Study 0106R143W
Measurement of Concentration in Body Fluids and Tissues (Surgery)

Dates: March 2002 – January 2003

Study Sites: / / / /

Objective:
To investigate the distribution of doripenem in peritoneal exudate in patients undergoing abdominal surgery.

Methods:
Study Design
This was an open-label study conducted in patients undergoing abdominal surgery and who are expected to require antimicrobial therapy (N = 5). Following surgery in patients with a confirmed negative result on the intradermal test for doripenem, a single dose of doripenem 250 mg was administered by intravenous infusion over 30 minutes. Samples of peritoneal exudate and blood samples were simultaneously obtained at 30 minutes after the start of infusion (at the end of infusion), and at 2.5, 4.5, and 6.5 hours after the start of infusion for measurement of doripenem.

Test Product
Doripenem: 250 mg (potency)/vial: CF0063
Intradermal test agent: 300 μg (potency)/ampule (Solvent/control solution: physiological saline 1.3 mL/ampoule): CF1022

Inclusion criteria
Male or female patients aged 20 to 79 years (inclusive) from whom it is possible to obtain peritoneal exudate following abdominal surgery, and who are expected to require antimicrobial therapy. Patients with evidence of renal or hepatic dysfunction or a history of chronic illness were excluded.

Pharmacokinetic assessment
Peritoneal exudate and blood samples were simultaneously obtained at 30 minutes after the start of intravenous infusion (at the end of intravenous infusion), and at 2.5, 4.5, and 6.5 hours after the start of infusion.

Analytical Methods
Plasma and peritoneal doripenem concentrations were analyzed by a validated HPLC assay. The LLOQ for plasma and exudate samples was — μg/mL. Exudate samples were prepared and assayed in the same manner as the plasma samples.

Pharmacokinetic Methods
For each patient, the time course of the concentration in peritoneal exudate and concentration in plasma were plotted. Furthermore, the time course of the overall mean concentration in peritoneal exudate and overall mean concentration in plasma for all patients were plotted. For each patient, the peritoneal exudate/plasma concentration ratio at each time was calculated, and the mean and standard deviation of the concentration ratio at each time were calculated. The maximum concentration in plasma (Cmaxplasma) and the maximum concentration in peritoneal exudate (Cmaxexudate) at the end of intravenous infusion were determined from actual measured

values. The maximum concentration ratio (Cmax Ratio) was calculated from Cmax(exudate)/ Cmax(plasma). The area under the plasma concentration-time curve from the start of infusion until the final time of measurement ($AUC_{0\text{-}6.5hr}$) was calculated by the trapezoidal method. The elimination half-life (t1/2) was calculated by linear regression for the logarithmically-transformed data obtained for the elimination phase. Model analysis of the plasma concentration data was performed using NONMEM® software.

Results:

Study Population

Six patients were enrolled in the study, and 5 completed participation. One subject was excluded following surgery due to the presence of highly advanced cancer discovered intraoperatively. The diagnoses in the 5 completed patients consisted of stomach cancer in 2 patients, and sigmoid colon cancer, ulcerative colitis, and Crohn's disease in 1 patient each.

Table 2. Demographic Details

Background factor	Category	No. of patients
Sex	M	4
	F	1
Age (y)	≥30 and <40 y	1
	≥50 and <60 y	1
	≥60 and <70 y	2
	≥70 and <80 y	1
	Mean	59.4
	S.D.	15.1
Body weight (kg)	<40 kg	1
	≥40 and <50 kg	1
	≥50 and <60 kg	2
	≥70 and <80 kg	1
	Mean	53.8
	S.D.	13.5
Inpatient/outpatient	Inpatient	5
Presence/absence of underlying disease/concurrent illness	Absent	2
	Present	3
Antimicrobial chemotherapy within 7 days before the start of treatment with the investigational product	Absent	5
	Present	0

Analytical Performance

Pharmacokinetic Analysis

All 5 subject who completed participation are included in the analyses. PK parameter values for individual subjects are listed in Table 1. Doripenem exposure in peritoneal exudate ranged from a mean of 3.18 μg/mL at 0.5 hr to 0.30 μg/mL at 6.5 hr. Two of the 5 subjects had undetectable levels at 6.5 hr. Exudate/plasma concentration ratios at each collected time point are presented in Table 3. Ratios ranged from 14 to 47% at 0.5 hours (Cmax), but increased to > 100% (mean) by 2.5 hours, and were maintained at over 100% (mean) at 4.5 hours. Overall, the peritoneal exudate/plasma concentration ratio for ranged from 0.0 to 322.2% for all subjects. The mean plasma elimination half-life (t1/2) of doripenem was 1.19 hours.

Table 1. Individual doripenem PK parameters in plasma and peritoneal exudate

Subject ID	$Cmax_{plasma}$ [1] (μg/mL)	$Cmax_{exudate}$ [2] (μg/mL)	Cmax Ratio (%)	$AUC_{0\text{-}6.5hr}$ (μg·hr/mL)	$t_{1/2}$ (hr)
1			17.0	36.79	0.95
2			27.4	35.58	1.48
3			14.4	31.10	1.59
4			47.3	16.99	1.11
6			19.4	35.26	0.80
Mean	18.1	4.13	25.1	31.14	1.19
S.D.	5.1	1.20	13.3	8.20	0.34
Min			14.4	16.99	0.80
Max			47.3	36.79	1.59

[1] Observed at 0.5 hr
[2] Observed values

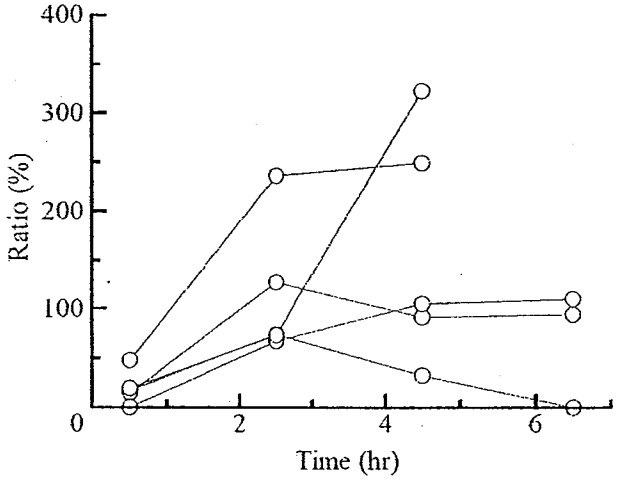
Table 2. Concentrations of doripenem in peritoneal exudate

Subject ID	Concentration in peritoneal exudate (μg/mL)			
	0.5 hr	2.5 hr	4.5 hr	6.5 hr
1				
2				
3				
4				
6				
Mean	3.97	3.18	1.11	0.50
S.D.	1.05	1.39	0.46	0.24

Table 3. Peritoneal exudate/plasma concentration ratio of doripenem

Subject ID	Ratio (%) 0.5 hr	2.5 hr	4.5 hr	6.5 hr
1				
2				
3				
4				
6				
Mean	19.7	115.3	159.8	67.8
S.D.	17.2	71.7	120.7	59.3

Figure 1. Time profile of peritoneal exudate/plasma concentration ratio of doripenem



Safety

There were no adverse symptoms occurring among the 5 patients evaluated for adverse drug reactions (symptoms). There were a total of 4 abnormal changes in laboratory values reported in 2 patients. These consisted of increased serum bilirubin in 1 patient, and 1 event each of increased GOT (AST), increased GPT (ALT), and positive urinary glucose in the other patient. These abnormal changes were mild in all instances, and the values returned to normal without treatment. All of the abnormal changes were assessed as being attributable to surgical invasion, and none of the abnormal changes was assessed as an adverse drug reaction.

Sponsor's Conclusions

- The mean Cmax in plasma was 18.1 μg/mL.
- The mean peritoneal exudate concentration reached a maximum of 3.97 μg/mL at 30 minutes (at the end of infusion) and 3.18 μg/mL at 2.5 hours after the end of infusion, and thereafter decreased gradually to 0.30 μg/mL at 6.5 hours.
- The peritoneal exudate/plasma concentration ratio for was 0.0 - 322.2% for all time points. The Cmax Ratio was 14.4 - 47.3%.
- The time profiles of the concentration in peritoneal exudate and concentration in plasma in 2 patients who used other concomitant antimicrobials (Subjects 3 and 4) were not different from those obtained for the patients who did not.
- The exudate/plasma ratio value of 67.8% at 6.5 hours after the start of infusion was low, but this may have been due to the fact that in 1 patient the plasma concentration was 0.24 μg/mL and the peritoneal exudate concentration was below the lower limit of detection (0.20 μg/mL) at 6.5 hours, leading to a calculated concentration ratio of 0. Despite the fact that the peritoneal exudate/plasma concentration ratio at 6.5 hours was low for calculation-related reasons, the actual mean concentrations in peritoneal exudate and plasma were both 0.30 μg/mL. Hence, it would appear that the concentrations in peritoneal exudate and plasma showed similar profiles from 2.5 hours to 6.5 hours after the start of infusion.
- The mean half-life of elimination from plasma (t1/2) was 1.19 hours, approximately the same as the value in healthy adult males, notwithstanding the presence of differences in the method of calculation.
- The mean value of Cmaxplasma was 18.1 μg/mL, and while the variation in surgical subjects was greater, this was almost the same as the result obtained in healthy adult males.
- The major bacterial organisms in gastroenterological surgery are the intestinal bacteria such as *E. coli, K. pneumoniae,* and *B.fragilis.* The MIC90 values of doripenem against these clinical isolates are 0.05, 0.05, and 1.56 μg/mL, respectively. Since the concentration of doripenem in peritoneal exudate measured in this study exceeded these MIC90 values, even at 2.5 hours after the start of intravenous infusion (over 30 minutes), it can be inferred that doripenem will be effective against abdominal infections.

Reviewer assessment:

Assuming linear pharmacokinetics of doripenem in peritoneal exudate, based on the results of the limited number of subjects included in this study, concentrations of doripenem in peritoneal exudate would be approximately 6 μg/mL and 2 μg/mL at 2.5 hours and 4.5 hours, respectively, following a 500 mg intravenous dose. Nearly 95% of the 1936 strains reported in the Phase III cIAI studies had MIC values ≤ 1 μg/mL. Taken together, this would suggest that doripenem exposure in the peritoneum exceeds the MIC of most cIAI pathogens for over half of the dosing interval.

4.2.6. In vitro Studies

Study S-4661-B-05-N(1)
Studies on the Pharmacokinetics of S-4661 in Experimental Animals: Protein Binding Rates of S-4661 in Various Animal Plasmas

Dates: September 1991 – April 1993

Study Sites: Developmental Research Laboratories, Shionogi & Co., Ltd. 101, Futaba-cho 3-chome, Toyonaka, Osaka 561-0825, Japan

Objective:
To evaluate the protein binding rates of S-4661 in various animal plasmas (mouse, rat, rabbit, dog, monkey, human), in comparison with imipenem (IPM) and meropenem (MEPM).

Method:
Sample measurement
Concentrations of S-4661, IPM and MEPM were measured in filtrate samples by band-culture, using *E. coli* 7437 for the test organism and Mueller-Hinton agar for the assay medium

Results:
Study results are shown in Table 1. The protein binding rates of S-4661 in animal plasmas were 10.2 – 35.2% for mice, rats, rabbits and dogs; these rates were higher than those of IPM and

MEPM, which ranged from not detectable (dog) to 22.4% in rabbit (MEPM) and 4.13 in rat (IPM). The binding rate in human plasma was 8.1% for S-4661, versus 4.7% and 6.1% for IPM and MEPM, respectively.

The value of S-4661, IPM and MEPM binding to the MPS-membrane was 0 – 1.11%, which suggests that the MPS-3 membrane did not greatly effect protein binding rates.

Table 1. Protein Binding Rate in Various Animal Plasmas

	Protein binding rate, %		
Plasma	S-4661	Imipenem	Meropenem
Mouse	25.2±0.95	2.49±2.16	18.9±1.27
Rat	35.2±3.45	4.13±4.54	22.4±3.52
Rabbit	11.8±3.48	0.57±0.99	10.5±1.74
Dog	10.2±3.52	0	0
Monkey	6.13±1.48	2.52±4.36	11.7±2.49
Human	8.11±1.31	4.73±0.53	6.12±1.33
0.05M MOPS,pH7	1.11±1.92	0.91±1.57	0

Means +/- SD (n = 3)

Precision and reproducibility:

Reviewer assessment:
The results of this study indicate that the protein binding of doripenem is similar to that of imipenem and meropenem. The protein binding rate of imipenem in human plasma in this study was similar to what is reported in its product label (4.7 vs. 2%). However, the protein binding of meropenem in this study was significantly lower than what is reported its respective label (6.1% vs. 20%).

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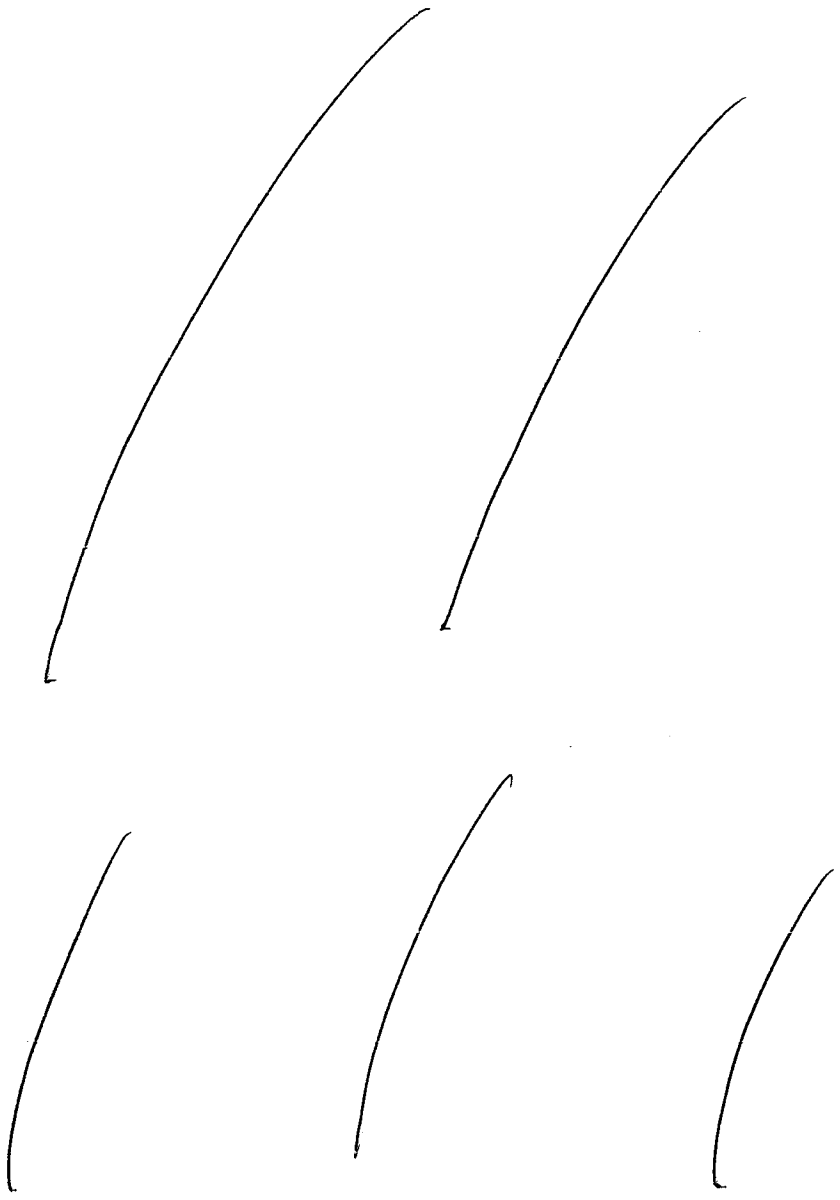
Study S-4661-EB-527-N
Studies on the Stability of S-4661 against Human DHP-1

Dates: February – June 2003

Study Sites: Shionogi & Co., Ltd., 1-1 Futaba-cho 3-chome, Toyonaka, Osaka, Japan

Objective:
To evaluate the stability of S-4661 against mouse and human renal dehydropeptidase-I (DHP-1), as compared to that of meropenem (MEPM) and imipenem (IPM).

Method:



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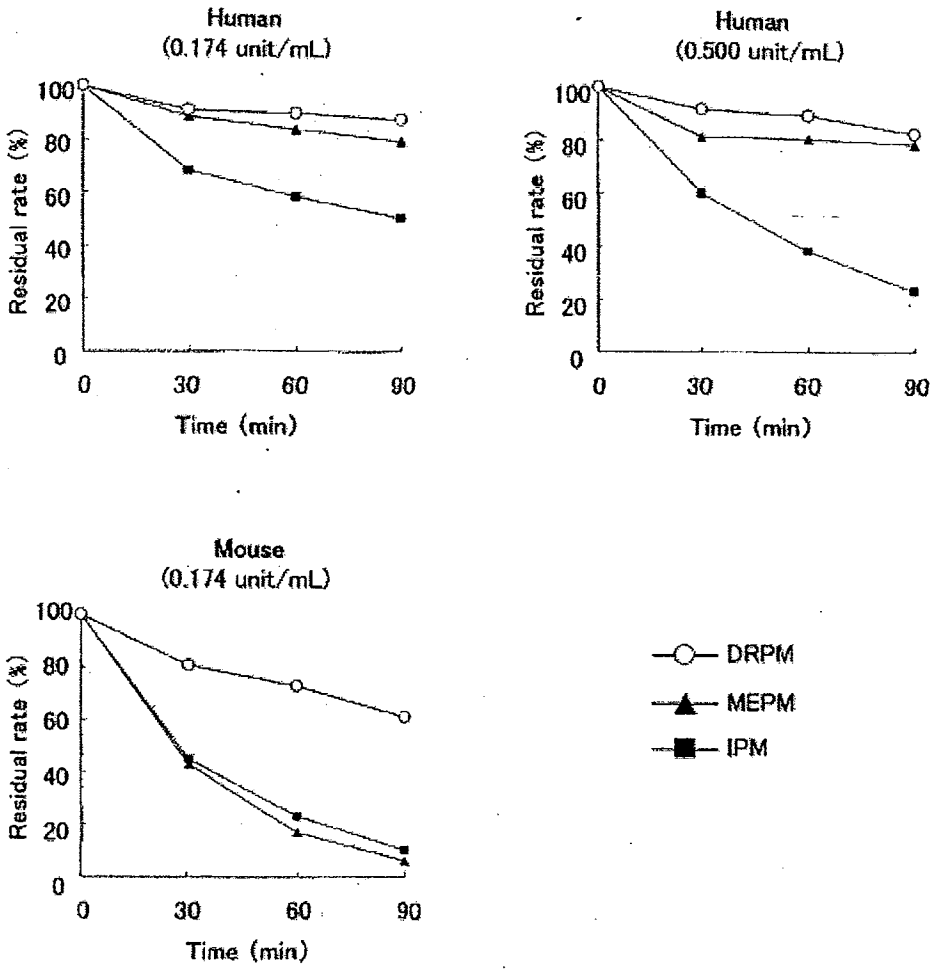
Results:
After incubating for 90 minutes in the presence of purified murine DHP-1, the residual concentrations of IPM and MEPM were 9.90% and 6.19%, respectively, compared with their initial concentrations. The residual concentration of S-4661 was 61.2% (Figure 1). The degree of stability was comparable to that of murine kidney homogenate, which was reported in Study No. S-4661-EB-526-N, suggesting that the stability in murine kidney homogenates reflects the stability against purified murine renal DHP-1.

After incubating for 90 minutes in the presence of purified human DHP-1 with a specific activity of 0.174 unit/mL, the residual concentration of IPM was 50.4% of the original concentration, while that of S-4661 and MEPM was 87.5% and 79.1%, respectively (Figure 1). Stability tests were also performed under conditions containing purified human renal DHP-1 with a specific activity of 0.5 unit/mL. After incubating for 90 minutes, the residual concentrations of S-4661, MEPM, and IPM were 82.4%, 78.1% and 23.2%, respectively.

Figure 1. Stability of doripenem (DRPM), meropenem (MEPM) and imipenem (IPM) against purified renal DHP-1.



Sponsor's Conclusions:
The stability of S-4661 against murine renal DHP-1 was significantly higher than that of IPM or MEPM, and the stability corresponded with that of the murine kidney homogenate. With respect to all of the test and reference substances, the stability against human renal DHP-1 was higher than that against murine renal DHP-1, although the enzymes had almost the same specific activity. The stability of S-4661 against human renal DHP-1 was significantly higher than that of IPM and was comparable to that of MEPM. These results suggest that S-4661, like MEPM, would exhibit good pharmacokinetic profiles in humans without the use of DHP-1 inhibitors.

Reviewer assessment:
The concentration of Test substance in the reaction mixture was 100 +/- 20 µg/mL. This concentration is greater than the C_{max} of a 500 mg IV dose of doripenem (33.1 µg/mL). As such, the concentrations studied were adequate for determining the stability of doripenem at clinically relevant doses.

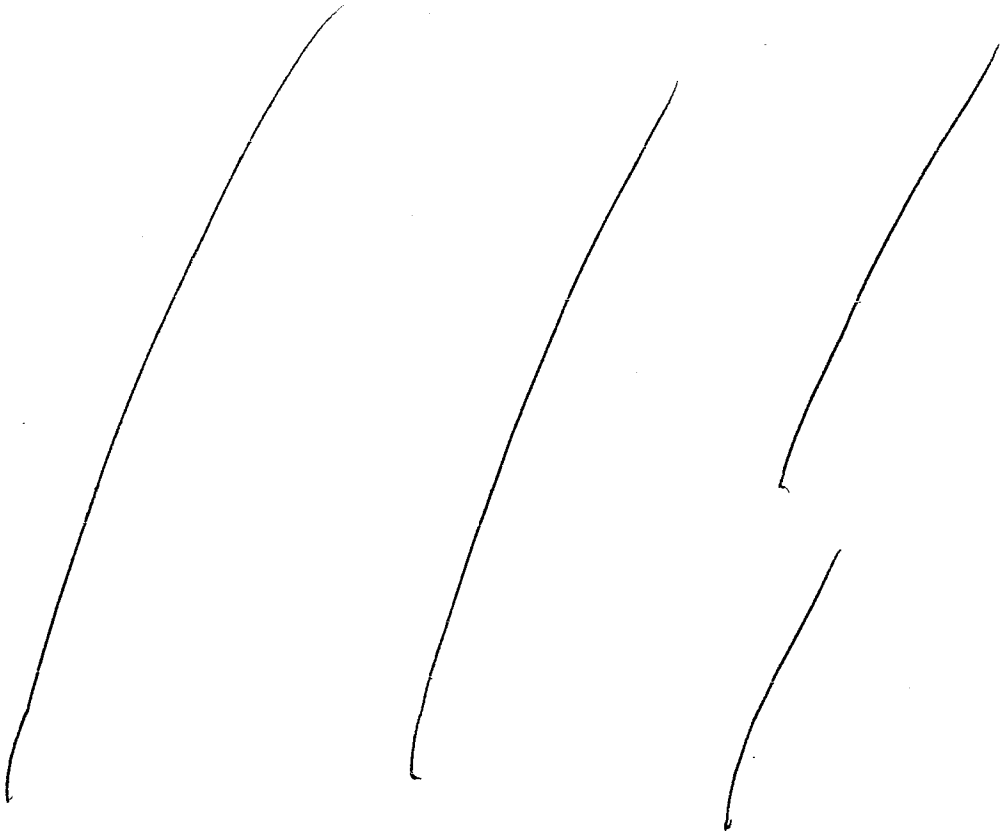
Study DORI-PK-001 (PPI)
Cytochrome P450 Reaction Phenotyping for Radio-labeled Doripenem

Dates: June 2003 – September 2003

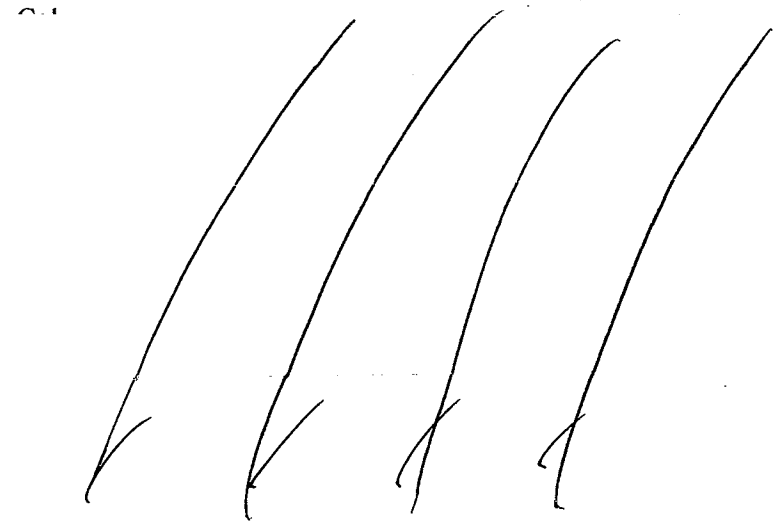
Study Sites:

Objective:
To determine whether doripenem is a major substrate for CYP450 metabolism, and if so, which isoenzyme may be responsible for its metabolism.

Method:



Analytical Method:
Analysis of doripenem was performed using HPLC with radiometric detection.



Results:
Doripenem was incubated for 0, 30 and 120 minutes. In the 0 min. injection, approximately 99% of the radioactivity eluted at ~~min., along with much smaller peaks a~~ .nin. and ~~min.~~ The ~~ min. peak increased over the course of the incubation, and also increased proportional to the length of sample storage in the HPLC, suggesting the formation was non-enzymatic and was likely a result of parent degradation.

There was no evidence of doripenem metabolism, as indicated by the lack of parent drug depletion and absence of metabolites as compared to the control. Incubations conducted with 5 μM doripenem also indicate no metabolism. Incubations of doripenem with or without NADPH showed no obvious differences.

The results of the positive control, testosterone 6β-hydroxylation, shown in Table 1, were consistent with historical values observed in the laboratory performing the study.

Table 1. Testosterone 6β-hydroxylation activity in human liver microsomes

Sample number	pmol/min/mg
1	7300
2	7117
3	7104

Sponsor's Conclusions
In the well-established human liver microsome test system, the *in vitro* hepatic metabolism of doripenem was examined. It is concluded that doripenem is not a major substrate for human hepatic CYP450 enzymes and that P450-dependent hepatic metabolic pathway plays little role in the degradation of doripenem. This conclusion is supported by the evidence below:

1) In incubations of doripenem with human liver microsomes in the presence of an NADPH-regenerating system, no metabolite peaks were found. This was the case when both microsomal

protein and substrate concentrations were varied in an effort to enhance the rate of reaction or avoid the possibility of substrate inhibition.

2) Chromatograms from incubations of doripenem with human liver microsomes with or without NADPH showed no obvious differences.

3) Positive controls are consistent with a properly functioning model.

Reviewer assessment:

Details of the analytical validation and assay performance were not included in the study report; therefore, assay performance could not be reviewed.

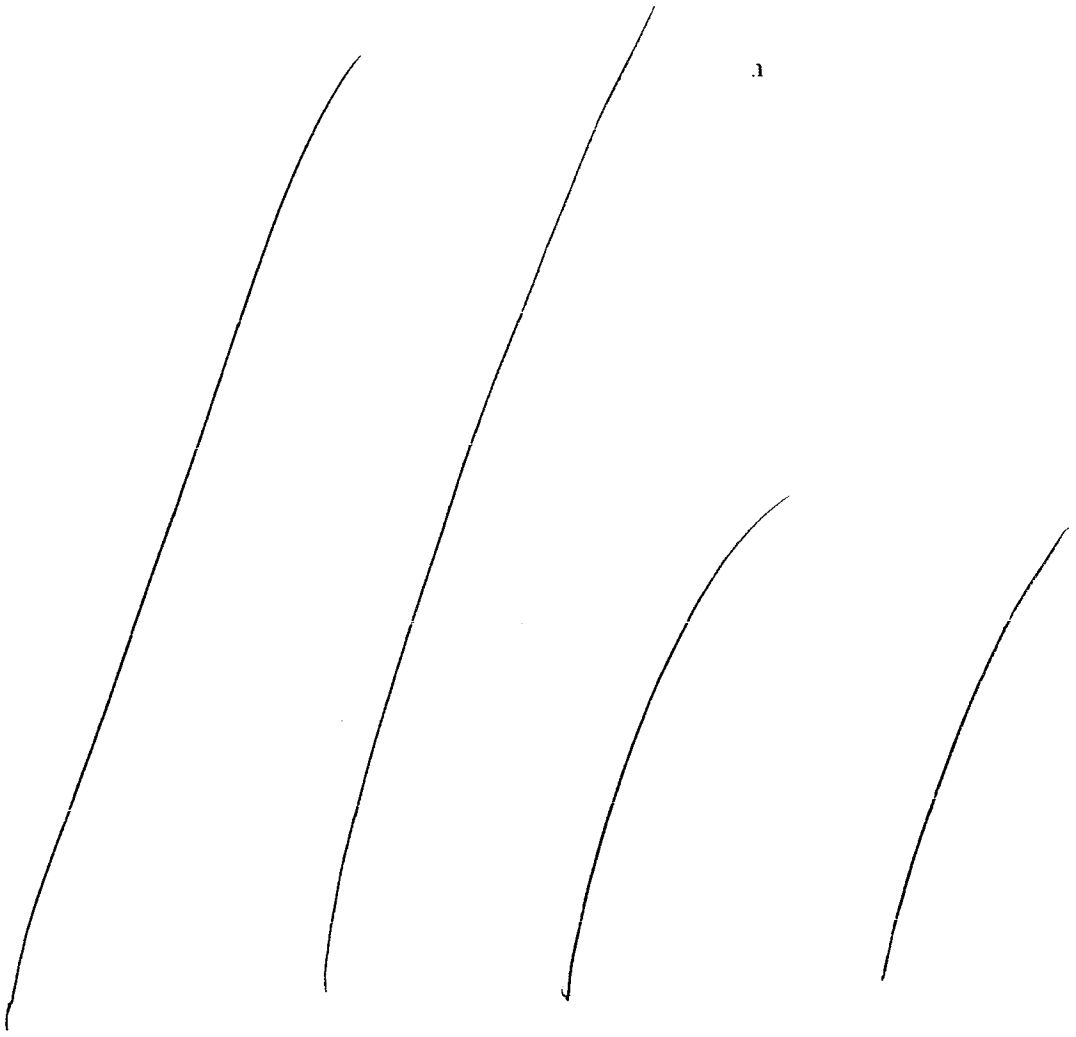
Study XT025016
***In Vitro* Enzyme Inhibition of S-4661 in Human**

Dates: December 2002 – January 2003

Study Sites: [redacted]

Objective:
To evaluate the *in vitro* ability of doripenem to inhibit the major CYP enzymes in human liver microsomes.

Method:
Doripenem was evaluated for its ability to inhibit the following CYP enzymes both directly and in a metabolism-dependent manner:



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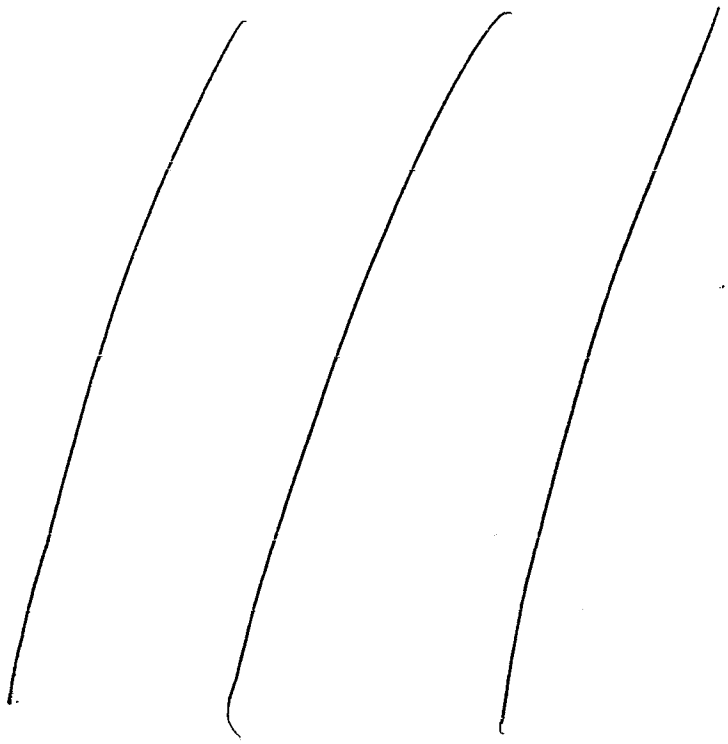


Results:
Under the experimental conditions, doripenem did not inhibit any of the following enzymes: CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4/5 (using four probe substrates) and CYP4A11. Therefore, the IC50 value for inhibition of these enzymes is greater than 300 μM, the highest concentration of doripenem studied. Based on a conservative assumption that 10% inhibition of the enzyme activity by 300 μM doripenem may have been masked by experimental error, the IC50 values for doripenem are estimated to be > 2700 μM, or > 1800 μM for CYP2B6. In the bupropion hydrolysis experiment (CYP2B6), the concentration of marker substrate was approximately equal to 2 x Km, instead of Km, due to a calculation error.

In addition, doripenem did not cause metabolism-dependent inhibition of any of the CYP enzymes examined. It should be noted that the experiments involved pre-incubating human liver microsomes in the presence of an NADPH-generating system but in the absence of marker substrate. In some cases, when such incubations were carried out, some loss in activity of the enzyme tested was observed regardless of the presence of doripenem. This loss in enzyme activity is attributed to inactivation of CYP enzymes (e.g., by reactive oxygen species).

Sponsor's Conclusions:
In summary, doripenem does not have the capacity to function as a direct inhibitor of any of the CYP enzymes examined. The IC50 values estimated for the enzymes studied, with the exception of CYP2B6, were greater than 2700 μM. Because the marker substrate concentration was approximately equal to 2 x Km in the CYP2B6 experiment, the IC50 value estimated for this enzyme is greater than 1800 μM. Under the experimental conditions examined, there was no evidence that doripenem caused metabolism-dependent inhibition of any of the CYP enzymes examined.

Reviewer assessment:
The concentration range of doripenem used to assess potential CYP450 inhibition in this study was 0 – 300 μM (0 – 126 μg/mL). The Cmax value following a 500 mg intravenous dose of doripenem is 33.1 μg/mL; therefore, the maximum concentration studied was sufficient for assessing potential CYP450 inhibition by doripenem.

The assay procedures (HPLC with UV detection) for each of the experimental substrates are provided in the study report. However, details of analytical validation and assay performance were not included; therefore, assay performance could not be reviewed.

FK5908

***In Vitro* Evaluation of Doripenem and Doripenem Carboxylic Acid as Inducers of Cytochrome P450 and UDP-Glucuronosyltransferase Expression in Cultured Human Hepatocytes**

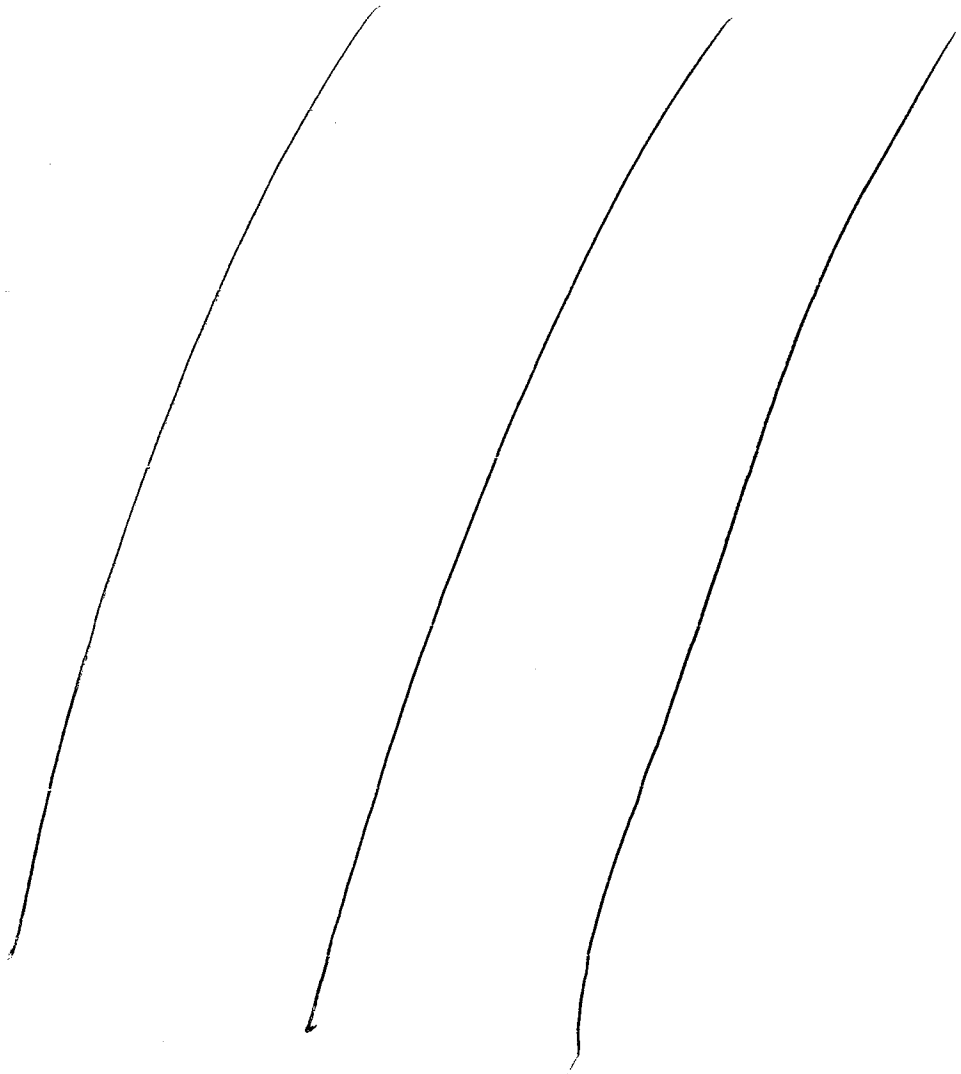
Dates: February – March 2006

Study Sites: [redacted]

Objective:

To investigate the effect of doripenem and doripenem carboxylic acid on the expression of cytochrome P450 (CYP) and UDP-glucuronosyltransferase (UGT) enzymes in primary cultures of human hepatocytes.

Method:



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Results

Within 24 hours after the final treatment, hepatocytes were photographed to document their morphological integrity. The photographs indicate that, in general, the hepatocytes treated with vehicle (DMSO), doripenem, doripenem carboxylic acid, or known CYP inducers exhibited normal hepatocyte morphology.

The results of the study were reported as both enzymatic activity (pmol/mg protein/min) and fold increase for each of the enzymatic reactions. Fold increase over control (the corresponding vehicle-treated samples) are presented in Table 3.

Table 3. Fold induction of CYP450 enzymes[a]

Probe Substrate/Reaction	Dimethyl sulfoxide 0.1%(v/v)	Doripenem 1 µg/mL	Doripenem 10 µg/mL	Doripenem 100 µg/mL	Doripenem Carboxylic acid 1 µg/mL	Doripenem Carboxylic acid 10 µg/mL	Doripenem Carboxylic acid 100 µg/mL
Phenacetin O-dealkylation (CYP1A2)	1.00 ± 0.43	1.17 ± 0.40	1.04 ± 0.18	1.12 ± 0.09	1.13 ± 0.11	1.16 ± 0.38	1.07 ± 0.07
Bupropion hydroxylation (CYP2B6)	1.00 ± 0.18	1.13 ± 0.21	1.01 (n = 2)	1.12 ± 0.10	1.03 ± 0.03	1.09 (n = 2)	1.06 ± 0.02
Diclofenac 4'-hydroxylation (CYP2C9)	1.00 ± 0.80	0.989 ± 0.236	0.932 (n = 2)	0.944 ± 0.279	0.926 ± 0.069	0.921 ± 0.189	0.894 ± 0.135
S-Mephenytoin 4'-hydroxylation (CYP2C19)	1.00 (n = 1)	0.935 (n = 1)	0.893 (n = 1)	1.11 (n = 1)	0.996 (n = 1)	0.749 (n = 1)	0.936 (n = 1)
Testosterone 6β-hydroxylation (CYP3A4/5)	1.00 ± 0.69	1.17 ± 0.32	0.997 ± 0.124	1.28 ± 0.16	1.12 ± 0.10	1.03 ± 0.26	0.943 ± 0.117
β-Estradiol 3-glucuronidation (UGT1A1)	1.00 ± 0.04	1.08 ± 0.33	0.923 ± 0.101	1.03 ± 0.16	0.989 ± 0.082	1.14 ± 0.38	1.10 ± 0.13

Table 3, cont.

Probe Substrate/Reaction	Omeprazole 100 µM	β-Naphthoflavone 33 µM	Phenobarbital 750 µM	CITCO 100 nM	Rifampin 10 µM
Phenacetin O-dealkylation (CYP1A2)	25.2 ± 21.5	18.4 ± 5.0	2.38 ± 0.45	1.02 ± 0.14	1.92 ± 0.36
Bupropion hydroxylation (CYP2B6)	5.50 ± 3.36	3.18 ± 0.80	10.6 ± 3.1	0.958 ± 0.166	7.34 ± 3.24
Diclofenac 4'-hydroxylation	2.27 ± 1.78	1.46 ± 0.34	3.34 ± 2.50	0.792 ± 0.116	4.25 ± 3.37

(CYP2C9)					
S-Mephenytoin 4'-hydroxylation (CYP2C19)	3.19 (n = 1)	2.87 (n = 1)	2.71 (n = 1)	0.845 (n = 1)	7.16 (n = 1)
Testosterone 6β-hydroxylation (CYP3A4/5)	2.23 (n = 2)	0.455 (n = 2)	7.88 ± 6.02	0.875 ± 0.159	10.2 ± 10.2
β-Estradiol 3-glucuronidation (UGT1A1)	2.15 ± 0.59	1.62 ± 0.14	1.73 ± 0.26	1.01 ± 0.08	1.74 ± 0.36

[a]Values are the mean rate ± standard deviation of 3 human hepatocyte preparations (H646, H650 and H651, unless otherwise indicated (e.g. n = 1)

S-mephenytoin 4'-hydroxylase activity was very low (BLQ) in several samples (including the vehicle control, 0.1% DMSO) in two (H650, H651) of the three preparations of human hepatocytes examined. Thus, fold induction could not be calculated for these two preparations. However, data from H646 indicate that treatment of cultured human hepatocytes with doripenem or doripenem carboxylic acid had little or no effect on microsomal *S*-mephenytoin 4'-hydroxylase (CYP2C19) activity at the concentrations tested.

Sponsor's Conclusions:
Under conditions where the prototypical inducers produced anticipated increases in CYP and UGT1A1 activity, doripenem and doripenem carboxylic acid did not cause an increase in CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP3A4/5 or UGT1A1 enzyme activity.

Reviewer assessment:
The concentration range of doripenem used to assess potential CYP450 induction in this study was 1 – 100 µg/mL. The Cmax value following a single dose of 500 mg intravenous doripenem is 33.1 µg/mL; therefore, the maximum concentration studied was sufficient for assessing potential CYP450 induction by doripenem.

Details of analytical validation and assay performance were not included; therefore, assay performance could not be reviewed.

DORI-M-002
The Pharmacodynamic Activities of Doripenem

Dates: July – September 2002

Study Sites: [redacted]

Objective:
To characterize the *in vivo* pharmacodynamic characteristics of doripenem in experimental thigh and lung infections in neutropenic mice.

Method:
Doripenem pharmacokinetic (PK) and pharmacodynamic (PD) characteristics were determined using the neutropenic mouse thigh and lung infection models. The impact of dosing regimen on *in vivo* efficacy was evaluated. In addition, the studies identified which PK parameter value best predicts doripenem efficacy and whether the magnitude of the PK/PD parameter is similar among common gram-positive and gram-negative bacteria.

MIC determination
The study organisms and their MIC values to doripenem are listed in Table 1. The MICs were determined in Mueller Hinton broth (MHB) using standard NCCLS microdilution techniques. MHB was supplemented with 3% lysed horse blood for MIC determinations with *S. pneumoniae*. All MICs were performed at least in duplicate. The *S. pneumoniae* group included strains with penicillin, erythromycin, ciprofloxacin and cephalosporin resistance.

Table 1. Doripenem In Vitro Activity Against Multiple Organisms

Organism	Doripenem MIC (mg/L)	Penicillin MIC (mg/L)	Methicillin MIC (mg/L)	Ciprofloxacin MIC (mg/L)	Cefotaxime MIC (mg/L)
S. pneumoniae 6301	0.004	0.008		-	-
S. pneumoniae MNO418	0.004	0.06		8.0	-
S. pneumoniae 10813	0.004	0.008		-	-
S. pneumoniae 1396	0.12	0.50		-	-
S. pneumoniae 1293	0.25	2.0		-	-
S. pneumoniae 145	0.50	4.0		-	-
S. pneumoniae 146	0.50	4.0		-	-
S. aureus 25923	0.015	-	0.25	-	-
S. aureus smith	0.015	-	0.25	-	-
S. aureus 307192	4.0	-	>64	-	-
E. coli 25922	0.015	-	-	-	-
E. coli 145	0.03	-	-	-	64
E. coli 154	0.06	-	-	-	0.5
K. pneumoniae 43816	0.06	-	-	-	1.0
K. pneumoniae 51504	0.06	-	-	-	-
K. pneumoniae 149	0.06	-	-	-	>256
K. pneumoniae 152	0.06	-	-	-	32
E. cloacae 31-59a	0.25	-	-	-	-
E. cloacae 31-54a	0.50	-	-	-	-
P. aeruginosa 27853	0.50	-	-	-	4.0

Animal models
Female Swiss ICR mice were used in all experiments. Neutropenia was induced by giving two injection of cyclophosphamide – 150 mg/kg 4 days prior to the study and 100 mg/kg 1 day prior to the study – which induces < 100 neutrophils/mL for at least 96 hours.

Pharmacokinetics
The pharmacokinetic properties of doripenem were assessed in the neutropenic mouse thigh-infection model. Drug was administered by subcutaneous injection in a 0.2 mL volume. Doses of 9.38, 37.5 and 150 mg/kg were administered in 2 to 4 mice per dose. Blood was drawn from groups of 3 mice by retroorbital aspiration into heparinized capillary tubes at 0.25, 0.5, 0.75, 1, 1.5, 2, 3 and 4 hours post-dose. Plasma was separated and doripenem plasma concentrations were measured using a microbiologic assay with *S. aureus 6538p* as the test organism. The lower limit of detection for the assay was 0.12 μg/mL. Intraday variation was < 10%. Doripenem half-life was determined by linear least-squares regression in individual mice. The AUC was calculated by the trapezoidal rule using mean concentrations.

In Vivo killing and post-antibiotic effects
The effect single doses of doripenem on the *in vivo* killing and regrowth of strains of *S. aureus* and *S. pneumoniae* was studied using the murine thigh infection model. Single doses of 2.34, 9.38 and 37.5 mg/kg were evaluated in the mice.

Dosing studies
Multiple dosing regimens were evaluated in these studies, with varying doses and dosage intervals administered to groups of mice for 24 hours. The dosing intervals studied were 3, 6, 12 and 24 hours. Five total doses of four-fold increases were studied for each dosing regimen, enabling characterization of each dosing interval.

Each of the dose-response curves generated was mathematically characterized using a maximum effect model. The model used the Hill equation to estimate by non-linear regression the maximum effect (Emax), the dose required to obtain 50% of the Emax (P50), and the slope of the dose-response relationship. From these parameters, the dose required to produce a net bacteriostatic effect over 24 hours (static dose) was estimated, as well as the 1- and 2-log kill doses for each of the drug-organism combinations.

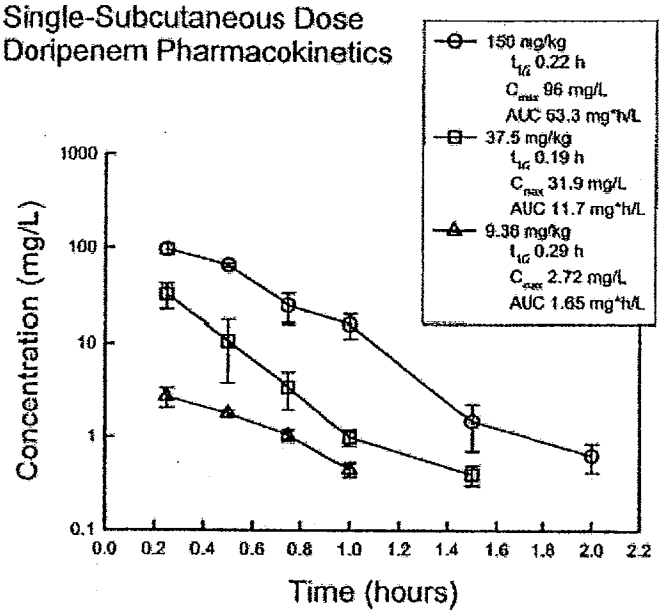
PK/PD parameter determining efficacy
In order to determine which PK/PD parameter best correlated with efficacy, the number of bacteria in the thigh at the end of 24 hours of drug therapy was related to (1) peak/MIC ratio, (2) 24-hour AUC/MIC ratio, and (3) % of dosing interval serum levels were above the MIC (%T>MIC).

In order to determine whether the %T>MIC required for a static effect was similar for multiple pathogens, the activity of 6-hourly dosing regimens of doripenem were studied against 7 strains of *S. pneumoniae* (penicillin-susceptible [PSSP], penicillin-resistant [PRSP] and quinolone resistant strains), 3 strains of *S. aureus*, (methicillin-susceptible [MSSA] and methicillin-resistant strains [MRSA]), and 10 strains of Gram-negative bacilli (cephalosporin-susceptible and ESBL-producing strains). The doses necessary to produce a bacteriostatic effect, 1- and 2-log reductions, and the corresponding %T>MIC values were evaluated.

Results:
The pharmacokinetics of doripenem in thigh-infected neutropenic mice are shown in Figure 1 below. Peak levels occurred by 15 minutes after injection. The elimination half-life in mice

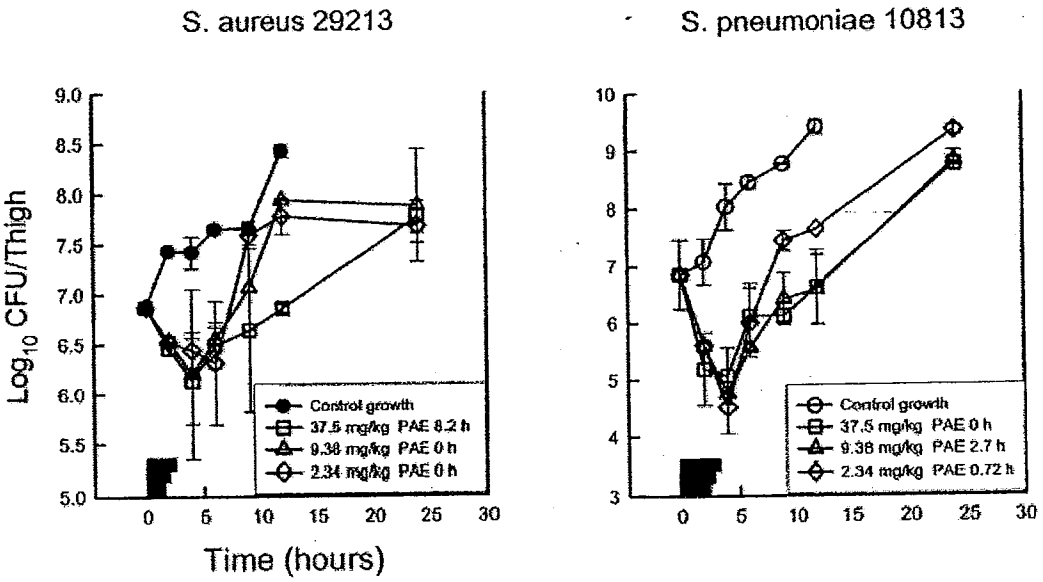
ranged from 0.19 to 0.29 hours. The mean AUC values were 1.65, 11.7 and 53.3 mg*hr/L for each dose group, respectively. The mean AUC/dose ratio was 0.28 (range 0.18 – 0.36). The mean peak/dose ratio was 0.59 (range 0.29 – 0.84). The protein binding of doripenem in mouse plasma was < 5%, as determined by ultrafiltration.

Figure 1. Doripenem pharmacokinetics following subcutaneous single-dose administration to thigh-infected neutropenic mice.



The effects of single doses of doripenem at 2.35, 9.38 and 37.5 mg/kg on the *in vivo* killing and regrowth of bacteria are shown in Figure 2. Significant organism killing ranged from 0.56 to 0.74 $\log_{10}$ cfu/thigh against *S. aureus* and 1.9 to 2.3 $\log_{10}$ cfu/thigh against *S. pneumoniae* at each of the dose levels studied. Organism killing was not dependent on the drug concentration delivered. Re-growth of both organisms began after a short to moderate delay.

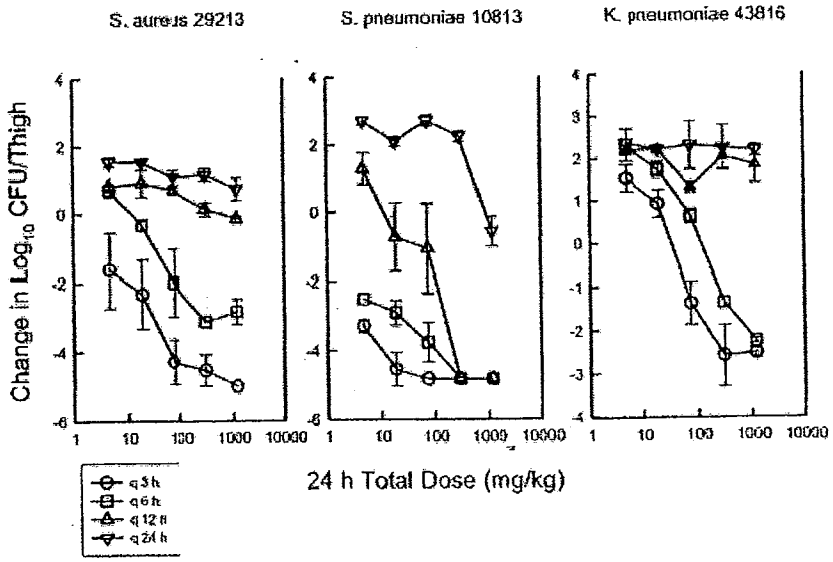
Figure 2. Doripenem *In Vivo* Post-Antibiotic Effect (PAE) Against strains of S. Aureus and S. pneumoniae in a murine thigh infection model[a]



[a] *Each point on the graphs represents a mean of 2 thighs.*

Figure 3 illustrates the dose response curves at the different dosing intervals studied for strains of *S.aureus*, *S. pneumonia* and *K. pneumoniae*. As the dosing interval increases, the dose-response curve is shifted to the right, indicating less efficacy with larger, less frequent dosing. The static doses, 1- and 2-log kill doses for each of the drug-organism combinations is shown in Table 2.

Figure 3. Relationship Between Doripenem Dosing Interval and *In Vivo* Efficacy Against S. aureus, S. pneumoniae and K. Pneumoniae in a Murine Thigh Infection Model[a]



[a] *Each point on the graphs represents a mean of 2 thighs.*

Table 2. Doripenem In Vivo Activity in a Murine Thigh Infection Model

Organism	MIC (mg/L)	SD (mg/kg)	%T>MIC	1 Log Kill (mg/kg)	%T>MIC	2 Log Kill (mg/kg)	%T>MIC
S. pneumoniae 6301	0.004	0.01	2.3	0.06	15	0.59	31
S. pneumoniae MNO418	0.004	0.21	15	0.89	34	5.42	47
S. pneumoniae 10813	0.004	0.08	17	0.21	24	0.49	30
S. pneumoniae 1396	0.12	1.22	12	2.45	17	6.86	24
S. pneumoniae 1293	0.25	11.0	21	93	31.3	na	na
S. pneumoniae 145	0.50	2.40	7.3	3.19	10	4.54	12
S. pneumoniae 146	0.50	4.97	12.2	10.5	16.7	25.2	20
mean ± SD			12.4 ± 6.2		21.1 ± 8.9		27.3 ± 11.9
S. aureus 25923	0.015	4.09	35	12.9	40	57.9	41
S. aureus smith	0.015	1.05	25	1.82	29	3.18	34
S. aureus 307192	4.0	362	27	494	28	882	31.3
mean ± SD			29 ± 5.3		32.3 ± 6.7		35.4 ± 5.0
E. coli 25922	0.015	22.1	38	113	47	na	na
E. coli 145	0.03	6.15	33	12.4	35	253	51
E. coli 154	0.06	7.32	28	24.7	30	97.4	38
K. pneumoniae 43816	0.06	29	29	75.3	35	210	46
K. pneumoniae 51504	0.06	55.6	34	216	49	na	na
K. pneumoniae 149	0.06	26.3	28	56.4	34	116	40
K. pneumoniae 152	0.06	12.6	31	98	39	1111	54
E. cloacae 31-59a	0.25	38.3	26	158	37	1074	47
E. cloacae 31-54a	0.50	23.7	20	78.4	27	276	36
P. aeruginosa 27853	0.50	46	23	100	28	245	35
mean ± SD			29 ± 5.3		36.1 ± 7.4		43.3 ± 7.1

The relationship between $\log_{10}$ cfu per thigh and the peak/MIC ratio, the 24-hour AUC/MIC ratio and the %T>MIC are illustrated in Figures 4-6. For each of the organisms studied, the best correlation was observed for %T>MIC. The coefficient of determination (R^2) was used to assess the relationship between efficacy and each PK/PD parameter. The highest R^2 values were observed for %T>MIC, from 75% for K. pneumoniae, 80% for S. pneumoniae, and 92% for S. aureus.

The time above MIC required to produce a static effect for the multiple pathogens studied ranged from 2.3 to 38%. The static doripenem doses varied from 0.01 mg/kg to 55.6 mg/kg every 6 hours (> 5000-fold difference in dose). There was no major difference in %T>MIC for the different strains of *S. pneumoniae*, regardless of penicillin or quinolone resistance. Gram-negative bacilli and *S. aureus* required a greater %T>MIC than *S. Pneumoniae*. However, the presence of methicillin resistance or ESBL production did not significantly impact the magnitude of the PK/PD parameter required for efficacy. The %T>MIC required to produce a 2-log kill ranged from a mean of 27% for *S. pneumonia* to a mean of 43% for gram-negative bacilli.

The relationship between doripenem %T>MIC and efficacy against each of the 20 strains of bacteria studied is shown in Figure 7. The relationship between the PK/PD parameter and efficacy is strong, as demonstrated by R^2 values of 0.69 to 0.79.

Figure 4. Relationship Between Doripenem PK/PD Parameters and *In Vivo* Efficacy Against *S. aureus* 29213.

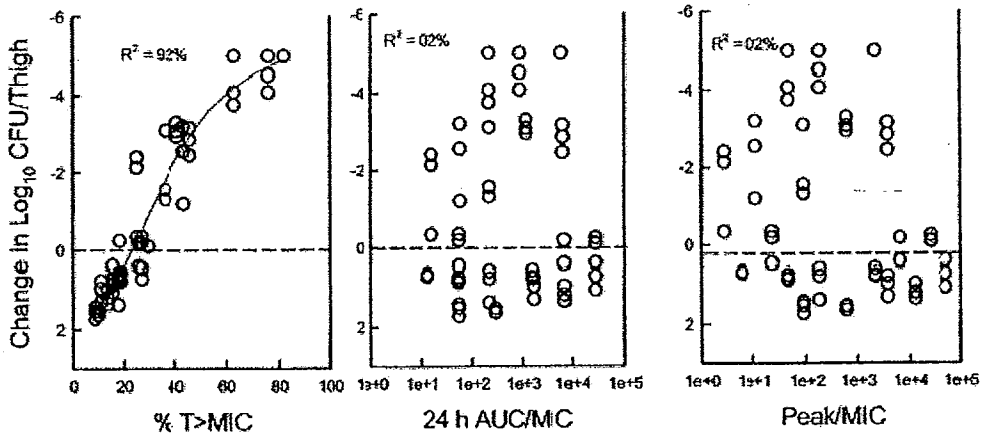


Figure 5. Relationship Between Doripenem PK/PD Parameters and *In Vivo* Efficacy Against *S. pneumoniae* 10813.

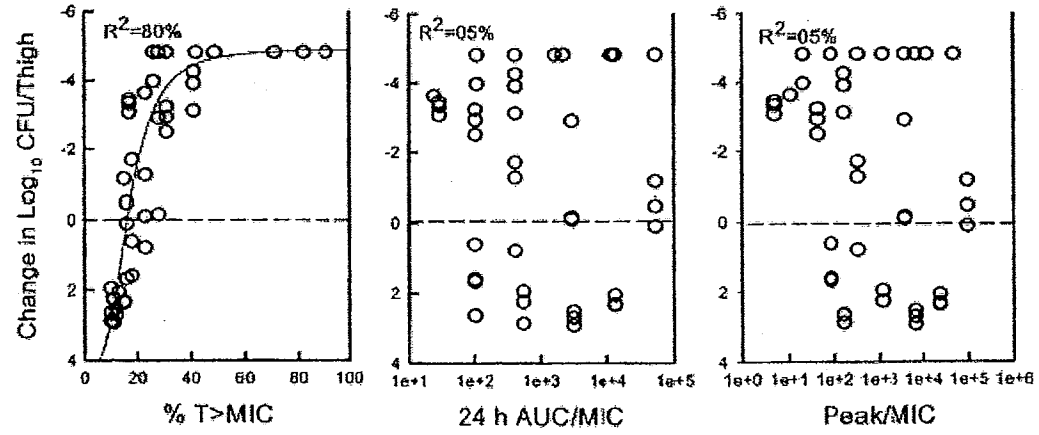


Figure 6. Relationship Between Doripenem PK/PD Parameters and *In Vivo* Efficacy Against *K. pneumoniae* 43816.

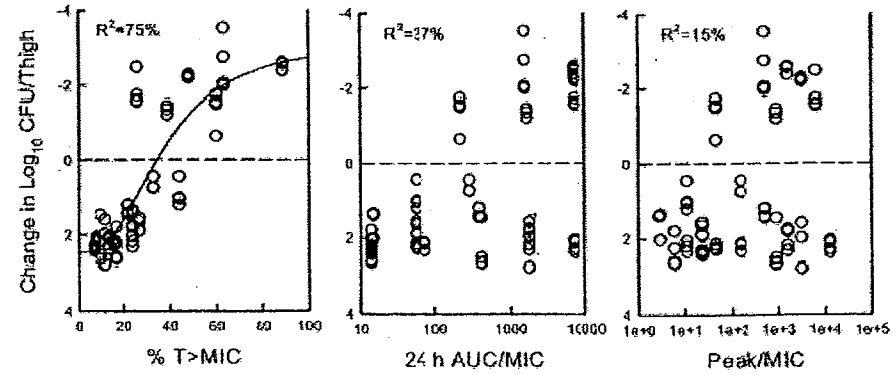
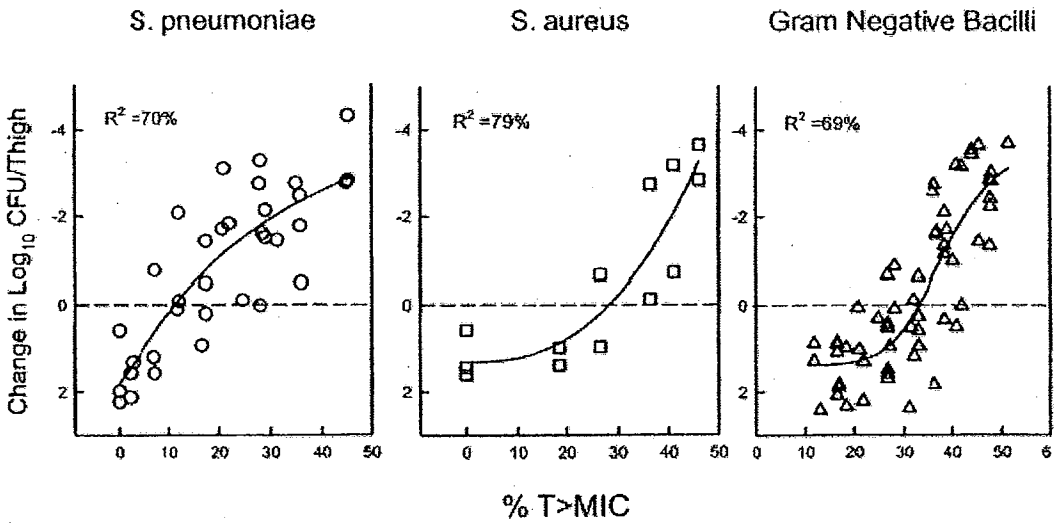


Figure 7. Relationship between Doripenem %T>MIC and *In Vivo* Efficacy Against Multiple Strains of Bacteria.



Sponsor's Conclusions:

1. Doripenem produced modest post-antibiotic effects with *S. aureus* and *S. pneumoniae*.
2. As with other carbapenem antibiotics, the time above MIC, rather than the amount of drug administered was the main determinant of *in vivo* efficacy.
3. The magnitude of the PK/PD parameter for a static effect was similar for doripenem as that previously observed with other carbapenems. The magnitude of the PK/PD parameter required for a static effect was relatively similar for *S. pneumoniae, S. aureus* and gram-negative bacilli. Strains of *S. aureus* and gram-negative bacilli appeared to require more drug for efficacy in relationship to the MIC (similar to other carbapenems).
4. Drug resistance did not impact the magnitude of the PK/PD parameter required for efficacy.

Reviewer assessment:

The time above MIC required to produce a static effect for the multiple pathogens studied ranged from 2.3 to 38%. Static doripenem doses varied from 0.01 mg/kg to 55.6 mg/kg every 6 hours (> 5000-fold difference in dose). There was no major difference in %T>MIC for the different strains of *S. pneumoniae*, regardless of penicillin or quinolone resistance. Further, the presence of methicillin resistance or ESBL production did not significantly impact the magnitude of the PK/PD parameter required for efficacy for *S. aureus* and gram-negative bacilli, respectively. The %T>MIC required to produce a 2-log kill ranged from a mean of 27% for *S. pneumonia* to a mean of 43% for gram-negative bacilli.

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