ORIGINAL ARTICLE

Absorption, distribution, metabolism, and excretion of [14C]-dasotraline in humans

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Abstract

Dasotraline is a dopamine and norepinephrine reuptake inhibitor, and the early clinical trials show a slow absorption and long elimination half-life. To investigate the absorption, distribution, metabolism, and excretion of dasotraline in humans, a single dose of [14C]-dasotraline was administered to eight healthy male adult volunteers. At 35 days, 90.7% of the dosed radioactivity was recovered in the urine (68.3%) and feces (22.4%). The major metabolic pathways involved were: (1) amine oxidation to form oxime M41 and sequential sulfation to form M42 or glucuronidation to form M43; (2) N-hydroxylation and sequential glucuronidation to form M35; (3) oxidative deamination to form (S)-tetralone; (4) mono-oxidation of (S)-tetralone and sequential glucuronidation to form M31A and M32; and (5) N-acetylation to form (1R,4S)-acetamide M102. A total of 8 metabolites were detected and structurally elucidated with 4 in plasma (M41, M42, M43, and M35), 7 in urine (M41, M42, M43, M31A, M32, M35, and (S)-tetralone), and 3 in feces (M41, (S)-tetralone, and (1R,4S)-acetamide). The 2 most abundant circulating metabolites were sulfate (M42) and glucuronide (M43) conjugates of the oxime of dasotraline, accounting for 60.1% and 15.0% of the total plasma radioactivity, respectively; unchanged dasotraline accounted for 8.59%. The oxime M41 accounted for only 0.62% of the total plasma radioactivity and was detected only at early time points. M35 was a minor glucuronide metabolite, undetectable by radioactivity but identified by mass spectrometry. The results demonstrate that dasotraline was slowly absorbed, and extensively metabolized by oxidation and subsequent phase II conjugations. The findings from this study also demonstrated that metabolism of dasotraline by humans did not produce metabolites that may cause a safety concern.

Abbreviations

ADHD, attention deficit/hyperactivity disorder; AUC, area under the concentration-time curve; C_{max}, maximum concentration; DAT, dopamine transporters; ESI+/−, electrospray ionization positive/negative mode; FDA, Food and Drug Administration; F, feces; FT-MS/MS, Fourier Transform tandem mass spectrometry; HPLC, high-performance liquid chromatography; IC_{50}, 50% inhibitory concentration; ICH, International Conference on Harmonization; ICRP, International Commission on Radiological Protection; IS, internal standard; LC, liquid chromatography; LLQ, lower limit of quantitation; LSC, liquid scintillation counting; mrem, unit of absorbed radiation dose; MS, mass spectrometry; NET, norepinephrine transporters; NME, new molecular entity; PK, pharmacokinetic; P, plasma; SERT, serotonin transporters; SLE, supported liquid extraction; t_{max}, time to maximum concentration; TRA, total radioactivity; U, urine.

Introduction

Dasotraline (SEP-225289; (1R,4S)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydronaphthalen-1-6 amine) is a diastereomer of the desmethyl metabolite of sertraline, but is not a metabolite of sertraline, nor is it converted to the demethylated metabolite of sertraline in vivo. In contrast to sertraline, dasotraline preferentially occupies dopamine transporters (DAT) relative to serotonin transporters (SERT) (DeLorenzo et al. 2011). Dasotraline is a potent inhibitor of human DAT (dopamine uptake 50% inhibitory concentration [IC_{50}] 3 nmol/L) and norepinephrine transporters (NET; norepinephrine uptake IC_{50} 4 nmol/L), and a weaker inhibitor of human SERT (serotonin uptake IC_{50} 15 nmol/L) (Hopkins et al. 2016). Dasotraline is slowly absorbed by humans (time to maximum concentration [t_{max}] of 10–12 h) and is characterized by slow elimination ($t_{1/2}$ of 47–77 h) with nearly dose-proportional maximum concentration (C_{max}) and area under the concentration–time curve (AUC) (Hopkins et al. 2016). A double-blind placebo-controlled proof-of-concept study demonstrated the potential for dasotraline to be efficacious for the treatment of subjects with attention deficit/hyperactivity disorder (ADHD) (Koblan et al. 2015).

An understanding of the disposition and metabolism of a new molecular entity (NME) in humans is an integral part of its comprehensive safety evaluation. For the vast majority of NMEs, the information on the drug disposition and metabolic fate and exposure of metabolites is required for regulatory approval (Prakash et al. 2012). To examine the absorption, metabolism, and excretion of dasotraline in humans, a single oral dose of radiolabeled [14C]-dasotraline was administered to 8 healthy male volunteers to follow the recovery of radioactivity over time (mass balance) in excreted urine and feces; meanwhile, radio profiling and the most advanced mass spectrometry (MS) technology Orbitrap™ MS, which has been widely used for unknown structure identification (Liang et al. 2011; Zhu et al. 2011; Cho et al. 2012), were employed to search for and elucidate the molecular structure of specific metabolites of dasotraline in human plasma, and excreted urine and fecal samples.

Materials, Study Conduct and Methods

Chemicals, radiolabeled test article and reference standards

The test articles were manufactured according to the guidelines of the Good Manufacturing Practices. [14C]-Dasotraline hydrochloride was synthesized in 10 steps from [14C]-succinic anhydride by Selcia Ltd (Essex, UK)

and stored at −20°C. Unlabeled dasotraline hydrochloride was synthesized from (S)-tetralone at Siegfried LLC (Pennsville, NJ) and provided by Sunovion Pharmaceuticals, Inc. (Marlborough, MA). The structure of [14C]-dasotraline is shown in Figure 1. A complete list of reference standards that were used to assist metabolite searching and structure elucidation are given in Table 1. All of these reference materials were synthesized and purified by in-house chemists at Sunovion Pharmaceuticals, Inc. and were stored at ambient temperature. Other chemical reagents such as formic acid, ammonium formate, acetic acid, and ammonium hydroxide were purchased from J.T. Baker (Center Valley, PA). Acetonitrile and methanol were obtained from EMD Science (Phoenix, AZ). Water used in this study was produced on site with a NANOPure® II (Barnstead Co.) water purification system. Liquid scintillation cocktails were obtained from R. J. Harvey Instrument Corporation (Hillsdale, NJ) or MP Biomedical (Solon, OH). Carbo-Sorb and Perma Fluor Model 307 from PerkinElmer (Akron, OH) were used for oxidizing samples. ACS aqueous counting scintillant was purchased from Amersham Biosciences (Piscataway, NJ).

Clinical study and sample collection

Eight healthy male adult volunteers were administered a single oral dose of a solution containing 8 mg/150 μCi [14C]-dasotraline (actual dose was found to be close to 6 mg/110 μCi [14C]-dasotraline), in an open-label clinical study. The 8 mg dose was selected as a clinically relevant and well-tolerated dose level with sufficient and lasting circulating drug concentrations to ensure the ability to detect measurable levels of dasotraline and its metabolites. Actual doses, as detailed in Table 2, were below the target dose due to the high levels of residual radioactivity measured in the dose vials. All subjects received similar dose concentrations with a mean dose of 6.08 mg/110 μCi of [14C]-dasotraline. This unintentional negative bias in dose (~25% lower than the target dose) given to study subject should not cause any safety concern or affect the study
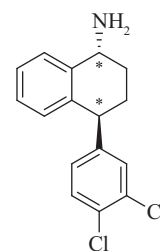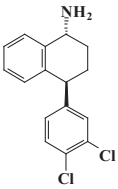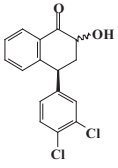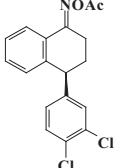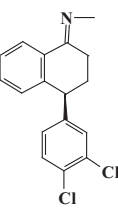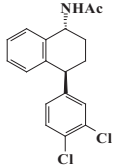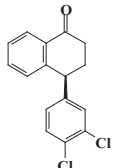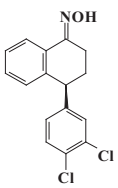

Figure 1. Chemical structure of [14C]-dasotraline (SEP-225289). *, 14C-labeled

Table 1. Information of reference standards.

Reference Standard	Structure	Monoisotopic Mass (Da) ^{35}Cl isotope mass	R_t (min)
Dasotraline (SEP-225289)		291.058	29.8[1]
SEP-227391		306.021	62.8[1]
(S)-Oxime Acetate		347.048	63.0[1]
Sertraline Imine		303.058	27.2[1]
(1R,4S)-Acetamide (M102)		333.069	58.4[1]
(S)-Tetralone		290.027	63.1[1]
SEP-0380096 (SEP-225289-oxime, M41)		305.0347	47.5[2]

[1]Retention time was obtained using HPLC method 1.
[2]Retention time was obtained using HPLC method 2.

conduct and data interpretation. The methodology for calculation of radiation dosimetry is based on publications from the Oak Ridge National Laboratory (Snyder et al. 1975), the International Commission on Radiological Protection (ICRP Publication 30 (Part 1) 1979; ICRP publication 89 2002), the Society of Nuclear Medicine (Weber et al. 1989), and the US Food and Drug Administration (1999). Selection of the total dose of radioactivity was based on dosimetry calculations using the quantitative whole body autoradiography data obtained from Long Evans (partially pigmented) male rats following a single oral administration of 2 mg/kg [^{14}C]-dasotraline. Based on the data from the rat study, it was calculated that the overall exposure for a 70-kg man, receiving a single oral dose of 150 μCi (5.6 MBq) [^{14}C]-dasotraline was 12.2 mrem (122 μSv), which was just approximately 0.407% of the allowable 3000 mrem exposure limit established by the FDA (U.S. Food and Drug Administration, Center for Drug Evaluation and Research and Center for Biologics Evaluation and Research 2010). This dose level would not be expected to represent a significant radiation exposure risk in man. The study was approved by an institutional review board at the investigational site and was conducted in accordance with the International Conference on Harmonization (ICH) Good Clinical Practices guidelines and with the ethical principles of the Declaration of Helsinki. Prior to study entry, all subjects reviewed and signed an informed consent form explaining study procedures and potential risks. The 8 volunteers enrolled in the study were aged 21–34 years old. One subject was withdrawn after the 168 h sample collection due to a family emergency. This subject's samples were not used for sample pooling for metabolite profiling, identification, or pharmacokinetic analysis.

During the clinical study, serial blood samples for plasma were collected at predose, at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 48, 72, 120, 168, 216, 336, 384, 480 h postdose, and at every 24-h time points until discharge (8 mL of blood per time point; a total quantity of 272 mL blood was collected if a subject was in study for 35 days); serial urine samples were collected at predose (−12–0 h), and for 0–6, 6–12, 12–24 h postdose, then at 24-h intervals until the subject was discharged; fecal samples, including toilet tissue were collected predose and at 24-h intervals postdose until the subject was discharged.

The study duration was between 23–35 days. Subjects were confined to the clinic for a minimum of 23 days (Day 1 to Day 22) until the criteria for discharge were met, that is, ≥90% of the radioactive dose was recovered from the sum of urine and feces; and plasma, urine, and fecal radioactivity levels were <2 times the background radioactivity level for 2 consecutive time

Table 2. Radioactive dose administered to individual subjects.

Subject	Dose weight (g)	Dose vial (dpm)	Dose administered (dpm/subject)	(mg/subject)	(μCi/subject)
001	10.0762	101969001	231286774	5.73	104
002	10.1007	86541686	247524391	6.14	111
003	10.0798	84525624	248849216	6.17	112
004	10.1083	86120486	248196950	6.15	112
005	10.1092	96033572	238313630	5.91	107
006	10.0701	102029924	231024102	5.73	104
007	10.1185	93763613	240891174	5.97	109
008	10.0757	58478869	274760369	6.81	124
Mean				6.08	110
S.D.				0.345	6.39

dpm, disintegrations per minute.

points up to 35 days postdose. Subjects were contacted 7 ($\pm$2) days after discharge to collect any relevant safety information. Selected time points of blood samples at predose, at 1, 4, 10, 24, 72, 168, 336, 504, 672, and 840 h postdose (less if subject was discharged earlier than Day 36; data shown below detection limit after 336 h) were collected for the evaluation of blood/plasma distribution of radioactivity.

Total blood volume requirement from each subject for pharmacokinetic (PK), total radioactivity (TRA), blood/plasma distribution of radioactivity, metabolite profiling, and structure elucidation was about 400 mL if a subject completed the full 35 days of study duration and was discharged on Day 36.

TRA measurements

Samples were analyzed for content of radioactivity by liquid scintillation counting (LSC) (Tri-Carb 2900 or 2910TR; PerkinElmer, Inc. Akron, OH). Each sample was analyzed in duplicate and each aliquot counted for at least 5 min or 100,000 counts. For plasma and urine, the samples were mixed and duplicate weighed aliquots (approximately 0.2 g each) were analyzed directly by LSC. For blood, red-blood cells, and feces, the samples were mixed well or homogenized (feces with a certain amount of water) and duplicate weighed aliquots (approximately 0.2 g) were combusted in Biological Sample Oxidizers (PerkinElmer, Inc.), and the resulting $^{14}CO_2$ was trapped in Carbo-Sorb E (PerkinElmer, Inc.) then mixed with Perma Fluor (PerkinElmer, Inc.), and analyzed by LSC. Upon completion of radioactivity measurement for the mass balance calculation, subsets of plasma, urine, and homogenized fecal samples were transferred to designated labs for metabolite profiling, identification, and pharmacokinetic analysis.

For total radioactivity measurements in this study, the used sample amounts were indicated by weight (g), therefore the unit of calculated concentration was supposed to be "ng-Eq/g". For comparison of PK parameters, it was often required to be converted to volume-based unit. In this study, an assumption of weight-to-volume conversion factor of 1 was made for plasma. Thus, all C_{max} and AUC values obtained based on radioactivity measurements are presented as "ng-Eq/mL" and "ng-Eq*h/mL" here.

The cumulative urine and feces radioactivity recovery results of all individual subjects and statistics are summarized in Table 3 and mean recovery time profile is shown in Figure 2.

Table 3. Recovery data of a single oral administration of [^{14}C]-dasotraline in healthy humans.

Subject	% of dosed radioactivity recovered Urine	Feces	Total recovery (%) Urine + Feces
001[1]	41.4	7.57	49.0
002	61.5	30.8	92.3
003	63.5	23.0	86.4
004	71.5	18.7	90.1
005[2]	69.4	20.6	90.0
006[2]	73.2	19.4	92.6
007	69.2	21.1	90.3
008	69.9	22.9	92.8
N	7	7	7
Mean	68.3	22.4	90.7
S.D.	4.24	4.06	2.22
CV%	6.21	18.1	2.45

[1]Withdrawn on Day 8 due to family emergency and excluded from statistics.

[2]Subjects 005 and 006 met discharge criteria early and discharged on Day 33 and Day 30, respectively; the other 5 subjects were discharged on Day 36.
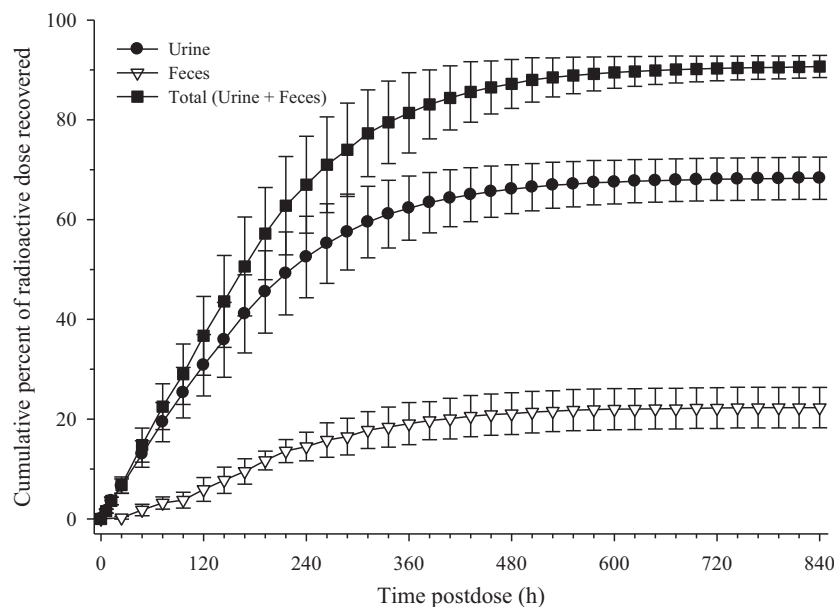
Figure 2. Mean (±SD, $N = 7$) cumulative percent of radioactive dose recovered in urine and feces at specified intervals after a single oral dose of [^{14}C]-dasotraline to healthy male subjects (mean dose: 6.08 mg and 110 μCi).

Metabolite profiling and identification

Due to a low dose given to humans and known long half-lives of unchanged drug and total radioactivity (from time-course plasma unchanged dasotraline exposure measurement and plasma TRA measurement), extremely low plasma concentration of dasotraline and its metabolites were expected, which created a substantial challenge for metabolite profiling and structure elucidation by use of even the most sensitive MS. To avoid further dilution of testing samples, different plasma pooling strategies were implemented to fulfill different purposes. For urine and feces, a typical sample pooling approach was used. The details are described below.

Plasma sample preparation

Plasma samples at predose, 1, 4, 12, 24, 48, 120, 216, 384, and 504 h postdose (10 mL of blood per time point; a total of 100 mL blood was collected for this purpose), which covered greater than 95% of total radioactivity in plasma, were selected for radioprofiling of individual subjects. One set of plasma samples was created by equal volume pooling among all 7 completed subjects for the above-mentioned time points except 1 h and 504 h samples, were used for exploratory PK analysis of TRA and circulating metabolites (Table 7 and Fig. 12). The most accurate PK parameters of dasotraline and TRA in plasma are summarized in Table 5, as the full time-course plasma dasotraline concentrations were measured, using a fully

validated quantitative LC-MS/MS method and TRA concentrations by LSC. Seven AUC plasma pools (1 per subject) were prepared, using the Hamilton method (Hamilton et al. 1981; Hop et al. 1998) for metabolite profiling, structure identification, and quantitative profile of radiolabeled components (Table 6). Some early time point samples, for example, up to 4 h samples from Subjects 002 and 004, were used for the structure elucidation of a low abundance metabolite M41. Plasma samples were extracted by fivefold volume of 0.1% acetic acid in methanol three times (each extraction vortexing 2 min followed by 10 min of centrifugation) and the methanol extracts were combined and evaporated to dryness under a nitrogen stream by an *N*-Evaporator at ambient temperature. The residues were reconstituted with one tenth of the original sample volume (ca. 0.2–0.5 mL) of 0.1% acetic acid in methanol–water mixture (2:1, v/v) for liquid chromatography (LC)-radioprofiling and LC-MS analysis. The recovery of radioactivity from plasma through the above extraction procedure was 89.0%.

Urine sample preparation

Equal percentages by volume of individual human urine samples were pooled from the 7 subjects for the 0–432 h time period (representing 95% of total urine radioactivity), resulting in one pooled urine sample. In addition, some specific time intervals might be pooled for metabolite structure elucidation as needed. Due to low dose and low total radioactivity, each urine sample was

concentrated by drying under nitrogen stream at ambient temperature and reconstituted with one-tenth of the original sample volume (ca. 0.4–0.5 mL) of 0.1% acetic acid in methanol–water (1:3, v/v). The reconstituted samples were subjected to LC-radioprofiling and LC-MS analysis. The recovery of radioactivity from urine through the above procedure was 98.5%.

Fecal sample preparation

Equal percentages by weight of individual human fecal samples were pooled across 7 subjects for the 0–504 h time period (representing 96% of total feces radioactivity) resulting in one pooled fecal sample. In addition, some specific time intervals might be pooled for metabolite structure elucidation as needed. The pooled fecal homogenate was extracted with fivefold (v/w) of 0.1% acetic acid in methanol for three times (each extraction vortex mixing (2 min) and shaking 20 min in a shaker followed by 10 min of centrifugation). The extracts were combined and the total volume was measured, and then evaporated to dryness under a nitrogen stream by an *N*-Evaporator (Organomation Associates, Berlin, MA) at ambient temperature. The resulting residues were reconstituted with an appropriate volume (ca. 0.25–0.45 mL for 3 g fecal extracts) of methanol: 0.1% acetic acid in water (4:6, v/v) for LC-radioprofiling and LC-MS analysis. The recovery of radioactivity from feces through the above procedure was 98.0%.

LC-radioprofiling and LC-MS/MS analysis

Plasma extracts, urine concentrates, and fecal extracts were subjected to LC separation and fraction collection with ISCO Foxy 200® Fraction Collector (Lincoln, NE, USA). [14C]-dasotraline and [14C]-related materials were detected by solid scintillation counting, using a Packard TopCount® NXT™ Microplate Counter (Meriden, CT). Metabolite separation and characterization were accomplished by LC-MS/MS in conjunction with an appropriate radioactivity monitor to facilitate metabolite peak detection. The structures of the metabolites were characterized by their retention times, accurate mass measurement, mass spectral fragmentation patterns, and comparisons with reference standards if feasible. The following LC procedures were used in separation of metabolites of dasotraline in different matrices.

HPLC method 1

A Shimadzu HPLC (high-performance liquid chromatography) system consisting of LC-20AD pumps, SIL-HTC autosampler, and system controller (Columbia,

MD) was used for this study. Separation was achieved on an ACE®Excel, C18-AR column (2 μm, 150 × 4.6 mm) with Ace 3, C18, 3 μm, 10 × 4.6 mm guard column, using a gradient mobile phase at a flow rate of 0.7 mL/min. The column was operated at 35°C and the autosampler was set at 4°C. Postcolumn LC flow was split into 4:1 with 1 to MS and 4 to dynamic flow radioactive detector (v.ARC™ Radio-LC System) or waste. Mobile phase A was 0.4% formic acid in water (pH = 3.2 with ammonium hydroxide), and B was 0.4% formic acid in acetonitrile:methanol (1:1. v/v). Gradient time profiles for B% were as in the following: 0–2 min 0%, 2–10 min going up to 45%, 10–25 min further up to 55%, 25–55 min climbing up to 60%, 55–55.5 min up to 75%, 55.5–64 min up to 100% and holding for 6 min, then 70–72 min from 100 to 0%, and equilibrating for 15 min. The above conditions were used for the majority of metabolite profiling experiments.

HPLC method 2

A modified chromatographic method was developed to separate and identify the metabolites (S)-tetralone and oxime (M41) in the fecal isolate, metabolites in urine, and in vitro incubation samples. In this method, the analytical column was ACE® 3, C18 (3 μm, 150 × 4.6 mm) and three mobile phases were used. Mobile phase A was 0.4% formic acid in water (pH = 3.2 with ammonium hydroxide); B was 0.4% formic acid in acetonitrile:methanol (1:1, v/v). The flow rate was 0.7 mL/min. Gradient time profiles for B% were as in the following: 0–2 min 0%, 2–4 min up to 40%, 4–62 min to 90%, 62–66 min to 100% and holding for 4 min, then 70–72 min back to 0%, and re-equilibrating for 15 min. Other detection conditions were the same as those described in HPLC method 1.

MS conditions

LTQ Orbitrap XL™ MS (Thermo Scientific™, San Jose, CA) with electrospray at positive or negative ion mode, depending on efficiency of ionization of each analyte, was used for detection. Ionspray voltage was 4.0 kV (or −4.0 kV), capillary voltage was 40 V (or −40 V), and tube lens voltage was 60 V (or −40 V). Capillary temperature was 150°C (or 200°C). Sheath gas flow, auxiliary gas flow, and sweep gas flow were set at 50, 50, and 5 units N2, respectively. The collision gas was helium. Resolutions were 60,000 for Fourier Transform (FT)-MS and 7500 for FT-MS/MS. Mass scan range was from 70 to 1000 Da. The software used for data processing and analysis was Xcalibur 2.0.7 from Thermo.

Unknown metabolite structure elucidation

Each unknown metabolite peak from radioprofiling was first compared by its chromatographic retention time with metabolite reference standards whenever available. Collected pure peak fractions were measured for its accurate mass to deduce its molecular formula, through their MS/MS spectral data, which were compared with possible fragmentation pathways to further identify the definite chemical structures of detected metabolites.

Human plasma PK analysis for TRA, unchanged dasotraline and metabolites

Unchanged dasotraline concentrations in serial human PK plasma samples (lithium heparin as anticoagulant) were determined, using a validated enantioselective LC-MS/MS method (curve range: 0.0200–20.0 ng/mL) with a lower limit of quantitation (LLQ) of 0.0200 ng/mL in a 0.200-mL sample. Dasotraline and $[^{13}C_4, D_4]$-labeled internal standard (IS) were extracted by supported liquid extraction (SLE+, 400 mg, 96-well plate) from plasma with 3×0.60 mL of the mixture MtBE/MeCl$_2$/Hexane (1:1:1, v/v/v). The eluate was evaporated to complete dryness and the residue was reconstituted with 0.20 mL methanol/water (30:70, v/v). An aliquot of 75 μL of the resulting sample was injected into the LC-MS/MS system (API 5000, Sciex) for analysis. The ion source for MS was atmospheric pressure chemical ionization operated in positive mode. Separation was achieved on a Supelco (Astec), Chirobiotic V2, 4.6 × 250 mm, 5 μm analytical column, using an isocratic mobile phase of 50% A:50% B at a flow rate of 1.00 mL/min, where A = 10:5:85 (v/v/v) acetonitrile/MeOH/10 mmol/L ammonium acetate (pH 4.0), and B = 70:20:10 (v/v/v) acetonitrile/MeOH/10 mmol/L ammonium acetate (pH 4.0). The retention times of both dasotraline and the internal standard were ~8.0 min. The MS/MS transitions used for monitoring were 275.0→159.0 for dasotraline and 283.0→160.0 for IS, respectively. A linear, 1/concentration weighted, least-squares regression algorithm was used to generate the calibration curve and then its parameters were used to quantitate unknown samples based on peak area ratio of dasotraline versus IS. For dasotraline measurements in this study, the inter-assay coefficient of variation for quality control samples ranged from 0.64 to 2.75%. The above human plasma samples (unextracted) were also analyzed by LSC for the concentration of TRA (ng-Eq/mL) as described in the previous section. PK parameters of TRA (by LSC) and unchanged dasotraline in plasma (by a validated LC-MS/MS method) were obtained using noncompartmental pharmacokinetic analysis with Phoenix® WinNonlin™ software (version 6.2.1 and 6.3, Pharsight Corporation) and the results are presented in Table 5.

AUC$_{TRA}$ and relative percent AUC(%) values of detected drug-related substances (unchanged dasotraline and metabolites in circulation) in the AUC$_{0–504 h}$ pooled plasma samples from 7 subjects (1 pool per subject) were obtained from TRA measurement and radioprofiling of each plasma pool (Table 6).

An exploratory PK analysis of circulating metabolites was also conducted by measuring seven time point plasma pools at 4, 12, 24, 48, 120, 216, and 384 h (each pool was created by an equal volume pooling for the same time point across 7 subjects, and two samples [i.e., 1–h and 504-h plasma] were not used as the selected seven time points provided a good coverage of the entire range). The concentrations (ng-Eq/mL) of TRA, unchanged dasotraline, and its major metabolites in plasma were measured by radio-profiling method. The exploratory PK parameters including AUC between 0 and 384 h (AUC$_{0–384 h}$), C_{max}, t_{max}, and $t_{1/2}$ for TRA, unchanged dasotraline, and individual metabolites were estimated when data points were sufficient (Table 7). These data were obtained based on a limited number of selected time point samples to derive unchanged dasotraline and circulated metabolites' PK parameters according to their radioprofiles. Such analysis was more for the exploratory system PK comparison purpose, but the results provided insights into each metabolite's relative percent AUC, C_{max}, and $t_{1/2}$, and therefore helped us to understand the mechanism of metabolism, PK, and safety of our investigational drug.

Results

Recovery of radioactivity

The amounts of radioactivity counted and recovered in human urine and feces after a single oral dose of $[^{14}C]$-dasotraline are summarized in Table 3. The overall mean (±S.D.) recovery of radioactivity in urine and feces samples was 90.7 ± 2.22 (%) ($N = 7$; %CV 2.45). A mean (±S.D.) of 68.3 ± 4.24 (%) ($N = 7$; %CV 6.21) of the dose was recovered in urine and 22.4 ± 4.06 (%) ($N = 7$; %CV 18.1) was recovered in feces through to the last collection interval. Two subjects were discharged on Day 30 and Day 33, respectively, as they met the discharge criteria earlier than the planned maximum study duration of 35 days. The remaining 5 subjects were discharged on Day 36 according to the study protocol. The mean cumulative percent recovery of the dosed radioactivity across 7 completed subjects over time is shown in Figure 2. The majority of the administered radioactivity was recovered (83.1%) within 384 h (16 days) postdose. The mean total

amount of drug excreted in the urine was 4180 μg equivalents and the mean total amount of drug excreted in feces was 1370 μg equivalents.

Distribution of radioactivity in blood, plasma and red blood cells

Following a single oral dose of [^{14}C]-dasotraline of ca. 6 mg and 110 μCi, the maximum mean concentration of drug-derived radioactivity in plasma was observed at 48 h postdose, with a value of 20.7 ng-Eq/mL. Maximum plasma radioactivity concentrations in individual subjects were observed from 24 to 168 h postdose and ranged from 11.7 to 33.1 ng-Eq/mL. The maximum mean radioactivity concentration in blood was observed at 72 h postdose with a value of 12.4 ng-Eq/mL. Maximum blood concentrations in individual subjects were observed from 10 to 72 h postdose and ranged from 7.40 to 18.9 ng-Eq/mL. Mean radioactivity concentrations in blood and plasma versus time course are shown in Figure 3. Mean blood-to-plasma radioactivity concentration ratios across subjects were ranging from 0.553 to 0.683 through 336 h postdose (individual data not presented). The concentration of radioactivity in red blood cells was calculated, using collected whole blood and plasma samples, in conjunction with corresponding hematocrit values. The maximum mean calculated concentration of radioactivity in red blood cells was observed at 10 h postdose, with a value of 4.24 ng-Eq/mL. Mean red blood cell-to-plasma concentration ratios across subjects ranged from 0.0901 to 0.312 through 336 h (14 days) postdose (individual data not presented).
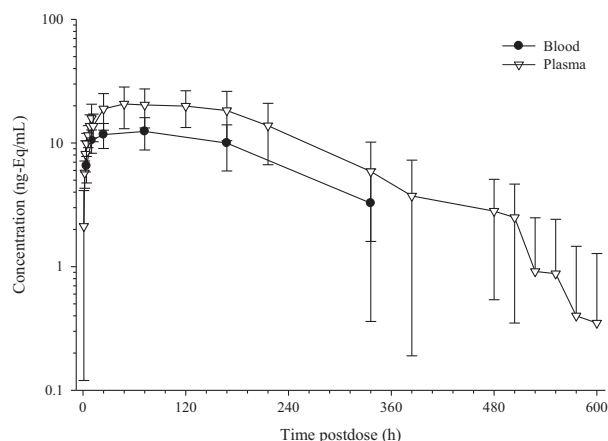

Figure 3. Mean ($\pm$SD, $N = 7$) concentrations of radioactivity in blood and plasma at selected time points.

Metabolite searching results and their relative abundances

A total of 8 metabolites were found and structurally identified through our metabolite profiling and identification efforts from plasma, urine, and fecal samples. The results are presented in Table 4 and the metabolic pathways in Figure 4.

In plasma, besides the parent analyte, four metabolites (M41, M42, M43, and M35) were detected and their structures were identified as the oxime of dasotraline (M41, or SEP-380096), the sulfate conjugate of the oxime (M42), the glucuronide conjugate of the oxime (M43), and the glucuronide of N-oxidation of dasotraline (M35). As summarized in Table 4, mean results ($N = 7$) of unchanged dasotraline, M41, M42, M43 were accounting for 8.59%, 0.62%, 60.1%, and 15.0% of the TRA, respectively, in the AUC-pooled plasma across seven subjects. Typical radio-chromatograms are shown in Figure 5, where Figure 5A, B, and C are radioprofiles of the 4-h, 24-h, and 120-h plasma pools by equal volume pooling across the seven subjects, and Figure 5D is the representative radioprofile of an individual subject's $AUC_{0-504\ h}$ plasma pool, covering the full time course of radioactivity (Subject 004). Two conjugates M42 and M43 were the most abundant metabolites and accounted for 75.1% of the total AUC-pooled plasma radioactivity. M41 (dasotraline-oxime) was a very minor metabolite and was only detected at an early time point (e.g., 4 h plasma) and accounted for 0.62% of the total plasma radioactivity. M35 was at negligible levels, not detected by radioactivity, but only detected and identified by MS.

In urine, besides dasotraline, seven metabolites (M41, M42, M43, M31A, M32, M35, and (S)-tetralone) were identified. In addition to those metabolites detected in plasma, (S)-tetralone (SEP-227390) was likely formed by oxidative deamination of dasotraline or from the hydrolysis of M41, M42 or M43, and M31A, and M32 were the glucuronides of SEP-227391, that is, mono-oxidation of (S)-tetralone. Urine was a major excretion route for dasotraline-derived TRA. Profiling of the urine samples pooled from the 0 to 432 h (65.0% of the dosed radioactivity) indicate that unchanged dasotraline, M31A, M32, M35, M42, and M43 accounted for 2.51, 4.96, 3.43, 13.5, 22.2, and 6.37% of the dosed radioactivity, respectively. Minor metabolites M41 and (S)-tetralone were also detected with a combined amount of 0.37% of the dose radioactivity. Typical radio-chromatogram is shown in Figure 6A.

In feces, unchanged dasotraline and three metabolites, that is, (S)-tetralone, M41, and (1R,4S)-acetamide (M102) were identified by comparing LC-MS/MS spectra with corresponding authentic standards. Feces were a minor excretion route for dasotraline-derived TRA. Profiling of the fecal samples pooled from 0 to 504 h (21.4% of the dosed

Table 4. Mean (*N* = 7) radioactivity distributions of dasotraline and its metabolites in plasma, urine and feces.

Compound name or code	Modification	Plasma %AUC[1]	Urine (0–432 h) %Dose	Feces (0–504 h) %Dose
Dasotraline (SEP-225289)	Parent (unchanged drug)	8.59	2.51	4.33
M31A	Mono-oxidation of (S)-tetralone	ND[2]	4.96	ND
M32	and glucuronidation	ND	3.43	ND
M41	Amine oxidation (oxime of dasotraline)	0.62	0.37 (M41 and (S)-	1.78
(S)-Tetralone (SEP-227390)	Oxidative deamination	ND	Tetralone co-eluted)	6.95
M42	Sulfation of M41	60.1	22.2	ND
M43	Glucuronidation of M41	15.0	6.37	ND
M35	*N*-hydroxylation and glucuronidation	Only detected by MS	13.5	ND
(1R,4S)-Acetamide (M102)	*N*-Acetylation	ND	ND	0.97

[1]Mean of seven individual subjects' relative percent AUCs (see Table 6).
[2]ND: nondetectable by radioactivity.
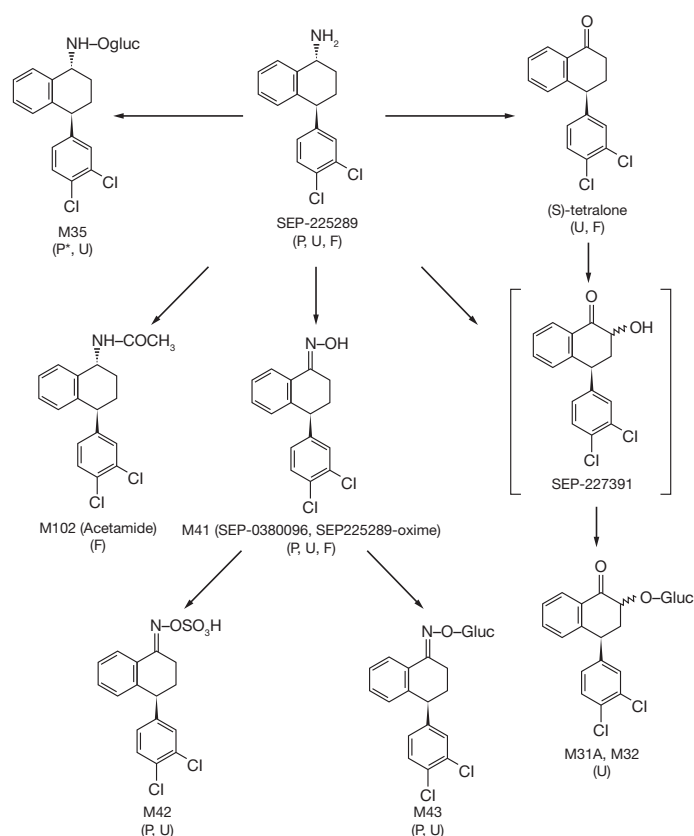
Figure 4. Proposed metabolic pathways of dasotraline (SEP-225289) in humans. *, only detected by MS

radioactivity) revealed that unchanged dasotraline, (S)-tetralone, M41, and (1R,4S)-acetamide (M102) accounted for 4.33, 6.95, 1.78, and 0.97% of the dosed radioactivity, respectively. Typical radio-chromatogram obtained under the HPLC method 1 is shown in Figure 6B, and the separation of (S)-tetralone and M41 under the optimized condition HPLC method 2 is shown in Figure 6C.

The structures of major circulating metabolites in plasma were elucidated by their MS/MS fragmentation patterns. M41, M42, and M43 were identified as the oxime of dasotraline, sulfate of dasotraline-oxime, and glucuronide of dasotraline-oxime, respectively. The MS/MS spectral data and proposed fragmentation pathways are shown in Figure 7 for M41, Figure 8 for M42, and
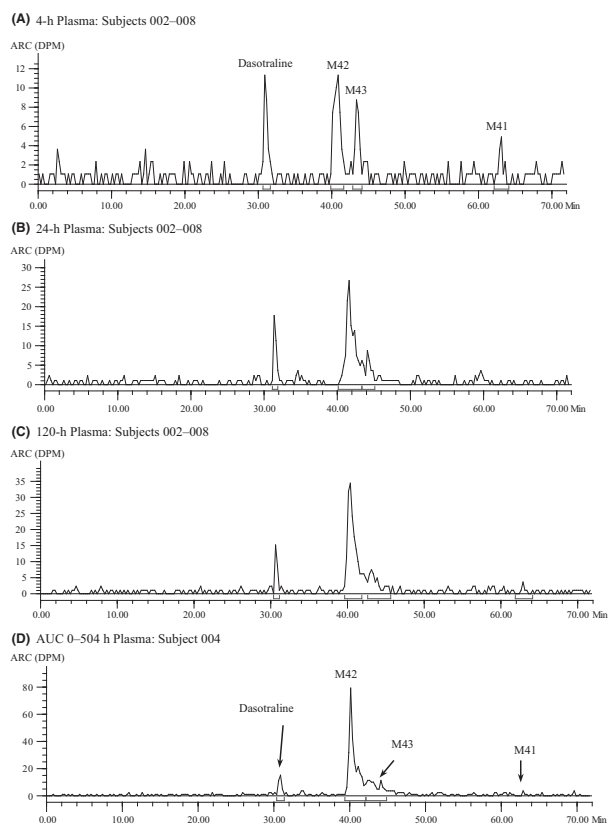
Figure 5. Representative LC radio-chromatograms of pooled 4-h (A), 24-h (B), and 120-h (C) human plasma samples across 7 subjects, and the AUC-pool (0–504 h; D) from one subject (Subject 004) (HPLC Method 1).
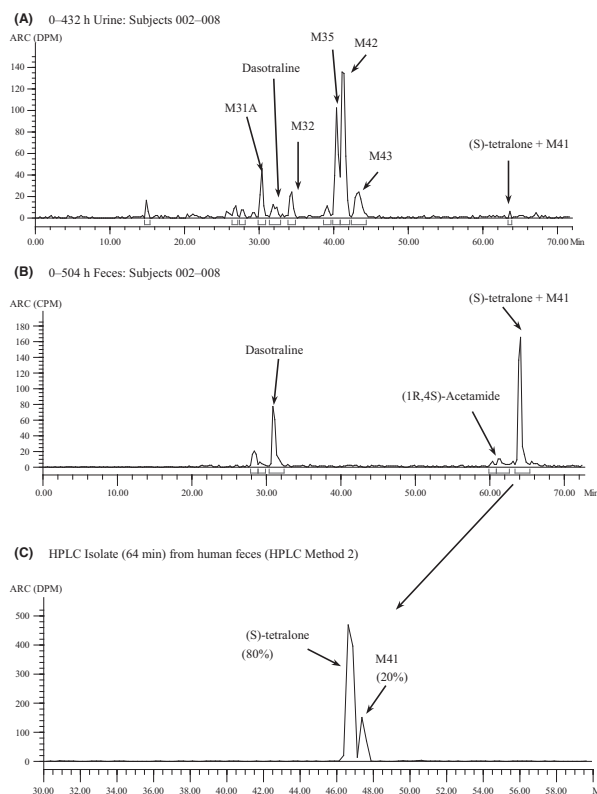

Figure 6. Representative LC radio-chromatograms of pooled 0–432 h urine (A), 0–504 h feces (B) across Subjects 002 to 008 (HPLC Method 1), and separation of M41 (oxime) from (S)-Tetralone using HPLC Method 2 (C).

Figure 9 for M43, respectively. M35 was a minor metabolite in plasma (only detected by MS) and was identified as glucuronide of *N*-hydroxylated dasotraline, and its MS/MS spectral data and proposed fragmentation pathways are shown in Figure 10. The structures of other identified metabolites in urine or feces were determined in the same way, but MS/MS data are not shown.

PK analysis of TRA and unchanged dasotraline in human plasma

PK analysis of plasma TRA concentrations (by LSC) and plasma unchanged dasotraline concentrations (by a validated LC-MS/MS method) were performed, using WinNonlin™ software. The PK profiles of TRA and unchanged dasotraline are shown in Figure 11A (linear scale) and Figure 11B (semi-log scale), respectively. PK parameters for both TRA and unchanged dasotraline in plasma are summarized in Table 5. Following a single oral dose of 6 mg/110 μCi dasotraline/[^{14}C]-dasotraline, dasotraline, and TRA were characterized by slow absorption, with median (min, max) t_{max} values of 10 h (8, 24)

for unchanged dasotraline and 60 h (24, 120) for TRA, and the geometric mean elimination $t_{1/2}$ values were 61.3 h for dasotraline and 114 h for TRA in plasma. The mean exposure values (C_{max} and AUC) for TRA in plasma was approximately ten- to twenty-fold higher than for dasotraline, which indicated the extensive metabolism of dasotraline. The geometric means (%CV) for C_{max} and AUC between 0 and infinity (AUC$_{0-inf}$) in plasma were 2.02 ng/mL (18.4) and 248 ng*h/mL (36.6) for dasotraline and 20.6 ng-Eq/mL (32.0) and 5840 ng-Eq*h/mL (36.6) for TRA, respectively.

TRA and its metabolite distribution in each individual subject's AUC-pooled plasma are presented in Table 6. The mean ($\pm$S.D.) value of AUC of TRA was 5613 ($\pm$2558) ng-Eq*h/mL across 7 subjects. The mean radioactivity distributions, that is, percent of the total AUC for each [^{14}C]-derived entity in pooled plasma, were 8.59% ($\pm$4.74) for unchanged dasotraline, 0.62% ($\pm$1.07) for M41 (oxime), 60.1% ($\pm$5.84) for M42 (oxime-sulfate), and 15.0% ($\pm$5.99) for M43 (oxime-glucuronide). M41 was only detectable in early time points (4 h) in 2 out of 7 subjects. The exploratory PK analysis of its metabolites based
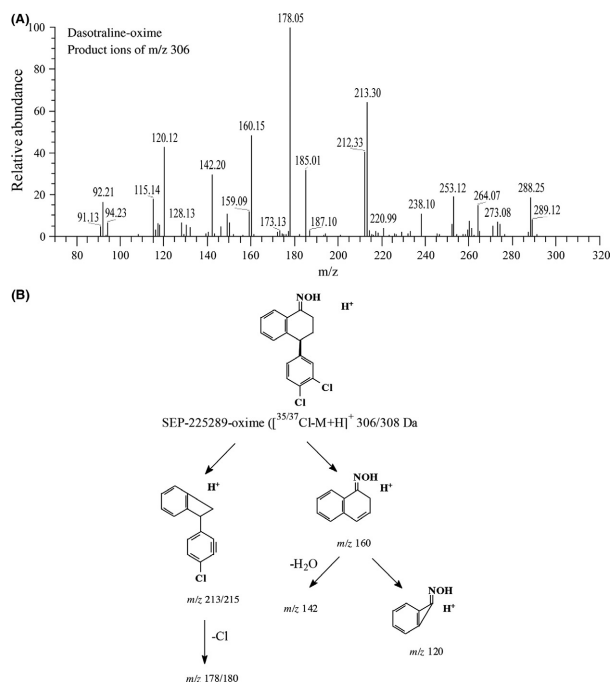
Figure 7. MS/MS Spectral data (only shown ^{35}Cl isotope ESI+ peak) (A) and proposed fragmentation pathways of dasotraline-oxime, that is, M41 (B).
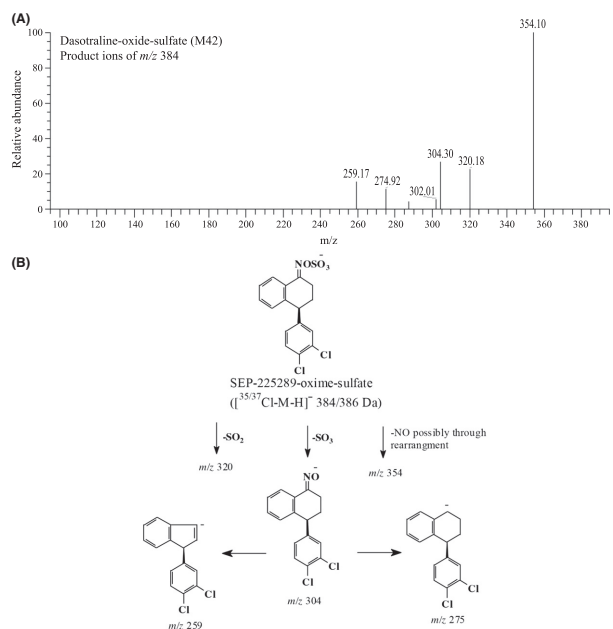

Figure 8. MS/MS Spectral data (only shown ^{35}Cl isotope ESI– peak) (A) and proposed fragmentation pathways of sulfate of dasotraline-oxime, that is, M42 (B).

Figure 9. MS/MS Spectral data (only shown ^{35}Cl isotope ESI+ peak) (A) and proposed fragmentation pathways of glucuronide of dasotraline-oxime, that is, M43 (B).
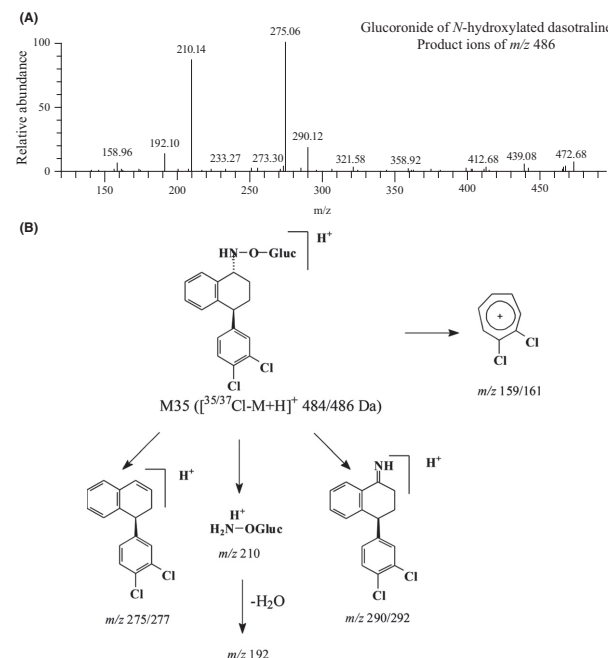
Figure 10. MS/MS Spectral data (only shown ^{35}Cl isotope ESI+ peak) (A) and proposed fragmentation pathways of glucuronide of *N*-hydroxylated dasotraline, that is, M35 (B).

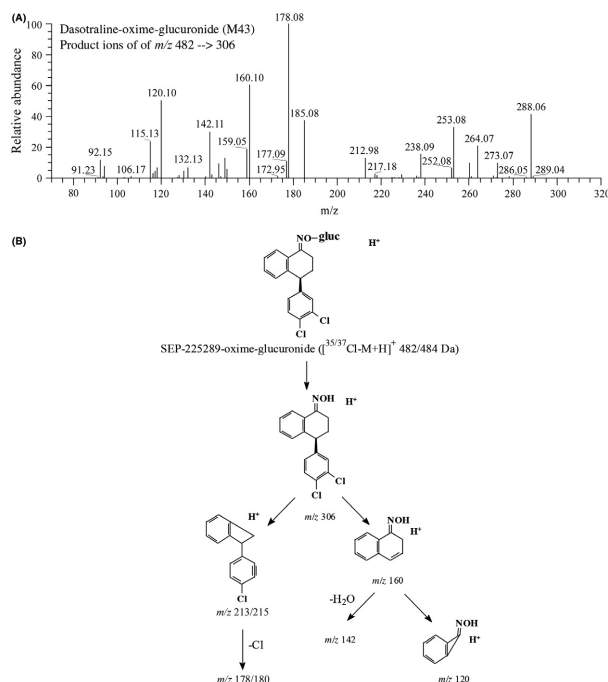on selected seven time points samples (pooled across 7 subjects) are summarized in Table 7. Mean plasma concentrations of TRA, unchanged dasotraline, M42, and M43 in plasma were plotted as a function of time in Figure 12. Although only a limited number of time point samples were used for this experiment, this exploratory analysis was

valuable to understanding of human system PK of dasotraline and its circulating metabolites.

Discussion

Mass balance

Mass balance excretion studies in humans using radiolabeled compounds represent a standard practice in drug development process for new drugs (Roffey et al. 2007), therefore the scope of this study is to understand and determine the drug disposition and metabolism of dasotraline through a single oral dose of $[^{14}C]$-dasotraline to healthy male volunteers. In this study, although the subjects were administered a dose amount (ca. 6 mg, 110 μCi of $[^{14}C]$-dasotraline) lower than the designed dose (8 mg, 150 μCi of $[^{14}C]$-dasotraline) due to the substantial amount of residue that remained in containers, the study was successfully conducted and yielded a 90.7% average recovery of total dosed radioactivity from 7 completers within 35 study days, which is above the commonly accepted 90% cutoff threshold. As shown in Table 3, TRA recovery in each individual subject ranged from 86.4–92.8% with a 2.22% S.D. ($N = 7$) and only one subject had lower than 90% recovery. The radioactivity recovery in urine and feces representing 68.3% (4.24 S.D.) and 22.4% (4.06 S.D.) of the dose, respectively, indicated that urine is a primary excretion pathway for the dosed drug and its metabolites. The data presented in Table 3 also demonstrate the high quality of this study conduct, supported by the small intersubject variation for radioactivity recovery in urine, feces, and combined overall recovery. The study found that >90% of the total recovered radioactivity was excreted from humans over the first 16 days (data not shown). A total of 2.51% and 4.33% of unchanged dasotraline were excreted through urine and feces, respectively, indicating that the majority of the dosed dasotraline was excreted through extensive metabolism. The mass balance results also demonstrated that the dasotraline was slowly, but highly, absorbed when it was orally administered to humans with dasotraline median t_{max} at 10 h postdose.

Radioactivity distribution in blood, plasma and red blood cells

As reported in the results section and in Figure 3, mean maximum radioactivity concentrations in blood, red blood cells, and plasma were 12.4 ng-Eq/mL at 72 h, 4.24 ng-Eq/mL at 10 h, and 20.7 ng-Eq/mL at 48 h, respectively. Both the concentration ratios of blood/plasma and red blood cell/plasma indicate a low association of radioactivity with blood cells

and the majority of $[^{14}C]$-derivative components were preferably circulating in plasma.

Metabolite profiling and structure elucidation

In the plasma samples, radio-chromatograms obtained with the HPLC method 1 are shown in Figure 5 with 4-h (5A), 24-h (5B), 120-h (5C) time point pools from 7 subjects and the AUC-pool created from one subject's 0–504 h (Subject 004) plasma (5D). Four peaks were detected from plasma metabolite profiling with respective retention times of ~31, ~41, ~43, and ~63 min. These four peaks were identified as unchanged dasotraline, a sulfate of the oxime of dasotraline, a glucuronide of the oxime of dasotraline, and the oxime of dasotraline, respectively. The observation of sulfonation of monoaryl oximes was reported by Mangold et al. (1986, 1989). The structures of dasotraline and the oxime of dasotraline were identified by comparing these unknown peaks with their reference standards' retention times, molecular weights, and the MS/MS fragmentation patterns. The structures of the oxime-sulfate and the oxime-glucuronide were identified by interpreting their accurate mass measurements, MS/MS patterns, and comparing with the products obtained from the in vitro incubation (at 37°C for 4 h) of the dasotraline-oxime standard with human liver microsomes to generate the glucuronide (in 0.1 mol/L phosphate buffer pH 7.4, containing 50 μmol/L of the oxime of dasotraline, 0.025 mg/mL alamethicin, 4 mmol/L magnesium chloride, 1 mmol/L EDTA, 1 mg/mL microsome protein, 1.0 mmol/L uridine-diphosphate-glucuronic acid, i.e., UDPGA) and human liver cytosol fractions to generate the sulfate (in 0.1 mol/L phosphate buffer pH 7.4 containing 50 μmol/L of the oxime of dasotraline, 4 mmol/L magnesium chloride, 1 mmol/L EDTA, liver cytosol protein (1 mg/mL), adenosine 3′-phosphate 5′-phosphosulfate, 1.0 mmol/L). Accurate mass measurement results of these identified peaks are presented in Table 8. Differences between the measured mass and its theoretical mass were −2.05, 0, −3.39, and −3.11 ppm for dasotraline, M41, M42, and M43, respectively, indicating a perfect match of their assigned structures. The MS/MS spectral data of M41, M42, and M43, as shown in Figure 7–9 fully support their assigned structures.

In the pooled urine sample from 0 to 432 h across all 7 subjects, the radio-chromatograms obtained with the HPLC method 1 is given in Figure 6A. Peaks of multiple metabolites were detected including the parent and 3 identified ones in plasma, and 4 other metabolites with respective retention times of ~30, ~34, ~40, and ~63 min. These four newly identified structures were assigned as M31A, M32, M35, and (S)-tetralone, respectively. Here, M31A
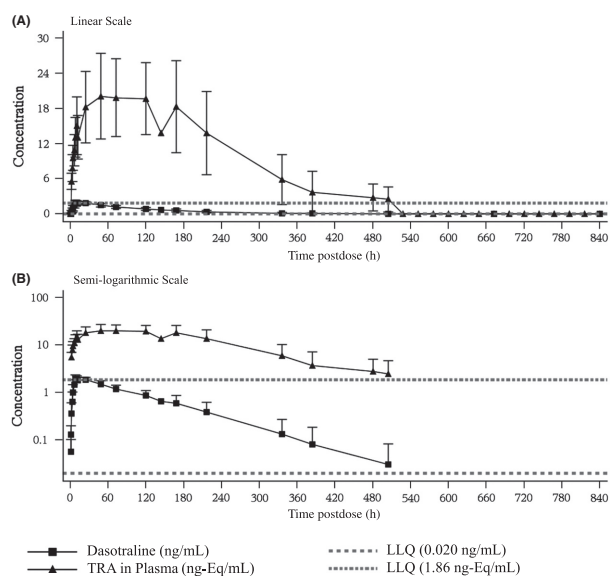
(A) Linear Scale

(B) Semi-logarithmic Scale

■ Dasotraline (ng/mL)   ⋯⋯ LLQ (0.020 ng/mL)
▲ TRA in Plasma (ng-Eq/mL)   ⋯⋯ LLQ (1.86 ng-Eq/mL)

Figure 11. Arithmetic mean plasma concentration profiles of dasotraline (ng/mL) and TRA in plasma (ng-Eq/mL) in linear (A) and semi-logarithmic scale (B).

Table 5. PK parameters for unchanged dasotraline and TRA in plasma, Geometric Mean (CV%).

PK Parameter	Dasotraline in Plasma[1] (N = 7)	TRA in Plasma[2] (N = 7)
C_{max}[3] (ng/mL)	2.02 (18.4)	20.6 (32.0)
t_{max}[3] Median (Min, Max) (h)	10.0 (8.00, 24.0)	60.0 (24.0, 120)
AUC_{0-last} (ng*h/mL)	243 (37.6)	5220 (43.7)
AUC_{0-inf} (ng*h/mL)	248 (36.6)	5840 (36.6)
$t_{1/2}$ (h)	61.3 (34.2)	114 (32.2)
λ_Z (h^{-1})	0.0113 (34.2)	0.00611 (32.2)
CL/F (L/h)	32.3 (36.6)	NA
V_Z/F (L)	2860 (21.5)	NA
$R_{Cmax\ drug/rd}$[3]	NA	0.0982 (33.9)

[1]Measured by a validated quantitative LC-MS/MS method.
[2]Measured by LSC. Units for TRA C_{max} and AUC are ng-Eq/mL and ng-Eq*h/mL, respectively.
[3]N = 8 for C_{max}, t_{max}, and $R_{Cmax\ drug/rd}$. only.

and M32 were a pair of diastereomers and both were glucuronides of hydroxylated dasotraline. M35 was the glucuronide of *N*-hydroxylated dasotraline and M35 was also later detected in plasma by MS though was not detectable by radioprofiling. The peak at retention time at ~63–64 min was identified as (S)-tetralone by comparing with its reference standard. This peak was later confirmed to contain M41 co-elution while the HPLC method 1 conditions were used. As presented in Table 8, mass differences between accurate mass measurements and their assigned

structures were −2.40, −4.00, −2.27, and −1.45 ppm for M31A, M32, M35, and (S)-tetralone, respectively. The MS/MS fragmentation patterns of identified metabolites were in good accordance with their corresponding assigned structures. As M35 was also detected in plasma by MS, its structure assignment was supported by MS/MS spectral data and proposed fragmentation pathways, shown in Figure 10. A few minor peaks were also detected but structures were not identified.

In the pooled fecal sample from 0 to 504 h across all 7 subjects, radio-chromatograms obtained with the HPLC method 1 are given in Figure 6B. Besides the unchanged dasotraline peak (~31 min), a dominant peak at retention time of ~63–64 min was detected. In addition, a couple of minor peaks (~28 min and ~61 min) were also detected in fecal samples. The peak at ~61 min was assigned as M102 and its structure was identified as a form of (1R,4S)-acetamide via comparison with its reference material. The dominant peak fraction from the HPLC method 1 was further investigated with an improved chromatographic gradient procedure. As shown in Figure 6C, with the HPLC method 2, it was able to successfully separate M41 (retention time of 47.5 min) from (S)-tetralone (retention time of 46.5 min). This condition also confirmed both M41 and (S)-tetralone existed in urine samples. In feces, (S)-tetralone was about 80% and M41 about 20% in the co-eluted peak from the HPLC method 1. M41 was unambiguously identified as the oxime of dasotraline by comparing retention time, accurate mass measurement, and MS/MS fragmentation pattern with the reference standard.

Proposed metabolic pathways

According to the mass balance data and metabolite profiling and structure elucidation result, dasotraline was extensively metabolized by oxidation and subsequent phase II conjugation. A total of 8 metabolites were structurally identified, 7 of which existed in urine (M41, M42, M43, M31A, M32, M35, and (S)-tetralone), 3 in feces (M41, (S)-tetralone, and (1R,4S)-acetamide), and 4 were circulating in plasma (M41, M42, M43, and M35; Table 4). The proposed metabolic pathways were described in Figure 4. The major biotransformation pathways of dasotraline involved: (1) amine oxidation to form M41 (dasotraline-oxime) and sequential sulfation to form M42 (a sulfate conjugate) or glucuronidation to form M43 (a glucuronide conjugate); (2) *N*-hydroxylation and sequential glucuronidation to form M35; (3) oxidative deamination to form (S)-tetralone; (4) mono-oxidation of (S)-tetralone and sequential glucuronidation to form M31A and M32; and (5) *N*-acetylation to form (1R,4S)-acetamide (M102).

Table 6. Percent AUC values of dasotraline-derived metabolites relative to TRA AUC in the AUC-pooled plasma samples from individual subjects.

	%AUC in individual subjects							Mean %AUC	S.D.
Analyte	002	003	004	005	006	007	008	($N = 7$)	
1 (Dasotraline)	3.83	6.33	9.20	7.95	8.53	18.5	5.79	8.59	4.74
2 (M42)	69.7	53.6	59.0	54.6	59.7	57.9	65.9	60.1	5.84
3 (M43)	4.88	23.2	14.9	20.8	14.2	11.6	15.2	15.0	5.99
4 (M41)	2.44	ND	1.92	ND	ND	ND	ND	0.62	1.07
AUC_{TRA} (ng-Eq*h/mL)	5539	10,498	5201	3871	3087	3846	7248	5613	2558

Table 7. Exploratory PK parameters of TRA, unchanged dasotraline and major metabolites in plasma based on selected time points pooling across 7 subjects at (predose), 4, 12, 24, 48, 120, 216, and 384 h (1-h and 504-h time points were not used for this measurement).

Analyte	$AUC_{0-384\ h}$ (ng-Eq*h/mL)	%AUC[1]	C_{max} (ng-Eq/mL)	t_{max} (h)	$t_{1/2}$ (h)
TRA	5460	100	20.7	48	135
Dasotraline	342	6.28	2.96	12	89.6
M42	3280	60.1	12.1	48	138
M43	1020	18.6	3.76	120	149[2]

[1]Relative to AUC_{TRA} (assume AUC_{TRA} as 100%). As only limited time points were used, AUC values were just for reference. The PK profiles are presented in Figure 12.

[2]The data at t_{max} was also used for estimation of $t_{1/2}$ due to insufficient data points.
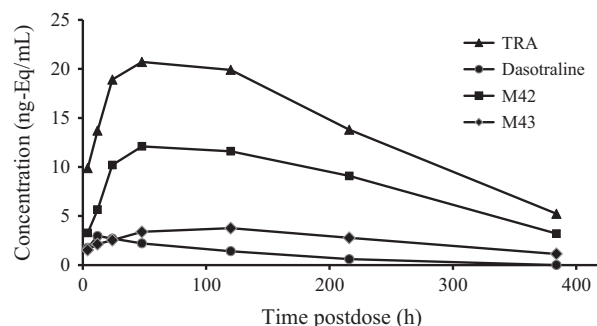
Figure 12. Mean plasma (pooled same time points across 7 subjects) concentration (ng-Eq/mL) – time profiles of TRA, dasotraline, sulfate of dasotraline-oxime (M42) and glucuronide of dasotraline-oxime (M43).

Circulating metabolites in plasma and their PK characteristics

The exploratory PKs of the circulating metabolites was calculated based on the selected time points pooling across 7 subjects at 4, 12, 24, 48, 120, 216, and 384 h and are presented in Table 7. The mean radioactivity C_{max} and AUC values showed that 2 phase II conjugated metabolites M42 and M43 were the major circulating components, in which AUC of M42, the sulfate conjugate, is the most abundant one and is about tenfold higher than that of the unchanged dasotraline along with an approximate twofold difference in elimination $t_{1/2}$. There was no evidence that the back conversion of these highly abundant phase II conjugates would contribute to the long half-life of dasotraline in plasma as the oxime of dasotraline had an extremely low exposure and short half-life in circulation. According to MIST guidance (U.S. Food and Drug Administration 2008), phase II conjugates generally render a compound more water soluble and pharmacologically inactive; thus, further evaluation is not warranted.

The only oxidative metabolite detected in plasma was the oxime of dasotraline (M41), accounted for a 0.62% of the plasma TRA and was detected at early time points (~4 h) from only 2 of 7 completed subjects and quickly disappeared likely due to subsequent conjugations. As M41 is a very minor metabolite with a short half-life, it is not expected to accumulate and cause a safety concern. According to ICH guidance (ICH 2010), nonclinical characterization of a human metabolite(s) is only warranted when that metabolite(s) is observed at exposures >10% of total drug-related exposure and at significantly greater levels in humans than the maximum exposure seen in the toxicity studies. Therefore, the further characterization of M41 for safety concern will not be necessary.

In conclusion, dasotraline is well but slowly absorbed, and extensively metabolized after a single oral dose of [14C]-dasotraline in healthy male adults. The TRA recovery is >90% in urine and feces over the course of 35 study days and the main excretion is through urine. In human plasma, unchanged dasotraline accounted for 8.59% of the TRA in AUC-pooled plasma samples. The 2 most abundant metabolites were sulfate and glucuronide conjugates of the oxime of dasotraline, accounting for 60.1 and 15.0% of TRA in pooled plasma, respectively.

Table 8. Accurate mass measurements of dasotraline and its metabolites.

Compound Code	m/z [M+H]+	Elemental Composition	Theoretical mass (based on 35Cl)	Measured Mass	Mass Difference (ppm)
Dasotraline	292	$C_{16}H_{15}Cl_2N$	292.0654	292.0648	−2.05
(S)-Tetralone	291	$C_{16}H_{12}Cl_2O$	291.0338	291.0338	0
M31A	500 (NH$_4$+- adduct)	$C_{22}H_{24}Cl_2O_8N$	500.0874	500.0862	−2.40
M32	500 (NH$_4$+- adduct)	$C_{22}H_{24}Cl_2O_8N$	500.0874	500.0854	−4.00
M35	484	$C_{22}H_{23}Cl_2NO_7$	484.0924	484.0913	−2.27
M41	306	$C_{16}H_{13}Cl_2NO$	306.0447	306.0447	0
M42	384 (M-1, negative)	$C_{16}H_{12}Cl_2NO_4S$	383.9870	383.9857	−3.39
M43	482	$C_{22}H_{21}Cl_2NO_7$	482.0768	482.0753	−3.11
(1R,4S)-Acetamide (M102)	344	$C_{18}H_{18}Cl_2NO$	344.0760	344.0755	−1.45

The oxime of dasotraline was at the minimal level (accounting for 0.62% of TRA in pooled plasma) and only detectable in two subjects' early time points (4 h) and not detectable afterwards. Those two dominant metabolites, that is, sulfate and glucuronide of dasotraline-oxime are phase II conjugates that are known to be pharmacologically inactive and not toxic. Per MIST guidance, these inactive phase II conjugates do not require for further evaluation. The findings from the current trial such as a consistent high recovery (>90%), major metabolic pathways (oxidation followed by conjugations), main excretion route (through urine), and unambiguous identification of major circulating metabolites (oxime-sulfate and oxime-glucuronide) provided rich knowledge and insight to help understand the mechanism of metabolism, PK, and safety of dasotraline. According to the findings from this study, metabolism of dasotraline by humans did not produce metabolites that may cause a safety concern.

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Authorship Contributions

Participated in research design: Chen, Lin, Skende, Hopkins, Koblan. Conducted experiments: Wills, Yi, Wang. Contributed new reagents: Wilkinson. Performed data analysis: Chen, Skende, Lin, Wilkinson, Hopkins. Wrote or contributed to the writing of the manuscript: All Authors (Chen, Skende, Lin, Yi, Wang, Wills, Wilkinson, Koblan, and Hopkins).

Disclosures

YLC, ES, JL, HSW, KSK, and SCH are employees of Sunovion Pharmaceuticals, Inc. YY and PLW are employees of XenoBiotic Laboratories, Inc. SW is an employee of Covance Laboratories, Inc.

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