Research article

Drug Testing and Analysis

Received: 1 February 2012 Revised: 24 February 2012 Accepted: 24 February 2012 Published online in Wiley Online Library: 19 April 2012

(www.drugtestinganalysis.com) DOI 10.1002/dta.1344

Metabolism and disposition of *N,N*-dimethyltryptamine and harmala alkaloids after oral administration of ayahuasca

Jordi Riba,[a,b]* Ethan H. McIlhenny,[c] Marta Valle,[b,d] José Carlos Bouso[a,b] and Steven A. Barker[c]

Ayahuasca is an Amazonian psychotropic plant tea obtained from *Banisteriopsis caapi*, which contains β-carboline alkaloids, chiefly harmine, harmaline and tetrahydroharmine. The tea usually incorporates the leaves of *Psychotria viridis* or *Diplopterys cabrerana*, which are rich in *N,N*-dimethyltryptamine (DMT), a psychedelic 5-HT$_{2A/1A/2C}$ agonist. The β-carbolines reversibly inhibit monoamine-oxidase (MAO), effectively preventing oxidative deamination of the orally labile DMT and allowing its absorption and access to the central nervous system. Despite increased use of the tea worldwide, the metabolism and excretion of DMT and the β-carbolines has not been studied systematically in humans following ingestion of ayahuasca. In the present work, we used an analytical method involving high performance liquid chromatography (HPLC)/electrospray ionization (ESI)/selected reaction monitoring (SRM)/tandem mass spectrometry(MS/MS) to characterize the metabolism and disposition of ayahuasca alkaloids in humans. Twenty-four-hour urine samples were obtained from 10 healthy male volunteers following administration of an oral dose of encapsulated freeze-dried ayahuasca (1.0 mg DMT/kg body weight). Results showed that less than 1% of the administered DMT dose was excreted unchanged. Around 50% was recovered as indole-3-acetic acid but also as DMT-*N*-oxide (10%) and other MAO-independent compounds. Recovery of DMT plus metabolites reached 68%. Harmol, harmalol, and tetrahydroharmol conjugates were abundant in urine. However, recoveries of each harmala alkaloid plus its *O*-demethylated metabolite varied greatly between 9 and 65%. The present results show the existence in humans of alternative metabolic routes for DMT other than biotransformation by MAO. Also that *O*-demethylation plus conjugation is an important but probably not the only metabolic route for the harmala alkaloids in humans. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: ayahuasca; DMT; harmala alkaloids; urine; metabolism; disposition

Introduction

Ayahuasca is a psychotropic plant tea obtained from the stems of the jungle liana *Banisteriopsis caapi* and usually the leaves of *Psychotria viridis* or *Diplopterys cabrerana*.[1,2] The tea is used by many Amazonian peoples to attain a modified state of consciousness, which is a central element of rites of passage, religious ceremonies, and shamanic medicine.[2] In recent years, the firmly established ancestral uses of ayahuasca have given way to new forms of consumption. Syncretic religious groups using ayahuasca as a sacrament have appeared and have expanded their activities to the urban areas of South America and also to Europe and North America. An increasing number of foreigners travel to the Amazon to participate in ayahuasca retreats and traditional healers travel to Europe to organize ayahuasca ceremonies. The growing attention ayahuasca is attracting worldwide has raised public health concerns.[3]

The powerful psychotropic effects of ayahuasca arise from the pharmacological interaction between the β-carboline alkaloids present in *B. caapi* and the tryptamines found in *P. viridis* and *D. cabrerana*. On the one hand, the β-carbolines, mainly harmine, harmaline, and tetrahydroharmine, are reversible inhibitors of the enzyme monoamine-oxidase A (MAO-A).[4,5] On the other hand, *P. viridis* and *D. cabrerana* contain DMT,[5] a potent psychedelic,[6–8] which is a priori not active orally[7] due to extensive first-pass metabolism by MAO-A. Both the β-carbolines, also known as harmala alkaloids, and the DMT present in the plants are extracted into the ayahuasca infusion and ingested by users.[9] The blockade of visceral MAO brought about by the β-carbolines is believed to render DMT orally active, allowing its access to systemic circulation and subsequently to the central nervous system.[10] There, DMT interacts with serotonergic 5-HT$_{2A}$, 5-HT$_{1A}$ and 5-HT$_{2C}$ and other receptor sites,[11–15] eliciting psychedelic effects in humans.[16,17]

* *Correspondence to: Jordi Riba. Human Experimental Neuropsychopharmacology, IIB Sant Pau, Hospital de la Santa Creu i Sant Pau. St. Antoni Maria Claret, 167. Barcelona 08025, Spain. E-mail: jriba@santpau.cat*

a *Human Experimental Neuropsychopharmacology, Institute for Biomedical Research IIB Sant Pau, Barcelona, Spain*

b *Centre d'Investigació de Medicaments, Servei de Farmacologia Clínica, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, and Departament de Farmacologia, de Terapèutica i de Toxicologia, Universitat Autònoma de Barcelona, and Centro de Investigación Biomédica en Red de Salud Mental, CIBERSAM*

c *Department of Comparative Biomedical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, USA*

d *Pharmacokinetic and Pharmacodynamic Modelling and Simulation, Institute for Biomedical Research IIB Sant Pau, Barcelona, Spain*

The chemical structures of ayahuasca alkaloids and their metabolites are shown in Figure 1.

Early studies involving the administration of pure DMT to humans had already observed that it lacked psychoactive effects after oral administration.[7] Following parenteral DMT, Szára failed to find the unmetabolized drug in urine and identified indole-3-acetic acid (IAA), formed by oxidative deamination, as the drug's degradation product.[6] Kaplan *et al*. found that following an intramuscular injection, DMT disappeared from plasma very rapidly. They reported that less than 0.1% was recovered in 24 h urine but they did not attempt to identify the putative metabolites.[18]

The role of MAO in the metabolic breakdown of DMT has been stressed in the literature based in the aforementioned presence of IAA in urine after DMT and in the efficacy of the harmala alkaloids and other MAO-inhibitors to render DMT psychoactive *per os*.[19] However, oxidative deamination by MAO may not be the sole metabolic pathway in humans. *In vitro* and animal studies have described *N*-oxidation, *N*-demethylation and cyclization as alternative metabolic routes,[20–22] as depicted in Figure 2.

To date, no study has addressed the fate of DMT and the harmala alkaloids when administered in combination in ayahuasca. In a preliminary assessment conducted by our group in the course of analytical method validation, DMT-*N*-oxide (DMT-NO) and harmol and harmalol, the *O*-demethylation products of the harmine and harmaline, respectively, were detected in the urine and blood of three individuals after ayahuasca intake.[23,24] This paper describes the assessment of the metabolism and urinary disposition of DMT and the harmala alkaloids in a group of healthy volunteers following ayahuasca administration.

Materials and methods

Volunteers

Ten young healthy male volunteers were recruited. Participants were experienced psychedelic drug users. The most commonly used substances were psilocybin mushrooms and LSD, followed by ketamine, peyote, and mescaline. None of the participants had used ayahuasca before. Volunteer mean age was 29.0 years (range 20–38); mean weight was 67.0 kg (range 60–85); and mean height was 1.77 m (range 1.69–1.96). Volunteers underwent a structured psychiatric interview (DMS-IV) to exclude current or past history of Axis-I disorders and alcohol or other substance dependence. General good health was confirmed by medical history, laboratory tests, and ECG.

The study was conducted in accordance with the Declarations of Helsinki and Tokyo concerning experimentation on humans, and was approved by the hospital's ethics committee and the Spanish Ministry of Health. All volunteers gave their written informed consent to participate.

Drugs

Ayahuasca was administered orally as an encapsulated lyophilizate. The freeze-dried material was obtained from a Brazilian batch of ayahuasca and contained 8.33 mg DMT, 14.13 mg harmine, 0.96 mg harmaline, and 11.36 mg tetrahydroharmine (THH) per

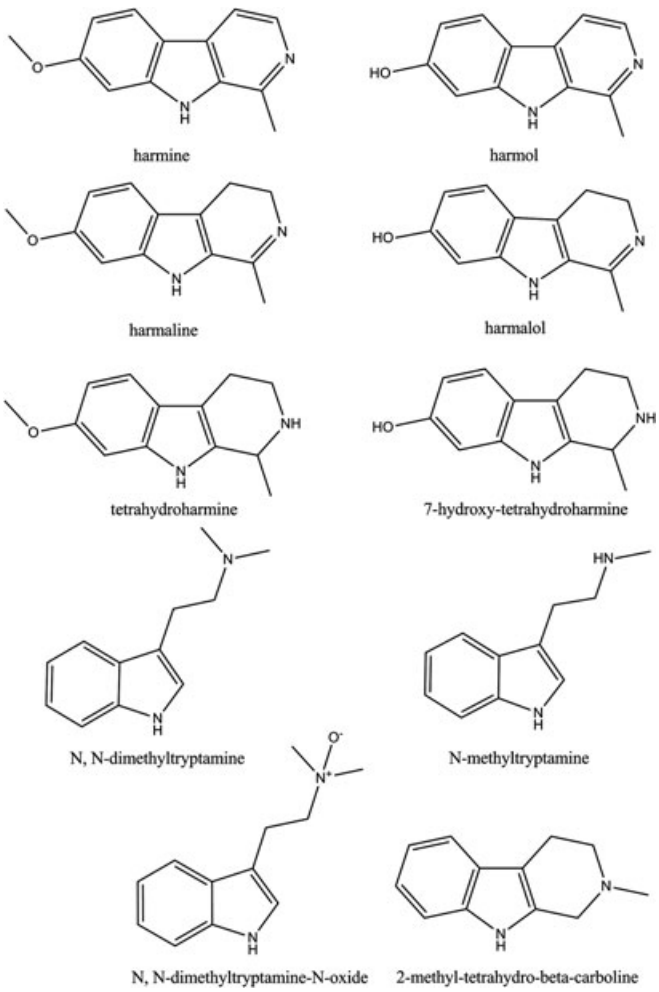

Figure 1. Chemical structures of ayahuasca alkaloids and their metabolites.

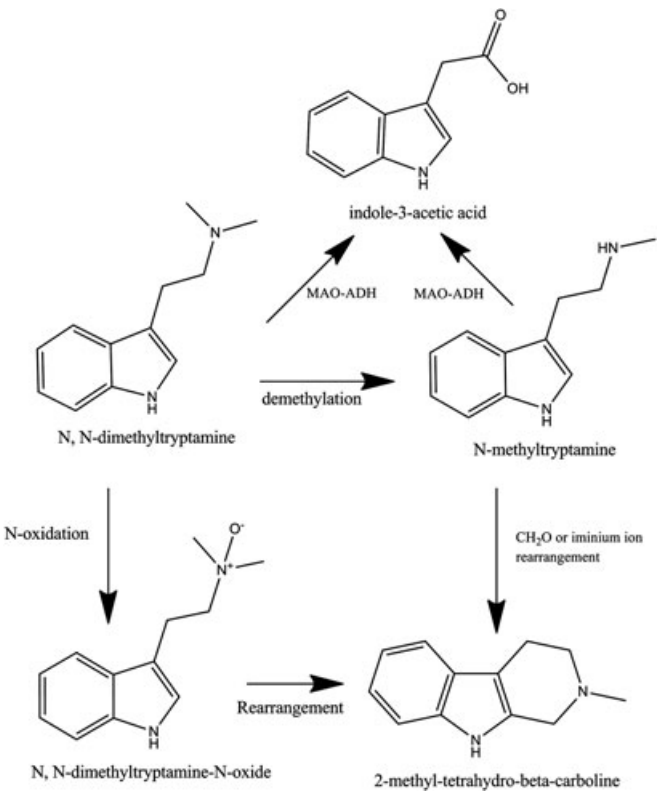

Figure 2. Metabolic pathways of *N*,*N*-dimethyltryptamine. MAO = monoamine-oxidase; ADH = aldehyde-dehydrogenase.

gram. The lyophilizate was also tested for harmol and harmalol and was found to contain 0.30 mg/g harmol and 0.07 mg/g harmalol. Freeze-dried ayahuasca was administered in doses equivalent to 1.0 mg DMT/kg body weight.

Study design and sample collection

Urine samples were obtained in the course of a clinical trial involving three experimental sessions. In a double-blind cross-over balanced design, participants received in each experimental session one of the following treatments: a lactose placebo, 20 mg *d*-amphetamine, and 1.0 mg DMT/kg body weight ayahuasca. In addition to urine collection, the study involved the measurement of various pharmacodynamic parameters including subjective, neuroendocrine, and immunomodulatory data. A detailed description of the methods used and the results concerning these variables have been published elsewhere.[25] In this paper, we report only the data obtained from the analyses of urine samples collected following ayahuasca administration. The amounts of harmine, harmaline, and tetrahydroharmine recovered in urine are reported together with the amounts of their potential *O*-demethylated metabolites, harmol, harmalol, and tetrahydroharmol (7-hydroxy-tetrahydroharmine). Samples were also analyzed for DMT and its potential biotransformation products IAA, DMT-NO, *N*-methyltryptamine (NMT), and 2-methyl-tetrahydro-betacarboline (2MTHBC). Additionally, samples collected after placebo administration were also quantified for IAA, which is known to be excreted under normal physiological conditions. In each experimental session, 24-h urine was collected, subdivided into the following time intervals relative to ayahuasca (and placebo) administration: 0–4 h, 4–8 h, 8–16 h and 16—24 h. The collected urine volume at each time interval was noted, the pooled urine was well mixed, and 50-ml aliquots were separated and stored at −80 °C until analysis. Samples underwent a single freeze-thaw cycle prior to analysis. Samples were analyzed with and without enzyme hydrolysis. Enzyme hydrolysis was achieved using β-glucuronidase/sulfatase from limpets (*Patella vulgata)* Type L-II (Sigma-Aldrich, St Louis, MO, USA) as described by McIlhenny *et al*.[23]

Analytical method

Urine sample analyses were conducted by the methods of McIlhenny *et al*., which uses high performance liquid chromatography (HPLC) with electrospray ionization (ESI) and tandem mass spectrometry (MS/MS).[23] Thus, 100 µl of well-mixed urine were diluted to a volume of 1.0 ml (900 µl of LC mobile phase; 97:3 water with 0.1% formic acid:acetonitrile with 0.1% formic acid), and filtered.[23] A volume of 10 µl was injected for the analysis.

The liquid chromatography-tandem mass spectrometry (LC-MS/MS) method had been validated for the determination of the following compounds: DMT, IAA, DMT-NO, NMT, 5-hydroxy-DMT, dimethylkynuramine, 2MTHBC, 5-methoxy-DMT, harmine, harmaline, tetrahydroharmine, harmol, harmalol, and tetrahydroharmol. Thus, analyses were conducted using an Agilent 1200 series LC system (Agilent Technologies, Palo Alto, CA, USA) equipped with an Agilent G1367A HiP ALS autosampler, an Agilent G1311A Quaternary micropump, and an Agilent G1332A degasser. An Agilent G131gA TCC column oven operating at 25° C was interfaced to a TSQ Quantum Access 1.5 SP1 tandem MS (Thermo Fisher Scientific, Waltham, MA, USA) with ESI operated in the positive ion mode.

Chromatographic separation was achieved on a 1.8 µm 4.6 × 50 mm (i.d.) Agilent ZORBAX Eclipse Plus C18 rapid resolution HT threaded column with an Alltech Direct-Connect Column 2 µm pre-filter (Deerfield, IL, USA) using gradient elution. The MS/MS analysis was performed using selected reaction monitoring (SRM) of the protonated molecular ions for the analytes. The spray voltage was 4000 V, sheath gas (nitrogen) pressure 50 psi, capillary temperature 310° C, and collision pressure was 1.5 psi. of high purity argon. Generation of detection data and integration of chromatographic peaks were performed by Xcalibur 2.0.7 Thermo Fisher Scientific (Waltham, MA, USA) LCquan 2.5.6 QF 30115 software.

Identification of the compounds was based on the presence of the molecular ion at the correct retention time, the presence of three transition ions and the correct ratio of these ions to one another (+/− 25% relative). The proven limit of quantitation (LOQ) was 5 ng/ml for all compounds. The limits of detection for the compounds examined were comparable to results previously attained,[23] ranging from 0.07 ng/ml for DMT-NO to 0.57 ng/ml for harmol. Tetrahydroharmol was observed to have a LOD of 0.17 ng/ml.

Statistics

Descriptive statistics (mean and standard deviation) were used to report the amounts of the different compounds measured. Percentage recoveries were calculated relative to the amount of parent compound administered. Differences in percentage recoveries between enzymatically treated and non-treated samples were analyzed using paired-samples *t*-tests. Pearson's correlation coefficient was used to explore potential linear relationships between measures. All comparisons were considered statistically significant for p values <0.05.

Results and discussion

Mean (SD) urine volume collected was 1632 (519) ml after placebo and 1535 (366) ml after ayahuasca. These volumes did not differ statistically [$t(9) = 0.62$]. Mean excreted creatinine was 5537(1368) mg/dl after placebo and 6525(1303) mg/dl after ayahuasca. Despite the larger values after ayahuasca, differences were not statistically significant [$t(9) = -2.02$, $p = 0.074$].

After placebo administration, concentrations for all measured compounds fell below the limit of detection (LOD) except for IAA.

The amounts of DMT and its potential metabolites measured at the different collection intervals are presented in Table 1. To control for physiological IAA, amounts after placebo have been substracted from amounts after ayahuasca. As shown in the table, less than 1% of the administered DMT dose was recovered as the unmetabolized parent compound. Recovery was significantly less following enzymatic treatment. This may be due to degradation of DMT produced by heating and hydrolysis (1 h at 65 °C).[23] The main DMT metabolite found in the urine was IAA, the oxidative deamination product obtained from the MAO pathway. Recovery was increased after enzymatic treatment, although the difference was not statistically significant. The second highest concentration metabolite detected was DMT-NO, with recoveries around 10%. Another 0.2% was made up by 2MTHBC and NMT. The cyclization product 2MTHBC accounted for 0.13-0.16% of the administered DMT dose. Enzymatic treatment increased the amount of NMT by a factor of 1.5.

Table 1. Mean (SD) DMT and metabolite amounts excreted in each collection interval. Amounts are expressed as micrograms (μg) and micromoles (μmol). Percent recovered relative to the administered DMT dose. Percent recoveries were compared using paired samples *t*-tests between non-enzyme and enzyme-treated samples.

	Time Interval				Total		
Non-Enzyme treated	**0-4 h (μg)**	**4-8 h (μg)**	**8-16 h (μg)**	**16-24 h (μg)**	**0-24 h (μg)**	**0-24 h (μmol)**	**% Recovered**
DMT	237.1(282.2)	224.7(120.1)	22.0(41.3)	0.4(1.2)	484.1(323.1)	2.6(1.7)	0.8(0.5)
IAA	8785.8(6358.6)	8476.9(6310.6)	6534.3(5790.6)	1768.3(6398.6)	25565.3(11068.8)	146.1(63.3)	44.2(19.6)
DMT-NO	3631.5(2356.4)	2535.6(1697.6)	597.1(440.2)	238.2(216.2)	7002.5(3429.1)	34.3(16.8)	10.2(4.8)
2MTHBC	28.5(24.3)	24.5(20.7)	12.4(16.7)	29.8(38.7)	95.3(79.8)	0.5(0.4)	0.16(0.1)
NMT	9.4(15.0)	4.7(8.4)	0.8(1.9)	3.8(5.1)	18.7(27.3)	0.1(0.2)	0.03(0.05)
Parent & Metabolites							*55.4(22.5)*
Enzyme treated							
DMT	186.2(211.7)	181.5(111.1)	10.4(23.7)	0.0(0.0)	378.0(242.4)	2.0(1.3)	0.6(0.4)**
IAA	8497.6(7423.4)	12226.5(6763.6)	9234.0(7890.5)	1519.3(10101.2)	31477.3(12390.5)	179.9(70.8)	54.9(22.8)
DMT-NO	4143.3(2188.5)	2829.1(1438.9)	768.3(640.6)	184.1(163.2)	7924.8(2560.6)	38.8(12.6)	11.6(3.5)
2MTHBC	29.4(31.8)	18.9(15.6)	9.2(17.1)	22.9(35.2)	80.4(80.0)	0.4(0.4)	0.13(0.1)
NMT	13.8(12.1)	9.7(11.0)	2.6(3.3)	4.6(5.6)	30.8(25.0)	0.2(0.1)	0.05(0.04)**
Parent & Metabolites							*67.3(23.7)*

** $p < 0.01$

DMT and metabolite excretion was maximal during the first third of the 24 h collection period. Thus, in the first 8 h after ingestion, 95–97% of all measured DMT (free/total) was excreted, 68/66% of all measured IAA, 88/88% of all measured DMT-NO, 57/60% of all measured 2MTHBC and 73/77% of all measured NMT.

In order to address the relative contribution of oxidative deamination and of MAO-independent metabolism, mainly *N*-oxidation, to the biotransformation of DMT we performed additional calculations. In addition to calculating the percentage of each metabolite relative to administered DMT dose, we assessed the percentage relative to the overall DMT plus metabolites recovered in urine. This way we controlled for potential individual differences in DMT absorption. Table 2 shows the amounts of DMT and metabolites found in 24-h urine for each participant expressed in micromoles and percentage. As shown therein, IAA was roughly 80% of all substances measured. Individual values were as low as 43% and as high as 88%. DMT-NO made up around 20% of the compounds measured, varying between 10% and 50%.

Potential linear relationships between excreted DMT, DMT-NO, and IAA were explored. We had hypothesized that an inverse relationship might exist between the amounts of DMT-NO and IAA excreted. However, no statistically significant correlation was found.

Results for the harmala alkaloids and their metabolites are shown in Table 3. THH was the most abundant alkaloid in urine followed by harmaline and harmine, both prior to and after enzymatic treatment. Relative to the respective administered dose, the highest recovery rates were found for harmaline and THH. Recovery for harmine was two orders of magnitude lower. Enzymatic treatment caused a 3-fold increase in the amount of recovered harmine. However, this procedure decreased the amounts of recovered harmaline and THH.

Before enzymatic treatment, the most abundant *O*-demethylated product in urine was tetrahydroharmol, followed by harmalol and harmol. However, the largest recovery was obtained for harmalol. Following enzymatic hydrolysis with glucuronidase/sulfatase, large increases were seen in the measured amount of these three compounds. A near 50-fold increase in the amount of harmol was observed. Harmalol levels were increased by a factor of 3 and tetrahydroharmol by a factor of 1.5. Thus, the degree of conjugation varied greatly from one metabolite to another. Whereas only 2% of harmol was present in free form, free harmalol was 36%, and free tetrahydroharmol as high as 68%. The combined recoveries of harmine plus harmol, harmaline plus harmalol, and THH plus tetrahydroharmol after enzymatic treatment were approximately 28%, 65%, and 9%, respectively.

Given that the ayahuasca used in the study contained small amounts of harmol and harmalol, we calculated also the percent recoveries of these two compounds relative to the amounts present in the tea. The obtained values were 1028% for harmol and 516% for harmalol, that is 10-fold and 5-fold the amounts ingested with ayahuasca. These figures show that the vast majority of harmol and harmalol recovered in urine after ayahuasca ingestion must necessarily be formed through the metabolic breakdown of harmine and harmaline. These calculations could not be performed for tetrahydroharmol because the analytical standard for this compound was not available prior to the start of the clinical trial, when the ayahuasca batch was analyzed for alkaloid content. Analyses of other preparations of ayahuasca have indicated, however, that tetrahydroharmol is not present in such extracts (preliminary data, Barker).

Disposition of the harmala alkaloids and their metabolites was more evenly distributed throughout the 24-h collection period than that of DMT and its breakdown products. In the first 8 h after dosing, 53/45% of all measured harmine (free/total) was excreted. Lower recoveries were obtained in this initial 8-h period for harmaline, 39/35%, and THH, 32/33%. Consistent with these differences, recovery in the first 8 h was 68% for total harmol, 47% for total harmalol, and 44% for total tetrahydroharmol.

No statistically significant correlations were found between the amounts of harmala alkaloids and their metabolites in 24-h urine and the amounts of DMT and its metabolites recovered.

The amounts of β-carbolines recovered in the global 24-h collection period expressed in micromoles were used to compute the following metabolic ratios: harmol/harmine, harmalol/harmaline, and tetrahydroharmol/tetrahydroharmine. Statistically significant correlations were found between them: harmol/harmine

Table 2. DMT and metabolite amounts measured for each study participant in 24 h urine in the absence (Non-Enz.) and presence (Enzyme) of enzymatic hydrolysis. Percent values refer to the total amounts measured. Mean = average for the 10 study participants. SD = standard deviation for the 10 study participants.

	Micromoles in 24 h						Percentage				
Subject	**DMT**	**IAA**	**DMT-NO**	**2MTHBC**	**NMT**	**Total**	**DMT**	**IAA**	**DMT-NO**	**2MTHBC**	**NMT**
Non-Enz.											
1	1.6	262.5	70.8	0.9	0.5	336.3	0.5	78.1	21.0	0.3	0.1
2	5.3	42.3	49.3	0.6	0.0	97.6	5.5	43.3	50.6	0.6	0.0
3	0.8	143.3	21.8	0.5	0.2	166.6	0.5	86.0	13.1	0.3	0.1
4	3.9	154.9	23.7	0.1	0.0	182.6	2.1	84.8	13.0	0.1	0.0
5	1.6	199.1	31.7	1.3	0.2	233.8	0.7	85.1	13.6	0.6	0.1
6	5.5	139.1	30.4	1.0	0.2	176.1	3.1	79.0	17.2	0.5	0.1
7	1.4	145.1	21.1	0.1	0.0	167.8	0.8	86.5	12.6	0.1	0.0
8	1.4	72.6	24.1	0.2	0.0	98.3	1.4	73.8	24.6	0.2	0.0
9	2.5	110.5	20.7	0.1	0.0	133.8	1.8	82.6	15.5	0.1	0.0
10	1.8	191.5	49.6	0.3	0.0	243.2	0.7	78.7	20.4	0.1	0.0
Mean	**2.6**	**146.1**	**34.3**	**0.5**	**0.1**	**183.6**	**1.7**	**77.8**	**20.1**	**0.3**	**0.0**
SD	**1.7**	**63.3**	**16.8**	**0.4**	**0.2**	**72.4**	**1.6**	**12.8**	**11.4**	**0.2**	**0.1**
Enzyme											
1	1.1	193.6	53.3	0.6	0.4	249.1	0.4	77.7	21.4	0.3	0.2
2	3.6	49.9	55.6	0.8	0.2	110.1	3.3	45.3	50.5	0.8	0.2
3	0.9	96.6	21.8	0.4	0.3	120.0	0.7	80.5	18.1	0.3	0.3
4	2.6	239.0	34.0	0.0	0.1	275.7	1.0	86.7	12.3	0.0	0.0
5	1.4	189.0	25.8	1.1	0.3	217.5	0.6	86.9	11.8	0.5	0.1
6	4.7	259.8	30.2	1.0	0.2	296.0	1.6	87.8	10.2	0.3	0.1
7	1.1	183.3	38.6	0.0	0.1	223.1	0.5	82.2	17.3	0.0	0.0
8	1.0	128.1	39.1	0.1	0.0	168.3	0.6	76.1	23.2	0.1	0.0
9	2.3	188.0	33.1	0.0	0.0	223.4	1.0	84.2	14.8	0.0	0.0
10	1.3	271.3	57.1	0.2	0.1	330.1	0.4	82.2	17.3	0.1	0.0
Mean	**2.0**	**179.9**	**38.8**	**0.4**	**0.2**	**221.3**	**1.0**	**79.0**	**19.7**	**0.2**	**0.1**
SD	**1.3**	**70.8**	**12.6**	**0.4**	**0.1**	**72.0**	**0.9**	**12.4**	**11.6**	**0.3**	**0.1**

Table 3. Mean (SD) amounts of excreted harmala alkaloids and their metabolites. Amounts are expressed as micrograms (μg) and micromoles (μmol). Percent recovered relative to the respective parent comound, i.e. harmine for harmol, harmaline for harmalol and tetrahydroharmine for tetrahydroharmol. Percent recoveries were compared using paired samples *t*-tests between non-enzyme and enzyme-treated samples

	Time Interval				Total		
Non-Enzyme treated	**0-4 h (μg)**	**4-8 h (μg)**	**8-16 h (μg)**	**16-24 h (μg)**	**0-24 h (μg)**	**0-24 h (μmol)**	**% Recovered**
Harmine	9.2(10.9)	11.9(9.2)	13.3(16.9)	5.6(8.0)	40.0(35.2)	0.2(0.2)	0.04(0.04)
Harmaline	48.9(50.2)	150.4(102.4)	140.0(157.9)	174.6(142.3)	513.8(399.8)	2.4(1.9)	8.5(6.3)
Tetrahydroharmine	472.4(546.8)	1456.2(965.3)	1921.5(2149.5)	2106.4(1363.0)	5956.5(4178.2)	27.6(19.3)	6.6(4.1)
Harmol	224.3(177.7)	191.9(187.6)	86.3(67.1)	98.4(49.5)	600.8(324.9)	3.0(1.6)	0.6(0.2)
Harmalol	260.2(164.1)	296.8(144.9)	187.2(116.7)	262.7(133.6)	1006.9(248.2)	5.0(1.2)	17.7(4.3)
Tetrahydroharmol	370.6(319.6)	438.6(389.7)	393.0(502.4)	502.2(384.1)	1704.4(1199.5)	7.3(5.2)	1.7(1.1)
Enzyme treated							
Harmine	20.6(20.1)	32.1(21.7)	34.8(25.5)	31.8(24.1)	119.4(74.6)	0.6(0.4)	0.1(0.1)***
Harmaline	40.6(41.0)	124.2(76.4)	131.6(139.2)	168.3(105.9)	464.7(317.0)	2.2(1.5)	7.7(5.0)
Tetrahydroharmine	448.2(516.8)	1348.8(846.0)	1624.9(1749.5)	1952.8(1261.7)	5374.7(3742.7)	24.9(17.3)	6.0(3.7)**
Harmol	10965.8(5057.1)	7984.9(3505.9)	3543.9(1791.5)	5360.4(2162.6)	27855.0(6010.2)	140.7(30.4)	27.8(5.3)***
Harmalol	625.6(434.2)	917.6(505.4)	778.2(712.5)	973.9(391.4)	3295.3(1378.3)	16.5(6.9)	56.9(21.1)***
Tetrahydroharmol	534.8(532.3)	656.8(547.6)	693.3(898.4)	820.6(738.7)	2705.5(2320.0)	11.7(10.0)	2.7(2.3)*

* $p < 0.05$,
** $p < 0.01$,
*** $p < 0.001$.

vs harmalol/harmaline, $r = 0.800$, $p < 0.01$; harmol/harmine vs. tetrahydroharmol/THH, $r = 0.803$, $p < 0.01$; harmalol/harmaline vs. tetrahydroharmol/THH, $r = 0.967$, $p < 0.001$.

These metabolic ratios were also tested for correlations with excreted DMT, DMT-NO, IAA, and the metabolic ratios IAA/DMT and DMT-NO/DMT. However, no statistically significant results were found.

In the present study we assessed the urinary disposition of ayahuasca alkaloids and their metabolites following administration of a lyophilized sample of the tea to humans. In line with previous findings after intramuscular administration,[18] DMT was found to undergo extensive metabolism, with less than 1% being detected unchanged in urine and increased IAA excretion.

Early investigations in humans had found a lack of psychoactivity when pure DMT was administered alone *per os* in doses as high as several hundred milligrams.[26] These studies had found IAA as a degradation product of DMT in urine.[6] A more recent study assessing human plasma concentrations of DMT following its i.v. administration found DMT to be measurable in blood at 30 min but almost undetectable at 1 h.[27] Our present findings support the notion that MAO plays a prominent role in the degradation of DMT, as previously noted by other researchers.[21,22,28–32] However, MAO-inhibition after ayahuasca appears to be either incomplete or short-lived, as large amounts of IAA were already found in the first 4-h collection interval. Partial inhibition of MAO by the harmalas in ayahuasca appears to be sufficient to allow psychoactive effects.

Another interesting finding is that MAO-catalyzed oxidative deamination is not the only metabolic pathway available to DMT when administered together with the β-carbolines in ayahuasca. We found DMT-NO to be a major metabolite accounting for 20% of all tryptamine derivatives measured in urine and 10% of the administered DMT dose. The cyclization product 2MTHBC and the *N*-demethylation derivative NMT were also found. Studies with pure tryptamines and tryptamine derivatives, including DMT, 5-MeO-DMT, and 5-OH-DMT, have found oxidative deamination by MAO-A to be a major metabolic route in brain, liver and kidney *in vitro*, producing the corresponding indoleacetic acid.[32–35] IAA has been identified in both rodent[35] and human urine[6] following DMT administration but the amount recovered represented only 2.7% and 8.3%, respectively, of the dose administered, with no detectable DMT being observed. In another study, [^{14}C]-IAA was identified as the major metabolite representing up to 23% of the radioactivity in the blood of rabbits 60 min after i.v. injection of [^{14}C]DMT. However, the majority of radioactivity, and thus other possible metabolites, was not successfully identified.[36]

In line with our findings after ayahuasca, *in vitro* studies have shown that other pathways besides oxidative deamination also contribute to DMT metabolism. Studies of DMT metabolism in vitro have identified DMT-NO as a major NADH dependent metabolite using mouse liver homogenates,[20] liver microsomal fractions from rabbits,[37] as well as rat brain homogenates.[21] Two of these studies also identified NMT as a metabolite of DMT.[21,37] *N*-oxidation has also been identified as an important metabolic pathway of DMT *in vivo*,[31] while *N*-demethylation seems to function as a minor degradation route.[31] NMT could also act as a substrate for MAO and become further metabolized to IAA. Cyclization to form the beta-carboline species 2-MTHBC has also been shown to be an alternative metabolic pathway in vivo and in vitro.[21,38]

DMT-NO does not seem to function as an intermediate during the formation of IAA by MAO-A but it does appear to represent the major metabolite of DMT in the absence of or after inhibition of mitochondrial MAO.[20,30] MAO inhibition could consequently shift metabolism from oxidative deamination to *N*-oxidation and the above-mentioned alternative routes as a compensatory metabolic mechanism. Sitaram's group demonstrated that iproniazid inhibited the formation of IAA from DMT in liver homogenates although not the formation of DMT-NO.[32] Iproniazid was found to increase the levels of DMT *in vivo* in rat brain, liver, kidney, and blood as well as DMT-NO in rat liver,[31] and urinary excretion of unmetabolized DMT, DMT-NO, and NMT.[30] In the present study, we found a 80:20 ratio for IAA: DMT-NO after ayahuasca. A future study could address the metabolism of DMT in humans after oral administration without the harmalas and evaluate whether this ratio is shifted toward lower DMT-NO formation in the absence of MAO inhibition.

With regard to the β-carbolines, they appeared to undergo also extensive metabolism with low urine recoveries. Harmine appeared to be the most metabolically labile of the three, as only 0.1% of the parent compound was recovered unchanged. In agreement with previous research that had shown harmine and harmaline to undergo *O*-demethylation,[39–42] we found large amounts of harmol and harmalol in urine. These findings are in line with results from a clinical study involving oral dosing with ayahuasca and in which these compounds were measured in plasma.[17] More recently, harmol and harmalol were found in urine in a three-subject sample following ayahuasca intake.[23] Enzymatic treatment of the samples in the present study showed that most harmol and harmalol was excreted as sulfate and glucuronide conjugates, as previously found by McIlhenny *et al*.[23] Here we also found for the first time that tetrahydroharmol, the *O*-demethylation product of tetrahydroharmine, is also formed in vivo following ayahuasca.

Recoveries of each harmala alkaloid plus its *O*-demethylation product were lower than expected. Whereas harmaline + harmalol in urine accounted for 65% of the total harmaline dose administered with ayahuasca, harmine + harmol only reached 28% of the administered parent comopound; and percentage recovery for THH + tetrahydroharmol was as little as 9%. A potential explanation is that THH undergoes an intense first pass effect and does not reach systemic circulation. Although low plasma concentrations have been observed for harmine after oral administration of ayahuasca,[17] THH levels in blood have consistently been found to be quite high.[17,43] A plausible alternative explanation is that the harmalas, and particularly THH, are degraded by other metabolic routes. In this respect, various hydroxylated metabolites have been described following incubation of harmine in mouse liver microsomes.[40] Despite this possibility and the large variation in recoveries observed, it is worth noting that the calculated metabolic ratios in the present study showed a high degree of correlation, suggesting a common enzymatic route for the *O*-demethylation of the parent compounds.

Conclusion

To conclude, the present results show that *N*-oxidation is also a major degradation pathway of DMT in humans when administered together with β-carbolines in ayahuasca. This finding demonstrates the existence of an alternative metabolic route to biotransformation by MAO. Also that *O*-demethylation plus conjugation is an important but probably not the only degradation route for the harmala alkaloids in humans. Finally, we propose that future investigations address the metabolism of oral DMT in humans in the absence of β-carbolines, in order to assess the contribution of the different metabolic pathways to its degradation under physiological conditions. This would allow us to estimate the degree of metabolic 'shift' induced by the harmala alkaloids in ayahuasca.

Acknowledgements

The authors wish to acknowledge the support of the Cottonwood Research Foundation (www.cottonwoodresearch.org). They are also indebted to THC Pharm (Frankfurt am Main, Germany), who generously provided the tetrahydroharmol analytical standard. Marta Valle was supported by FIS trough grant CP04/00121 from the Spanish Health Ministry in collaboration with Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau, Barcelona.

References

[1] R.E. Schultes, A. Hofmann. *The Botany and Chemistry of Hallucinogens*, Charles C. Thomas, Springfield, **1980**.
[2] R.E. Schultes, A. Hofmann. *Plants of the Gods: Origins of Hallucinogenic Use*, A. van der Marck, New York, **1987**.
[3] K.W. Tupper. The globalization of ayahuasca: Harm reduction or benefit maximization. *Int. J. Drug Policy* **2008**, *19*, 297l.
[4] N.S. Buckholtz, W.O. Boggan. Monoamine oxidase inhibition in brain and liver produced by β-carbolines: Structure-activity relationships and substrate specificity. *Biochem. Pharmacol.* **1977**, *26*, 1991.
[5] D.J. McKenna, G.H.N. Towers, F. Abbott. Monoamine oxidase inhibitors in South American hallucinogenic plants: Tryptamine and β-carboline constituents of ayahuasca. *J. Ethnopharmacol.* **1984**, *10*, 195.
[6] S. Szára. Dimethyltryptamine: Its metabolism in man; The relation of its psychotic effect to the serotonin metabolism. *Experientia* **1956**, *12*, 441.
[7] S. Szára. The comparison of the psychotic effect of tryptamine derivatives with the effects of mescaline and LSD-25 in self-experiments, in *Psychotropic Drugs*, (Eds: S. Garattini, V. Ghetti), Elsevier, Amsterdam, **1957**, pp. 460–467.
[8] R.J. Strassman, C.R. Qualls, E.H. Uhlenhuth, R. Kellner. Dose–response study of *N*,*N*-dimethyltryptamine in humans, II. Subjective effects and preliminary results of a new rating scale. *Arch. Gen. Psychiatry* **1994**, *51*, 98.
[9] J. Riba. Human Pharmacology of Ayahuasca, doctoral thesis, Universitat Autònoma de Barcelona, **2003**. Available at: http://www.tdx.cat/handle/10803/5378 [23 January 2012] .
[10] J. Riba, S. Romero, E. Grasa, E. Mena, I. Carrió, M.J. Barbanoj. Increased frontal and paralimbic activation following ayahuasca, the pan-Amazonian inebriant. *Psychopharmacology* **2006**, *186*, 93.
[11] R.A. Glennon, M. Dukat, B. Grella, S. Hong, L. Costantino, M. Teitler, *et al*. Binding of β-carbolines and related agents at serotonin (5-HT(2) and 5-HT(1A)), dopamine (D(2)) and benzodiazepine receptors. *Drug Alcohol Depen.* **2000**, *60*, 121.
[12] P.A. Pierce, S.J. Peroutka. Hallucinogenic drug interactions with neurotransmitter receptor binding sites in human cortex. *Psychopharmacology* **1989**, *97*, 118.
[13] D.J. McKenna, D.B. Repke, L. Lo, S.J. Peroutka. Differential interactions of indolealkylamines with 5-hydroxytryptamine receptor subtypes. *Neuropharmacology* **1990**, *29*, 193.
[14] D. Fontanilla, M. Johannessen, A.R. Hajipour, N.V. Cozzi, M.B. Jackson, A.E. Ruoho. The hallucinogen *N*,*N*-dimethyltryptamine (DMT) is an endogenous sigma-1 receptor regulator. *Science* **2009**, *116*, 1591.
[15] T.S. Ray. Psychedelics and the human receptorome. *PLoS One* **2010**, *5*, e9019. DOI:10.1371/journal.pone.0009019
[16] J. Riba, A. Rodriguez-Fornells, G. Urbano, A. Morte, R. Antonijoan, M. Montero, *et al*. Subjective effects and tolerability of the South American psychoactive beverage Ayahuasca in healthy volunteers. *Psychopharmacology* **2001**, *154*, 85.
[17] J. Riba, M. Valle, G. Urbano, M. Yritia, A. Morte, M.J. Barbanoj. Human pharmacology of ayahuasca: subjective and cardiovascular effects, monoamine metabolite excretion, and pharmacokinetics. *J. Pharmacol. Exp. Ther.* **2003**, *306*, 73.
[18] J. Kaplan, L.R. Mandel, R. Stillman, R.W. Walker, W.J.A. VandenHeuvel, J.C. Gillin, *et al*. Blood and urine levels of N,N-dimethyltryptamine following administration of psychoactive dosages to human subjects. *Psychopharmacologia* **1974**, *38*, 239.
[19] J. Ott. *Pharmacotheon: Entheogenic Drugs, their Plant Sources and History*, Natural Products Co., Kennewick, Washington, **1993**.
[20] M.S. Fish, N.M. Johnson, E.P. Lawrence, E.C. Horning. Oxidative N-dealkylation. *Biochem. Biophys. Acta* **1955**, *18*, 564.
[21] S.A. Barker, J.A. Monti, T. Christian. Metabolism of the hallucinogen *N*,*N*-dimethyltryptamine in rat brain homogenates. *Biochem. Pharmacol.* **1980**, *29*, 1049.
[22] B.R. Sitaram, W.R. McLeod. Observations on the metabolism of the psychotomimetic indolealkylamines: implications for future clinical studies. *Biol. Psychiat.* **1990**, *28*, 841.
[23] E.H. McIlhenny, J. Riba, M.J. Barbanoj, R. Strassman, S.A. Barker. Methodology for the determination of the major constituents and metabolites of the Amazonian botanical medicine ayahuasca in human urine. *Biomed. Chromatogr.* **2011**, *25*, 970.
[24] E.H. McIlhenny, J. Riba, M.J. Barbanoj, R. Strassman, S.A. Barker. Methodology for determining major constituents of ayahuasca and their metabolites in blood. *Biomed. Chromatogr.* **2012**, *26*, 301.
[25] R.G. Dos Santos, M. Valle, J.C. Bouso, J.F. Nomdedéu, J. Rodríguez-Espinosa, E.H. McIlhenny, *et al*. Autonomic, neuroendocrine, and immunological effects of ayahuasca: A comparative study with d-amphetamine. *J. Clin. Psychopharm.* **2011**, *31*, 717.
[26] W.J. Turner, S. Merlis. Effect of some indolealkylamines on man. *Arch Neurol. Psychiat.* **1959**, *81*, 121.
[27] R.J. Strassman, C.R. Qualls. (1994) Dose–response study of N,N-dimethyltryptamine in humans, I. Neuroendocrine, autonomic and cardiovascular effects. *Arch Gen. Psychiat.* **1994**, *51*, 85.
[28] S.A. Barker, J.A. Monti, S.T. Christian. N,N-dimethyltryptamine: An endogenous hallucinogen. *Int. Rev. Neurobiol.* **1981**, *22*, 83.
[29] O. Suzuki, Y. Katsumata, M. Oya. Characterization of eight biogenic indoleamines as substrates for type A and type B monoamine oxidase. *Biochem. Pharmacol.* **1981**, *30*, 1353.
[30] B.R. Sitaram, L. Lockett, G.L. Blackman, W.R. McLeod. Urinary excretion of 5-methoxy-*N*,*N*-dimethyltryptamine, *N*,*N*-dimethyltryptamine and their *N*-oxides in the rat. *Biochem. Pharmacol.* **1987**, *36*, 2235.
[31] B.R. Sitaram, L. Lockett, R. Talomsin, G.L. Blackman, W.R. McLeod. *In vivo* metabolism of 5-methoxy-*N*,*N*-dimethyltryptamine and *N*,*N*-dimethyltryptamine in the rat. *Biochem. Pharmacol.* **1987**, *36*, 1509.
[32] B.R. Sitaram, R. Talomsin, G.L. Blackman, W.R. McLeod. Study of metabolism of psychotomimetic indolealkylamines by rat tissue extracts using liquid chromatography. *Biochem. Pharmacol.* **1987**, *36*, 1503.
[33] F. Raynaud, P. Pévet. 5-Methoxytryptamine is metabolized by monoamine oxidase A in the pineal gland and plasma of golden hamsters. *Neurosci. Lett.* **1991**, *123*, 172.
[34] R.W. Fuller, H.D. Snoddy, K.W. Perry. Tissue distribution, metabolism and effects of bufotenine administered to rats. *Neuropharmacol.* **1995**, *34*, 799.
[35] V. Ersparmer. Observations on the fate of indolealkylamines in the organism. *J. Physiol.* **1955**, *127*, 118.
[36] L.R. Mandel, R. Prasad, B. Lopez-Ramos, R.W. Walker. The biosynthesis of dimethyltriptamine in vivo. *Res. Commun. Chem. Pathol. Pharmacol.* **1977**, *16*, 47.
[37] S. Szára, J. Axelrod. Hydroxylation and N-demethylation of N,N-dimethyltryptamine. *Experientia* **1959**, *15*, 216.
[38] S.A. Barker, J.M. Beaton, S.T. Christian, J.A. Monti, P.E. Morris. In vivo metabolism of α,α,β,β-tetradeutero-N,N,dimethyltryptamine in rodent brain. *Biochem. Pharmacol.* **1984**, *33*, 1395.
[39] T.A. Slotkin, V. DiStefano, W.Y.W. Au. Blood levels and urinary excretion of harmine and its metabolites in man an rats. *J. Pharmacol. Exp. Ther.* **1970**, *173*, 26.
[40] D.J. Tweedie, M.D. Burke. Metabolism of the beta-carbolines, harmine and harmol, by liver microsomes from phenobarbitone- or 3-methylcholanthrene-treated mice. Identification and quantitation of two novel harmine metabolites. *Drug Metab. Dispos.* **1987**, *15*, 74.
[41] A. Yu, J.R. Idle, K.W. Krausz, A. Küpfer, F.J. Gonzalez. Contribution of individual cytochrome P450 isozymes to the *O*-demethylation of the psychotropic β-carboline alkaloids harmaline and harmine. *J. Pharmacol. Exp. Ther.* **2003**, *305*, 315.
[42] A. Yu. Indolealkylamines: Biotransformations and potential drug–drug interactions. *AAPS J.* **2008**, *10*, 242.
[43] J.C. Callaway, D.J. McKenna, C.S. Grob, G.S. Brito, L.P. Raymon, R.E. Poland, *et al*. Pharmacokinetics of *Hoasca* alkaloids in healthy humans. *J. Ethnopharmacol.* **1999**, *65*, 243.