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ORIGINAL RESEARCH ARTICLE

Pharmacokinetics of Escalating Doses of Oral Psilocybin in Healthy Adults

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Abstract

Introduction Psilocybin is a psychedelic tryptamine that has shown promise in recent clinical trials for the treatment of depression and substance use disorders. This open-label study of the pharmacokinetics of psilocybin was performed to describe the pharmacokinetics and safety profile of psilocybin in sequential, escalating oral doses of 0.3, 0.45, and 0.6 mg/kg in 12 healthy adults.

Methods Eligible healthy adults received 6–8 h of preparatory counseling in anticipation of the first dose of psilocybin. The escalating oral psilocybin doses were administered at approximately monthly intervals in a controlled setting and subjects were monitored for 24 h. Blood and urine samples were collected over 24 h and assayed by a validated liquid chromatography-tandem mass spectrometry (LC–MS/MS) assay for psilocybin and psilocin, the active metabolite. The pharmacokinetics of psilocin were determined using both compartmental (NONMEM) and noncompartmental (WinNonlin) methods.

Results No psilocybin was found in plasma or urine, and renal clearance of intact psilocin accounted for less than 2% of the total clearance. The pharmacokinetics of psilocin were linear within the twofold range of doses, and the elimination half-life of psilocin was 3 h (standard deviation 1.1). An extended elimination phase in some subjects suggests hydrolysis of the psilocin glucuronide metabolite. Variation in psilocin clearance was not predicted by body weight, and no serious adverse events occurred in the subjects studied.

Conclusions The small amount of psilocin renally excreted suggests that no dose reduction is needed for subjects with mild–moderate renal impairment. Simulation of fixed doses using the pharmacokinetic parameters suggest that an oral dose of 25 mg should approximate the drug exposure of a 0.3 mg/kg oral dose of psilocybin. Although doses of 0.6 mg/kg are in excess of likely therapeutic doses, no serious physical or psychological events occurred during or within 30 days of any dose.

Clinical Trials Identifier NCT02163707.

Key Points

Psilocybin, as its active metabolite psilocin, demonstrates linear pharmacokinetics over the 0.3–0.6 mg/kg oral dose range tested.

Less than 2% of the psilocin in plasma is excreted in urine in that form, suggesting minimal effect of renal dysfunction in elimination of the active metabolite.

A fixed oral dose of 25 mg is expected to approximate the area under the concentration–time curve and concentration profile of the 0.3 mg/kg oral dose used in this study.

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1 Introduction

Psilocybin (4-phosphoryloxy-*N,N*-dimethyltryptamine) is a naturally-occurring tryptamine contained within *Psilocybe mexicana* Heim and other species of psychoactive mushrooms used for centuries by native cultures for shamanic or spiritual purposes. Psilocybin produces remarkable effects on consciousness, often described as 'psychedelic' or 'hallucinogenic' [1]. The effects have been characterized as an intense dream-like state with colorful visual illusions, changes in auditory, tactile, olfactory, gustatory, and kinesthetic perceptions, altered perceptions of time and space, changes in body image and sensations, and intense mood changes.

Interest in using psilocybin as a therapeutic agent has been rekindled based on clinical data indicating that, with appropriate preparation and dosing conditions, psilocybin has safety and efficacy in alleviating existential anxiety and depression associated with a diagnosis of terminal disease [2]. Concurrent placebo-controlled, phase II clinical trials at New York and Johns Hopkins Universities found clinically significant improvement in anxiety and depression in patients with incurable disease even 6 months after a single oral dose of psilcocybin [3, 4]. In addition, Carhart-Harris, et al. reported similar results in an open-label trial of patients with treatment-resistant depression in which two oral doses of psilocybin 1 week apart resulted in an improvement in depression in all subjects, with a sustained benefit measured 3 months after treatment in 67% of these previously refractory patients [5].

Following oral or parenteral administration, psilocybin is rapidly dephosphorylated to psilocin [6, 7], and it is believed that it is the psilocin that is responsible for the psychoactive effects of ingested psilocybin. Binding and partial agonist activity at serotonin 5 HT_{2A} receptors are required for the manifestation of psychoactive effects from psilocin and other tryptamines [8], although other serotonin and nonserotonergic receptors appear to be involved in the psychoactive effects of these substances [1, 9–16].

One requirement for developing psilocybin as a therapeutic agent will be an assessment of the pharmacokinetic profiles of psilocybin and psilocin in humans. Two studies have previously been published regarding the human pharmacokinetics of orally administered psilocybin. In one study, six volunteers were administered a single oral dose of psilocybin of approximately 0.2 mg/kg, and plasma concentrations of psilocin were determined over a period of 6.5 h [7]. In the second study, eight subjects received a single oral dose of psilocybin of approximately 0.2 mg/kg, and plasma and urine concentrations of psilocin were assessed over a 24-h period [17]. These studies demonstrated the immediate hydrolysis of psilocybin after oral dosing to its active metabolite psilocin, which demonstrated a half-life of 163 min, and the presence of approximately 67% of the psilocin present as the glucuronide.

The primary objective of this pharmacokinetic analysis was to develop a population pharmacokinetic model for psilocybin administered to healthy adults who received sequential, escalating oral doses. Secondarily, we sought to identify covariates that are predictive of the pharmacokinetic behavior of oral psilocybin, such as renal function and dose, and to characterize adverse events (AEs) associated with these doses. The earlier work of Hasler et al. [7] used a mass spectrometry assay that had a higher lower limit of quantitation (LLOQ) than was available for this study (0.8 vs. 0.5 ng/mL), and carried out their pharmacokinetic sampling for only 390 min after oral administration of psilocybin. In addition to extending the sampling duration and determining the renal excretion of psilocin, this study was performed following current Good Clinical Practices (cGCP) and current Good Laboratory Practices (cGLP) to support future attempts to obtain US FDA approval of psilocybin as a drug.

2 Materials and Methods

2.1 Study Design and Subject Selection

After approval by the University of Wisconsin-Madison Health Sciences Institutional Review Board, 12 healthy adults were recruited for the study by word of mouth and from inquiries from the ClinicalTrials.gov website (NCT02163707). Subjects were recruited and administered over a 15-month period. Subjects were first screened by telephone for general eligibility, and were then met in person for an in-depth screening visit. Eligible subjects were required to have had at least one substantial prior experience with a psychedelic due to concerns about the acceptance to the repeated blood sampling from an indwelling catheter for this pharmacokinetic study during the peak effects of psilocybin, which also required shifting to phlebotomy for each sample if the catheter patency was lost. No subject had used a psychedelic within 1 month of accrual to the study and all had negative urine drug screen results on each day of psilocybin dosing.

Subjects underwent a clinical interview with a licensed clinical psychologist using the Structured Clinical Interview for DSM-4 (SCID) [18] and were excluded if they met criteria for substance use disorder, seizure, or a history of bipolar disorder, psychosis, anxiety disorder, or major depressive disorder within the past 5 years. A first-degree relative with bipolar or psychotic spectrum diagnoses also

rendered the subject ineligible. Tobacco users not on a nicotine patch were excluded, as were individuals who would typically need to take medications within the 8-h period of drug action. Subjects were also excluded if they were taking antidepressants or monoamine oxidase inhibitors.

Each subject met with a trained pair of guides for 6–8 h of preparatory counseling prior to receiving the first dose of psilocybin. The same guide pair (male and female dyad) attended the subjects during each of their 8-h psilocybin sessions. The preparatory counseling sessions involved a review of relevant personal history, meditation and grounding exercises, preferred method for initiating communication (e.g. requesting assistance, alerting participant to an impending blood sample), and strategies for optimizing the potential personal benefit of the psychedelic experience. The same guide dyad met with the subject the morning after each dosing session for debriefing and integration of the experience. Separate, trained study staff performed the blood sampling, collection of vital signs, and reattachment and operation of a laptop-based 12-lead electrocardiograph (CardioCard, Nasiff Associates, Central Square, NY, USA).

Synthetic psilocybin was prepared under appropriate federal and state controlled substance permits. A certificate of analysis was prepared by the Zeeh Pharmaceutical Experiment Station housed in the University of Wisconsin-Madison School of Pharmacy. The analysis included purity by high-performance liquid chromatography (HPLC), water content, and demonstration of the absence of residual organic solvents or metals. The purity of the psilocybin was consistently over 99%, with approximately 1–6% residual water. Using actual body weight from their most recent visit or treatment, individual psilocybin doses of 0.3, 0.45, or 0.6 mg/kg were prepared 1–4 days prior to the date of the intended dose, and were corrected for water content. The psilocybin was added to one-half of an opaque #0 methylcellulose capsule, with lactose USP completing the fill of that capsule half. The study dose was not masked.

At a minimum of 4-week intervals, subjects received single, oral doses of psilocybin at escalating doses of 0.3, 0.45, or 0.6 mg/kg. On the morning of each dosing day, the subject arrived at the University of Wisconsin Clinical Research Unit (CRU) for placement of electrocardiography (ECG) electrodes, collection of vital signs, urine drug screen testing, pregnancy testing (if applicable), and placement of the intravenous catheter. The subject was then walked approximately 500 meters to the study room in the School of Pharmacy. After approximately 15 min of centering meditation, the subject emptied their bladder, a baseline 12-lead ECG was obtained along with vital signs, and the baseline blood sample was collected. Subjects were allowed a standardized breakfast in the CRU, and ingested the psilocybin capsule with 360 mL of water.

Anticoagulated (K_2 EDTA) blood samples for determination of plasma psilocybin and psilocin concentrations were collected from subjects just prior to each dose and at 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 12, 18, and 24 h postdose. Urine was collected for 24 h after the dose with the use of a commode insert. To stabilize psilocin, 5% ascorbic acid was added to each plasma and urine aliquot to make a final 25 mM ascorbate concentration [17]. After addition of the ascorbic acid solution, the aggregated urine collection was stored in crushed ice until aliquots were removed and frozen at the end of the 24-h collection period. Aliquoted and stabilized plasma and urine samples were stored in a −70 °C freezer until thawed for assay.

2.2 Liquid Chromatography–Tandem Mass Spectrometry Analysis

The analyses for psilocybin and psilocin were performed by Covance Laboratories (Madison, WI, USA) following cGLP methods. The assay included a solid-phase extraction step using a C18 matrix, with elution performed by a Luna Phenyl-Hexyl, 50 × 2 mm, 5 µm particle size column. The HPLC assay used an ammonium formate buffer (10 mM) and a gradient from 2 to 95% acetonitrile. Plasma and urine samples were treated with 1:1 methanol:acetonitrile to precipitate protein, and after centrifugation of the deep-well 96-well plates an aliquot was transferred to a new plate, dried under nitrogen, and reconstituted for injection. Psilocybin and its metabolite psilocin were quantified using mass-spectrometry [19, 20]. The Sciex API spectrometer used positive ion electrospray at 1200 V and 600C, with nitrogen gas used throughout. No parent psilocybin was found in any urine or plasma sample. The lower limit of quantitation (LLOQ) for psilocin in plasma and urine was 0.5 and 5.0 ng/mL, respectively. Loss of psilocin at room temperature for 24 h and over five freeze–thaw cycles was less than 3%.

2.3 Noncompartmental Pharmacokinetics

WinNonlin (v6.4; PharSight, Certara Corp, Princeton, NJ, USA) was used to determine maximum concentration (C_{max}), time to reach C_{max} (T_{max}), and area under the concentration–time curve from zero to 24 h (AUC_{24}) of psilocin in plasma. AUC was determined using the default trapezoidal-up, log-trapezoidal-down approach.

2.4 Population Pharmacokinetic Modeling

Model building and evaluation was conducted using NONMEM Version 7 level 3 (NONMEM; ICON

Development Solutions, Ellicott City, MD, USA), and followed standard model building approaches to define the structure, intersubject variability, and covariate dependence of the model [21, 22]. Modeling was performed on a Dell Inspiron 6420 i7 laptop running the current GFortran compiler. R version 3.2.3 was used for statistical modeling and graphics in combination with XPOSE4 [23, 24]. Wings for NONMEM was used as an interface for the model fitting and bootstrap simulations [25].

2.4.1 Base Model Development

A value of 0.71863 was given to F1 in the NONMEM model to assume that all psilocybin had been converted to psilocin, and to relate the measured amount of psilocin to the equivalent molar amount of psilocybin. The resulting clearance and volume terms from the model still include an unknown extent of conversion of the psilocin to its glucuronide or other metabolites. Psilocin concentration data were log-transformed.

The subroutines ADVAN2, ADVAN4, and ADVAN6 were used to model one-, two-, and three-compartment models, respectively, and the estimation method used first-order conditional estimation (FOCE) with interaction. Covariates such as hepatic and renal function and weight were tested against both clearance and distribution volume. In addition to the use of the initially reported data, the M3 method was applied to plasma psilocin concentrations that were below the LLOQ (0.5 ng/mL) [26]. Between-subject (intraindividual) variability (IIV, ETA) was assessed on each pharmacokinetic parameter using an exponential model. Residual error was modeled as an additive error on the log scale, resulting in a proportional error model, although a combined proportional and additive error model was tested with less satisfactory results.

Selection of the structural model was made by inspection of residual error plots, normalized prediction distribution error (NPDE) distributions, and visual predictive check (VPC), as well as by minimization of the objective function. A decrease in the objective function value of ≥ 3.84 was considered a significant ($p < 0.05$) improvement by the addition of a covariate term. Model selection also included consideration of the condition number, calculated as the square root of the ratio of the highest and lowest values of the Eigen matrix from the covariance step. Although a condition number less than 20 was sought, condition numbers less than 100 were considered to have acceptable levels of colinearity.

Given a prolonged peak and suggestion of a biexponential decay curve in some, but not all, dosing sessions, conversion to a psilocin glucuronide pool was also tested. This allowed recirculation of the psilocin glucuronide from the plasma to the liver with hydrolysis and reabsorption.

2.4.2 Covariate and Error Model Development

Covariates that significantly contributed to the model when added singly were sought in a manner similar to that used to develop the structural model. These covariates were then all added to the model and individually removed to determine their impact on the model. In addition to reductions in the objective function, the residual plots of NPDE and ETA versus covariate were inspected for evidence of a covariate effect. Furthermore, available demographic and laboratory covariates were added to the structural model to determine if the objective function and residual plots were improved.

Continuous covariates such as weight were normalized to the median of the study population and included as a power function, where P_1 and P_2 are fixed effect parameters, and R_{ref} is a reference value of the covariate (Eq. 1) affecting the typical value (TV) of a given parameter:

$$P_{\text{TV}} = P_1 \left(\frac{R}{R_{\text{ref}}} \right)^{P_2} \tag{1}$$

Categorical covariates of sex, race, and dose number were tested using a proportional model, where sex is 0 or 1 (male, female) and race or dose number (e.g. 1, 2, or 3) activated potential parameters associated with that covariate, where P_1 and P_2 are fixed-effect parameters (Eq. 2).

$$P_{\text{TV}} = P_1 \times (1 + R \times P_2) \tag{2}$$

Alternatively, this relationship was tested using the following relationship:

$$P_{\text{TV}} = P_1 \times R^{P_2} \tag{3}$$

Creatinine clearance was measured with a monitored 24-h urine collection that was begun just prior to each dose of psilocybin. Five percent (v/v) of 1 M ascorbic acid in water was added immediately to each urine collection fraction to stabilize the psilocin. After subtracting the volume of ascorbic acid added to the urine, the creatinine clearance was determined from Eq. 4:

$$\text{CLCr (mL/min)} = \frac{\text{Urine creatinine (mg/dL)} \cdot \text{Urine volume (mL)}}{\text{Serum creatinine (mg/dL)} \cdot \text{Collection duration (1440 min)}} \tag{4}$$

Body surface area (BSA) was estimated from the Mosteller equation [27], ideal body weight (IBW) was calculated from the method of Devine [28], and lean body weight (LBW) was calculated from the method of Janmahasatian [29].

2.4.3 Model Evaluation

One thousand bootstrap runs were performed using the NMBS routine in Wings for NONMEM using the

parameters from the final model to provide 95% confidence intervals (CIs) for the parameters. Covariate factors that had 95% CIs that included null were removed and the bootstrap rerun [30]. Additionally, a VPC [31, 32] was conducted to compare the distribution of simulated observations from the final model with those obtained from the original data. In this visual check, the concentration–time profiles were simulated using the final model parameters. The VPC was stratified by the three administered dose levels. In addition to the plots of IIV (ETA), the NPDE was examined and plotted against covariates [33].

2.4.4 Renal Clearance

Renal clearance was determined from the ratio of the amount of excreted psilocin in the 24-h urine collection divided by the plasma psilocin AUC over the 24-h collection period. Measured creatinine clearance was also tested as a covariate for clearance in the NONMEM model.

2.4.5 Flat Dose Simulation

Consideration is being given to the use of a flat dose of psilocybin for future studies instead of the current, individualized dosing of the drug (e.g. 0.3 mg/kg). Using the parameters of the final model for psilocybin, a VPC was performed with simulation of the concentrations expected from a single 20 and 25 mg dose, and was compared with the actual concentrations observed after the 0.3 mg/kg dose. The psilocin AUC and calculated C_{max} and T_{max} arising from the VPC simulation of both the 0.3 mg/kg and 25 mg doses were compared using an unpaired *t* test.

2.5 Adverse Events

All expected and unexpected AEs occurring from the time of enrollment into the study through the 30-day visit following the last dose were recorded and will be reported in detail in a subsequent manuscript. Severity of the AEs was graded using the Common Terminology Criteria for Adverse Events (CTCAE) version 4 (http://evs.nci.nih.gov/ftp1/CTCAE/). Laboratory values that were out of normal limits were recorded as AEs, but determination of clinical significance and attribution was made by the study physician.

3 Results

The final dataset contained 353 evaluable measurable plasma psilocin concentration observations from 12 subjects. When values below the LLOQ were included for the M3 method, an additional 48 plasma samples were available. One subject was removed from the study and replaced because no blood samples could be obtained from the indwelling catheter or venipuncture at any timepoint after the first dose despite being normotensive. One subject received only one of the three planned doses due to 'white-coat' hypertension. A third subject received only two doses of psilocybin because of difficulty getting the time off from work to participate in the study (Tables 1, 2).

Table 1 Categorical demographics

	All subjects	
	N	%
Total	12	100
Sex		
Male	10	83
Race		
White	11	92
Native American	1	8
Hispanic	0	0

3.1 Structural Model

The final model for psilocin in serum following oral administration of psilocybin capsules was a one-compartment model with linear clearance and linear absorption. The model was parameterized in terms of absorption rate constant (Ka), clearance (CL/*F*), and volume of distribution of the central compartment (V_1/F). Although the prospective model-building criteria slightly favored the selection of a two-compartment model, the plots of concentration versus time for some subjects appeared, by inspection, to favor a one-compartment model. It was considered that the sensitivity of the assay might have limited the ability of the model to distinguish a two-compartment model; however, inclusion of the below limit of

Table 2 Summary of baseline continuous covariates for all subjects ($N = 12$)

	Units	Mean	Median	Range
Age	years	43	43	24–61
Height	cm	179.1	177.5	169.3–187.7
Weight	kg	78.1	71	60.9–119.8
BMI	kg/m^2	24.2	23.5	19.4–34.0
BSA	m^2	1.96	1.86	1.73–2.50
IBW	kg	73.4	72.8	60.8–82.0
LBW	kg	60.0	56.5	51.1–79.7
CLCr	mL/min	126	123	71–177
CLCr/1.73 m^2	mL/min/1.73 m^2	73	71	41–102

BMI body mass index, *BSA* body surface area, *IBW* ideal body weight, *LBW* lean body weight, *CLCr* creatinine clearance

quantitation (BLOQ) plasma psilocin concentrations in the range of 0.25–0.5 ng/mL (LLOQ) using the M3 method did not improve the fit of the model and was not used in covariate screening. For those subjects for whom a two-compartment model provided a better fit than a one-compartment model, the volume of the peripheral compartment was over 20,000 L.

As an alternative to a second tissue compartment, the inclusion of a compartment for psilocin glucuronide improved the model. The inclusion of a bile-gut transit process did not improve the model over the addition of rate constants to a 'compartment' of psilocin glucuronide. Stepwise forward and backward removal of covariates suggested no effect of weight on CL/*F*, but normalized dose, total bilirubin, and albumin appeared to improve the fit of CL/*F*, and normalized weight affected apparent volume of distribution (*V*/*F*). When the bootstrap of this combined model was performed, the 95% CIs for these covariate factors included null, suggesting that their inclusion in the model was not needed. Additionally, a bootstrap of the model with and without normalized weight as a covariate of *V*/*F* did not significantly alter the estimates for *V*/*F* or CL/*F*. The final model therefore included no covariates, but did incorporate a compartment in which psilocin glucuronide could be formed and hydrolyzed to reform psilocin. This psilocin glucuronide compartment provided the allowance for apparent biexponential decay of psilocin concentrations noted in some subjects. Inclusion of renal function as a covariate of the loss of psilocin glucuronide did not improve the fit of the model.

Between-subject variability was applied to both the clearance and distribution volume terms (CL/*F* and *V*/*F*), and allowing for the incorporation of the off-diagonal of the covariance matrix (BLOCK 2) improved the fit. The limited number of subjects and sampling strategy impaired the ability to estimate Ka, and without assays of psilocin glucuronide the kinetics of this compartment were only indirectly estimated. Confidence in the final model estimates for other parameters improved when the interindividual variation (ETA) for Ka and K32 (glucuronide to psilocin) were fixed at null.

The renal clearance of psilocin was 1.7% of the total clearance of this active metabolite, and renal function was not a useful covariate in the pharmacokinetic model of clearance.

3.2 Final Compartmental Model

The final pharmacokinetic model included a linear one-compartment and bidirectional compartment of psilocin glucuronide (Fig. 1). The pharmacokinetic parameters for the model are provided in Table 3.

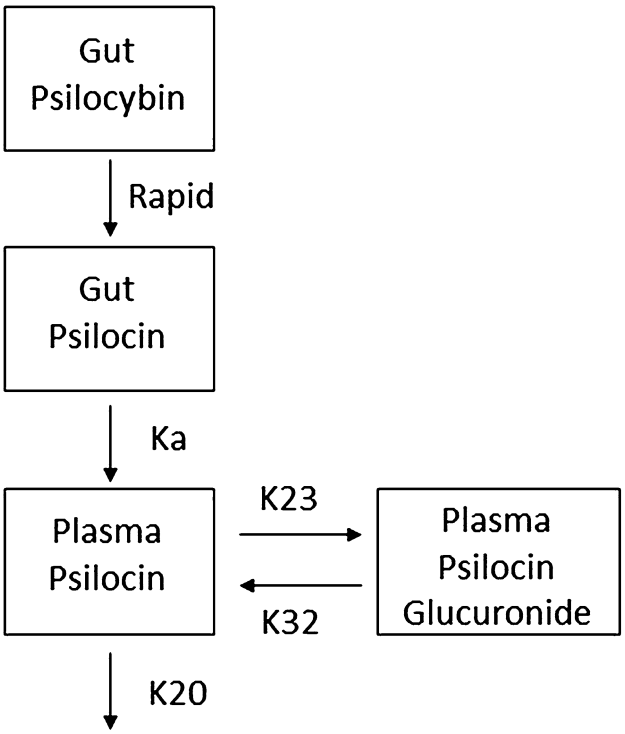

Fig. 1 Psilocybin pharmacokinetic model. *Ka* first-order rate constant

3.3 Noncompartmental Analysis

The pharmacokinetics of psilocin over a twofold dosing range were linear. This was indicated both by the structural model and its diagnostic plots, and by the noncompartmental evaluation of dose-normalized AUC and C_{max} (Fig. 2). The slope of AUC/dose or C_{max}/dose versus dose was not significantly different than 0, with 95% CIs of −308 to 222 (μg·h/L)/(mg/kg) and −43.6 to 61.3 (μg/L)/(mg/kg), respectively (Table 4).

3.3.1 Renal Clearance

Renal clearance of psilocin was calculated from the ratio of the amount of this active metabolite excreted in urine over 24 h divided by the AUC for the same 24-h interval, as reported by WinNonlin. Only 1.7% (95% CI 1.4–1.9%) of the administered dose of psilocybin was found as psilocin in the urine. When calculated as the amount of renally excreted psilocin with respect to the plasma psilocin AUC_{24}, the renal clearance of psilocin was 1 mL/min/kg, or 58% that of the corresponding measured creatinine clearance. The addition of measured creatinine clearance as a covariate in the NONMEM mixed-effects model did not improve the fit of the data, and did not lead to a successful convergence of the model.

Table 3 Parameter estimates for final pharmacokinetic model: psilocin in serum

Parameter (units)	PK parameter mean (SE %)	IIV (%CV; % shrinkage)	Bootstrap median (95% CI)	Bootstrap IIV [%CV] (95% CI)
CL/F (L/h)	164 (23.2)	31.4% (61.9; 1.5)	164.5 (29.0–224)	31.9 (15.8–145)
V/F (L)	298 (20.2)	50.4% (51.2; 2.6%) CL:V covariance: −0.269	305 (212–465)	47.3 (24.8–72.2) Covariance: −0.387 (−100 to 61.9)
Ka (1/h)	0.367 (9.3)	NE	0.372 (0.322–0.482)	NE
K23 (1/h)	0.212 (39.6)	NE	0.198 (0.1–0.576)	NE
K32 (1/h)	0.0175 (55.9)	NE	0.017 (0.006–0.068)	NE
Residual error	0.43 (6.3)	NE	0.425 (0.375–0.48)	NE

PK pharmacokinetic, *SE* standard error of the mean, *CV* coefficient of variation, *NE* not estimated, *CI* confidence interval, *IIV* interindividual variability, *CL/F* apparent clearance, *V/F* apparent volume of distribution, *Ka* first-order rate constant, *K23* rate of formation of psilocin glucuronide from psilocin, *K32* rate of hydrolysis of psilocin glucuronide back to psilocin

3.4 Safety

Detailed presentation of the adverse effects noted during and after the dosing sessions with psilocybin will be published elsewhere. No serious AEs were reported. The side effects of the doses reflected previously reported findings, and included mild, transient hypertension and tachycardia [34, 35]. As previously reported, mild headaches were common in the second 12 h of the 24-h study periods, and were successfully treated with acetaminophen [36]. No reports of hallucinogen persisting perception disorder (HPPD) were reported during study or at follow-up.

3.5 Simulation of Fixed Psilocybin Doses

Simulations ($N = 500$) were performed using the final pharmacokinetic model to evaluate the effect of administering a fixed dose of psilocybin (20 or 25 mg) instead of administering a 0.3 mg/kg dose to subjects included in the present study. Simulations were plotted using a VPC with an overlay of the actual concentrations observed after the 0.3 mg/kg oral dose. The VPC plots demonstrate that the expected concentrations from the 25 mg fixed dose overlap with the observed concentrations (Fig. 3).

Psilocin AUC and C_{max} expected from a fixed 20 and 25 mg dose are compared with the actual data points for AUC and C_{max} for study subjects who received the 0.3 mg/kg dose (Fig. 4). The plotted AUC values are derived from the dose and estimated clearance from the compartmental model. The outliers from the box and whisker plot reflect the influence of the subject weighing 120 kg, and the large number of these outliers ('+' symbol) arises from the 500 iterations performed for the VPC test. The interquartile range (box region) of AUC and the C_{max} estimated for a fixed psilocybin 25 mg dose approximates the range of concentrations following the actual 0.3 mg/kg dose (blue circles).

Figure 5 compares the AUC and C_{max} of psilocin following a fixed psilocybin dose of 25 mg versus a weight-based dose of 0.3 mg/kg. Although the regression lines suggest that the weight-based dosing of psilocybin may result in higher than average exposures in heavier patients, the slopes of these lines are not significantly different than null. Instead, the extensive overlap of the respective prediction CIs supports the use of a fixed dose of psilocybin over this dose and weight range.

4 Discussion

Our findings corroborated previous reports of the pharmacokinetics of psilocybin and its active metabolite, psilocin [7, 18]. No parent psilocybin was detectable in plasma or urine, arguing for the rapid luminal and first-pass dephosphorylation of psilocybin to psilocin. Although psilocybin metabolites psilocin-*O*-glucuronide and 4-hydroxyindole alcohol have been reported to be present in concentrations several-fold greater than psilocin in plasma [17], we did not determine their concentrations in this study due to funding constraints. The activity of the metabolites of psilocin has not been described.

The pharmacokinetics following the administration of escalating doses of oral psilocybin were best fit with a two-compartment model of elimination, but the large distribution volume of the tissue compartment suggested from a two-compartment fit (21,500 L) is difficult to justify on a physiologic basis. Rather than presume a tissue compartment of psilocin, the model was adapted to allow the reversible glucuronidation of psilocin. No separate compartment was assumed for the glucuronide, but it was instead assumed to share the volume of the central compartment. Although multiple organs have β-glucuronidase activity, the cleavage of the sugar to reform psilocin was assumed to occur in the the vascular compartment [37]. This 'two-compartment' model does not necessarily

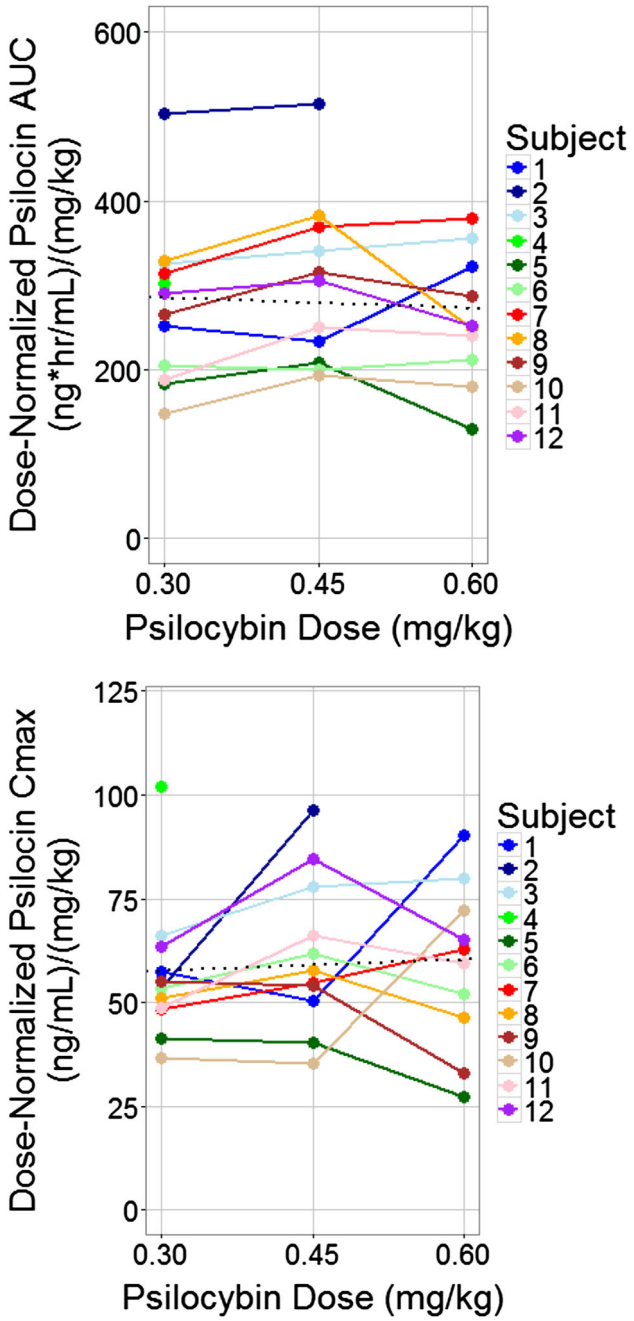

Fig. 2 Dose-normalized plasma psilocin AUC and C_{max}. The *dotted black line* represents the least squares regression. *AUC* area under the concentration–time curve, C_{max} maximum concentration

contradict the monoexponential model described by Hasler et al. [7], which utilized a slightly less sensitive assay and collected samples for only 6.5 h instead of over 24 h.

A confounder of the incorporation of a reversible psilocin-glucuronide 'compartment' is that we did not have the resources to synthesize standard psilocin glucuronide or other metabolites that would have provided support for this component of the model. Additionally, the report by Hasler et al. [17] suggested that after enzymatic hydrolysis of plasma, the concentration of psilocin approximately doubled at most of the sampling time points. In contrast, the stoichiometric amount of psilocin estimated to be in the glucuronide compartment in the present model is 29.4% (SD 8.7) of the psilocin concentration. In addition, although the present model does fit the observed psilocin concentrations well, it does not include the direct elimination of the glucuronide, which, although presumed to occur, cannot be fitted with the data available.

The peak concentration of psilocin was more gradually attained in some subjects than in others, but the broad peak psilocin concentrations did not appear to be a function of dose. Multiple alternative models were tested to attempt to characterize these findings, including an intermediate absorption compartment of enterochromaffin cells that might temporarily sequester and release the serotonin analog psilocin. Additional models included transit compartments of psilocin to bile and gut, and mixed order (first and mixed order) elimination of psilocin. Such models did not yield improved objective functions, and were uniformly poorly conditioned and parameters were indeterminately resolved. Although the unavailability of plasma psilocin-*O*-glucuronide concentrations made it impossible to confirm the glucuronide as the source of the apparent two-compartment pharmacokinetics, the premise of an exchangeable 'reservoir' of psilocin was considered to be plausible and explained the plasma psilocin concentrations well.

The 24-h collection of urine after the administration of psilocybin allowed the renal clearance of psilocin to be determined from the amount of psilocin in the aggregate 24-h urine collection and the plasma psilocin AUC_{24}. We chose to add stabilizing ascorbic acid to the aggregate urine collection kept in crushed ice, rather than to add ascorbate and immediately freeze individual urine collections. The use of DL-dithiothreitol as a reducing agent was not considered necessary due to the excellent peak resolution in the presence of ascorbate [38]. The renal clearance of psilocin in the present study was less than 2% of total clearance, which is similar to the 3.4% renal excretion reported by Hasler et al. [17]. The renal clearance of psilocin was 58% that of measured creatinine clearance. Based on these findings and the lack of influence of measured creatinine clearance on the pharmacokinetic model, no adjustment of the dose of psilocybin appears warranted in subjects with impaired renal function. Furthermore, moderately impaired renal function does not appear to be a justifiable criterion for exclusion of subjects from future studies, but this presupposes that psilocin-*O*-glucuronide and the 4-hydroxyindole metabolites are neither active nor renally excreted. No data were found to support or refute activity of these metabolites.

Only two women and two non-White subjects were recruited to this study. Advertising for the study was by

Table 4 Plasma psilocin AUC and C_{max} by dose level

Dose level (mg/kg) [doses]	Plasma psilocin AUC (μg·h/L)	Dose-adjusted plasma psilocin AUC (μg·h/L)/(mg/kg)	Plasma psilocin C_{max} (μg/L)	Dose-adjusted plasma psilocin C_{max} (μg/L)/(mg/kg)	T_{max} (h)
0.3 [$N = 12$]	140 (102–175)	6.06 (4.61–7.34)	16 (14.5–17.2)	0.7 (0.584–0.779)	2.03 (1.15–2.07)
0.45 [$N = 11$]	213 (150–261)	6.84 (4.61–8.13)	26 (22.7–35.1)	0.838 (0.781–0.875)	2.03 (1.3–3)
0.6 [$N = 10$]	267 (201–356)	6.84 (4.61–8.13)	37.6 (27.7–43.2)	0.799 (0.645–1.096)	2.05 (1.55–2.08)

Data are expressed as median (25th and 75th percentiles) [number of doses administered]

AUC area under the concentration–time curve, C_{max} maximum concentration, T_{max} time to reach C_{max}

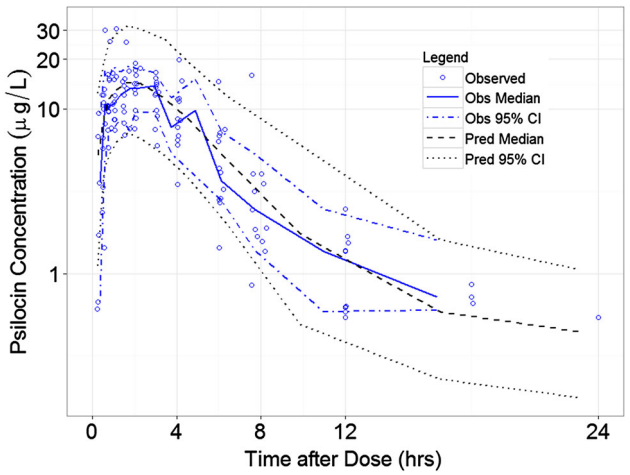

Fig. 3 VPC plot comparing observed results from psilocybin 0.3 mg/kg oral dose versus simulated 25 mg oral dose. The *open*, *blue circles* are the observed concentrations after a psilocybin 0.3 mg/kg oral dose; the *solid* and *dotted-dashed blue lines* are the median and 95% CI of these observed concentrations; and the *dashed* and *dotted black lines* are the predicted median and 95% CI of the concentrations from a flat 25 mg dose administered to the same subjects. *VPC* visual predictive check, *CI* confidence interval, *Obs* observed, *Pred* predicted

word of mouth and this, plus the imbalanced ethnic demographics of Madison, Wisconsin, led to a low representation of non-White races and ethnicities. Although we found no evidence of a sex or race effect on the pharmacokinetics of psilocybin, our ability to make such distinctions was limited. Similarly, the psilocybin dose range of 0.3–0.6 mg/kg was only twofold, and limited our ability to identify nonlinear pharmacokinetics. Higher doses were considered an excessive risk, but evaluation of lower doses such as 0.2 mg/kg or even 0.1 mg/kg psilocybin would have improved the confidence of a finding of linearity.

Future progress to phase III trials of psilocybin will require the use of an oral formulation meeting the FDA and European Medicines Agency (EMA) expectations of current Good Manufacturing Practice (cGMP). All recent clinical studies of oral doses of psilocybin have utilized individually prepared doses based on body weight (e.g. 0.3 mg/kg or 21 mg/70 kg). Oral psilocybin doses greater than approximately 0.3 mg/kg, such as those evaluated in this phase I trial, are not expected in future clinical trials. Weight-based dose individualization would dramatically complicate the standardization and validation of a cGMP product and its distribution, particularly for a scheduled drug. An alternative is to utilize a fixed dose of psilocybin. Simulations were performed for single 20 and 25 mg oral doses of psilocybin, and, in the present study, were compared with the actual concentrations observed after a single 0.3 mg/kg

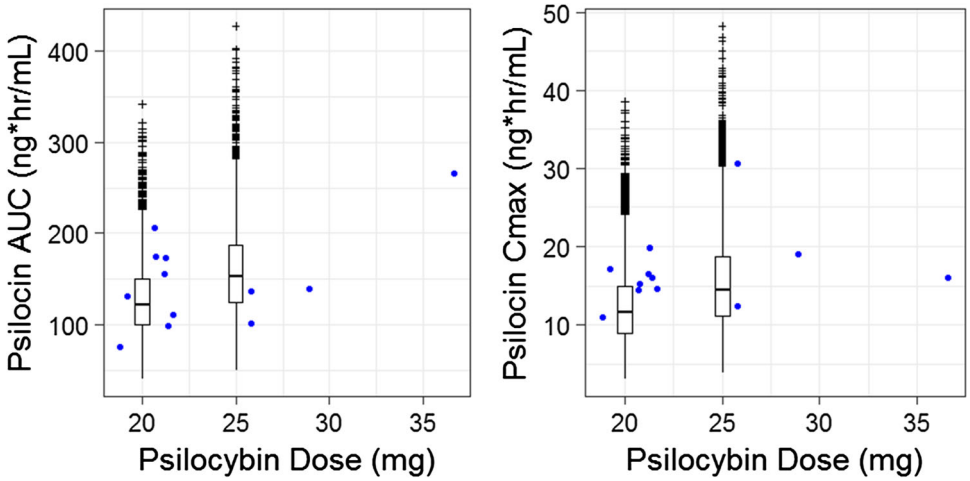

Fig. 4 Comparison of psilocin AUC and C_{max} following fixed psilocybin 20 and 25 mg doses versus a 0.3 mg/kg dose. *Blue circles* represent the observed AUC and C_{max} in subjects; the *box* represents the first and third quartiles (25th and 75th percentile) of 500 simulations of AUC and C_{max} from psilocybin 20 and 25 mg oral doses; and the *black crosses* represent outliers beyond the whisker denoting 1.5 times the upper quartile range. *AUC* area under the concentration–time curve, C_{max} maximum concentration

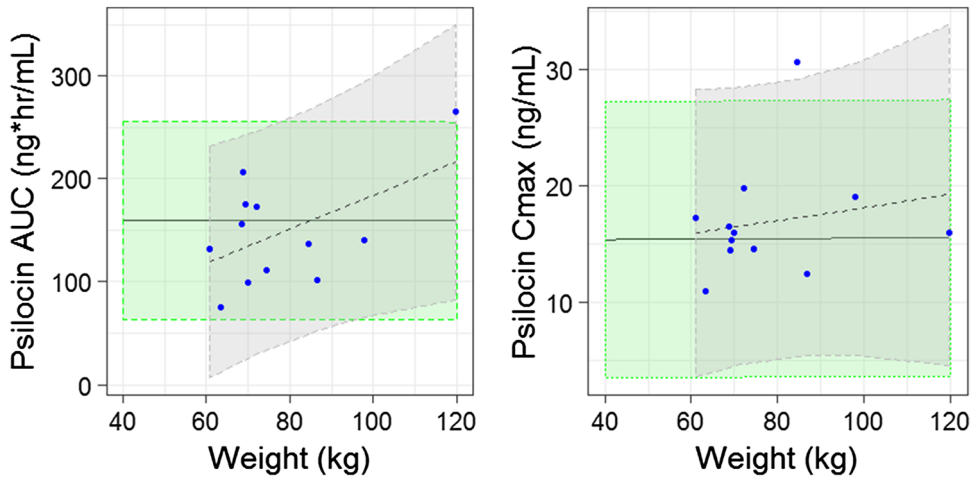

Fig. 5 Comparison of psilocin AUC and C_{max} by weight for the psilocybin 25 mg versus 0.3 mg/kg dose. *Blue circles* represent the observed AUC and C_{max} in subjects; the *dashed*, *black lines* and *grey shading* show the least-squares fit and 95% prediction interval for the subject results following their psilocybin 0.3 mg/kg oral dose; and the *solid black lines* and *green shading* show the respective AUC and C_{max} following the psilocybin 25 mg dose, based on the 500 simulations of the final pharmacokinetic model. *AUC* area under the concentration–time curve, C_{max} maximum concentration

dose. Although there is good overlap for both fixed doses with the observed concentrations, the correspondence is better with the 25 mg fixed dose at the time of peak plasma concentration and in the first 6 h, corresponding with the peak effect of the drug.

No serious AEs were noted in the 12 subjects treated with oral psilocybin. One subject was removed from the study before receiving the second dose because his predose blood pressure (BP) exceeded initial eligibility criteria. After several months of comparing recorded home BP measurements versus those obtained at the CRU, it was determined that the subject demonstrated 'white-coat' hypertension. In consultation with CRU staff, it was learned that multiple BP readings were taken on the initial eligibility screening visit in the successful hope that the initially elevated BP might fall to acceptable concentrations. Given that pharmacokinetic sampling was obtained after the first dose, this subject was considered eligible.

Another subject withdrew after completing the components of the second dose of psilocybin due to difficulty in getting off work midweek for the study. Although not demonstrating adverse effects, another subject was declared unevaluable after all attempts at drawing blood after the first dose of psilocybin or at phlebotomy were unsuccessful. Given that collection of postdose blood samples was not feasible, the IRB permitted this subject to be replaced. A subsequent report will describe relationships between plasma psilocin concentrations and the psychological effects of psilocybin, and associations of psilocin concentration with mild but reportable AEs such as transient hypertension and tachycardia.

5 Conclusions

This study demonstrated the linearity of psilocin exposure over the oral psilocybin dose range of 0.3–0.6 mg/kg in healthy adults, and suggests that the formation and hydrolysis of psilocin glucuronide may explain a biexponential decay curve of psilocin. All doses were well tolerated. Less than 5% of the oral psilocybin dose was excreted in the urine as psilocin, suggesting no need for dose adjustment in patients with mild to moderate renal impairment. Lastly, the use of a fixed oral psilocybin dose of 25 mg is expected to result in psilocin AUC and C_{max} exposures similar to those demonstrated after the individualized 0.3 mg/kg oral dose.

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Compliance with Ethical Standards

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Conflicts of interest Randall T. Brown, Christopher R. Nicholas, Nicholas V. Cozzi, Michele C. Gassman, Karen M. Cooper, Daniel

Muller, Chantelle D. Thomas, Scott J. Hetzel, Kelsey M. Henriquez, Alexandra S. Ribaudo, and Paul R. Hutson declare that they have no conflicts of interest that might be relevant to the contents of this article.

Ethical approval All procedures performed in these studies involving human subjects were in accordance with the ethical standards of the institutional research committee, and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in this study. Consent was re-established after any change in protocol while the subject was still on study.

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