pm 4.2

Actively sensitized guinea pigs were exposed to aerosolized antigen on days 8, 15 and 22. Dexamethasone and HQL-79 were given orally 4 times from days 20 to 22. Each values represents the mean \pm S.E.M. of 6 or 7 guinea pigs. *P < 0.05 vs vehicle-treated group (Dunnett's test).

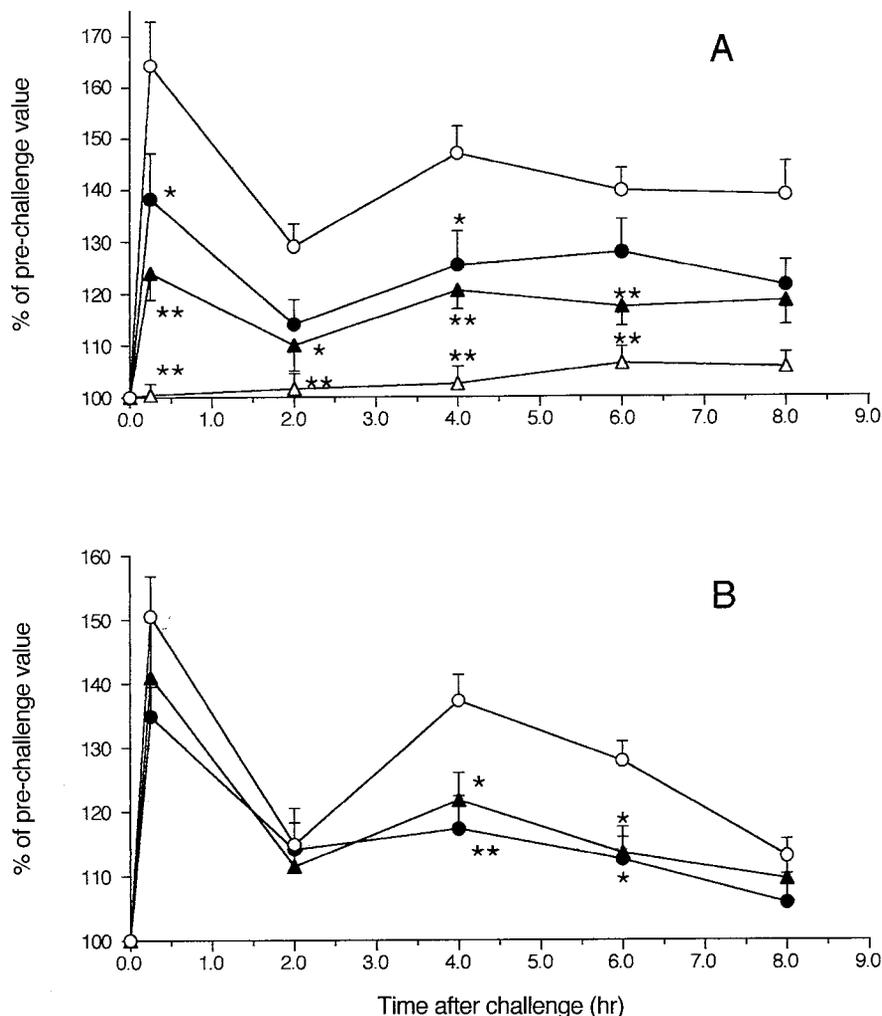


Fig. 5. Effects of HQL-79 (A) and dexamethasone (B) on antigen-induced R_{rs} increase in metyrapone-treated guinea pigs. Actively sensitized guinea pigs were exposed to aerosolized antigen on days 10, 17 and 24. HQL-79 was given orally 13 times from days 10 to 24. Dexamethasone was given orally 3 times from days 22 to 24. Each point represents the mean \pm S.E.M. of 10 guinea pigs. * $P < 0.05$, ** $P < 0.01$ vs vehicle-treated group (Dunnett's test). ○: Vehicle control, ●: HQL-79, 10 mg/kg, ▲: HQL-79, 30 mg/kg, △: Normal (A); ○: Vehicle control, ●: Dexamethasone, 3 mg/kg, ▲: Dexamethasone, 10 mg/kg (B). Pre-challenge values in vehicle-control, HQL-79 (10 mg/kg), HQL-79 (30 mg/kg) and normal groups were 0.509 ± 0.012 , 0.526 ± 0.015 , 0.533 ± 0.023 and 0.573 ± 0.017 cmH₂O/ml/sec, respectively (A); and those in the vehicle-control, dexamethasone (3 mg/kg) and dexamethasone (10 mg/kg) groups were 0.537 ± 0.013 , 0.541 ± 0.024 and 0.537 ± 0.024 cmH₂O/ml/sec, respectively (B).

Effects of HQL-79 on DTH reaction in mice

TDI-induced contact dermatitis in mice was used as a model for examining the effect of HQL-79 on the DTH reaction. As shown in Fig. 6, the chronic treatment with HQL-79 (1–30 mg/kg) during the 7-day immunization period partially, but significantly inhibited the DTH reaction. Single dosing of dexamethasone (0.3 mg/kg) suppressed the DTH reaction to a similar degree. On the other hand, single dosing of HQL-79 (3, 30 mg/kg) did not inhibit the DTH reaction (Table 3). The chronic administration of epinastine had no significant effect on the DTH reaction (Table 4).

DISCUSSION

In the present study, the effects of the newly developed antiallergic drug HQL-79 on immediate hypersensitivity reactions, airway inflammatory responses and DTH were investigated using various experimental models. It was found that the oral administration of HQL-79 was effective in inhibiting not only immediate hypersensitivity reactions but also airway inflammatory responses such as airway eosinophilia and LAR, and the DTH reaction.

HQL-79 showed substantially higher potency in inhibiting the PCA reaction in rats than did epinastine and

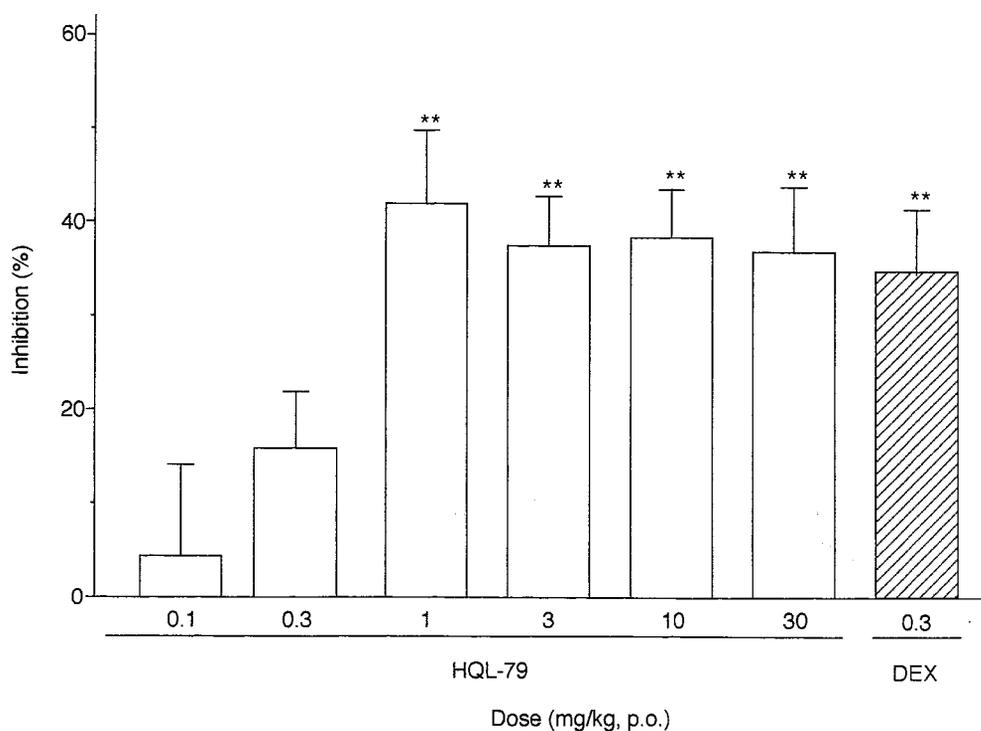


Fig. 6. Effects of HQL-79 and dexamethasone on TDI-induced DTH reaction in mice. The animals were sensitized with TDI for 7 days. HQL-79 was administered daily (7 times) during the immunization period. Dexamethasone (DEX) was administered once 1 hr before antigen challenge. Each column represents the mean \pm S.E.M. of 13–15 mice. ** $P < 0.01$ vs vehicle-treated group (Dunnett's test). The effect of 0.1 and 0.3 mg/kg HQL-79 was tested in an experiment independent of that for testing the effects of the higher doses of HQL-79 and 0.3 mg/kg dexamethasone.

Table 3. Effects of single administration of HQL-79 on the TDI-induced DTH reaction in mice

Drugs	Dose (mg/kg)	n	Dye leakage (μ g/ear)	Inhibition (%)
Vehicle	—	15	11.4 \pm 0.96	—
HQL-79	3	15	10.4 \pm 1.12	8.7
	30	16	11.9 \pm 1.06	–4.6

The animals were sensitized with TDI for 7 days. HQL-79 was administered once at 1 hr before the antigen challenge. Each value represents the mean \pm S.E.M. of 15 or 16 mice.

ketotifen. The inhibitory effects of HQL-79 on antigen-induced bronchoconstriction and nasal vascular permeability in guinea pigs were more potent than those of epinastine, and comparable to those of ketotifen. These results suggest that HQL-79 is highly effective against the immediate hypersensitivity reactions occurring in skin, airway and nasal cavity. Epinastine and ketotifen have been established as potent antiallergic drugs with histamine- H_1 blocking activities (6, 7), and the drugs are used for the treatment of type I allergy related diseases such as bronchial asthma and allergic rhinitis. It is there-

Table 4. Effects of chronic administration of epinastine on the TDI-induced DTH reaction in mice

Drugs	Dose (mg/kg)	n	Dye leakage (μ g/ear)	Inhibition (%)
Vehicle	—	11	11.0 \pm 0.7	—
Epinastine	1	10	10.2 \pm 0.9	7.2
	10	10	10.8 \pm 0.9	1.2

The animals were sensitized with TDI for 7 days. Epinastine was administered daily (7 times) during the immunization period. Each value represents the mean \pm S.E.M. of 10 or 11 mice.

fore possible that the potent antiallergic drug HQL-79 will be clinically useful for treating allergic diseases.

HQL-79 showed a stronger inhibitory effect on histamine-induced skin reaction in rats than the reference drugs (16). The higher potency of HQL-79 in inhibiting the PCA reaction may be ascribed to the stronger antihistaminic activity. However, the PCA reaction in rats was not completely inhibited by HQL-79, but was completely inhibited by epinastine. Epinastine inhibited bradykinin-induced bronchoconstriction in guinea pigs, and the inhibitory action was substantially stronger than

that of ketotifen (17). HQL-79 did not show any significant anti-bradykinin effect in guinea pigs (16). These observations suggest the anti-bradykinin effect as well as antihistaminic effect contribute to the inhibitory action of epinastine on the PCA reaction.

The chronic administration of HQL-79 (10 or 30 mg/kg, 2 weeks) dose-dependently inhibited antigen-induced airway eosinophilia in actively sensitized guinea pigs, and the effect was stronger than that of epinastine. The eosinophil infiltration was clearly inhibited by the administration of dexamethasone for a shorter duration (3 days). It was shown that epinastine inhibited antigen-induced airway eosinophilia in passively sensitized guinea pigs (18). It has been also reported that other histamine- H_1 blocking agents such as KW-4679 (19) and ZCR-2060 (20) inhibited antigen-induced migration of inflammatory cells into guinea pig airway.

In general, experiments for evaluating antigen-induced airway inflammation in guinea pigs have been conducted under the cover of H_1 antagonists such as mepyramine to prevent death from anaphylaxis. It is therefore suggested that these antihistaminics including HQL-79 and epinastine have some non-antihistaminic activities that could be related to the inhibitory actions on antigen-induced airway inflammation in guinea pigs. Leukocyte chemotactic factors such as platelet activating factor (PAF) and leukotriene (LT) B_4 have been shown to induce eosinophil infiltration in vivo (21, 22). Epinastine has been reported to inhibit PAF-induced bronchoconstriction in guinea pigs (23) and LTB_4 -induced chemotaxis of granulocytes in guinea pig dermal tissue (18). KW-4679 inhibited PAF-induced bronchoconstriction and airway eosinophilia in guinea pigs (24). These reports suggest that the anti- H_1 antiallergics affect antigen-induced airway eosinophilia through the antagonistic effects on the eosinophil chemotactants. On the other hand, HQL-79 exhibited neither PAF- nor LTB_4 -antagonism in a receptor binding study. It has been shown that the chronic administration of HQL-79 significantly reduced prostaglandin (PG) D_2 contents and enhanced PGE_2 contents in the lungs of antigen-challenged guinea pigs (16). The differential effects on the prostaglandins might be related to the anti airway-inflammation effect of HQL-79, since PGD_2 is known to activate isolated human eosinophils (25) and PGE_2 is known for its anti-inflammatory and immunomodulatory effects (26, 27).

In the metyrapone-treated guinea pigs, the incidence of LAR was obviously improved. Using this model, it was found that the chronic administration of HQL-79 (10 or 30 mg/kg, 2 weeks) significantly suppressed not only the immediate asthmatic response (IAR) but also the LAR, and dexamethasone selectively inhibited the LAR. This is an important finding concerning the pharmacological

features of HQL-79, because improvement of LAR has been thought to be of therapeutic benefit in human bronchial asthma. Inhaled PGE_2 caused marked inhibition of the immediate and late bronchial obstructions in asthmatic subjects (28). The inhibitory effect of HQL-79 on the antigen-induced IAR and LAR might be ascribed to the increased PGE_2 production in guinea pig lungs.

The effect of HQL-79 on the TDI-induced DTH reaction in mice is one of the unique pharmacological features of this drug. It is well known that TDI, which is widely used in the manufacture of polyurethane foams and other elastomers, is highly irritating to the respiratory organs and skin. It has been reported that low molecular weight antigens such as TDI and picryl chloride induce airway hyperresponsiveness (29, 30) and ear edema (14) in mice. The chronic treatment with HQL-79 (1–30 mg/kg) during the 7-day immunization period partially, but significantly inhibited the TDI-induced DTH reaction in mice, although single dosing of HQL-79 up to 30 mg/kg did not suppress the DTH reaction. These results suggest that HQL-79 affected the immune systems working in the process of sensitization. The inhibitory effect of HQL-79 on the DTH reaction might be also ascribed to the stimulatory effects of the compound on the production of the immunomodulatory PGE_2 (16).

In conclusion, HQL-79 markedly inhibited allergic immediate hypersensitivity reactions such as the antigen-induced cutaneous reaction, bronchoconstriction and nasal vascular permeability. HQL-79, when administered chronically, was also effective in inhibiting airway inflammatory responses and the DTH reaction, similarly to corticosteroids. These effects of HQL-79 will be beneficial for the treatment of allergic inflammatory diseases such as bronchial asthma, allergic rhinitis and allergic dermatitis in humans.

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