Effect of KW-4679, an Orally Active Anti-allergic Drug, on Antigen-Induced Airway Hyperresponsiveness in Actively Sensitized Guinea Pigs

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ABSTRACT—We investigated the effect of KW-4679 ((Z)-11-[(3-dimethylamino)propylidene]-6,11-dihydrodibenz[b,e]oxepin-2-acetic acid monohydrochloride), an orally active anti-allergic drug, on antigen-induced airway hyperresponsiveness using two different indicators, pulmonary resistance (R_L) and dynamic lung compliance (C_{dyn}), in actively sensitized guinea pigs. Oral administration of KW-4679 (0.1 and 1 mg/kg) 1 hr before aerosolized antigen exposure significantly inhibited the development of airway hyperresponsiveness to inhaled acetylcholine in R_L and C_{dyn} in a dose-dependent manner. Terfenadine (10 mg/kg) also inhibited the development of airway hyperresponsiveness. These results indicate that KW-4679 could be useful in the treatment of bronchial asthma.

Keywords: KW-4679, Airway hyperresponsiveness

Airway hyperresponsiveness (AHR) is one of the most important pathophysiological features of asthma. Accordingly, it is very essential to find a way to alleviate the AHR. To date, virus (1)-, ozone (2)-, platelet activating factor (PAF) (3)-, lipopolysaccharide (4) and antigen (5)-induced AHR models in various animals are widely used in many laboratories. The model of antigen-induced AHR in particular is an essential model for evaluating the efficacy of drugs.

KW-4679 ((Z)-11-[(3-dimethylamino)propylidene]-6,11-dihydrodibenz[b,e]oxepin-2-acetic acid monohydrochloride) is an orally active anti-allergic drug that inhibits both the release and the action of histamine (6, 7). KW-4679 also inhibits the production and/or release of PAF and leukotriene B_4 (L_{TB_4}) in human neutrophils (8). KW-4679 reduces the electrical field stimulation-induced tachykininergic contraction through reduction in the release of transmitters from sensory nerve endings in the isolated guinea pig main bronchus (9). Receptor binding studies have shown this compound to be a histamine H_1-receptor antagonist with a K_i value of 16 nM (10). KW-4679 had neither anti-cholinergic, ganglion blocking, α-adrenergic blocking nor β-adrenergic receptor stimulating properties.

In the present study, we investigated the effect of KW-4679 on antigen-induced AHR to inhaled acetylcholine by using R_L and C_{dyn} as indicators of functional pulmonary alterations in actively sensitized guinea pigs under anesthetization.

Active sensitization and antigen exposure were performed as previously described by Boichot et al. (5), with a slight modification. For active sensitization, male Hartley guinea pigs (Charles River, Yokohama), weighing 350 to 500 g, were exposed twice for 30 min to an aerosol of 0.2% ovalbumin (OA, chicken egg, grade III; Sigma, St. Louis, MO, USA) at a 48-hr interval in a plastic chamber (30 x 50 x 30 cm). The aerosol was generated by an ultrasonic nebulizer (Ultra-NEB 99; DeVilbiss, Sommerset, PA, USA). Under these conditions, the aerodynamic diameter of the nebulized particles was between 0.5 and 3 μm. At 15–20 days after the initial sensitization procedure, the guinea pigs were challenged in the plastic chamber as described above by exposure to aerosols of five successive increasing concentrations of OA (0.01, 0.1, 0.5, 1 and 5 mg/ml) for 15 min each. One hour after the last challenge with the highest concentration of OA, no apparent sign of respiratory failure was observed in the guinea pigs.

KW-4679, which was synthesized in our laboratories, was dissolved in distilled water. Terfenadine (Sigma), which is a non-sedative histamine H_1 antagonist, was suspended in 0.3% carboxymethylcellulose (CMC) solution. Drugs were administered orally 1 hr before the first antigen challenge. As a vehicle-treated group, sensitized guinea pigs were administered distilled water or 0.3% CMC solution instead of drugs.
Twenty-four hours after the antigen challenge, the guinea pigs were anesthetized with urethane (1.2 g/kg, i.p.; Tokyo Kasei Kogyo, Tokyo) and artificially ventilated by a respiratory pump (Ugo Basile, Varese, Italy) with a constant volume of 1 ml laboratory air/100 g body weight at 60 breaths/min. Spontaneous breathing was abolished by injection of gallamine triethiodide (Sigma, 3 mg/animals) into the jugular vein. After a sufficient stabilization period, five successive 1-min aerosol administrations of acetylcholine chloride (ACh; Daiichi Pharmaceutical Co., Ltd., Tokyo; 20, 50, 100, 150 and 200 µg/ml) were performed. The aerosol was generated by an ultrasonic nebulizer connected to the inspiratory line of the respiratory pump using three-way breathing valves. The airflow signal and the pressure signal measured by pressure transducers were electronically integrated using a computerized pulmonary function monitoring system (Model 6; Buxco Electronics, Sharon, CT, USA). RL and Cdyn were simultaneously calculated by this system according to the method of Aoki et al. (11).

Results are each expressed as the mean±S.E.M. of the indicated number of experiments. The statistical differences were analyzed by Student’s t-test or the Aspin-Welch’s test. In the case of the experiment of KW-4679 pretreatment, Dunnett’s or Steel’s multiple comparison test followed by the Bartlett test was used. A value of P less than 0.05 was accepted as statistically significant.

First, the following four groups were examined: unsensitized and saline-challenged animals (saline-saline), unsensitized and OA-challenged animals (saline-OA), sensitized and saline-challenged animals (OA-saline) and sensitized and OA-challenged animals (OA-OA). The groups of unsensitized animals and saline-challenged animals were exposed to saline instead of OA under the same conditions in the sensitization procedure and in the challenge procedure, respectively. Inhalation of ACh produced a concentration-dependent increase in RL and reduction in Cdyn (Fig. 1). In the group of OA-OA, the AHR to ACh was clearly observed; that is, the alterations in both RL and Cdyn were significantly enhanced at 20, 50 and 100 µg/ml of ACh compared to the other groups. In contrast, no significant alterations were observed in both RL and Cdyn at each concentration of ACh between the groups of saline-saline, saline-OA and OA-saline. In addition, there were slight but significant reductions in the basal Cdyn values measured just before ACh exposure in the group of OA-OA.

The oral treatment of 0.1 and 1 mg/kg of KW-4679 1 hr before antigen challenge significantly prevented the development of AHR (Fig. 2). A significant inhibition was observed in RL in a dose-dependent manner. In the experiment on the OA-saline group, treatment of 1 mg/kg of KW-4679 1 hr before the saline challenge almost never influenced the airway responsiveness to ACh in both RL and Cdyn (data not shown). It is therefore considered that the alterations of airway responsiveness caused by pretreatment of KW-4679 were due to the prevention of the development of AHR, not a bronchodilative action or an anti-cholinergic action. The oral pretreatment of 10 mg/kg of terfenadine also significantly inhibited the development of AHR (Fig. 3).

In the present study, we demonstrated that KW-4679 inhibited the development of AHR to inhaled ACh elicited by the five successive antigen challenges in actively
sensitized guinea pigs. The present experimental model was developed without any pharmacological modulation, which involved various mediators including histamine. In addition, we measured RL and Cdyn simultaneously to assess airway responsiveness separately in the more central airways and in the more peripheral airways. Accordingly, we considered that it is a more favorable experimental model of asthma as a pulmonary disease and a good model for evaluation of drugs that possess antihistaminic properties such as KW-4679 and terfenadine. From our observations, Cdyn was more sensitive than RL as an indicator of airway responsiveness to ACh.

In a recent study, Mauser et al. (12) reported that bronchoscopy at 24 hr after antigen challenge showed clear signs of inflammation in peripheral airways such as tissue edema and hyperemia. Moreno et al. (13) demonstrated by morphological and physiological studies that airway wall thickness is one of the mechanisms of the development of AHR. We also observed a significant baseline reduction in Cdyn. Accordingly, it is likely that successive antigen exposures induce the development of AHR through the manifestation of edema in peripheral airways, although it has not been fully elucidated.

It has been demonstrated that a variety of mediators such as thromboxanes (TXs), leukotrienes, PAF and tachykinins participate in the development of AHR.
Recently, Santing et al. (14) reported that histamine may contribute to the antigen-induced AHR in guinea pigs. The development of AHR, not surprisingly, was markedly reduced by drugs that inhibit both the release and the action of histamine. It is therefore likely that the inhibitory effect of drugs such as KW-4679 and terfenadine on the development of AHR is based on their anti-histaminic properties. We also found that KW-4679 (1 mg/kg, p.o.) inhibited the production and/or release of TXB2 from leukocytes in bronchial alveolar lavage fluid of guinea pigs (Y. Sasaki, unpublished data). On the other hand, we recently demonstrated that KW-4679 inhibits the antigen-induced development of AHR through the prevention of eosinophil infiltration into guinea pig airways using a mild-symptoms experimental model that utilizes a single-antigen challenge with metyrapone (15). Consequently, although the anti-asthmatic mechanism of KW-4679 has not been fully elucidated, it is considered that not only the inhibition of leukocyte infiltration but also the prevention of the action of chemical mediators (e.g., edema formation) such as TXA2, LTB4, PAF, tachykinins and especially histamine could contribute to the inhibitory effect of KW-4679 on the development of AHR.

In conclusion, we demonstrated that pretreatment by the oral administration of KW-4679 markedly inhibited the development of AHR elicited by the successive antigen challenge in actively sensitized guinea pigs. These findings suggest that KW-4679 will be beneficial for the treatment of bronchial asthma.

REFERENCES


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