CNS Neuroscience & Therapeutics

ORIGINAL ARTICLE

CNS Neuroscience & Therapeutics

Exploratory Biomarker Study of the Triple Reuptake Inhibitor SEP-432 Compared to the Dual Reuptake Inhibitor Duloxetine in Healthy Normal Subjects

John J. Sramek,[1] Larry W. Hardy,[2] Peter Bieck,[1] Cynthia Zamora,[3] Mark Versavel,[2] Jahnavi Kharidia,[2] Todd Grinnell,[2] Yu-Luan Chen,[2] Michael Sullivan,[3] Hong Ding[1] & Neal R. Cutler[1]

1 Worldwide Clinical Trials, Beverly Hills, CA, USA
2 Sunovion Pharmaceuticals, Marlborough, MA, USA
3 Worldwide Clinical Trials, Drug Development Solutions, San Antonio and Austin, TX, USA

Keywords
Biomarkers; Cerebrospinal fluid; Duloxetine; Monoamine reuptake inhibitor; SEP-432.

Correspondence
Dr. Neal R. Cutler, Worldwide Clinical Trials, 401 N Maple Dr., Beverly Hills, CA 90210, USA.
Tel.: +1-310-7869100;
Fax: +1-310-7869101;
E-mail: neal.cutler@wwctrials.com
Received 15 October 2015; revision 10 December 2015; accepted 11 December 2015

doi: 10.1111/cns.12513

SUMMARY

Introduction: SEP-432 is a triple monoamine reuptake inhibitor of norepinephrine (NE), serotonin (5-HT), and dopamine (DA), based on *in vitro* binding studies. We sought evidence that SEP-432 engages these monoamine systems by measuring concentrations of monoamines and/or their main metabolites in cerebrospinal fluid (CSF) and plasma and comparing results to duloxetine, a dual reuptake inhibitor of NE and 5-HT. **Methods:** Eighteen healthy normal subjects received either SEP-432 (300 mg/day), duloxetine (60 mg/day), or placebo for 14 days in-clinic (double blind) with CSF and plasma collections at baseline (single lumbar puncture) and Day 14 (24-h CSF and plasma collection). Concentrations of monoamines and their metabolites, as well as pharmacokinetic concentrations of SEP-432 and metabolite, were quantified by liquid chromatography–tandem mass spectrometry. **Results:** Compared to placebo in the Day 14 area under the curve 24-h ($AUC_{0-24\ h}$) analysis, SEP-432 significantly ($P < 0.05$) decreased the NE metabolite dihydroxyphenylglycol (DHPG) in CSF and plasma, decreased 5-HT in plasma, and did not affect DA metabolites, while duloxetine had significant effects on DHPG and 5-HT. Time-matched baseline to Day 14 biomarker comparisons confirmed these findings. **Conclusion:** CSF monoamine biomarkers confirmed central NET activity for SEP-432 and duloxetine's dual reuptake inhibition.

Introduction

Monoamine biomarkers have been used to elucidate the mechanism of action of antidepressants [1,2]. Previous patient studies have documented drug-induced effects on CSF concentrations of 5-hydroxyindoleacetic acid (5-HIAA, the primary metabolite of serotonin, 5-HT), dihydroxyphenylglycol and 3-methoxy-4-hydroxyphenylglycol (respectively, DHPG and MHPG—norepinephrine [NE] metabolites), and homovanillic acid (HVA, metabolite of dopamine [DA]) [3–8]. However, with a few exceptions, measurement of CSF monoamines has generally not been applied to early drug development [9].

The drug candidate SEP-432 ((1s,4s)-4-(3,4-dichlorophenyl)-4-((dimethylamino)methyl)-1-methylcyclohexanol) has been shown *in vitro* to be an unbalanced triple reuptake inhibitor [10] and was under development for the potential indications of depression and neuropathic pain. SEP-432 is extensively metabolized to SEP-431 by demethylation. The metabolite is also a reuptake inhibitor *in vitro*, although less potent than the parent compound. Both SEP-432 and its metabolite exert their greatest potency effect on NE reuptake, followed by reduced effects on 5-HT and DA [10]. Increasing activity of all three biogenic amine neurotransmitters simultaneously through monoamine transporter inhibition could provide advantages in efficacy and speed of onset over currently marketed antidepressants. Single and multiple dose studies were initially conducted to determine the safety and tolerability of SEP-432. A maximally tolerated dose (MTD) of 300 mg/day was previously defined in healthy subjects.

This study was carried out to characterize the monoamine profile in CSF and plasma of SEP-432 compared to those effects following duloxetine administration. Duloxetine was selected as the comparator due to its specificity of action as a norepinephrine and serotonin reuptake inhibitor [11]. It was hypothesized that SEP-432 would demonstrate biomarker changes consistent with NE transporter (NET) and 5-HT transporter inhibition similar to duloxetine, as well as DA transporter inhibition as evidenced by modulation of DA metabolites.

Materials and Methods

The study was approved by the institutional review board Integ-Review in Austin, Texas. Each study subject signed an IRB-approved informed consent form prior to study participation.

Study Design

Up to 30 healthy subjects could be enrolled to receive SEP-432, duloxetine, or placebo (QD in the morning) for 14 days in an inpatient research unit, with the goal of completing at least seven subjects on each of the active treatments and 4 subjects on placebo. Treatment was given for 14 days to allow sufficient time to attain steady state in the central nervous system. The study was conducted on a double-blind basis to subjects and clinical staff; the pharmacist who prepared study drug was unblinded. The dosing schedule, which employed a 3-day initial titration for both active compounds, was as follows:

1. SEP-432 was dosed at 40 mg QD for 3 days, then at 300 mg QD for 11 days
2. Duloxetine was dosed at 30 mg QD for 3 days, then 60 mg QD for 11 days
3. Placebo was dosed QD for 14 days

Subjects underwent daily safety and routine psychiatric scale evaluations at set times during the inpatient period.

Plasma biomarkers were collected in the supine position, predose, on days 1 and 14, and NE and DHPG were collected in the standing positions predose on days 1, 7, and 13 to coincide with postural testing [12]. Postural testing (subjects standing still, as motionless as possible, for 10 min after being supine for 5 min) was carried out predose on days 1, 7, and 13. On Day 14, plasma biomarkers were collected in the supine position only. Blood samples for SEP-432 and SEP-431 pharmacokinetic (PK) analysis were drawn predose and 0.25, 0.5, 0.75, 2, 3, 4, 6, 8, 10, 12, 16, 18, 20, and 24 h postdose on Day 14. Blood samples were collected from all subjects, but only the samples from subjects dosed with SEP-432 were analyzed for SEP-432 and SEP-431 PK as duloxetine PK is well known [13]. The clinic sent all PK samples to an independent laboratory, which unblinded samples prior to the analysis. On Day 0 and Day 14, lumbar puncture was performed and an indwelling catheter was inserted for repeated CSF sampling on Day 14 predose and 2, 4, 6, 8, 10, 12, 16, 18, 20, and 24 h postdose for analysis of CSF PK and CSF biomarkers.

Subjects were discharged from the clinical research unit after completing a 3-day observation period and safety evaluations. Subjects later returned to the clinic for three outpatient safety assessments.

Inclusion and Exclusion Criteria for Subjects

This study was conducted in healthy male and nonpregnant, nonnursing female subjects between 21 and 50 years old. Subjects were screened by medical history, physical examination, electrocardiogram (ECG), routine laboratory tests, and urine drug screen and could not have any clinically relevant medical conditions, psychiatric disorder (including assessment with the C-SSRS; retrieved from http://www.cssrs.columbia.edu/), or blood donation. They were screened to ensure no history of substance abuse in the past 12 months, and no recent use of prescription or nonprescription drugs, vitamins, dietary, or herbal supplements. Subjects were also screened to ensure that they had no conditions or surgeries that would complicate lumbar puncture; this included an X-ray of the lumbar spine to rule out any anatomical abnormalities, and a careful history for any recent febrile illnesses/inflammations near the lumbar puncture site, or a history of headaches. Subjects were required to refrain from taking alcohol, acetaminophen, and caffeine within 48 h prior to in-clinic admission and to refrain from smoking 30 days prior to signing the informed consent and for the entire duration of participation. Subjects refrained from exercise from the time of in-clinic admission until the end of the study. Subjects fasted from 8:00 pm the night before laboratory sample collections were planned.

Study Drug Materials

The study drug was a solid formulation of SEP-432 (maleate salt) blended with excipients and filled into a Swedish orange capsule. Placebo was a matching capsule. Duloxetine was sourced as commercial Cymbalta® 30 mg oral capsules and was over-encapsulated into a dark brown capsule. Each subject received the same number of orange and brown capsules.

Plasma and CSF Sample Collections

Plasma Monoamine Sample Collection

For each time point, two K2-EDTA Vacutainer tubes (6 and 10 mL) were used to draw whole blood via venipuncture. The whole blood tubes were kept on wet ice for no more than 30 min before they were centrifuged at 1100 g for 10 min at 4°C to produce platelet-poor plasma. The resulting plasma from the 6-mL tube was removed and stored in a polypropylene cryovial at −70°C. For each 1 mL of whole blood collected, about 0.4 mL of plasma was extracted. The resulting plasma from the 10 mL tube was used for the acid stabilized samples. Four milliliters of plasma was removed and added to a polypropylene cryovial containing 0.2 mL of 2.4N hydrochloric acid. The sample was capped, mixed briefly, and stored at −70°C.

CSF Sample Collection

CSF sampling was performed for PK and biomarkers on Day 0 and Day 14. All subjects underwent PK draws, but only SEP-432 and its metabolite concentrations were analyzed. Tygon tubing was used for CSF collection. The total CSF volume collected for each sample for the biomarker assays was 6 mL (2 mL of untreated, 4 mL of stabilized CSF). A single lumbar puncture in the L-3 or L-4 space was performed in the supine position predose on Day 0 after 8 h of IV fluids for the baseline CSF PK and CSF biomarkers. The IV fluids were discontinued 12 h after completion of CSF collection procedures. On Day 14 in the morning, a CSF catheter was placed in the L-3 or L-4 space after 8 h of IV fluids overnight, and CSF sampling was conducted in the supine position at predose and at 2, 4, 6, 8, 10, 12, 16, 18, 20, and 24 h postdose for PK and

biomarker samples. The first 1 mL of CSF fluid was sent to the laboratory for immediate analysis of protein, glucose, and cell count with differential. For each time point, a 2 mL untreated CSF sample was collected into a polypropylene cryovial kept on wet ice. This sample was capped and placed at −70°C within 30 min of collection. A second 4 mL CSF sample was collected into a polypropylene cryovial containing 0.2 mL of 2.4N hydrochloric acid kept on wet ice. This sample was capped, mixed briefly, and placed at −70°C within 30 min of collection.

Extraction and Analysis of Monoamines and Monoamine Metabolites

Quantitative analyses employed 10 validated liquid chromatography–tandem mass spectrometry (LC-MS-MS) methods: five distinct methods were used for plasma and CSF biomarker extraction, including: one method for NE and DHPG extraction [14–16]; one method for 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) extraction [15–17]; one method for 5-HIAA extraction [15–17]; and one method for 5-HT extraction [15,16].

Qualifying quality control (QC) samples from high, medium, and low pools were processed along with each study sample run. Samples were extracted and injected onto a Sciex API 5000 LC-MS-MS equipped with an HPLC column. For the analysis, the peak areas of each analyte were compared to their corresponding stable labeled internal standards to provide a normalized instrument response. The concentrations of the calibration standards were compared with their peak responses and fitted by a weighted linear least-squares regression analysis. Only data with accuracy between 85% and 115% of theoretical concentrations for internal standards and QC were used. Monoamine biomarkers were analyzed within several weeks of their collection, except in a few instances when repeats were necessary, and were well within validated storage stability timeframes (ranging from 15.6 weeks for 5-HIAA in CSF to 164.6 weeks for DOPAC and HVA in CSF).

LC-MS/MS Method for Measurement of SEP-432 and SEP-431 in Human Plasma and CSF

An LC-MS/MS method (API 4000; SCIEX, AB Sciex LLC, Framingham, MA, USA) for simultaneously determining SEP-432 and SEP-431 concentrations in lithium heparin-anticoagulated human plasma was developed and validated for a range of 0.05–50 ng/mL. The peak area ratio of the compound to the corresponding internal standard was used in calculating analyte concentrations in plasma using a concentration-inverted linear least-squares regression algorithm. Analyst software 1.4.2 (AB Sciex LLC, Framingham, MA, USA)was used for data acquisition, peak integration, and concentration calculation. Human CSF samples were measured using a validated curve range of 0.05–25 ng/mL by LC-MS/MS (API 5000; Sciex) with plasma as surrogate matrix.

Statistical Analysis

For continuous measures, statistical summaries for absolute value and change from baseline at each time point included the number of subjects, mean, and standard deviation. Change from baseline values (including plasma NE and DHPG in the postural test) and changes in $AUC_{0-24\ h}$ (ten values per subject on Day 14) biomarker values (comparing active groups to placebo) were calculated as follow-up value minus baseline value and analyzed by the Wilcoxon signed rank test (two tailed with significance at $P < 0.05$ using the GraphPad Prism version 6.0f program for Macintosh, GraphPad Software, La Jolla, CA, USA, www.graphpad.com). The Day 14 CSF and plasma $AUC_{0-24\ h}$ analyses were conducted for subjects who had complete data collections, while the Day 1–14 comparisons utilized all available data, including from subjects who ended the study early. Hypothesis tests and associated *P*-values are considered exploratory and were not adjusted for multiplicity.

The plasma and CSF PK parameters for SEP-432 were estimated using a noncompartmental method with WinNonlin software, version 5.2, Pharsight Corporation (Princeton, NJ, USA).

Results

Twenty-six (26) healthy normal subjects (19 males, 7 females; mean age 36 years) were randomized in the study. Sixteen (61.5%) of the 26 subjects were Caucasian, eight (30.8%) were African American, and 2 (7.7%) were American Indian. The mean age was 31.3 years (range 21–50 years). Mean BMI was 26 kg/m^2 (range 19–31 kg/m^2).

Eighteen subjects randomized to 300 mg SEP-432 (7 subjects), 60 mg duloxetine (7 subjects), or placebo (4 subjects), completed the study through Day 14 collections. Of the 26 randomized subjects, 8 (34.6%) subjects discontinued from the study due to the following reasons: vomiting (1), lack of CSF flow on Day 14 (4), voluntary withdrawal from study (1), and other reasons not related to study drug (2).

Monoamine Biomarkers

The end of treatment (Day 14) CSF and plasma biomarker concentrations obtained during the 24-h period were analyzed, and the area under curve values ($AUC_{0-24\ h}$) were calculated. Values for SEP-432 were compared to those for placebo and for duloxetine. As seen in Table 1, the concentrations of DHPG and of 5-HIAA were 2- to 4-fold higher in the CSF than in plasma ($P < 0.05$ & $P < 0.01$, respectively). In the case of DOPAC, the results showed the opposite effect.

In the CSF, both SEP-432 and duloxetine significantly increased NE and decreased DHPG compared to placebo, with SEP-432 having a greater effect than duloxetine. SEP-432 decreased DHPG 39% compared to placebo, while duloxetine decreased DHPG 28% compared to placebo. The maximal decrease in DHPG for SEP-432 occurred at approximately 6 h postdose on Day 14 in CSF (Figure 1). Only duloxetine significantly increased 5-HT concentrations in CSF, with maximal increase in 5-HT concentrations occurring 4 h postdose (Figure 2). Compared to placebo values, mean plasma DHPG and 5-HT decreased significantly on Day 14 for subjects treated with SEP-432 (300 mg/day) or duloxetine (60 mg/day). DOPAC and HVA were unaffected in CSF and plasma.

Comparison of the single CSF time points drawn at the same time on Day 1 (predose) and Day 14 (just prior to the last dose)

Table 1 Day 14 CSF and plasma catecholamine exposure ($AUC_{0–24\ h}$) for DHPG, NE, 5-HIAA, 5-HT, DOPAC, and HVA after steady-state dosing of placebo, SEP-432 (300 mg), or Duloxetine (60 mg)

		CSF		Plasma	
		DHPG $AUC_{0–24}$ (ng × h/mL)	NE $AUC_{0–24}$ (ng × h/mL)	DHPG $AUC_{0–24}$ (ng × h/mL)	NE $AUC_{0–24}$ (ng × h/L)
Duloxetine (60 mg)	Mean	30.9*	2.92*	16.8*	3.74^{ns}
N = 7	SD	3.48	0.93	2.9	1.46
Placebo N = 4	Mean	42.7	1.75	20.9	3.88
	SD	11.3	0.36	2.4	1.61
SEP-432 (300 mg)	Mean	26.0*	3.81**	14.8**	4.37^{ns}
N = 7	SD	6.8	1.54	3.2	1.42
		CSF		Plasma	
		5-HIAA $AUC_{0–24}$ (pg × h/mL)	5-HT $AUC_{0–24}$ (pg × h/mL)	5-HIAA $AUC_{0–24}$ (pg × h/mL)	5-HT $AUC_{0–24}$ (pg × h/mL)
Duloxetine (60 mg)	Mean	386^{ns}	1495**	117^{ns}	87**
N = 7	SD	82	534	28	58
Placebo N = 4	Mean	457	302	126	930
	SD	95	141	8.2	502
SEP-432 (300 mg)	Mean	472^{ns}	436^{ns}	102^{ns}	224*
N = 7	SD	173	153	19	107
		CSF		Plasma	
		DOPAC $AUC_{0–24}$ (pg × h/mL)	HVA $AUC_{0–24}$ (μg × h/mL)	DOPAC $AUC_{0–24}$ (pg × h/mL)	HVA $AUC_{0–24}$ (ng × h/mL)
Duloxetine (60 mg)	Mean	15^{ns}	1.42^{ns}	35^{ns}	190^{ns}
N = 7	SD	3	0.34	9	43
Placebo N = 4	Mean	13	1.15	35	187
	SD	3	0.35	4.3	52
SEP-432 (300 mg)	Mean	15^{ns}	1.14^{ns}	34^{ns}	203^{ns}
N = 7	SD	5.3	0.57	6	72

*$P < 0.05$; **$P < 0.01$; ns, not significant.

also showed a significant decrease in DHPG for both SEP-432 and duloxetine, and an increase in 5-HT for duloxetine (see Table 2). Duloxetine also significantly increased CSF DOPAC concentrations between days 1 and 14. The placebo group showed no significant changes in any CSF biomarkers between days 1 and 14. In plasma, SEP-432 significantly decreased DHPG, 5-HT, 5-HIAA, and HVA between days 1 and 14. Duloxetine significantly decreased plasma DHPG, 5-HT, and HVA between days 1 and 14. Placebo decreased HVA between days 1 and 14 (see Table 3).

A significant decrease in the standing plasma DHPG/NE ratio was seen for SEP-432 on days 7 and 13 compared to baseline (Day 1), but only a trend ($P = 0.078$) for duloxetine was seen on Day 13. However, standing DHPG alone showed a significant decrease on both days for SEP-432 and duloxetine.

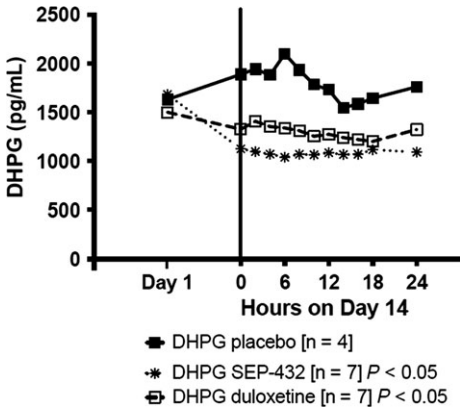

Figure 1 Average Values of Hourly DHPG Concentration in CSF, Comparing Day 1 (Predose Baseline) to Day 14 (the 0–24 Hour Time Points) after Treatment with Placebo, Duloxetine (60 mg), or SEP-432 (300 mg).

Pharmacokinetics

Time courses of the mean plasma and CSF SEP-432 concentration over 24 h on Day 14 are presented in Figure 3. These data were used to derive values for the pharmacokinetic parameters for SEP-432 and SEP-431. As seen in Figure 3, maximum concentration (C_{max}) for SEP-432 in CSF is much lower (approximately 10-fold) than C_{max} in plasma. The CSF exposure of SEP-432 was 22% that of plasma, while SEP-431 was 46% of

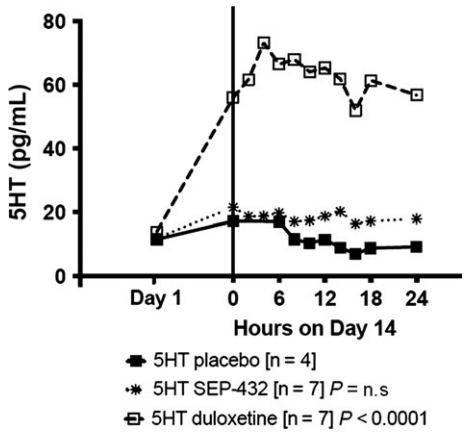

Figure 2 Average Values of Hourly 5-HT Concentrations in CSF, Comparing Day 1 (Predose Baseline) to Day 14 (the 0–24 Hour Time Points) after Treatment with Duloxetine (60 mg), SEP 432 (300 mg), and Placebo (n.s., nonsignificant).

plasma (ratio of CSF/plasma AUC_{0-24}). In CSF, the median SEP-432 T_{max} at the 300 mg dose was 4.0 (2.0–6.0) h and 6 (4.0–8.0) h for the metabolite SEP-431. In CSF, the apparent $t_{1/2}$ was approximately 18 h for SEP-432 and 32 h for SEP-431. These values represent an increase over the previously determined plasma $t_{1/2}$ for both the parent and metabolite (15.5 and 21.5 h, respectively).

Safety and Tolerability

There were no serious AEs and no severe AEs that were considered to be related to study drugs. Overall, no trends or clinically relevant changes were observed in hematology or serum chemistry parameters, and there were no abnormal ECG findings. There were also no clinically relevant changes in safety tests and psychiatric ratings. The most common moderate AEs in the SEP-432 group included headache (50%), nausea (30%), and decreased appetite (30%). In the duloxetine group, the most common moderate AEs were nausea, headache, and vomiting (all at 44% incidence). In the placebo group, the most common moderate AE was headache (57%).

Orthostatic hypotension (a decrease of ≥20 mmHg in SBP or ≥10 mmHg in DBP after the subject had been standing for at least 2–4 min compared to the respective values measured in the supine position) was exhibited by 5 of 10 (50%) SEP-432 subjects, 1 of 9 (11.1%) duloxetine subjects, and 3 of 7 (42.9%) placebo subjects, but none were clinically significant. Mean heart rate, comparing values on Day 13 minus Day 1, using all subjects in the safety data analysis, increased by 22.4 (SD 13.2) beats per minute (bpm) in SEP-432-treated subjects (n = 9), versus an increase of 2.4 (SD 7.2) bpm in duloxetine-treated subjects (n = 9). Mean heart rate decreased by 1.1 (SD 10.1) bpm in the placebo group (n = 7). Following completion of the 24-h CSF collection, four subjects developed headaches, which were judged to be related to the sampling procedure.

Discussion

The main goals of this study were to provide evidence of central engagement of monoamine transporters by measuring monoamine biomarkers in CSF, to evaluate the effect on NET by measuring plasma concentrations of DHPG and NE during postural testing, and to characterize the PK parameters of SEP-432 in CSF relative to plasma at steady state. In CSF, both SEP-432 and

Table 2 Comparison of day 1 versus day 14 CSF biomarkers

CSF Data								
		Day 1			Day 14			
Treatment	Biomarker	Mean	SD	N	Mean	SD	n	Statistical result
Duloxetine	NE (pg/mL)	117	57	9	143	62	7	ns
Placebo	NE	109	52	7	80	35	4	ns
SEP-432	NE	101	65	9	147	70	7	ns
Duloxetine	DHPG (ng/mL)	1.57	0.25	9	1.32	0.17	7	*
Placebo	DHPG	1.50	0.36	7	1.97	0.61	4	ns
SEP-432	DHPG	1.79	0.67	9	1.15	0.34	7	*
Duloxetine	5-HT (pg/mL)	12.8	7.2	9	55.9	17.6	7	*
Placebo	5-HT	14.1	7.2	7	11.1	6.0	4	ns
SEP-432	5-HT	10.0	5.5	9	13.7	4.1	7	ns
Duloxetine	5-HIAA (ng/mL)	21.3	7.0	9	16.8	4.4	6	ns
Placebo	5-HIAA	20.4	9.8	7	18.6	3.5	4	ns
SEP-432	5-HIAA	17.0	6.9	9	18.1	5.7	7	ns
Duloxetine	DOPAC (ng/mL)	0.5	0.1	9	0.7	0.2	7	*
Placebo	DOPAC	0.6	0.4	7	0.5	0.1	4	ns
SEP-432	DOPAC	0.5	0.2	9	0.5	0.1	7	ns
Duloxetine	HVA (ng/mL)	45.4	13.6	9	58.4	24.6	7	ns
Placebo	HVA	41.3	22.5	7	44.5	7.7	4	ns
SEP-432	HVA	37.5	15.3	9	42.5	10.8	7	ns

*$P < 0.05$; ns, not significant.

Table 3 Comparison of day 1 versus day 14 plasma biomarkers

Plasma data								
		Day 1			Day 14			
Treatment	Biomarker	Mean	SD	n	Mean	SD	n	Statistical result
Duloxetine	NE (pg/mL)	205	79	9	193	92	8	ns
Placebo	NE	248	83	7	201	122	6	ns
SEP-432	NE	301	185	9	202	121	8	ns
Duloxetine	DHPG (ng/mL)	0.908	0.148	9	0.721	0.123	8	**
Placebo	DHPG	1.02	0.29	7	0.851	0.085	6	ns
SEP-432	DHPG	0.955	0.394	9	0.613	0.183	8	**
Duloxetine	5-HT (ng/mL)	19.3	7.7	9	6.6	4.5	8	**
Placebo	5-HT	21.6	4.6	7	23.7	3.2	6	ns
SEP-432	5-HT	22.5	5.8	9	8.2	9.1	8	*
Duloxetine	5-HIAA (ng/mL)	5.2	1.3	9	4.6	1.5	8	ns
Placebo	5-HIAA	6.2	1.4	7	5.6	1.1	6	ns
SEP-432	5-HIAA	5.4	1.2	9	4.3	0.9	8	**
Duloxetine	DOPAC (ng/mL)	1.4	0.2	9	1.3	0.2	8	ns
Placebo	DOPAC	1.5	0.3	7	1.4	0.2	6	ns
SEP-432	DOPAC	1.6	0.7	9	1.4	0.2	8	ns
Duloxetine	HVA (ng/mL)	10.5	2.5	9	7.0	1.8	8	**
Placebo	HVA	11.0	2.4	7	8.2	2.5	6	*
SEP-432	HVA	10.6	3.9	9	7.2	2.0	8	**

*$P < 0.05$; **$P < 0.01$; ns, not significant.

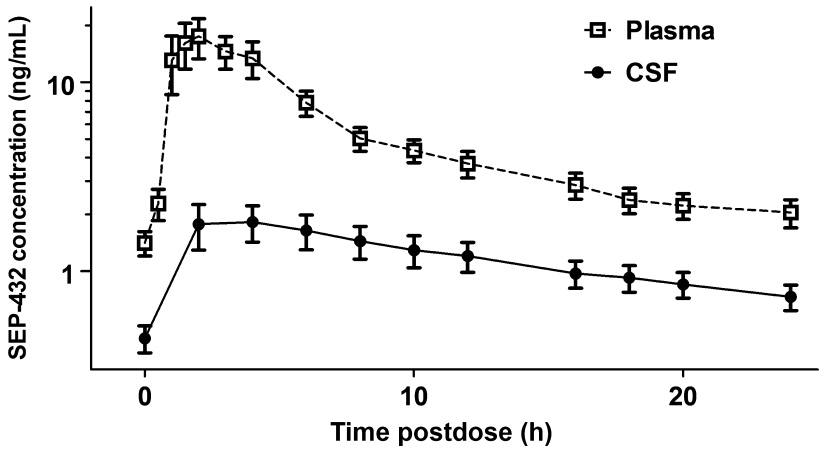

Figure 3 Mean Values for SEP-432 Plasma and CSF Concentrations over 24 h (Day 14) after 300 mg Dosing to Steady State (note that the SEP-432 concentrations are displayed on a log10 scale; error bars indicate SEM).

duloxetine significantly decreased DHPG relative to placebo in both analyses (i.e., Day 14 $AUC_{0-24\ h}$, and comparison of Day 1 to Day 14 time-matched values), confirming NET inhibition [18, 19]. Both compounds also increased 5-HT in CSF, but only the results with duloxetine were significant. SEP-432 had no significant effects on dopamine metabolites. The DHPG/NE ratio in the postural test also confirmed potent NET inhibition for SEP-432. Comparison of the Day 14 SEP-432 CSF and plasma PK profiles confirmed that the compound crosses the blood–brain barrier. Of interest, the maximal decrease in CSF DHPG occurred close to the T_{max} for both SEP-432 and its metabolite.

Plasma monoamines and their metabolites were measured to allow comparisons with those obtained in the CSF, but CSF concentrations are preferred as they reflect cerebral metabolism of the neurotransmitters [20,21]. Plasma monoamine results generally confirmed those in CSF, with the exception that plasma 5-HT showed a decrease as opposed to an increase found in CSF 5-HT. This occurs as a consequence of 5-HT reuptake inhibition in platelets, with depletion of 5-HT by Day 14, even in platelet-poor plasma. Also, a decrease in plasma HVA was seen in the single time point analysis (comparing days 1 and 14) for all treatments; interpretation of this finding is unclear.

SEP-432 300 mg/day is a more potent NET inhibitor than duloxetine at 60 mg/day, as evidenced by a 36% decrease in CSF DHPG compared to a 16% decrease for duloxetine in the baseline

to Day 14 analysis. A 27% decrease in CSF DHPG was reported in a recent study in which duloxetine was administered at 60 mg/day for 11 days [22]. Duloxetine was found earlier to decrease plasma DHPG as predicted by a compound that inhibits NE reuptake, resulting in decreased NE turnover [12]. Changes in heart rate from baseline to end of treatment are also consistent with NET inhibition [23,24].

The effect of SEP-432 on 5-HT was mixed. A significant decrease in plasma 5-HT was found; however, SEP-432 did not significantly increase CSF 5-HT and failed to decrease 5-HIAA as well. Duloxetine was a useful comparator and demonstrated effects centrally on both norepinephrine and serotonin. Duloxetine significantly increased CSF 5-HT but showed only a modest (16%) and nonsignificant decrease in CSF 5-HIAA (inhibition of 5-HT reuptake centrally would have been expected to decrease neuronal conversion of 5-HT to 5-HIAA) [25]. SEP-432 had no effect on DA metabolites in CSF or plasma.

The monoamine biomarker results in this study suggest that clinically meaningful central serotonin or dopamine inhibition by SEP-432 appears unlikely. The lack of translation of the preclinical profile as a triple reuptake inhibitor from *in vitro* studies to humans may be due to a disadvantageous ratio of peripheral to central distribution of SEP-432, consistent with ten-fold lower C_{max} found for SEP-432 in CSF than in plasma. It seems unlikely therefore that SEP-432 can be utilized to increase the activity of three biogenic amine neurotransmitters simultaneously.

In CSF, combining all postdose time points over 24 h was useful to increase the number of values given the high variability in monoamine values observed. Also, the 24-h profile controls for diurnal variation [26,27]. Ideally, a 24-h CSF monoamine profile would be taken at baseline. However, this was considered unsuitable for the evaluation of safety effects in this study. The biomarker analyses in this study were exploratory, given the small number of subjects in each dose group. While some prior investigations of CSF monoamines used sample sizes that were similar to our study [28,29], others had larger sample sizes [30]. Although small numbers of subjects were given placebo, the aggregated results of the 24-h CSF sampling period were sufficient to demonstrate activity of the two compounds relative to placebo.

Two different approaches to analysis of the CSF data (i.e., intergroup changes in the Day 14 $AUC_{0–24}$ values, versus intragroup changes between days 1 and 14) showed similar significant results for DHPG and 5-HT in CSF, but the $AUC_{0–24}$ analysis was also able to demonstrate a significant increase in NE for both SEP-432 and duloxetine. The days 1–14 analysis also showed a significant increase in CSF DOPAC with duloxetine, but interpretation of this finding is unclear, as duloxetine does not interact directly with dopamine receptors.

Of interest, the apparent $t_{1/2}$ in CSF was longer for both SEP-432 and SEP-431 than the $t_{1/2}$ in plasma. Prior studies have shown that the apparent $t_{1/2}$ of marketed CNS drugs in the CSF is longer than that in plasma [9]. Therefore, to achieve steady-state concentrations in CSF, one should administer a compound longer than that necessary to achieve steady state in plasma, which was carried out in the present study. For example, in another study, healthy subjects were given a single dose of paroxetine and CSF measurements of 5-HIAA were taken 3 h postdose; the unexpected finding of increased 5-HIAA levels was most likely the result of not dosing to steady state [7]. However, reaching equilibrium in the brain cannot be precisely predicted currently, as this is determined by both blood–brain barrier penetration and brain tissue binding [31,32].

An occupancy study of SEP-432 utilizing SPECT imaging with the competitive radioligand [123I]-2β-carbomethoxy-3β-(4-iodophenyl) tropane [33] was conducted in healthy volunteers who were dosed to steady state with SEP-432 300 mg/day. The mean values for 5-HT and DA transporter occupancies were 31% and 25%, respectively (L.W. Hardy & M. Versavel, unpublished data), which are lower than the approximately 80% occupancy rate associated with efficacious treatment of depression with SSRIs and SNRIs [34]. As we found in the present study, a disadvantageous PK (ratio of peripheral to central distribution) for SEP-432 is likely responsible for the lack of significant central 5-HT and DA reuptake inhibition. Thus, while SEP-432 may indeed be a triple reuptake inhibitor based on *in vitro* data, it seems unlikely to reach its full potential at doses that are well tolerated in man.

Healthy subjects were evaluated in this study instead of depressed patients. While differences in tolerability between healthy subjects and patients have been reported in studies with antipsychotic and Alzheimer's disease agents [35–40], no clear difference has been shown for altered dose tolerability in depression [9]. Also, using depressed patients could present confounding effects, such as altered levels of centrally acting monoamines, even after washout of prior treatments. The highest safe dose of SEP-432 was employed in this study to maximize its central pharmacodynamics, as most CNS compounds display dose-proportional clinical efficacy within the safe dose range. Nortriptyline is an exception, showing increased efficacy within a narrow plasma therapeutic window [41–43].

Consistent with a prior safety study in which the MTD had been defined at 300 mg/day, SEP-432 300 mg/day was found to be well tolerated in this study, with mild-to-moderate AEs of nausea, headache, and dizziness. However, the noted increased HR, consisting of an approximately 9-fold greater increase with SEP-432 than duloxetine, could limit the dose range in certain populations, such as the elderly. Nausea and vomiting may reflect the compound's activity on serotonin reuptake, as similar effects have been reported both with SSRIs [44,45] and with SNRIs [46,47]. Dizziness may reflect HR and BP changes associated with NET inhibition [27,48,49].

In summary, SEP-432 was found to be an effective central NET reuptake inhibitor and also inhibited the serotonin transporter, but this effect was seen more in the periphery than centrally. Duloxetine's effects as a centrally active dual uptake inhibitor were substantiated by biomarker measurements in this study. The results of this study support the usefulness of monoamine biomarkers in early drug development of potential antidepressants.

Acknowledgments

The authors would like to thank the participants of this study and Sunovion colleagues who assisted with study design and execution. Editorial assistance with manuscript preparation was pro-

vided by Andrew Kuhlman and paid for by Worldwide Clinical Trials, Inc.

The authors are entirely responsible for the scientific content of the article.

Conflict of Interest

This study was sponsored by Sunovion Pharmaceuticals Inc.

Drs. Hardy, Versavel, Kharidia, Grinnell, and Chen are (or were at the time the studies described were conducted) employees of Sunovion Pharmaceuticals Inc. Drs. Sramek, Bieck, Zamora, Cutler, and Mr. Sullivan and Mr. Ding are employees of Worldwide Clinical Trials, Inc.

Drs. Sramek, Bieck, Zamora, Cutler, and Mr. Sullivan and Mr. Ding, in the past 2 years, have not received any direct research support and/or consulting fees.

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