RESEARCH ARTICLE

Development of a physiologically based pharmacokinetic (PBPK) model of psilocybin and psilocin from magic mushroom in rats and humans [version 1; peer review: 1 approved with reservations, 1 not approved]

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Abstract

Background: Psilocybin (PB) is a psychoactive compound commonly found in magic mushroom (*Psilocybe cubensis*). PB is quickly converted by the body to psilocin (PI), which has a psychedelic effect through the activation of the 5-HT_{2A} receptor in the brain. The objective of this study is to develop a physiologically based pharmacokinetic (PBPK) model of PB and PI in rats and humans for predicting concentrations of the psychoactive substance in the brain.
Methods: Following a search in PubMed, three studies were retrieved and information concerning concentration-time profiles of PI were extracted from the selected studies. In the study in rats, PI was orally administered with a dose of 10.1 mg/kg. There were two studies in humans following a single intravenous dose of PB (1 mg) and oral dose of PB (0.224 mg/kg and 0.3 mg/kg). Berkeley Madonna software was used for computer coding and simulations. The developed PBPK model consisted of seven organ compartments (i.e. lung, heart, brain, fat, muscle, kidney, and liver).
Results: The simulations show a good agreement between observed and simulated data, although results for oral administration in rats and humans showed under-predictions and results for intravenous administration in humans showed over-predictions.
Conclusions: A PBPK model of PB and PI in rats and humans was developed and could predict concentration-time profiles of PI in plasma, particularly in the brain, following intravenous and oral

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administration of PB. This model may be useful for a safer dosage regimen of PB for patients with some disorders.

Keywords
Psilocybin, Psilocin, Magic mushroom, Gold cap mushroom, PBPK model

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Introduction

Magic mushroom (*Psilocybe cubensis*), also called gold cap mushroom, can be found in tropical and subtropical areas around the world. Magic mushroom is a psychedelic mushroom that can cause euphoria, hallucinations (mental, auditory) and changes in perception[1]. Psilocybin (PB) and psilocin (PI) are the major psychoactive compounds in the mushrooms[2].

PB (4-phosphoryloxy-N,N-dimethyltryptamine) is a compound found in the mushroom in a psychologically inactive form. It can be orally absorbed (F = 0.5) following single oral administration in humans[2]. After being orally absorbed, PB is rapidly dephosphorylated by alkaline phosphatase and non-specific esterase to produce an active metabolite, namely PI (4-hydroxy-N,N-dimethyltrypt-amine)[2,3]. Since PI is structurally similar to serotonin, PI can bind and activate several receptors in the brain such as 5-HT_{2A}, 5-HT_{1A}, 5-HT_{1D}, and 5-HT_{2C} receptors[3]. Activation through the 5-HT_{2A} receptor can lead to the psychedelic effects of the mushrooms[4]. PI can be glucoronidated by UDP-glucuronosyltransferase (UGT) 1A10 in the liver and intestinal mucosa[5].

There have been studies of PB for treatment of some psychological disorders such as depression, suicidal attempts, obsessive-compulsive disorder (OCD), alcohol use disorder, tobacco use disorder and resistant depression[5,6]. However, knowledge of how PB and PI behave in the body is still incomplete, particularly information concerning PI concentration levels in the brain, the major target organ of the magic mushroom. As a result, dosing of PB and PI in humans can lead to inadequate outcomes. Physiologically-based pharmacokinetic (PBPK) modeling is a quantitative tool capable of predicting concentration-time profiles of chemicals including drugs, toxicants and natural products[7–12]. Any biologically relevant process related to the PK of the drugs can be incorporated into the model. To be able to quantify brain concentration levels of PI in human brains following PB administration, a PBPK model of PB and PI with a description of a brain compartment can be useful in understanding the disposition of PI. Therefore, the objective of this study was to develop a PBPK model of PB and PI in rats and humans.

Methods

PBPK model development

PBPK model structure. A PBPK model structure consists of seven compartments including the lung, heart, brain, fat, muscle, kidney, and liver. All of the organ compartments are linked together by the blood circulation system. The lung is the site of an interchange of the arterial and venous blood. The brain is the major site of action of PI. PI is assumed to solely be eliminated from the body through the liver. Adipose tissue is the storage organ for PI in the body. Additional organs including the heart and kidney are included in the model regarding their role in the pharmacokinetics and pharmacodynamics of PI. The structure of our PBPK model of PB and PI is depicted in Figure 1.

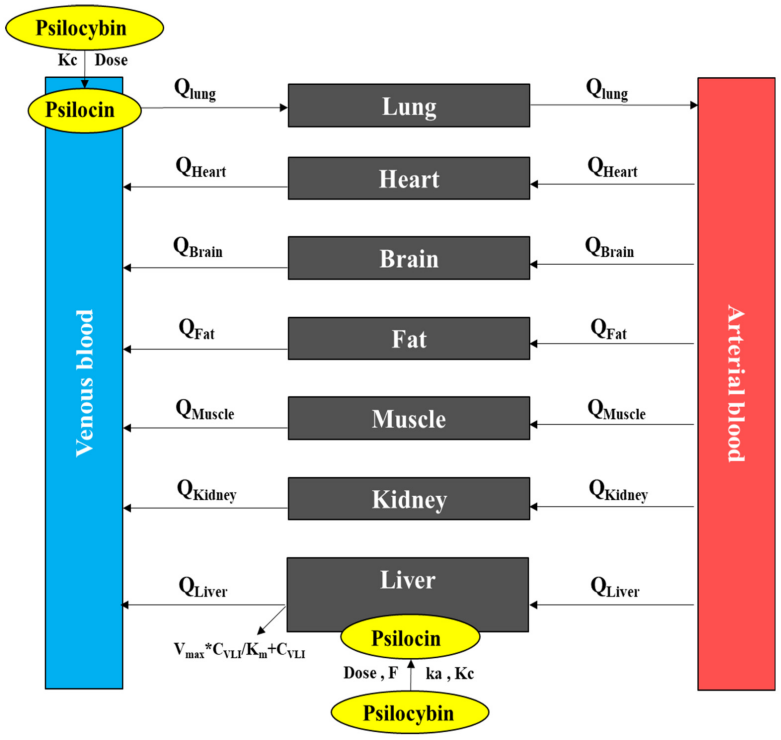

Figure 1. Structure of a physiologically based pharmacokinetic model of psilocybin (PB) and psilocin (PI) in rats and humans.

Tissue model specification. A perfusion rate-limited and well-stirred tissue model hypothesis was assumed for all organs.

Model parameterization and equations. Physiological parameters including organ blood flow (Q) and organ volume (V) were obtained from published literature[13–15]. Pharmacokinetic parameters including bioavailability and absorption rate constant were acquired from published literature[16,17] and optimized using Berkeley Madonna Software. Physicochemical parameters including partition coefficient of each compartment were calculated using an approach from the literature[18]. A PBPK model was coded and performed using Berkeley Madonna Software version 8.3.18 (developed by Robert Macey and George Oster of the University of California at Berkeley). Replication of the simulation results from our PBPK model using an alternative and freely available software is possible; recently, Zurlinden T.J. *et al.*[19] have developed a physiologically based pharmacokinetic model of a rifapentine model using the Python software (version 2.7.2) as their computational tool along with these numby, scipy and matplotlib packages[20–23]. All of the parameters used in the PBPK model are summarized in Table 1.

Table 1. Model parameters in the PBPK model of psilocybin (PB) and psilocin (PI).

Parameters	Rats	Reference	Humans	Reference
Blood flow fraction				
Fat (Q_{FC})	0.07	13	0.021	14
Liver (Q_{LIC})	0.183	13	0.1195	14
Muscle (Q_{MUC})	0.278	13	0.0606	14
Kidney (Q_{KC})	0.141	13	0.1001	14
Brain (Q_{BRC})	0.02	13	0.0565	14
Heart (Q_{HC})	0.051	13	0.0194	14
Tissue volume fraction				
Fat (V_{FC})	0.076	15	0.1429	14
Liver (V_{LIC})	0.0366	15	0.0241	14
Muscle (V_{MUC})	0.404	15	0.5	14
Lung (V_{LUC})	0.005	15	0.0167	14
Kidney (V_{KC})	0.0073	15	0.004	14
Brain (V_{BRC})	0.0057	15	0.0207	14
Heart (V_{HC})	0.0033	15	0.0044	14
Vein (V_{VC})	0.0544	15	0.0496	14
Artery (V_{AC})	0.0272	15	0.0247	14
Partition coefficients				
Fat (P_F)	0.1484	18	0.1818	18
Liver (P_{LI})	1.0749	18	1.5383	18
Muscle (P_{MU})	0.8781	18	1.1732	18
Lung (P_{MU})	1.2035	18	0.7665	18
Kidney (P_{KJ}	1.0799	18	1.1873	18
Brain (P_{MU})	1.8662	18	2.1357	18
Heart (P_H)	1.0046	18	0.9673	18
Molecular weight (g/mole)	204.27 (PI)	24	284.25 (PB)	25
Maximum velocity (V_{max}), µmole/h	270*	Optimized	1,450-2,080*	Optimized
Michaelis constant (K_m), µM	1,080*	Optimized	50*	Optimized
Bioavailability (F)	0.52*	Optimized	0.527[17]-0.9*	Optimized
Absorption rate constant (k_a), 1/h	0.16*	Optimized	0.367[16]-0.67*	Optimized
Conversion rate constant (K_C)	-	Optimized	0.4*	Optimized

Note: * Optimized value using Berkeley Madonna software.

Routes of PI administration. In this development of the PBPK model of PB and PI in rats and humans, there were two different routes of administration. Those included 1) intravenous (IV) administration and 2) oral administration. Differential equations (Equation 1 – Equation 3) describing the respective routes of administration are as follows:

1) Intravenous administration:

$$Input_{IV} = Dose * BW * Kc / Time \quad \text{Eq. 1}$$

where Dose is an administered dose of PI (mg), BW is the body weight (kg), Time is the duration period of the injection (h), and Kc is the conversion rate constant.

2) Oral administration:

$$Input_{oral} = K_a * A_{GU} \quad \text{Eq. 2}$$

$$A_{GU} = Dose * BW * F * K_c \quad \text{Eq. 3}$$

where A_{GU} is the amount of PI in the gut lumen after an oral administration (μg), k_a is the absorption rate constant (1/h), F is the bioavailability, and Kc is the conversion rate constant.

Organ compartments. In this PBPK model, there were seven organ compartments. Differential equations (Equation 4 – Equation 20) describing concentration-time profiles of PI in each of seven compartments are as follows:

1) Lung:

$$dA_{Lu} / dt = Q_c * (C_V - C_{ALU}) \quad \text{Eq. 4}$$

$$C_{ALU} = C_{LU} / P_{LU} = A_{LU} / V_{LU} * P_{LU} \quad \text{Eq. 5}$$

where A_{LU} is the amount of PI in the lung (μg), QC is the cardiac output (l/h), C_V is the concentration of PI in venous blood (μg/l), C_{ALU} is the concentration of PI in arterial blood leaving the lung (μg/l), C_{LU} is the concentration of PI in the lung (μg/l), V_{LU} is the volume of the lung (l), and PLU is the lung/blood partition coefficient.

2) Heart:

$$dA_H / dt = Q_H * (C_A - C_{VH}) \quad \text{Eq. 6}$$

$$C_{VH} = C_H / P_H = A_H / V_H * P_H \quad \text{Eq. 7}$$

where A_H is the amount of PI in the heart (μg), Q_H is the blood flow to the heart (l/h), C_A is the concentration of PI in arterial blood (μg/l), C_{VH} is the concentration of PI in venous blood leaving from the heart (μg/l), C_H is the concentration of PI in the heart (μg/l), V_H is the volume of the heart (l), and P_H is the heart/blood partition coefficient.

3) Brain:

$$dA_{BR} / dt = Q_{BR} * (C_A - C_{VBR}) \quad \text{Eq. 8}$$

$$C_{VBR} = C_{BR} / P_{BR} = A_{BR} / V_{BR} * P_{BR} \quad \text{Eq. 9}$$

where A_{BR} is the amount of PI in the brain (μg), Q_{BR} is the blood flow to the brain (l/h), C_A is the concentration of PI in arterial blood (μg/l), C_{VBR} is the concentration of PI in venous blood leaving from the brain (μg/l), C_{BR} is the concentration of PI in the brain (μg/l), V_{BR} is the volume of the brain (l), and P_{BR} is the brain/blood partition coefficient.

4) Fat:

$$dA_F / dt = Q_F * (C_A - C_{VF}) \quad \text{Eq. 10}$$

$$C_{VF} = C_F / P_F = A_F / V_F * P_F \quad \text{Eq. 11}$$

where A_F is the amount of PI in the fat (μg), Q_F is the blood flow to the fat (l/h), C_A is the concentration of PI in arterial blood (μg/l), C_{VF} is the concentration of PI in venous blood leaving from the fat (μg/l), C_F is the concentration of PI in the fat (μg/l), V_F is the volume of the fat (l), and P_F is the fat/blood partition coefficient.

5) Muscle:

$$dA_{MU} / dt = Q_{MU} * (C_A - C_{VMU}) \quad \text{Eq. 12}$$

$$C_{VMU} = C_{MU} / P_{MU} = A_{MU} / V_{MU} * P_{MU} \quad \text{Eq. 13}$$

where A_{MU} is the amount of PI in the muscle (μg), Q_{MU} is the blood flow to the muscle (l/h), C_A is the concentration of PI in arterial blood (μg/l), C_{VMU} is the concentration of PI in venous blood leaving from the muscle (μg/l), C_{MU} is the concentration of PI in the muscle (μg/l), V_{MU} is the volume of the muscle (l), and P_{MU} is the muscle/blood partition coefficient.

6) Kidney:

$$dA_K / dt = Q_K * (C_A - C_{VK}) \quad \text{Eq. 14}$$

$$C_{VK} = C_K / P_K = A_K / V_K * P_K \quad \text{Eq. 15}$$

where A_K is the amount of PI in the kidney (μg), Q_K is the blood flow to the kidney (l/h), C_A is the concentration of PI in arterial blood (μg/l), C_{VK} is the concentration of PI in venous blood leaving from the kidney (μg/l), C_K is the concentration of PI in the kidney (μg/l), V_K is the volume of the kidney (l), and P_K is the kidney/blood partition coefficient.

7) Liver:

$$dA_{LI} / dt = Q_{LI} * (C_A - C_{VLI}) - LI_{Met} \quad \text{Eq. 16}$$

$$C_{VLI} = C_{LI} / P_{LI} = A_{LI} / V_{LI} * P_{LI} \quad \text{Eq. 17}$$

$$LI_{Met} = (V_{max} * C_{VLI}) / (K_m + C_{VLI}) \quad \text{Eq. 18}$$

where A_{LI} is the amount of PI in the liver (μg), Q_{LI} is the blood flow to the liver (l/h), C_A is the concentration of PI in arterial blood (μg/l), C_{VLI} is the concentration of PI in venous blood leaving from the liver (μg/l), C_{LI} is the concentration of PI in the liver (μg/l), V_{LI} is the volume of the liver (L), P_{LI} is the liver/blood partition coefficient, LI_{Met} is the amount of PI

excreted via liver metabolism, Vmax is the maximum velocity of UGT1A10 (μmol/h), and Km is Michaelis-Menten constant of UGT1A10 (μM).

8) Blood:

$$dA_V / dt = Q_H * C_{VH} + Q_{BR} * C_{VBR} + Q_F * C_{VF} + Q_{MU} * C_{VMU} + Q_K * C_{VK} + Q_{LI} * C_{VLI} - QC * C_V \quad \text{Eq. 19}$$

$$dA_A / dt = QC * C_{ALU} - C_A * (Q_H + Q_{BR} + Q_F + Q_{MU} + Q_K + Q_{LI}) \quad \text{Eq. 20}$$

where A_V and A_A are the amount of PI in the venous and arterial blood, Q_H, Q_{BR}, Q_F, Q_{MU}, Q_K, Q_{LI} are the blood flow rate into the heart, brain, fat, muscle, kidney, and liver (l/h), and C_{VH}, C_{VBR}, C_{VF}, C_{VMU}, C_{VK}, C_{VLI} are the concentration of PI in the venous blood leaving from the heart, brain, fat, muscle, kidney, and liver (μg/).

Model development and validation

From a search in PubMed from inception to July 2019, the search terms "psilocybin; psilocin; magic mushroom; pharmacokinetic" were used as keywords. Studies were included if they met the selection criteria as follows: pharmacokinetic studies of psilocybin or psilocin that were conducted in animals or humans and different of routes of administration. Studies were excluded if they conducted pharmacokinetic studies in unhealthy or pregnancy animals or humans. From the following information in the literature, three studies were retrieved and were subsequently used for further model development. The PBPK model was evaluated based on visual inspection of goodness of fit. A summary of the selected studies is as follows:

In the study by Chen *et al.*[26], Sprague-Dawley rats (n = 10, BW = 220 – 250 g) were orally administered with a single dose of *G. spectabilis* extract, which is equivalent to 10.1 mg/kg of PI. Then, a serial of blood samples was collected at 5, 10, 20, 30, 45, 60, 90, 120, 180, 240, 360, and 420 minutes after oral administration. Then, the samples were analysed for PI using ultra-performance liquid chromatography coupled to photodiode array detection (UPLC-PDA).

In the study by Hasler *et al.*[17], nine healthy male volunteers (BW = 56 – 72 kg) were intravenously administered with a single dose of 1 mg PB. Subsequently, blood samples were collected at 0.75, 1.5, 2.5, 3.75, 5, 6.75, 10, 15, 20, 30, 60, and 120 minutes. In addition, six healthy male and female volunteers (BW = 53 – 88 kg) were orally administered with a single oral dose of 0.224 mg/kg PB. Blood samples were collected at 15, 30, 4.5, 60, 7.5, 90, 105, 120, 150, 180, 220, 300, 350, and 390 min after the oral PB administration. Then, the samples were analysed using high performance liquid chromatography coupled with electrochemical detection (HPLC-ECD).

In Brown *et al.*[16], 12 healthy volunteers were given a single oral administration of PB 0.3 mg/kg. Blood collections were performed at 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 12, 18, and 24 hours. Subsequently, the samples were analysed for PI concentration using a HPLC technique.

PI concentration levels shown in figures for the selected studies were extracted using WebPlotDigitizer version 4.4 (Free Software Foundation, Inc., Boston, MA). Assessment of model qualification was based on visual inspection of the agreement between observed and simulated PI concentrations. Regression analysis between the simulated and observed PI levels was also performed in Microsoft Excel version 2019.

Results

Single oral administration of PB in rats

Following a single oral administration of PI (10.1 mg/kg) in rats, the simulated results of PI concentration profiles in plasma from the developed model compared to observed data acquired from the study by Chen *et al.*[26] is demonstrated in Figure 2A with the simulated PI concentration-time profiles in the brain[27]. Subsequently, simulated results of PI concentration-time profiles in the lung, heart, muscle, kidney, and liver are illustrated in Figure 2B.

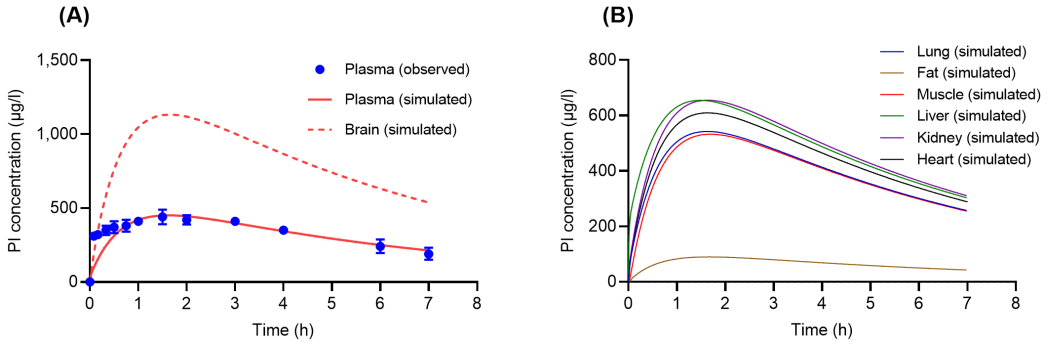

Figure 2. (**A**) Concentration-time profiles of psilocin (PI) in rat plasma and brain following a single oral administration of psilocin (PI) (10.1 mg/kg). Solid and dashed lines are concentration-time profiles of PI from the developed physiologically based pharmacokinetic (PBPK) model, whereas closed circles with error bars (S.E.) are experimental data acquired from Chen *et al.*[26]. (**B**) Simulated concentration-time profiles of PI following a single oral dose of PI (10.1 mg/kg) in rat's tissues such as lung (blue solid line), fat (brown solid line), muscle (red solid line), liver (green solid line), kidney (purple solid line), and heart (black solid line).

Single intravenous administration in humans

Following a single IV administration of PB (1 mg) in humans, simulated PI concentration-time profiles in plasma compared to the observed data acquired from Hasler *et al.*[17] are demonstrated in Figure 3A. In addition, PI concentration-time profiles in the human brain were simulated and are presented in Figure 3A. Simulated PI concentration-time profiles of other tissues are illustrated in Figure 3B.

Single oral administration of PB in humans

In the selected studies, two different doses of PB (0.224 mg/kg and 0.3 mg/kg) were administered to humans via a single oral administration. Simulated results of PI concentration-time profiles in plasma following oral administration of PB (0.224 mg/kg and 0.3 mg/kg) compared to the observed data acquired from Hasler *et al.*[17] and Brown *et al.*[16] are presented in Figure 4A and Figure 5A, respectively. Simulated brain concentration-time profiles of PI for both doses (0.224 mg/kg and 0.3 mg/kg) are also presented in Figure 4A and Figure 5A, respectively. Then, from the developed model following oral administration of PB (0.224 mg/kg and 0.3 mg/kg), the simulated results of PI in organs of interest including the lungs, fat tissue, muscle, kidney, and liver are depicted in Figure 4B and Figure 5B.

Our model simulations in both species included different routes of administration including an oral dose of PI in rats (10.1 mg/kg), IV dose of PB in humans (1 mg), and different oral doses of PB in humans (0.224 mg/kg and 0.3 mg/kg). Key parameters including maximum concentration (C_{max}), time to reach maximum concentration (T_{max}), and area under the curve from time equals zero to the last time point (AUC_{0-t}) from the developed PBPK model and experimental data in both rats and humans are demonstrated in Table 2.

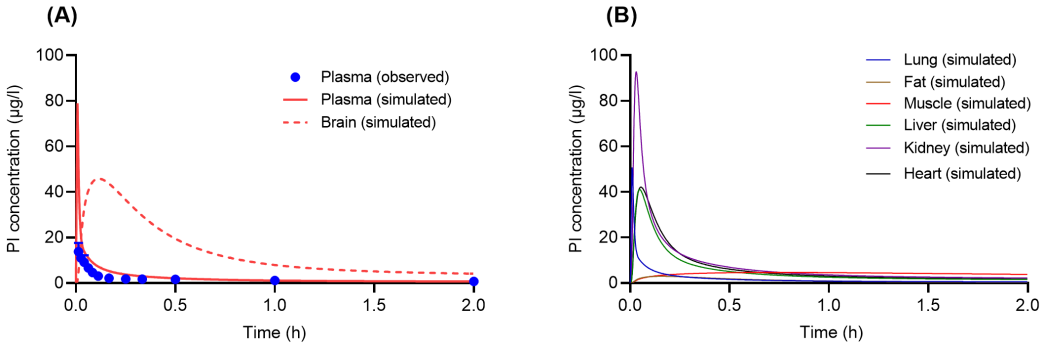

Figure 3. (**A**) Concentration-time profiles of psilocin (PI) in human plasma and brain following a single intravenous administration of psilocybin (PB) (1 mg). Solid and dashed line are concentration-time profiles of PI from the developed physiologically based pharmacokinetic (PBPK) model, whereas closed circles with error bars (S.E.) are experimental data acquired from Hasler *et al.*[17]. (**B**) Simulated concentration-time profiles of PI following a single intravenous dose of PB (1 mg) in human tissues such as lung (blue solid line), fat (brown solid line), muscle (red solid line), liver (green solid line), kidney (purple solid line), and heart (black solid line).

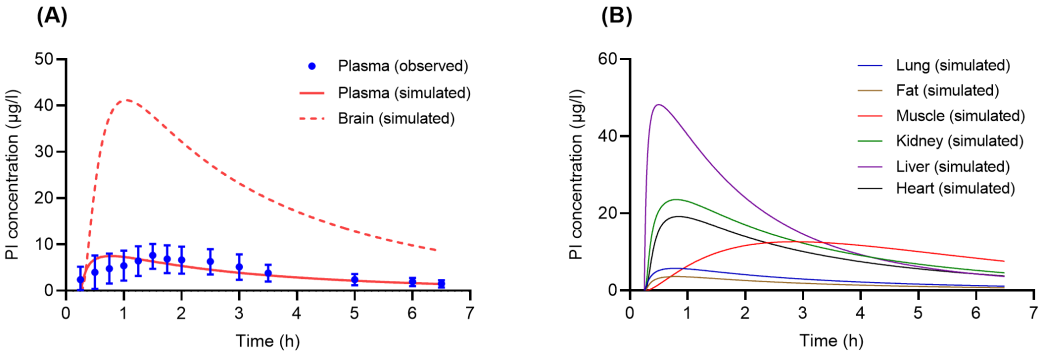

Figure 4. (**A**) Concentration-time profiles of psilocin (PI) in human plasma and brain following a single oral administration of psilocybin (PB) (0.224 mg/kg). Solid and dashed lines are concentration-time profiles of PI from the developed physiologically based pharmacokinetic (PBPK) model, whereas closed circles with error bars (S.E.) are experimental data acquired from Hasler *et al.*[17]. (**B**) Simulated concentration-time profiles of PI following a single oral dose of PB (0.224 mg/kg) in human tissues such as lung (blue solid line), fat (brown solid line), muscle (red solid line), liver (green solid line), kidney (purple solid line), and heart (black solid line).

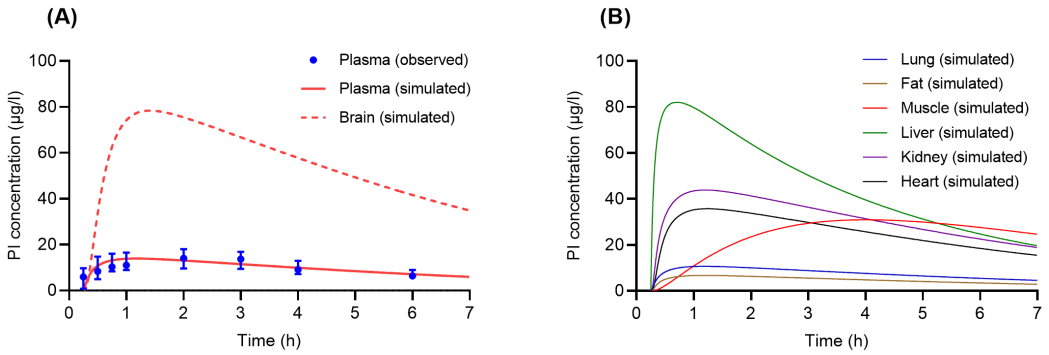

Figure 5. (**A**) Concentration-time profiles of psilocin (PI) in human plasma and brain following a single oral administration of psilocybin (PB) (0.3 mg/kg). Solid and dashed lines are concentration-time profiles of PI from the developed physiologically based pharmacokinetic (PBPK) model, whereas closed circles with error bars (S.E.) are experimental data acquired from Brown *et al.*[16]. (**B**) Simulated concentration-time profiles of PI following a single oral dose of PB (0.3 mg/kg) in human tissues such as lung (blue solid line), fat (brown solid line), muscle (red solid line), liver (green solid line), kidney (purple solid line), and heart (black solid line).

Discussion

To our knowledge, this current PBPK model of PB and PI is the first model with the capacity to describe PI concentration-time profiles following an oral administration of PI in rats as well as an intravenous and oral administration of PB in humans. The developed PBPK model could describe PI concentration-time profiles in plasma from both species (Figure 2A, Figure 3A, Figure 4A and Figure 5A). Furthermore, the developed PBPK model could simulate PI concentration-time profiles in other tissues, particularly in the brain, which is the major target organ of the psychoactive compound in magic mushrooms.

However, under-predicting of blood PI levels at early time points after PB oral administration was observed (Figure 4A and Figure 5A). These under-predictions could be a result of the conversion rate constants used in the current PBPK model of PB and PI. In this model, the conversion rate constant was assumed as a constant in a zero-ordered kinetic process. However, the enzymatic reaction by the esterase enzyme system in the blood could be either a first-ordered kinetic process or Michaelis-Menten process. Nonetheless, our model outputs after two hours post-administration could capture most of the data points acquired from the selected studies reasonably well (Figure 2A, Figure 4A, and Figure 5A).

Following IV administration in human subjects, from time equals zero to about half an hour, model over-predictions were observed (Figure 3A). These over-predictions might be a result of 1) over-simplification from the well-stirred model hypothesis and 2) at the beginning of the IV administration, the administrated amount of PB could saturate the available enzymatic process for changing PB to PI in the body.

Our developed PBPK model of PB and PI is capable of making predictions of PI concentration-time profiles in the brain of both rats and humans. However, data concerning brain PI concentration-time profiles are lacking. From our literature search, there was a study by Law *et al.*[28]. In this study, pregnant rats were intravenously administrated with PI. Subsequently, tissue samples (i.e. brain, lung, fat, muscle, kidney, liver, spleen, placenta, and fetus) were harvested at different time points and PI concentration levels in the tissue samples were analyzed. However, this study was conducted in pregnant rats which, due to potential changes as a result of the physiology of the pregnancy, may significantly influence the pharmacokinetics of PB and PI. Consequently, we could not use this dataset in the current model development. Thus, to have more confidence in the developed PBPK model, further PI concentration-time profiles in tissue organs including the brain are necessary. Therefore, a new study in rats intravenously and orally administered with PB with an additional design of tissue harvesting (e.g. brain, fat, muscle, kidney, and liver) is warranted. In addition, to further extend the model's application and to make an adequate prediction between the administered dose of PB and PI and its psychoactive effects, a physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) model for PB and PI in humans should be developed.

Conclusions

A PBPK model of PB and PI in rats and humans was developed. The PBPK model could predict concentration-time profiles of PI following intravenous and oral administration of PB. In addition, concentration-time profiles of PI in the brain, the major target organ of PI, could be simulated. The PBPK model of PB and PI may be useful for a safer dosage regimen for

Table 2. Pharmacokinetic parameters of psilocybin (PB) and psilocin (PI) following intravenous and oral administration from model simulations.

Species	Study	Route of administration	Dose	Observed C_{max} (µg/l)	Simulated C_{max} (µg/l)	Observed T_{max} (h)	Simulated T_{max} (h)	Observed AUC_{0-t} (µg.h/l)	Simulated AUC_{0-t} (µg.h/l)	$\%AUC_{0-t}$ deviation	R-square
Rats	Chen *et al.*[26]	Oral	10.1 mg/kg (PI)	430	450.7	1.5	1.625	2,375	2,342	1.38	0.6
Humans	Hasler *et al.*[17]	IV	1 mg (PB)	12.9	78.46	0.031	0.008	4	5	25	0.8
		Oral	0.224 mg/kg (PB)	8.2	7.5	1.75	0.78	32.71	24.2	26	0.57
	Brown *et al.*[16]	Oral	0.3 mg/kg (PB)	16	13.95	2.03	1.17	140	93.81	33	0.76

Note: C_{max}, maximum plasma concentration; T_{max}, time to reach maximum concentration; AUC, area under the curve.

patients with some disorders for which PB or magic mushrooms may be therapeutically effective.

Data availability

Underlying data

Zenodo: Heang60/PBPK-of-Magic-Mushroom: Coding for PBPK of Magic Mushroom. https://doi.org/10.5281/zenodo.4536617[27].

This project contains the following underlying data:

- Raw data (Figures 2–5)_magic mushroom.xlsx (raw data underlying Figures 2–5)

Extended data

Zenodo: Heang60/PBPK-of-Magic-Mushroom: Coding for PBPK of Magic Mushroom. https://doi.org/10.5281/zenodo.4536617[27].

This project contains the following extended data:

- Code availability_magic mushroom.pdf (parameters and code for model reproducibility)

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Open Peer Review

Current Peer Review Status: ✗ ?

Version 1

Reviewer Report 08 April 2021

https://doi.org/10.5256/f1000research.31120.r81474

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Quoc Ba Tran
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Major issues:
Could you please explain why the PBPK model structure consists of seven compartments: the lung, heart, brain, fat, muscle, kidney, and liver?

This study simulated the concentration-time courses of psilocin (PI) following a single oral/single intravenous administration of psilocybin (PB) in human/rat tissues (the lung, heart, brain, fat, muscle, kidney, and liver). However, the whole study only gives data/information on the relationship between PB adsorption and PI concentration in plasma, and just a few data on the relationship between PB adsorption and PI concentration in other components of the human body/rat. Therefore, the study should find more solid scientific evidence, that mentions PI concentration will be distributed in those components of the human body/rat if PB is absorbed.

One of the main objectives of this study is to quantify brain concentration levels of PI in human brains following PB administration. Therefore, in the Discussion session, please carefully discuss the relationship between PI concentration in brain and PB absorption.

Minor issues:
I have read reference number [18] of this study but have not found the corresponding data, which you acquired for the section “Partition Coefficients" of Table 1. Please explain how did you acquire the data for the parameters: Fat (PF); Liver (PLI); Muscle (PMU); Lung (PMU); Kidney (PK); and Brain (PMU) in Table 1.

Part of the information in Table 1 (Blood flow fraction and Tissue Volume fraction) is the basic information that has been published by many studies, so this study may consider incorporating this information into the SI section.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Environmental health, Environmental modeling, PBPK modeling, Air pollution

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 31 March 2021

https://doi.org/10.5256/f1000research.31120.r81816

Michael Dzierlenga
United States Environmental Protection Agency, Durham, NC, USA

Musikaphongsakul *et al.* present the first PBPK model for psilocin (PI), the active metabolite of psilocybin (PB). The model is described as a model of PB and PI, but PB is not included in the PBPK as it is assumed to be instantaneously metabolized to PI. The model and its presentation are incomplete, and the manuscript is not yet ready to be indexed.

The key inadequacies are a disagreement between the way that the conversion from PB to PI is described and modeled, a poor fit to the experimental plasma time-courses at early times, lack of an analysis of the accuracy of the predicted partition coefficients, lack of consideration of urinary clearance of unmetabolized PI, lack of an analysis of the results of parameter optimization and the sensitivity of model predictions to those parameters, and a lack of distinction between PI in blood

and plasma. These inadequacies need to be addressed before the model is suitable forindexing because they will help readers to understand the limitations of the model. As the paper stands, I have concerns that others might apply the model to predict brain concentrations without understanding how uncertain those measurements are.

Major Issues:

I have concerns about the way that the conversion from PB to PI is implemented. The paper states that this conversion occurs "rapidly" after oral absorption. In the model, this is captured as an instantaneous and incomplete process, where Kc simply scales the dose. For example, in an oral experiment, the initial amount in the oral uptake compartment is the dose multiplied by F, the fraction bioavailable, and Kc. Kc is described in the paper as a "*conversion rate constant*" and as a *"zero-ordered kinetic process".* This description does not agree with the model implementation.

To justify the way that Kc is included in the model as written, the paper needs to describe Kc as the fraction of PB that is converted to PI, provide justification that the conversion between PB and PI is incomplete, and justify that this conversion is instantaneous both in oral and IV exposure. The correct way to model a *"conversion rate constant"* and a *"zero-ordered kinetic process"* would be to track the amount of PB as a state variable and describe decreases in PB and increases in PI as rates in differential equations.

The only data which was used to inform the model was PI plasma data from 3 experiments. 5 parameters were optimized using this data by a visual fitting process. Visual fitting is not a recommended method but can be overlooked if the outcome of the fit is satisfactory. The model fit to the plasma time-courses were poor at early times, especially for the oral rat time-course and the IV human time-course. A poor model fit to data after optimization suggests that a better optimization scheme is need or that something about the model structure itself is insufficient.

The use only of plasma data to inform the model is problematic because there is no way to determine the accuracy of the predicted tissue-blood partition coefficients for PI. The authors mention that tissue specific concentrations do exist, but only for pregnant rats (Law *et al.* 2014; reference 28 in manuscript). Pregnant animals will have some PK differences relative to non-pregnant animals, but a comparison of that data to the model prediction may still prove fruitful. A quick look shows that that maternal blood concentration was roughly twice the maternal brain concentration in Law et al (a partition coefficient of roughly 0.5). The predicted brain:blood partition coefficient was 2, which is substantially different, and this discrepancy should be discussed and explained. Furthermore, the paper being reviewed states in the first paragraph of the methods that adipose tissue is a storage organ for PI. The predicted partition coefficient is around 0.15, which suggests that PI has a low affinity for adipose tissue compared to blood or other organs. Suggesting that most of the chemical is not stored in fat. Another way to evaluate whether the predicted partition coefficients were reasonable would be to calculate the volume of distribution of the model (as the weighted sum of organ volumes and partition coefficients) and compare that to Vd from PK studies (if available). The clearance rate of the model should also be compared to published clearance or half-life values (if available).

Kalberer *et al.* (1962)[1] reported that approximately 25% of PI dosed is excreted unchanged in urine. Urinary clearance is not captured in the model, which assumes PI is metabolized prior to excretion. This is a flaw of the model that should be addressed. In addition, PI is described to be

metabolized by UGT 1A10 in the liver and intestinal mucosa, but metabolism in the model only takes place in the liver, which seems to disagree with the description in the introduction. This may be especially important for oral dosage since metabolism in the intestine would increase initial first-pass metabolism.

Table 1 shows the optimized parameters for rat and human. There needs to be some analysis of the parameter values that were reached after optimization, including a comparison between rat and human values. In addition, I think a sensitivity analysis is warranted to show the relative impact of the different parameters on the plasma and brain concentrations. Table 1 also has some typographical errors. Partition coefficients for lung and brain have the symbol for muscle. For human bioavailability and k_a, there is some issue with the presentation of the human values. Also, molecular weight is put in columns for rat and humans when the molecular weight of PI and PB is species independent.

There needs to be some description of the ratio of PI between whole blood and plasma. It seems like organ blood flow and partition coefficients are for whole blood, but chemical concentrations are treated as plasma concentrations. I believe this assumes that the whole blood to plasma ratio is 1, for which evidence is not provided.

Minor Issues:

- Dose should have units of mg/kg in the equations in the methods section.
- Absorption rate constant is formatted as K_a in the oral equation, but ka in Table 1.
- C_v equations need a parenthesis: C_v = A/(V*P).
- LI_{met} should be described as the rate of metabolism of PI, not the amount of PI metabolized. The amount metabolized is the integral of LI_{met}.
- In the Blood equation description, the unit at the end is “ug/” instead of ug/l.
- In the first subheading of the Results section, “PB” is listed as the chemical, when PI is dosed.
- Concentration figures are not color-blind friendly. Ideally, a palette optimized for color-blindness or different line-types should be used.
- The times for the Hasler experiment should be listed in increasing order.
- In Zurlinden, *et al.* (reference 19 in the manuscript), the model was implemented and run in MCSim, not in Python. Data processing and analysis was performed in Python.
- There should be some description of why a perfusion limited model is appropriate.
- The discussion needs to better place the work in context. How will the brain concentration predicted in the model help to aid dosimetry and the potential application to mental health treatment?

- Ensure that the MW parameter in the code is correctly specified for each chemical. For example, when PB is dosed is the MW for PB being used to scale that dose initially and then the MW for PI is being used in subsequent conversions?

- The concentration unit in comments in the code is sometimes specified as "mcmole" instead of mcmole/L.

References

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Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
No

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Pharmacokinetics and PBPK modeling.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.