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Determination of psilocin and 4-hydroxyindole-3-acetic acid in plasma by HPLC-ECD and pharmacokinetic profiles of oral and intravenous psilocybin in man

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Abstract

In order to investigate the pharmacokinetic properties of psilocybin (PY), the main psychoactive compound of *Psilocybe* mushrooms, high performance liquid chromatographic procedures with column-switching coupled with electrochemical detection (HPLC-ECD) for reliable quantitative determination of the PY metabolites psilocin (PI) and 4-hydroxyindole-3-acetic acid (4HIAA) in human plasma were established. Sample work-up includes protection of the highly unstable phenolic analytes with ascorbic acid, freeze-drying and in-vitro microdialysis. The data of two controlled clinical studies with healthy volunteers are presented. The subjects ($N = 6$ for both studies) received single oral PY doses of 0.224 ± 0.02 mg/kg b.wt. (10–20 mg) and intravenous doses of 1 mg PY, respectively. Peak plasma levels of PI after oral administration of PY were measured after 105 ± 37 min showing an average concentration of 8.2 ± 2.8 ng PI/ml plasma. 4HIAA peak concentrations of 150 ± 61 ng/ml plasma were found 113 ± 41 min after ingestion of PY. After intravenous administration, a mean PI maximum plasma concentration of 12.9 ± 5.6 ng/ml plasma was found 1.9 ± 1.0 min after injection. The maximum plasma levels appearing within a very short period indicate a rapid dephosphorylation of PY also when administered systemically. 4HIAA was not detected after 1 mg of intravenous PY. Estimates for the absolute bioavailability of PI after oral administration of PY were $52.7 \pm 20\%$ ($N = 3$).

Keywords: Psilocybin; Psilocin; HPLC-ECD; Pharmacokinetics

1. Introduction

Psilocybin (PY), the main psychoactive compound of *Psilocybe* mushrooms, represents one of the 'classic' hallucinogens, known in South American traditional medicine for centuries. Nowadays, apart from the wide-spread use as a psychotropic substance, this controlled indole alkaloid became an important model in research to investigate psychiatric disorders such as acute states of schizophrenia (Hermle et al., 1992; Vollenweider et al., 1994), taking into account a common underlying pathophysiology. In order to study the relationship between PY-induced psychotic symptoms and cerebral energy metabolism, positron emission tomography (PET) and the radioligand ^{18}F-fluorodeoxyglucose (FDG) was used. An important experimental issue was to correlate PY-induced psychosis and the plasma levels of the active PY-metabolite psilocin (PI) as well as to elucidate the pharmacokinetic properties of PY in man. Therefore, procedures for sensitive determination of PI and its first metabolite 4-hydroxyindole-3-acetic acid (4HIAA) in plasma samples were established.

The metabolism of PY in animals has been investigated by several authors. The rapid and extensive cleavage of the phosphoric ester group of PY by alkaline phosphatase and unspecific esterases of the intestine mucosa has been shown (Kalberer et al., 1962; Horita and Weber, 1961, 1962;

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Eivindvik and Rasmussen, 1989). These findings indicate that PY acts as a prodrug and that its hydroxy metabolite PI represents the true pharmacologically active agent. Kalberer et al. showed in 1962 that PI undergoes demethylation and consecutive deamination and oxidation to 4HIAA presumably by the influence of liver enzymes such as monoamino-oxidases and aldehyde dehydrogenase. Recently Holzmann identified 4-hydroxy-tryptophole (4HT) as a further metabolite of PY in man and postulated 4-hydroxyindole-acetaldehyde (4HIA) to be the intermediate metabolite from PI to 4HIAA (Fig. 1) (Holzmann, 1995). Up to now the presumably existing corresponding glucuronides have not yet been identified.

Several chromatographic methods for the determination of PY and PI in seizures and mushrooms have been published. Thin-layer chromatography is useful for simple qualitative detection of tryptamine derivatives (Brown et al., 1972; Tanimukai, 1967; Beug and Bigwood, 1981). Methods involving high performance liquid chromatography (HPLC) in combination with either UV photometric detection (Beug and Bigwood, 1981; Wurst et al., 1984; Borner and Brenneisen, 1987; Perkal et al., 1980; Vanhaelen-Fastré and Vanhaelen, 1984; Thomson, 1980; White, 1979; Sottolano and Lurie, 1983) or UV photometric and electrochemical detection (ECD) (Kysilka et al., 1985; Kysilka and Wurst, 1988; Kysilka and Wurst, 1989; Christiansen and Rasmussen, 1983) as well as GC-MS techniques (United Nations, 1989; Unger and Cooks, 1979; Timmons, 1984) were mainly established and optimized for quantitation of PY and related compounds in solvent extracts from *Psilocybe* mushrooms. For our clinical samples these methods were either not suited due to interfering plasma compounds and stabilizing agents or they lack of the sensitivity required to determine the expected low ng/ml concentrations. The presented HPLC methods with column-switching and ECD enabled us to establish plasma profiles of PY metabolites after stabilization of the phenolic analytes with ascorbic acid. Due to the high polarity of PY and the ensuing difficulty of separating this analyte from interfering endogenous plasma compounds of comparable polarity, the determination of PY in plasma samples was not possible in default of a reliable analytical method.

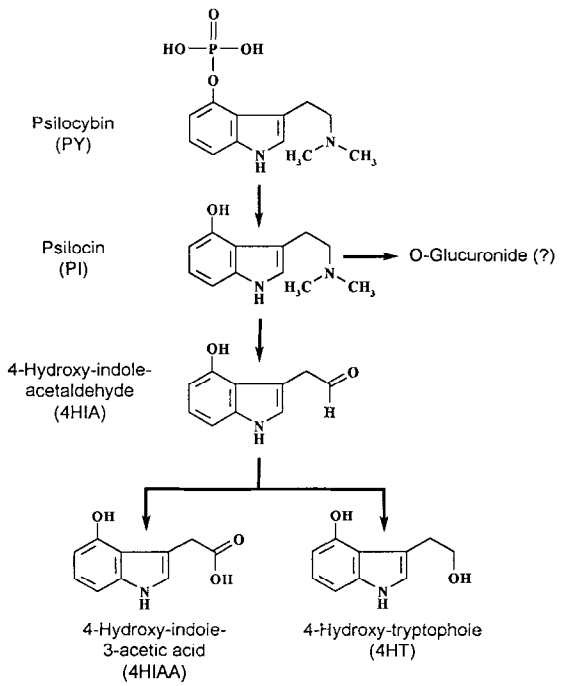

Fig. 1. Metabolism of psilocybin.

2. Experimental

2.1. Study design

Two controlled clinical studies in humans (one with oral and one with intravenous PY) were authorized by the Ethics Committee of the Psychiatric University Hospital Zürich (PUK-ZH) and conducted at PUK-ZH in April/May 1995 and August/September 1995, respectively, under permanent supervision of at least one experienced psychiatrist during all experiments. The subjects were one female and eight male volunteers who agreed to participate with informed consent. The subjects were screened by psychiatric interview to assure that the subjects had neither personal nor family histories of major psychiatric disorders in first-degree relatives. Subjects with a history of illicit drug abuse were excluded from the study. The 'openness' and 'neuroticism' scales of the Freiburg personality inventory (FPI) were also used as exclusion criteria. Subjects were healthy according to physical examination, electrocardiogram, blood and urine analysis. The detailed data of the nine subjects are listed in Table 1. After an overnight fast, the six participants of the oral study received doses of 0.224 mg/kg b.wt. $\pm$0.02 mg PY as capsules of 1% PY and 99% of lactose. For the intravenous study, PY was dissolved in a physiological saline solution to give a concentration of 0.05% (w/v) and sterile-filtered. HPLC monitoring of the injection solution was performed to ensure the stability of the formulation. The six subjects of the intravenous study received absolute doses of 1 mg PY in a 30 s bolus injection. Venous blood samples for both studies were drawn from an indwelling antecubital catheter into vacuum tubes immediately before drug administration and at time points set after evaluating the data of the pilot studies (i.e. 15, 30, 45, 60, 75, 90, 105, 120, 150, 180,

Table 1
Subjects, route of administration and dosage of PY

Subject	Sex	Age (years)	Weight (kg)	Route of administration	Dosage of PY p.o. (mg/kg b.wt.)	Dosage of PY p.o. (mg)	Dosage of PY i.v. (mg)
A	female	32	53	p.o.	0.226	12	—
B	male	27	56	p.o./i.v.	0.178	10	1
C	male	27	70	p.o.	0.229	16	—
D	male	41	88	p.o. / i.v.	0.227	20	1
E	male	30	59	p.o. / i.v.	0.254	15	1
F	male	25	65	p.o.	0.231	15	—
G	male	41	80	i.v.	—	—	1
H	male	33	90	i.v.	—	—	1
I	male	26	72	i.v.	—	—	1
Mean	—	31	70	—	0.224	15	1
SD	—	6	14	—	0.020	3	0

220, 300, 350, 390 min after oral administration of PY and 0.75, 1.5, 2.5, 3.75, 5, 6.75, 10, 15, 20, 30, 60, 120 min after intravenous administration of PY, respectively). The blood sampling protocols had to be altered occasionally due to clogging of the catheter. Besides collecting blood samples, the APZ (altered states of consciousness) questionnaire and the EWL rating scale (profile of mood states) were answered at the beginning, after 90 min and at the end of the session.

2.2. Sample preparation

Without adding an anticoagulant, plasma was separated by centrifugation (15 min at 3000 rpm) immediately after sampling. 3.0 ml of the clear supernatant plasma were used for analysis and transferred to polypropylene test tubes in order to avoid drug adsorption to glassware (Peng and Chiou, 1990). 150 μl of a concentrated solution of ascorbic acid (940 mg/10 ml water) were added to give a final concentration of 25 mM of ascorbic acid, a sufficiently high concentration to stabilize the analytes and the mixtures were vortexed for 30 s. All samples were stored at −78°C over dry ice and freeze-dried overnight. After reconstitution of the residue with 700 μl of water, the analytes of interest were separated from plasma proteins by in vitro-microdialysis using two polycarbonate-membrane probes per sample. Bidistilled water was used as perfusion liquid at a flow rate of 2 μl/min for a total perfusion time of 2.5 h. The total volume of perfusate (600 μl) was collected in light-protected HPLC vials and concentrated by freeze-drying. The residue was redissolved in 60 μl of mobile phase prior to HPLC analysis.

2.3. Chemicals and materials

PY and PI base were obtained from the Swiss Federal Office of Public Health (Bern, Switzerland) and checked for identity and purity by IR-, NMR-, MS- and HPLC analysis. 4HIAA was synthesized in our laboratory according to the procedure of Stoll et al. (1955) and its structure confirmed by ^{1}H-NMR, ^{13}C-NMR and MS analysis. Ascorbic acid (Microselect, > 99.5% pure), hexylamine (puriss.) and ammonia solution 25% in water (Microselect, puriss. p.a.) were purchased from Fluka BioChemika (Buchs, Switzerland). The HPLC grade methanol and acetonitrile as well as ammonium acetate (puriss. p.a.) and ortho-phosphoric acid 85% (puriss. p.a.) were obtained from Merck (Zürich, Switzerland). Bidistilled water was used for all purposes. Standard solutions of PI and 4HIAA were prepared in water containing 25 mM of ascorbic acid and stored in light-protected vials at −25°C. These solutions were found to be stable for at least 3 weeks. For microdialysis CMA/10 polycarbonate-membrane probes (membrane diameter 0.5 mm, length 16 mm, molecular weight cut off 20,000 Da) from Schmidlin (Neuheim, Switzerland) were used. HPLC separation of PI was performed on Spherisorb RP-8 cartridges (particle size 3 μm, 50 × 4.6 mm I.D. and 150 × 4.6 mm I.D., respectively) supplied by Chemie Brunschwig (Basel, Switzerland). 4HIAA was determined by use of a Lichrospher 100 RP-18 column (particle size 5 μm, 125 × 4.6 mm) from Merck (Zürich, Switzerland) fitted with a RP-18 guard column (particle size 5 μm, 4 × 4 mm I.D.).

2.4. Instrumentation

Microdialysis for sample preparation was performed using CMA microdialysis pumps Mod. 102 from Schmidlin (Neuheim, Switzerland). Freeze-drying was achieved on a Lyo-System GT 2 supplied by Leybold Heraeus (Köln, Germany). The HPLC-ECD system consisted of an Altex LC pump Mod. 100 from Beckman (Zürich, Switzerland) with additional external pulsation damper, two Rheodyne injection valves Mod. 7125 from Kontron (Zürich,

Switzerland), an ESA Coulochem II electrochemical detector from Stagroma (Wallisellen, Switzerland) and a Kontron CT-10 integrator coupled with a plotter Mod. 800 from Kontron (Zürich, Switzerland).

2.5. Analytical procedure for PI quantification

For PI quantification 20 μl of the reconstituted freeze-dried plasma dialysate were injected into the HPLC system. The mobile phase consisted of 47% (v/v) water containing 0.3 M ammonium acetate buffered to pH 8.3 by addition of ammonia solution 25% and 53% (v/v) of methanol and was used at a flow rate of 450 μl/min. In order to avoid excess plasma matrix and ascorbic acid reaching the detector cell, the following column-switching step was introduced. The outlet of the injection valve was connected to a 5 cm Spherisorb RP-8 column to achieve a pre-separation of the injected dialysate. By linking the pre-column outlet with the inlet of a second six-port Rheodyne valve, the flow of the eluate could be directed either to the waste or to the 15 cm Spherisorb RP-8 analytical column by simple valve switching. Exactly 2.2 min after sample injection, the separator valve was switched to 'analyze' position in order to achieve a separation of PI from remaining endogenous plasma compounds without any loss of analyte. The time of switching was determined by repeated injection of stabilized standard solutions and consecutive monitoring of the PI recovery. In order to obtain an optimal baseline stability, the electrochemical detector cell was set at 35°C. The recording of a hydrodynamic voltammogram (detection voltage versus current response plot) of PI in the mobile phase showed the best compromise between optimal sensitivity (high working potential with maximum signal) and optimal selectivity (low working potential with minimal noise and best signal/noise ratio) at a detection voltage of +150 mV. At this working potential 76% of the maximum signal (found at +650 mV) is achieved, but the voltammogram shows only 0.7% of the baseline signal at +650 mV. If detection voltages above +300 mV are applied, the baseline signal increases dramatically due to oxidation of the water in the mobile phase. The detector was run at +150 mV for all PI determinations and the 100% detector response range was set at 1 μA. Representative chromatograms of blank, spiked (10 ng PI/ml plasma) and post-dose plasma samples are presented in Fig. 2. Due to the column-switching process, the retention time of PI varies in the range of ±5%.

2.6. Analytical procedure for 4HIAA quantification

Chromatographic separation of 4HIAA was achieved on a Lichrospher 100 RP-18 column using a mobile phase of 70 mM ortho-phosphoric acid containing 5.5% (v/v)

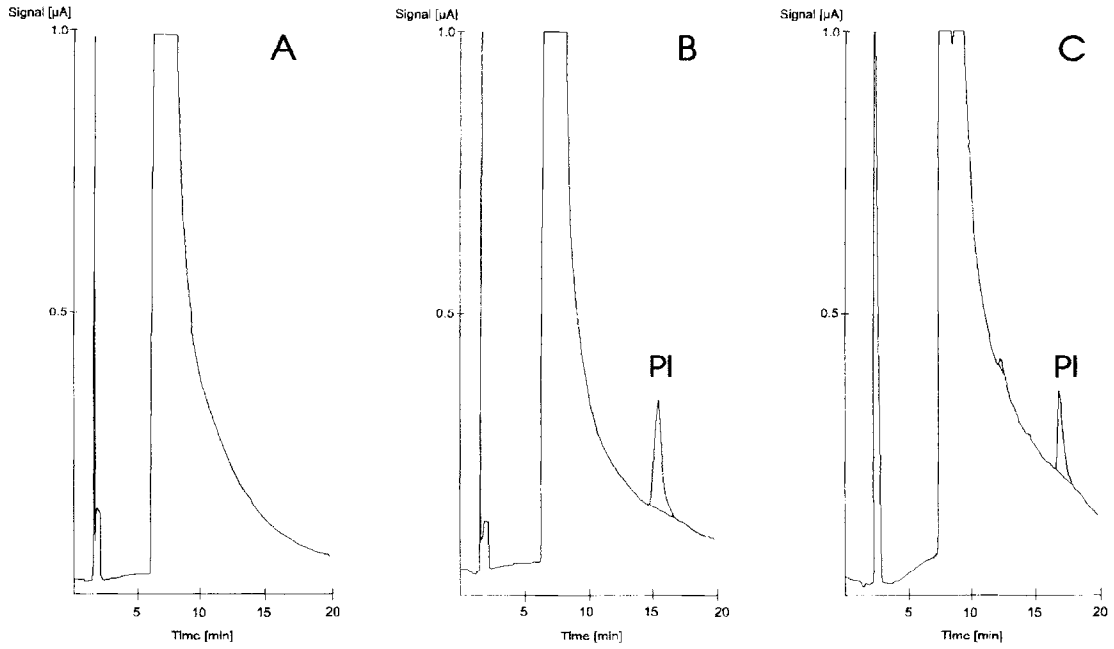

Fig. 2. HPLC-ECD determination of PI. Representative chromatograms obtained from blank (A), spiked (B, 10 ng PI/ml plasma) and post-dose plasma samples (C, subject E, 220 min after oral administration of 15 mg PY).

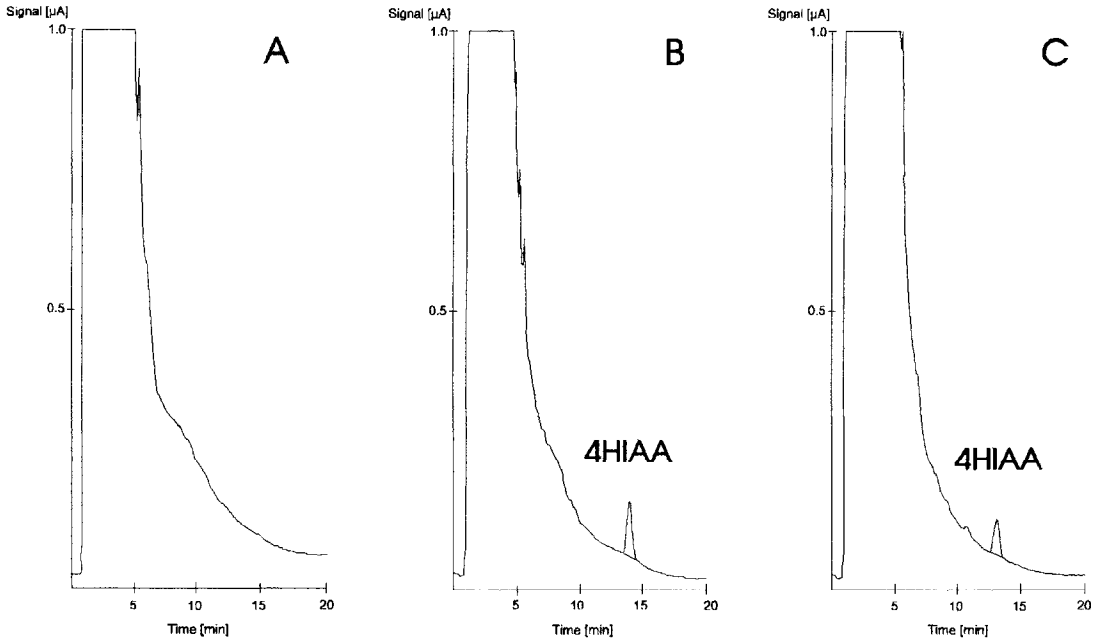

Fig. 3. HPLC-ECD determination of 4HIAA. Representative chromatograms obtained from blank (A), spiked (B, 50 ng 4HIAA/ml plasma) and post-dose plasma samples (C, subject E, 150 min after oral administration of 15 mg PY).

acetonitrile and 300 µl/l hexylamine at a flow rate of 1 ml/min. For quantification a sample volume of 10 µl was injected and 4HIAA was detected by applying a working potential of +175 mV. At this detection voltage the maximum signal response for 4HIAA is achieved without noticeable increase of the background current. The 100% response range of the detector was set at 1 µA. Representative chromatograms of blank, spiked (50 ng 4HIAA/ml plasma) and post-dose plasma samples are presented in Fig. 3.

2.7. Stabilization of PI and 4HIAA

Experiments with PI and 4HIAA showed that these substances are highly unstable in aqueous solutions. HPLC chromatograms from unstabilized solutions usually showed decreasing signals over the time as well as additional peaks most presumably from degradation products. The rate of degradation showed to be strongly dependent on the concentration of the analytes. In ng/ml ranges, as PI and 4HIAA were expected to be present in plasma samples, the oxidation rate increases dramatically compared to µg/ml or mg/ml concentrations. These facts indicate the necessity of stabilizing the analytes during sample processing and analysis. Ying and Ming (1994) used ethylenediaminetetraacetate disodium salt dihydrate (EDTA) to protect dopamine and related compounds from oxidation by scavenging metal ions which are considered to potentiate autooxidation of indole compounds. However, EDTA completely failed to protect PI and 4HIAA and also experiments with sodium sulfite and sodium hydrogen sulfite were not successful. Finally, ascorbic acid used in concentrations higher than 10 mM showed to be an effective compound to stabilize the phenolic analytes. Stress tests with unstabilized aqueous PI and 4HIAA standard solutions and solutions containing 1, 6, 10 and 25 mM of ascorbic acid were performed. All solutions were stored in an ultrasonic bath under light exposure to accelerate the oxidation rate of the analytes. The time dependent degradation process of PI and 4HIAA was monitored by HPLC-ECD. Test results from unstabilized PI solutions and solutions containing 25 mM of ascorbic acid are shown in Fig. 4. Since ascorbic acid is also dialyzed from the plasma

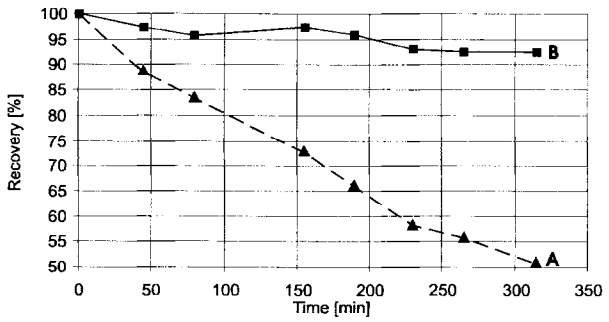

Fig. 4. Stabilization of PI with ascorbic acid. Comparison of PI recoveries from a 10 nM aqueous standard solution (A) and an equimolar PI solution containing 25 mM of ascorbic acid (B).

samples, PI and 4HIAA are stabilized throughout the sample work-up until HPLC analysis.

2.8. Calibration

The quantitative determination of PI and 4HIAA was based on calibration curves established by use of the external standard method. All calibration graphs were obtained by spiking blank plasma with four different amounts of analyte (covering the concentration range of PI and 4HIAA in the plasma samples of the clinical study) and final linear regression analysis of the corresponding detector signal areas. The relationship of detector response and analyte concentration was found to be linear in the range of 0.8–50 ng/ml plasma for PI and 5–500 ng/ml plasma for 4HIAA, respectively. Coefficients of determination (r^2) of the least-squares linear regressions were ≥ 0.995 for both PI and 4HIAA. Interday precisions of the assays were evaluated by measuring spiked blank plasma (0.8, 5, 10, 50 ng PI/ml plasma and 5, 50, 100, 500 ng 4HIAA/ml plasma, respectively) in triplicate and determination of the coefficients of variation (CV). At the low end of the standard curve (0.8 ng PI/ml plasma and 5 ng 4HIAA/ml plasma, respectively) CV were 10.9% and 14.2%, and at the high end (50 ng PI/ml plasma and 500 ng 4HIAA/ml plasma, respectively), CV were 4.8% and 6.5%. Recovery, precision and limits of quantitation of both methods are summarized in Table 2.

2.9. Pharmacokinetic analyses

Analysis of the plasma samples provided individual plasma concentration–time profiles for PI and 4HIAA for each subject. All PI and 4HIAA plasma concentrations were analyzed by use of the TOPFIT Vers. 2.0 computer software (Heinzel et al., 1993) using standard noncompartmental methods. Maximum plasma concentrations (c_{max}) and time to reach maximum plasma concentrations (t_{max}) were found by visual inspection of the raw data. Total area under the curve values ($AUC_{0-\infty}$) after oral administration of PY were dose-normalized for comparison across subjects. For estimation of the terminal elimination half-life ($t_{1/2\beta}$) the following time intervals were used for calculations: 220–390 min for PI and 4HIAA after oral administration of PY and 20–120 min for PI after intravenous dosing of PY, respectively. Estimates of PI bioavailability (F_{abs}) after oral administration of PY were calculated as follows: $F_{abs} = (AUC_{p.o.}/Dose_{p.o.})/(AUC_{i.v.}/Dose_{i.v.}) \times 100$. Values for total plasma clearance (CL), mean residence time (MRT) and steady-state volume of distribution (VD_{ss}) were calculated with the PI plasma concentration data resulting from the intravenous mode of administration. In the calculation of CL and VD_{ss} adjustment for molecular weight differences between PI and PY were considered.

3. Results

3.1. Plasma concentrations and pharmacokinetic profiles of PI and 4HIAA after a single oral dose of PY

Oral doses of 10–20 mg PY resulted in varying intensity in derealisation and depersonalisation phenomena including virtual hallucinations, thought disorders and changes in affect and mood. Reported psychopathological alterations arose with PI plasma levels ranging from 4–6 ng/ml (20–90 min after oral administration of PY and within 2 min after intravenous dosing, respectively) and were tolerated well by the subjects. No physical adverse effects were observed. Detailed correlation data between changes of psychopathological scores and PI plasma levels will be presented elsewhere. PI peaked at an average of 105 ± 37 min ($N = 6$), reaching a maximum concentration of 8.2 ± 2.8 ng/ml plasma. After a slower elimination phase with a terminal elimination half-life $t_{1/2\beta}$ of 163.3 ± 63.5 min the PI concentration usually approached the limit of quantitation of the assay (0.8 ng/ml) within 400 min. The average $AUC_{0-\infty}$ was 1963 ± 659 ng × min/ml. No significant indication for an enterohepatic cycling of PI was found in any plasma profile. 4HIAA attained a maximum concentration of 150 ± 61 ng/ml plasma ($N = 5$)

Table 2
Recovery, precision and limits of quantitation

Compound	Recovery [a] (mean (%) ± SEM [b], $n = 4$)	Precision (mean (%) ± SEM, $n = 4$ [c])	Limit of quantitation (abs. (ng); rel. (ng/ml plasma))
PI	15.1 ± 0.85	7.0 ± 1.3	0.05; 0.8
4HIAA	11.0 ± 1.10	9.1 ± 1.7	0.50; 5.0

[a] The low recoveries are due to the microdialysis work-up technique showing typical recoveries < 25%.
[b] Standard error of the mean.
[c] Mean of coefficients of variation of 4 concentrations.

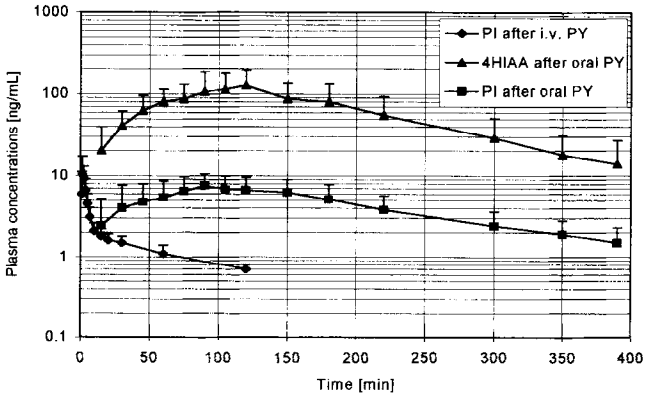

Fig. 5. Plasma concentration–time profiles (mean + S.D.) of PI and 4HIAA after oral administration of 0.224 ± 0.02 mg PY/kg b.wt. and PI plasma concentrations (mean + S.D.) after intravenous administration of 1 mg PY.

113 ± 41 min after oral administration of PY. Statistical comparison of the values of t_{max} and $t_{1/2\beta}$ did not show significant differences for PI and 4HIAA at a significance level of $\alpha = 0.05$. Though the time to peak concentration of 4HIAA is not reached significantly later compared to the t_{max} of PI, the hypothesis of a first pass effect explaining the very low PI concentrations in plasma is supported. One volunteer (subject E) showed a first 4HIAA concentration plateau (82 ng/ml) after 30 min but the maximum plasma concentration of 335 ng/ml was not reached before 290 min. This metabolic behavior was not seen in any other subject, therefore the data of subject E were rejected for calculations of means. Mean plasma concentration-time profiles of PI and 4HIAA after oral administration of PY are shown in Fig. 5 and detailed pharmacokinetic data of PI and 4HIAA for all subjects are listed in Table 3.

3.2. Plasma concentrations and pharmacokinetic profiles of PI after a single intravenous dose of PY

Pharmacokinetics of systemically administered PY were investigated after single intravenous doses of 1 mg PY to six subjects. In contrast to oral administration, moderate vegetative effects such as nausea, vertigo and fatigue were reported by two subjects during the first 5 min following administration. Psychopathological symptoms were similar, but somewhat less pronounced than those seen after oral administration. Somatic and psychotropic effects after higher intravenous doses of PY are reported in Section 4. The mean plasma concentration-time profile for PI resulting from i.v. administration of PY is shown in Fig. 5. An almost immediate dephosphorylation of PY is seen in all subjects, leading to a maximum PI plasma concentration of 12.9 ± 5.6 ng/ml after 1.9 ± 1.0 min ($N = 6$). After peaking, the PI plasma concentration decreased rapidly with a mean $t_{1/2\beta}$ of 74.1 ± 19.6 min, this half-life being significantly shorter than after oral administration of PY (163.3 ± 63.5 min). The limit of quantitation of the assay was reached generally within 120 min. An average mean residence time (MRT) of 90.9 ± 28.2 min, a mean total plasma clearance (CL) of 3126 ± 719 ml/min and a mean volume of distribution at steady-state (Vd_{ss}) of 277 ± 92 l was calculated. The time dependent plasma concentrations of PI are in good agreement with the drug effects reported by the subjects, the latter being of short duration and subsiding within 15 to 30 min after injection. Detailed data for PI

Table 3
Pharmacokinetic data of PI and 4HIAA following a single oral dose of PY

Subject	Pharmacokinetic parameters of PI					Pharmacokinetic parameters of 4HIAA			
	C_{max} [a] (ng/ml plasma)	t_{max} (min)	$AUC_{0-\infty}$ (ng·min/ml)	$t_{1/2\beta}$ (min)	F_{abs} [b] (%)	C_{max} (ng/ml plasma)	t_{max} (min)	$AUC_{0-\infty}$ (ng·min/ml)	$t_{1/2\beta}$ (min)
A	9.1	85	1971	106.0	—	117	125	24,537	180.0
B	7.9	88	1757	182.9	59.7	238	88	57,429	234.4
C	4.8	100	1455	272.2	—	154	170	23,880	42.7
D	5.5	90	1184	111.0	29.7	73	60	6,343	39.5
E	12.3	90	2988	183.0	68.6	335	290	—	—
F	9.6	180	2425	124.5	—	167	120	42,108	226.0
Mean [c]	8.2	105	1963	163.3	52.7	150	113	30,859	144.5
SD [c]	2.8	37	659	63.5	20.4	61	41	19,508	96.7

[a] Pharmacokinetic parameters are defined in Section 2.
[b] Subjects B, D and E received PY by oral and intravenous route of administration.
[c] The mean and SD in the calculation of the kinetic parameters of 4HIAA are based on $N = 5$, excluding subject E who showed a unique second redistribution phase not seen in any other subject.

Table 4
Pharmacokinetic data of PI following a single intravenous dose of 1 mg PY

Subject	C_{max} [a] (ng/ml plasma)	t_{max} (min)	$AUC_{0-\infty}$ (ng·min/ml)	$t_{1/2\beta}$ (min)	CL (ml/min)	MRT (min)	VD_{SS} (l)
B	12.6	2.5	234	59.6	3068	70.0	213
D	12.4	1.5	202	97.5	3557	123.4	440
E	8.4	1.6	329	91.1	2185	114.9	252
G	13.9	0.7	168	49.8	4276	59.8	254
H	7.1	3.7	245	85.3	2925	110.2	322
I	23.1	1.5	262	61.2	2745	67.0	183
Mean	12.9	1.9	240	74.1	3126	90.9	277
SD	5.6	1.0	55	19.6	719	28.2	92

[a] Pharmacokinetic parameters are explained in Section 2.

in all subjects are listed in Table 4. The plasma samples of two subjects were also examined for the presence of 4HIAA, but in none of the samples 4HIAA was found. Fittings of PI plasma concentration-time data sets resulting from i.v. and oral dosing of PY to pharmacokinetic models are reported in Section 4.

Three subjects (B,D and E) who participated in the study with intravenous PY, received oral doses of PY, too. With the known individual doses and the corresponding $AUC_{0-\infty}$ values calculated from the plasma concentration versus time data of these subjects, the absolute bioavailability (F_{abs}) of PI (liberated from the orally administered prodrug PY) was found to be $52.7 \pm 20\%$. Individual values for these subjects are listed in Table 3.

4. Discussion

4.1. Analytical method

The analytical challenge of this study was to solve the problems of the high instability of the analytes and their low ng/ml concentrations in plasma. Immediate addition of ascorbic acid to the plasma samples proved to be an effective step to prevent oxidation and to achieve accurate determination of PI and 4HIAA. Despite this stabilization, the often useful sample work-up method of solid phase extraction (SPE) using reversed phase material (RP-18), strong cation exchange phase (SCX) or aminobonded material (NH_3) turned out to provide irreproducible results, presumably due to promoted oxidation processes on the extended surface of these column materials, also under nitrogen. When the technique of microdialysis is applied, the analytes are continuously stabilized by the presence of ascorbic acid and no loss of PI or 4HIAA is observed. On the other hand this procedure is time-consuming (depending on the number of microdialysis pumps available for simultaneous work-up of several samples) and shows much lower recoveries (typically $< 25\%$) than SPE methods. The sensitivity required to quantitatively determine PI and 4HIAA in biological samples can only be achieved by application of ECD. Optimized UV detection showing a quantitation limit of approximately 10 ng PI/ml would only allow measurement of the peak concentration of a sample series. Also the GC-MS technique showed insufficient sensitivity with the additional disadvantage of the necessary derivatizing step and low precision in quantitation. The addition of hexylamine as a masking agent to the mobile phase used for HPLC separation of 4HIAA leads to a quantitative saturation of remaining unsilanized silanol groups in the RP-18 material of the analytical column. Hexylamine leads to an improved peak shape and a better signal to noise ratio. None of the tested structurally related compounds (e.g. 5-hydroxy-N,N-dimethyltryptamine, 2-methylindole, 4-hydroxyindole, 4-hydroxy-N-methyltryptamine and 5-hydroxy-N,N-diethyltryptamine) was suited as an internal standard, either because these substances required oxidation voltages highly different to those of the analytes to be detected or because they showed inappropriate chromatographic behavior and would be at least partially lost in the column switching process.

4.2. Galenic preparation and dose finding for the intravenous PY study

Stability tests investigating the thermal stability of PY showed that a rapid and extensive dephosphorylation to PI occurs when aqueous PY injection preparations are sterilized by heating in an autoclave (121°C; 20 min). Addition of ascorbic acid in different amounts did not sufficiently reduce the degradation process. The method of sterile filtration was then chosen to ensure absence of pathogenic agents in the formulation. These preparations were found to be stable for at least 1 month when stored at 5°C.

As, to our best knowledge, PY has never before been administered intravenously to humans under controlled

clinical conditions, dose-finding studies were necessary starting with an amount of 50 μg PY. The doses were then increased step by step up to 1 mg, where distinct hallucinogenic effects were reported by the subjects without appearance of physical adverse reactions. One trial with 3 mg PY, injected within a time range of 90 s, was found to produce strong physical symptoms such as vertigo, vomiting and cardiovascular side effects. At this dosage level the reported derealisation and depersonalisation phenomena were characterized as fearful and very unpleasant due to almost complete loss of contact with reality. However, 10 min after injection these more fearful psychotomimetic effects disappeared, the subject gained surveillance and recovered completely within 40 min. Throughout the clinical study with intravenous PY, the chosen threshold dosage of 1 mg PY was found to be well tolerated by all subjects.

4.3. Pharmacokinetic findings

The judgement of the pharmacokinetic findings is difficult due to the obligation of handling data resulting from drug metabolites. Strongly supported by earlier in vivo and in vitro metabolism experiments with PY performed on mice (Horita and Weber, 1962) and rodent tissues (Horita and Weber, 1961; Eivindvik and Rasmussen, 1989), it is assumed that PY is completely converted to PI before entering the systemic circulation. This assumption is supported by the finding that equimolar amounts of PY and PI evoke qualitatively and quantitatively comparable psychotropic effects in man (Laatsch, 1996). However, in default of a reliable analytical method to determine PY in plasma samples, it was not yet possible to proof this assumption by showing the absence of parent drug in plasma after PY administration. Although the rate of formation of PI is very fast, PY is most presumably measurable just after an intravenous bolus injection. The observed differences in $t_{1/2\beta}$ for PI between oral and intravenous dosing of PY (163 ± 63 min following oral application and 74 ± 20 min following i.v. application, respectively) seems to be a dose-dependent effect. From the i.v. experiment with 3 mg PY mentioned in the upper part of Section 4 resulted a calculated $t_{1/2\beta}$ of 146.6 min, a value similar to the one seen after oral administration of PY. Since the 3 mg dose evoked too strong physical and psychotropic effects, repetition of this experiment to support the assumption of dose-dependent kinetics of PI after intravenous PY was not possible. The absence of 4HIAA in the plasma samples from the study with 1 mg of intravenous PY is explained by metabolically formed 4HIAA concentrations below the limit of quantitation of our analytical method. PI plasma concentration–time curves for both intravenously and orally administered PY can be described by two exponential components, one for metabolite formation (k_f, PI after i.v. PY), or summarizing metabolite formation and absorption ($k_{f,a}$, PI after oral PY) and one for elimination (k_e). Whether the metabolite formation or absorption is the rate-limiting step concerning the rate of entry of PI into the systemic circulation after oral PY cannot be determined. Kinetically, administering an i.v. bolus of drug and measuring the plasma metabolite concentration is similar to giving an oral dose of drug and measuring its plasma concentration (Rowland and Tozer, 1995). In terms of a model, the plasma concentration-time profiles of PI after i.v. PY can be fitted to a one-compartment model with (very fast) first-order absorption and ('standard') first-order elimination. Using the standard one compartment model of the TOPFIT data analysis software (Heinzel et al., 1993), a formation rate constant k_f of 45.8 min^{-1} was estimated for PI after i.v. PY (data sets treated as p.o. administered PI) and a summarized formation and absorption rate constant $k_{f,a}$ of 0.0107 min^{-1} was calculated for the appearance of PI in plasma after oral administration of PY. The corresponding elimination rate constants k_e were 0.0105 min^{-1} for the systemic elimination of PI after oral PY and 0.0174 min^{-1} for PI following i.v. application of PY; the latter estimation was not affected by the input model chosen. The better accuracy of the 'PI p.o. input model' compared to a standard i.v. bolus model is supported by the calculated mean b-values (fit criterion used in the TOPFIT software) of the objective functions (0.9095 for the p.o. input model compared to 0.7725 for the standard i.v. bolus model). An incomplete conversion from PY to PI may explain the finding of an extremely high plasma clearance of PI after intravenous administration of PY (3.1 ± 0.7 l/min). Although the current status of PY metabolism (Fig. 1) doesn't propose an alternative metabolic pathway (e.g. excretion as unchanged compound or formation of PY-glucuronides), only the determination of PY in plasma and urine (or the proof of absence) can elucidate this point at issue.

4.4. Further investigations

Studies investigating the pharmacokinetic behavior of orally and intravenously administered PI as well as clinical research projects examining the pharmacokinetic properties of PI after ingestion of *Psilocybe* mushrooms are in progress. The authors hope that the presented study may contribute to further clinical research projects with PY such as positron emission tomography-assisted studies with labelled PY in order to better understand the mechanisms and sites of action of PY in the human brain.

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References

Beug, M.W., Bigwood, J., 1981. Quantitative analysis of psilocybin and psilocin in *Psilocybe baeocystis* (Singer and Smith) by high-performance liquid chromatography and by thin-layer chromatography. J. Chromatogr. 207, 379–385.

Borner, S., Brenneisen, R., 1987. Determination of tryptamine derivatives in hallucinogenic mushrooms using high-performance liquid chromatography with photodiode array detection. J. Chromatogr. 408, 402–408.

Brown, J.K., Shapazian, L., Griffin, G.D., 1972. A rapid procedure for some 'street drugs' by thin-layer chromatography. J. Chromatogr. 64, 129–133.

Christiansen, A.L., Rasmussen, K.E., 1983. Screening of hallucinogenic mushrooms with high- performance liquid chromatography and multiple detection. J. Chromatogr. 270, 293–299.

Eivindvik, K., Rasmussen, K.E., 1989. Handling of psilocybin and psilocin by everted sacs of rat jejunum and colon. Acta Pharm. Nord. 1, 295–302.

Heinzel, G., Woloszczak, R., Thomann, P., 1993. Topfit, Version 2.0: Pharmacokinetic and Pharmacodynamic Data Analysis System for the PC. Gustav Fischer, Stuttgart, Jena, New York.

Hermle, L., Spitzer, M., Borchardt, D., Gouzoulis, E., 1992. Beziehungen der Modell- bzw Drogenpsychose zu schizophrenen Erkrankungen. Fortschr. Neurol. Psychiat. 60, 383–392.

Holzmann, P.P., 1995. Bestimmung von Psilocybin-Metaboliten im Humanplasma und -urin. Ph.D. Thesis, Eberhard-Karls-Universität, Tübingen, Germany.

Horita, A., Weber, L.J., 1961. The enzymic dephosphorylation and oxidation of psilocybin and psilocin by mammalian tissue homogenisates. Biochem. Pharmacol. 7, 47–54.

Horita, A., Weber, L.J., 1962. Dephosphorylation of psilocybin in the intact mouse. Toxicol. Appl. Pharmacol. 4, 730–737.

Kalberer, F., Kreis, W., Rutschmann, J., 1962. The fate of psilocin in the rat. Biochem. Pharmacol. 11, 261–269.

Kysilka, R., Wurst, M., Pacakova, V., Stulik, K., Haskovec, L., 1985. High-performance liquid chromatographic determination of hallucinogenic indoleamines with simultaneous UV photometric and voltametric detection. J. Chromatogr. 320, 414–420.

Kysilka, R., Wurst, M., 1988. Optimized determination of indole derivatives by high-performance liquid chromatography with selective detection. J. Chromatogr. 446, 315–321.

Kysilka, R., Wurst, M., 1989. High-performance liquid chromatographic determination of some psychotropic indole derivatives. J. Chromatogr. 464, 434–437.

Laatsch, H., 1996. Zur Pharmakologie von Psilocybin und Psilocin. In: Liggenstorfer, R., Rätsch, C. (Eds.), Maria Sabina, Botin der heiligen Pilze. Nachtschatten Verlag, Solothurn, Switzerland, pp. 193–202.

Peng, G.W., Chiou, W.L., 1990. Analysis of drugs and other toxic substances in biological samples for pharmacokinetic studies. J. Chromatogr. 531, 3–50.

Perkal, M., Blackman, G.L., Ottrey, A.L., Turner, L.K., 1980. Determination of hallucinogenic components of psilocybe mushrooms using high-performance liquid chromatography. J. Chromatogr. 196, 180–184.

Rowland, M., Tozer, N.T., 1995. Clinical Pharmacokinetics. Concepts and Applications. Williams and Wilkins, Baltimore, Philadelphia, Hong Kong.

Sottolano, S.M., Lurie, I.S., 1983. The quantitation of psilocybin in hallucinogenic mushrooms using high performance liquid chromatography. J. Forens. Sci. 28, 929–935.

Stoll, A., Troxler, F., Peyer, J., Hofmann, A., 1955. Eine neue Synthese von Bufotenin und verwandten Oxy-tryptaminen. Helv. Chim. Acta 38, 1452–1472.

Tanimukai, H., 1967. Modifications of paper and thin layer chromatographic methods to increase sensitivity for detecting N-methylated indoleamines in urine. J. Chromatogr. 30, 155–163.

Thomson, B.M., 1980. Analysis of psilocybin and psilocin in mushroom extracts by reversed-phase high-performance liquid chromatography. J. Forens. Sci. 25, 779–785.

Timmons, J.E., 1984. The identification of psilocin and psilocybin using gas chromatography-mass spectrometry. Microgram 17, 28–32.

Unger, S.E., Cooks, R.G., 1979. Application of mass spectrometry/mass spectrometry (ms/ms) to the identification of natural products in *Psilocybe cyanensis*. Anal. Lett. 12, 1157–1167.

United Nations, 1989. Recommended Methods for Testing Peyote Cactus (Mescal Buttons)/Mescaline and Psilocybe Mushrooms/Psilocybin. Manual ST/NAR/19, New York.

Vanhaelen-Fastré, R., Vanhaelen, M., 1984. Qualitative and quantitative determination of hallucinogenic components of psilocybe mushrooms by reversed-phase high-performance liquid chromatography. J. Chromatogr. 312, 467–472.

Vollenweider, F.X., Antonini, A., Scharfetter, C., Angst, J., Leenders, K.L., 1994. Hyperfrontality and psychopathology in the acute ketamin and psilocybin model of schizophrenia using PET and FDG. J. Neurol. 241, 262.

White, P.C., 1979. Analysis of extracts from *Psilocybe semilanceata* mushrooms by high-pressure liquid chromatography. J. Chromatogr. 169, 453–456.

Wurst, M., Semerdzieva, M., Vokoun, J., 1984. Analysis of psychotropic compounds in fungi of the genus *Psilocybe* by reversed-phase high-performance liquid chromatography. J. Chromatogr. 286, 229–235.

Ying, S., Ming, Y.Y., 1994. Determination of the stability of dopamine in aqueous solutions by high performance liquid chromatography. J. Liq. Chromatogr. 7, 1557–1565.