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RESEARCH PAPER

Effects of KP-496, a novel dual antagonist at the cysteinyl leukotriene receptor 1 and the thromboxane A_2 receptor, on airway obstruction in guinea pigs

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Background and purpose: KP-496 is a novel dual antagonist for cysteinyl leukotriene receptor 1 ($CysLT_1$) and thromboxane A_2 (TXA_2) receptor (TP). The aim of this study was to evaluate the pharmacological profile of inhaled KP-496 and its effects on airway obstruction.
Experimental approach: Antagonist activities of inhaled KP-496 were investigated using bronchoconstriction induced in guinea pigs by LTD_4 or U46619, a stable TXA_2 mimetic. Guinea pigs sensitized with injections of ovalbumin were used to assess the effects of inhaled KP-496 on bronchoconstriction induced by antigen (i.v.). Another set of guinea pigs were sensitized and challenged with ovalbumin by inhalation and the effects of inhaled KP-496 on immediate and late airway responses and airway hyperresponsiveness were investigated.
Key results: KP-496 significantly inhibited LTD_4- and U46619-induced bronchoconstriction in a dose-dependent manner. The inhibitory effects of KP-496 (1%) were comparable to those of montelukast (a $CysLT_1$ antagonist, p.o., 0.3 mg kg^{-1}) or seratrodast (a TP antagonist, p.o., 3 mg kg^{-1}). KP-496 (1%) and oral co-administration of montelukast (10 mg kg^{-1}) and seratrodast (20 mg kg^{-1}) significantly inhibited antigen-induced bronchoconstriction, whereas administration of montelukast or seratrodast separately did not inhibit antigen-induced bronchoconstriction. KP-496 exhibited dose-dependent and significant inhibitory effects on the immediate and late airway responses and airway hyperresponsiveness following antigen challenge.
Conclusions and implications: KP-496 exerts effects in guinea pigs which could be beneficial in asthma. These effects of KP-496 were greater than those of a $CysLT_1$ antagonist or a TP antagonist, in preventing antigen-induced airway obstruction.
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Keywords: dual antagonist; leukotriene D_4; thromboxane A_2; bronchoconstriction; guinea pig

Abbreviations: AHR, airway hyperresponsiveness; $CysLT_1$, cysteinyl leukotriene receptor 1; IAR, immediate airway response; LAR, late airway response; LT, leukotriene; OA, ovalbumin; sRaw, specific airway resistance; TP, TXA_2 receptor

Introduction

Bronchial asthma is characterized by reversible airway obstruction, airway hyperresponsiveness (AHR) and chronic airway inflammation. Allergen provocation causes a biphasic airway obstruction, with an immediate asthmatic response and a late asthmatic response, in atopic asthmatics (Pepys and Hutchcroft, 1975). The immediate response peaks 15–30 min after provocation and disappears within 1–3 h. The late response appears 4–8 h after antigen provocation and lasts several hours. The late response is accompanied by AHR (Cartier *et al*., 1982), increased airway responsiveness to non-specific stimulation, and by an influx of inflammatory cells (De Monchy *et al*., 1985).

Various chemical mediators and cytokines are involved in the onset and development of the pathogenesis of asthma. Among these mediators, leukotriene D_4 and thromboxane A_2 are potential targets of antiasthmatic drugs. They are thought to play important roles in airway smooth-muscle contraction (Svenssen *et al*., 1977; Dahlen *et al*., 1980), development of mucosal oedema (Camp *et al*., 1983; Inoue

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et al., 2002), mucosal secretions (Coles *et al*., 1983), and AHR (Fujimura *et al*., 1986; Abraham *et al*., 1993), and to lead to narrowing of the airways. There is some evidence that production of LTs and TXA_2 is increased in asthmatics (Creticos *et al*., 1984; Smith *et al*., 1992). Currently, some cysteinyl leukotriene receptor 1 ($CysLT_1$) antagonists, such as montelukast (Jones *et al*., 1995), pranlukast (Obata *et al*., 1992) and zafirlukast (Krell *et al*., 1990), and a TXA_2 receptor (TP) antagonist, seratrodast (Ashida *et al*., 1989), are being used in the treatment of asthma.

Leukotrienes and TXA_2 exert biological activities via different pathways. Regulation of the activities of both mediators would thus provide more potent and beneficial therapeutic effects in asthmatics than the $CysLT_1$ antagonists or the TP antagonist that have been launched. On the basis of this concept, KP-496 (2-{*N*-[4-(4-chlorobenzenesulphonylamino) butyl]-*N*-{3-[(4-isopropylthiazol-2-yl)methoxy]benzyl} sulphamoyl}benzoic acid), a novel dual antagonist at $CysLT_1$ and TP receptors (Ishimura *et al*., 2006; Kurokawa *et al*., 2007), was synthesized. KP-496 exhibited potent antagonistic activities against leukotriene D_4 (LTD_4) and U46619 and, *in vitro*, its antagonistic activities were comparable to those of $CysLT_1$ antagonists and a TP antagonist.

In this study, we evaluated, *in vivo*, the antagonist activities of inhaled KP-496, expecting that inhalational administration would maximize the pharmacological properties of KP-496. To clarify the advantages of dual antagonist activities, the effect of inhaled KP-496 on antigen-induced bronchoconstriction was investigated in guinea pigs sensitized with ovalbumin (OA) and the effects of KP-496 were compared with those of a $CysLT_1$ antagonist (montelukast) and a TP antagonist (seratrodast). The effects of inhaled KP-496 on airway responses (immediate airway response (IAR), late airway response (LAR) and AHR), following antigen challenge were also evaluated in guinea pigs sensitized by inhalation of OA.

Methods

Animals

The handling and treatment of the animals were in accordance with the guidelines of the Japanese Association for Laboratory Animal Science (1987). Male Hartley guinea pigs (5 weeks old, 300–400 g) were purchased from Kyudo (Kumamoto, Japan) and JAPAN SLC (Hamamatsu, Japan).

Drug treatment

The dry powder formulation, KP-496DPI, was suspended in an aqueous solution of carboxymethyl cellulose (0.5%) to prepare the 1% KP-496 suspension. The carboxymethyl cellulose containing vehicle for KP-496DPI was used as vehicle suspension. The 1% KP-496 suspension was diluted with the vehicle suspension to prepare 0.03, 0.1 and 0.3% KP-496 suspensions before use.

KP-496 suspensions or vehicle were administered as suspension aerosols generated by a pressure nebulizer (Pari GmbH, Starnberg, Germany). The suspension aerosol was introduced into an exposure chamber (MIPS, Osaka, Japan) and the animals were exposed for 10 min. Montelukast and seratrodast were suspended in 0.5% MC and given orally.

Measurement of mediator-induced bronchoconstriction

Animals were anaesthetized with urethane. Cannulas were inserted into the trachea and external jugular vein. Spontaneous respiration was stopped with succinylcholine chloride ($2\,mg\,kg^{-1}$, intravenous (i.v.)) and the tracheal cannula was connected to a constant volume respirator. Artificial respiration was performed at a rate of 60 strokes per minute with a tidal volume of 3 ml. Bronchoconstriction was measured according to the overflow method described by Konzett and Rössler (1940), using a bronchospasm transducer (Ugo Basile, Milano, Italy) connected to the tracheal cannula. Bronchoconstriction was induced by U46619 ($0.6\,\mu g\,kg^{-1}$, i.v.) and LTD_4 ($3\,\mu g\,kg^{-1}$, i.v.) 54 and 60 min after drug administration, respectively. The animals were pretreated with indomethacin ($5\,mg\,kg^{-1}$, i.v.), a cyclooxygenase inhibitor, 5 min before LTD_4 challenge. U46619-induced bronchoconstriction was a transient response, and overflow volume returned to baseline within 1 min. It was confirmed that pretreatment with U46619 had no effect on LTD_4-induced bronchoconstriction. Bronchoconstriction was expressed as the percentage of maximum overflow volume obtained by clamping the tracheal cannula. The effects of drug treatments on U46619- and LTD_4-induced bronchoconstriction were evaluated by the maximum bronchoconstriction occurring within 1 and 15 min after challenge, respectively.

Measurement of antigen-induced bronchoconstriction in guinea pigs sensitized by injected antigen

Animals were sensitized with 5 mg OA by intraperitoneal and subcutaneous injection. Three days later, animals were given a booster injection of 5 mg antigen by intraperitoneal injection. Twenty-one days after the first sensitization, drugs were administered to sensitized animals, as described above. The animals were anaesthetized with urethane and antigen-induced bronchoconstriction was measured as described above. Bronchoconstriction was induced by OA ($4\,mg\,kg^{-1}$, i.v.) 1 h after drug administration and measured continuously for 15 min. The animals were pretreated with pyrilamine ($1\,mg\,kg^{-1}$), an H_1 receptor antagonist, 5 min before antigen challenge. The effect of drug treatment on antigen-induced bronchoconstriction was evaluated by the area under the curve for bronchoconstriction for 15 min.

Measurement of antigen-induced airway responses, IAR, LAR and AHR after sensitization by inhaled antigen

Animals were sensitized by exposure to aerosolized OA (1% in saline) for 10 min once a day for eight consecutive days. The aerosol was generated by an ultrasonic nebulizer (NE-U12, OMRON, Tokyo, JAPAN). Seven days after the last sensitization, drugs were administered as described above. The animals were then challenged by exposure to aerosolized antigen (2% OA in saline) for 5 min, 1 h after drug administration. The animals were pretreated with pyrilamine ($10\,mg\,kg^{-1}$, i.p.) 30 min before antigen challenge and

metyrapone (10 mg kg^{-1}, i.v.), a cortisol synthesis inhibitor, 24 and 1 h before antigen challenge.

Airway resistance, specific airway resistance (sRaw), in conscious guinea pigs was measured with a two-chambered, restrained, whole-body plethysmograph. The conscious animal was positioned with the head extending through the partition of the two-chambered, rectangular plastic box. The two chambers were joined by spring clamps, and a latex collar between chambers formed a seal around the neck. Each chamber was fitted with an identical wire screen and identical flow sensors. These flow sensors were connected to an airway resistance measuring system (MIPS, Osaka, Japan) to determine sRaw. Airway resistance was monitored before and 1 min, 2, 4, 5, 6, 7, 8 and 24 h after antigen challenge. The animals were removed from the plethysmograph between measurements. The effects of drugs on IAR and LAR were evaluated by percent changes in sRaw 1 min after antigen challenge from that before antigen challenge (baseline), and area under the curve for percentage change in sRaw between 4 and 8 h after antigen challenge, respectively.

Twenty-four hours after antigen challenge, animals were exposed to increasing concentrations of aerosolized acetylcholine (ACh) (0.0625–1 mg ml^{-1}) for 1 min, and then sRaw was measured with the airway resistance measuring system. AHR was evaluated by the concentration of ACh required to produce a 100% increase in sRaw, determined with each concentration–response curve to ACh.

Measurement of KP-496 in the lungs

To measure the concentration of KP-496 in the lungs, animals were exposed to the drug (KP-496 suspension, 0.1–1%) in the exposure chamber, as described above, and immediately after the exposure, they were anesthetized with urethane and the lungs were removed. The amount of KP-496 in the lungs was determined by LC-MS/MS methods. Estimated whole-body doses were calculated by dividing the amount of KP-496 in the lungs by the body weight of animals.

Statistical analysis

Values shown are the mean ± s.d. Area under the curve was calculated based on the percent-change curve of sRaw between 4 and 8 h by trapezoidal method. The percent-change curve of sRaw was obtained by plotting the percent change of sRaw versus time (Figure 3). Differences between means were assessed by one-way analysis of variance followed by Dunnett's multiple range test or Student's *t*-test, as appropriate. Values of $P<0.05$ were considered significant.

Drugs and chemicals

KP-496, montelukast and seratrodast were synthesized by Kaken Pharmaceutical Co. Ltd (Tokyo, Japan). A dry powder formulation of KP-496 (KP-496DPI, dry powder inhaler of KP-496) and vehicle for KP-496DPI were also prepared by Kaken Pharmaceutical Co. Ltd. Methylcellulose was purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan). Urethane, indomethacin, OA (Grade V), pyrilamine maleate and metyrapone were purchased from Sigma (St Louis, MO, USA). Succinylcholine chloride was purchased from Tokyo Kasei (Tokyo, Japan). U46619 and LTD_4 were purchased from Cayman Chemical Co. (Ann Arbor, MI, USA). Acetylcholine chloride (Ovisot for injection) was purchased from Daiichi Pharmaceutical Co. Ltd (Tokyo, Japan). Carboxymethyl cellulose was purchased from Nacalai Tesque Inc. (Kyoto, Japan).

Results

Effects of KP-496 on LTD_4- and U46619-induced bronchoconstriction

Challenge with LTD_4 (3 μg kg^{-1}, i.v.) induced a biphasic bronchoconstriction that reached a peak in 2 min and increased gradually again after the first peak disappeared. Inhaled KP-496 or montelukast (per OS (p.o.)) significantly inhibited LTD_4-induced bronchoconstriction in a dose-dependent manner (Figure 1). KP-496 and montelukast reached maximum inhibitory effects at doses of 1% and 0.3 mg kg^{-1}, respectively, and had maximum percent inhibition of 82 and 88%, respectively.

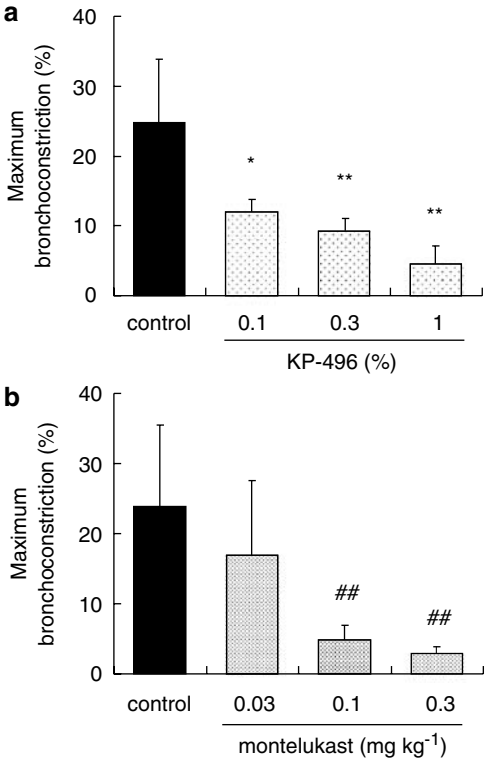

Figure 1 Effects of KP-496 (**a**) and montelukast (**b**) on LTD_4-induced bronchoconstriction in guinea pigs. KP-496 (0.1–1%, inhalation) and montelukast (0.03–0.3 mg kg^{-1}, p.o.) were administered 60 min before challenge with LTD_4 (3 μg kg^{-1}, i.v.). Results are expressed as maximum bronchoconstriction. Each value represents the mean ± s.d. of 8–9 animals. *$P<0.05$ and **$P<0.01$ compared with inhalation control, $^{\#\#}P<0.01$ compared to oral control (Dunnett's multiple range test). i.v., intravenous; LTD_4, leukotriene D_4.

Challenge with U46619 (0.6 μg kg^{-1}, i.v.) also induced bronchoconstriction, which reached a peak in 1 min. Inhaled KP-496 and seratrodast (p.o.) significantly inhibited U46619-induced bronchoconstriction in a dose-dependent manner (Figure 2). KP-496 and seratrodast reached maximum inhibitory effects at doses of 1% and 3 mg kg^{-1}, respectively, and had maximum percent inhibition of 89 and 94%, respectively.

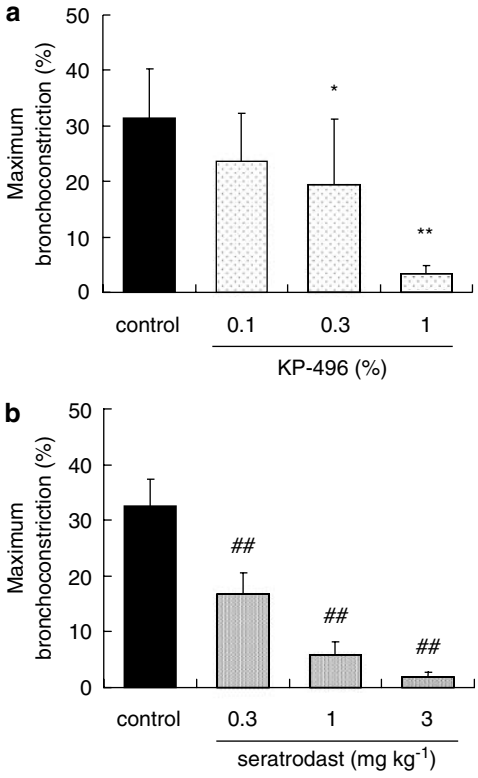

Figure 2 Effects of KP-496 (**a**) and seratrodast (**b**) on U46619-induced bronchoconstriction in guinea pigs. KP-496 (0.1–1%, inhalation) and seratrodast (0.3–3 mg kg^{-1}, p.o.) were administered 54 min before U46619 challenge (0.6 μg kg^{-1}, i.v.). Results are expressed as maximum bronchoconstriction. Each value represents the mean ± s.d. of eight animals. *$P<0.05$ and **$P<0.01$ compared with inhalation control, $^{\#\#}P<0.01$ compared with oral control (Dunnett's multiple range test). i.v., intravenous.

Effect of KP-496 on antigen-induced bronchoconstriction

Antigen challenge (4 mg kg^{-1}, i.v.) induced a biphasic bronchoconstriction that reached a peak in 2 min and increased gradually again after the peak disappeared in actively sensitized animals. However, saline challenge (i.v.) did not induce bronchoconstriction in actively sensitized animals (data not shown). Inhaled KP-496 at a concentration of 1% inhibited antigen-induced bronchoconstriction significantly, with 47% inhibition (Figure 3). Administration of montelukast (10 mg kg^{-1}, p.o.) or seratrodast (20 mg kg^{-1}, p.o.) separately did not inhibit antigen-induced bronchoconstriction. However, combination of these doses of montelukast and seratrodast inhibited antigen-induced bronchoconstriction significantly, by 39% (Figure 3).

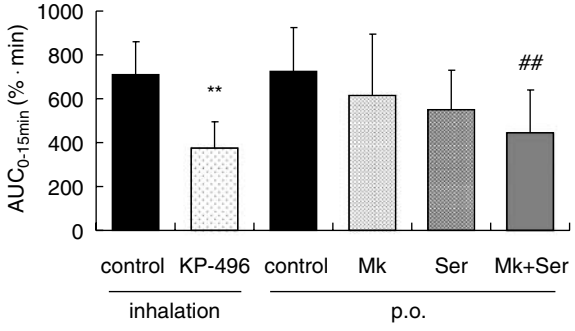

Figure 3 Effects of KP-496, montelukast (Mk), and seratrodast (Ser) on antigen-induced bronchoconstriction in actively sensitized guinea pigs. KP-496 (1%, inhalation), Mk (10 mg kg^{-1}, p.o.) and Ser (20 mg kg^{-1}, p.o.) were administered 1 h before antigen challenge (4 mg kg^{-1}, i.v.). Results are expressed as area under the curve for bronchoconstriction over 15 min ($AUC_{0-15\,min}$). Each value represents the mean ± s.d. of 9–10 animals. **$P<0.01$ compared with inhalation control (Student's *t*-test), $^{\#\#}P<0.01$ compared with oral control (Dunnett's multiple range test). i.v., intravenous.

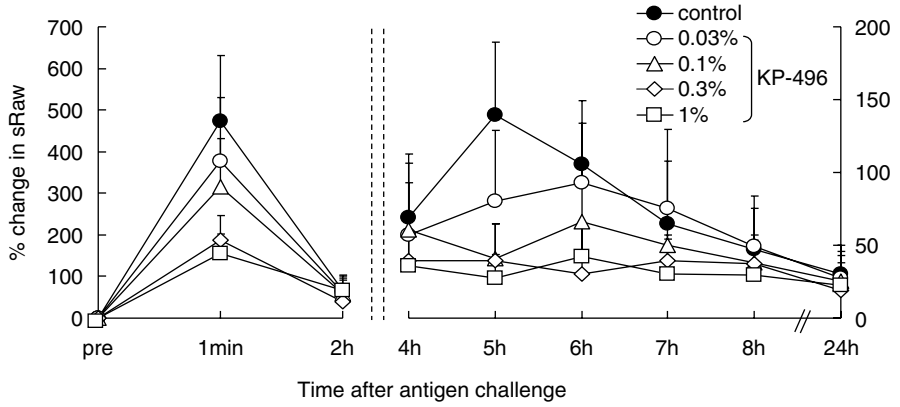

Figure 4 Effect of KP-496 on the increase in sRaw induced by antigen challenge in actively sensitized guinea pigs. KP-496 (0.03–1%, inhalation) was administered 1 h before antigen challenge (2%, inhalation). The increase in sRaw is shown as the the IAR (0–2 h; left hand graph) and the LAR (4–8 h; right hand graph). Each value represents the mean ± s.d. of 10 animals. IAR, immediate airway response; sRaw, specific airway resistance; LAR, late airway response.

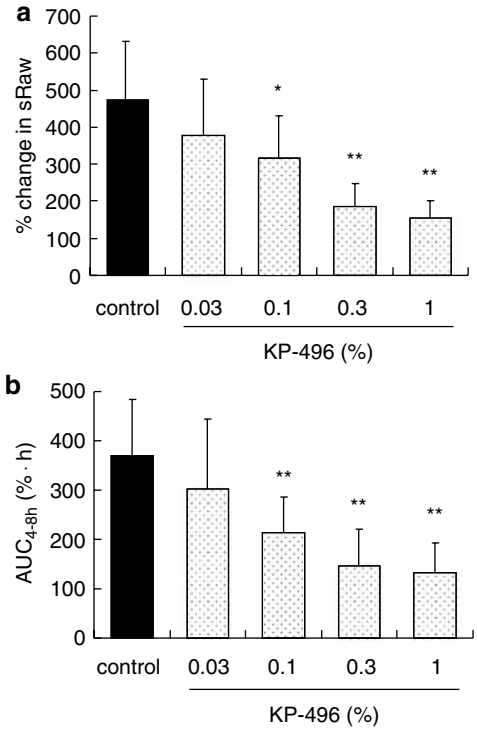

Figure 5 Effects of KP-496 on the IAR (**a**) and the LAR (**b**) induced by antigen challenge in actively sensitized guinea pigs. KP-496 (0.03–1%, inhalation) was administered 1 h before antigen challenge (2%, inhalation). The IAR is expressed as percent change in sRaw 1 min after antigen challenge. The LAR is expressed as area under the curve for percent change in sRaw from 4–8 h after antigen challenge ($AUC_{4-8\,h}$). Each value represents the mean ± s.d. of 10 animals. $^{*}P<0.05$ and $^{**}P<0.01$ compared with control (Dunnett's multiple range test). AUC, area under the curve; IAR, immediate airway response; LAR, late airway response; sRAW, specific airway resistance.

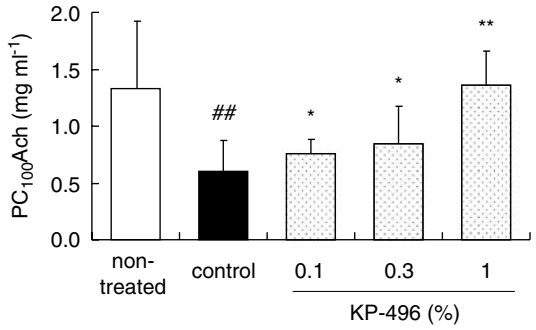

Figure 6 Effect of KP-496 on antigen-induced AHR in actively sensitized guinea pigs. KP-496 (0.1–1%, inhalation) was administered 1 h before antigen challenge (2% OA, inhalation). Animals were exposed to increasing concentrations of aerosolized ACh (0.0625–1 mg ml^{-1}) for 1 min, 24 h after antigen challenge. Results are expressed as the concentration of ACh to induce 100% increase in sRaw (PC_{100}ACh). Each value represents the mean ± s.d. of 10 animals. $^{*}P<0.05$ and $^{**}P<0.01$ compared with control (Dunnett's multiple range test), $^{\#\#}P<0.01$ compared with the non-treated group (Student's *t*-test). ACh, acetylcholine; sRaw, specific airway resistance.

Table 1 Amount of KP-496 in the lungs of guinea pigs, and the estimated doses

Dose (%)	n	*Amount of KP-496 in the lung (μg per lung)*	*Estimated dose*[a] *(μg kg^{-1})*
0.1	4	0.3 ± 0.04	0.55 ± 0.09
0.3	4	1.24 ± 0.28	2.27 ± 0.52
1	4	8.18 ± 3.38	14.54 ± 5.23

[a]The estimated doses were calculated by dividing the amount of KP-496 in the lungs by the body weight of animals. Dose refers to the concentration of KP-496 in the aerosol to which the guinea pigs were exposed (see Methods).

Effects of KP-496 on antigen-induced IAR and LAR

Inhalation of aerosolized antigen (2% OA) induced a biphasic airway response in actively sensitized animals (Figure 4). The IAR was a marked increase in sRaw within 1 min after antigen challenge. sRaw gradually decreased and returned to the baseline by 2 h after antigen challenge. The LAR was observed from 4 to 8 h after antigen challenge. Inhalation of aerosolized saline did not induce IAR and LAR in actively sensitized animals (data not shown).

Inhaled KP-496 inhibited the IAR in a dose-dependent manner (Figure 5a); thus, inhaled KP-496 at doses of 0.1, 0.3 and 1% reduced IAR by 33, 61 and 67%, respectively, and exhibited statistically significant effects.

Inhaled KP-496 also inhibited the LAR in a dose-dependent manner (Figure 5b), with inhaled KP-496 at doses of 0.1, 0.3 and 1% reducing LAR by 44, 60 and 64%, respectively, and exhibited statistically significant effects.

Effect of KP-496 on antigen-induced airway hyperresponsiveness

Inhalation of aerosolized ACh induced concentration-dependent increases in sRaw, when measured 24 h after antigen challenge in actively sensitized animals. Significant enhancement of AHR was observed in the antigen-challenged control group, compared with the saline-challenged non-treated group. Inhaled KP-496 inhibited the decrease of the provocation concentration causing 100% increase (PC_{100}ACh) in a dose-dependent manner. PC_{100}ACh was improved to the level of the non-treated group in the antigen-challenged sensitized animals treated with 1% KP-496 group (Figure 6).

Amount of KP-496 in lungs

The amount of KP-496 found in the lungs of guinea pigs immediately after exposure to a range of concentrations, was increased in a dose-dependent manner (Table 1).

Discussion

In this study, we evaluated the antagonist activities of inhaled KP-496 *in vivo* on bronchoconstriction induced in guinea pigs by exogenous mediators or by antigen challenge. Inhaled KP-496 inhibited LTD_4- and U46619-induced bronchoconstriction in a dose-dependent manner. Inhaled KP-496 also inhibited airway responses (IAR, LAR and AHR) to endogenous mediators generated as a result of antigen challenge. Furthermore, inhaled KP-496 exhibited a

significant inhibitory effect on antigen-induced bronchoconstriction, which was not inhibited by montelukast or seratrodast given separately.

Inhaled KP-496 significantly inhibited LTD_4- and U46619-induced bronchoconstriction at doses of 0.1 and 0.3%, respectively. It is difficult to compare the antagonist activities of KP-496 for $CysLT_1$ and TP, since LTD_4 caused persisting bronchoconstriction and U46619 caused transient bronchoconstriction. However, these results indicated that the antagonist activities of KP-496 for $CysLT_1$ and TP were exhibited in the same dose range.

In asthmatic patients, a variety of chemical mediators, including histamine, LTs, TXA_2 and platelet-activating factor, are involved in bronchoconstriction. These mediators are released from mast cells after IgE-dependent activation and interact with each other. LTD_4- or platelet-activating factor-induced bronchoconstriction was inhibited by a TP antagonist (Aizawa *et al*., 1996) and a TXA_2 synthetase inhibitor (Sakurai *et al*., 1994). Bronchoconstriction induced by a TXA_2 analogue was also found to be partly mediated by LTD_4 or platelet-activating factor (Kawikova *et al*., 1996). It is known that these mediators are involved in antigen-induced bronchoconstriction in actively sensitized guinea pigs. In our model, inhaled KP-496 or combined administration of montelukast and seratrodast, significantly inhibited antigen-induced bronchoconstriction. However, montelukast or seratrodast, given separately, did not significantly inhibit antigen-induced bronchoconstriction at doses sufficient to suppress LTD_4- and U46619-induced bronchoconstriction, respectively. This result is consistent with results obtained by other groups (Kagoshima *et al*., 1997; Arakida *et al*., 2000a). These findings suggest that antagonists for more than one mediator, such as KP-496, are more effective inhibitors of bronchoconstriction than antagonists of single mediators, such as $CysLT_1$ antagonists or TP antagonists.

We used actively sensitized guinea pigs to investigate the effects of inhaled KP-496 against airway response induced by endogenous mediators. In this model, antigen challenge induced IAR, LAR and AHR, which are also characteristic features of allergic asthma. The IAR is believed to result from the same mechanisms as antigen-induced bronchoconstriction. The effects of $CysLT_1$ antagonists and a TP antagonist on IAR in animal models have been investigated by many groups. Some groups have reported that these antagonists did not inhibit IAR (Tohda *et al*., 1997; Ihaku *et al*., 1999; Arakida *et al*., 2000b; Yamada *et al*., 2003). Others have reported that these antagonists inhibited IAR significantly, but they required larger doses to inhibit IAR than LAR (Matsumoto *et al*., 1994; Aoki *et al*., 2000). In this study, 0.1% of inhaled KP-496 was the lowest dose to inhibit IAR and LAR. These results together suggest that inhibition of several mediators is needed for significant effects on IAR.

In humans, the late response to antigen challenge is slow and persistent airway obstruction caused by chemical mediators released from inflammatory cells, such as eosinophils, following their accumulation and activation locally. LTs and TXA_2 are involved in mucosal oedema, mucosal secretions and bronchoconstriction. In asthmatic patients, such responses are thought to lead to serious and chronic asthma. Many groups have reported that $CysLT_1$ antagonists and a TP antagonist inhibited LAR in animal models, and have demonstrated the clinical usefulness of $CysLT_1$ antagonists and a TP antagonist. KP-496 thus could be of therapeutic benefit in the treatment of asthma.

Airway hyperresponsiveness is also one of the important features of asthma and the degree of AHR is related to severity of asthma symptoms (Bernstein *et al*., 1992). In our animal model, a significant increase in airway responsiveness to ACh was observed in antigen-challenged animals. Inhaled KP-496 at concentrations of 0.1 and 0.3% inhibited AHR significantly, although these doses were not enough to return airway responsiveness to the level of the non-treated group, and a concentration of 1% KP-496 was needed to reverse the AHR completely. The lower concentrations of KP-496 (0.1 and 0.3%) were enough to inhibit the LTD_4-mediated response, but not enough to inhibit the TXA_2-mediated response completely. The highest level of KP-496 (1%) was needed to inhibit completely the response to TXA_2. These results indicate that TXA_2 is a major mediator of AHR in our model. AHR is known to be suppressed by seratrodast and the importance of TXA_2 in AHR has already been demonstrated (Aizawa *et al*., 1998).

In addition to a late response and AHR, airway inflammation is another characteristic of asthma. Although airway inflammation was observed in our model, the degree of airway inflammation was not sufficient to evaluate the effects of KP-496 on airway inflammation. In another guinea pig asthma model, which exhibits chronic airway inflammation, KP-496 did show anti-inflammatory effects (manuscript in preparation). Further study is needed to determine the anti-inflammatory effects of KP-496 and their mechanisms. However, methods available to measure cytokines and chemokines in guinea pigs are limited and we are therefore investigating the anti-inflammatory effects of KP-496 in rats.

It has been thought that the airway responsiveness of guinea pigs is very similar to that of human airways. The maximum antagonist activities of KP-496 for LTD_4- and U46619-induced bronchoconstriction were comparable to those of montelukast and seratrodast, respectively. KP-496 exhibited dose-dependent inhibitory effects on antigen-induced airway responses (IAR, LAR and AHR). On the basis of these results, KP-496 could become a useful therapeutic agent for asthma.

Although $CysLT_1$ antagonists and a TP antagonist are used clinically, these antagonists do not always provide adequate therapeutic effects and there are both responders and non-responders to these antagonists (Drazen *et al*., 1999; Tanaka *et al*., 1999; Asano *et al*., 2002). These findings reflect the fact that asthma is a complex disease in which many mediators are involved. Therefore, inhibition of a single mediator is not enough to control asthma. In this study, KP-496 exhibited advantages as a dual antagonist in antigen-induced bronchoconstriction. LTs and TXA_2 are involved in not only bronchoconstriction but also in mucosal secretion, AHR and airway inflammation. It is thus likely that KP-496 could provide more potent therapeutic effects for asthmatic patients than the $CysLT_1$ antagonists and the TP antagonist already in clinical use.

Conflict of interest

The authors state no conflict of interest.

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