Abstract

Background: TP-434 is a broad-spectrum fluoroufonyl for treatment of serious hospital infections caused by tetracycline-susceptible and tetracycline-resistant bacteria. Methods: Metabolite profiles after incubation of 10 µM TP-434 with hepatocytes from rats, dogs, monkeys, and humans for 4 hours were determined by mass spectrometry and selective ion monitoring analysis. The potential for TP-434 to inhibit the activity of human hepatic microsomal enzymes was determined using CYP-selective substrates and LC/MS/MS detection. The bloodplasma (B:P) ratio of 0.5, 1.0, and 10 µM TP-434 in pooled human plasma in the presence of EDTA, heparin, and sodium citrate was determined. The apparent passive permeability (Papp; A–B) and potential transport (Bapp; B–A) of 1 µM TP-434 in MDCK cell cultures over-expressing Multi-Medication Resistance 1 gene (MDR1) was determined by adding TP-434 to apical (A) or basolateral (B) sides of the cultures, incubation at 37°C, and measurement of concentrations at 1 & 2 hours by LC/MS/MS. Results: The metabolic stability of TP-434 was high, with 103.2%, 95%, 94.5%, and 85.5% remaining after 4 hours incubation with rat, dog, monkey, and human cells, respectively, suggesting that hepatic metabolism of TP-434 is not significant. (IC50) values against relevant human MCF7 cells expressing different IGF1R values were 285 µM, suggesting that CYP-mediated drug-drug interactions are unlikely. The permeability of TP-434 was determined to be highly permeability through different cell lines, with values ranging from 1.25 to 0.5, which demonstrates that TP-434 has the potential for distribution to red blood cells (RBCs). Permeability in MDCK-MDR1 cultures was low, with mean Papp; A–B of 0.44 x 10(-6) cm/sec. The mean efflux Papp; A–B was 1.57 x 10(-6) cm/sec.

Introduction

The need for new antibacterial agents capable of treating multidrug-resistant gram-positive and gram-negative bacterial infections is well recognized. TP-434 is a novel, synthetic tetracycline indicated for the parenteral treatment of hospital infections, with activity against isolates containing tetracycline-specific resistance and adenosine resistance mechanisms (poster F1-215, F1-215:F1-216). TP-434 is not subject to mechanisms conferring specific resistance to other classes of antibiotics. The pharmacokinetics, safety and tolerability of TP-434 have been evaluated in Phase I single- and multiple-dosing dose trials (A1-G27, A1-S28). Prior to Phase I trials, studies to characterize the disposition of TP-434 and to better understand the potential for this drug to be used in the treatment of human plasma protein binding, the bloodplasma (B:P) ratio, metabolic stability in animal and human hepatocytes, human microsomal CYP inhibition, and permeability and efflux potential.

PLASMA PROTEIN BINDING

The unbound fraction of TP-434 (µM) in human plasma was 11.5 ± 0.4% in human plasma by ultracentrifugation. For the indicated human plasma samples, ultracentrifugation equilibrium dialysis in plasma collected with heparin, concentrations of heparin-dependent protein binding (µM) have been observed by reporting for tetracycline (Muranishih, Alam et al. Antimicrob Agents Chem). Results: TP-434 was determined to be highly penetrant (A–B) and preferentially distributed to red blood cells (B:RBCs). Permeability in MDCK-MDR1 cultures was low, with mean Papp; A–B of 0.44 x 10(-6) cm/sec. The mean efflux Papp; A–B was 1.57 x 10(-6) cm/sec.

PLASMA PROTEIN BINDING AND B:P RATIO

TP-434 was determined to be highly penetrating (A–B) and preferentially distributed to red blood cells (B:RBCs). Permeability in MDCK-MDR1 cultures was low, with mean Papp; A–B of 0.44 x 10(-6) cm/sec. The mean efflux Papp; A–B was 1.57 x 10(-6) cm/sec.

Methods

PLASMA PROTEIN BINDING AND B:P RATIO

TP-434 was determined to be highly penetrating (A–B) and preferentially distributed to red blood cells (B:RBCs). Permeability in MDCK-MDR1 cultures was low, with mean Papp; A–B of 0.44 x 10(-6) cm/sec. The mean efflux Papp; A–B was 1.57 x 10(-6) cm/sec.

Conclusions

• TP-434 exhibits concentration and ciprofloxacin-dependent protein binding in human plasma
• The metabolic stability and lack of CYP inhibition are consistent with low potential for metabolic instability
• TP-434 is not sequestered in RBCs
• TP-434 is a poor substrate for MDR1-mediated transport and has low potential for distribution into brain

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TP-434 is Metabolically Stable and Has Low Potential for Drug-Drug Interactions

Abstract

Background: TP-434 is a broad-spectrum fluoroufonyl for treatment of serious hospital infections caused by tetracycline-susceptible and tetracycline-resistant bacteria.

Methods: Metabolite profiles after incubation of 10 µM TP-434 with hepatocytes from rats, dogs, monkeys, and humans for 4 hours were determined by mass spectrometry and selective ion monitoring analysis. The potential for TP-434 to inhibit the activity of human hepatic microsomal enzymes was determined using CYP-selective substrates and LC/MS/MS detection. The bloodplasma (B:P) ratio of 0.5, 1.0, and 10 µM TP-434 in pooled human plasma in the presence of EDTA, heparin, and sodium citrate was determined. The apparent passive permeability (Papp; A–B) and potential transport (Bapp; B–A) of 1 µM TP-434 in MDCK cell cultures over-expressing Multi-Medication Resistance 1 gene (MDR1) was determined by adding TP-434 to apical (A) or basolateral (B) sides of the cultures, incubation at 37°C, and measurement of concentrations at 1 & 2 hours by LC/MS/MS.

Results: The metabolic stability of TP-434 was high, with 103.2%, 95%, 94.5%, and 85.5% remaining after 4 hours incubation with rat, dog, monkey, and human cells, respectively, suggesting that hepatic metabolism of TP-434 is not significant. (IC50) values against relevant human MCF7 cells expressing different IGF1R values were 285 µM, suggesting that CYP-mediated drug-drug interactions are unlikely. The permeability of TP-434 was determined to be highly permeability through different cell lines, with values ranging from 1.25 to 0.5, which demonstrates that TP-434 has the potential for distribution to red blood cells (RBCs). Permeability in MDCK-MDR1 cultures was low, with mean Papp; A–B of 0.44 x 10(-6) cm/sec. The mean efflux Papp; A–B was 1.57 x 10(-6) cm/sec.

Conclusions

• TP-434 exhibits concentration and ciprofloxacin-dependent protein binding in human plasma
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