

Investigation of SEP-227900 metabolites in Humans from the First-in-Man Study Samples by LC-MS and NMR

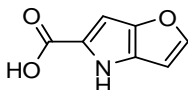
Yu-Luan Chen^a, Estela Skende^a, Gary Maier^a, Li-Quan Wang^b, Zhe-ming Gu^b
^aClinical Pharmacology, Sunovion Pharmaceuticals Inc. ^bXenobiotic Laboratory

Overview

- Four metabolites were identified: SEP-227900 glycine and glucuronide conjugates, and a mono-oxidative metabolite and its glycine conjugate.
- The glycine conjugate of SEP-227900 was found to be the most abundant metabolite in human urine that was ~3-fold as the glucuronide level. All together it gives the 900 urine excretion of > 80% of the dosed amount.
- The glucuronide metabolite was identified as acyl glucuronide based on the migration phenomenon, the methylation data, and NMR analysis.
- The oxidative-metabolite has significantly longer half-life than the parent drug and its conjugated metabolites.

Introduction

SEP-227900 (structure shown below) is an D-amino-acid oxidase (DAO) inhibitor. In the previous *in vitro* and animal *in vivo* DMPK studies, a glucuronide metabolite was found to be a major metabolite for SEP-227900. Little other metabolites were identified. The position of glucuronidation in SEP-227900 remains unknown. According to FDA guidance regarding "Safety Testing of Drug Metabolites" (MIST), it was strongly recommend *in vivo* metabolic evaluation in humans be performed as early as feasible. The FIM study is the earliest opportunity to explore human *in vivo* metabolism and obtain preliminary human metabolite profiling and identification information. In this study, plasma and urine samples from 80-mg cohort were utilized to search metabolites of SEP-227900 in humans. LC-MS/UV were used for metabolite profiling. Accurate mass measurement by Orbitrap MS was employed to identify the structure of each metabolite. The structure of the SEP-227900-glucuronide metabolite was characterized by accurate mass measurement, migration experiment, NMR analysis, and methylation coupled with MS measurement.



Clinical Samples

- Study 900-001: 80-mg cohort; urine and plasma**
 - Number of subject: 9
 - Urine samples: pre-dose, 0-2 h, 2-4 h, 4-6 h, 6-12 h, 12-24 h
 - Plasma samples: 0.25 h, 0.50 h, 0.75 h, 1 h, 3 h, 5 h, 12 h

Sample Pooling

- Urine samples were pooled across 9 subjects by equal volume to get pre-dose urine and 0-24 h urine
- Plasma samples were pooled by equal volume across 9 subjects to get 0-12 h plasma for metabolite searching.
- Plasma samples were pooled by time points across 9 subjects to get 0.5-, 5-, and 12-h plasma for semi-quantitation.

Methods

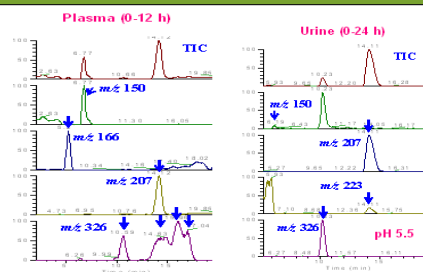
- Sample preparation:** The pooled plasma was deproteinized by acetonitrile then supernatant was dried down and reconstituted and injected into LC-MS for analysis. The pooled urine sample was centrifuged before injected into LC-MS for analysis.
- LC-MS/UV:** Predictable metabolite searching, and semi-quantitation
- Accurate mass measurement:** Metabolite identification
- Structural characterization of SEP-227900 glucuronide:**
 - Migration phenomena
 - Methylation by CH₂N₂
 - NMR

Results and Discussion

Accurate mass measurement by Orbitrap-MS

Metabolite Code	Proposed Structure	(M-H) ⁻ Theoretical	(M-H) ⁻ Orbitrap MS (4.8 ppm)	R _t (min)	Sources
SEP-227900 (m.w. 151)		150.01837	150.01929 (4.8 ppm)	6.5	Plasma Urine
M166/1 (m.w. 167)		166.01348	166.01419 (4.25 ppm)	5.4	Plasma
M207/1 (m.w. 203)		207.04003	207.04009 (0.29 ppm)	14.2	Plasma Urine
M223/1 (m.w. 224)		223.03495	223.03529 (1.5 ppm)	14.2	Urine
M326/1 (m.w. 327)		326.05066	326.04987 (-2.4 ppm)	10.5	Plasma Urine

Metabolites detected in human plasma and urine



Semi-quantitation of SEP-227900 metabolites in plasma

Time (h)	MS peak area		
	0.5	5	12
SEP-227900	6659686	145672	0
M166/1	181614	80297	137848
M207/1	11012733	146233	0
M326/1	2451490	101639	0

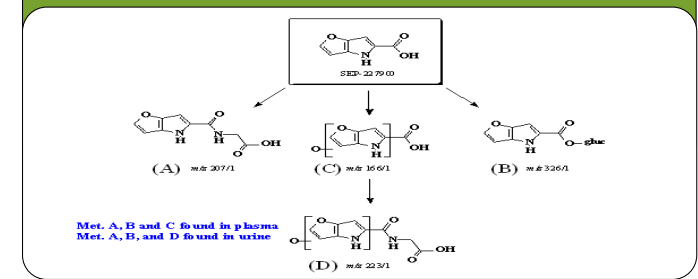
Characterization of SEP-227900 glucuronide

* The urine sample (pH 5.5) had little migration, but the migration was observed when 0.1% NH₄OH was added to the urine sample.

* This confirms only one free -COOH group in molecule.

* The Hc shift is indicative of the acyl glucuronidation.

Proposed metabolic pathways in humans



Summary

Four metabolites of SEP-227900 were identified in humans from the 80-mg cohort plasma and urine samples: SEP-227900 glycine and glucuronide conjugates, mono-oxidative metabolite and its glycine conjugate. SEP-227900-glucuronide was an acyl glucuronide, rather than an N-glucuronide. Greater than 80% of the dosed compound was excreted in urine.

