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Skin and soft tissue concentrations of tedizolid (formerly torezolid), a novel oxazolidinone, following a single oral dose in healthy volunteers

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Abstract

Plasma concentrations of antimicrobial drugs have long been used to correlate exposure with 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 are localised in tissues. Therefore, a clinical microdialysis study was conducted to evaluate the distribution of tedizolid (TR-700), the active moiety of the antimicrobial prodrug tedizolid phosphate (TR-701), into interstitial fluid (ISF) of subcutaneous adipose and skeletal muscle tissues following a single oral 600 mg dose of tedizolid phosphate in fasting conditions. Twelve 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 h following a single oral dose of 600 mg tedizolid phosphate, and blood samples were drawn over 24 h. Unbound tedizolid levels in plasma were similar to those in muscle and adipose tissue. The ratios of unbound (free) AUC in tissues over unbound AUC in plasma ($fAUC_{tissue}/fAUC_{plasma}$) were 1.1 ± 0.2 and 1.2 ± 0.2 for adipose and muscle tissue, respectively. The median half-life was 8.1, 9.2 and 9.6 h for plasma, adipose tissue and muscle tissue, respectively. Mean protein binding was 87.2 ± 1.8%. The study drug was very well tolerated. The results of this study show that tedizolid distributes well into ISF of adipose and muscle tissues. Unbound levels of tedizolid in plasma, adipose tissue and muscle tissue were well

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Competing interests

CdA and PP are employees of Trius Therapeutics, Inc. (San Diego, CA). All other authors declare no competing interests.

Ethical approval

The study was approved by the University of Florida Institutional Review Board (IRB protocol #680-2007).

correlated. Free plasma levels are indicative of unbound levels in the ISF of muscle and adipose tissues.

Keywords

Microdialysis; Tissue distribution; Tedizolid; Pharmacokinetics

1. Introduction

There has been a steady increase in the number of infections caused by meticillin-resistant *Staphylococcus aureus* (MRSA), meticillin-resistant coagulase-negative staphylococci and vancomycin-resistant enterococci [1]. Although there appears to be a trend towards decreasing numbers of hospital-onset and hospital-associated MRSA infections with community onset [2], there is still a need for novel treatments with an optimised efficacy, safety and pharmacodynamic profile to bolster the armamentarium against potentially severe or fatal *S. aureus* and *Enterococcus* spp. infections.

The first oxazolidinone drug to enter the market was linezolid in 2000. Linezolid has good in vitro and in vivo properties against staphylococci, enterococci and streptococci [3]. Linezolid also shows good pharmacokinetic properties with an oral bioavailability of ca. 100%, and tissue penetration following multiple and single doses that are close to the free concentration in plasma [4,5]. One disadvantage of linezolid is that it has to be administered twice daily [6]. Furthermore, linezolid pharmacokinetics have been shown to have considerable interindividual variability and there are safety concerns due to monoamine oxidase interactions and potential myelosuppression [5,6].

Tedizolid is a novel oxazolidinone compound with four to eight times improved antibacterial potency compared with linezolid [7].

The rationale behind studying tissue concentrations is the understanding that for most antibiotics it is the free drug available at the site of action, the biophase, that is responsible for the antibacterial effect [8]. Moreover, most bacteria cause infection not in the bloodstream but in the tissue itself, therefore measuring concentrations in tissue should give greater clarity on the amount of drug available for action [9,10]. One method that can easily be used for measuring drug concentrations in tissue is microdialysis [11]. It has been widely used to measure tissue concentrations, for example, in lungs, soft tissues, and skin and soft-tissue infections [12–16]. Measurement of biophase concentrations is also recommended by regulatory authorities [17,18].

The purpose of this study was to assess the tissue distribution of tedizolid, the microbiologically active moiety, following a single oral dose of tedizolid phosphate prodrug.

2. Materials and methods

This clinical study was conducted according to the Declaration of Helsinki and Good Clinical Practices. Approval for the study was obtained from the institutional review board of Shands Hospital at the University of Florida (Gainesville, FL) before any volunteers were recruited for the study.

2.1. Healthy volunteers

Fifteen healthy volunteers (ten female and five male) participated in the study. To confirm eligibility of the subjects, a physical examination and electrocardiography were performed and urinalysis, haematology and blood chemistry laboratory samples were evaluated.

Females also had to have a negative serum β-human chorionic gonadotropin pregnancy test at screening and a negative urine pregnancy test on Day 1. Eligible subjects had to be between 18 years and 50 years of age, healthy and not receiving any other medication; hormonal contraception was allowed for females. The body mass index had to range from 20 kg/m^2 to 29 kg/m^2.

2.2. Study design

This study was an open-label, single-dose, single-centre study in 15 healthy volunteers; 3 volunteers were enrolled into a pilot study to confirm the feasibility of the microdialysis method in vivo, and 12 volunteers were enrolled into the main part of the study that included a single oral dose of tedizolid. For subjects in the pilot study, one microdialysis membrane each was inserted into the muscle and subcutaneous adipose tissue of the upper thigh. After feasibility was confirmed, subjects were enrolled and screened for the main part of the study. Once eligibility was confirmed, subjects were admitted to the General Clinical Research Unit at Shands Hospital. On the first study day, microdialysis probes were placed and perfused with lactated Ringer's solution for 30 min, before probe calibration by retrodialysis was performed. For this, the probe was connected to a syringe containing tedizolid at a concentration of 2 μg/mL, which was perfused for 30 min before a sample was collected for another 30 min. Thereafter, the probe was perfused again with lactated Ringer's solution with a washout period of 4 h. Microdialysis samples were collected every 20 min for 12 h after study drug administration, and blood samples were collected for 24 h post dose. Subjects had to remain on bed rest for the duration of active microdialysis and had to remain fasted for 5 h before dose administration followed by a fast from food for ≥3 h post dose.

2.3. Study drug

Study drug was supplied in 200 mg capsules (Trius Therapeutics, Inc., San Diego, CA). Subjects were administered a single oral dose of 600 mg tedizolid phosphate (the prodrug) and a hand and mouth check was performed for verification. A 600 mg dose was selected as early dosing estimates projected that it could be in the range of the therapeutic dose for the treatment of skin infections. However, results of a recent Phase 2 study showed a high degree of efficacy at lower doses [19].

2.4. Clinical microdialysis

The microdialysis method has been described in detail and has been used in many clinical studies [5,11,12,20]. Briefly, to assess interstitial fluid (ISF) concentrations in the tissues of interest, two microdialysis probes are placed, one into the subcutaneous adipose tissue and one into the muscle. The probes were perfused with lactated Ringer's solution at a steady rate of 1.5 μL/min. This constant perfusion results in an incomplete equilibrium between the ISF of the tissue and the inside of the microdialysis probe. The factor that correlates the drug measured in the dialysate to the actual concentration in the ISF is the recovery factor. One method to assess this in vivo is the retrodialysis method, which was first described by Ståhle et al. [20]. For this method, tedizolid was perfused through the probe at a concentration of 2 μg/mL and the recovered amount of tedizolid in the dialysate was analysed. The recovery is then calculated as the recovered amount divided by the nominal amount. The recovery factor is used to adjust the measured tissue concentrations.

2.5. Analysis

2.5.1. Microdialysis sample analysis—Microdialysis samples were collected every 20 min for a total of 12 h post dose and were placed on ice and frozen at −80 °C within 2 h of collection. For analysis, samples were thawed at room temperature and were analysed using

high-performance liquid chromatography with ultraviolet light detection. The method was validated according to US Food and Drug Administration (FDA) and Good Laboratory Practice guidelines. The limit of quantitation was 50 ng/mL. The accuracy and precision of the microdialysis samples ranged from −5.4% to 4.2% and 1.1% to 7.3%, respectively.

2.6. Plasma and protein binding samples

Blood samples were collected pre dose and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 18 and 24 h post dose. For protein binding, additional blood was drawn at 0, 2 and 12 h post dose. Samples were centrifuged and frozen immediately at −80 °C.

2.7. Data analysis

A non-compartmental analysis of the data from the three sampling sites was conducted using WinNonlin® v.5.2 software (Pharsight, St Louis, MO). Pharmacokinetic parameters included the area under the concentration–time curve (AUC), calculated by linear trapezoidal rule, the maximum concentration (C_{max}), the time to C_{max} and the terminal half-life ($T_{1/2}$). Apparent oral clearance (CL/F) was calculated as dose/AUC, where CL is drug clearance and F is the fraction absorbed. The apparent volume of distribution (V_z/F) was calculated as $CL \times T_{1/2}/\ln_2$. To derive free plasma concentrations, each subject's total values were adjusted by their individual protein-binding results by multiplying the total tissue concentrations by the subject's individual fraction of unbound drug in plasma (f_u = 1–fraction bound). Adipose and muscle tissue concentrations were derived from the measured concentrations in tissue and were adjusted by the measured recovery value. The calculation was done as follows: concentration in tissue = 100 × (sample concentration/in vivo recovery [%]).

3. Results

The results of the pilot study indicated that tedizolid recovery from the microdialysis probe was very high at 87.0% and 94.7% for adipose and muscle tissue, respectively. The pilot phase also revealed that a washout period of 4 h was sufficient to ensure that there was no leftover drug from probe calibration in the tissue.

Mean recovery values (± standard deviation) for the 12 volunteers in the main study were 95.3 ± 2.8% for adipose tissue and 96.3 ± 1.9% for muscle tissue. For one subject the probe in the adipose tissue malfunctioned, therefore calculations for adipose tissue are based on 11 subjects. The mean protein binding of tedizolid was measured at 87.3 ± 1.3%. The pharmacokinetic results of the study are in good agreement with a previous study of the same dose [21]. Mean concentration–time profiles for total and free plasma and free tissue concentrations are given in Figs 1 and 2. Table 2 shows a summary of estimated pharmacokinetic parameters. The ratios of unbound AUC in tissues over unbound (free) AUC in plasma ($f\text{AUC}_{tissue}/f\text{AUC}_{plasma}$) were 1.1 ± 0.2 and 1.2 ± 0.2 for adipose and muscle tissue, respectively, indicating slightly higher tedizolid tissue distribution (at least for the two tissues measured) relative to plasma. Wilcoxon matched pairs tests showed that the $AUC_{0–12h}$ (AUC over 0–12 h) for muscle tissue was statistically significantly ($P < 0.05$) higher than the $AUC_{0–12h}$ in free plasma and the $AUC_{0–12h}$ in adipose tissue. The $AUC_{0–12h}$ values of adipose tissue and free plasma were not statistically significantly different from each other.

4. Discussion

This study shows that the concentrations of tedizolid in adipose and muscle tissue following a single oral dose are similar to the free plasma concentrations, indicating that tedizolid can freely distribute into the tissues. In addition to having similar concentrations over time, the

$T_{1/2}$ values obtained for adipose and muscle tissue were similar to the $T_{1/2}$ in plasma, suggesting that plasma is a good surrogate for tissue concentrations and that these single-dose results would be predictive of multiple dosing. The distribution of tedizolid was found to be similar to that of the currently marketed oxazolidinone linezolid, which shows ratios of $f\text{AUC}_{\text{tissue}}/f\text{AUC}_{\text{plasma}}$ of 0.9 and 1.0 for adipose and muscle tissue, respectively [5]. The bioavailability of tedizolid was recently reported to be 91.7% [22], which is comparable with the reported value for linezolid [23].

One limiting factor of the microdialysis method is that it cannot measure intracellular concentrations of the drug. Yet intracellular colonisation of neutrophils has been associated with recurrent disease [24]. In a study by Lemaire et al. [25], at pH 7.4 intracellular concentrations of tedizolid were ca. 10–15 times those of extracellular concentrations and accumulation was rapid, whereas linezolid concentrations in the cell equilibrated slowly to those measured extracellularly. This could be an explanation as to why tedizolid showed an excellent effect in a clinical study in patients with drug-resistant skin and soft-tissue infections [26] at a dose of 200 mg once daily, yet when examining the pharmacokinetic/pharmacodynamic (PK/PD) index ($f\text{AUC}_{\text{tissue}}$/minimum inhibitory concentraiton) for both linezolid and tedizolid (data not shown) for clinically relevant strains one would not expect this high effect. Indeed, PK/PD studies and clinical studies performed subsequent to the present study support the selection of a 200 mg once-daily therapeutic dose for the treatment of skin infections [19,27]. However, there is no reason to speculate that the results of the present study using a 600 mg single dose cannot be extrapolated to what would be expected at the 200 mg dose selected for therapeutic use.

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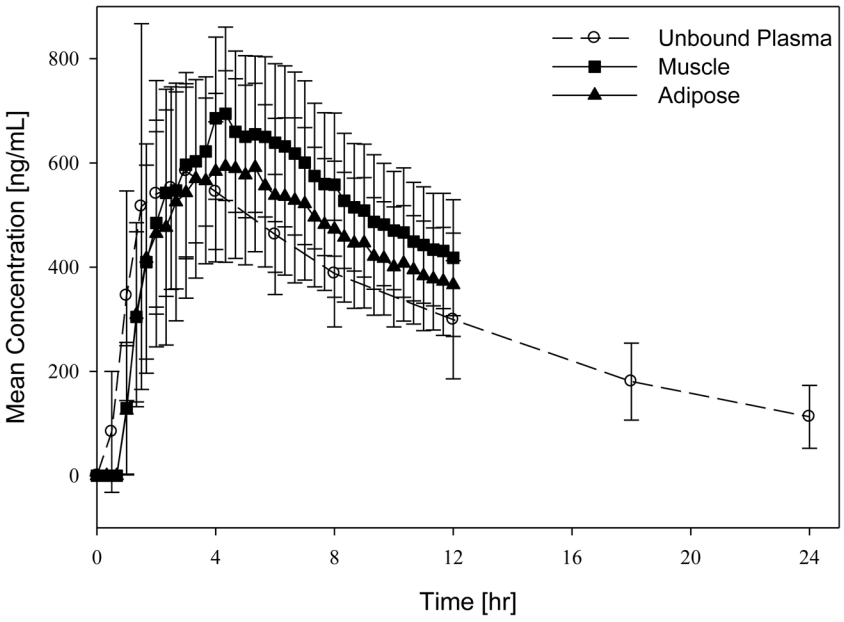

Fig. 1.
Mean concentration–time profiles for free (unbound) plasma and tissue concentrations.

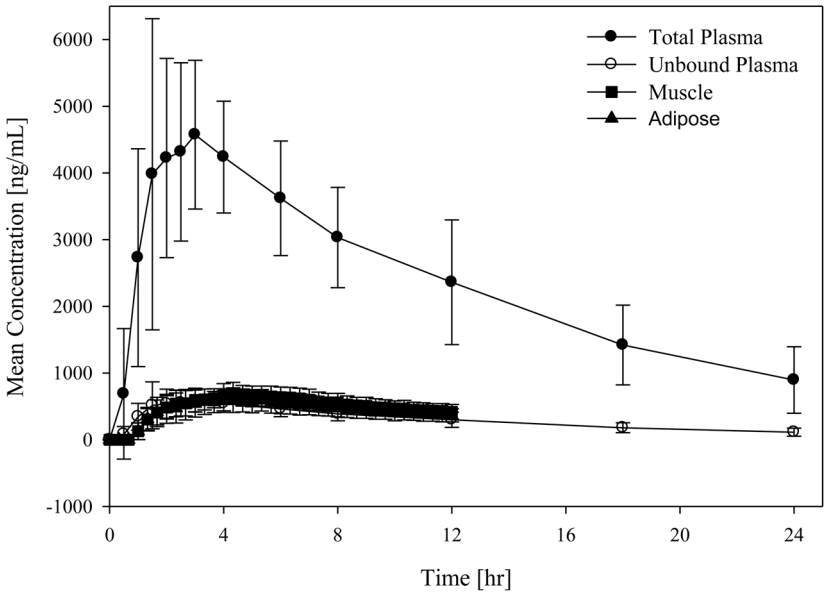

Fig. 2.

Mean concentration–time profiles for total plasma concentrations and free (unbound) plasma and tissue concentrations.

Table 1

Study demographics (main study)

Parameter	
No. of subjects	12
Age (years) [mean (± S.D.)]	24 ± 4
Height (cm) [mean (± S.D.)]	172 ± 11
Weight (kg) [mean (± S.D.)]	72 ± 16
Race/ethnicity [*n* (%)]	
Caucasian	9 (75)
African-American	2 (17)
Hispanic	1 (8)
Sex [*n* (%)]	
Female	7 (58)
Male	5 (42)

S.D., standard deviation.

Table 2

Estimated pharmacokinetic parameters

Parameter	Mean ± standard deviation			
	Total plasma	Free plasma	Adipose tissue	Muscle
C_{max} (mg/L)	5.4 ± 1.5	0.69 ± 0.20	0.66 ± 0.16	0.74 ± 0.15
T_{max}(h)	2.4 ± 1.1	N/D	4.3 ± 2.4	3.7 ± 1.5
$T_{1/2}$ (h)[a]	8.1 (5.9–12.8)	N/D	9.2 (5.9–85.9)	9.6 (6.2–48.2)
$AUC_{0–12h}$ (mg h/L)	38.8 ± 7.5	4.9 ± 1.1	5.3 ± 1.3	5.9 ± 1.1
$AUC_{0–24h}$ (mg h/L)	57.1 ± 14.7	7.3 ± 1.9	N/A	N/A
CL/F (L/hr)	9.5 ± 2.9	N/D	N/D	N/D
V_z/F (L)	113.3 ± 19.3	N/D	N/D	N/D
$f AUC_{tissue}/f AUC_{plasma}$			1.1 ± 0.2	1.2 ± 0.2

C_{max}, maximum concentration; T_{max}, time to C_{max}; $T_{1/2}$, terminal half-life; AUC, area under the concentration–time curve over the specified time interval; CL/F, apparent oral clearance; V_z/F, apparent volume of distribution; $f AUC_{tissue}/f AUC_{plasma}$, ratio of $AUC_{0–12h}$ between specified matrices; N/D, not determined; N/A, not available.

[a] $T_{1/2}$ is shown as median (range).