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In Vivo Pharmacodynamics of MK-2764 / PTK 0796 Against Various Gram-positive and Gram-negative Bacteria in the Thighs of Neutropenic and Normal Mice

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ABSTRACT

Background MK-2764 / PTK 0796 (PTK) is an aminomethylcycline antibiotic, derived from the tetracycline class, with potent activity against gram-positive cocci and many *Enterobacteriaceae*. We used the murine thigh-infection model in neutropenic and normal mice to determine [1] the PK/PD indice driving *in vivo* efficacy and its magnitude with different bacteria, and [2] the effect of neutrophils on the magnitude of the PK/PD indice. PTK was compared with tigecycline (TG), a glycylcycline antibiotic also derived from the tetracycline class and recently FDA approved, in some experiments.

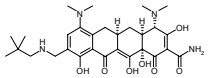
Methods Neutropenic and normal mice were exposed to $10^{5.9\text{-}7.8}$ cfu/thigh of 21 strains [3 MSSA, 2 CA-MRSA, 12 *S. pneumoniae* (SP), 3 *E. coli* (EC) and 1 *K. pneumoniae* (KP)] and then treated sc for 24 hrs with PTK. Dose fractionation studies were performed with 4 strains (MSSA, SP, and 2 EC). Dosing in normal and neutropenic mice was compared with SP and KP. Dose-response data using CFUs at 24 hrs were analyzed by an Emax model using non-linear regression. Static doses for each strain were determined. The pharmacokinetics (PK) of PTK were determined by LC/MS/MS assay at doses of 1.25, 5 and 20 mg/kg. PK/PD indices were calculated using total drug concentrations, as protein binding of PTK is very low.

Results *In vitro* MICs ranged from 0.03 to 2 mg/L. PK of PTK was linear for all doses with peak/dose values of 0.10-0.15 and AUC/dose values of 0.84-0.94. 24-hr AUC/MIC was the PK/PD indice that best correlated with efficacy with all 4 strains (R^2 = 81-85%). The presence of neutrophils enhanced the activity of PTK with SP more than 6-fold, but KP only about 2-fold. PTK was more potent against EC and KP than TG even though MICs of PTK were 4-fold higher than MICs of TG.

Conclusions PTK exhibited potent *in vivo* activity against MSSA, MRSA, SP, EC and KP. The increased potency over TG despite higher MICs suggests that PTK has more favorable PK or PD characteristics.

INTRODUCTION

Structure of MK-2764 / PTK 0796



The following studies were designed to characterize the *in vivo* pharmacodynamic characteristics of MK-2764. The impact of the dosing regimen on the *in vivo* efficacy of MK-2764 in experimental thigh infections in neutropenic mice was determined. Studies were also performed to investigate [1] which pharmacokinetic parameter (peak serum level, area under the concentration-versus-time curve (AUC), the duration of time serum levels exceed the MIC) best predicts efficacy of MK-2764 and [2] whether the magnitude of the PK/PD parameter required for efficacy is similar among organisms with varing susceptibility to MK-2764.

METHODS

Study Organisms and MICs to MK-2764 MICs were determined in MHB by standard CLSI microdilution techniques. MHB was supplemented with 3% lysed horse blood for MIC determinations with *S. pneumoniae*.

Pharmacokinetics The pharmacokinetics of MK-2764 in thigh-infected neutropenic Swiss ICR mice were determined by HPLC/MS/MS on serum samples collected over 8 hours.

***In Vivo* Efficacy** The neutropenic murine thigh-infection model was the standard model used for all organisms throughout the various studies. In this well-established model, neutropenia was produced by two injections of cyclophosphamide, 150 mg/kg four days prior to study and 100 mg/kg one day prior to study. Approximately $10^{6\text{-}7}$ cfu/ml of the study organisms were injected into one or both thighs two hours before starting therapy.

RESULTS

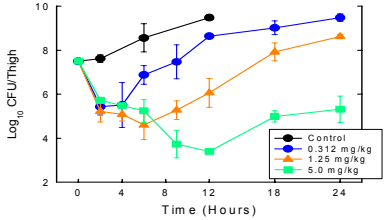
Table 1: Pharmacokinetic parameters of MK-2764 after subcutaneous administration

Dose	Tmax (Hr)	Cmax (ug/ml)	AUC (0-24 Hr)	Half-life (Hr)
1.25	2.0	0.188	1.06	2.4
5.0	0.5	0.585	4.71	3.4
20.0	2.0	1.957	16.7	5.1

The drug is also reported to have very low protein binding to mouse plasma proteins. Thus, total drug concentrations were used in all PK/PD calculations.

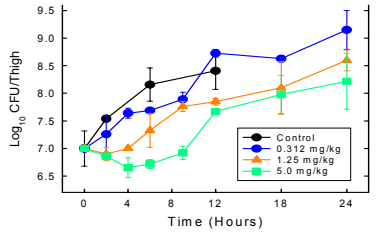
The effect of single doses of 0.312, 1.25 and 5 mg/kg of MK-2764 on the *in vivo* killing and regrowth of *S. pneumoniae* ATCC 10813 are shown in Figure 1. Each point represents the mean of 3 mice. The extent of killing of the strain of *S. pneumoniae* was moderate and similar over the first 2 hours with all three doses. Organism regrowth began soon after total drug concentrations fell below the MIC of the organism.

Figure 1: Effect of single doses of MK-2764 on time course of antimicrobial activity with *Streptococcus pneumoniae* ATCC 10813 in the thighs of neutropenic mice.



The effect of single doses of 0.312, 1.25 and 5 mg/kg dose of MK-2764 on the *in vivo* killing and regrowth of *S. aureus* ATCC 29213 are shown in Figure 2.

Figure 2: Effect of single doses of MK-2764 on time course of antimicrobial activity with *Staphylococcus aureus* ATCC 29213 in the thighs of neutropenic mice



Comparing the time for growth of one $\log_{10}$ in untreated mice with the treated mice after estimated total serum concentrations fall below the MIC results in *in vivo* PAEs of 0-1 hr for *S. pneumoniae* and about 4 hours for *S. aureus*. Sub-MIC concentrations also delayed regrowth of *S. aureus* for 2 to 4 hours with the lower doses.

Parameters Correlating with Efficacy

The PK/PD indice that correlated best with efficacy was determined by relating the number of bacteria in the thigh at the end of 24 hours of therapy with [1] the peak/MIC ratio, [2] the 24-hour AUC/MIC ratio, and [3] the percentage of the dosing interval that serum levels exceed the MIC for each of the dosage regimens studied. The PK/PD indice values for those doses not specifically studied were linearly extrapolated from the data provided by the sponsor. The best correlations were observed with the 24-hr AUC/MIC ratio and the time above the MIC for free drug.

Table 2 lists the coefficient of determination observed for the relationship between efficacy and each PK/PD parameter. The coefficient of determination (or R^2) represents the percentage of the variance in bacterial numbers that can be attributed to each PK/PD parameter. The 24-hr AUC/MIC had the highest coefficient of determination, followed closely by the time above the MIC for three of the four organisms.

Table 2: Coefficients of Determination for Relationship between Efficacy and PK/PD Parameters of MK-2764 against four organisms in the Thighs of Neutropenic Mice

Organism	Site	Data Used	Coefficient of Determination for:		
			Peak/MIC	24-Hr AUC/MIC	Time > MIC
S. pneumoniae	Thigh	3-,6-,12-,24-hr	57.9	81.3	79.4
S. aureus	Thigh	3-,6-,12-,24-hr	68.5	82.8	44.6
E. coli 1-741	Thigh	3-,6-,12-,24-hr	43.5	85.2	84.1
E. coli 1-894	Thigh	3-,6-,12-,24-hr	63.0	82.9	72.8

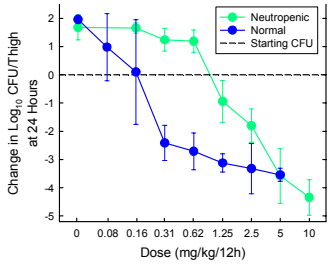
To determine if the 24-hr AUC/MIC required for a static effect was similar for multiple pathogens, we studied the activity of 12-hourly dosing regimens of MK-2764 against 12 strains of *S. pneumoniae* with MICs of 0.03-0.06 mg/L, 4 strains of *S. aureus* with MICs of 0.25-0.5 mg/L, and 4 Enterobacteriaceae with MICs of 0.5-2.0 mg/L (Table 3). Two of the pneumococcal strains were intermediate to penicillin, while four strains were resistant to penicillin. Four of the strains were also resistant to trimethoprim/sulfamethoxazole. Three of the staphylococcal strains were MRSA with two being community-acquired MRSA (CAMRSA).

Table 3: The Static Dose, Maximum Killing and 24-Hr AUC/MIC Required for a Static Effect for 12-hourly Dosing of MK-2764 against 11 Organisms

Organism	MIC MK-2764 / TG (µg/ml)	Static Dose (mg/kg/12h)	24-Hr AUC/MIC	Maximal Killing (Log_{10} cfu/thigh)
S. pneumoniae ATCC 10813	0.06	0.900	25.4	5.24
S. pneumoniae ATCC 49619	0.06	0.799	22.6	3.37
S. pneumoniae CDC 1020 tetR	0.06	1.25	35.3	3.77
S. pneumoniae CDC 1199 tetR	0.03	0.368	20.8	3.92
S. pneumoniae CDC 1293 tetR	0.06	1.45	38.7	3.95
S. pneumoniae CDC 1329	0.03	0.561	31.7	3.61
S. pneumoniae CDC 1396 tetR	0.06	0.820	23.2	2.54
S. pneumoniae CDC 146	0.06	0.798	22.6	5.31
S. pneumoniae CDC 673	0.03	0.408	23.1	4.22
S. pneumoniae CDC 1325 tetR	0.06	0.636	19.1	5.16
S. pneumoniae ATCC 6301	0.03	0.540	30.5	4.00
S. pneumoniae ATCC 6303	0.06	0.526	14.9	2.93
S. aureus ATCC 29213	0.25	11.9	81.8	0.79
S. aureus ATCC Smith	0.5	11.4	39.3	0.61
S. aureus ATCC 33591	0.5	13.6	46.3	0.86
S. aureus CAMRSA MW2	0.25	8.88	62.5	1.19
S. aureus CAMRSA R2527 tetR	0.25	5.02	37.8	1.68
E. coli ATCC 25922	0.5/0.12	7.03	25.3	2.13
E. coli 1-741 tetR	0.5	5.86	21.6	1.61
E. coli 1-894 tetR	0.5	6.00	22.0	1.32
K pneumoniae ATCC 43816	2.0/0.5	71.1	59.4	0.25

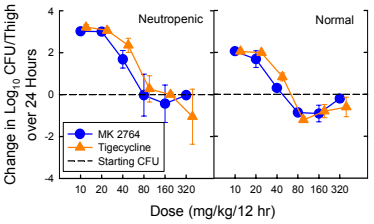
S. pneumoniae ATCC 10813 and *K. pneumoniae* ATCC 43816 are capable of infecting the thighs of normal non-neutropenic mice. The dose-response relationship for MK-2764 against *S. pneumoniae* ATCC 10813 indicates the presence of WBCs markedly enhanced the activity of MK-2764 against *S. pneumoniae*.

Figure 3: Dose-response relationships for 12-hourly dosing of MK-2764 against *Streptococcus pneumoniae* ATCC 10813 in the thighs of neutropenic and normal mice



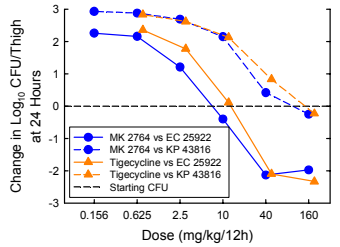
The presence of WBCs had only a modest effect on the activity of MK-2764 and tigecycline against the strain of *K. pneumoniae*. This has been observed previously with fluoroquinones, beta-lactams and aminoglycosides. MK-2764 was slightly more potent than tigecycline in both neutropenic or normal mice.

Figure 4: Dose-response relationships for 12-hourly dosing of MK-2764 and Tigecycline against *Klebsiella pneumoniae* ATCC 43816 in the thighs of neutropenic and normal mice



Despite having *in vitro* activity less than that of tigecycline (Table 1), MK-2764 exhibited comparable, if not slightly superior efficacy in the neutropenic thigh model against *E. coli* and *K. pneumoniae* (Figure 5).

Figure 5: Dose-response relationships for 12-hourly dosing of MK-2764 and Tigecycline against *Escherichia coli* ATCC 25922 and *Klebsiella pneumonia* ATCC 43816 in the thiehs of neutronenic mice



The impact of WBC on the efficacy of MK-2764, the relative efficacy of MK-2764 compared to tigecycline, and the PK/PD requirements for both compounds are summarized in Table 4.

Table 4: Static Dose and 24-Hr AUC/MIC Required for the Static Dose for MK-2764 and Tigecycline against *S. pneumoniae* and *K. pneumoniae* in thighs WBC of normal and neutropenic mice

Drug	Organism	WBC Present	Static Dose (mg/kg/12h)	24-Hr AUC/MIC
MK 2674	*S. pneumoniae*	No	1.02	28.8
		Yes	0.150	4.24
	K. pneumoniae	No	77.8	65.0
		Yes	44.9	37.5
Tigecycline	*K. pneumoniae*	No	121	184
		Yes	59.8	91

CONCLUSIONS

The above studies have characterized the *in vivo* pharmacodynamic activity of MK-2764 against various strains of *S. pneumoniae*, *S. aureus* and a few gram-negative bacilli.

- MK-2764 produced a modest postantibiotic effect with *S. aureus*.
- The 24-hr AUC/MIC was the PK/PD indice most important for efficacy.
- The magnitude of the 24-hr AUC/MIC required for the various strains of pneumococci was 23.8. This value is very similar to the 24-hr AUC/MIC values obtained with fluoroquinolones, other tetracyclines, clindamycin and macrolides with *S. pneumoniae*. The 24-hr AUC/MIC values were similar for *E. coli*, but *S. aureus* and *K. pneumoniae* appeared to require a slightly higher AUC/MIC for a static effect.
- The presence of WBCs had a major impact in reducing the magntiude of the 24-hr AUC/MIC over 6-fold with one strain of *S. pneumoniae*.
- MK-2764 exhibited excellent killing of *S. pneumoniae* over 24 hours of therapy. Killing of *E. coli* was modest while the drug was primarily bacteriostatic against *S. aureus* and *K. pneumoniae*.
- Against *E. coli* and *K. pneumoniae*, MK-2764 was slightly more potent *in vivo* than tigecycline, despite being four-fold less active *in vitro* in two strains selected. This suggests that MK-2764 has more favorable pharmacologic characteristics (eg. longer half-life with resulting higher AUC, lower protein binding, or even more potent pharmacodynamics).