Plasma and Intrapulmonary Pharmacokinetics of Levonadifloxacin in Healthy Adult Subjects

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ABSTRACT

- **Background:** WCK 2349 is a novel L-alanine ester prodrug of levonadifloxacin being developed as an oral fluoroquinolone antibacterial agent that displays excellent coverage for methicillin-resistant *Staphylococcus aureus*. In addition, levonadifloxacin has an *in vitro* spectrum of activity for commonly encountered respiratory organisms such as *Streptococcus pneumoniae, Haemophilus influenzae*, and *Moraxella catarrhalis*. For pulmonary infections, concentrations of antibiotics in epithelial lining fluid (ELF) for extracellular pathogens and in alveolar macrophage (AM) cells for intracellular pathogens are thought to reflect antibiotic activity in pneumonia. The primary objective of this study was to determine and compare plasma, ELF, and AM concentrations of levonadifloxacin following oral administration of WCK 2349 in healthy adult male and female subjects.
- **Material/methods:** Levonadifloxacin concentrations in plasma, ELF, and AM of 30 healthy subjects were measured by LC-MS/MS following repeat oral dosing of WCK 2349 (1000 mg twice daily for 5 days). Subjects were enrolled into 5 groups with 6 subjects assigned to each bronchoalveolar lavage (BAL) sampling time. Noncompartmental pharmacokinetic (PK) parameters were determined from serial plasma samples collected over a 12-hour interval following the first and ninth oral doses. BAL samples were collected once in each subject, at 2, 4, 6, 8 or 12 hours after the ninth oral dose. To determine penetration ratios, the AUC_{0-12} for plasma, ELF, and AM were calculated using mean and median concentrations at the BAL sampling times. Unbound plasma concentrations were used to determine penetration ratios since plasma protein binding of levonadifloxacin is high (~85%).
- **Results:** Mean (± standard deviation) PK parameters, based total plasma levonadifloxacin concentrations, after the first dose included maximum concentration (C_{max}) of 16.5 ± 5.1 µg/mL, time to C_{max} (t_{max}) of 1.8 ± 0.7 h, apparent volume of distribution (V/F) of 58.0 ± 14.7 L, clearance (CL/F) of 9.11 ± 2.23 L/h, and elimination half-life ($t_{½}$) of 4.5 ± 0.9 h. Steady-state was achieved by the fifth oral dose and PK parameters after the ninth dose were C_{max} of 20.0 ± 4.3 µg/mL, t_{max} of 2.1 ± 1.4 h, V/F of 59.2 ± 16.0 L, CL/F of 8.17 ± 2.05 L/h, $t_{½}$ of 5.1 ± 1.3 h, and AUC_{0-12} of 129.8 ± 31.6 µg•h/mL. The respective AUC_{0-12} values based on mean and median ELF concentrations were 172.6 and 161.2 µg•h/mL, whereas AUC_{0-12} values based on AM concentrations were 35.3 and 30.6 µg•hr/mL.
- **Conclusions:** The ratio of ELF to unbound plasma levonadifloxacin concentrations based on the mean and median AUC_{0-12} values were 7.66 and 7.58, respectively, whereas the AUC_{0-12} ratios of AM to unbound plasma levonadifloxacin concentrations were 1.58 and 1.44, respectively. These data support further study of WCK 2349 for treatment of lower respiratory tract bacterial infections caused by susceptible pathogens.

BACKGROUND

- WCK 2349 is a novel L-alanine ester prodrug of levonadifloxacin being developed as an oral fluoroquinolone antibacterial agent that displays excellent coverage for methicillin-resistant *Staphylococcus aureus*.
- Epithelial lining fluid (ELF) and alveolar macrophages (AM) have been advocated as important sites of infection for common extracellular and intracellular pathogens, respectively, for lower respiratory tract infections.
- The objectives of this study were to: 1) determine and compare the plasma, ELF, and AM concentrations of levonadifloxacin following multiple oral doses of WCK 2349 (1000 mg twice daily for 5 consecutive days) in healthy adult subjects; and 2) determine the safety and tolerability of multiple oral doses of WCK 2349 in healthy adult subjects.

MATERIALS AND METHODS

- This was a Phase 1, multiple dose, open-label, pharmacokinetic study conducted in healthy male and female subjects who were between 18 to 55 years of age and had a body mass index between 18.5 and 30 kg/m^2.
- Each subject received WCK 2349 at an oral dose of 1000 mg twice daily within two hours after a meal for five consecutive days (10 doses).
- Blood samples for assay of plasma concentrations of levonadifloxacin were obtained before and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours after the first and ninth dose.
- Subjects were assigned to 1 of 5 bronchoscopy sampling times:

	Bronchoalveolar (BAL) Sampling Times after the Ninth Dose				
Time	**2-Hour**	**4-Hour**	**6-Hour**	**8-Hour**	**12-Hour**
Subjects (n)	**6**	**6**	**6**	**6**	**6**

- Assays for levonadifloxacin and urea concentrations were performed with validated HPLC tandem mass spectrometry (LC/MS/MS) methods.
- The concentration of levonadifloxacin (ABX_{ELF}) in ELF was determined as follows: $ABX_{ELF} = ABX_{BAL} \times (Urea_{Plasma} / Urea_{BAL})$. The concentration ($ABX_{AM}$) in alveolar macrophages (AM) was determined as follows: $ABX_{AM} = ABX_{M} / V_{AM}$, where ABX_{M} is the measured concentration of levonadifloxacin and VAM is the volume of alveolar cells in the 1-ml cell suspension.
- The mean and median concentrations of levonadifloxacin from the plasma and bronchopulmonary sampling times were used to estimate the AUC_{0-12} by the linear trapezoidal method. The ratio of AUC_{0-12} of ELF and AM to unbound plasma were calculated.

RESULTS

- A total of 31 subjects received oral doses of WCK 2349. Thirty (30) subjects (**Table 1**) completed the study and received repeat oral dosing of WCK 2349 (1000 mg twice daily for 5 days).

Table 1. Characteristics of 30 Study Subjects

Sampling Time	Sex	Age (years)	Weight (kg)	Total Cell Count in BAL Fluid (mm^3)	Macrophages (%)
2-hour	2 M, 4 F	39 ± 6	78.3 ± 12.2	106 ± 22	84 ± 8
4-hour	3 M, 3 F	37 ± 9	74.6 ± 7.0	163 ± 92	86 ± 6
6-hour	4 M, 2 F	31 ± 8	70.5 ± 9.0	96 ± 25	84 ± 7
8-hour	6 M	41 ± 12	83.0 ± 9.6	105 ± 35	85 ± 11
12-hour	6 M	36 ± 9	85.7 ± 11.6	118 ± 65	89 ± 6

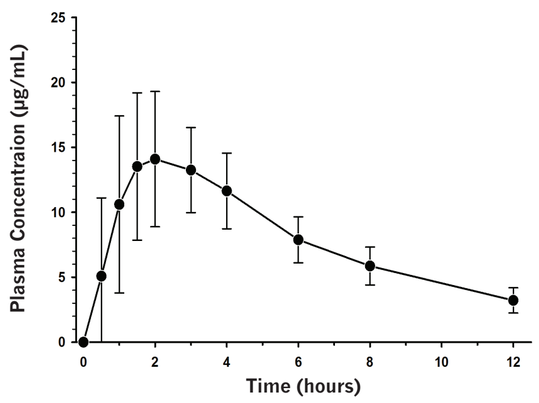
Data expressed as mean ± SD except for sex; M = males; F = females; 6 subjects per sampling time

- The mean (± SD) total plasma concentrations of levonadifloxacin in the 12-interval following the first and ninth doses are displayed in **Figures 1 and 2**. Noncompartmental pharmacokinetic parameters in plasma are presented in **Table 2.**
- The mean (± SD) concentrations of levonadifloxacin in plasma, ELF, and AM during the intrapulmonary sampling times are presented in **Table 3**.
- The mean (± SD) unbound plasma, ELF, and AM concentrations of levonadifloxacin following the 9th dose are displayed in **Figure 3**.
- The AUC_{0-12} values based on mean and median ELF and AM concentrations and the site-to-unbound plasma ratios based on AUC_{0-12} values are shown in **Table 4**.

Table 2. Noncompartmental Pharmacokinetic Parameters of Levonadifloxacin in Plasma after the 1st and 9th Oral Dose

	C_{max} (µg/mL)	T_{max} (hours)	AUC_{0-12} (µg•h/mL)	CL/F (L/h)	V/F (L)	$t_{½}$ (hours)
1st Dose	16.5 ± 5.1	1.8 ± 0.7	116 ± 29	9.11 ± 2.23	58.0 ± 14.7	4.5 ± 0.9
9th Dose	20.0 ± 4.3	2.1 ± 1.4	130 ± 32	8.17 ± 2.05	59.2 ± 16.0	5.1 ± 1.3

Figurre 1. Mean (±SD) Plasma Concentrations of Levonadifloxacin durng the 12-hours Interval Following the First Dose



Figurre 2. Mean (±SD) Plasma Concentrations of Levonadifloxacin durng the 12-hours Interval Following the Ninth Dose

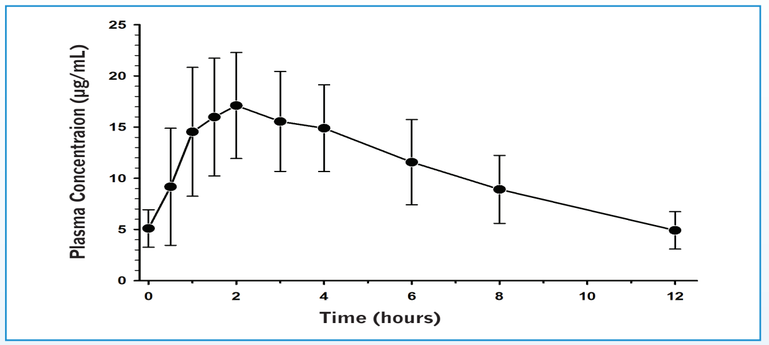


Table 3. Levonadifloxacin Concentrations in Plasma, ELF, and AM

Sampling Time	Plasma (µg/mL)	ELF (µg/mL)	AM (µg/mL
2-hour	20.5 ± 6.3	26.0 ± 15.9	3.91 ± 1.14
4-hour	17.6 ± 6.1	9.7 ± 7.2	3.64 ± 1.63
6-hour	16.4 ± 4.7	19.9 ± 11.1	4.79 ± 4.85
8-hour	9.49 ± 4.25	10.6 ± 6.0	2.10 ± 0.79
12-hour	4.88 ± 1.09	4.28 ± 1.40	1.59 ± 1.47

Data are expressed as mean ± SD; 6 subjects per sampling period for all matrices

Figurre 3. Mean (±SD) Plasma Concentrations of Levonadifloxacin durng the 12-hour Interval Following the Ninth Dose

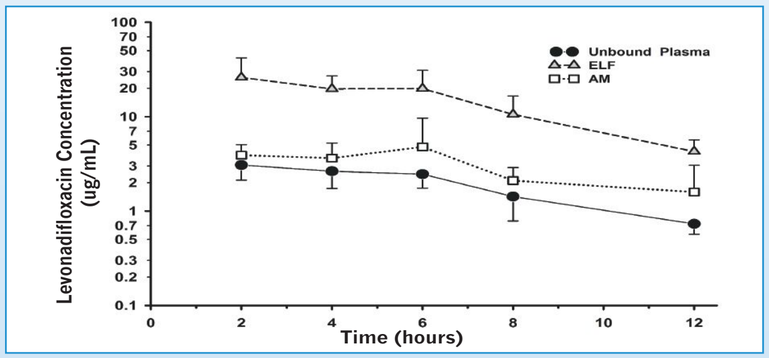


Table 4. AUC_{0-24} and Site-to-Unbound Plasma Ratios for ELF and AM

	ELF	AM
Mean Values:		
AUC_{0-12} (µg-h/mL)	**172.6**	**35.3**
Ratio Site:Unbound Plasma	**7.66**	**1.58**
Median Values:		
AUC_{0-12} (µg-h/mL)	**161.2**	**30.6**
Ratio Site:Unbound Plasma	**7.58**	**1.44**

Unbound plasma AUC_{0-12} determined with concentrations obtained at bronchoscopy sampling times. The unbound plasma levonadifloxacin concentration were calculated from the total concentration by assuming 85% plasma binding.

CONCLUSIONS

- **WCK 2349 administered orally to 31 heathly adult subjects at 1000 mg for 5 consecutive days was safe and well tolerated. The most frequent occurring treatment-emergent adverse events included photophobia (4/17) in 4 subjects and dysgeusia (4/17) reported in four subjects. All adverse events were mild in severity.**
- **Overall, concentrations in ELF and AM were higher than unbound plasma concentrations of levonadifloxacin (ELF-to-unbound plasma ratio [>7.5 times] and AM-to-unbound plasma ratio [>1.4 times] based on AUC_{0-12} values) after oral administration of WCK 2349.**
- **These data support further study of WCK 2349 for treatment of lower respiratory tract bacterial infections caused by susceptible pathogens.**

DISCLOSURES

This study was sponsored by Wockhardt Bio AG, Switzerland.
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