In Vitro Investigations on the "Atypical" Protein Binding Behaviour of Tigecycline Using Ultrafiltration

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Introduction

Tigecycline is a glycyclcycline antibiotic with activity against a variety of Gram-positive and Gram-negative bacteria including those with resistance to many existing antibiotics.

- Protein binding of tigecycline is 71-89% according to the Summary of Product Characteristics (08Jan2015, Pfizer Ltd), i.e. the unbound fraction (fu) is 10-30%.
- An atypical behaviour of tigecycline, i.e. increasing fu with decreasing plasma concentrations, has been reported using ultrafiltration [1] or microdialysis [2].
- However, a great impact of the experimental conditions on the measured protein binding has been shown for several antibiotics [3].

The aim of the present study was

- to describe the influence of the experimental conditions during ultrafiltration on the protein binding of tigecycline.
- to further characterise the atypical protein binding behaviour of tigecycline.

Results I

The binding of tigecycline in plasma of healthy volunteers

- was much higher at pH > 8 in agreement with the lipophilic nature of the tetracycline albumin interaction [4].
- was similar in plain plasma and in plasma buffered to 8.2.

Fig. 1: Unbound fraction of tigecycline at 0.3 mg/L (mean, SD, n = 8) in plasma buffered with phosphate or HEPES (finally 0.1 M) and in unbuffered plasma, respectively.

The atypical binding behaviour of tigecycline: increasing unbound fraction with decreasing concentration

- was not confined to tigecycline, but also seen with minocycline.
- was attenuated in phosphate buffered plasma.

Fig. 2: Unbound fraction of tigecycline or minocycline (mean, SD) in plasma buffered with HEPES (n = 3) or phosphate (n = 4) to pH 7.4.

HPLC analysis: Tigecycline and minocycline were determined by RP-HPLC with UV detection at 350 nm.

Methods

Ultrafiltration: The standard procedure [3] included ultrafiltration (20 min, 1000xg, 37˚C using Nanosep Omega 10K centrifugal devices) of 300 µL heparin plasma from healthy volunteers buffered with 10 µL 3 M potassium phosphate, pH 7.5. The resulting pH was 7.3 before and 7.5-7.6 in the remaining plasma after ultrafiltration. HEPES 3 M was used alternatively or to achieve pH 8.2. The ultrafiltrate (70-85 µL) was acidified with 50 µL 0.2 M hydrochloric acid containing minocycline 0.5 mg/L as internal standard.

Binding studies: Studies in human serum albumin (HSA, lyophilised powder, essentially fatty acid free, A1887, Sigma-Aldrich) were performed in 70-100 mM potassium phosphate or HEPES buffer made isotonic with sodium chloride. Calcium chloride 2.5 mM and/or EDTA 2.5-3.0 mM were added to investigate the influence of calcium ions and/or of a metal ion chelating agent on the protein binding of tigecycline.

References


Conclusions

- Protein binding of tigecycline strongly increases with pH: maintaining a physiological pH is mandatory to get reliable results.
- The atypical binding behaviour
  - is not unique for tigecycline, but also seen with other tetracyclines such as minocycline.
  - is compatible with a saturable cooperative binding type incorporating polyvalent metal ions.
- EDTA or (to a minor extent) phosphate in high concentrations hide the atypical binding behaviuor.
- Binding results obtained by ultrafiltration or equilibrium dialysis using phosphate buffer are probably biased.
- However, the neutrality of HSA buffer has still to be verified.

Fig. 1: Unbound fraction of tigecycline (mean, SD, n = 6 if not mentioned otherwise) in HSA 4% buffered with isotonic 70 mM HEPES (H) or 70 mM phosphate (P) to pH ca. 7.4, with/without 2.5 mM Ca++ and/or 5.0 mM EDTA.