Pharmacokinetics and Anti-asthmatic Potential of Non-parenterally Administered Recombinant Human Interleukin-1 Receptor Antagonist in Animal Models

Ting Li¹, Wan-Liang Lu¹,*, Hai-Yan Hong², Yan-Sheng Yao², Pu Han², Zhong-Kun Li¹, Gui-Ling Wang¹, Yi Cao¹, Xiang-Rui Liu¹, Jian-Cheng Wang¹, Xuan Zhang¹, and Qiang Zhang¹

¹State Key Laboratory of Natural and Biomimetic Drugs and School of Pharmaceutical Sciences, Peking University, Beijing 100083, China
²Beijing Medical University United Biological Engineering Co., Beijing 100083, China

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Abstract. The objectives of this study were to define the pharmacokinetics of recombinant human interleukin-1 receptor antagonist (rhIL-1ra) and its effects on allergic asthma, cell adhesion molecules, and upper respiratory tract following non-parenteral administration in animals. Pharmacokinetics and immunomodulating effects of rhIL-1ra were investigated in Sprague-Dawley rats and asthmatic guinea pigs, respectively. Effects on the upper respiratory tract following the applications of rhIL-1ra were investigated on the ex vivo nasal mucosa of Sprague-Dawley rats and in situ in the upper palate of Chinese toads. Absolute bioavailabilities after intratracheal and intranasal administrations of rhIL-1ra were 94.3% and 24.8%, respectively. After administration of rhIL-1ra solution as ultrasonic spraying, the asthmatic symptom in guinea pigs was obviously attenuated. The plasma soluble intercellular cell adhesion molecule (sICAM-1) and P-selectin levels in asthmatic guinea pigs were each dose-dependently reduced with the increase of rhIL-1ra dose. The rhIL-1ra solution after administration via the airway seemed to have no impact on the integrity of nasal mucosa and mucocilia clearance in the upper respiratory tract. The present study provides evidence that rhIL-1ra effectively suppresses allergen-induced asthmatic symptoms through spraying, which corresponds to nasal and pulmonary absorption or both, and the efficacy is associated with downregulation of sICAM-1 and P-selectin expressions.

Keywords: recombinant human interleukin-1 receptor antagonist (rhIL-1ra), pharmacokinetics, asthma, soluble intercellular cell adhesion molecule (sICAM-1), P-selectin

Introduction

Interleukin-1 (IL-1) family genes encode three different peptides, IL-1α, IL-1β, and IL-1 receptor antagonist (IL-1ra), respectively (1). In normal homeostasis, the actions of IL-1 are maintained in balance by IL-1ra and other natural IL-1 inhibitors such as a network of anti-inflammatory cytokines. A variety of diseases, which include autoimmune diseases, infection, solid tumors, leukemia, Alzheimer’s disease, trauma, hemodialysis, ischemic myocardial infarction, asthma, and graft versus host disease, are associated with increased IL-1 production (2).

IL-1ra was previously found in the glycosylated form in the urine of febrile patients or of children with rheumatoid arthritis (3) and in experimental inflammation. It may play a role in modulating the systemic host responses to a variety of non-lethal disease states by altering the balance between cytokines and their antagonists (4). Under most circumstances, it limits the extent of the potentially deleterious effects of IL-1 (5, 6). Therefore, delivery of an exogenous specific inhibitor IL-1ra could be useful for rectifying the imbalance.

Recombinant human IL-1ra (rhIL-1ra) that is pro-
duced in *Escherichia coli* (*E. coli*) is a non-glycosylated, N-terminal methionyl form of the human naturally occurring polypeptide chain containing 153 amino acids and having a molecular mass of 17 kDa. The clinical efficacy of rhIL-1ra has been defined in rheumatoid arthritis, and studies on the effectiveness of rhIL-1ra cerebral ischemia, severe sepsis, and acute stroke are being performed in the laboratory or in clinical trials (7–9).

Asthma and chronic obstructive pulmonary disease (COPD) are chronic respiratory diseases that affect millions of people in the world, especially children and the elderly. Asthma and COPD are characterized by an uncontrolled inflammation of the airways (10). One approach to treat such diseases is to reduce the number of inflammatory cells that infiltrate the airway. The recruitment of leukocytes to sites of inflammation is dependent on the expression and function of a class of receptors called cell adhesion molecules. They are expressed on both the surface of leukocytes as well as the endothelial cells that line blood vessel walls. Leukocytes loosely associate and ‘roll’ on endothelial cell layers that have been activated by cytokines such as IL-1 or tumor necrosis factor receptor (TNF-α) (11, 12). The key cell adhesion molecules that are involved in the recruitment and migration of cells to sites of inflammation include selectins, integrins, and the respective counter-receptors for each.

However, the effect of rhIL-1ra on the cell adhesion molecules has not been defined. In the present study, we hypothesize that rhIL-1ra may improve the asthmatic symptom through regulating the P-selectin and intercellular cell adhesion molecule (ICAM-1, an integrin ligand) expression levels. P-selectin is stored in α-granules of platelets and in Weibel-Palade bodies of endothelial cells and translocated to the cell surface of activated endothelial cells and platelets (13). ICAM-1 is constitutively expressed on the endothelial cells and can be further upregulated by the inflammatory cytokines IL-1 and TNF-α (14).

In clinical therapy, rhIL-1ra is mainly administered intravenously or subcutaneously, and a multi-center clinical study showed that injection-site reactions were the most common adverse events in patients treated with rhIL-1ra (15). As an alternative to parenteral application, the patient-friendly delivery of active peptides by mucosal routes of entry is of major interest. The objectives of this study were to define the pharmacokinetics of rhIL-1ra and its effects on allergic asthma, cell adhesion molecules, and upper respiratory tract following the parenteral administration in animals.

### Materials and Methods

#### Materials and animals

rhIL-1ra (lot No. 20050307) was supplied by Center for Human Disease Genomics of Peking University (China); sodium deoxycholate (SDch), ephedrine hydrochloride, and ovalbumin (OVA, Grade II) were purchased from Sigma-Aldrich (local agent, Shanghai Branch, China); isoprenaline hydrochloride injection was produced by Harvest Pharmaceutical Co., Ltd. (Shanghai, China); urethane was from Beijing Chemical Reagent Plant (Beijing, China); all other chemicals and reagents were of analytical grade.

Human IL-1ra/IL-1F3 immunoassay kit (catalog No.: DRA00) was purchased from R&D System, Inc. (Minneapolis, MN, USA); sICAM-1 kit (catalog No.: SX1061) and P-selectin kit were (catalog No.: SX1025) were from BioSource International, Inc. (Camarillo, CA, USA).

Male Sprague-Dawley rats weighing 200–220 g, male guinea pigs weighing 300–330 g, and male or female Chinese toad weighing 30–40 g were obtained from Experimental Animal Center of Peking University, and maintained on a light/dark cycle. Rats and guinea pigs were acclimatized for 7 days prior to the experiments, and they were allowed free access to standard chow and water. Temperature and relative humidity were maintained at 25°C and 50%, respectively. All care and handling of animals were performed with the approval of Institutional Authority for Laboratory Animal Care of Peking University.

#### Preparation of solutions

RhIL-1ra was diluted with 20 mM phosphate buffer solution (PBS, pH 7.2, consisting of 14.4 mM dibasic sodium phosphate and 5.6 mM sodium dihydrogen phosphate) for intranasal (i.n.), intratracheal (i.t.), and intravenous (i.v.) administration in the pharmacokinetic study. Similarly, for treatment of allergic asthma, rhIL-1ra solutions were prepared by the same procedures as above. In addition, isoprenaline hydrochloride injection was diluted using PBS to a final concentration of 0.025% (w/v) as a positive control.

In the experiments to study treatment of allergic asthma, guinea pigs were sensitized and challenged with 5% (w/v) and 1% (w/v) of OVA solutions, respectively, each prepared in physiological saline.

In the study on the effects on the upper respiratory tract mucosa, SDch (5%, w/v) was dissolved in PBS as a positive control and 5% (w/v) of ephedrine hydrochloride was dissolved in PBS as a negative control. RhIL-1ra was diluted to a concentration of 7.8 mg/ml using physiological saline as a sample.
**Pharmacokinetics**

**Administrations:** Male Sprague-Dawley rats were divided into three groups of 6 rats each, as follows: Group 1, i.v. administration; Group 2, i.t. administration; and Group 3, i.n. administration. Rats were fasted for 12–16 h prior to the experiment. The rats in the Group 1 were given 0.5 ml of rhIL-1ra PBS solution by tail vein injection at a single dose of 2.5 µg/kg, and those in the Groups 2 and 3 were with given 20 µl of rhIL-1ra PBS solution by i.t. and i.n. administration at the same dose, respectively.

I.t. administration of rhIL-1ra was performed as reported previously (16). The rats were anesthetized with intraperitoneal injection of urethane (1.5 g/kg), and the trachea was exposed by operation. A microliter syringe was inserted into trachea at the location between the fifth and sixth tracheal ring. rhIL-1ra solution was instilled into the lung through the microliter syringe. The rats were maintained in a supine position at an angle of 90° to a horizontal plane for 30 s after administration and then at 30° during the experiment.

I.n. administration was performed as reported previously (17). Briefly, the rats were anesthetized by intraperitoneal injection of urethane (1.5 g/kg) and restrained in a supine position. The rats were tracheotomised to divert the airflow from the nasal passages and aid breathing. The esophagus was closed by ligation onto the tracheal cannula. A 20-µl aliquot of rhIL-1ra solution (25 µg/ml) was carefully injected into nasal cavity using a microliter syringe with a blunt needle. During the experiment, no visible leakage of solution was observed from the administration sites.

**Sampling and measurements:** For sampling in Group 1 or Group 2 after administration, a 0.5-ml blood sample was drawn at 0, 2, 5, 15, 30, 60, 120, 240, 360, and 480 min from the orbit venous plexus of the rats. For sampling in Group 3 after dosing, 0.5-ml blood sample was drawn at 0, 10, 15, 30, 60, 120, 240, 360, and 480 min from the orbit venous plexus of the rats. The blood samples were immediately put in heparinized tubes, stored at 4°C for 10 min, and then centrifuged at 5000 revolutions per min for 10 min at room temperature. The plasmas were collected and stored at −70°C until analysis.

For measuring rhIL-1ra concentration in plasma, a 200-µl aliquot of kit standard solution or plasma sample was processed according to the human IL-1ra/IL-1F3 immunoassay kit instructions. The lower limit of quantification (LOQ) of the assay was 22 pg/ml, and linearity was obtained for rhIL-1ra/IL-1ra concentrations between 0.09 and 6.5 ng/ml (r² = 0.9992). The coefficients of variation of the inter-day and intra-day precision of the quality control samples ranged from 3.1% – 6.7%, and the average recovery was 93%.

**Calculations:** Non-compartmental pharmacokinetics was used for calculating the parameters by 3P87 software, a practical pharmacokinetic program compiled by the Chinese Association of Mathematic Pharmacology (Beijing, China). The area under the plasma concentration-time curve (AUC) was calculated according to the trapezoidal rule. The absolute bioavailability (F) was calculated with the formula:

\[ F_{i.n.} = \frac{AUC_{0,8h, i.n.}}{AUC_{0,8h, i.v.}} \]

or \[ F_{i.t.} = \frac{AUC_{0,8h, i.t.}}{AUC_{0,8h, i.v.}} \]

where i.n., i.t., and i.v. represent intranasal, intratracheal, and intravenous, respectively. T_max, C_max, 1/2, and MRT were obtained from the non-compartmental analyses and denote the time to reach maximal concentration, maximal concentration, terminal half-life, and mean residence time of rhIL-1ra, respectively.

**Treatment of allergic asthma and effects on the cell adhesion molecules**

**Asthma model:** The asthma guinea-pig model was established with the OVA sensitization and challenge method as reported previously (18). Briefly, the procedures were divided into two steps: sensitizing and challenging for asthma, as follows: 1) for sensitizing, 40 normal male guinea pigs were intramuscularly injected with 5% (w/v) of OVA solution in the two crural legs (0.4 ml for each leg), followed by intraperitoneal administration of 1 ml of the same OVA solution to each guinea pig, respectively; 2) 15 days after sensitizing, each guinea pig was further challenged for asthma by an ultrasonic spraying approach. In brief, each guinea pig was placed in an airtight glass bell mantle (4 liter in volume), in which 1% (w/v) of OVA solution was sprayed at a speed of 2 ml/min for 30 s for challenging asthma using a medical ultrasonic atomizer (YC-Y800; Beijing Yadu Science and Technology Co., Ltd, Beijing, China). The asthmatic latent period, ≤180 s, was used as a criterion for inclusion of a guinea pig for the further experiments described below.

**Treatment of allergic asthma:** Six normal guinea pigs (not sensitized) were included as a normal control (Group I). Thirty of the sensitized guinea pigs were equally divided into 5 groups and administered by an ultrasonic spraying approach, as above. Briefly, each guinea pig in the Group II was given physiological saline, as a pathological control; each guinea pig in the Group III administered 2 mg/kg of isoprenaline hydrochloride, as a positive control; guinea pigs in Groups IV, V, and VI were administered 1.0 (low-dose), 3.0 (mid-dose), or 9.0 mg/kg (high-dose) of rhIL-1ra, respectively. One minute after administration, each guinea pig in the Group II – VI was challenged for asthma with 1%
(w/v) of OVA using the ultrasonic spraying method for 30 s. The asthmatic latent period (the time from starting challenge to the moment when asthmatic symptom was observed), and the tumbling animal numbers for guinea pigs in each group were recorded. As for the normal control in Group I, each guinea pig was given physiological saline instead of ovalbumin solution for challenging. The above procedures were repeated once daily for 8 consecutive days.

**Measurements of soluble ICAM (sICAM-1) and P-selectin:** On the 23rd day, the guinea pigs were anesthetized by intraperitoneal injection of urethane (1.5 g/kg) and restrained in a supine position. A 1-ml blood sample was directly drawn from the heart of each guinea pig using a syringe and put into a tube containing anticoagulant, stored at 4°C for 10 min, and then centrifuged at 5,000 revolutions per min for 10 min at room temperature. The plasmas were collected and stored at −70°C until analysis.

Plasma sICAM-1 level was measured according to the sICAM-1 kit instructions. Similarly, P-selectin level was assayed by following the P-selectin kit instructions. Both measurements are based on the enzyme-linked immunoassay.

**Effects on the upper respiratory tract mucosa**

**Nasal mucosa:** The rat model for observing the effect of drug on the nasal mucosa was established as reported previously (19). Twelve normal male Sprague-Dawley rats were equally divided into 3 groups for i.n. administrations. Briefly, each rat in Group A was given 0.3 ml of physiological saline as a negative control; each rat in Group B was given 0.3 ml of 5% (w/v) SDch as a positive control; and each rat in Group C was given 0.3 ml rhIL-1ra (7.8 mg/ml). The i.n. administration for each rat was repeated thrice daily for 8 consecutive days. On the 9th day, the rats were sacrificed. The nasal septum mucosa was removed for morphological examination according to the previous reports (20, 21). Briefly, the nasal septum mucosa was washed with cold saline, fixed with 2.5% glutaraldehyde solution, and then with 1% osmic acid. The sample was dehydrated by serial concentrations of ethanol, replaced by n-amyl acetate, dried at critical pointer of carbon dioxide, and coated with gold by an ion coater. The processed nasal mucosa was examined with a scanning electron microscope (JSM-5600 LV; JEOL, Tokyo).

**Mucocilia clearance:** Mucocilia clearance was estimated as reported previously (22). Twenty Chinese toads were equally divided into 4 groups and restrained in a supine position. In Group a, 0.2 ml of physiological saline was applied to the upper palate of each toad using a pipettor, as a blank control; in Group b, 0.2 ml of 1% (w/v) ephedrine hydrochloride solution was applied to each toad, as a negative control; in Group c, 0.2 ml of 1% (w/v) SDch solution was applied to each toad, as a positive control; in Group d, 0.2 ml of 7.8 mg/ml rhIL-1ra solution was applied to each toad. A plumbago particle was placed on the anterior part of the palate 5 min after drug application. The particle slowly moved towards the pharyngeal portion direction along the mucosa surface, and the time for moving 1 cm of distance was recorded. The relative percentage for the velocity of the ciliary movement was calculated by taking the ratio of the velocity for a test group to the velocity for the blank group.

After drug application at 30 min, the palate of the toad was rinsed with physiological saline, and the mucosa was removed (3 × 3 mm). The mucocilia were examined with an optical microscope and duration of the time of ciliary movement was recorded from right after rinsing until the ciliary movement stopped. The relative percentage for the duration of ciliary movement was calculated by taking the ratio of test group duration to the blank group duration.

**Statistical analyses**

Data are presented as the mean ± S.D. One-way analysis of variance (ANOVA) was used to determine significance among groups, after which post-hoc tests with the Bonferroni correction were used for comparison between individual groups. A value of $P<0.05$ was considered to be significant.

**Results**

**Pharmacokinetics**

The mean plasma rhIL-1ra concentration versus time profiles following i.v., i.t., and i.n. administrations of rhIL-1ra are illustrated in Fig. 1. 1) After i.v. administration at a dose of 2.5 µg/kg to rats, the mean rhIL-1ra concentration was 6.279 ± 0.262 ng/ml at 2 min, and the concentration dropped to 0.206 ± 0.041 ng/ml at 480 min. 2) After i.t. administration of rhIL-1ra solution at the same dose, the mean maximal concentration was 1.860 ± 0.148 ng/ml, and the mean concentration of rhIL-1ra at 480 min still remained at the level of 0.267 ± 0.038 ng/ml. The time to reach the peak concentration was 60 min, suggesting that rhIL-1ra was slowly absorbed intratraceally. 3) After i.n. administration at the same dose of rhIL-1ra, the mean maximal concentration was 0.580 ± 0.045 ng/ml, and time to reach the peak concentration was 15 min, indicating rhIL-1ra was quickly absorbed intranasally. The mean concentration of rhIL-1ra at 480 min was 0.191 ± 0.014 ng/ml.
The pharmacokinetic parameters using non-compartmental analysis are presented in Table 1. 1) Results showed that the rank order of mean $C_{\text{max}}$ value was $7.27 \pm 0.69$ ng/ml for i.v. administration $> 2.06 \pm 0.17$ ng/ml for i.t. administration $> 0.54 \pm 0.10$ ng/ml for i.n. administration, and the rank order of mean AUC$_{0-8h}$ value was $7.25 \pm 0.49$ h·ng/ml for i.v. administration $> 6.84 \pm 0.20$ h·ng/ml for i.t. administration $> 1.80 \pm 0.14$ h·ng/ml for i.n. administration. Comparing the two non-parenteral administrations, the mean $T_{\text{max}}$ value of i.n. administration was significantly lower than that of i.t. administration ($0.23 \pm 0.04$ h vs $0.92 \pm 0.20$ h). 2) The rank order of mean $t_{1/2}$ value was $9.84 \pm 2.65$ h for i.n. administration $> 2.75 \pm 0.59$ h for i.t. administration $> 2.64 \pm 0.58$ h for i.v. administration. Accordingly, the MRT$_{0-8h}$ showed the same order for the three administration groups. 3) The absolute bioavailability was 94.34% for i.t. administration, and it was 24.79% for i.n. administration group.

Table 1. The pharmacokinetic parameters followed intravenous (i.v.), intratracheal (i.t.), or intranasal (i.n.) administration of rhIL-1ra at a single dose of 2.5 $\mu$g/kg

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>—</td>
<td>0.92 ± 0.20</td>
<td>0.23 ± 0.04*</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (ng/ml)</td>
<td>7.27 ± 0.69</td>
<td>2.06 ± 0.17b</td>
<td>0.54 ± 0.10ab</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>2.64 ± 0.58</td>
<td>2.75 ± 0.59</td>
<td>9.84 ± 2.65ab</td>
</tr>
<tr>
<td>AUC$_{0-8h}$ (ng·h/ml)</td>
<td>7.25 ± 0.49</td>
<td>6.84 ± 0.20</td>
<td>1.80 ± 0.14ab</td>
</tr>
<tr>
<td>MRT$_{0-8h}$ (h)</td>
<td>3.30 ± 0.58</td>
<td>4.01 ± 0.55</td>
<td>14.83 ± 3.52ab</td>
</tr>
<tr>
<td>F (%)</td>
<td>—</td>
<td>94.3</td>
<td>24.8</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± S.D. (n = 5 – 6). *P<0.05, vs i.t. administration group; bP<0.05, vs i.v. administration group.

The pharmacokinetic parameters using non-compartmental analysis are presented in Table 1. 1) Results showed that the rank order of mean $C_{\text{max}}$ value was $7.27 \pm 0.69$ ng/ml for i.v. administration $> 2.06 \pm 0.17$ ng/ml for i.t. administration $> 0.54 \pm 0.10$ ng/ml for i.n. administration, and the rank order of mean AUC$_{0-8h}$ value was $7.25 \pm 0.49$ h·ng/ml for i.v. administration $> 6.84 \pm 0.20$ h·ng/ml for i.t. administration $> 1.80 \pm 0.14$ h·ng/ml for i.n. administration. Comparing the two non-parenteral administrations, the mean $T_{\text{max}}$ value of i.n. administration was significantly lower than that of i.t. administration ($0.23 \pm 0.04$ h vs $0.92 \pm 0.20$ h). 2) The rank order of mean $t_{1/2}$ value was $9.84 \pm 2.65$ h for i.n. administration $> 2.75 \pm 0.59$ h for i.t. administration $> 2.64 \pm 0.58$ h for i.v. administration. Accordingly, the MRT$_{0-8h}$ showed the same order for the three administration groups. 3) The absolute bioavailability was 94.34% for i.t. administration, and it was 24.79% for i.n. administration group.

Treatment efficacy
In contrasting to the normal rats, the challenged rats showed obvious allergic symptoms including cough, wheezing, accelerated breathing, nodding, jumping, and tumbling. However, the symptoms among of the groups administrered rhIL-1ra were obviously diminished compared with the pathological control group.

The mean asthmatic latent time and tumble ratio on the first day are presented in Table 2. The result showed that the mean latent time for rats administrered low-dose,
mid-dose, or high-dose of rhIL-1ra was obviously extended. Accordingly, the mean tumble ratio after administration was also significantly decreased as compared to that of the pathological control group. The rank order for the latent time among groups was as follows: 325.8 ± 18.6 s for high-dose group > 285.4 ± 17.1 s for mid-dose group > 229.4 ± 26.8 s for low-dose group. The tumble ratios were 0% for high-dose group, 40% for the mid-dose group, and 60% for the low-dose group, respectively.

Effects on the sICAM-1 and P-selectin levels

The mean plasma sICAM-1 levels of asthmatic guinea pigs in Group I – VI are illustrated in Fig. 2. 1) As compared to the normal control, the mean plasma sICAM level of guinea pigs in the pathological control was significantly increased. However, after they were given the positive drug isoprenaline hydrochloride, the mean plasma sICAM level was significantly decreased as compared to that in the pathological control not given isoprenaline, indicating that the pathological model was established successfully. 2) When the groups given the low-dose, mid-dose, and high-dose of rhIL-1ra were compared, the mean sICAM levels were significantly different and decreased with the increase of rhIL-1ra dose. 3) As compared to the pathological control, the mean sICAM level after administration of low-dose rhIL-1ra was slightly lowered while that after administration of mid-dose or high-dose rhIL-1ra was significantly decreased. The mean sICAM level after administration of high-dose rhIL-1ra was lowered more than that of the positive drug.

The mean plasma P-selectin levels of asthmatic guinea pigs in Groups I – VI are shown in Fig. 3. The results showed that the P-selectin levels exhibited the same trend as those of the mean plasma sICAM levels.

Table 2. Effect of rhIL-1ra on the asthma latent time and tumble ratio of asthmatic guinea pigs sensitized by 5% (w/v) of ovalbumin and challenged with 1% (w/v) of ovalbumin

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>Latent time (s)</th>
<th>Tumble ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I, as a normal control</td>
<td>0</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>Group II, as a pathological control</td>
<td>0</td>
<td>85.8 ± 15.6</td>
<td>100.0</td>
</tr>
<tr>
<td>Group III, as a positive control</td>
<td>2.0</td>
<td>325.0 ± 31.8a</td>
<td>20.0</td>
</tr>
<tr>
<td>Group IV</td>
<td>1.0</td>
<td>229.4 ± 26.8ab</td>
<td>60.0</td>
</tr>
<tr>
<td>Group V</td>
<td>3.0</td>
<td>285.4 ± 17.1abc</td>
<td>40.0</td>
</tr>
<tr>
<td>Group VI</td>
<td>9.0</td>
<td>325.8 ± 18.6abcde</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± S.D. (n = 6). aP<0.05, vs Group II; bP<0.05, vs Group III; cP<0.05, vs Group IV; dP<0.05, vs Group V.
Effects on the upper respiratory tract mucosa

After i.n. administration of various drug solutions to rats thrice daily for 8 consecutive days, the scanning electron micrographs of rat nasal mucosal cilia are shown in Fig. 4. As a negative control group, cilia after i.n. administration of physiological saline were orderly arranged on the surface of the mucosa (Fig. 4A). A similar phenomenon was observed in the group after i.n. administration of 7.8 mg/ml rhIL-1ra (Fig. 4C). In contrast, cilia shedding and obvious erosion were observed on the nasal mucosa of rats after i.n. administration of 5% SDch as a positive control (Fig. 4B).

To understand the effects of rhIL-1ra on the cilia in upper respiratory tract following spraying via nasal or oral delivery, the duration time and velocity for the ciliary movement were performed on Chinese toad upper palate. Results showed that the duration time and velocity of the ciliary movement after applying rhIL-1ra solution was similar to those after applying ephedrine hydrochloride solution (negative control). On the contrary, the movement of the cilia after applying the SDch solution (positive control) totally ceased, as shown in Table 3.

Discussion

For the active peptides like rhIL-1ra, very little pharmacokinetic information is available owing to the absence of a suitable measurement method. Limited information regarding the pharmacokinetics of rhIL-1ra was related to the evaluation of the clearance and tissue distribution using the $^{35}$S-labeling technique (23). In the present study, we first defined the pharmacokinetics of rhIL-1ra administered by the intranasal and intratracheal routes using the human IL-1ra/IL-1F3 immunoassay kit assay. This characterization may provide useful information for future clinical regimens.

As compared to the injection administration, delivery of active peptides by the respiratory airway, which involves pulmonary and nasal sites, is clinically interesting due to better patient compliance and less adverse events at the injection-site, as mentioned in the introduction section. The pulmonary route has been reported to be one of most promising alternatives for delivering therapeutic peptides since many such drugs, which are poorly absorbed from the enteral and other routes, are well absorbed from the lung due to the large surface area of the alveolar epithelium and the short distance of the air to blood pathway (24 – 26). In view of the pharmacokinetic results of rhIL-1ra, the i.t. administration that corresponds to the pulmonary absorption through oral spraying appears to reach an approximately equivalent absorption extent to the i.v. injection, suggesting that
from the viewpoint of systemic availability, such an administration route could be used as an alternative to the injection.

A nasal delivering peptide drug has been reported to be another promising alternative to parenteral administration (27, 28). This is because there are three different nasal epithelia that line the cavities: the vestibule, olfactory, and airway regions in which the epithelium is covered with numerous microvilli that increase the surface area. The pharmacokinetic results from the present study indicate that the intranasal administration of rhIL-1ra exhibits a lower absorption extent as compared to the i.t. dosing. This may be due to the fact that it is difficult for a high molecular weight drug like rhIL-1ra (17-kDa) to cross biological membranes. Higher-molecular-weight drugs (>1000 Da) may need some form of drug delivery system with penetrating enhancers in order to achieve clinical availability (29).

Nowadays, the standard treatment for asthma, which is one of the leading causes of childhood hospitalization and its incidence is rising, is the use of corticosteroids to inhibit the inflammatory reaction in the bronchial tree (30). Using a murine model employing guinea pigs sensitized and challenged with OVA, the treatment experiment in the present study showed that the asthmatic latent times and tumble ratios of asthma guinea pigs after administration of rhIL-1ra were obviously and dose-dependently reduced as compared to those of the blank control. The previous study showed that the IL-1ra secreted from monocytes was obviously decreased in asthma patients (31), suggesting that the endogenously lowered expressed IL-1ra may be associated with the asthma disease. The present results provide direct evidence that rhIL-1ra administered by ultrasonic spraying can effectively suppress the asthma symptoms through elevating the IL-1ra level.

To further elucidate the mechanism of rhIL-1ra in asthma, the effects on the cell adhesion molecules, including plasma sICAM-1 and P-selectin, were estimated in the present study. Results of the present study indicate that the plasma sICAM-1 levels in asthmatic guinea pigs are reduced with an increase of rhIL-1ra dose, showing a dose-dependent relationship, suggesting that the sICAM-1 levels are associated with treatment effects including the extended asthmatic latent time and reduced tumble ratio. Although the pathophysiological function of the soluble form of ICAM-1 remains unclear (32), the plasma sICAM-1 may have a role in promoting migration of eosinophils and neutrophils cells into the airway and be involved in inflammatory cell recruitment (33). A similar relationship was observed for the P-selectin levels in the present study. P-selectin is known to play a role in the allergen-induced peribronchial inflammation and airway hyperreactivity, and it is postulated to be an important controller of the inflammation by mediating selective eosinophil cell influx to the lung (34). As the severity of allergic asthma is associated with the intensity of peribronchial inflammation, a specific inhibitor of P-selectin-mediated leukocyte endothelial-cell interactions like rhIL-1ra may therefore attenuate the inflammatory processes associated with allergic airway disease (35). Taken together, the present study indicates the efficacy of rhIL-1ra for treating allergic asthma is associated with downregulation of the expression of the sICAM-1 and P-selectin molecules.

Since rhIL-1ra was administered by the ultrasonic spraying approach, it could be considered as intranasal administration, pulmonary administration, or both. Accordingly, the tolerance of rhIL-1ra when administered by nasal delivery or oral spraying needs to be evaluated as the formulation may potentially affect the upper respiratory tract mucosa. Results from the TEM (transmission electron microscopy) and ciliary movement examinations in the present study demonstrate that the rhIL-1ra formulation used can be safely applied via
the respiratory airway.

The present pharmacokinetic study showed that the absolute bioavailabilities after i.t. and i.n. administrations of rhIL-1ra were 94.3% and 24.8%, respectively. After administration of rhIL-1ra solution by ultrasonic spraying, the asthmatic symptoms in guinea pigs were obviously attenuated. The plasma sICAM-1 and P-selectin levels in asthmatic guinea pigs were each reduced with the increase of rhIL-1ra dose; the reduction was dose-dependent in both cases. Nasal or pulmonary delivery via spraying could be effective, thus providing new alternatives to the parenteral administration route. The present study provides evidence that rhIL-1ra effectively suppresses allergen-induced asthmatic symptoms through spraying, and the efficacy is associated with downregulation of sICAM-1 and P-selectin expression.

References

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