

EVALUATION of TISSUE DISTRIBUTION of TR-700 IN HEALTHY VOLUNTEERS, USING MICRODIALYSIS

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ABSTRACT

Background: Plasma concentrations of antimicrobial drugs have long been used to correlate exposure to effect, yet one cannot always assume that unbound plasma and tissue concentrations are similar. Knowledge about unbound tissue concentrations is important in the development of antimicrobial drugs, since most infections occur localized in tissues. Therefore, a clinical microdialysis study was conducted to evaluate the distribution of TR-700, the active moiety of the antimicrobial prodrug TR-701, into interstitial fluid (ISF) of subcutaneous adipose and skeletal muscle tissues following a single, oral 600 mg dose of TR-701 in fasting conditions.

Methods: 12 healthy, adult subjects were enrolled. Two microdialysis probes were implanted into the thigh of each subject, one into the vastus medialis muscle and one into subcutaneous adipose tissue. Probes were calibrated using retrodialysis. Dialysate samples were collected every 20 min for 12 hours after a single, oral dose of 600 mg TR-701 and blood samples were drawn at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 18, and 24 h post dose.

Results: Unbound TR-700 levels in plasma were similar to those in muscle and adipose tissue. The median ratios of $fAUC_{0-12, \text{tissue}}/fAUC_{0-12, \text{plasma}}$ were 1.08 (0.22) and 1.22 (0.18) for adipose and muscle tissues, respectively. The median half-life was 8.13, 9.22 and 9.59 h for plasma, adipose and muscle tissues, respectively. Mean protein binding was $87.2 \pm 1.3\%$. The study drug was very well tolerated.

Conclusions: The results of the study show that TR-700 distributes well into ISF of adipose and muscle tissues. Unbound levels of TR-700 in plasma, adipose and muscle tissues were well correlated. Free plasma levels are indicative of unbound levels in the ISF of muscle and adipose tissues.

INTRODUCTION

TR-700 is a novel oxazolidinone intended for the use against infections caused by Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE) as well as some Gram-negative pathogens.

Often, plasma concentrations are used for PK/PD indices such as AUC/MIC even though they rely on the assumption that drug distributes equally in and into all tissues (1). Both FDA (2,3) and EMA (4) suggest the use of target site concentrations for the use with these indices. Microdialysis provides a useful and valuable tool for the access to tissue concentrations.



Figure 1: Catheter Placement

METHODS

Design: The study was an open-label, single dose study with 15 healthy volunteers. Three volunteers were enrolled into a Pilot Study, twelve were enrolled into the Main Study. Recovery of TR-700 *in vivo* and washout duration was assessed during the Pilot Study. In both study phases, two microdialysis probes were inserted into subcutaneous adipose tissue and vastus medialis muscle tissue, respectively (Figure 1). The probes were then calibrated using the retrodialysis method by perfusing the catheters with a 2 µg/mL solution of TR-700 for 60 min and collecting a cumulative sample after which a washout period was observed. The subjects in the Main Study then received a single, oral dose of 600 mg TR-701. Microdialysis samples were taken every 20 minutes for a total of 12 hours and plasma samples were taken at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 18 and 24 hours after dosing. At 0, 2 and 12 hours additional blood for determination of protein binding was taken.

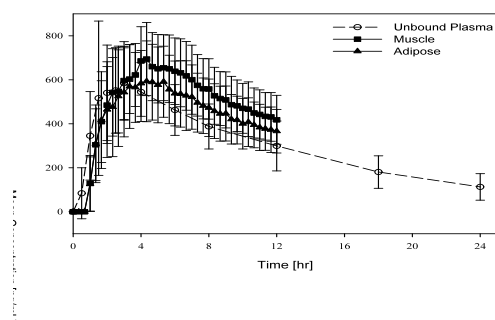
Sample Analysis: Microdialysis and plasma samples were frozen at -80°C and analyzed using a valid HPLC/UV and LC-MS/MS method, respectively.

Data Analysis: *In vivo* recovery was calculated as follows: Recovery [%] = $100 \cdot c(\text{dialysate}) \cdot c(\text{perfusate})^{-1}$. Observed concentrations were then adjusted for recovery using the following formula: $c(\text{final}) = c(\text{observed}) \cdot \text{Recovery}^{-1}$. Free plasma concentrations were calculated by multiplying each individual's fraction unbound with the observed total plasma concentration.

Results were analyzed using WinNonlin[®] 5.2 software. Statistical comparisons were made using a one way ANOVA method and Bonferroni method for multiple comparison.

RESULTS

Figure 2: Mean (SD) unbound TR-700 concentration



RESULTS

Pilot Study:

The *in vivo* recovery in the pilot study was approximately 90%. In order for the method to be feasible, recovery had to be $>10\%$. The necessary duration for washout was found to be four hours.

Main Study:

TR-700 pharmacokinetics in plasma and tissue were analyzed using non-compartmental analysis. The median parameter estimates (range) are shown in Table 1 and the time course of drug exposure can be seen in Figure 2.

Table 1: Mean (SD) Pharmacokinetic parameters of TR-700 in Plasma and Tissues

Parameter	Unit	Plasma	f/Plasma	Adipose Tissue	Muscle Tissue
C_{max}	(ng/mL)	5373 (1514)	*	664 (164)	742 (155)
$T_{\text{max}}^{\ddagger}$	(hr)	2 (1-4)	*	3.33 (2.33-10.3)	3.67 (1.67-7)
AUC_{0-12}	(ng*hr/mL)	38813 (7548)	4959 (1093)	5266 (1279)	5948 (1109)
AUC_{last}	(ng*hr/mL)	57105 (14740)	7276 (1906)	5266 (1279)	5948 (1109)
AUC_{∞}	(ng*hr/mL)	70021 (24784)	8895 (3059)	16634 (15802)	14582 (10494)
$t_{1/2}^{\ddagger}$	(hr)	8.13 (5.94-12.8)	*	9.22 (5.98-85.9)	9.59 (6.15-48.2)
λ_z	(hr ⁻¹)	0.08 (0.02)	*	0.07 (0.04)	0.07 (0.03)
CL/F	(L/hr)	9.46 (2.91)	*	*	*
V_z/F	(L)	113 (19.3)	*	*	*
$fAUC_{0-12, \text{tissue}}/fAUC_{0-12, \text{plasma}}$		*	*	1.08 (0.22)	1.22 (0.18)
Protein Binding	%	87.2 (1.3)	*	*	*

* Not applicable; [†] Median (Range); λ_z : terminal elimination rate constant

CONCLUSIONS

TR-700 appears in tissue in levels comparable to free, unbound plasma concentrations. The median tissue to plasma ratios of free AUC_{0-12} are 1.16 and 1.26 for adipose and muscle tissue, respectively, indicating that TR-700 may penetrate into muscle tissue to a slightly higher extent. No statistically significant differences can be found between the three tissue groups. AUC ratios were comparable to those found in a microdialysis study of linezolid (5). TR-700 was well tolerated and adverse events were mild and usually related to the microdialysis procedure.

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