

Pharmacodynamics of TR-701 in a Mouse Thigh Infection Model Against *Staphylococcus aureus* MRSA Strain ATCC33591

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Abstract

Background: TR-701 is an agent of the oxazolidinone class being developed by Trius Therapeutics and would be a valuable addition to the therapeutic armamentarium. We examined TR-701 against a strain of MRSA in a mouse thigh infection model.

Methods: The mouse thigh infection model of Craig was employed. Mice were made neutropenic by cyclophosphamide, 150 mg/kg day -4 and 100 mg/kg day -1. An inoculum of 5.82×10^8 CFU of MRSA ATCC 33591 was injected into the posterior thighs of female Swiss-Webster mice. TR-701 was examined over a dose range of 10 mg/kg/day to 240 mg/kg/day along with a no-treatment control and a positive control of linezolid at 120 mg/kg/day. All doses were administered twice daily. Cohorts were terminated at 24 and 48 h. An inhibitory sigmoid-Emax model was fit to the data using ADAPT II. Weighting was inverse of observation variance. PK parameters were identified with NPAG. Quantization was by LC/MS/MS.

Results: At therapy initiation, there were 5.66×10^8 CFU/g. The model fit the data well for the 24 and 48 h cohorts ($r^2 = 0.998$ and 0.999). Emax (24 and 48 h) was 4.59 & 5.97 \log_{10} CFU/g decline; ED_{50} was 25.2 & 31.7 mg/kg/day. H (Hill's Constant) was 2.02 & 2.56; $E_{control}$ was 8.87 & 8.79 \log_{10} CFU/g. Calculation revealed a stasis dose (24 & 48 h) of 38.5 & 33.6 mg/kg/day, with 1 Log kill of 82.6 and 44.4 mg/kg/day. At 48 hours a 2 log kill required 65.2 mg/kg/day. Linezolid at 120 mg/kg/day at 24 & 48 h produced colony counts of 7.2 and 7.49 \log_{10} CFU/g, which did not achieve stasis.

Conclusions: TR-701 performed well in this experiment, generating greater than (relative to stasis) 1 log kill at 24 h and almost 3 log kill at 48 h. Linezolid at 120 mg/kg/day did not achieve stasis. At both 24 and 48 h, TR-701 required 1/6th the dose to produce the same microbiological activity as linezolid. This indicates that when MIC, PK and protein binding are integrated, TR-701 is 6 times more potent than linezolid. TR-701 is a promising compound requiring further experimentation with more strains and other modes of administration.

Background

From 1992 to 2003, the proportion of hospital-acquired *Staphylococcus aureus* isolates that were methicillin-resistant within intensive care units of U.S. hospitals increased from 35.9% to 64.4%.¹ Furthermore, in 2004 community-associated MRSA (CA-MRSA) represented 59% of all acute, purulent skin and soft tissue infections seen in 11 emergency rooms across the U.S.A.² Often, hospital-acquired MRSA are resistant to multiple antibiotics, including all beta-lactam antibiotics, trimethoprim-sulfamethoxazole, tetracycline, clindamycin, the quinolones, and the aminoglycosides.³ Although CA-MRSA tends to be more susceptible to non-beta-lactam antibiotics, 94% are resistant to erythromycin and 40% are resistant to fluoroquinolones.³ In some geographic locations up to 79% of isolates are resistant to clindamycin.² Vancomycin is considered the "drug of choice" for the treatment of MRSA. However, this drug is only slowly bactericidal. Also, strains of *S. aureus* with decreased susceptibility to vancomycin have been described.⁴

TR-701 is a phosphate monoester prodrug of TR-700, a new oxazolidinone antibiotic that has potent *in vitro* activity against Gram-positive bacteria, including MRSA. The *in vivo* activity of TR-701 against MRSA is incompletely defined.

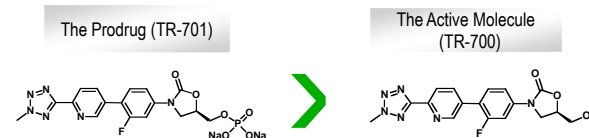
In this study we conducted pharmacokinetic and dose-range studies in a neutropenic mouse thigh model of MRSA infection to define the pharmacodynamics of TR-701 against this important pathogen.

Methods

Bacterial isolate. *Staphylococcus aureus* ATCC 33591 (American Type Culture Collection, Manassas, VA), is a MRSA strain that was used throughout the study. Stock specimens that were stored at -80°C .

Antibiotic agent. TR-700 and its phosphate monoester prodrug, TR-701, were provided by Trius Therapeutics (San Diego, CA). Prior to each study a fresh solution of TR-700 was dissolved in DMSO and then serially diluted in Mueller-Hinton II broth. TR-701 was dissolved in sterile water.

Figure 1: Structure of the phosphate monoester prodrug TR-701 and the active molecule TR-700.



MIC determination. The MIC to TR-700 was determined using a macrobroth dilution method in Mueller-Hinton II broth as described by CLSI.⁵ The bacterial inoculum was 5×10^5 cfu/ml. The MIC was read after the cultures were incubated at 35°C for 24 hrs. The MICs were conducted in duplicate on 3 different days.

Neutropenic animal infection model. Female, 24-26 gram outbred Swiss Webster mice (Taconic Farms, Taconic, NY) were used. Transient neutropenia was induced by injecting mice with cyclophosphamide 150 mg/kg IP four days prior to bacterial inoculation and 100 mg/kg of cyclophosphamide IP one day prior to infection, as described by Craig et al.⁶ With this regimen the mice were neutropenic for 4 days, beginning on the day the second dose of cyclophosphamide was administered. One day after the second dose of cyclophosphamide was given, the mice were infected IM in each posterior thigh muscle with 10^8 cfu of the MRSA isolate. The animal procedures used in this study were approved by our IACUC.

Pharmacokinetics of single dose TR-700 and TR-701 in infected mice. Neutropenic mice were infected in each posterior thigh muscle with the MRSA strain. Two hours later, mice were given 10, 20, 40, or 80, mg/kg of TR-701, IP, as a single dose. At 0.5, 1, 2, 3, 4, 6, and 8 hours after drug administration, 2 to 3 mice per group were sacrificed. The blood was collected and placed on ice in vials containing EDTA. The plasma was collected. Then 1 N HCl was added to the plasma to stabilize the TR-701 within the sample before the plasma was frozen at -80°C . The concentration of drug in plasma was measured by LC/MS/MS. All pharmacokinetic data were population modeled with the computer program the Non-Parametric Adaptive Grid (NPAG) with adaptive γ (of Leary, Jelliffe and Schumitzky)⁷ using a 2 compartment model with first order input. The clearance and plasma terminal half-lives were determined. Simulations were conducted to determine the cumulative AUCs generated in each of the dosing groups from the time of drug administration to 24 hr using the Adapt II package of software programs of D'Argenio and Schumitzky.⁸ Cumulative plasma AUCs, peak concentrations, and Time > MIC were calculated for each dose examined.

Dose-range studies. Dose-range studies were conducted in infected, neutropenic mice. Two hours after neutropenic mice were inoculated with MRSA, the animals were injected IP with 0 to 240 mg/kg of TR-701 or 120 mg/kg of linezolid given as two divided doses every 12 hours for 48 hours. This dose of linezolid produced a free 24h-AUC in mice that was more than twice the reported free 24h-AUC in humans who were treated with linezolid 600 mg q 12 hr.⁹ At 24 and 48 hours after treatment was initiated, the mice were sacrificed and both posterior thigh muscles were aseptically collected. Homogenates of the thigh muscles were washed twice with sterile saline and were quantitatively cultured onto Mueller-Hinton II agar. After 48 hours of incubation at 35°C the plates were enumerated. The relationship between dose and the reduction in bacterial densities in thigh muscles was determined using an inhibitory sigmoid Emax analysis.

Results

MIC results. The MIC of TR-700 was 0.5 mg/L for the MRSA isolate used in these studies. The MIC to linezolid was 2.0 mg/L. The MBC for each drug was >256 mg/L. The MIC of TR-701 was >256 mg/L.

TR-700 and TR-701 pharmacokinetics. TR-700 and TR-701 concentrations from plasma samples, collected over 8 hrs were modeled using a population pharmacokinetic analysis using the software program BigNPAG. The mean parameter values for TR-700, the active compound, are shown in Table 1.

Table 1. Mean Parameter Values for the Active Compound (TR-700)

Parameter (units)	Vc (L)	Kapd (hr^{-1})	Kcp (hr^{-1})	Kpc (hr^{-1})	CL (L/hr)
Mean	0.0295	37.4	14.2	1.48	0.00384
Stand Dev.	0.0116	47.0	2.70	0.561	0.00214

V_c = volume of the central compartment; Kapd = first order absorption rate constant for prodrug; Kcp and Kpc = first order intercompartmental transfer rate constants; CL = Clearance.; Terminal T_{1/2} = 5.7 hrs

The fit of the models to the data after the MAP-Bayesian step for TR-700 and TR-701 were good ($r^2 \geq 0.982$). The Observed-Predicted plots for plasma concentrations for TR-700 and TR-701 are presented below in Figure 2.

Fig 2A. TR-700 Mouse Population PK Predicted - Observed Plot

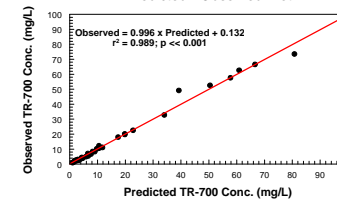
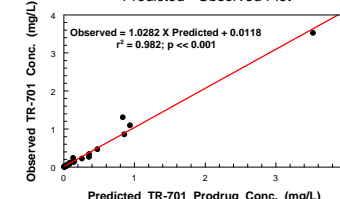


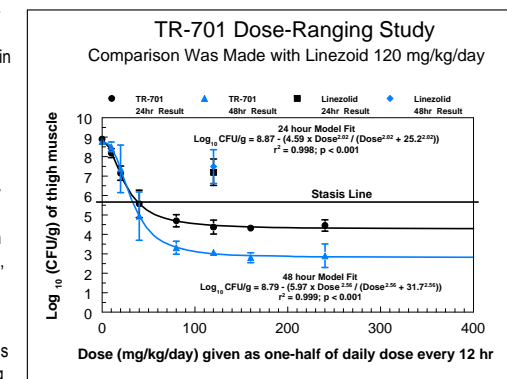
Fig 2B. TR-701 Prodrug Mouse Population PK Predicted - Observed Plot



Previously we showed that the plasma clearance for linezolid in infected mice was 0.00384 ± 0.00393 L/hr and the terminal half-life was 3.4 hr. The dose of linezolid 120 mg/kg/day examined in this project produced a free 24h-AUC in mice that was more than twice the reported free 24h-AUC in humans who were treated with the clinical regimen of linezolid 600 mg q 12 hr.⁹

References: (1) Klevens et al. Clin Infect Dis. 2006;43:387. (2) Moran et al. N Engl J Med. 2006;355:666. (3) Diekema et al. Clin Infect Dis. 2001;32(Suppl 2): S114. (4) Hiramatsu et al. J Antimicrob Chemother. 1997;40:135. (5) CLSI. Document M7-A7. 2006. Wayne PA. (6) Craig et al. J Antimicrob Chemother. 1991.;27(Suppl C):29. (7) Leary et al. Proceedings 14th IEEE Symposium on Computer Based Systems. 2001:389. (8) D'Argenio et al. Adapt II User manual. 1997. <http://bmsr.usc.edu>. (9) Louie et al. ICAAC 2004 abstr. A-1865, p 37.

Fig. 3. Dose-response effect of TR-701 (mg/kg, IP), total doses given twice-daily, on the density of MRSA in thigh muscles of mice. The dose-response relationship is well described by an inhibitory sigmoid Emax curve.



After 24 and 48 hrs, TR-701 therapy was associated with an Emax reduction in MRSA densities in thigh muscles of 4.59 and 5.97 \log CFU/g, respectively, compared with no treatment.

The bacterial densities in the controls arms were 8.87 and 8.79 \log CFU/g at 24 and 48 hours, respectively.

Table 2. At 24 and 48 hours, the following doses of TR-701 (mg/kg/day) were associated with stasis effect, a 1 log CFU/g kill, and a 2 log CFU/g kill of MRSA:

Microbiological Effect	24 hr	48 hr
Stasis effect:	38.5	33.6
1 log CFU/g kill:	82.6	44.4
2 log CFU/g kill:	Not achieved	65.2

At 48 hr, TR-701 doses of > 100 mg/kg/day were able to achieve almost a 3 \log CFU/g kill of MRSA compared with stasis and almost a 6 \log CFU/g kill compared with untreated controls.

In contrast, linezolid at 120 mg/kg/day resulted in only a 1.5 \log CFU/g reduction on bacterial counts at 24 and 48 hours of therapy compared with untreated controls. This linezolid regimen did not achieve stasis.

Conclusions

- TR-701 was highly active versus linezolid (6-fold when MIC, PK and protein binding are integrated) against the MRSA isolate evaluated in a neutropenic murine thigh infection model.
- Increased effect of TR-701 was seen when treatment duration was extended from 24 to 48 hrs.
- TR-701 was superior to linezolid and achieved stasis in 24 hrs at 38.5 mg/kg/day and almost a 3 \log CFU/g kill of MRSA at doses >100 mg/kg/day at 48 hrs. In contrast, a 120 mg/kg/day dose of linezolid generated a free 24h-AUC in mice that was more than twice the free 24h-AUC reported in the serum of humans treated with linezolid 600 mg q 12 hr. This dose of linezolid did not achieve a stasis effect in mice.
- Extending the duration of TR-701 therapy from 24 to 48 hrs had a positive effect in clearing MRSA from the infection site, but for linezolid it had only a minimal effect in clearing MRSA.