

Inhibitory Effect of AL-3264, a New Antiallergic Agent, on the Prausnitz-Küstner Reaction and Leukotriene Production in Monkeys

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ABSTRACT—To clarify the antiallergic effect and antiallergic mechanism of AL-3264 (*N*-[4-[4-(diphenylmethyl)-1-piperazinyl]butyl]-3-(6-methyl-3-pyridyl)acrylamide) in monkeys, its effects on the Prausnitz-Küstner (P-K) reaction, the histamine skin reaction and leukotriene production were examined. In contrast to ketotifen and mepyramine, AL-3264 inhibited the P-K reaction, which is mainly mediated by leukotriene and histamine, more clearly than the skin reaction evoked by histamine alone. AL-3264 also inhibited the leukotriene (LT) production in the broncho-alveolar cells, suggesting that the inhibition of LT production actually contributes to the antiallergic effect of AL-3264.

Keywords: AL-3264, Prausnitz-Küstner reaction, Monkey

N-[4-[4-(Diphenylmethyl)-1-piperazinyl]butyl]-3-(6-methyl-3-pyridyl)acrylamide (AL-3264) is a new antiallergic agent that possesses a 5-lipoxygenase (5-LO) inhibiting property together with the abilities to block histamine H₁-receptors and inhibit histamine release (1), but has very little sedative effect (2). The metabolism of AL-3264 in humans has also been assessed to be similar to that in monkeys, but not in rats.

The monkey is thought to be a good experimental animal in which to develop allergic reactions, i.e., asthma, cedar pollinosis and the Prausnitz-Küstner (P-K) reaction (3–5), because its mast cells and basophils can be passively sensitized with human IgE antibody (6, 7). We recently revealed that the P-K reaction in monkeys is mediated by leukotriene (LT) and histamine, similar to the mediation of human allergic diseases, and proposed that it is useful for the evaluation of antiallergic drugs (unpublished data, I. Yakuo and H. Nakamura).

Some antiallergic drugs with multiple mechanisms of action have been developed, but these *in vivo* mechanisms of action have hardly been compared at the same time and at the effective dose. To assess the antiallergic effect and antiallergic mechanism of AL-3264 in monkeys, the effect of AL-3264 on the P-K reaction was compared with that on the histamine skin reaction in the same monkeys.

Furthermore, its effect on LT production was examined using the broncho-alveolar cells of monkeys pretreated with AL-3264. Broncho-alveolar lavage is a useful

method for the collection of proinflammatory cells and for the diagnosis or treatment of respiratory diseases. The method that is generally used in clinical studies is applied to experimental animals such as monkeys.

Young adult cynomolgus monkeys (Japan SLC, Inc., Hamamatsu) were used for the experiments at an interval of more than a month. The P-K reaction was performed as follows: Forearm skin sites of monkeys (female, 2.5–4 kg) were sensitized with an intradermal injection (20 μ l/site) of 5 times-diluted serum from an asthmatic patient. Two days later, the monkeys were challenged with dust mite antigen extract by an intradermal injection (0.2 μ g/20 μ l/site) into the 6 sensitized skin sites. In the same monkeys, the histamine skin reaction was also induced by an intradermal injection of histamine solution (0.2 μ g/20 μ l/site) into 5 unsensitized forearm skin sites. After 20 min (antigen injection) or 10 min (histamine injection), the wheal diameters at the dermal injection sites were measured by dial calipers, and the wheal size was expressed as the product (mm²) of the longest diameter and the line perpendicular to it. To eliminate non-specific skin reactions, the size of blank wheals, which were induced by an intradermal injection of dust mite extract or saline into unsensitized skin sites, was subtracted from each wheal size. The P-K reaction and the histamine skin reaction were induced in each monkey before and at 1 and 3 hr after the oral administration of test drugs. The inhibitory rate (%) was calculated from the pre-drug value and

the value at 1 or 3 hr after the drug administration.

The ex vivo study of LT production was performed as follows: AL-3264 (5 mg/kg) or vehicle (0.5% tragacanth solution) was orally administered to 5 monkeys (male, 4–6 kg), and the opposite treatment was carried out in each monkey after a month. One hour after the administration, a pediatric fiberoptic bronchoscope with a cannula (BF-P20 type; Olympus, Tokyo) was introduced into the third bronchi of the right lung lobe under anesthesia with ketamine (10 mg/kg, i.m.). Saline (10 ml) warmed at 37°C was instilled into the lobe through the cannula using a syringe and then aspirated gently to collect the bronchoalveolar lavage fluids (BALF). This procedure was repeated 3 times, and the 2nd and 3rd BALF were used for the experiment. Exudated cells in the BALF were centrifuged at 1540 rpm at 4°C for 10 min, washed with Ca^{2+} -free Tyrode solution 2 times, and resuspended into Tyrode solution at a density of 10^5 cells/ml. More than 80% of the cells were confirmed to be macrophages by Giemza staining. The cell suspension was incubated with ionophore A23,187 (0.5 μM) at 37°C for 15 min and then centrifuged at 2,000 rpm at 4°C for 10 min. The level of LTB_4 in the supernatant was measured by RIA.

The following drugs and agents were used: histamine di-

hydrochloride (Nacalai Tesque, Kyoto), dust mite antigen (crude extract) and serum of an asthmatic patient who was sensitized with dust mite (Andard-Mount Ltd., London, UK), mepyramine (Sigma, St. Louis, MO, USA) and LTB_4 RIA kit (Amersham, Tokyo) were purchased. *N*-[4-[4-(Diphenylmethyl)-1-piperazinyl]butyl]-3-(6-methyl-3-pyridyl)acrylamide (AL-3264), oxatomide and ketotifen fumarate were synthesized at Dainippon Pharmaceutical Co., Ltd. The doses of drugs and agents described in this paper refer to the forms presented above.

Statistical significance between groups was analyzed by Student's paired *t*-test.

Figures 1 and 2 show the inhibitory effects of AL-3264, oxatomide, ketotifen and mepyramine on the P-K and histamine skin reactions in monkeys. AL-3264 (1 to 20 mg/kg, p.o.) dose-relatedly inhibited the P-K and histamine skin reactions at 1 and 3 hr after oral administration. The inhibitory effect of AL-3264 on the P-K reaction was more clearly evident than that on the histamine reaction. Oxatomide (5 and 20 mg/kg, p.o.) also inhibited both reactions in a dose-related manner, but its effects were less potent than those of AL-3264. On the contrary, ketotifen (0.2 and 1.0 mg/kg, p.o.) and mepyramine (5 and 10 mg/kg, p.o.) tended to inhibit the histamine skin

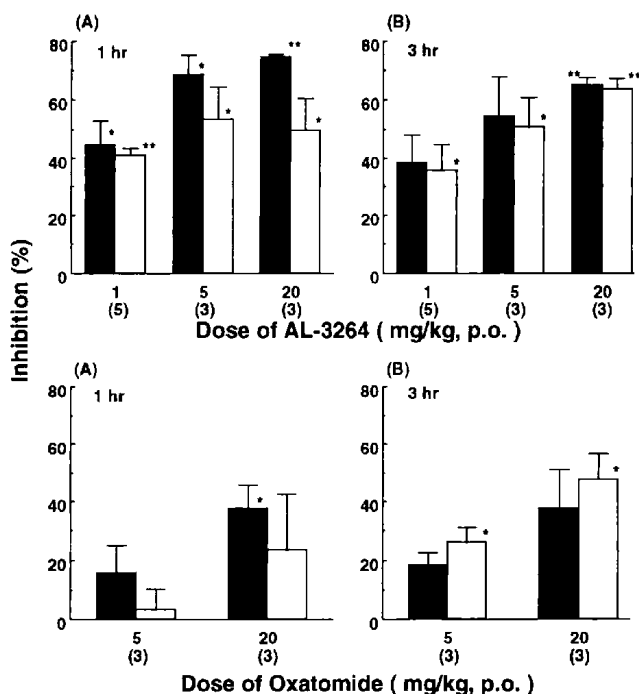


Fig. 1. Effects of AL-3264 and oxatomide on P-K and histamine skin reactions in monkeys. The P-K and histamine skin reactions were caused at 1 hr (A) and 3 hr (B) after drug administration. Each column and bar represents a mean and S.E.M. Closed column: P-K reaction, open column: histamine skin reaction. * $P < 0.05$ and ** $P < 0.01$, significantly different from each pre-drug value. (): number of monkeys.

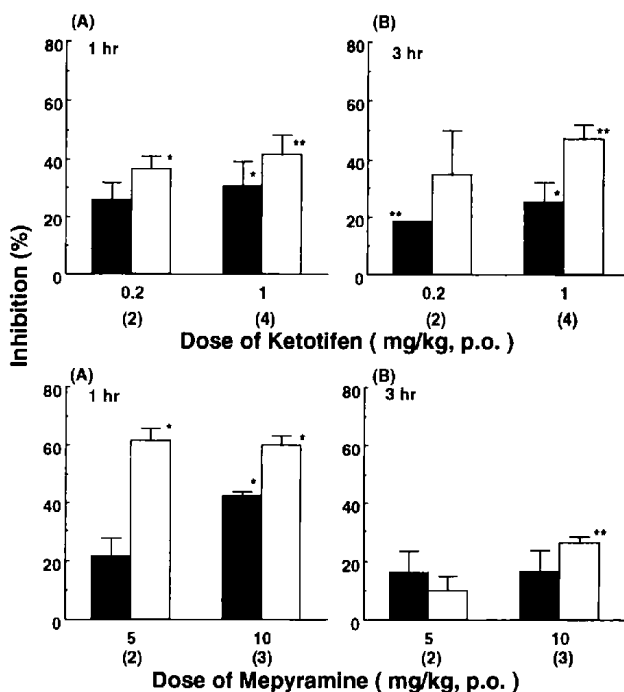


Fig. 2. Effects of ketotifen and mepyramine on P-K and histamine skin reactions in monkeys. The P-K and histamine skin reactions were caused at 1 hr (A) and 3 hr (B) after drug administration. Each column and bar represents a mean and S.E.M. Closed column: P-K reaction, open column: histamine skin reaction. * $P < 0.05$ and ** $P < 0.01$, significantly different from each pre-drug value. (): number of monkeys.

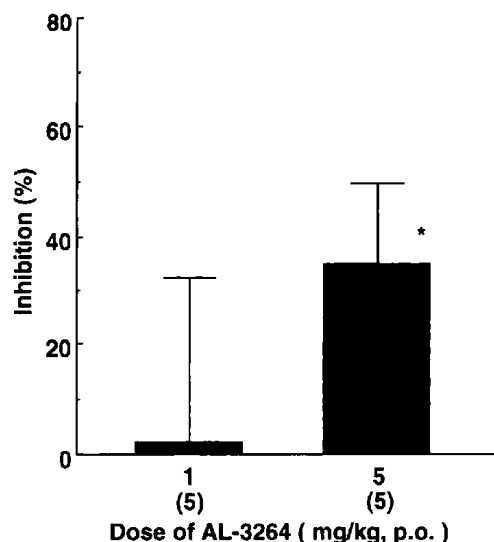


Fig. 3. Effect of AL-3264 on A23,187-induced LTB₄ production from the broncho-alveolar cells obtained from monkeys. The cells were collected 1 hr after administration of AL-3264 or vehicle. Each column and bar represents a mean and S.E.M. * $P < 0.05$, significantly different from each control value. (): number of monkeys.

reaction more clearly than the P-K reaction.

As shown in Fig. 3, AL-3264 (5 mg/kg, p.o.) reduced A23,187-induced LTB₄ production in isolated broncho-alveolar cells obtained from monkeys at 1 hr after oral administration. The levels of LTB₄ in the vehicle control and AL-3264 were 779 ± 164 and 762 ± 235 ng/ 10^5 cells at 1 mg/kg (inhibitory rate: 2.2%) and 757 ± 107 and 494 ± 115 ng/ 10^5 cells at 5 mg/kg (34.7%, $P < 0.05$), respectively. AL-3264 did not affect the cell population in BALF.

Since histamine and LT, especially LT, have been thought to be important mediators of the human allergic reaction, many antiallergic drugs designed to inhibit both mediators have been developed, but not all of them possess sufficient antiallergic effect because of a shortage of inhibitory activity towards LT. To study the balance between the inhibitory effect on LT and the antihistaminic effect, we tried to compare the inhibitory effects of test drugs on the P-K reaction, which was mainly mediated by LT and histamine, similar to the mediation of human allergic diseases (unpublished data, I. Yakuo and H. Nakamura) and on the histamine skin reaction caused simultaneously in the same monkeys. AL-3264 and oxatomide which has multiple actions (8) inhibited the P-K reaction more clearly than the histamine skin reaction at 1 and 3 hr and at 1 hr, respectively. On the contrary, both ketotifen, which has extremely potent antihistaminic action (9), and mepyramine, an antihistamine, showed the opposite inhibitory pattern. These results suggest that AL-3264 potently inhibits the P-K reaction in monkeys

via not only its antihistaminic action but also other actions. The intradermal injection of specific LT antagonists, such as FPL-55712 (20–200 ng/site) and ONO-1078 (0.2–20 ng/site), dose-relatedly and potently inhibited the P-K reaction in monkeys (unpublished observation, I. Yakuo and H. Nakamura), supporting the participation of LT in this reaction. So, it is very likely that the inhibition of the LT system is much involved in the mechanism by which AL-3264 inhibits the P-K reaction. Because it has been reported that AL-3264 inhibits 5-LO activity (1) and reduces LT production in rodents (10), we investigated the effect of AL-3264 on the LT production in monkeys. AL-3264 (5 mg/kg at which the P-K reaction was inhibited) significantly reduced the A-23,187-induced LTB₄ production (inhibitory rate: 34.7%). This effect on the LT production may result in the clearer inhibition of the P-K reaction than that of the skin reaction evoked by histamine alone.

In conclusion, AL-3264 potently inhibited the P-K reaction in monkeys, which is similar to human allergic diseases. It was suggested that AL-3264 inhibited the P-K reaction via its inhibitory effects on both LT production and the histamine reaction.

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