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EXPERIMENTAL THERAPEUTICS

Pharmacokinetics and Safety of Intravenous Murepavadin Infusion in Healthy Adult Subjects Administered Single and Multiple Ascending Doses

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ABSTRACT Murepavadin is the first in class of the outer membrane protein-targeting antibiotics (OMPTA) and a pathogen-specific peptidomimetic antibacterial with a novel, nonlytic mechanism of action targeting *Pseudomonas aeruginosa*. Murepavadin is being developed for the treatment of hospital-acquired bacterial pneumonia (HABP) and ventilator-associated bacterial pneumonia (VABP). The pharmacokinetics (PK) and safety of single and multiple doses of murepavadin were investigated in healthy male subjects. Part A of the study was a double-blind, randomized, placebo-controlled, single-ascending-dose investigation in 10 sequential cohorts where each cohort comprised 6 healthy male subjects; 4 subjects were randomized to murepavadin, and 2 subjects were randomized to placebo. Part B was a double-blind, randomized, placebo-controlled, multiple-ascending-dose investigation in 3 sequential cohorts. After a single dose of murepavadin, the geometric mean half-life (2.52 to 5.30 h), the total clearance (80.1 to 114 ml/h/kg), and the volume of distribution (415 to 724 ml/kg) were consistent across dose levels. The pharmacokinetics of the dosing regimens evaluated were dose proportional and linear. Murepavadin was well tolerated, adverse events were transient and generally mild, and no dose-limiting toxicity was identified.

KEYWORDS phase 1 study, murepavadin, pharmacokinetics

Bacterial resistance has become a major public health problem. In recent years, there have been frequent publications regarding superbacteria, while there has been no increase in the development of new antibiotics (1). Current antibiotics are less effective due to the expression of various resistance mechanisms, which are having a major impact clinically, epidemiologically, and microbiologically worldwide. Multidrug-resistant organisms (MDROs) are those that exhibit a particular decreased susceptibility to antibiotics (2). The currently available options to treat *Pseudomonas aeruginosa* ventilator-associated bacterial pneumonia (VABP) include β-lactam antibiotics (e.g., meropenem), aminoglycosides (e.g., amikacin), quinolones (e.g., levofloxacin), fosfomycin, and polymyxins (e.g., colistin). However, *P. aeruginosa* has an intrinsic resistance to many antibiotics due to high cellular impermeability and efficient drug efflux mechanisms, and the recent increase in the prevalence of multidrug-resistant (MDR) *P. aeruginosa* infections is particularly threatening in intensive care unit settings (3, 4). It is estimated that at least 30% of the *P. aeruginosa* strains retrieved from respiratory specimen in patients with nosocomial pneumonia are MDR (5). Consequently, the treatment of hospital-acquired bacterial pneumonia (HABP)/VABP caused by *P. aeruginosa* is becoming more challenging (6), and new treatment options are urgently needed.

Murepavadin (formally known as POL7080) is an antimicrobial peptidomimetic with a novel, nonlytic mechanism of action and is the first in class of the outer membrane protein-targeting antibiotics (OMPTA) being developed by Polyphor Ltd. (7, 8). Murepavadin functions through a novel mechanism of action by binding to the lipopolysaccharide (LPS) transport protein D (LptD), an outer membrane protein involved in

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lipopolysaccharide biogenesis in Gram-negative bacteria (8). By binding to LptD, murepavadin inhibits the LPS transport function of LptD and causes lipopolysaccharide alterations in the outer membrane of the bacterium and, ultimately, cell death (9). Mutations conferring resistance to murepavadin have been localized in the N-terminal domain of LptD, which in the case of *P. aeruginosa* consists of about 300 residues, whereas it consists of only 180 residues in *Escherichia coli*, which partially explains why murepavadin's activity is specific for *P. aeruginosa* and murepavadin has no activity against other Gram-negative bacteria (10, 11). Given this specific mechanism of action, murepavadin is unlikely to generate resistance or negatively impact the patient's native bacterial flora, each of which is an unintended sequela of treatment with broad-spectrum antibiotics.

Nonclinical studies have demonstrated the selective and potent bactericidal antimicrobial activity of murepavadin against *P. aeruginosa in vitro*, including MDR strains. When tested against over 1,200 *P. aeruginosa* isolates from the United States, Europe, and China, including MDR isolates, the MIC_{90} was 0.12 to 0.25 mg/liter (H. S. Sader, G. E. Dale, P. Rhomberg, and R.K. Flamm, submitted for publication). Murepavadin had a low propensity to induce resistance *in vitro*, and the induction of resistance to murepavadin resulted in no cross-resistance to the other antibiotics tested. On the basis of nonclinical pharmacodynamic (PD) models, the area under the plasma concentration-versus-time curve (AUC)/MIC ratio was determined to be the pharmacokinetic (PK)/PD index that best predicts the efficacy of murepavadin (M. J. Melchers, J. J. Teague, P. Thommes, P. Warn, J.-U. Hansen, C. Vingsbro-Lundberg, P. Smith, F. Bernardini, A. Wach, D. Obrecht, G. E. Dale, and J. W. Mouton, submitted for publication). In the present study, the PK and safety of single and multiple doses of murepavadin were investigated in healthy male subjects.

(Parts of this research was previously presented in poster P1421 at the 22nd European Congress on Clinical Microbiology and Infectious Diseases [ECCMID], London, United Kingdom [12].)

RESULTS

Demographics and disposition. A total of 52 subjects received the study drug. In part B of the study, three subjects discontinued the study. One subject withdrew consent after study day 3, and two subjects were withdrawn due to treatment-emergent adverse events (AEs; one infusion site reaction and one event of gingivitis). These subjects' PK samples were obtained up to the time of withdrawal and were included in the PK analysis. The demographic characteristics of the subjects included in the PK analyses were similar between the single- and multiple-ascending-dose groups. Demographic data are presented in Table 1.

Safety. No serious adverse events or deaths were reported in the study. In part A of the study, 3-h infusions of murepavadin were well tolerated at doses up to and including 4.5 mg/kg of body weight. The highest single dose of 4.5 mg/kg was also well tolerated when administered as a 2-h infusion; although the 2-h infusion resulted in a higher mean maximum plasma concentration (C_{max}; the mean C_{max} was 12,800 ng/ml after the 2-h infusion and 9,470 ng/ml after the 3-h infusion), there was no appreciable qualitative or quantitative difference in the AE profile between the 2 treatment groups. Few treatment-emergent adverse events (TEAEs) were reported at the lower doses (0.05 to 2.2 mg/kg murepavadin), and those reported at the higher doses were transient and generally mild in intensity (Table 2). The most common adverse event observed at the higher doses was paresthesia or perioral paresthesia. There were few local-site reactions following single infusions of murepavadin (7/40 subjects), and these were generally mild and short-lived. No local reactions were reported in either of the groups receiving the highest dose (4.5 mg/kg murepavadin). In part B, in both groups receiving murepavadin doses every 12 h (q12h) (1.0 mg/kg and 5.0 mg/kg) the subject incidence of TEAEs was 100%; the number of TEAEs was greatest after the 5.0-mg/kg dose (24 TEAEs in the 5.0-mg/kg group, compared to 10 TEAEs in the 1.0-mg/kg group). The number and incidence of TEAEs in the group receiving the 2.0-mg/kg murepavadin dose every 8 h

TABLE 1 Baseline demographics of subjects in the study

Parameter	Value(s) for subjects receiving[a]:	
	SAD (n = 40)	MAD (n = 12)
Age (yr)		
Mean ± SD	27 ± 7	29 ± 8
Median	26	27
Range	19–44	18–43
Ht (cm)		
Mean ± SD	177 ± 8	174 ± 8
Median	178	177
Range	166–178	158–183
Body wt (kg)		
Mean ± SD	76 ± 10.8	76.6 ± 5.3
Median	75.6	78.4
Range	63.5–87.2	62.7–83.2
Body mass index (kg/m^2)		
Mean ± SD	24.1 ± 2.5	25.1 ± 2.0
Median	24.4	25.1
Range	19.6–30.0	22.0–28.9
No. (%) of patients of the following race:		
Caucasian	27 (67.5)	8 (66.7)
Black or African American	8 (20)	2 (16.7)
Asian	4 (10)	2 (16.7)
Other	1 (2.5)	0 (0)

[a]SAD, single ascending dose; MAD, multiple ascending dose.

(q8h) were similar to those in the group receiving placebo. In part B, the most frequently reported TEAEs were infusion site reaction, catheter site-related reaction, dizziness, paresthesia, and perioral paresthesia, and they were transient and generally mild in intensity (Table 3). The TEAEs relating to paresthesia and perioral paresthesia developed earlier in the dosing regimen (within the first 2 days of dosing), whereas the TEAEs relating to infusion site reactions and catheter site reactions developed over days 3 to 6. The single severe-intensity TEAE (infusion site reaction) was reported by a subject in the 1.0-mg/kg murepavadin q12h dose group and led to withdrawal of the subject from further treatment. There were no clinically significant findings in clinical laboratory safety test results, vital signs, 12-lead electrocardiogram (ECG), 24-h Holter ECG, physical examination findings, or neurological examination findings.

Pharmacokinetic summary. The values of the PK parameters for murepavadin when given as single and multiple ascending doses are given in Tables 4 and 5, respectively. All mean murepavadin plasma concentration profiles (part A and part B) showed a multiphasic decline following drug administration by intravenous (i.v.) infusion over the 0.05-mg/kg to 5-mg/kg dose range studied, with the terminal elimination phase commencing after 6 to 9 h postdose at the lower part of the dose range and extending to greater than 24 h at the higher doses with higher concentrations. In part B, the mean trough plasma concentrations (day 2 to day 5) of murepavadin suggested that steady-state plasma concentrations were likely achieved on day 3 following repeated dosing by the 1.0- and 5.0-mg/kg q12h dose regimens or the 2.0-mg/kg q8h dose regimen. For the highest dose in part B (5.0 mg/kg q12h), PK data were limited to 2 subjects. The mean concentration-time profiles of murepavadin in plasma in part A are shown in Fig. 1.

Single ascending doses. The geometric mean AUC from time zero extrapolated to infinity (AUC_{0-inf}) ranged from 438 ng · h/ml following the 0.05-mg/kg murepavadin dose to 56,200 ng · h/ml following the 4.5-mg/kg murepavadin dose; increases in AUC_{0-inf} were generally dose proportional. Cohorts A to I had an infusion time of 3 h; in cohort J, 4.5 mg/kg was infused for 2 h, and that cohort showed the highest geometric mean C_{max} (12,800 ng/ml). Cohort I (which received 4.5 mg/kg as a 3-h

TABLE 2 Summary of TEAEs ranked by overall frequency and preferred term in part A (SAD)[d]

Preferred term	No. of subjects in the following murepavadin dose group (mg/kg)[a,b]:									
	0.50 (cohort C, *n* = 4)	1.0 (cohort D, *n* = 4)	1.5 (cohort E, *n* = 4)	2.2 (cohort F, *n* = 4)	2.8 (cohort G, *n* = 4)	3.5 (cohort H, *n* = 4)	4.5 (cohort I, *n* = 4)	4.5[c] (cohort J, *n* = 4)	Total (*n* = 40)	Placebo[a,c] (*n* = 20)
Paresthesia	0	0	0	0	1	0	2	3 (1 M)	6 (1 M)	0
Paresthesia, oral	0	0	0	0	1	3	1	1	6	0
Dizziness	0	0	0	0	0	1	1	2	4	0
Headache	0	0	0	0	1	1	1	0	3	2
Catheter site pain	0	0	0	0	1 (M)	0	0	0	1 (M)	0
Dry mouth	0	0	0	0	0	0	1	0	1	0
Eye irritation	0	0	0	0	1	0	0	0	1	0
Infusion site erythema	0	0	0	0	0	1	0	0	1	0
Pain in extremity	0	0	1	0	0	0	0	0	1	0
Pharyngeal hypoesthesia	0	0	0	0	0	1	0	0	1	0
Presyncope	0	0	0	0	1 (M)	0	0	0	1 (M)	0
Procedural site reaction	1	0	0	0	0	0	0	0	1	0
Pruritus	0	0	0	0	0	0	0	1	1	0
Sensation of heaviness	0	0	0	0	0	1	0	0	1	0
Sensory disturbance	0	0	1	0	0	0	0	0	1	0
Catheter site-related reaction	0	0	0	0	0	0	0	0	0	1
Nasal obstruction	0	0	0	0	0	0	0	0	0	1

[a]Cohorts A to I received a 3-h infusion.

[b]Subjects in the 2 lowest-dose groups (cohort A [0.05 mg/kg murepavadin] and cohort B [0.15 mg/kg murepavadin]) did not report any TEAEs, and so data for those subjects were have been omitted from this table.

[c]Cohort J received a 2-h infusion. All treatment-emergent adverse events were mild, unless they are indicated to have been of moderate intensity (M).

[d]SAD, single ascending dose. Adverse events were coded using MedDRA (version 14.0).

TABLE 3 Summary of TEAEs ranked by overall frequency and preferred term in part B (MAD)[a]

Preferred term	No. of subjects in the following murepavadin dose group (mg/kg):				
	1.0 q12h (cohort 1, *n* = 4)	2.0 q8h (cohort 2, *n* = 4)	5.0 q12h (cohort 3, *n* = 4)	Total (*n* = 12)	Placebo (*n* = 6)
Catheter site-related reaction	2 (1 M)	2 (1 M)	0	4 (2 M)	1 (M)
Infusion site reaction	2 (1 M, 1S)	0	2	4 (1 M, 1 S)	0
Dizziness	0	0	3 (M)	3 (1 M)	0
Paresthesia	1	0	2	3	0
Paresthesia, oral	1	0	2	3	0
Asthenia	0	0	1 (M)	1 (M)	0
Catheter site pain	0	1	0	1	0
Dermatitis contact	1	0	0	1	0
Eye pain	0	0	1	1	0
Eyelid ptosis	0	0	1	1	0
Feeling hot	0	0	1	1	0
Gait disturbance	0	0	1	1	0
Gingival pain	0	0	1	1	0
Gingival swelling	0	0	1	1	0
Gingivitis	0	0	1 (M)	1 (M)	0
Headache	0	0	1 (M)	1 (M)	0
Lethargy	0	0	1	1	0
Lip dry	1	0	0	1	0
Mouth ulceration	0	0	1	1	0
Oropharyngeal pain	0	1	0	1	0
Phlebitis	1	0	0	1	0
Pleuritic pain	1	0	0	1	0
Rhinorrhea	0	1	0	1	0
Skin reaction	0	0	1	1	0
Tongue ulceration	0	0	1 (M)	1 (M)	0
Fatigue	0	0	0	0	1
Rhinitis	0	0	0	0	1

[a]MAD, multiple ascending dose. Adverse events were coded using MedDRA (version 14.0). All treatment-emergent adverse events were mild, unless they are indicated to have been of moderate intensity (M) or severe intensity (S).

infusion) had the highest geometric mean AUC_{0-inf} (56,200 ng · h/ml), but the values of the C_{max} as well as the AUC_{0-inf} parameters were within the standard deviation for either of the two cohorts. No major differences outside of C_{max} were observed with the two different infusion times in patients receiving the 4.5-mg/kg doses. Increases in C_{max} were dose proportional over the 0.05-mg/kg to 4.5-mg/kg dose range for the 3-h infusion time. Following single-dose administration, geometric mean murepavadin plasma exposure parameters (AUC_{0-inf} and C_{max}) generally increased in a dose-proportional manner from 0.05 to 4.5 mg/kg (Fig. 2). The geometric mean terminal elimination half-life ($t_{1/2}$) was similar (range, 2.66 to 5.43 h) through the dose range. While $t_{1/2}$ was apparently lower at the lowest two doses, it appeared to be dose

TABLE 4 Mean PK values for murepavadin after a single dose in part A[a]

Cohort	Dose (mg/kg)	No. of subjects	C_{max} (ng/ml)	AUC_{0-inf} (ng · h/ml)	$t_{1/2}$ (h)	CL (ml/h/kg)	V_z (ml/kg)	f_e[b] (%)	CL_R (ml/h)
A	0.05	4	104 (20.1)	438 (17.9)	2.52 (42.2)	114 (17.7)	415 (42.2)	ND	ND
B	0.15	3	353 (9.7)	1,750 (12.9)	3.49 (7.8)	85.6 (13.2)	431 (19.2)	ND	ND
C	0.5	4	992 (23.9)	4,690 (25.0)	4.43 (24.3)	107 (25.0)	681 (11.2)	ND	ND
D	1	4	2,600 (13.0)	11,400 (5.3)	5.30 (8.8)	87.3 (5.5)	667 (13.8)	ND	ND
E	1.5	4	3,660 (15.4)	13,900 (15.9)	4.55 (12.3)	108 (16.1)	707 (15.3)	ND	ND
F	2.2	4	4,960 (25.3)	24,200 (13.7)	5.39 (14.0)	91.0 (13.6)	708 (16.2)	ND	ND
G	2.8	4	65,230 (21.8)	32,600 (30.6)	5.26 (15.0)	85.8 (30.5)	652 (19.1)	ND	ND
H	3.5	4	7,250 (6.7)	35,600 (7.6)	5.10 (10.2)	98.3 (7.5)	724 (7.9)	ND	ND
I	4.5	4	9,470 (4.9)	56,200 (7.3)	5.15 (9.5)	80.1 (7.3)	595 (11.8)	3.06 (28.8)	197 (29.4)
J	4.5[c]	4	12,800 (15.8)	53,000 (15.0)	4.69 (12.9)	85.0 (15.1)	575 (20.1)	5.59 (31.1)	393 (26.5)

[a]Values represent the geometric mean (coefficient of variation, in percent). Parameter values were rounded to three significant digits. Many samples from cohorts A to I were analyzed outside of the stability date and are not reported. ND, not determined.

[b]f_e, cumulative fraction of the dose excreted in urine through 24 h postdose.

[c]Two-hour infusion.

TABLE 5 Mean PK values for murepavadin after the first dose (day 1) and multiple doses (day 6) in part B[a]

Dose (mg/kg)	Day	No. of subjects	C_{max} (ng/ml)	AUC_{0-inf} or AUC_{0-tau}[b] (ng · h/ml)	$t_{1/2}$ (h)	CL (ml/h/kg)	V_z or V_{ss}[b] (ml/kg)	f_e[c] (%)	CL_R (ml/h)
1 q12h	1	4	2,280 (14.5)	12,500 (7.8)	3.38 (16.7)	79.9 (8.0)	390 (21.0)	ND	ND
	6	3	2,320 (30.8)	11,400 (26.7)	6.17 (7.8)	88.0 (26.6)	410 (30.0)	ND	ND
2 q8h	1	4	5,360 (18.8)	22,400 (17.4)	3.36 (17.9)	89.3 (17.3)	432 (16.9)	0.292 (105)	54.3 (90.9)
	6	4	6,650 (16.1)	29,300 (18.4)	5.23 (2.6)	68.3 (18.3)	379 (23.6)		
5 q12h	1	4	14,000 (13.2)	65,300 (12.0)	3.24 (13.0)	76.6 (12.0)	358 (9.0)	9.56 (16.7)	218 (26.5)
	6	2	15,900	74,500	7.15	67.2	389	21.8	67.2

[a]Values represent the geometric mean (coefficient of variation, in percent). Parameter values were rounded to three significant digits. ND, not determined.
[b]AUC_{0-inf} and V_z are used for day 1, and AUC_{0-tau} and V_{ss} are used for day 6.
[c]f_e, cumulative fraction of the dose excreted in urine through 24 h postdose.

independent over the dose range of 0.5 mg/kg to 4.5 mg/kg due to the scarce plasma levels at the terminal phase. In part A, after a single dose, cohorts I and J had notable murepavadin urine concentrations up to 24 h postdose; in these cohorts, less than 6% of the dose was excreted as unchanged murepavadin. The mean renal clearance of the drug from plasma (CL_R) was 197 ml/h in cohort I (3-h infusion), and with the same dose in cohort J (2-h infusion), the mean CL_R was 393 ml/h, indicating some degree of variability associated with the low urinary excretion of murepavadin.

Multiple ascending doses. In part B, cohort 1 (1.0 mg/kg) and cohort 3 (5.0 mg/kg) received the murepavadin dose as a 3-h infusion q12h and cohort 2 received the murepavadin dose at 2.0 mg/kg as a 3-h infusion q8h. The results are summarized in Table 5. On day 1 in cohort 1 (1.0 mg/kg), the geometric mean AUC_{0-inf} was 12,500 ng · h/ml, in cohort 2 (2.0 mg/kg) the geometric mean AUC_{0-inf} increased 1.8-fold to 22,400 ng · h/ml, and in cohort 3 (5.0 mg/kg) it increased 2.9-fold to 65,300 ng · h/ml. On day 1 in cohort 1 (1.0 mg/kg), the geometric mean C_{max} was 2,280 ng/ml, in cohort 2 (2.0 mg/kg) the geometric mean C_{max} increased 2.4-fold to 5,360 ng/ml, and in cohort 3 (5.0 mg/kg) it increased 2.6-fold to 14,000 ng/ml. The geometric mean $t_{1/2}$ was consistent for all doses in part B, ranging from 3.24 to 3.38 h. The geometric mean clearance (CL) and volume of distribution based on the terminal phase following intravenous administration (V_z) values appeared to be dose independent.

Geometric mean exposure and CL results were comparable between part B (day 1) and part A; however, in part B (day 1), estimates for the geometric mean $t_{1/2}$ and V_z were slightly less than those reported in the part A single-dose cohorts. This is likely due to the multiphasic decline in the plasma concentrations and a more robust determination of the elimination rate constant (λ_z) over the entire terminal phase in part A. The sampling scheme was up to 27 h in part A and was not limited to sampling time points stopping at the dose interval (12 or 8 h), as scheduled in part B (day 1).

In part B (day 1), urinary excretion results for murepavadin were estimated in cohorts 2 and 3 with reportable concentrations up to the dose interval (Table 5). In cohort 1,

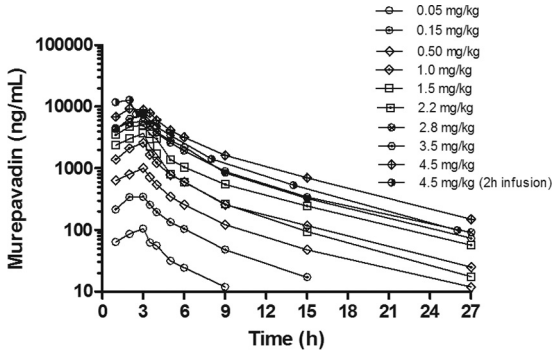

FIG 1 Mean concentration-time profiles of murepavadin in plasma (semilog plot, part A).

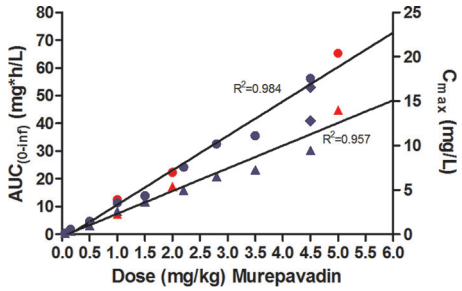

FIG 2 Geometric mean AUC_{0-inf} (blue closed circles) and C_{max} (blue closed triangles) versus dose. Blue diamonds, cohort J (4.5 mg/kg, 2-h infusion); red circles, day 1 AUC_{0-inf} from part B; red triangles, day 1 C_{max} from part B.

murepavadin was not quantifiable in urine at a lower limit of quantitation (LLOQ) of 100.0 ng/ml. In cohort 2 (2.0 mg/kg q8h), the cumulative fraction of the dose excreted in urine (f_e; in percent) over the 8-h sampling period was less than 1%, and less than 10% was excreted in cohort 3 (5.0 mg/kg q12h) over the 12-h sampling period. The geometric mean CL_R was 54.3 ml/h in cohort 2 (q8h), and in cohort 3 (q12h) the mean CL_R was 218 ml/h.

A summary of the values for the plasma murepavadin PK parameters on day 6 is presented in Table 5. The geometric mean area under the plasma concentration-versus-time curve during a dosing interval (AUC_{0-tau}) for cohort 1 (1.0 mg/kg q12h) was 11,400 ng · h/ml with no perceptible accumulation (1.05-fold increase) following q12h dosing. In cohort 2 (2.0 mg/kg q8h), the mean AUC_{0-tau} was 29,300 ng · h/ml with an accumulation ratio of 1.58 following q8h dosing. The mean C_{max} was 2,320 ng/ml in cohort 1 with no perceptible accumulation (1.02-fold increase), and it was 6,650 ng/ml in cohort 2 with little accumulation (a 1.24-fold increase) from day 1. The mean $t_{1/2}$ (6.17 and 5.23 h, respectively) was similar in both dose groups. The mean $t_{1/2}$ was higher following dosing on day 6, which was most likely a result of a more complete characterization of the elimination phase. The mean clearance at steady state was lower in cohort 2 (68.3 ml/h/kg) than in cohort 1 (88.0 ml/h/kg), while the mean volume of distribution at steady state (V_{ss}) was similar between cohort 1 and cohort 2 (410 and 379 ml/kg, respectively), and similar results were seen for V_z on day 1 for the corresponding doses. Results determined from renal clearance (CL_R) data were inconclusive for day 6 due to minimal excretion, as less than 2% of the dose was excreted unchanged over the 8-h dosing interval (LLOQ = 100 ng/ml) for cohort 2 (2 mg/kg q8h).

As expected, the accumulation parameters R_{AUC} and $R_C$max were higher following a q8h regimen (2.0 mg/kg q8h) than a q12h regimen (1.0 mg/kg q12h). The q12h dosing resulted in essentially no accumulation, while the q8h dosing showed a slight accumulation for C_{max} (24%) and somewhat greater accumulation for AUC_{0-tau} (58%). Accumulation ratios for the two individual subjects receiving the 5.0-mg/kg q12h dose were highly variable and were between those for the other 2 multiple-dose cohorts. The ratio of the day 6 AUC_{0-tau}/day 1 AUC_{0-inf} (linearity index [LI]) was close to unity for all dose groups in part B, with a slight increase being detected in cohort 2 (2.0 mg/kg) compared to that seen in the other cohorts.

DISCUSSION

Doses of murepavadin up to 4.5 mg/kg as a single dose and 5 mg/kg as multiple doses for up to 6 days were safe and generally well tolerated. The nature and incidence of adverse events did not appear to be dose related, except for paresthesia and perioral paresthesia, which were observed only at doses of 2.8 mg/kg and above. No dose-limiting toxicities were identified for murepavadin at single doses up to 4.5 mg/kg (median dose, 376 mg) and multiple doses up to 5 mg/kg q12h (median dose, 766 mg/day). In part B, the 1.0-mg/kg murepavadin q12h and 2.0-mg/kg murepavadin q8h

dose regimens were generally well tolerated, but 1 subject was withdrawn from the 1.0-mg/kg murepavadin dose group due to a severe-intensity infusion site reaction (Table 3). Although the 5.0-mg/kg q12h dose of murepavadin was considered by the investigator to be tolerable to the healthy subjects, the investigator and sponsor decided not to escalate the dose further in this healthy population due to the frequency of mild to moderate TEAEs and the fact that the upper limit of the projected dose range for the treatment of infections had been reached. There were a greater number of TEAEs in the 5.0-mg/kg murepavadin q12h dose group than in the other groups (Table 3), and 1 subject was withdrawn due to moderate-intensity gingivitis, which was considered to have a possible relationship to the treatment. Although it is possible that this subject's symptoms were due to an infection, it was noted that 2 other subjects in the 5.0-mg/kg murepavadin q12h dose group reported similar symptoms: mild intermittent gingival pain and mouth ulceration in one subject and moderate-intensity painful gum swelling and tongue ulceration in another. All of these TEAEs were considered to have a possible relationship to treatment. There were no clinically significant findings in the serum biochemistry, hematology, or urinalysis test results, vital signs, 12-lead ECG, 24- Holter ECG, or the results of physical or neurological examinations.

All single- and multiple-dose murepavadin mean plasma concentration profiles showed a multiphasic decline in the plasma concentrations following i.v. infusion administration. Following single i.v. dose administration by infusion, evaluation of the mean exposure parameters of the increases in C_{max} and AUC suggested a nearly linear dose-exposure correlation from 0.15 mg/kg to 5 mg/kg, suggesting dose proportionality. Drug accumulation determined from the accumulation ratios $R_{ac(AUC)}$ and $R_{ac(Cmax)}$ in the part B multiple-dose PK study was minor with the 2.0-mg/kg q8h dose but was not evident in the q12h dose regimen. The mean plasma $t_{1/2}$ was similar (range, 2.66 to 5.43 h) through the dose range. Due to the scarce terminal plasma level data and the putative overestimation of $t_{1/2}$ at the two lowest doses, robust dose independency was deduced only from the dose range of 0.5 mg/kg to 5 mg/kg. In part A, cohorts I and J receiving a single dose had notable murepavadin urine concentrations up to 24 h postdose; in these cohorts, less than 6% of the dose was excreted as unchanged murepavadin. According to the compound plasma concentration-time profiles during the terminal elimination period, murepavadin appeared to be cleared from the systemic circulation at glomerular filtration rates. On the other hand, the fraction of the administered murepavadin dose excreted unchanged in urine was low in part A (for cohort I [4.5 mg/kg, 3-h infusion], 3.0%; for cohort J [4.5 mg/kg, 2-h infusion], 5.6%) following single-dose i.v. administration by infusion. Thus, glomerular filtration might be only the primary elimination process in the terminal phase, while during the infusion and distribution phase, proteolytic degradation might be the principal cause of murepavadin elimination (13).

The PK of murepavadin were predictable and dose proportional over the doses administered in this phase 1 study. The PK/pharmacodynamic index that best correlates with murepavadin efficacy is the AUC/MIC; thus, the predictable and linear exposures observed with the murepavadin doses tested are advantageous (Melchers et al., submitted). The murepavadin V_{ss} (approximately 400 ml/kg) suggests that most of the drug is located in the extracellular volume (250 to 300 ml/kg in human), indicating that it distributes well into the extracellular space of infection. These potential PK advantages, in addition to the reported *in vitro* activity of murepavadin toward *P. aeruginosa*, including strains which are extensively drug resistant, support the use of murepavadin as an option for the treatment of those infections caused by organisms for which there are currently limited treatment or no treatment options. The results of the present study support the further clinical development of murepavadin.

MATERIALS AND METHODS

This study was conducted according to good clinical practices and the ethical principles of the Declaration of Helsinki. The study was conducted at a single center and was approved by an independent institutional review board (National Research Ethics Service [NRES] South East London Research Ethics Committee 1). Each participating subject provided written informed consent prior to enrollment. This clinical trial was conducted

and essential study documentation has been archived, in compliance with International Conference on Harmonization Guidelines and Good Clinical Practice (ICH-GCP; CPMP/ICH/135/95/Step5, Explanatory Note and Comments to the above, issued as CPMP/768/97).

Study design. The study was divided into 2 parts. Part A was a single-ascending-dose study and part B was a multiple-ascending-dose study, as described below. Single doses up to and including 4.5 mg/kg were investigated in ascending order in part A (starting with the lowest 0.05-mg/kg dose) prior to the start of part B. Part A was a double-blind, randomized, placebo-controlled, single-ascending-dose investigation in 10 sequential cohorts (cohorts A to J). Each cohort comprised 6 healthy male subjects; 4 subjects were randomized to murepavadin, and 2 subjects were randomized to placebo. Subjects in cohorts A to I received a single i.v. infusion of murepavadin or placebo over 3 h (doses of 0.05 mg/kg to 4.5 mg/kg). Cohort J received a single i.v. infusion of murepavadin or placebo (4.5 mg/kg) over 2 h. Part B was a double-blind, randomized, placebo-controlled, multiple-ascending-dose investigation in 3 sequential cohorts (cohorts 1 to 3). Each cohort comprised 6 healthy male subjects; 4 subjects were randomized to murepavadin, and 2 subjects were randomized to placebo. Subjects in each cohort received i.v. infusions over 3 h either twice daily (q12h) or 3 times daily (q8h) on days 1 to 5, with a final single 3-h infusion occurring in the morning of day 6. Doses of 1.0 mg/kg q12h, 2.0 mg/kg q8h, and 5.0 mg/kg q12h were investigated. Safety data from each cohort were reviewed prior to dosing of the next cohort. Subjects remained in the clinical research center for 3 days after the last dose, and pharmacokinetic assessments were conducted for up to 24 h after the last dose.

Subject selection. Healthy men aged 18 to 45 years with a body mass index (BMI) of between 19 and 30 kg/m^2 were eligible. Vital signs had to be in the normal range. Subjects were excluded if they had used any prescribed systemic or topical medication within 4 weeks of administration of the first dose; had used any nonprescribed systemic or topical medication (including herbal remedies) within 7 days of the first dose administration, unless, in the opinion of the investigator, the medication would not interfere with the study procedures or compromise safety; had clinically significant electrocardiogram (ECG) abnormalities; had significant allergies requiring intranasal or systemic corticosteroids during any time of the year, a history of any anaphylactic reaction, or an eosinophil count outside the reference range; consumed more than 28 units of alcohol per week or had a significant history of alcoholism or drug/chemical abuse; smoked or had smoked in the previous 6 months; were not willing to use appropriate contraception; or had a medical history which, in the opinion of the investigator, might interfere with study procedures or safety.

Safety monitoring. During the course of the study, all subjects were monitored for the occurrence of adverse events by the use of physical examinations, vital sign assessments, neurological examinations, 12-lead ECG, 24-h Holter ECG, and laboratory assessments, including hematology, serum, coagulation, and urinalysis tests.

Pharmacokinetic evaluations. In part A, plasma samples were collected prior to the dose, at 1, 2, and 3 h during the infusion, and at 0.5, 1, 2, 3, 6, 12, and 24 h postinfusion. For cohort J, peripheral blood samples were collected prior to the dose, at 1 and 2 h during the infusion, and at 0.5, 1, 2, 3, 6, 12, and 24 h postinfusion. On the same day, urine samples for PK analysis were collected over a 24-h period.

In part B, plasma samples were collected on day 1 and day 6 as well as 30 min prior to each dose to collect trough values. For the q12h dosing, on day 1 samples were collected prior to the dose, at 1, 2, and 3 h during the infusion, and at 0.5, 1, 2, 3, and 6 h postinfusion, and on day 6 plasma samples were collected prior to the dose, at 1, 2, and 3 h during the infusion, and at 0.5, 1, 2, 3, 6, 12, and 24 h postinfusion. For the q8h cohort, plasma samples were collected on day 1 prior to the dose, at 0.5, 1, 2, and 3 h during the infusion, and at 0.5, 1, 2, 3, and 5 h postinfusion and on day 6 prior to the dose, at 0.5, 1, 2, and 3 h during the infusion, and at 0.5, 1, 2, 3, 5, 8, 13, and 24 h postinfusion. Urine samples were collected on days 1 and 6 (in the multiple-ascending-dose study only) over a 24-h period.

The plasma and urine concentrations of murepavadin were determined by liquid chromatography (LC)-tandem mass spectrometry (MS/MS) by Quintiles AB (Uppsala, Sweden). Murepavadin was extracted from plasma into 2% formic acid in dimethyl sulfoxide-acetonitrile (25:75, vol/vol) by protein precipitation. The supernatant was diluted 1:1 with 0.1% formic acid, injected onto an allure biphenyl column, and detected by MS/MS with positive electrospray ionization. The method was validated in the range of 10 to 2,000 ng/ml, with the lower limit of quantification being 10 ng/ml using a 0.1-ml volume. The assay accuracy and precision values for murepavadin were 3.3 to 9.5%. The urine samples were diluted 20 times prior to injection onto an allure biphenyl column and detected by MS/MS with positive electrospray ionization. The method was validated in the range of 100 to 4,000 ng/ml, with the lower limit of quantification being 100 ng/ml using a 0.05-ml volume. The assay accuracy and precision values for murepavadin were 3.3 to 5.8%.

Plasma and urinary PK parameters were determined using standard noncompartmental methods (model 202; constant infusion for plasma data and SAS [version 9.1] for urinary data) using WinNonlin Professional software (version 5.2 or later). The following parameters were determined: for all evaluable plasma profiles, the maximum plasma concentration (C_{max}) observed, the area under the plasma concentration-versus-time curve from time zero to the last quantifiable sampling point (AUC_{0-t}), the time of the last quantifiable concentration (t_{last}), the area under the plasma concentration-versus-time curve during a dosing interval (AUC_{0-tau}), the terminal elimination half-life ($t_{1/2}$), systemic plasma clearance (CL), the volume of distribution at steady state (V_{ss}), the volume of distribution based on the terminal phase following intravenous administration (V_z), and the mean residence time (MRT). The following parameters were determined only for a subset of subjects: the area under the plasma concentration-versus-time curve from time zero extrapolated to infinity (AUC_{0-inf}) (only in part A and part B, cohort 1), the renal clearance of the drug from plasma (CL_R), if possible (part A), and the amount of unchanged drug excreted

into the urine as the amount (A_e) and the percentage of the dose (f_e) (part A and part B when urine was collected). The following PK parameters were assessed for murepavadin in part B (day 6): C_{max}, AUC_{0-inf}, AUC_{0-t}, AUC from time zero to 3 h after dosing (AUC_{0-3}), AUC_{0-tau}, λ_z, $t_{1/2}$, CL, V_{ss}, V_z, MRT, CL_R, A_e, f_e, minimum concentration (C_{min}), average concentration (C_{avg}), the time of the minimum concentration (t_{min}), the correlation of x and y for λ_z (Corr), the linearity index (LI), and the accumulation index. The AUC was determined using the linear trapezoidal method.

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