Epithelium-dependent and -independent inhibitory effects of sivelestat, a neutrophil elastase inhibitor, on substance P-induced contraction of airway smooth muscle in lipopolysaccharide-treated guinea-pigs

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Abstract

The underlying mechanism involved in the interaction between neutrophil elastase inhibitors and tachykinins has not been elucidated. In this study we have examined the effects of sivelestat, a neutrophil elastase inhibitor, on the in vitro responses of airways from lipopolysaccharide (LPS)-untreated or -treated guinea-pigs to substance P. Substance P (0.01-30 µmol/l) produced concentration-dependent contractions of both tracheal and bronchial ring preparations of LPS-untreated or -treated guinea-pigs. Responsiveness to substance P in these isolated airway preparations was augmented by either epithelium removal or LPS treatment. In epithelium-intact tracheal ring preparations isolated from LPS-untreated guinea-pigs, sivelestat (100 µmol/l) significantly inhibited substance P-induced contractions. The inhibitory action was markedly attenuated by pretreatment with L-NAME (100 µmol/l) or indomethacin (2 µmol/l), and was almost undetected following removal of the epithelium. On the other hand, in bronchial ring preparations isolated from LPS-untreated guinea-pigs, sivelestat had only a very slight effect on substance P-induced contraction of the epithelium-intact preparation, whereas sivelestat greatly inhibited contraction in epithelium-removed bronchial ring preparations. In LPS-treated guinea-pigs, whether the epithelium was intact or not, sivelestat significantly inhibited the substance P-induced contraction of bronchial ring preparations. Pretreatment with L-NAME (100 µmol/l) or indomethacin (2 µmol/l) did not affect the inhibitory effect of sivelestat in bronchial ring preparations. In conclusion, epithelium removal or LPS treatment induced hyperreactivity to substance P in the guinea-pig airway. Sivelestat caused epithelium-, nitric oxide- and prostaglandin-dependent inhibition of the substance P-induced contraction of isolated guinea-pig tracheal ring preparations. In contrast, the inhibitory effect of sivelestat on substance P-induced contraction of guinea-pig bronchial ring preparations is mediated by epithelium-, nitric oxide- and prostaglandin-independent mechanisms. Sivelestat may be effective in reducing the airway hyperresponsiveness to tachykinins induced by epithelial injury as occurs in LPS-mediated inflammatory lung diseases.

Key words: sivelestat, neutrophil elastase inhibitor, substance P, lipopolysaccharide, airway smooth muscle
Introduction

Neutrophil elastase plays a major role in the pathogenesis of acute lung injury, and is considered to be one of the progressive factors (Kawabata et al., 2002; Kawabata et al., 2003). Elastase released from activated neutrophils damages lung tissues, and causes epithelial injury (Amitani et al., 1991), vascular hyperpermeability (Hagio et al., 2001) and airway hyperresponsiveness (Suzuki et al., 1996). Suzuki et al. (1996) reported that aerosol inhalation of human neutrophil elastase increases airway resistance and induces airway hyperresponsiveness to 5-hydroxytryptamine. These workers suggested that human neutrophil elastase plays a role in the induction of airway hyperresponsiveness in various inflammatory lung diseases. It is of interest that a neutrophil elastase inhibitor, sivelestat, inhibited the antigen-induced early and late asthmatic responses in allergic sheep (Fujimoto et al., 1995). Sivelestat also improved the airway hyperresponsiveness caused by ozone exposure in guinea-pigs (Matsumoto et al., 1999). In addition, ozone exposure causes a significant increase in the concentration of substance P in the airway lavage of human subjects (Hazbun et al., 1993), and CP 99994, a NK1 antagonist, attenuates the increased contraction to electric field stimulation after ozone exposure in the ferret (Wu et al., 2003). Capsaicin-pretreatment, which depletes tachykinins from C-fibers, prevents ozone-induced (Koto et al., 1995) and antigen-induced airway hyperresponsiveness in guinea-pigs (Matsuse et al., 1991; Ladenius and Nijkamp, 1993). Tachykinins are considered to contribute to antigen-induced bronchoconstriction and to ozone-induced airway hyperresponsiveness by activating tachykinin receptors. However, the underlying mechanism for the interaction between neutrophil elastase inhibitor and substance P has not yet been studied.

Endotoxin (lipopolysaccharide, LPS) is a potent proinflammatory toxin found in the outer membrane of gram-negative bacteria. LPS induces the release of chemical mediators which trigger the inflammatory cascade in airways (Brigham and Meyrick, 1986). LPS also induces a significant expression of preprotachykinin mRNA in the lung and causes an increase in the concentration of substance P in bronchoalveolar lavage fluid (BALF) (Haung and Lai, 2003). Substance P causes airway hyperresponsiveness (Advenier et al., 1997), plasma exudation and extravascular accumulation of granulocytes in airways (Maggi, 1997). Combined treatment with both NK1 and NK2 antagonists leads to a significant reduction in the LPS-induced increase in neutrophils in the BALF of the mouse (Veron et al., 2004). In addition, capsaicin-pretreatment significantly reduced LPS-induced airway hyperresponsiveness to bronchoconstrictive agents in the guinea-pig (Jarreau et al., 1994; Loeffler et al., 1997). Furthermore, Kuo et al. (1998) demonstrated that LPS enhances the neurogenic exudation of plasma via NK1 receptors. These observations strongly suggest that endogenous substance P is implicated in the leukocyte activation, airway inflammation and hyperresponsiveness induced by LPS. However, the effect of a neutrophil elastase inhibitor on the airway hyperresponsiveness induced by substance P after LPS treatment is not known.

In the present study, we have examined the effect of sivelestat, a neutrophil elastase inhibitor, on the substance P-induced airway response of LPS-untreated or -treated guinea-pigs in vitro. Here we report new data that sivelestat inhibited the substance P-induced contraction of guinea-pig airway smooth muscle by both epithelium-dependent and -independent mechanisms.
Methods

Animals

Adult male guinea-pigs (Hartley strain; 300–500 g in weight) were used in the present study. The experimental animals were divided into a non-LPS group that were not given LPS and an LPS group that were injected with an intraperitoneal injection of LPS (8 mg/kg). The mortality 24 h following the LPS injection was 19.4%. Tracheal and bronchial ring preparations were isolated from the surviving animals 24 h after the intraperitoneal injection. This study was approved by the Dokkyo University School of Medicine Animal Ethics Committee.

Tension measurements

Guinea-pigs were anesthetized with enflurane and were exsanguinated via the carotid artery. The tracheo-bronchial tree was rapidly excised and placed in a modified Krebs bicarbonate solution with the following composition (mmol/l): NaCl 120, KCl 4.7, CaCl2 2.5, MgCl2 1.2, NaHCO3 25, KH2PO4 1.2, glucose 14 and ascorbic acid 0.12. Two cervical tracheal and two main bronchial ring preparations, each with a length of 1.5–2.0 mm, were prepared from each animal, according to our previously described method (Kamikawa and Takayama, 2005). Each tracheal or bronchial ring preparation was suspended in an organ bath filled with 5 ml modified Krebs bicarbonate solution. The solution was bubbled with 95% O2 and 5% CO2, and was maintained at 37°C. The ring preparation was suspended under a load of 0.5 g and the tension responses recorded on a polygraph (RJG-4124, Nihon Kohden, Tokyo, Japan) by an isometric transducer (TB-651T, Nihon Kohden). The preparation was equilibrated for 60 min and was washed with a fresh Krebs solution every 15 min. At the end of equilibration, the preparation was maximally contracted with a single application of carbachol (CCh, 10 µmol/l). After washout and equilibration for a further 30 min, the experiments were started.

The responsiveness of the tracheal or bronchial ring preparations to substance P was examined. Each ring preparation was exposed to a cumulatively increasing concentration of substance P. After washing out the substance P, the preparation was incubated for 120 min with sivelestat (30–100 µmol/l). Then, the preparation was exposed to substance P again. Some experiments were conducted after removal of the epithelium by gently rubbing the mucosal surface with a cotton wool probe (Uchida and Kamikawa, 1995). The preparations were also pretreated with L-NAME (100 µmol/l) or indomethacin (2 µmol/l) for 120 min together with sivelestat.

Data analysis

Each experimental group consisted of 6–22 preparations. Both the efficacy (Emax as % of 10 µmol/l carbachol-induced contraction) and potency (pEC50 as –log molar EC50) were calculated from the individual concentration-response curves. The EC50 was determined as the molar concentration eliciting 50% of the carbachol (10 µmol/l)-induced maximal contraction. Data were compared using either paired or unpaired Student’s t-test or by ANOVA followed by the Scheffe test post hoc to determine statistical differences. Differences were considered to be significant when P was less than 0.05. Data are expressed as the mean ± SEM while n indicates the number of preparations.
Drugs

The following drugs and substances were used: sivelestat (sodium N-[2-[4-(2,2-dimethylpropionyloxy)phenylsulfonyl-amino]benzoyl]amino-acetate tetrahydrate: ONO-5046-Na, donated by Ono Pharmaceutical Co., Osaka, Japan), substance P (Peptide Institute, Inc., Osaka, Japan), lipopolysaccharide (LPS: Escherichia coli 0111:B4), carbamylcholine chloride (carbachol), indomethacin, Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME) (Sigma, St. Louis, USA). Sivelestat was dissolved in and diluted with a solution containing 24.5 mg of Na₂CO₃ and 20 ml of water. Substance P was dissolved in and diluted with distilled water. Indomethacin was dissolved in distilled water containing equimolar concentration of Na₂CO₃ and diluted with 0.9% saline. Other drugs were dissolved in and diluted with 0.9% saline. The molar concentration of drugs described in this paper refers to the final bath concentration.

Results

Responsiveness to substance P

Substance P produced a concentration-dependent contraction of both the tracheal and bronchial ring preparations in the non-LPS group (Figs. 1, 2). The efficacy (Emax) and the potency (pEC₅₀) of the response to substance P for both the trachea and the bronchus are shown in Table 1. The substance P-induced contraction showed a tendency to increase
Sivelestat inhibits substance P-induced contraction following the removal of the epithelium. The efficacy was significantly greater in the epithelium-removed preparations compared to that in the epithelium-intact preparations.

In the LPS group, substance P also caused a concentration-dependent contraction of both ring preparations from both the trachea and bronchus (Figs. 3, 4). Application of LPS induced the augmentation of substance P-induced contraction. The efficacy was significantly increased by pretreatment with LPS (Table 1).

**Table 1.** Responsiveness of guinea-pig airway smooth muscle to substance P

<table>
<thead>
<tr>
<th>Group</th>
<th>Epithelium-intact</th>
<th>Epithelium-removed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Emax (%)</td>
</tr>
<tr>
<td>non-LPS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>trachea</td>
<td>21</td>
<td>61.8 ± 3.67</td>
</tr>
<tr>
<td>bronchus</td>
<td>8</td>
<td>61.5 ± 3.59</td>
</tr>
<tr>
<td>LPS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>trachea</td>
<td>22</td>
<td>66.5 ± 4.20</td>
</tr>
<tr>
<td>bronchus</td>
<td>8</td>
<td>77.5 ± 4.31*</td>
</tr>
</tbody>
</table>

Each value represents mean ± SEM. Emax values show the maximal amplitude of contraction as a percentage of that of carbachol (10 μmol/l)-induced contraction. pEC_{50} values shown as –log molar concentration eliciting 50% of the carbachol (10 μmol/l)-induced contraction. *P<0.05, **P<0.01, ***P<0.001. *: vs. epithelium-intact, #: vs. non-LPS group.
Effect of sivelestat on the contractile response to substance P in the non-LPS group

In epithelium-intact tracheal ring preparations from non-LPS animals, the concentration-response curve to substance P was significantly shifted to the right by pretreatment with sivelestat (100 μmol/l) (Fig. 1A). Removal of epithelium from the tracheal ring preparations noticeably attenuated the inhibitory effect of sivelestat on the substance P-induced contraction (Fig. 1B). In contrast to the tracheal preparations, in epithelium-intact bronchial ring preparations from non-LPS animals, pretreatment with sivelestat (100 μmol/l) only slightly inhibited the responses to substance P (Fig. 2A), whereas for epithelium-removed bronchial ring preparations, the concentration-response curve was greatly shifted to the right and downwards (Fig. 2B). Sivelestat at less than 30 μmol/l caused only a slight downward shift at the lower range (10–30 nmol/l) of the concentration-response curve to substance P in both preparations (data not shown). The solvent had no effect on the concentration-response curve to substance P.

Effect of sivelestat on the contractile response to substance P in the LPS group

In the LPS group, substance P also produced contraction of the tracheal and bronchial ring preparations in a concentration-dependent manner (Figs. 3, 4). Sivelestat (100 μmol/l) partly inhibited the response to substance P in the epithelium-intact tracheal ring preparations (Fig. 3A). Epithelium removal slightly modified the inhibitory effect of sivelestat on the substance P-
Sivelestat inhibits substance P-induced contraction

Influence of indomethacin or L-NAME on the inhibitory effect of sivelestat

It is generally accepted that the epithelium modulates the reactivity of airway smooth muscle by releasing relaxing factors such as prostaglandins (PGs), nitric oxide (NO) or epithelium-derived hyperpolarizing factor (EpDHF) (Morrison et al., 1990; Raeburn, 1990; Gaston et al., 1994; Ito and Tashiro, 1994). To determine the possible involvement of epithelium-derived relaxing factors on the inhibitory effect of sivelestat, tracheal ring preparations were treated with either L-NAME or indomethacin. L-NAME (100 μmol/l) and indomethacin (2 μmol/l) markedly attenuated the inhibitory effect of sivelestat as seen in the concentration-response curve for substance P in epithelium-intact tracheal ring preparations from non-LPS animals (Fig. 1A). In the epithelium-intact tracheal ring preparations isolated from LPS-treated guinea-pigs, L-NAME (100 μmol/l) and indomethacin (2 μmol/l) only slightly attenuated the inhibitory effect of sivelestat (Fig. 3A). As NO and PGs have been shown to be released from non-epithelial cells in the lung (Jorens et al., 1992; Ghosh et al., 2004), the effects of either L-NAME or indomethacin were investigated in bronchial ring preparations to confirm the involvement of NO and PGs in the inhibition induced by sivelestat. Pretreatment with L-NAME (100 μmol/l) or indomethacin (2 μmol/l) did not significantly shift the concentration-
response curve to substance P as compared with that for sivelestat alone (Fig. 2B). The inhibitory effect of sivelestat in the epithelium-removed bronchial ring preparations was not affected by pretreatment with L-NAME or indomethacin.

**Effect of sivelestat on KCl-induced contraction of the bronchial ring preparations**

KCl (20–60 mmol/l) produced a sustained contraction of epithelium-removed bronchial ring preparations. The magnitude of the sustained contraction induced by 20 mmol/l KCl was 54.5 ± 9.43% of that of the maximal carbachol (10 µmol/l)-induced contraction, while that of the contraction induced by 60 mmol/l KCl was 117.1 ± 9.42% (Fig. 5). As shown in Fig. 5, sivelestat (100 µmol/l) had no effect on the sustained KCl-induced contraction of the epithelium-removed bronchial ring preparations.

**Discussion**

In the present study, sivelestat inhibited the substance P-induced contractile response of the epithelium-intact tracheal ring preparations isolated from guinea-pigs that were untreated by LPS. This inhibitory effect of sivelestat was significantly attenuated by the removal of the epithelium from the preparation. Moreover, L-NAME, a NO synthase inhibitor, and indomethacin, a cyclooxygenase (COX) inhibitor, significantly attenuated the inhibitory effect of sivelestat in the epithelium-intact tracheal ring preparations. It has been shown that the respiratory epithelium modulates the responsiveness of airway smooth muscle by releasing epithelium-derived relaxing factors (Morrison et al., 1990; Raeburn, 1990; Gaston et al., 1994; Ito and Tashiro, 1994). Our present results suggest that in the tracheal ring preparations isolated...
from the LPS-untreated guinea-pigs, the inhibitory effect of sivelestat is mainly epithelium-dependent, and is mediated by NO and inhibitory PGs. It is well known that NO and PGs can be released from airway epithelium. NO is also considered to be an endogenous neurotransmitter of non-adrenergic, non-cholinergic inhibitory nerves in the guinea-pig trachea (Li and Rand, 1991), and appears to play an important role in the regulation of airway smooth muscle tone (Gaston et al., 1994). The epithelium-associated PGs also have a significant role in regulating basal tone in guinea-pig airways (Raeburn, 1990; Kohno and Ohata, 1993). It has also been shown by Okajima et al. (2004) that sivelestat reduces ischemia/reperfusion-induced liver injury and that the protective effect of sivelestat was completely inhibited by pretreatment with L-NAME or indomethacin. They suggested that the protective effect of sivelestat is mediated by enhancing the production of NO and PGs from the vascular endothelium. Their report is consistent with our present results. Our results suggest that the inhibitory effect of sivelestat is mediated by enhancing the epithelial production of NO and PGs in the tracheal ring preparations isolated from guinea-pigs not treated with LPS.

A recent report provides further evidence to suggest that there is considerable cross-talk between the production of NO and the synthesis of PGs by COX (Mollace et al., 2005). Salvemini et al. (1993) suggested that nitroprusside directly stimulates PGE₂ formation via the activation of COX. Mollace et al. (2005) also suggested that endogenous or exogenous NO enhances COX activity and leads to the release of PGs. Based on this evidence and our present results, it seems possible that sivelestat facilitates NO production from the tracheal epithelium and that the NO then causes the release of PGs (probably PGE₂), which inhibit the substance P-induced contraction of the guinea-pig trachea, by activating COX in the tracheal epithelium.

In contrast to the trachea, while sivelestat had a weak influence on the substance P-induced contraction of the epithelium-intact bronchial ring preparations isolated from LPS-untreated guinea-pigs, it strongly inhibited contraction in the epithelium-removed bronchial ring preparations from these animals. These results indicate that sivelestat causes inhibition of the substance P-induced contraction of bronchial ring preparations through epithelium-independent mechanisms. It is generally accepted that both NO and PGs may also be released from non-epithelial cells in the lung such as fibroblasts (Jorens et al., 1992; Ghosh et al., 2004). Compared with the effect of sivelestat alone, pretreatment with L-NAME or indomethacin did not modify the concentration-response curve obtained for substance P in the epithelium-removed bronchial ring preparations. The inhibitory effect of sivelestat observed here did not seem to be mediated by NO and PGs released from non-epithelial cells. Sivelestat did not inhibit the KCl-induced contraction of the epithelium-removed bronchial ring preparations. KCl-induced contraction of smooth muscle is generally explained by Ca²⁺-influx through voltage-dependent Ca²⁺ channels (VDCs), activation of the Ca²⁺-calmodulin-myosin light chain kinase process and phosphorylation of contractile proteins (Mitchell, 1996). Therefore, the epithelium-independent inhibitory effect of sivelestat is unlikely to be mediated by the blockade of VDCs or the inhibition of the Ca²⁺-calmodulin-phosphorylation process. Further studies are needed to explain the mechanisms involved in the epithelium-independent inhibitory effect of sivelestat.

In the LPS treated guinea-pigs, the slight inhibitory effect of sivelestat on the substance P-induced contraction of the tracheal ring preparations was unchanged by the removal of the
Sivelestat significantly inhibited the substance P-induced contraction of the bronchial ring preparations whether the epithelium was intact or not. These results suggest that in the LPS treated animals, the inhibitory effect of sivelestat is mainly mediated by epithelium-independent mechanisms. Airway epithelium is a physiological barrier that protects smooth muscle from various stimuli and has a significant role in the modulation of airway responsiveness to various drugs (Sparrow et al., 1995). Some reports indicate that LPS induces an increase in pulmonary epithelial permeability (Li et al., 1998; Han et al., 2004). Sivelestat possibly permeates the airway epithelium and causes the epithelium-independent inhibition on the substance P-induced contraction of both tracheal and bronchial ring preparations isolated from LPS-treated guinea-pigs. LPS has been associated with airway hyperresponsiveness to endogenous bronchoconstrictive mediators (Jarreau et al., 1994; Loeffler et al., 1997). In the guinea-pig, Folkerts et al. (1989) have reported that pretreatment with LPS increases airway responsiveness to arecoline and histamine, and that histamine-induced PGE2 formation of the epithelium-intact tracheal preparation is reduced by LPS pretreatment. They concluded that airway hyperresponsiveness induced by LPS is due to a decrease in the production of PGE2 from the airway epithelium. Michel et al. (1989) have exhibited that inhaled LPS causes bronchial hyperreactivity in asthmatic subjects, but not in normal subjects. LPS induces a significant expression of preprotachykinin mRNA (Huang and Lai, 2003) and substance P receptor mRNA (Bost et al., 1992). Tachykinins are involved in LPS-induced airway hyperresponsiveness to spasmogens (Jarreau et al., 1994; Loeffler et al., 1997). In our present experiments, ring preparations from LPS-treated guinea-pigs exhibited a similar hyperresponsiveness to substance P as observed in ring preparations denuded of epithelium. Sivelestat more significantly inhibited the substance P-induced contraction of bronchial ring preparations than that of tracheal ring preparations isolated from LPS-treated guinea-pigs. Sivelestat may be useful in the inhibition of peripheral airway hyperresponsiveness in either sepsis or asthma.

Neutrophil-derived elastase causes epithelial damage (Amitani et al., 1991). In addition, inhalation of neutrophil elastase increases airway hyperresponsiveness to spasmogens (Suzuki et al., 1996). Furthermore, removal of the airway epithelium in vitro causes an increase in responsiveness to contractile agents (Morrison et al., 1990; Ito and Tashiro, 1994; Knight et al., 1994). In the present experiments, removal of the epithelium significantly enhanced the contractile response to substance P. Sivelestat may be effective in reducing the airway hyperresponsiveness to tachykinins caused by epithelium injury in various respiratory diseases. Substance P activates leukocytes via mainly NK1 and partly NK2 receptors, and triggers inflammation in airway (Frossard and Advenier, 1991; Montuschi et al., 2000). Yoshitake et al. (1995) reported that substance P-activated polymorphonuclear leukocytes in cultured hamster tracheal epithelial cells were inhibited by sivelestat. They suggest that neutrophil elastase brings about a mucus secretion which is induced by substance P-activated polymorphonuclear leukocytes. The elastase released from activated neutrophils belongs to a group of endoproteolytic enzymes with a comparatively broad substrate specificity, that are capable of cleaving nearly all proteins in an unspecific manner. On the other hand, protease-activated receptors (PARs) are a unique subfamily of G-protein-coupled and seven-transmembrane domain
receptors, which are cleaved at an activation site within the N-terminal exodomain by a variety of protease (Macfarlane et al., 2001). PARs currently include four receptor subtypes. PAR-1, PAR-3 and PAR-4 are activated by thrombin, whereas trypsin can activate PAR-2 and PAR-4. The reactivity or sensitivity of the PAR family to elastase is likely to differ among cell types. Recently, in lung epithelial cells, Dulon et al. (2003) reported that human neutrophil elastase disarms PAR-2 by preventing its activation by trypsin. In addition, Suzuki et al. (2005) reported that human leukocyte elastase mediates apoptosis of lung epithelial cells through PAR-1 activation. Furthermore, Cocks et al. (1999) propose that activation of epithelial PAR-2 initiates important PGE2-dependent bronchoprotection by antagonizing increased airway tone. In guinea-pig airways, PAR-2 activation causes an epithelium-dependent relaxation by NO and PGs released from epithelial cells (Ricciardolo et al., 2000), while activation of PAR-1 on smooth muscle cells induces bronchoconstriction (Carr et al., 2000). From these observations, we speculate that elastase released from neutrophils activated by substance P impedes the epithelium-dependent bronchoprotection mediated by the activation of epithelial PAR-2, potentiates bronchoconstriction and exacerbates epithelial destruction by activating PAR-1. Sivelestat may exhibit the bronchoprotective effect by preventing the elastase-induced epithelial PAR-2 inactivation, and inhibit bronchoconstriction by preventing the elastase-induced PAR-1 activation.

The present results have indicated that there was a regional difference in the inhibitory mechanism of sivelestat. Some possible explanations for the cause of this regional difference are as follows: 1) Regional difference of sensitivity to drugs in airway smooth muscle, as relaxation induced by NO donors or PGE2 vary depending upon the region of the airways (Moore et al., 1986; Gruetter et al., 1989). 2) Inhibitory nitrergic nerves are more abundantly distributed in proximal than distal airway of guinea-pigs (Kamikawa, 1994). 3) Regional heterogeneity in epithelial modulation of airway responsiveness (Stuart-Smith and Vanhoutte, 1987; Uchida and Kamikawa, 1995).

In conclusion, responsiveness of guinea-pig airways to substance P was augmented by epithelium removal or LPS pretreatment. Sivelestat caused the epithelium-, NO- and PGs-dependent inhibition on the substance P-induced contraction of the isolated guinea-pig tracheal ring preparations. In contrast, the inhibitory effect of sivelestat on the substance P-induced contraction of guinea-pig bronchial ring preparations is mediated by epithelium-, NO- and PGs-independent mechanisms. Thus, airway hyperresponsiveness induced by epithelial injury or LPS may be prevented by sivelestat treatment.

Acknowledgements

We thank Professor Kamikawa and Professor Sakio for their helpful suggestions. We also thank Ono Pharmaceutical Co. for supplying sivelestat.

References


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Okajima, K., Harada, N., Uchiba, M. and Mori, M. (2004). Neutrophil elastase contributes to the development of ischemia-reperfusion-induced liver injury by decreasing endothelial production...


(Received August 23, 2005; Accepted September 28, 2005)