PHARMACOKINETICS OF N,N-DIMETHYLTRYPTAMINE FUMARATE IN HUMANS

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Abstract

Aim: N,N-dimethyltryptamine (DMT) is a psychedelic compound under development for the treatment of major depressive disorder (MDD). This study evaluated the in vitro metabolism and clinical pharmacokinetics (PK) of DMT fumarate (SPL026) in healthy subjects. Methods: Results are from the Phase I component of an ongoing Phase I/IIa randomised, double-blind, placebo-controlled, parallel-group, dose-escalation trial. Healthy adults received escalating doses of SPL026 via a 2-phase intravenous (IV) infusion. Dosing regimens were calculated based on PK modelling and predictions, with progression to each subsequent dose level according to safety and tolerability. In vitro experiments assessed hepatic clearance and metabolism of DMT by monoamine oxidase (MAO) and cytochrome P450 enzymes. Results: 24 healthy subjects received escalating doses of SPL026 which were safe and well-tolerated. Dose-proportional increases in DMT exposure were observed over the range of 9–21.5 mg. For all doses, median time to peak plasma concentration was ˜10 min and mean elimination half-life was 9–12 min. There was no relationship between peak DMT plasma concentration and body mass index, weight or age. In vitro hepatic mitochondrial fraction clearance of SPL026 was inhibited by MAO-A, but not MAO-B, inhibition. CYP2D6 and CYP2C19 modified SPL026 clearance in vitro. The unbound fraction of SPL026 was approximately 70%. Conclusion: This is the first study to determine, in detail, the full PK profile of DMT in humans, confirming rapid attainment of peak plasma concentrations followed by accelerated clearance. These findings provide evidence which support the development of novel DMT infusion regimens for the treatment of MDD.

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Running title: DMT fumarate human pharmacokinetics

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What is already known about this subject

N,N-dimethyltryptamine (DMT) is a psychedelic compound in clinical development for the treatment of major depressive disorder

It has been previously established that DMT is rapidly metabolised following intravenous (IV) administration; however, the complete pharmacokinetic (PK) profile of DMT in humans has not been fully characterised

What this study adds

This is the first study to determine the full PK profile of DMT in humans, confirming the rapid attainment of peak plasma levels and subsequent clearance, with dose proportionality observed between 9–21.5 mg IV doses

Human cytochrome P450 isozymes 2D6 and 2C19 may contribute to the metabolism of DMT

ABSTRACT

Aim: N,N-dimethyltryptamine (DMT) is a psychedelic compound under development for the treatment of major depressive disorder (MDD). This study evaluated the *in vitro* metabolism and clinical pharmacokinetics (PK) of DMT fumarate (SPL026) in healthy subjects.

Methods: Results are from the Phase I component of an ongoing Phase I/IIa randomised, double-blind, placebo-controlled, parallel-group, dose-escalation trial. Healthy adults received escalating doses of SPL026 via a 2-phase intravenous (IV) infusion. Dosing regimens were calculated based on PK modelling and predictions, with progression to each subsequent dose level according to safety and tolerability. *In vitro* experiments assessed hepatic clearance and metabolism of DMT by monoamine oxidase (MAO) and cytochrome P450 enzymes.

Results: 24 healthy subjects received escalating doses of SPL026 which were safe and well-tolerated. Dose-proportional increases in DMT exposure were observed over the range of 9–21.5 mg. For all doses, median time to peak plasma concentration was ˜10 min and mean elimination half-life was 9–12 min. There was no relationship between peak DMT plasma concentration and body mass index, weight or age. *In vitro* hepatic mitochondrial fraction clearance of SPL026 was inhibited by MAO-A, but not MAO-B, inhibition. CYP2D6 and CYP2C19 modified SPL026 clearance *in vitro* . The unbound fraction of SPL026 was approximately 70%.

Conclusion: This is the first study to determine, in detail, the full PK profile of DMT in humans, confirming rapid attainment of peak plasma concentrations followed by accelerated clearance. These findings provide evidence which support the development of novel DMT infusion regimens for the treatment of MDD.

1 INTRODUCTION

A number of clinical trials have supported the use of psychedelics for the treatment of major depressive disorder (MDD).[1]Psilocybin, an alkaloid found in certain types of mushroom, has shown efficacy in patients with treatment-resistant depression (TRD)[2,3] and comparable efficacy to escitalopram in a randomised controlled trial in patients with MDD.[4] In studies of patients with recurrent MDD or TRD, a single dose of ayahuasca, a natural psychedelic-containing plant brew was associated with improvements in depressive symptoms.[5,6]

N,N-dimethyltryptamine (DMT) is the principal psychedelic compound contained in ayahuasca.[7] DMT is not orally active as it is rapidly metabolised: its primary metabolite is indole-3-acetic acid (IAA), produced by monoamine oxidase (MAO) via oxidative deamination.[8] The production of DMT-N-oxide (DMT-NO; the second most abundant metabolite) and other minor metabolites is thought to be independent of MAO, and it has been suggested that enzymes of the cytochrome family may play a key role in this.[7–10]

A number of studies have evaluated the pharmacokinetics (PK) of DMT in humans, although these have largely been limited to investigations following oral ingestion of ayahuasca.[10–14] A single study showed that following bolus intravenous (IV) administration of DMT in healthy volunteers, peak blood concentrations were reached at approximately 2 minutes; however, no other PK variables were reported.[15] Similarly, after bolus or rapid IV infusion of DMT, psychedelic effects are apparent almost immediately and resolve within approximately 30 minutes. Dose dependency has been observed–the psychedelic experience is more intense with higher doses of DMT.[16,17] It has therefore been suggested that a more complete psychedelic experience can be achieved with modified IV infusion regimens to maintain therapeutic concentrations of DMT over a longer period.[18]

To date, the studies using DMT have been conducted in healthy psychedelic-experienced subjects.[19,20] However, no assessment of DMT in 'psychedelic-naïve' healthy subjects (i.e., subjects who have never taken a serotonergic psychedelic drug, in any form) has been made. This has potential bias as experienced users will tend to be a self-selected group who tolerate such substances well. A study in psychedelic-naïve healthy

subjects offers an advantage when determining a safe and well tolerated dose regimen of DMT, particularly as many patients in the target MDD population will also be psychedelic-naïve.

However, because of the potential psychological intolerance of an extended infusion procedure[21] and potential sensitivity of psychedelic-naïve individuals described above, a better understanding of the relationship between dosing regimen and psychedelic effects is warranted.

The purpose of the studies presented here was to investigate the PK of DMT fumarate (SPL026). We report results evaluating: 1) *in vitro*metabolism of SPL026 and the enzymes involved; and for the first time, 2) the PK profile of DMT following a slow IV infusion in psychedelic-naïve healthy subjects, which was conducted in the Phase I component of a Phase I/IIa clinical trial hereinafter, referred to as Phase I.

The objective of the Phase I study was to determine a safe and well-tolerated infusion dose of SPL026 that elicits a breakthrough psychedelic experience to give to patients with MDD in the Phase II component of the clinical trial. Phase I clinical outcome results and Phase II study details will be reported elsewhere.

2 METHODS

2.1 *In vitro* studies

Methods for the *in vitro* studies are summarised briefly here; full details can be found in the Supplementary Information. All analyses were performed using ultra-high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS).

The contribution of MAO vs other Phase I and II drug metabolising enzymes on SPL026 metabolism was investigated in human whole cell hepatocytes. *In vitro* metabolic stability of SPL026 was determined in the presence and absence of an irreversible and combined MAO-A/MAO-B inhibitor (100 nM clorgyline and 100 nM deprenyl/selegiline). Mitochondrial preparations contain a higher proportion of MAO enzymes relative to other Phase I and II enzymes and were therefore pre-incubated with and without separate MAO-A and MAO-B inhibitor solutions (clorgyline and deprenyl, respectively) to further assess the contribution of MAO[8] and its specific isoform with respect to SPL026 stability i*n vitro*. *In vitro* CYP phenotyping was performed to characterise the metabolism of SPL026 by 8 human CYP isozymes: CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2D6, CYP3A4 and CYP3A5 (Table S1).

The blood:plasma ratio of SPL026 was determined using fresh human blood and plasma samples from a donor panel. Plasma protein binding was assayed using pooled donor plasma and a Rapid Equilibrium Dialysis (RED) plate (Thermo Fisher Scientific, UK).

The distribution coefficient $\log D_{7.4}$ measures the partition of substances in octanol and aqueous solutions in a solution at pH 7.4, i.e., approximating that of blood. SPL026 was assayed with octanol and buffer prior to extraction and analysis.

2.2 Phase I/IIa clinical study

2.2.1 Subjects

To be eligible to participate in the Phase I clinical study male and female subjects were required to be aged 25 years and above, with no previous exposure to serotonergic psychedelic substances, and registered with a general practitioner within the UK. Subjects were also required to have a body mass index (BMI) 18.0–30.9 kg/m^2, no clinically relevant physical findings, electrocardiogram (ECG) or clinical laboratory parameters at the screening visit.

Subjects with a current or past diagnosis of a mental health disorder as defined by the American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5), a history of suicide attempts, or any first- or second-degree relative with a psychotic or bipolar disorder were excluded from the study.

2.2.2 Study design and treatment

The Phase I study was a randomised, double-blind, placebo-controlled, parallel-group dose-escalation trial (ClinicalTrials.gov: NCT04673383; EudraCT: 2020-000251-13). The study was conducted to meet criteria of European Medicines Agency (EMA) guidelines and Good Clinical Practice. The study was approved by the UK Medicines and Healthcare Products Regulatory Agency (MHRA) and London Brent ethics committee.

Due to the psychedelic effects of DMT and the absence of comprehensive safety and tolerability data from previous clinical studies, EMA guidelines for risk identification and risk mitigation were followed,[22] together with scientific advice from the MHRA. Additionally, the study was designed in accordance with published guidelines regarding the clinical assessment of hallucinogenic substances in humans.[23]

At the screening visit, subjects provided written informed consent and attended a structured interview with the study psychiatrist using the Mini-International Neuropsychiatric Interview. All subjects participated in individual or group preparation sessions, including advice on what to expect and how to respond to the psychedelic experience at screening and again the day prior to study drug administration.

Subjects were admitted to the clinical pharmacology unit (Hammersmith Medicines Research [HMR], London, UK) the day before study drug administration. No intake of alcohol or caffeine for 24 hours before and during the treatment period was permitted. On the day of treatment, study drug was administered in a room set up according to best practice principles for psychedelic studies,[23] including soft lighting, soft furnishings, music and photographs/art depicting scenes of nature; subjects were also asked to wear an eye mask during study drug administration. A therapist and a psychiatrist were present in the room, with additional clinical staff supervising study drug administration and blood sampling.

Subjects were required to remain in the clinical unit overnight for further psychological and safety evaluations and discharged the next morning dependent on satisfactory assessment outcomes. Follow-up was conducted by phone or video call for up to 3 months after study treatment.

Four single ascending dose levels of SPL026 were studied, dependent on tolerability and the PK profile of each dose. Further details of the clinical study and psychotherapy methodology will be published elsewhere.

SPL026 drug substance and drug product were manufactured in the UK in accordance with Good Manufacturing Practice (GMP) with 2.5 mg/mL DMT free base in 10 ml aqueous sterile solution; placebo consisted of the same ingredients and volume with the exception of the active substance. Active and placebo treatments were identical in appearance and administered in the same volume.

2.2.3 Dosing procedure

Study drug was administered as a continuous 10-minute IV infusion, split into 2 phases. A single cannula was inserted in the forearm vein with two separate syringes and two separate syringe pumps, connected by a 3-way tap in order to provide different infusions rates for the 2 phases of infusion. The first syringe pump infused study drug over 5 minutes (Phase 1), followed by another 5-minute infusion from the second syringe pump (Phase 2).

There were 4 dosing regimens of SPL026 (Table 1) and each dose cohort was randomised so that 6 subjects received SPL026 and 2 placebo (doses are stated as free base DMT unless otherwise indicated). Following the completion of each dose level, the infusion regimen for the next dose level was assessed according to PK modelling (described below).

2.2.5 PK modelling

The dosing regimen used in this study was selected based on PK modelling with the aim of generating a slower onset of the psychedelic experience, whilst achieving the same peak plasma concentrations attained in previous clinical studies. A one-compartment model was determined to best describe the PK of IV administered DMT fumarate data.[22,23] The resulting model parameters were used to simulate multiple infusion regimens of total DMT doses ranging from 9–21.5 mg. Each infusion regimen comprised a 6 mg dose given over the first 5 min to support the intent of a gradual onset of psychedelic experience, followed by a second infusion designed to elicit further psychedelic effects (see the Supplementary Information for full details).

Simulated PK profiles and associated peak plasma concentration (C_{max}) predictions are presented in Figure S1 and Table S2.

The plasma concentration data from Cohorts 1, 2 and 4 were also modelled using a one-compartment infusion model. The model was considered an appropriate fit for the individual plasma-concentration data from Cohort 1 (mean parameter errors <15%), Cohort 2 (mean parameter errors <20%) and Cohort 4 (mean parameter errors <20%) (Table S2, Table S3). The resulting mean dataset parameters were used to generate predictions at different infusion times and rates prior to dose escalation (Figure S2). Cohort 3 data was deemed inappropriate for such modelling due to multiple missing samples (described in the Results section).

Compartmental and simulated PK analyses were conducted using PCModfit version 6.9 for Windows running with Microsoft Excel (2019).[24]

2.2.6 PK

Blood samples were drawn using a cannula inserted in the forearm vein (the opposite side to where study treatment was administered) pre-dose and at a nominal 2, 5, 6, 7, 10 (end of IV infusion), 11, 13, 15, 30, 60, 120 and 240 min after the start of the infusion.

Plasma concentrations at actual timepoints for each subject were analysed. The following PK parameters were calculated using all available data for each evaluable subject: C_{max}; time to peak plasma concentration (T_{max}), area under the plasma concentration–time curve from time zero to time of last measurable concentration (AUC_{last}); area under the plasma concentration–time curve from time zero to infinity (AUC_{inf}); terminal half-life ($t_{1/2}$); clearance (CL); apparent volume of distribution during terminal phase after IV administration (V_z); volume of distribution at steady state after IV administration (V_{ss}); and mean residence time after IV administration (MRT_{inf}).

Determination of SPL026 and IAA in human plasma was conducted using a validated ultra high-performance LC-MS/MS system (Pharmaron UK Ltd., Hoddesdon, UK). The internal standard used for DMT was DMT-d_8 and control blank was human plasma (K_2EDTA; BiolVT Ltd.). The calibration range to be validated for DMT was 0.0619 ng/mL (lower limit of quantification [LLOQ]) to 310 ng/mL, and 2.00–1000 ng/mL for IAA.

2.2.7 Statistical analyses

The PK concentration population comprised subjects who received at least 1 dose of study treatment and for whom a blood sample had been analysed. The PK parameter population comprised subjects in the PK concentration population for whom PK parameters could be derived.

Actual sampling times were used to derive PK parameters and missing data were not imputed. Plasma concentrations of DMT below LLOQ of the LC-MS/MS were either treated as zero (if they occurred before T_{max}) or considered as missing.

Plasma concentrations and PK parameters were summarised by treatment, using descriptive statistics. For log-transformed parameters, the primary measure of central tendency was the geometric mean and other parameters, it was the arithmetic mean or median.

Dose proportionality was assessed using analysis of variance (ANOVA), linear regression and the power model for C_{max} and AUC_{last} parameters. Dose-normalised and log-transformed PK parameters were compared between dose cohorts using one-way ANOVA. The linearity between log transformed C_{max}, and AUC_{last} vs SPL026 was determined[25]using a linear regression model, setting the administered dose as the independent variable and each PK parameter as the dependent variable. The linear relationship between C_{max} and AUC_{last} dose were assessed using the power model (log PK parameter = $\alpha + \beta * \log(\text{dose}) + \varepsilon$), where α=intercept, β=slope. The power model was fitted by restricted maximum likelihood (REML) mixed effect model, with intercept and log(dose) as fixed effects. The dose proportionality of each PK parameter was confirmed if the 90% confidence interval (CI) of β (log PK parameter vs log dose) included the value 1.0.

The relationship between BMI, weight and age vs dose-normalised C_{max} and concentration at 5 minutes (C_{5min}) was determined by linear regression analysis to confirm that flat dosing (rather than weight-based dosing) of SPL026 was appropriate.

PK analysis was conducted by the Statistics and Data Management Department at HMR, using WinNonlin version 8.1 or higher. Descriptive statistics were derived using SAS version 9.4 or higher, including mean, standard deviation (SD), median, minimum and maximum values. Additionally, for PK variables percent coefficient of variation (%CV) and 95% CI of the arithmetic mean were derived. The adjusted coefficient of determination (R^2) measured the goodness-of-fit for the linear regression model (where values range from 0-1, 0 indicating that the predictor variable accounts for no variation in the dependent variable, and 1 predictor variable accounts for all variation in dependent variable values). Post-hoc analyses were performed using IBM SPSS version 28.0 and tested at the $p<0.05$ significance level.

3 RESULTS

3.1 *In vitro* experiments

Mean (SD) intrinsic hepatocyte clearance (n=2) of SPL026 was 19.4 (0.8) µL/min/million cells, with a half-life of 98.9 (3.9) min. Separately, MAO inhibition had a minimal impact on the intrinsic hepatocyte clearance rate of SPL026, 13.8 µL/min/million cells (half-life, 92.4 min) with MAO inhibitors vs 13.24 µL/min/million cells (half-life, 96.1 min) in the absence of MAO inhibition.

The contribution of MAO in the metabolism of SPL026 was further assessed in human liver mitochondrial fractions. The intrinsic clearance of SPL026 was reduced by approximately 11-fold with MAO-A inhibition compared to vehicle control (<3.9 vs 42.9 µL/min/mg protein), with an accompanying increase in half-life (>373.7 vs 33.7 min); however, there was no influence of MAO-B inhibition (intrinsic clearance, 42.7 µL/min/mg protein; half-life, 32.5 min). Blood:plasma ratio was 1.53 for SPL026 (Table 2). Around 70% of SPL026 was not bound to plasma proteins, suggesting greater efficiency for diffusion within the blood plasma, with a large proportion of the free drug available and subject to both circulation and metabolism. In addition, a mean $logD_{7.4}$ value of 0.15 indicated that the lipophilicity of SPL026 was very low.

CYP phenotyping using 8 human enzymes showed that the intrinsic clearance of SPL026 was <29 µL/min/nmol CYP. No turnover of SPL026 with CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP3A4 and CYP3A5 was detected for the duration of the study indicating that SPL026 is not a substrate for these isozymes. SPL026 intrinsic clearance was 801 µL/min/nmol CYP (half-life, 9 min) for CYP2D6 and 37 µL/min/nmol CYP (half-life, 189 min) for CYP2C19. This data corresponds to an estimated clearance rate of 9.5 and 0.3 µL/min/mg microsomal protein with CYP2D6 and CYP2C19, respectively, indicating a role for CYP2D6 and minor role of CYP2C19 in DMT metabolism in human liver microsomes.

3.2 Clinical study

3.2.1 Safety and tolerability

A total of 32 subjects received study treatment and were assessed for safety. Baseline demographics were similar across dosing cohorts, with a mean age of 36.4 years (range, 25–76 years), mean BMI of 25.0 kg/m^2 and 24 subjects were male (75%) (Table 3). SPL026 was found to be safe and well tolerated with treatment-emergent adverse events categorised as either mild or moderate and all were transient in nature. Further details of the SPL026 safety profile will be reported elsewhere.

3.2.2 PK analysis

As mean measured levels of DMT at the 120 min and 240 min timepoints were low (<0.1 ng/mL) (Table 4), these timepoints were excluded from (Figure 1) to improve graphical data representation. Due to dosing errors, 1 subject from Cohort 1, and 1 subject from Cohort 3 were excluded from PK analyses. The PK parameters from each dosing group are presented in Table 5.

PK analysis of 5 evaluable subjects from the first dosing cohort (Cohort 1) that received a total dose of 9

mg SPL026 (Table 1) revealed a large observed inter-individual variability for both C_{max}(mean, 20.8 ng/mL; range, 5.0–34.9) and AUC_{last} (mean, 349 ng.min/mL; range, 71–705) (Table 5, Figure 1). Median T_{max} was approximately 10 min (close to the end of the infusion), before an observed gradual decline over the remaining sampling period (Figure 1). Mean $t_{1/2}$ was 12.1 min (range, 5.8–18.3).

For the 6 subjects in Cohort 2 that received 12 mg SPL026 (6 mg over 5 min and then 6 mg over 5 min) mean DMT C_{max} = 30.6 ng/mL (range, 12.7–62.3), mean AUC_{last} = 451 ng.min/mL (range, 245–755) (Table 5, Figure 1) and mean $t_{1/2}$ was 9.5 min (range, 6.0–17.0).

With respect to the 5 subjects from Cohort 3 that received a dose of 17 mg SPL026 (6 mg over 5 min then 11 mg over 5 min), sampling issues for PK data resulted in 9 missing data points. It was therefore not possible to calculate any parameters for DMT other than C_{max}(mean, 72.1 ng/mL; range, 16.2–126.0), T_{max} (median, 9.8 min; range, 9.7–11.3 min) and AUC_{last} (mean, 842 ng.min/mL; range, 204–1298) (Table 5).

The final cohort (Cohort 4) received SPL026 infused as 6 mg over 5 min followed by 15.5 mg over 5 min (total dose 21.5 mg). For these 6 subjects mean C_{max} was 62.7 ng/mL (range, 29.0–107) (Table 5) and mean AUC_{last} was 835 ng.min/mL (range, 477–1052); the median T_{max} of 9.7 min and mean $t_{1/2}$ (12.1 min; range, 6.3–20.3) were in line with the previous dose cohorts.

Individual C_{5min} following a 6 mg dose, ranged from 3.32 ng/mL to 43.0 ng/mL across all dose cohorts. A one-way ANOVA revealed that there were no significant differences between cohorts ($F(3,20)=1.628$, $p=0.214$).

IAA concentrations were measured from 4 subjects from Cohort 2 and Cohort 4. However, IAA was detectable at 30 min for Cohort 4 only (1260–1780 ng/mL).

3.2.3 Dose proportionality

There was no statistical difference between cohorts in dose-normalised C_{max} ($p=0.346$) and AUC_{last} ($p=0.792$). Linear regression for log(dose) vs log(C_{max}) and log(AUC_{last}) were fit resulting in the following model parameters: C_{max} (adjusted $R^2=0.367$, $p=0.002$) and AUC_{last} (adjusted $R^2=0.330$, $p=0.003$). In the analysis of dose proportionality using the power model of C_{max} and AUC_{last}, each slope (β) was greater than 1, with corresponding CI crossing 1 (Table 6),[26] indicating dose proportionality for each of tested PK parameters.

Post-hoc analysis showed no relationship across all dose cohorts between BMI ($R^2=0.048$, $p=0.327$), weight ($R^2=0.009$, $p=0.681$) or age ($R^2=0.024$, $p=0.489$) with dose-normalised $C_{max,}$ and no relationship between BMI ($R^2=0.177$, $p=0.051$), weight ($R^2=0.061$, $p=0.269$) or age ($R^2=0.046$, $p=0.336$) with C_{5min}.

4 DISCUSSION

This is the first study to validly determine the elimination half-life and other PK parameters of DMT. This detailed PK profile of DMT was determined in healthy subjects following the administration of DMT fumarate (SPL026) as a slow IV infusion. The general PK profiles obtained from administration of different doses of SPL026 were similar to those obtained in previous studies,[15] demonstrating a very short mean plasma DMT half-life of 10–12 min; median T_{max} was 10–12 min, which differed significantly from that of other studies due to the slower IV infusion. Dose-proportional increases in C_{max} and AUC_{last} for doses of SPL026 between 9 mg and 21.5 mg were observed, although substantial inter-individual variability in C_{max} and AUC_{last} values was recorded across all dosing cohorts.

The variability observed in this study is in broad agreement with previously published data for DMT. In an early report of DMT administered by intramuscular injection at 0.7 mg/kg to healthy subjects, C_{max} values ranged from 30 to 150 ng/mL.[27] Similarly, in the study conducted by Strassman and Qualls, reported C_{max} for 10 subjects ranged from 32 to 204 ng/mL following an IV bolus of 0.4 mg/kg DMT.[14] It is possible that the PK variability may be explained, at least in part, by the rapid metabolism of DMT in the human body.

DMT administered by bolus IV injection[19,20] is calculated to have a half-life of approximately 5 min and

plasma clearance rate of 24,483 mL/min. Using the 1.53 blood:plasma ratio factor, this equates to a blood clearance rate of 16,002 mL/min, and 229 mL/min/kg based on a 70 kg person. This estimated clearance rate is much greater than average human liver blood flow (approximately 20 mL/min/kg with a cardiac output of 71 mL/min/kg). Based on these calculations, it can be expected that DMT is largely metabolised before reaching the human liver.

Despite a high level of inter-individual variability, the dose linearity and relative accuracy of prediction from infusion modelling confirms the development of an improved understanding of the PK profile of DMT in healthy individuals, which can be used to make estimations for different SPL026 dosing strategies. Our findings support a previous study of IV bolus of DMT, whereby a doubling of the dose administered was associated with an approximate doubling of C_{max}.[15]

The hepatic *in vitro* studies reported here with SPL026 confirm rapid clearance of DMT, with minimal impact of MAO inhibition. However, mitochondrial fraction clearance, i.e., with higher MAO enzyme levels, was substantially reduced by inhibition of MAO-A but not MAO-B, supporting our assumption that the primary route of DMT metabolism is extra-hepatic and via MAO-A *in vivo*.

These are the first experimental data to be published demonstrating CYP enzyme-mediated DMT metabolism, although this has previously been suggested.[8-10] Whilst it was found that DMT is a substrate for CYP2D6 (and to a lesser extent, for CYP2C19) *in vitro* , it has not yet been established whether this is relevant to the*in vivo* metabolism of DMT in humans due to its rapid clearance by MAO, as discussed above.

SPL026 exhibited low lipophilicity and plasma protein binding, indicating extremely high free drug concentrations and poor membrane permeability. Additionally, the *in vitro* human blood:plasma concentration ratio of DMT (1.53) suggests that a higher proportion of DMT is distributed in whole blood (e.g., erythrocytes) relative to plasma, indicating that plasma concentration data significantly underestimates overall blood concentrations and should be accounted for in blood clearance calculations.

Plasma concentration levels of IAA relative to DMT were similar to published data, which require further investigation.[10,28]

Administration of SPL026 was well-tolerated and there were no serious safety concerns, including minimal impact on blood pressure.

There are a number of study limitations. Firstly, missing samples from Cohort 1 and 3 resulted in inability to calculate PK parameters for all subjects. Secondly, DMT-NO metabolite analysis of the clinical plasma samples could not be completed as validated bioanalytical methods for its analysis could not be achieved.

In conclusion, this is the first study to determine the full PK profile and parameters of DMT in humans, confirming the rapid attainment of peak plasma levels and subsequent clearance. Additionally, these are the first published findings that identify specific human CYP isozymes (2D6 and 2C19) that can contribute to the metabolism of DMT. Moreover, it was demonstrated that when MAO-A and MAO-B enzymes are inhibited, an alternative CYP-mediated (and potentially other) metabolic routes can break down SPL026 without affecting its clearance rate in *in vitro* fractions. These findings contribute to the development of improved PK and metabolic models of DMT and to the design of IV infusion regimens for the treatment of mental health disorders.

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CONFLICT OF INTEREST STATEMENT

M.G., T.B., Z.J., C.R. and E.J. are all currently paid employees of Small Pharma and have owned stock in the company, C.T., D.E., G.A., R.W., C.H., H.T. and A.B., are all paid employees of contracted research

and academic organisations or independent consultants engaged by Small Pharma.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analysed during the current study are filed in EudraCT and are not publicly available [in accordance with the regulations for Phase 1 data]. Further information is available from the corresponding author on reasonable request.

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TABLES

TABLE 1 Final dosing regimens for SPL026 in healthy subjects

Cohort	Total dose (free base DMT)	Duration of IV infusion
1	9 mg	Phase 1: 6 mg over 5 min Phase 2: 3 mg over 5 min
2	12 mg	Phase 1: 6 mg over 5 min Phase 2: 6 mg over 5 min
3	17 mg	Phase 1: 6 mg over 5 min Phase 2: 11 mg over 5 min
4	21.5 mg	Phase 1: 6 mg over 5 min Phase 2: 15.5 mg over 5 min

DMT, N,N-dimethyltryptamine.

TABLE 2 Human blood:plasma (B:P) ratio of SPL026

Compound (concentration)[a]	Plasma MS response	Plasma MS response	Plasma MS response	Plasma
	Blood incubations Sample 1	Blood incubations Sample 2	Blood incubations Mean	Plasma Sample
SPL026 (0.156%)	114.24	116.69	115.47	180.46
Sumatriptan (0.05%)	177.90	172.01	174.96	230.21
Chloroquine (0.05%)	1.69	1.26	1.47	5.62
Chloroquine (0.156%)	1.01	1.10	1.05	5.26
Verapamil (0.05%)	32.41	34.81	33.61	27.60

Compound (concentration)[a]	**Plasma MS response**	**Plasma MS response**	**Plasma MS response**	**Plasma**
Verapamil (0.156%)	31.56	35.23	33.40	26.30
Diclofenac (0.05%)	1.38	1.58	1.48	1.04
Diclofenac (0.156%)	1.42	1.63	1.52	0.97

[a]All compounds were prepared in dimethylsulfoxide. MS, mass spectrometry.

TABLE 3 Subject characteristics (safety population)

Parameter

Placebo

(n=8)

SPL026 9 mg[a] (n=6)

SPL026 12 mg[a] (n=6)

SPL026 17 mg[a] (n=6)

SPL026 21.5 mg[a] (n=6)

Age, years

Mean (SD)

31.9 (6.7)

34.3 (7.1)

34.5 (8.7)

43.0 (17.4)

40.0 (8.5)

Range

26-42

27-43

25-44

28-76

28-50

Sex, n (%)

Male

7 (87.5)

5 (83.3)

2 (33.3)

5 (83.3)

5 (83.3)

Female

1 (12.5)

1 (16.7)

4 (66.7)

1 (16.7)

1 (16.7)

Race, n (%)

Asian

0

3 (50.0)

0

1 (16.7)

1 (16.7)

Black/ African American

1 (12.5)

0

1 (16.7)

1 (16.7)

1 (16.7)

Latin American

1 (12.5)

0

0

0

0

White

5 (62.5)

2 (33.3)

3 (50.0)

3 (50.0)

4 (66.7)

Other

1 (12.5)

1 (16.7)

2 (33.3)

1 (16.7)

0

Ethnicity, n (%)

Hispanic/ Latino

3 (37.5)

0

1 (16.7)

0

0

Non-Hispanic/ Latino

5 (62.5)

6 (100.0)

5 (83.3)

6 (100.0)

6 (100.0)

Weight, kg

Mean (SD)

84.5 (4.8)

79.5 (14.2)

59.6 (5.5)

74.2 (12.8)

80.6 (7.9)

Range

75.9-92.3

63.0-98.9

51.6-65.7

61.8-90.2

66.4-89.4

Body mass index, kg/m^2

Mean (SD)

26.5 (2.0)

25.7 (3.0)

21.4 (2.3)

25.0 (4.0)

26.0 (2.4)

Range

24.0-29.8

23.1-30.6

19.0-24.1

20.0-30.1

23.6-29.8

[a]Doses are free base DMT. SD, standard deviation.

TABLE 4 Summary of DMT concentration-time profiles after IV administration of SPL026

Mean (SD) DMT concentration (ng/ml)	**SPL026 9 mg[a] (n=5)**	**SPL026 12 mg[a] (n=6)**	**SPL026 17 mg[a] (**
Pre-dose	0	0	0
2 min	1.95 (1.57)	5.41 (1.80)	7.01 (11.75)
5 min	10.01 (7.01)	13.47 (3.29)	18.25 (16.57)
6 min	11.98 (8.69)	21.63 (18.63)	25.48 (23.75)
7 min	15.86 (13.24)	23.63 (19.55)	37.43 (37.13)
Immediately before infusion termination	16.27 (11.78)	26.05 (14.72)	62.28 (41.16)
11 min	17.47 (10.63)	23.14 (11.85)	62.80 (46.10)
13 min	14.22 (8.17)	20.50 (8.94)	22.55 (9.12)
15 min	7.77 (4.46)	16.73 (7.89)	18.30 (8.63)
30 min	7.02 (8.72)	3.78 (3.62)	3.68 (2.95)
60 min	0.43 (0.33)	0.65 (0.90)	0.49 (0.54)
120 min	0.05 (0.05)	0.06 (0.14)	0.05 (0.07)
240 min	0	0	0

[a]Doses are free base DMT. DMT, N,N-dimethyltryptamine; IV, intravenous; SD, standard deviation.

TABLE 5 Clinical pharmacokinetics of DMT following IV SPL026 administration

Parameter

SPL026 9 mg[a]

(n=5)

SPL026 12 mg[a] (n=6)

SPL026 17 mg[a,b] (n=5)

SPL026 21.5 mg[a] (n=6)

C_{max}, ng/mL

Mean (SD)

20.8 (12.9)

30.6 (18.1)

72.1 (47.1)

62.7 (25.8)

Range

5.0–34.9

12.7–62.3

16.2–126.0

29.0–107.0

CV (%)

0.62

0.59

0.65

0.41

T_{max}, min

Median (range)

9.6 (7.0–11.0)

10.5 (6.0–11.2)

9.8 (9.7–11.3)

9.7 (9.7–11.0)

AUC_{last}, ng.min/mL

Mean (SD)

349 (253)

451 (229)

842 (453)

835 (231)

Range

71–705

245–755

204–1298

477–1052

CV (%)

0.72

0.51

0.54

0.28

AUC_{inf}, ng.min/mL

Mean (SD)

352 (252)

455 (229)

-

837 (231)

Range

75–707

249–763

-

478–1054

CV (%)

0.72

0.50

-

0.28

$t_{1/2}$, min

Mean (SD)

12.1 (4.7)

9.5 (4.0)

-

12.1 (5.2)

Range

5.8–18.3

6.0–17.0

-

6.3–20.3

CV (%)

0.39

0.42

-

0.43

CL, L/min

Mean (SD)

46.0 (43.6)

32.4 (14.6)

-

27.9 (9.7)

Range

12.7–120.0

15.7–48.2

-

20.4–45.0

CV (%)

0.95

0.45

-

0.35

V_z, L

Mean (SD)

611 (308)

425 (214)

-

456 (157)

Range

241–996

172–683

-

218–653

CV (%)

0.50

0.50

-

0.34

V_{ss}, L

Mean (SD)

551 (346)

375 (173)

-

400 (149)

Range

246–1094

157–587

-

215–673

CV (%)

0.63

0.46

-

0.37

MRT_{inf}, min

Mean (SD)

14.6 (4.1)

12.4 (5.6)

-

15.0 (5.0)

Range

9.1–19.3

8.6–23.3

-

8.4–20.1

CV (%)

0.28

0.45

-

0.33

[a]Doses are free base N,N-dimethyltryptamine.[b]Insufficient data for calculation of other pharmacokinetic variables. DMT, N,N-dimethyltryptamine; IV, intravenous; SD, standard deviation, CV(%) coefficient of variation

TABLE 6 Dose proportionality of SPL026[a]

Pharmacokinetic parameter (dependent variable)	Model variable	Estimate (β)	90% CI
C_{max}	log(dose)	1.58	0.84–2.33
AUC_{last}	log(dose)	1.35	0.65–2.04

[a]Using the power model: log(PK parameter) = α + β * log(dose) + ε), where α=intercept β=slope. CI, confidence interval.

FIGURE LEGENDS

FIGURE 1 Mean plasma concentration–time profiles for SPL026[a] on a (A) linear and (B) logarithmic scale

[a]Doses are free base N,N-dimethyltryptamine.

FIGURE 2 C_{max} (A) and AUC_{inf}(B) for all doses of SPL026[a]

[a]Doses are free base N,N-dimethyltryptamine. DMT, N,N-dimethyltryptamine.