ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 2009, p. 3218–3225 Vol. 53, No. 8
0066-4804/09/$08.00+0 doi:10.1128/AAC.00022-09
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Pharmacokinetics of EDP-420 after Multiple Oral Doses in Healthy Adult Volunteers and in a Bioequivalence Study▽

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Received 6 January 2009/Returned for modification 15 April 2009/Accepted 12 May 2009

EDP-420 (also known as EP-013420, or S-013420) is a first-in-class bridged bicyclolide currently in clinical development for the treatment of respiratory tract infections (RTI) and has previously shown favorable pharmacokinetic (PK) and safety profiles after the administration of single oral doses of a suspension to healthy volunteers. Here we report its PK profile after the administration of multiple oral doses of a suspension to healthy adults. Bioequivalence between suspension and capsule formulations, as well as the effect of food, is also reported. The most important PK features of EDP-420 observed in these clinical studies are its long half-life of 17 to 18 h and its high systemic exposure, which support once-daily dosing and treatment durations potentially shorter than those of most other macrolide antibiotics. EDP-420 is readily absorbed following oral administration in both suspension and capsule formulations. In the multiple-oral-dose study, steady state was achieved on day 1 by using a loading dose of 400 mg/day, followed by 2 days of 200 mg/day. A high-fat meal had no effect on the bioavailability of EDP-420 administered in a capsule formulation. EDP-420 was well tolerated, with no serious or severe adverse events reported, and no subject was discontinued from the study due to an adverse event. Based on its human PK and safety profiles, together with its in vitro/in vivo activities against common respiratory pathogens, EDP-420 warrants further development, including trials for clinical efficacy in the treatment of RTI.

Macrolides are currently used as a first-line treatment for respiratory tract infections (RTIs), including community-acquired pneumonia, acute exacerbations of chronic bronchitis, acute sinusitis, pharyngitis/tonsillitis, and otitis media (5, 37). The extensive clinical usage of macrolides has resulted in the rapid emergence of macrolide resistance, particularly among streptococci, staphylococci, and enterococci (1, 13, 38). Thus, there is an urgent need to develop new antibiotics with activity against a broad spectrum of pathogens (especially resistant strains) commonly encountered in community-acquired RTIs. The design of EDP-420 (formerly known as EP-013420, or S-013420) was undertaken in response to this unmet medical need.

EDP-420 represents a novel structural class of bridged bicyclolide antibacterial agents (36) (the structure is shown in Fig. 1). It exhibits potent in vitro activities against RTI pathogens, including multidrug-resistant streptococci and the atypical pathogens (e.g., *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, and *Legionella pneumophila*) (4, 10, 19, 24, 27–32, 34, 39). In vivo, EDP-420 has also demonstrated efficacy against *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus aureus*, and *Mycobacterium avium* in mouse protection tests, against *S. pneumoniae* and *Haemophilus influenzae* in a rat lung infection model, and against penicillin- and quinolone-resistant pneumococci in a rabbit meningitis model (2–4, 22, 23, 25, 32–34). The nonclinical pharmacokinetics (PK) of EDP-420 across multiple species displayed a long half-life as well as extensive distribution and uptake into respiratory tissue and fluids (14–17, 32, 33). Moreover, EDP-420 has previously shown favorable PK and safety profiles in healthy volunteers after single oral doses given as a suspension (18). Here, the PK of EDP-420 after multiple oral suspension doses in healthy adult volunteers is reported. In addition, since a capsule formulation was developed for use in phase II clinical trials, a separate clinical study was conducted to compare the PK and safety/tolerability of two formulations (i.e., capsule versus suspension) of EDP-420 in healthy adult volunteers. The potential effects of food on the oral bioavailability and systemic exposure of EDP-420 capsules were also investigated.

(The results of this study were presented in part at the 47th Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, September 2007.)

MATERIALS AND METHODS

Subjects and study designs. Two sequential clinical studies were conducted at SeaView Research (Miami, FL) in accordance with good clinical practices as described in the Code of Federal Regulations, Title 21, parts 50, 56, and 312 subpart D. Each subject was provided a written informed-consent form after having been apprised of the nature and purpose of the study, the participation/termination conditions, and the potential risks and benefits of participating in the study. Subjects were required to read, understand, and sign the written informed-consent form before participating in the study.

(i) Multiple-oral-dose study. A single-center, randomized, double-blind, placebo-controlled, two-way crossover study was performed to determine the safety, tolerability, and PK of EDP-420 given in multiple doses compared with a placebo. A total of 38 eligible adult subjects aged 18 to 45 years participated in the study. All were in good general health as determined by medical history, physical examination (including vital signs), clinical laboratory tests, and a 12-lead electrocardiogram (ECG). These subjects were sequentially randomized into one of two study groups (18 and 20 subjects in groups 1 and 2, respectively) with equal numbers of subjects for the active-drug and placebo regimens. Group 1 received 200 mg of EDP-420 or a placebo administered as a single daily oral dose for 3 days. Group 2 received 400 mg of EDP-420 or a placebo administered as a single oral loading dose on day 1, and 200 mg of EDP-420 or a placebo was administered as a single daily oral dose on days 2 and 3. Within each group, subjects were

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▽ Published ahead of print on 18 May 2009.

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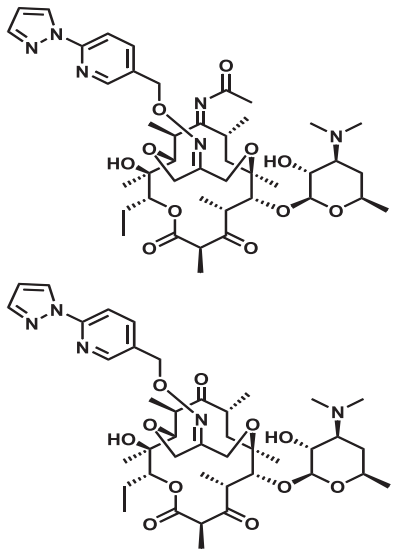

FIG. 1. Structures of EDP-420 (top) and its gastric acidic degradant, EDP-420 9-keto (bottom).

randomized to receive EDP-420 or a placebo in two separate treatment periods (period 1 and period 2). PK and safety were assessed throughout the study.

The study consisted of a screening period and two dosing periods. During the screening period (days −30 to −1), informed consent was obtained, eligibility for study entry was assessed, and screening evaluations were performed. On day −1 of period 1, subjects returned to the clinical research facility for final assessments before dosing. Those subjects who satisfied all of the inclusion criteria and none of the exclusion criteria qualified for the treatment period and were randomized. On the morning of the next day (day 1, the first dosing day), predose evaluations were obtained and the study medication was administered. After the last dose of the study medication on day 3, subjects remained in the clinical research facility for 7 days (a total of 10 days' confinement for each dosing period). Subjects with preentry baseline characteristics who continued to meet all the inclusion criteria and none of the exclusion criteria after the completion of period 1 were scheduled for period 2 and instructed to return to the clinical research facility the following week (a minimum of 7 days after discharge from period 1) to begin the confinement of the next dosing period. On day −1 of period 2, subjects returned to the clinical research facility, and similar procedures were repeated. Subjects were discharged following the completion of study assessments on day 10 of period 2.

The sample size for this study was not determined by formal statistical methods but was deemed a reasonable size to address the objectives of a multiple-oral-dose study.

(ii) Bioequivalence and food effect study. A single-center, randomized, open-label, three-way crossover study was conducted to determine the safety, tolerability, and PK of an EDP-420 suspension (fasting state) and capsule (fasting and fed states) administered as a single daily dose, as well as the effect of food on bioavailability. A total of 18 volunteers aged 18 to 44 years participated in the study. All were in good general health as determined by medical history, physical examination (including vital signs), clinical laboratory tests, and a 12-lead ECG. Three groups of six adult subjects were randomized to receive one of three EDP-420 dose regimens: a single dose of a 200-mg oral suspension of EDP-420 administered under fasting conditions (regimen A), a single dose of two 100-mg capsules of EDP-420 administered under fasting conditions (regimen B), or a single dose of two 100-mg capsules of EDP-420 administered under fed conditions (regimen C). Each group then successively crossed over to receive the remaining two dose regimens. Subjects receiving regimen A or B were fasted for a minimum of 8 h prior to dosing. Subjects receiving regimen C consumed a high-fat meal (containing approximately 150, 250, and 500 to 600 cal from protein, carbohydrate, and fat, respectively) 30 min before dosing. PK and safety were assessed throughout the study.

The study consisted of a screening period and three dosing periods. The study design was similar to that described for the multiple-oral-dose study, except that the subjects remained in the clinical research facility for a total of 6 days of each dosing period and were discharged following the completion of the 120-h post-dose study assessments on day 6 of each period.

The sample size for this study, i.e., 18 subjects, was not determined by formal statistical methods, due to a lack of intrasubject variability data from crossover studies. However, 18 subjects were considered to be sufficient to perform a preliminary assessment of bioequivalence between the suspension and capsule formulations and to evaluate the effect of food on the bioavailability of EDP-420.

Study drugs. Clinical trial materials, including the investigational product EDP-420 and a placebo (microcrystalline cellulose, USP), were manufactured according to current good manufacturing practices. In the multiple-dose study, the EDP-420 drug substance and the placebo were supplied as powders for reconstitution and were administered as suspensions. In the bioequivalence and food effect study, EDP-420 was supplied as 100-mg capsules or as a powder for reconstitution and was administered as a suspension. To prepare a suspension for oral dosing, the appropriate amount of EDP-420 or the placebo was weighed into a 75-ml high-density polyethylene bottle and reconstituted using simple syrup, USP. EDP-420 is stable in simple syrup, USP.

All study drugs were kept in a secure, limited-access storage area under recommended storage conditions (room temperature) until needed or until returned to the sponsor.

Blood sample collection. In the multiple-oral-dose study, blood samples (4 ml each) were collected in EDTA-containing tubes during each treatment period at predose (0 h) and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, and 12 h postdose on days 1 and 3, at predose on day 2, and 24, 48, 96, 120, 144, and 168 h after the last dose of day 3. In the bioequivalence and food effect study, blood samples (4 ml each) were collected at predose (0 h) and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12, 24, 48, 72, 96, and 120 h postdose. The tubes were immediately chilled in ice. Plasma was separated by refrigerated centrifugation at 3,000 rpm for approximately 10 min. Plasma was then transferred to appropriately labeled storage tubes and stored at approximately −20°C until analysis. Exact dosing times and blood collection times were recorded.

Analytical methodology. The concentrations of EDP-420 and its gastric acidic degradant, EDP-420 9-keto (structures shown in Fig. 1), in plasma were analyzed using a validated high-performance liquid chromatography (HPLC) method with mass spectrometric detection (liquid chromatography-tandem mass spectrometry).

EDP-420 and EDP-420 9-keto were extracted from human plasma samples by protein precipitation. A 200-μl volume of a blank, standard quality control (QC) or plasma sample was placed in a clean 2-ml Eppendorf tube, and then 400 μl of a methanol-containing internal standard (approximately 50 ng/ml of the EDP-420 core structure EP-01304 dissolved in methanol) was pipetted into each tube. All the tubes were capped and vortexed for 3 min, followed by centrifugation at 10,000 rpm for 10 min to remove the precipitate. Approximately 400 μl of supernatant was transferred to HPLC vials for liquid chromatography-tandem mass spectrometry analysis. Chromatography was performed on a reverse-phase C_{18} column (30 mm by 2.0 mm; 5 μm pore size; Luna C_{18}; Phenomenex) and an HPLC system consisting of a Shimadzu LC-10AD pump and a Shimadzu SCL-HT autosampler. The mobile phases were water with 25 mM ammonium acetate, pH 5.1 (buffer A), and methanol-water (90:10, vol/vol) with 1 mM ammonium acetate (buffer B), and chromatography was run in a linear gradient mode from 20% buffer B to 75% buffer B in 6.5 min at a flow rate of 0.8 ml/min. EDP-420, EDP-420 9-keto, and the internal standard were detected using a Sciex/Perkin-Elmer API-4000 mass spectrometer in the positive-ion multiple-reaction monitoring mode. Mass transitions (m/z) monitored were 841.3 → 158.1 for EDP-420, 800.5 → 158.3 for EDP-420 9-keto, and 667.7 → 158.1 for the internal standard. The line of best fit for calibration standards was calculated by weighted ($1/x^2$) linear regression based on analyte/internal-standard peak area ratios for two replicates of seven calibration standards using the Watson LIMS System. QC and unknown sample concentrations for the analyte were calculated from the calibration standard curve based on analyte/internal-standard peak area ratios. The quantification and calibration ranges were from 1 to 1,000 ng/ml. The interbatch accuracy and precision of validation QC samples ranged from 99.0 to 108.5% and 3.9 to 5.6% for EDP-420 and from 98.0 to 107.5% and 4.1 to 6.2% for EDP-420 9-keto, respectively. The intrabatch accuracy and precision of validation QC samples ranged from 94.3 to 110.1% and 2.2 to 7.6% for EDP-420 and from 95.0 to 110.9% and 1.7 to 6.9% for EDP-420 9-keto, respectively. No significant interference with EDP-420 or EDP-420 9-keto was found from endogenous components of plasma or other sources.

PK and statistical analyses. PK analyses were performed for EDP-420 in plasma and urine using noncompartmental methods with WinNonlin Professional software, version 4.1 (Pharsight Corp., Mountain View, CA). Descriptive statistics were prepared with SAS, version 9.1 (SAS Institute, Inc., Cary, NC). Prior to the estimation of the plasma PK parameters, drug concentrations below

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the limit of quantitation were assigned a value of zero if they preceded quantifiable samples prior to the maximum drug concentration (C_{max}) in plasma. A concentration below the limit of quantitation that occurred after the time to C_{max} (t_{max}) was assigned a value of "missing." The actual elapsed time from dosing was used in the final PK analysis to estimate all individual PK parameters. The following plasma PK parameters were estimated by noncompartmental methods: both C_{max} in plasma and t_{max}, obtained directly from the observed concentration-versus-time data; the area under the plasma concentration-time curve from time zero until the last measurable plasma concentration time point ($AUC_{0\text{-last}}$), calculated by linear up/log down trapezoidal summation; the plasma AUC from time zero to infinity ($AUC_{0\text{-}\infty}$), calculated by linear up/log down trapezoidal summation and extrapolated to infinity by addition of the last quantifiable plasma concentration divided by the elimination rate constant (i.e., $AUC_{0\text{-last}} + C_{last}/\lambda_z$); the elimination rate constant (λ_z), determined by linear regression of the terminal points of the log-linear plasma concentration-time curve; the terminal half-life ($t_{1/2}$), determined as $\ln 2/\lambda_z$; and the percentage of $AUC_{0\text{-}\infty}$ obtained by extrapolation, calculated as $[(AUC_{0\text{-}\infty} - AUC_{0\text{-last}})/AUC_{0\text{-}\infty}] \times 100$. In the multiple-dose study, the accumulation index was calculated as ($AUC_{0\text{-}24}$ on the last dosing day)/($AUC_{0\text{-}24}$ on day 1).

To determine bioequivalence between the suspension (reference) and capsule (test) formulations of EDP-420, and to determine the effect of a high-fat meal on the bioavailability of the capsule formulation of EDP-420, analysis of variance (ANOVA) with pairwise comparison was carried out. After $\log_e$ transformation, $AUC_{0\text{-}\infty}$, $AUC_{0\text{-last}}$, and C_{max} values for EDP-420 were analyzed by ANOVA using a mixed-effects model with sequence, period, and treatment as fixed effects and subject nested within sequence as a random effect. For each parameter, the point estimate and 90% confidence intervals were calculated for the difference between formulations or treatments (i.e., test-reference or fed-fasting), using the residual variance from the ANOVA model. The point estimate and associated 90% confidence intervals were then exponentially back-transformed to provide point estimates and 90% confidence intervals for the treatment ratio (the test/reference or fed/fasting ratio, i.e., the regimen B/regimen A ratio or the regimen C/regimen B ratio). ANOVA included data from subjects who completed at least two treatments that contributed to the pairwise comparison of interest.

Safety assessment. Safety was assessed throughout the study by physical examination, adverse-event monitoring, ECG, clinical laboratory tests, and vital-sign measurements.

RESULTS

Subjects. In the multiple-dose study, 18 subjects were randomized into group 1 and 20 subjects into group 2. One subject in group 2 was withdrawn during the placebo treatment period for personal reasons. All other subjects completed the study. Within each group, subjects randomized to EDP-420/placebo and placebo/EDP-420 treatment sequences were comparable with respect to demographic characteristics (sex, age, race, and weight). In group 1, the majority of subjects were male (94%) and Hispanic (83%), with a mean age of 35.4 years (range, 19 to 44 years) and a mean body mass index (BMI) of 25.7 kg/m^2 (range, 21.8 to 29.5 kg/m^2). In group 2, all subjects were male and the majority were Hispanic (90%), with a mean age of 33.2 years (range, 19 to 44 years) and a mean BMI of 25.8 kg/m^2 (range, 22.0 to 29.7 kg/m^2).

In the bioequivalence and food effect study, a total of 18 subjects were enrolled and received at least one dose of the study drug. One subject discontinued the study prematurely for personal reasons. The remaining 17 subjects completed the entire study treatments and assessments as planned. All the subjects in the study were equally distributed between males and females who had had a hysterectomy or used a highly effective method of birth control. The racial distribution of EDP-420-treated subjects was 61% Caucasian, 33% black, and 6% of other racial background. The subjects had a mean age of 32.5 years (range, 18 to 44 years) and a mean BMI of 25.2 kg/m^2 (range, 20.3 to 30.1 kg/m^2).

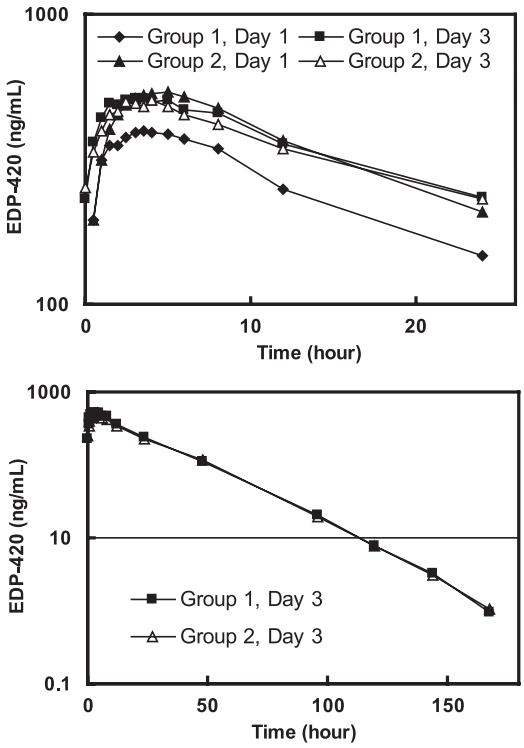

FIG. 2. Mean EDP-420 concentration-versus-time curves from 0 to 24 h (top) and from 0 to 168 h (bottom) in the multiple-oral-dose study.

During the study, no subject taking EDP-420 had a known concomitant illness, and/or received concomitant treatments or medicine, that was likely to interfere with the PK or safety of EDP-420.

PK. One subject from each study was excluded in the PK data analysis due to premature discontinuation. All the remaining subjects had evaluable data and were included in the PK analysis.

(i) EDP-420 PK. Figures 2 and 4 show the concentration-versus-time profiles of EDP-420 in the plasma of healthy volunteers after a single oral dose and/or multiple oral doses. No predose plasma samples had quantifiable EDP-420 concentrations (<1 ng/ml). EDP-420 was rapidly absorbed, with concentrations measurable at the first sampling time (0.5 h) postdose following administration of EDP-420 in suspension or capsule formulations under fasting conditions. Approximately 70% of subjects had measurable plasma EDP-420 concentrations at the first sampling time (0.5 h) postdose following administration of EDP-420 capsules with a high-fat meal (data not shown). All subjects had measurable plasma EDP-420 concentrations up to 96 h postdose. More than 80% of subjects had measurable plasma EDP-420 concentrations at 144 h postdose.

Table 1 shows the PK parameters of EDP-420 after multiple oral doses. The median t_{max} following multiple dosing was comparable to t_{max} on day 1, and t_{max} occurred at 3 to 4 h postdose after a single or multiple oral doses. The mean systemic exposure ($AUC_{0\text{-}24}$) was 6.20 or 8.10 μg · h/ml, with a mean C_{max} of 0.43 or 0.55 μg/ml after a single oral dose of 200 mg or 400 mg, respectively. A mean C_{max} of 0.56 μg/ml and a

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TABLE 1. PK parameters of EDP-420 in the multiple-oral-dose study

PK parameter and statistic[a]	Value for:			
	Group 1[b]		Group 2[b]	
	Day 1	Day 3	Day 1	Day 3
C_{max} (ng/ml)				
Geometric mean	432	559	548	514
CV (%)	23	19	39	33
t_{max} (h)				
Median	3.50	3.25	4.00	3.50
Minimum–maximum	1.5–8.0	1.5–8.0	2.5–6.0	2.5–6.0
$AUC_{0\text{-last}}$[c] (ng · h/ml)				
Geometric mean	6,200	16,356	8,103	15,009
CV (%)	22	23	41	44
$AUC_{0\text{-}\infty}$[d] (ng · h/ml)				
Geometric mean		16,419		15,059
CV (%)		23		44
Accumulation index				
Geometric mean	NA	1.41	NA	0.99
CV (%)		11		21
$t_{1/2}$[d] (h)				
Geometric mean		17.4		17.7
CV (%)		18		12

[a] CV, coefficient of variation.
[b] The dosing regimen for group 1 was 200 mg once daily for 3 days. The dosing regimen for group 2 was 400 mg once on day 1, followed by 2 days of 200 mg once daily. NA, not applicable.
[c] $AUC_{0\text{-last}}$ was $AUC_{0\text{-}24}$ on day 1 and $AUC_{0\text{-}168}$ on day 3.
[d] $t_{1/2}$ and $AUC_{0\text{-}\infty}$ could not be calculated accurately for day 1 due to a long $t_{1/2}$ and limited time points.

TABLE 2. PK parameters of EDP-420 in the bioequivalence and food effect study

PK parameter and statistic[a]	Value with the following regimen[b]:		
	A	B	C
C_{max} (ng/ml)			
Geometric mean	381	405	394
CV (%)	19	17	20
t_{max} (h)			
Median	3.50	3.50	4.00
Minimum–maximum	1.00–5.00	2.00–6.00	3.00–6.00
$AUC_{0\text{-last}}$ (ng · h/ml)			
Geometric mean	8,268	8,938	8,944
CV (%)	25	25	26
$AUC_{0\text{-}\infty}$ (ng · h/ml)			
Geometric mean	8,351	9,029	9,038
CV (%)	26	26	26
$t_{1/2}$ (h)			
Geometric mean	16.8	16.9	16.9
CV (%)	19	19	19

[a] CV, coefficient of variation.
[b] Regimen A, 200 mg of EDP-420 in a suspension taken orally under fasting conditions; regimen B, two 100-mg capsules of EDP-420 taken under fasting conditions; regimen C, two 100-mg capsules of EDP-420 taken with a high-fat meal.

mean $AUC_{0\text{-}\infty}$ of 16.42 μg · h/ml were achieved after three doses of 200 mg once daily. The mean C_{max} and mean $AUC_{0\text{-}\infty}$ were 0.51 μg/ml and 15.06 μg · h/ml, respectively, after a higher loading dose of 400 mg/day followed by 2 days of 200 mg/day. After absorption, EDP-420 was eliminated from the plasma with a mean $t_{1/2}$ of 17 to 18 h across the two treatment groups. The EDP-420 accumulation indices were approximately 1.4 and 1.0 after 3 days of 200-mg once-daily oral doses and after a higher loading dose of 400 mg/day followed by 2 days of 200 mg/day, respectively.

Table 2 shows the PK parameters of EDP-420 after single-dose administration in the bioequivalence and food effect study. The median t_{max} occurred at 3.5 h postdose for both formulations following oral administration of EDP-420 in a suspension or capsule formulation under fasting conditions, and the high-fat meal appeared to cause only a slight delay in the median t_{max}, to 4.0 h. The mean total systemic exposures ($AUC_{0\text{-}\infty}$) were 8.35, 9.03, and 9.04 μg · h/ml, with mean C_{max}s of 0.38, 0.40, and 0.40 μg/ml for regimens A, B, and C, respectively. After absorption, EDP-420 was eliminated from the plasma with a mean $t_{1/2}$ of approximately 17 h for all three treatment regimens (Table 2). The individual $t_{1/2}$ estimates ranged from approximately 11.0 to 24.0 h for all subjects across the three treatment regimens. Thus, blood sampling time in this study, lasting as long as 120 h postdose (covering approximately five times the estimated $t_{1/2}$), was sufficient for a reliable estimate of the terminal elimination rate constant for EDP-420. With a 120-h PK sampling time for each dose period, the percentage of $AUC_{0\text{-}\infty}$ obtained by extrapolation for all individual EDP-420 plasma profiles was <3% for treatment regimens A and B and <3.5% following treatment regimen C, indicating a sufficiently long sampling period for a reliable estimate of the total $AUC_{0\text{-}\infty}$.

There were no significant differences in EDP-420 PK profiles among the three treatment regimens (Fig. 4 and Table 2). The intersubject variability of EDP-420 PK parameters was generally in the range of approximately 20 to 25% across the three treatment regimens except at the beginning (0.5 h postdose) and toward the end (from 72 to 120 h postdose) of the concentration-versus-time profiles.

(ii) Plasma EDP-420 9-keto concentrations. Figures 3 and 4 show the concentration-versus-time profiles of EDP-420 9-keto in the plasma of healthy volunteers after single or multiple oral doses. No predose plasma samples had quantifiable EDP-420 9-keto concentrations (<1 ng/ml). EDP-420 9-keto appeared less rapidly in the plasma than the parent drug. It took 1.5, 2.0, or 2.5 h for all subjects to have measurable concentrations of EDP-420 9-keto in plasma following treatment regimen A, B, or C, respectively. All subjects had measurable plasma EDP-420 9-keto concentrations up to 72 h postdose.

Table 3 shows the PK parameters of EDP-420 9-keto after multiple oral doses. The median t_{max} of EDP-420 9-keto was delayed by 1 to 2 h compared to that for EDP-420; it occurred at 5 to 6 h postdose after a single or multiple oral doses. The mean $AUC_{0\text{–}24}$ was 0.46 or 0.76 μg · h/ml, with a mean C_{max} of 0.030 or 0.049 μg/ml after a single oral dose of 200 mg or 400 mg, respectively. A mean C_{max} of 0.046 μg/ml and a mean $AUC_{0\text{-}\infty}$ of 2.21 μg · h/ml were achieved after three doses of 200 mg once daily. The mean C_{max} and mean $AUC_{0\text{-}\infty}$ were 0.048 μg/ml and 2.23 μg · h/ml, respectively, after a higher loading dose of 400 mg/day followed by 2 days of 200 mg/day.

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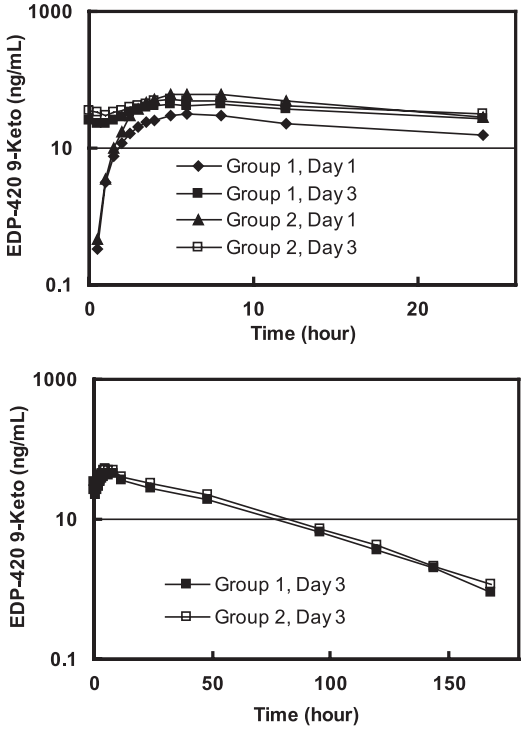

FIG. 3. Mean EDP-420 9-keto concentration-versus-time curves from 0 to 24 h (top) and from 0 to 168 h (bottom) in the multiple-oral-dose study.

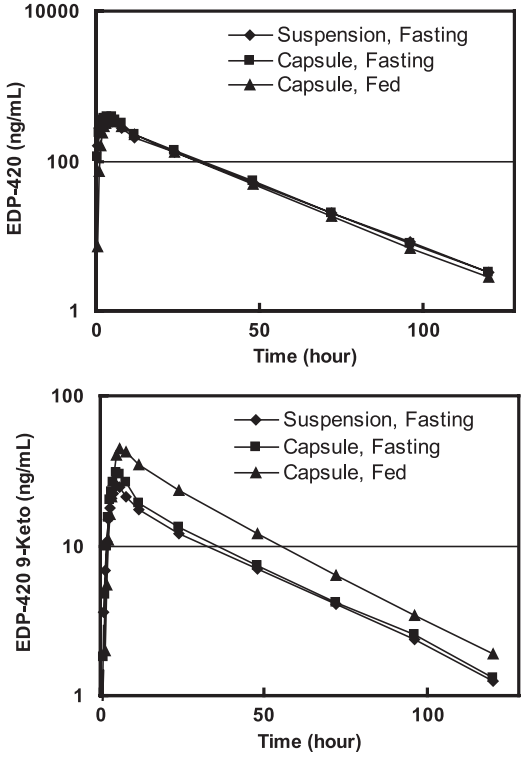

FIG. 4. Mean EDP-420 (top) and EDP-420 9-keto (bottom) concentration-versus-time curves in the bioequivalence and food effect study.

A mean $t_{1/2}$ of 28 to 30 h for EDP-420 9-keto was observed across the two treatment groups. The mean AUC_{0-24} of EDP-420 9-keto was 7 to 9% of the EDP-420 plasma exposure after a single dose of EDP-420 on day 1. Following multiple dosing, the EDP-420-9-keto $AUC_{0-\infty}$ as a proportion of the EDP-420 $AUC_{0-\infty}$ increased to 13 to 16% on day 3.

In the bioequivalence and food effect study, the median t_{max} of EDP-420 9-keto occurred at 5.0 h postdose following oral administration of EDP-420 in a suspension or capsule formulation under fasting conditions, and a high-fat meal appeared to cause only a slight delay in the median t_{max}, to 6.0 h (Table 4). The mean total systemic exposures (i.e., $AUC_{0-\infty}$) were 0.84, 0.84, and 1.33 μg · h/ml, with mean C_{max}s of 0.025, 0.024, and 0.036 μg/ml for regimens A, B, and C, respectively. EDP-420 9-keto was eliminated from the plasma with a mean $t_{1/2}$ of approximately 29 to 32 h across the three treatment regimens (Table 4). The individual $t_{1/2}$ estimates ranged from approximately 16 to 49 h for all subjects across the three treatment regimens. With a 120-h PK sampling time for each dose period, the percentage of $AUC_{0-\infty}$ obtained by extrapolation for the majority of individual EDP-420 9-keto plasma profiles was <20% following each treatment regimen.

No significant differences were observed in the plasma profiles of EDP-420 9-keto between suspension and capsule formulations administered under fasting conditions. The high-fat meal apparently increased plasma exposure to EDP-420 9-keto by ~58% over its exposure under fasting conditions.

The plasma exposure (i.e., C_{max} and $AUC_{0-\infty}$) ratios of EDP-420 9-keto versus the parent drug following each treatment regimen were calculated based on the data presented in Tables 3 and 4. The mean C_{max} ratios of EDP-420 9-keto to EDP-420 were 0.065, 0.058, and 0.092, and the mean $AUC_{0-\infty}$ ratios of EDP-420 9-keto to EDP-420 were 0.101, 0.093, and 0.147, respectively, for regimens A, B, and C.

Statistical analysis. The results of statistical analysis showed that the 90% confidence intervals of the geometric least-squares mean (GLSM) ratios for the bioequivalence parameters (i.e., $AUC_{0-\infty}$, AUC_{0-last}, and C_{max}) of EDP-420 were all within the 80-to-125% range, the criteria necessary to claim bioequivalence, when the capsule formulation was compared to the suspension formulation (Table 5). The capsule formulation administered under fasting conditions also produced a plasma EDP-420 9-keto profile that was almost superimposable on that obtained from the suspension formulation (Fig. 3). Administering EDP-420 capsules with a high-fat meal resulted in increases of approximately 58%, 64%, and 52% in the $AUC_{0-\infty}$, AUC_{0-last}, and C_{max} of EDP-420 9-keto, respectively, compared to the corresponding values from administration of EDP-420 capsules in the fasting state.

For comparison between fasting and fed states, the 90% confidence intervals of the GLSM ratios for the $AUC_{0-\infty}$, AUC_{0-last}, and C_{max} of EDP-420 were also all within the 80-to-125% range. The ANOVA results also showed that there were no differences in the $t_{1/2}$ of EDP-420 in plasma between the capsule and suspension formulations and that a high-fat meal had no effect on the $t_{1/2}$ of EDP-420 in plasma. The intrasubject variability of EDP-420 was determined to be approximately 17% for both the AUC and C_{max} values, based on the ANOVA results from this study. Specifically, intrasubject

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TABLE 3. PK parameters of EDP-420 9-keto in the multiple-oral-dose study

PK parameter and statistic[a]	Value for:			
	Group 1[b]		Group 2[b]	
	Day 1	Day 3	Day 1	Day 3
C_{max} (ng/ml)				
Geometric mean	30.5	46.0	49.0	47.6
CV (%)	37	25	56	43
t_{max} (h)				
Median	5.0	5.0	6.0	5.0
Minimum–maximum	3.5–8.0	4.0–8.0	3.5–12.0	4.0–8.0
$AUC_{0\text{-last}}$[c] (ng · h/ml)				
Geometric mean	461	2,134	756	2,154
CV (%)	36	27	57	48
$AUC_{0\text{-}\infty}$[d] (ng · h/ml)				
Geometric mean		2,209		2,228
CV (%)		27		48
Accumulation index				
Geometric mean	NA	1.76	NA	1.11
CV (%)		73		69
$t_{1/2}$[d] (h)				
Geometric mean		29.5		27.8
CV (%)		21		19

[a] CV, coefficient of variation.
[b] The dosing regimen for group 1 was 200 mg once daily for 3 days. The dosing regimen for group 2 was 400 mg once on day 1, followed by 2 days of 200 mg once daily. NA, not applicable.
[c] $AUC_{0\text{-last}}$ was $AUC_{0\text{-}24}$ on day 1 and $AUC_{0\text{-}168}$ on day 3.
[d] $t_{1/2}$ and $AUC_{0\text{-}\infty}$ could not be calculated accurately for day 1 due to a long $t_{1/2}$ and limited time points up to 24 h.

variability, expressed as the coefficient of variation, was 17.43% for $AUC_{0\text{-}\infty}$, 17.42% for $AUC_{0\text{-last}}$, 16.28% for C_{max}, and 3.75% for $t_{1/2}$.

Safety. EDP-420 was well tolerated. In the multiple-oral-dose study, there were no deaths, no reported serious or severe adverse events, and no adverse events resulting in discontinuation of the study medication. The most frequent adverse events reported by subjects on EDP-420 or the placebo were application site pruritis and erythema (which were related to the electrode site for performance of the ECGs) and headache. Mild alanine aminotransferase and/or aspartate transaminase elevations (≤1.6 times the upper limit of normal) were observed in five and two subjects on EDP-420 and the placebo, respectively, and the values returned to normal at the end of study. None of these elevations were associated with abnormal levels of total or direct bilirubin.

During the bioequivalence and food effect study, neither serious or severe adverse events nor any dose-limiting clinical or laboratory adverse events were reported. No subject was discontinued from the study due to an adverse event. No clinically significant changes in laboratory test results (including liver function), vital signs, ECG, or physical examinations were noted during the study following treatments with different formulations of EDP-420 under fasting/fed conditions.

TABLE 4. PK parameters of EDP-420 9-keto in the bioequivalence and food effect study

PK parameter and statistic[a]	Value with the following regimen[b]:		
	A	B	C
C_{max} (ng/ml)			
Geometric mean	24.9	23.7	36.1
CV (%)	47	61	47
t_{max} (h)			
Median	5.00	5.00	6.00
Minimum–maximum	3.50–6.00	3.50–8.00	5.00–8.00
$AUC_{0\text{-last}}$ (ng · h/ml)			
Geometric mean	754	745	1,224
CV (%)	58	52	47
$AUC_{0\text{-}\infty}$ (ng · h/ml)			
Geometric mean	842	837	1,326
CV (%)	57	49	47
$t_{1/2}$ (h)			
Geometric mean	29.5	30.5	28.4
CV (%)	27	31	27

[a] CV, coefficient of variation.
[b] Regimen A, 200 mg of EDP-420 in a suspension taken orally under fasting conditions; regimen B, two 100-mg capsules of EDP-420 taken under fasting conditions; regimen C, two 100-mg capsules of EDP-420 taken with a high-fat meal.

DISCUSSION

Two clinical studies, reported here, were conducted to investigate the safety/tolerability and PK of EDP-420 in healthy adult volunteers. EDP-420 is generally well tolerated. The most important PK features of EDP-420 observed in these clinical studies are its long $t_{1/2}$ and high systemic exposure compared to those of other macrolide antibiotics. The consistently long $t_{1/2}$ of 17 to 18 h has been observed in healthy adult volunteers after single and multiple oral doses of suspension and/or capsule formulations. Due to the long $t_{1/2}$ of EDP-420, >80% of subjects had measurable plasma EDP-420 concentrations at 144 h (6 days) after the last dose. In a study with community-acquired pneumonia patients, the $t_{1/2}$ was slightly prolonged, to 21 h (20). Thus, EDP-420 has a much longer $t_{1/2}$ than erythromycin or clarithromycin ($t_{1/2}$ values, 2.0 and 3.89 h, respectively) (7, 35). The $t_{1/2}$ of EDP-420 is comparable to the $t_{1/2}$ of 11 to 40 h for azithromycin (8, 21), which allows the latter drug to be used for a 1-day short-course therapy for the treatment of RTIs.

Also, EDP-420 has higher systemic drug exposure than other

TABLE 5. Statistical analysis of EDP-420 PK parameters

PK parameter	Ratio of GLSMs (90% confidence interval)	
	Regimen B/regimen A[a]	Regimen C/regimen B[a]
$AUC_{0\text{-}\infty}$	1.082 (0.977–1.197)	0.988 (0.893–1.094)
$AUC_{0\text{-last}}$	1.081 (0.977–1.197)	0.988 (0.893–1.094)
C_{max}	1.063 (0.967–1.169)	0.963 (0.876–1.059)

[a] Regimen A, 200 mg of EDP-420 in a suspension taken orally under fasting conditions; regimen B, two 100-mg capsules of EDP-420 taken under fasting conditions; regimen C, two 100-mg capsules of EDP-420 taken with a high-fat meal.

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macrolides. For example, the $AUC_{0-\infty}$ of EDP-420 at the 200 mg single oral dose of capsules was approximately 8.9 μg · h/ml (Table 2), which is 2.6-fold of the $AUC_{0-\infty}$ for azithromycin at the therapeutic dose of 500 mg (8, 21). Moreover, excellent distribution in the lung (the targeted site for RTIs) and penetration into epithelial lining fluid (ELF) and alveolar macrophages (AM) have been observed in a study with healthy Japanese subjects (12). Following a single oral dose of a 400-mg EDP-420 suspension in humans, mean AUC_{0-24} values in ELF and AM were 212.4 and 2,559 μg · h/ml, respectively, and the ratios of AUC_{0-24} in ELF and AM to AUC_{0-24} in plasma were 20.3 and 244.6, respectively. Significant amounts of EDP-420 were measurable in ELF and AM (6.5 and 68 μg/ml, respectively), even at 24 h postdose (12). The ELF and AM concentrations of EDP-420 at 24 h postdose were >72- and >2-fold, respectively, of the corresponding concentrations of azithromycin in ELF (below the detection limit of 0.09 μg/ml) and AM (30.8 μg/ml) after a single oral dose of 500 mg (9, 12). The efficiency of EDP-420 is driven by AUC/MIC ratios (23), and therefore, the high systemic exposure of EDP-420, its excellent penetration of ELF and AM, and its long $t_{1/2}$ in humans, plus its in vitro antimicrobial activity profiles and improved preclinical in vivo efficacy (including postantibiotic effect), support once-daily dosing and may allow for treatment durations shorter than those of most other macrolide antibiotics. Once-daily dosing with a potentially short treatment duration for EDP-420 is consistent with the current concept of "hit hard and stop early" for the treatment of RTIs (5, 11, 26), and it may provide advantages over other macrolide antibiotics (such as the widely used clarithromycin and erythromycin) in terms of patient convenience and compliance and, more importantly, minimization of the selection/emergence of resistance (26).

Due to its long $t_{1/2}$, there was significant systemic accumulation of EDP-420 following 3 days of 200-mg once-daily dosing; the AUC_{0-24} on day 3 was approximately 1.4 times that attained following a single dose (Table 1). When a higher loading dose of 400 mg/day was used, followed by 2 days of 200 mg/day, steady state was achieved on day 1 with high systemic exposure, which could eradicate bacteria early in the infection, when the burden is likely to be high, and could thus result in more-rapid resolution of infections (6).

The three-way crossover study demonstrated the bioequivalence between a new capsule formulation to be used in phase II clinical trials and a suspension formulation which was used in the first-in-humans study. Further, no effect of food on the systemic exposure of EDP-420 administered in the capsule formulation was observed. Based on a small intrasubject variability of 17% in the C_{max} and $AUC_{0-\infty}$ of EDP-420, a sample size of 17 subjects resulted in >90% power to detect a 20% difference in systemic exposure between treatment regimens. This means that the PK data obtained from 17 subjects in this study were sufficient to assess the bioequivalence between the suspension and capsule formulations and to evaluate the effects of food on the bioavailability of EDP-420.

The PK parameters of EDP-420 obtained from the bioequivalence and food effect study (a single oral dose) were comparable to those obtained in the previous first-in-humans study (18). EDP-420 was rapidly absorbed, with concentrations measurable as early as the first blood draw of 30 min postdose, and remained in the bloodstream as long as 144 h postdose (Fig. 2 and 4). In addition, a relatively small intersubject variability of ~20 to 25% in the EDP-420 C_{max}, $AUC_{0-\infty}$, and $t_{1/2}$ values was observed again in this study. The small intersubject and intrasubject variability might reduce the chance of unexpectedly high drug exposure (leading to toxicity) as well as the chance of insufficient drug exposure (leading to therapeutic failure).

Besides those of EDP-420, the concentrations of the EDP-420 gastric acidic degradant, EDP-420 9-keto, in plasma were also monitored in these two clinical studies. EDP-420 9-keto was the only major metabolite whose plasma exposure was ≥10% that of the parent, EDP-420, in the first-in-humans phase Ia clinical trial (L. J. Jiang, unpublished data). Like humans, preclinical species (such as rat and monkey) were also exposed to significant amounts of EDP-420 9-keto (Jiang, unpublished). More interestingly, EDP-420 9-keto exhibits potent in vitro activities against RTI pathogens, including multidrug-resistant streptococci and the atypical pathogens, and its in vitro potency is comparable to or even slightly better than that of EDP-420 itself (L. T. Phan, unpublished data).

In the bioequivalence and food effect study, the ratio of the exposure of EDP-420 9-keto to that of the parent drug was approximately 0.10 when EDP-420 formulations were administered under fasting conditions. A high-fat meal increased the mean metabolite-to-parent drug plasma exposure ratio to approximately 0.16. Although a high-fat meal increased plasma exposure to EDP-420 9-keto by ~58%, it is not known if this increase would be clinically significant because of the relatively low plasma exposure of EDP-420 9-keto (≤16%) compared to that of the parent drug. The contributions of EDP-420 9-keto to the clinical efficacy and safety of EDP-420 will be further evaluated in future clinical trials.

In conclusion, based on its human safety and PK profiles, together with its in vitro/in vivo activities against common respiratory pathogens, EDP-420 warrants further development, to include clinical efficacy trials for the treatment of RTIs using a capsule formulation. The lack of a significant food effect supports the administration of EDP-420 capsules without consideration of meal schedules in future clinical trials. In addition, the long half-life and high exposure of EDP-420 in the sites of infection support once-daily dosing and may allow for treatment durations shorter than those for most other macrolide antibiotics.

ACKNOWLEDGMENTS

We are grateful to the study participants, clinical investigators, and study coordinators and to Maria Paris, Ly Phan, Andrew Sonderfan, and David Brazier, who made this study possible.

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