International Journal of Antimicrobial Agents 49 (2017) 449–455

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: www.elsevier.com/locate/ijantimicag

A need to revisit clinical breakpoints of tigecycline: effect of atypical non-linear plasma protein binding

Ravi Shankar Prasad Singh, Jatinder Kaur Mukker, Stefanie K. Drescher, Amelia N. Deitchman, Hartmut Derendorf *

Department of Pharmaceutics, College of Pharmacy, University of Florida, Gainesville, FL 32610, USA

ARTICLE INFO

Article history:
Received 11 May 2016
Accepted 17 December 2016

Keywords:
Atypical non-linear plasma protein binding model
Mortality risk
Probability of target attainment
Pharmacodynamic index
Antibacterial

ABSTRACT

Tigecycline is highly active against various drug-resistant bacteria. The US Food and Drug Administration (FDA) recently issued a black box warning for tigecycline owing to an associated increase in all-cause mortality. Clinical breakpoints of antibiotics are vital in susceptibility testing of pathogens for the selection of antibiotic therapy; however, no consensus exists between different committees on the clinical breakpoints of tigecycline. Of note, tigecycline exhibits atypical non-linear plasma protein binding (PPB) behaviour, and the pivotal probability of target attainment (PTA) analysis for the determination of clinical breakpoints did not account for the PPB of tigecycline. In this work, the PTA analysis was performed with consideration of atypical non-linear PPB behaviour of tigecycline. A model describing atypical non-linear PPB was developed and validated. Monte Carlo simulations were performed to determine the target ratio of area under the free drug concentration–time curve to minimum inhibitory concentration (*f*AUC/MIC) for *Escherichia coli* and, subsequently, PTA analyses were performed. The target *f*AUC/MIC ratio for *E. coli* was determined as 2.05, whilst the target AUC/MIC ratio was 6.96. The PTA analyses suggest a lower clinical breakpoint of tigecycline against *E. coli*. This finding suggests that there is a need to revisit the current clinical breakpoints of tigecycline.

1. Introduction

Recently, the World Health Organization (WHO) [1,2] and the US Centers for Disease Control and Prevention (CDC) [1,3] have shown concerns regarding an imminent post-antibiotic era, requiring antibiotic stewardship measures including more appropriate use of currently approved antibiotics. Tigecycline, which is currently approved for the treatment of mild to moderately severe complicated intra-abdominal infections (cIAIs), complicated skin and skin-structure infections (cSSSIs) and community-acquired pneumonia, was found in meta-analyses to have higher all-cause mortality generally resulting from worsening infection, complications of infection, or other underlying medical conditions [4–6]. The greatest increased risk was found to be in tigecycline-treated patients with ventilator-associated pneumonia, an off-label use of the drug [4–7].

Despite some concerns raised about the validity and methodology of the meta-analyses [8–10], these results led to the issuing of a black box warning by the US Food and Drug Administration (FDA) [7], making tigecycline an option of last resort. A meta-analysis of randomised controlled trials (RCTs) found a higher risk for mortality with tigecycline treatment compared with other comparator antibiotic treatments (risk difference 0.7%) [11]. Yahav et al also reached a similar conclusion in a meta-analysis of 15 RCTs with 7654 patients [5]. Interestingly, the increased all-cause mortality was not associated with an increase in adverse events, which may lead one to believe that the increased mortality with tigecycline treatment was possibly due to treatment failure [12], likely due to suboptimal therapy.

Agreement on the clinical breakpoints of tigecycline against Enterobacteriaceae does not exist between different breakpoint committees. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) defined the clinical breakpoints for tigecycline against Enterobacteriaceae as ≤1 mg/L and >2 mg/L for susceptible and resistant, respectively [13]. The FDA has set higher breakpoints for tigecycline at ≤2 mg/L for susceptible and >8 mg/L for resistant [14], whilst the Clinical and Laboratory Standards Institute (CLSI) has not defined clinical breakpoints for tigecycline. A Monte Carlo simulation-based probability of target attainment (PTA) analysis [15], with a target area under the concentration–time curve over minimum inhibitory concentration ratio (AUC/MIC) of 6.86 [16], to establish clinical breakpoints did not consider plasma protein binding (PPB) of tigecycline, which shows a counterintuitive atypical non-linear behaviour.

* Corresponding author. Department of Pharmaceutics, College of Pharmacy, University of Florida, P.O. Box 100495, Gainesville, FL 32610-0495, USA. Fax: +1 352 392 3249.
E-mail address: hartmut@cop.ufl.edu (H. Derendorf).

http://dx.doi.org/10.1016/j.ijantimicag.2016.12.008
0924-8579/

Drug protein binding is a saturable process, with bound concentrations increasing proportionally with the increase in unbound concentrations and saturating at higher unbound concentrations. In typical PPB behaviour, the unbound fraction remains constant at lower total concentration but increases at higher concentration, whereas in atypical non-linear PPB behaviour the free fraction decreases as total concentration increases. Tigecycline has been reported to have atypical non-linear PPB behaviour [17–19]. Another drug to show atypical PPB behaviour is eravacycline, which is currently in phase 2/3 of drug development; atypical PPB behaviour has been observed in six different species [20].

Given that only unbound concentrations of antibiotics are responsible for the bacterial killing effect, it has been widely agreed that the ratio of free AUC (*f*AUC) to MIC is a preferred pharmacodynamic (PD) index for efficacy compared with the AUC/MIC ratio. However, the PTA analysis of tigecycline did not account for PPB. Unlike with linear PPB, where conversion of AUC/MIC to *f*AUC/MIC is rather straightforward, conversion of the AUC/MIC ratio into the *f*AUC/MIC ratio of tigecycline is complicated due to atypical non-linear PPB behaviour within the therapeutic range. The current work assessed the effect of atypical non-linear PPB on clinical breakpoints of tigecycline against Enterobacteriaceae.

2. Materials and methods

2.1. Chemicals, reagents and software

Pooled and individual, unfiltered, heparinised human plasma was obtained from Bioreclamation, LLC (Westbury, NY). The pooled plasma was from at least six individuals of each sex. Acetonitrile, methanol, formic acid and sodium chloride were obtained from Fisher Scientific (Pittsburgh, PA), whilst tigecycline was procured from TSZ CHEM (Framingham, MA). Millipore® Centrifree® ultrafiltration cartridges were obtained from EMD Millipore (Billerica, MA). NONMEM was obtained from ICON Development Solutions (Hanover, MD). Perl speaks NONMEM (PsN) and RStudio are free software and were obtained from their respective websites. Pirana was obtained free of charge under a Creative Commons license for academic users. The R 3.0.1 and R-packages Xpose4 and ggplot2 were downloaded from R-CRAN mirror free of charge. Plot Digitizer was downloaded for free from http://plotdigitizer.sourceforge.net.

2.2. In vitro plasma protein binding determination

The PPB of tigecycline in human plasma was determined in triplicate at different concentrations using an ultrafiltration technique. Tigecycline was added to human plasma to obtain desired concentrations and was equilibrated at 37 ± 0.1 °C for 15 min and then filtered through a Millipore® Centrifree® ultrafiltration device by centrifuging at 1000 × *g* for 15 min to collect the filtrate. Filtrates were stored at −80 °C until analysis using a validated liquid chromatography–tandem mass spectrometry (LC-MS/MS) method to determine the unbound concentration in the sample. The bound concentration was calculated as the difference between total and unbound concentration, whilst the unbound fraction (f_u) was calculated as the ratio between the unbound concentration and total concentration.

2.3. Ultrafiltrate sample analysis

Samples were thawed on ice and were analysed using a validated LC-MS/MS method as previously described [18]. In brief, an aliquot of 10 µL of samples was mixed with 10 µL of internal standard. The samples were separated on a Varian Polaris C18-A (4.6 × 50 mm, 5 µm) column with Varian Polaris MetaGuard (Agilent Technologies Inc., Santa Clara, CA) by a gradient method at a flow rate of 0.8 mL/min. The eluate was split into 1:1 before electrospray ionisation. The ion pairs for tigecycline and eravacycline were monitored in positive-ion MRM mode by an API-4000 LC-MS/MS system (AB Applied Biosystems, Foster City, CA). Each run consisted of a calibration curve with eight non-zero standards, a zero standard, a double blank and at least six quality control samples or >5% of samples. At least 66% of the quality control samples were within ±15% of their nominal concentrations for acceptance of the run.

2.4. Plasma protein binding: structural model development

PPB was determined using an ultrafiltration technique in pooled, heparinised, non-filtered, human plasma as described in Section 2.2 at 9 different concentrations, including 0.1, 0.2, 0.4, 0.8, 1.6, 3.1, 6.3, 12.5 and 25 µg/mL. The structural model developed for eravacycline [21] PPB (Eq. 1) was fitted for tigecycline unbound fraction (f_u) versus total concentration using non-linear regression modelling in RStudio 0.98.501. The linearised form of the model (Eq. 2) was also fitted to the observed data. External evaluation of the model was performed by fitting the linearised form of the model (Eq. 2) to the PPB data reported in tigecycline's New Drug Application (NDA) clinical pharmacology review (available on Drugs@FDA) [22] (Supplementary Fig. S1). In addition to the FDA reported data, the unbound fraction (f_u) observed in diabetic patients by Bulik et al [23] was digitised for external validation (Supplementary Fig. S2).

$$f_u = \beta \times C_t^{(-\alpha)} \quad (1)$$

$$\mathrm{Log}(f_u) = \mathrm{Log}(\beta) - \alpha \times \mathrm{Log}(C_t) \quad (2)$$

2.5. Population plasma protein binding model

PPB was determined in unfiltered, heparinised plasma collected from 12 individuals (6 males and 6 females) at three different concentrations including 0.1, 1 and 10 µg/mL as described in Section 2.2. A population model given by Eq. (3) was fitted between f_u (DV) and total concentration using NONMEM 7.2 with PsN and Pirana.

$$f_u = \beta \times C_t^{(-\alpha)} \times (1+\varepsilon)$$

$$\alpha = \theta_1; \beta = \theta_2 \times e^{\eta} \quad (3)$$

where f_u is the unbound fraction, θ_1 and θ_2 are structural parameters of atypical PPB model, η is interindividual variability of β, and ε is residual variability. The parameters of the atypical PPB model were estimated using first-order conditional estimation with interaction (FOCE+I) method. Selection of the final model was based on a significant ($P < 0.05$, χ^2, df = 1) decrease in the objective function value (ΔOFV > 3.84), goodness-of-fit plots, and successful convergence of the model with ≥3 significant digits. The models were evaluated by bootstrap method ($N = 1000$) and visual predictive checks ($N = 1000$) using PsN in Pirana. The model comparisons were made using Xpose4 in RStudio 0.98.501.

2.6. Population pharmacokinetic (PK) model

The published population PK model, derived from phase 2 clinical study with patients who had either cIAI or cSSSI [24], was used for simulation (Eq. 4). The PK model included two compartments with first-order elimination and zero-order input. The weight of the patient (kg) and sex were covariates for the typical value of clearance as shown in Eq. (4).