Pharmacokinetics of Laninamivir after a Single Administration of its Prodrug, Laninamivir Octanoate, a Long-Acting Neuraminidase Inhibitor, Using an Easy-to-Use Inhaler in Healthy Volunteers

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Abstract
Pharmacokinetic profiles of laninamivir after a single inhalation of laninamivir octanoate (LO), a prodrug of laninamivir, using a newly developed easy-to-use inhaler were evaluated in healthy volunteers. LO appeared rapidly in plasma after an inhaled administration in healthy volunteers with a median value of t_{1/2} of 0.25 hr, and the plasma concentrations decreased below the detection limit after 24 hr of inhalation. The median t_{1/2} of laninamivir was 4.0 hr and laninamivir slowly declined after C_{max} with a mean t_{1/2} of 66.6 and 74.4 hr at a dose of 20 mg and 40 mg, respectively. The average AUC_{0-144h} and C_{max} for LO and laninamivir almost increased proportionally with the dose. The mean cumulative excretion amounts of LO in urine for 144 hr after inhaling 20 mg or 40 mg dose of LO were 4.7 and 5.5% of the dose, respectively, and those of laninamivir were 19.2 and 23.3%, respectively. No clinical or laboratory adverse experiences were reported and no subject discontinued because of an adverse experience. As plasma concentrations of both LO and laninamivir revealed a similar pattern between using the prototype and this new inhaler, LO exhibited potential for long lasting anti-influenza activity using this easy-to-use inhaler.

Keywords: Laninamivir; Laninamivir octanoate; CS-8958; Pharmacokinetics; Human; Safety; Neuraminidase inhibitor

Abbreviations: LO: laninamivir octanoate; DPIs: Dry Powder Inhalers; BMI: Body Mass Index; AUC_{0-tz}: Area Under the Concentration-time curve up to the time of the last measurable concentration data; AUC_{0-144h}: AUC values extrapolated to infinity; C_{max}: Maximum Concentration; t_{1/2}: Time to C_{max}; t_{1/2}: Half-Life; CL/F: Apparent total body clearance; V/F: Apparent volume of distribution; Xu_{0-144h}: The cumulative percentage of dose excreted in urine up to 144 hours; CL_{r}: Renal clearance; APSD: Aerodynamic Particle Size Distribution

Introduction
Laninamivir (Figure 1) is a new neuraminidase inhibitor which has been shown to be sensitive against various influenza A and B viruses, including subtypes N1 to N9, oseltamivir-resistant viruses [1] and new swine-origin H1N1 strains like A/California/04/09 in vitro and in vivo [7, 8]. The prophylactic and therapeutic efficacy of laninamivir octanoate (LO, Figure 1), the octanoyl esterified form of laninamivir, against highly pathogenic H5N1 influenza viruses has been reported [15]. The increase of lipophilicity by acylating the active form provided prolonged the survival effects in accordance with the chain length in a mouse infection model [4]. Also, LO conferred more potent and long-lasting protection to mice against H5N1 influenza viruses, including subtypes N1 to N9, oseltamivir-resistant viruses [1] and those of laninamivir over 24 hours post-dose [9]. Long retention in the trachea and lung was also observed after intratracheal administration to rats, and plasma concentration of laninamivir in rats was slowly eliminated. Hydrolysis of LO in the respiratory tract was observed in human lung S9 in vitro by various kinds of esterase (unpublished data). In addition, its half-life was considerably longer than that after intravenous administration of laninamivir [10].

A double-blind, randomized controlled trial compared the efficacy of LO with oseltamivir in adult patients that have suffered from influenza virus has been conducted recently [18]. The time to illness alleviation after a single inhalation of LO was not inferior to that of multiple oral treatment of oseltamivir, and it was sufficient to treating adult seasonal influenza. LO was also an effective and well-tolerated treatment for children with oseltamivir-resistant influenza A (H1N1) virus infection [17].

The pharmacokinetics of LO and laninamivir after a single inhaled dose of LO in human volunteers were previously investigated [5, 6].

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AUC and C_{max} of laninamivir increased almost linearly with a dose administered up to 120 mg in healthy volunteers, and the plasma concentration of laninamivir slowly decreased from the body, even lasting for as long as 144 hours after administration with t_{1/2} for about 3 days apart from the renal insufficiency. These pharmacokinetic characteristics suggest the potential for long lasting anti-influenza activity.

Dry powder inhalers (DPIs) have become widely known as a very attractive platform for drug delivery. Many patients have traditionally used DPIs to treat asthma and chronic obstructive pulmonary disease. Recently, the development of new DPIs for delivering therapeutic proteins such as insulin has been accelerated through patient demand and innovative research [16]. A new, simple and easy-to-use inhaler was developed to reduce potential operational error and the number of inhalation of LO. It consists of a main body including a mouthpiece and a shuttle with two dose compartments for powder formulation. Powder formulation containing 20 mg of LO is directly filled in the dose compartments in the manufacturing process. This inhaler becomes ready to use by just pushing the shuttle sideways to align the dose compartment with the mouthpiece for the first dose, and then by pushing the shuttle in the other direction for the second dose. The prototype inhaler required changing the 5 mg capsule every one inhalation, therefore 4 or 8 times of changing capsule and inhalation were needed. On the other hand, the new inhaler requires no changing capsules and less inhalation is needed than the prototype inhaler. Due to the simplicity and low risk of operational error, this inhaler may be suitable for high dose and single treatment pharmaceutical products like LO. In addition, the reduced number of inhalation by its ease of use is another advantage for LO, especially for a pandemic requiring immediate treatment of large populations.

On the basis of this work, the pharmacokinetic of laninamivir in healthy volunteers was evaluated to characterize the feature of LO using a new easy-to-use inhaler.

Materials and Methods

Clinical pharmacokinetic study

A randomized, parallel study to evaluate the safety and pharmacokinetics of LO and laninamivir was conducted at Kyushu Clinical Pharmacology Research Clinic (Fukuoka, Japan). The protocol was approved by the institutional review boards of Kyushu Clinical Pharmacology Research Clinic (Fukuoka, Japan). The protocol was conducted in accordance with the guidelines on Good Clinical Practice and with ethical standards for human experimentation established by the Declaration of Helsinki Principles. Every subject gave written informed consent to participate in this study.

Subjects and study designs

Male subjects (aged 20~45 years) with a body mass index in the range of 18.5~25.0 kg/m² were eligible for inclusion if they were deemed healthy in terms of medical history, physical examination findings, 12-lead electrocardiogram findings and clinical laboratory evaluations. Evidence of organ dysfunction or any clinically significant deviation from the norm in a physical examination, vital signs, electrocardiogram were excluded. The protocol was approved by the institutional review boards of Kyushu Clinical Pharmacology Research Clinic (Fukuoka, Japan). The protocol was conducted in accordance with the guidelines on Good Clinical Practice and with ethical standards for human experimentation established by the Declaration of Helsinki Principles. Every subject gave written informed consent to participate in this study.

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<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Laninamivir octanoate dose</th>
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<tbody>
<tr>
<td>Age yrs</td>
<td>21 (20-24)</td>
</tr>
<tr>
<td>Height cm</td>
<td>170 (161-183)</td>
</tr>
<tr>
<td>Body weight Kg</td>
<td>63.3 (56.3-75.6)</td>
</tr>
<tr>
<td>BMI</td>
<td>21.9 (20.2-23.3)</td>
</tr>
</tbody>
</table>

Values are arithmetic mean (range). BMI, body mass index

Table 1: Demographic characteristics of the study subjects for pharmacokinetics.
The pharmacokinetic parameters were calculated by a non-compartment analysis using the computer software WinNonlin Professional (version 5.2.1, Pharsight Corp., CA). The maximum concentration ($C_{\text{max}}$) and the time to $C_{\text{max}}$ ($t_{\text{max}}$) were obtained by observation. The apparent elimination $t_{\text{1/2}}$ was obtained by a linear regression of 3 or more log-transformed data points in the terminal phase (calculated automatically by WinNonlin). The area under the concentration versus the time curve up to the time of the last measurable concentration data ($\text{AUC}_0-t_z$) was obtained by the linear trapezoidal method. The $\text{AUC}$ values were extrapolated to infinity ($\text{AUC}_{0-\infty}$) using the equation $\text{AUC}_{0-\infty} = C_{t_z}/\lambda$, where $C_{t_z}$ is the last measurable concentration and $\lambda$ is the terminal elimination rate. The urine concentrations, urine volumes from individual collection intervals and nominal times of the collection intervals were used to calculate the urinary pharmacokinetic parameters. The amount of LO and laninamivir excreted in urine in each collection interval was determined by the product of the urine concentration and the urine volume. The renal clearance ($\text{CL}_{\text{R}}$) was determined as the quotient of the cumulative excretion amount of the drug and $\text{AUC}$.

### Safety and tolerability

Each subject was confined to the Clinical Pharmacology Unit from the previous day of dosing through the completion of 144-hour post-dose procedures. Safety and tolerability were assessed in each study by clinical evaluation (including physical examinations, vital signs, lung function tests and 12-lead electrocardiograms) and laboratory measurements (including hematology, serum chemistry and urinalysis). A physical examination was performed at screening, 144 hours after drug administration and at the end-of-study visit. Vital signs (blood pressure and heart rate), temperature and a 12-lead electrocardiogram (ECG) were recorded. Standard clinical laboratory profiles for hematology, serum chemistry and urinalysis were assessed at screening, 24 and 144 hours after drug administration and at the end-of-study visit. Adverse experiences were monitored throughout the study. Subjects were questioned concerning their well being. Investigators evaluated all the clinical adverse experiences in terms of intensity (mild, moderate or severe), duration, severity, outcome and relationship to the study drug.

### Results

#### Clinical pharmacokinetic study

Sixteen participants received LO and all participants were included in the pharmacokinetic analyses. The demographic and baseline characteristics of the study populations are shown in Table 1. The plasma LO and laninamivir concentration profiles after a single inhaled administration of LO using the new inhaler are shown in Figure 2, and the pharmacokinetic parameters are summarized in Table 2.

<table>
<thead>
<tr>
<th>PK parameters</th>
<th>Laninamivir octanoate dose</th>
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<tbody>
<tr>
<td></td>
<td>LO (N=8)</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (ng/mL)</td>
<td>440 (82)</td>
</tr>
<tr>
<td>$t_{\text{max}}$ (h)</td>
<td>0.25 (0.25-0.25)</td>
</tr>
<tr>
<td>$t_{\text{1/2}}$ (h)</td>
<td>1.79 (0.11)</td>
</tr>
<tr>
<td>$\text{AUC}_{0-\infty}$ (h/mL)</td>
<td>693 (116)</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (ng/mL)</td>
<td>19.0 (3.1)</td>
</tr>
<tr>
<td>$t_{\text{max}}$ (h)</td>
<td>4.0 (3.0-6.0)</td>
</tr>
<tr>
<td>$t_{\text{1/2}}$ (h)</td>
<td>68.6 (9.1)</td>
</tr>
<tr>
<td>$\text{AUC}_{0-\infty}$ (h/mL)</td>
<td>558 (98)</td>
</tr>
</tbody>
</table>

Table 2: Pharmacokinetic parameters of laninamivir octanoate (LO) and laninamivir after a single inhaled administration of LO using a new easy-to-use inhaler in healthy human volunteers.

or clinical laboratory determinations were criteria for exclusion. A total of 16 young healthy male subjects were planned to be enrolled in this study. Subjects (N=8) were randomly assigned to receive a single inhaled dose of 20 or 40 mg of LO (DaiichiSankyo Co., Ltd., Tokyo, Japan). The subjects were instructed to inhale each dose using quiet tidal breathing. LO was administered in a seated position and the subjects were prohibited from resting in a supine position for 2 hours after inhalation.

### Blood and urine sampling for the pharmacokinetic analysis

Plasma and urine samples were collected and analyzed for LO and laninamivir concentrations. Blood (5 mL) was collected into vacutainers containing heparin as an anticoagulant at predose and 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, 120 and 144 hours after dosing. After the addition of acidic AEBSF (4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride) as an enzyme inhibitor to minimize degradation, followed by centrifugation, the plasma supernatants were stored at -20°C until the assay. LO and laninamivir concentrations in human plasma and urine were determined by the validated sensitive and specific LC-MSMS method as described previously [5].
inhalation of LO reduced the duration of influenza illness significantly, compared with oseltamivir, against H1N1 virus with the H274Y mutation in pediatric patients [17]. This long-acting characteristic of LO is supported by the long plasma half-life of laninamivir (~3 days) after a single inhaled administration, which might predominantly reflect the slow release from retaining tissues to plasma [5,6]. Plasma half-life of laninamivir after a single inhaled administration using this inhaler was rather long and almost comparable with that reported previously, suggesting potential for long-lasting anti-influenza activity using this inhaler.

Though plasma half-lives of LO and laninamivir after a single inhaled administration using this inhaler were almost comparable with those reported values, mean plasma concentrations, AUC, C_{max} and X_{t1/2} of LO and laninamivir using a new inhaler exhibited higher values than those using the prototype [5]. However, the emission performance of the inhaler used in this study evaluated by the cascade impactor was equivalent to that of the prototype inhaler (In-house data). Cascade impactor is the most widely encountered means for in vitro determination of the aerodynamic particle size distribution (APSD) from medical inhalers, both in product development, batch release and in applications with add-in devices [12]. Though it is reported that the links between laboratory-measured APSD data from the cascade impactor, particle deposition in the respiratory tract and clinical response are not straightforward [14]. APSD data may principally provide the information which might be helpful in estimating the likelihood of clinical response in studies on the efficacy and safety of inhaled drugs [13]. Though mean values of AUC, C_{max} and X_{t1/2} using a new inhaler were higher than those using the prototype, each parameter exhibited a relatively large inter-individual variation and the ranges of the individual parameters almost overlapped between the two inhalers. Future population pharmacokinetic analysis together with other pharmacokinetic results might be a powerful tool to evaluate the inter- and intra-individual variation and the covariates which affect the pharmacokinetics of LO and laninamivir, including the difference between the two inhalers.

The value of neuraminidase inhibitors was clearly established during the initial phases of the 2009 pandemic when vaccines were not available, i.e., stockpiles of anti-virals are valuable [1]. The development of high-potency drugs requiring less frequent administration is desirable because the amount of drug needed for pandemic stockpiling is tremendous. As an inhaled neuraminidase inhibitor, laninamivir was efficacious in twice daily treatment for 5 days [2], and was also efficacious for the prevention of influenza with self-administration once daily for up to 10 days in families [3]. In addition, laninamivir was successful in preventing infection with the predominant strains circulating in the 2000-2001 influenza season in high-risk community-dwelling subjects for up to 28 days [11]. It is reported that LO has substantial efficacy as both a therapeutic and a prophylactic agent against H5N1 influenza viruses in mice. LO is, therefore, a promising candidate antiviral for the prevention and treatment of influenza patients infected with H5N1 or other subtype viruses [7]. From the current study, it revealed that the plasma half-life of laninamivir, which might reflect the elimination of drug from retaining tissues, was long enough to prevent infection by a single inhaled LO administration in humans. However, whether laninamivir concentration in target tissues may be enough to prevent influenza virus infection was not clear. Further clinical investigation to evaluate laninamivir concentration in target tissues might be necessary to consider the appropriate dosage regimens for prevention of influenza infection by LO inhalation, especially for highly pathogenic viruses.

LO was well tolerated by all subjects following a single inhaled dose using the new easy-to-use inhaler. Similar pharmacokinetic profiles between the two inhalers indicate that a single inhaled dose of LO using the new easy-to-use inhaler may exhibit efficacy against influenza virus.

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References