INTRODUCTION

(E,2)-3-(4-hydroxy-3,5-dimethoxybenzylidene) indolin-2-one (indolinone) from wood (Isatis tinctoria L., Brassicaceae) was previously identified as a compound possessing histamine release inhibitory and anti-inflammatory properties.

AIMS OF THE STUDY

To further evaluate the potential of indolinone, a preliminary bioavailability study was carried out in male Sprague Dawley rats (2 mg/kg bw i.v.) with blood sampling up to 12 h after injection. Moreover, we analyzed the ability of indolinone to cross the blood-brain barrier (BBB). For this purpose, we screened indolinone (5 µM) in several in vitro cell-based human and animal BBB models. P-glycoprotein (P-gp) interaction for indolinone was studied with the aid of a calcein uptake assay, and by calculation of the efflux ratio (ER) from the bidirectional permeability assays.

METHODS

UPLC-MS/MS assay for indolinone

Both LC-MS/MS/analysis methods for indolinone in lithium heparinized rat plasma and Ringer HEPES buffer were validated according to current regulatory guidelines.

Extraction: Protein precipitation

UPLC: Waters ACQUITY UPLC®

Column: Waters ACQUITY TQD operating in MRM mode. ESI positive ion mode. MRM transitions for indolinone: 297.7→265.0 and 221.8→194.0

In vivo animal study

A preliminary bioavailability study was carried out in male Sprague Dawley rats (2 mg/kg bw i.v.), with blood sampling at 0, 5, 10, 20, 30 min, 1, 2, 4, 6, 8 and 12h after injection.

In vitro BBB models

Human BBB model

Imortalized monculture human BBB model

Apical (blood) (A) Basolateral (brain) (B)

Animal BBB models

Primary co-culture bovine and rat BBB model

Apical (blood) (A) Basolateral (brain) (B)

Primary triple culture rat BBB model

Apical (blood) (A) Basolateral (brain) (B)

Calcein uptake assay

The calcein accumulation assay was performed in primary porcine brain capillary endothelial cells seeded in 96-well plates. Indolinone was tested at 5, 50, and 500 µM. Verapamil (50 µM, P-gp inhibitor) was used as positive control.

RESULTS

LC-MS/MS assay for indolinone

Typical LC-MS/MS profile of E/Z-indolinone (top) and I.S. (bottom) (at 1000 ng/mL). The calibration curve of indolinone was in the range between 30.0 and 3000 ng/mL.

Indolinone permeability studies

Human BBB model

Transport direction Δt (min) Recovery (%) Papp (x10⁻⁹ cm/s) Efflux ratio

A → B 60 88.9 75.2 1.13
B → A 60 90.1

Bovine/ rat BBB model

Transport direction Δt (min) Recovery (%) Papp (x10⁻⁹ cm/s) Efflux ratio

A → B 60 73.6 56.2 0.61
B → A 60 70.6 34.6

Rat BBB model

Transport direction Δt (min) Recovery (%) Papp (x10⁻⁹ cm/s) Efflux ratio

A → B 60 73.0
B → A 60 70.6

For integrity control of the cell monolayers, Papp (C0 = 3.2 × 10⁻⁹ cm/s) of sodium fluorescein (fluorescent integrity marker) was assessed in parallel to indolinone.

Calcein uptake assay

The calcein accumulation assay showed that indolinone does not inhibit P-gp.

The validated LC-MS/MS assays will be used for further PK studies addressing oral bioavailability (in vitro caco-2 assay in vivo experiments in SD rats).

CONCLUSIONS

These validation studies demonstrated that the quantitative assays for indolinone in both matrices are specific, selective, precise, accurate, and able to produce reliable results.

The low t½ and the high clearance demonstrate the need of rat urine collection for next PK studies.

The data obtained with the human and animal BBB models showed good correlation and are indicative of a high BBB permeation potential of indolinone.

The efflux ratio below 2 indicates that indolinone is not subject to active mediated transport mechanism.

The calcein assay showed that indolinone does not inhibit P-gp.

REFERENCES


Eiseleman CE et al., J. Fluids Barriers 2013:1033-1033.


Nagasaki S et al., Nahrung 2006; 50: 253 – 263.