Pharmacokinetics of cathinone, cathine and norephedrine after the chewing of khat leaves

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Aim The stimulating herbal drug khat is habitually used in East Africa and the Arabian peninsula but is also imported into other countries. The aim was to study the pharmacokinetics of its alkaloids cathinone, cathine and norephedrine.

Methods Four volunteers chewed khat leaves in an amount equivalent to one-quarter of that used in a typical khat session. Blood samples were collected up to 80 h and the alkaloids were assayed using gas chromatography-mass spectrometry. The data were evaluated using computerized pharmacokinetic compartmental analysis.

Results The plasma concentration-time data for the alkaloids could be described using a two-compartment model with two-segment absorption. The mucosa of the oral cavity is considered to be the first absorption segment, where the major proportion of the alkaloids is absorbed (mean ± SD 59 ± 21% for cathinone and 84 ± 6% for cathine). The extraction of the alkaloids from the leaves by chewing was very effective with only 9.1 ± 4.2% remaining as a residue. Cathinone was eliminated from the central compartment with a mean half-life of 1.5 ± 0.8 h. The half-life of cathine was 5.2 ± 3.4 h. The metabolism of cathinone to norephedrine had a substantial influence on its plasma concentration profile. Psychophysical functions were essentially unaffected by the chewing of khat.

Conclusions The pharmacokinetics of khat alkaloids in humans explain why chewing is the preferred form of khat ingestion. Subjects absorbed a mean dose of 45 mg of cathinone, and did not suffer any severe adverse reactions.

Keywords: cathine (d-norpseudoephedrine), cathinone, khat, norephedrine (phenylpropanolamine), pharmacokinetics

Introduction

The psychostimulating herbal drug khat (*Catha edulis* Forsk.) is cultivated and used as a recreational drug in East Africa and the Arabian Peninsula. It is habitually used in formal meetings (khat sessions) where the participants are engaged in discussions and maintain social contact. During such sessions the leaves and the bark of the plant are chewed slowly over several hours and the juice of the masticated leaves is swallowed, but not the residues. Khat contains alkaloids of the phenylpropylamine type of which the main psychoactive constituent is S-(-)-α-aminopropiophenone (cathinone) [1], together with the less psychoactive phenylpropanolamine diastereomers S,S-(+)-norpseudoephedrine (cathine) and R,S-(-)-norephedrine [2, 3].

Cathinone has been determined in spiked human plasma [4], in the plasma of volunteers after administration of pure cathinone [5] or after chewing of khat leaves [6, 7]. The components of khat represent pertinent compounds for pharmacokinetic studies with respect to their absorption during chewing and the stereospecific metabolism of cathinone to norephedrine [8, 9]. Until now, the pharmacokinetic parameters for cathinone and other ingredients of khat leaves have only been determined over a short observation period of 8 h [6] and a pharmacokinetic model has not been developed. However, since khat chewing becomes more common in western countries, for example due to migration [10], and given the possible abuse potential of the herb, which is prohibited by federal law in several European countries, knowledge of its pharmacokinetics may be useful.

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Received 18 June 2001, accepted 12 December 2002.

The present paper describes the pharmacokinetic analysis of plasma concentration data for cathinone, cathine and norephedrine in volunteers chewing authentic khat leaves, based on a two-compartment model and two-segment absorption.

Methods

Chemicals, reference standards and apparatus

S-(-)-cathinone, (±)-phenylpropanolamine (norephedrine) and (±)-3,4-methylenedioxyamphetamine-d_5 (MDA-d_5) were purchased from Radian (Promochem, Wesel, Germany), D-norpseudoephedrine (cathine) was from Heinrich Mack Nachf. GmbH (Illertissen, Germany). The derivatization reagent N-methyl-bis(heptafluorobutyramide) (MBHFBA) was from Macherey & Nagel (Düren, Germany) and all other reagents and organic solvents were of analytical grade and from Merck (Darmstadt, Germany).

Gas chromatographic-mass spectrometric (GC/MS) analysis was performed on a Hewlett Packard (Waldbronn, Germany) GC/MS (HP 5890 Series II GC, HP 6890 ALS, HP 5972 MSD) using a HP-1 MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness), the carrier gas was helium with a flow rate of 1.0 mL min^{-1}. The MS conditions were 280 °C transferline temperature and 70 eV ionization energy. Data analysis was performed using HP ChemStation software (Rev. C.03.00).

Study design

The leaves of authentic khat material that had been confiscated at Frankfurt airport and stored at −20 °C, were used. The content of cathinone, cathine and norephedrine was determined to be 1.14, 0.83 and 0.44 mg g^{-1} khat, respectively, by the method of Widler *et al.* [6].

Four healthy, nondrug-using volunteers (two male, two female, age range 26-57 years) chewed four portions of a total of 0.6 g authentic khat leaves per kg body weight for 1 h, and collected the residue. This procedure was the same as in the two previously performed studies [6, 7]. Blood samples (5 mL) were taken using EDTA as anticoagulant. Plasma was separated immediately by centrifugation of each sample for 10 min at 2000 *g* and stored at −20 °C for analysis. Sampling times were: 0.0, 0.1, 0.4, 0.8, 1.1, 1.4, 1.8, 2.1, 2.4, 2.8, 3.1, 3.4, 3.8, 4.4, 5.3, 5.8, 6.5, 7.2, 7.8, 9.3, 25.1, 33.3 and 54.3 h after the start of chewing. Spontaneous urine samples were also collected [11]. During the study, tests were performed to assess the impairment of psychophysical functions as used for the assessment of driving ability in Germany. At the times of blood sampling, systolic and diastolic blood pressures, heart rate, pupil diameter/reactivity and rotatory nystagmus were measured and the mental state of the subjects was assessed using a list of paired terms describing opposite states of emotion ('Befindlichkeitsskala' [12]). Every 2 h the reaction to visual (four different colours) and acoustic (high and low sound) stimuli was tested using the Wiener Determinationstest [13], and 3 h after chewing the individual attention and concentration performance was tested using Test d2 [14], which is based on repeated discrimination of similar graphical patterns, and measures processing speed, rule compliance and quality of performance for a period of 280 s. To obtain reference values for each participant all tests were performed once before chewing the leaves except for the Test d2, which was carried out 2 weeks after the study.

The study protocol was approved by the Ethics Committee of the University of Frankfurt/Main (Germany), and written informed consent was obtained from the study participants.

Determination of khat alkaloids in plasma using GC/MS

Plasma (1 mL) was diluted with 4 mL of 0.1 M phosphate buffer pH 6.0 and 100 µL of internal standard solution (1 ng μL^{-1} of MDA-d_5 in methanol) were added and the mixture vortexed. The diluted samples were extracted using 3 mL Bond Elut Certify HF 300 mg solid-phase extraction (SPE) cartridges (Varian, Darmstadt, Germany) and with the robot RapidTrace (Zymark, Idstein, Germany). The extraction protocol was as follows: conditioning of the cartridge with 2 mL methanol and 3 mL phosphate buffer, introducing the sample onto the column at a flow of 1 mL min^{-1}, rinsing with 2 mL 0.1 M acetic acid and 3 mL methanol at a flow of 1.5 mL min^{-1}, and elution of the analytes with 3 mL of a freshly prepared mixture of methylene chloride : 2-propanol : ammonia (80 : 20 : 2, v/v/v) at a flow of 1 mL min^{-1}. The extracts were evaporated to dryness using a Zymark TurboVap LV at 25 °C. Residues were reconstituted in 2 × 100 µL methanol and 50 µL of 0.1 M hydrochloric acid in 2-propanol and evaporated again at 60 °C. Residues were derivatized with 40 µL MBHFBA. One mictolitre of these solutions was analysed by GC/MS set to the following conditions: injection port temperature 260 °C, temperature program at 120 °C for 0.5 min, increasing 10 °C min^{-1} to 170 °C, and then 30 °C min^{-1} to 310 °C, and held for 5 min. Quantification was performed in single ion monitoring (SIM) mode with MDA-d_5 as the internal standard. The following fragment ions were used in SIM mode (quantifier ion underlined): MDA-d_5 HFBA <u>136</u>, 166, 167, cathinone HFBA <u>105</u>, 77, 240, cathine 2HFBA <u>330</u>, 303, 240 and norephedrine 2HFBA <u>330</u>, 303, 240. Mean recoveries

for cathinone, cathine and norephedrine were 65 ± 14%, 84 ± 6%, 87 ± 9%, respectively (100 μg L^{-1}, $n = 6$). Calibration curves were linear over the range 1 to 1000 μg L^{-1} and all regression coefficients were >0.999. Limits of detection were 1 μg L^{-1} for the three analytes. Intraassay coefficients of variation for cathinone, cathine and norephedrine were 7%, 3% and 6%, respectively (100 μg L^{-1}, $n = 7$).

Pharmacokinetic analysis

The concentration-time data for cathinone, cathine and norephedrine were analysed with TopFit 2.0 software [15] and fits of different pharmacokinetic models to the data were obtained. The models incorporated one- or two-segment absorption in combination with one, two or three-compartment distribution and elimination. The Akaike information criterion and analysis of residuals were used to identify the best fit.

Results

On completion of khat leaf chewing residues were analysed for their cathinone, cathine and norephedrine content to calculate the ingested doses. The four participants ingested a mean ± SD khat dose equivalent to 0.63 ± 0.04 mg of cathinone, 0.45 ± 0.03 mg of cathine and 0.26 ± 0.01 mg of norephedrine per kg body weight (Table 1).

Plasma concentration-time data for cathinone, cathine and norephedrine are shown in Figure 1 and pharmacokinetic parameters in Table 1. The early phase was best characterized by a two-segment absorption model. The time until appearance of the compounds in the central compartment (tlag 1) was short (0.1–0.2 h) for the three alkaloids, whereas the lag time for the second segment (tlag 2) was more than 1.2 h.

Maximal plasma concentrations were reached (t_{max}) on average after 2.3 h for cathinone, 2.6 h for cathine and 2.8 h for norephedrine. Interindividual variabilities in t_{max} and C_{max} were less than 32%. The elimination phase of the plasma concentration-time data was described best by a two-compartment model. The mean ± SD terminal elimination half-life of cathinone was 1.5 ± 0.8 h (median 1.5 h) and that of cathine 5.2 ± 3.4 h (median 4.0 h). The mean residence time (MRT) for cathinone was short (4.5 ± 0.1 h) whereas that for cathine was twice this value (10.2 ± 2.6 h). MRT exhibited low interindividual variation (< 25%). The apparent volume of the central compartment for cathinone was 2.7 ± 1.6 L kg^{-1} and for cathine 0.7 ± 0.4 L kg^{-1}.

Systolic and diastolic blood pressures were increased for 3 h after chewing. Heart rate, pupil size and reaction to light showed no changes during the study, a rotatory nystagmus was not observed and an impairment of reaction or mental condition was not found. All participants reported the personal feeling of being alert and 'energetic'.

Discussion

During a typical drug-taking session 100–300 g khat are used over a period of 3–4 h [16–18]. In the present study 36–59 g were chewed during 1 h, and only the juice was swallowed as is the habit in Yemen [19]. The alkaloid composition of the khat in the present study was almost identical to the material used in the report of Widler *et al.* [6] and corresponds to other published data [20, 21].

During chewing, most of the alkaloids were extracted into the saliva, since only 10% of the original content was found in the leaf residues. It is concluded that the buccal mucosa plays a major role in the absorption of all three alkaloids. In particular the absorption of cathine in the early period shows very small variation between the four study participants. In this first absorption segment the mucous membranes in the oral cavity would probably be exposed to high concentrations of the alkaloids. The stomach and/or small intestine receive the swallowed juice and are probably responsible for the second phase of absorption. Our data rationalize the traditional method of khat use by chewing and masticating the material, effectively liberating the alkaloids from the leaves, and allowing rapid absorption into the systemic circulation.

The times to maximal plasma concentration (t_{max}) are in good agreement with the results of Halket *et al.* [7] and Widler *et al.* [6]. However, in these studies values for maximal plasma concentration (C_{max}) were smaller than our values, most probably due to the lower alkaloid doses used in the present study (0.6 mg kg^{-1} cathinone *vs.* 0.8–1 mg kg^{-1}). Our data were described best by a two-compartment distribution/elimination model, which was also used for the prediction of cathinone blood concentrations in the rabbit after intravenous dosing [4]. The mean terminal half-life of cathinone in the present study was 1.5 h, which is in precise agreement with data from Kalix [16]. This explains its short MRT and the finding that cathinone was only detectable in blood samples up to 10 h after ingestion. The latter observation was also made by Widler *et al.* [6], but a much longer mean ± SD half-life of 4.3 ± 1.7 h was reported. For cathine given in solution Hoogkamer *et al.* [22] found a mean ± SD half-life of 5.1 ± 2.0 h, which is similar to our results. The MRT of cathine was longer than that of cathinone, which can be explained by its longer elimination half-life. Hoogkamer *et al.* [22] reported a lower MRT of 7.9 ± 2.6 h, but this may be accounted for by a more rapid absorption from the dosing solution.

Table 1 Data on khat dosing and on the pharmacokinetic models for cathinone, cathine and norephedrine.

	Participant 1, male	*2, female*	*3, female*	*4, male*	*Mean ± SD*
Body weight (kg)	95.0	58.0	59.0	74.0	71.5 ± 17.3
Khat chewed (g)	59.2	36.1	36.1	43.6	43.8 ± 10.9
Cathinone					
Amount in residue (% of original content)	6.6	8.6	16.7	8.8	10.2 ± 4.5
Ingested dose (mg)	63.1	37.7	34.3	45.4	45.1 ± 12.8
Tlag 1 (h)	0.11	0.11	0.24	0.03	0.12 ± 0.09
Tlag 2 (h)	0.83	1.02	0.70	2.36	1.23 ± 0.77
Fabs 1 (%)	36	66	50	84	59 ± 21
Fabs 2 (%)	64	34	50	16	41 ± 21
t_{max} (h)	1.97	2.13	1.87	3.27	2.31 ± 0.65
C_{max} (μg L^{-1})	50.7	87.0	47.8	50.0	58.9 ± 18.8
AUC (μg min L^{-1})	229	308	190	253	245 ± 49
CL_{total}/F (mL min^{-1})	4590	2060	2990	2990	3158 ± 1051
MRT (h)	4.37	4.15	4.15	5.42	4.52 ± 0.61
Vc/F (L kg^{-1})	4.67	1.16	3.05	1.70	2.65 ± 1.57
$t_{1/2}$ α (h)	0.36	0.38	0.33	0.49	0.39 ± 0.07
$t_{1/2}$ β (h)	2.44	1.29	1.77	0.51	1.50 ± 0.81
Cathine					
Amount in residue (% of original content)	8.0	6.2	17.3	12.3	10.9 ± 5.0
Ingested dose (mg)	45.1	28.1	24.8	31.7	32.4 ± 8.9
tlag 1 (h)	0.13	0.001	0.52	0.20	0.21 ± 0.22
tlag 2 (h)	1.33	1.10	1.07	2.32	1.46 ± 0.59
Fabs 1 (%)	84	77	92	84	84 ± 6
Fabs 2 (%)	16	23	8	16	16 ± 6
t_{max} (h)	1.65	2.46	2.88	3.49	2.62 ± 0.77
C_{max} (μg L^{-1})	67.2	87.6	54.6	75.2	71.2 ± 13.9
AUC (μg min L^{-1})	598	881	620	753	713 ± 131
CL_{total}/F (mL min^{-1})	1250	530	672	710	791 ± 316
MRT (h)	7.13	10.70	13.30	9.70	10.21 ± 2.55
Vc/F (L kg^{-1})	1.08	0.67	0.28	0.94	0.74 ± 0.35
$t_{1/2}$ α (h)	0.08	0.37	0.11	0.41	0.24 ± 0.17
$t_{1/2}$ β (h)	2.72	4.71	10.10	3.34	5.22 ± 3.36
Norephedrine					
Amount in residue (% of original content)	4.8	5.9	7.2	6.3	6.1 ± 1.0
Ingested dose (mg)	25.0	15.1	14.9	18.2	18.3 ± 4.7
tlag 1 (h)	0.01	0.20	0.18	0.30	0.17 ± 0.12
tlag 2 (h)	1.03	1.03	0.99	2.22	1.32 ± 0.60
t_{max} (h)	2.48	2.92	2.56	3.41	2.84 ± 0.42
C_{max} (μg L^{-1})	76.3	84.2	55.3	72.7	72.1 ± 12.2
AUC (μg min L^{-1})	690	942	525	681	710 ± 173

tlag, Lag time until appearance of substance in the central compartment; Fabs, absorbed proportion; C_{max}, maximal plasma concentration; t_{max}, corresponding time to C_{max}; AUC, area under the concentration-time curve (by curve integration); CL_{total}/F, apparent total body clearance; MRT, mean residence time; Vc/F, apparent volume of the central compartment; $t_{1/2}$ α, half-life of the distribution phase; $t_{1/2}$ β, terminal elimination half-life.

S-(-)-Cathinone has been shown to be metabolized stereoselectively to R,S-(-)-norephedrine [8]. Since khat leaves also contain the latter compound, its plasma concentration may result from a combination of absorbed norephedrine and the product of cathinone metabolism. Therefore, no conclusions regarding the absorption, distribution and elimination of norephedrine could be made. However, since the mean AUCs of the diastereomers cathine and norephedrine were almost identical (713 *vs.* 710 μg min L^{-1}), whereas the mean ingested doses were quite different (32.4 *vs.* 18.3 mg), it can be concluded that the norephedrine formed from the metabolism of cathinone makes an important contribution to the plasma concentration of norephedrine. This is in accordance with our finding that only 7% or less of the absorbed cathinone was recovered in urine and that

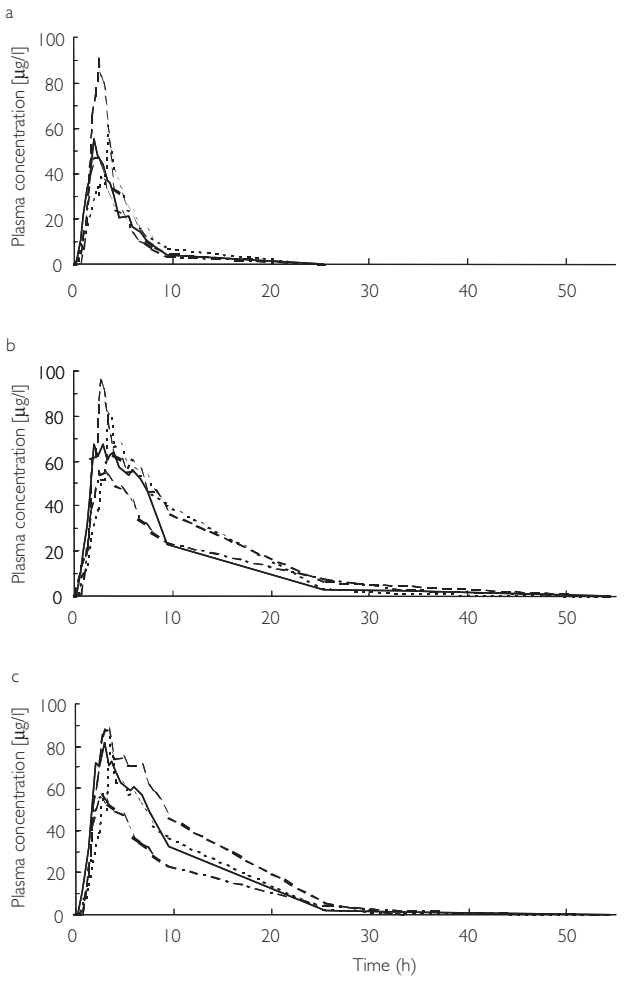

Figure 1 The plasma concentration-time profiles for cathinone (a), cathine (b) and norephedrine (c) in four subjects.

the mean ± SD amount of norephedrine excreted was much higher than the amount ingested (149 ± 32%) [11].

In the study by Widler *et al*. [6] no change of heart rate was found but a significant increase in systolic and diastolic blood pressures persisting for approximately 4 h after the onset of chewing. We made a similar observation for the diastolic and systolic blood pressures which were elevated for 3 h after chewing. The rise of blood pressures started sooner than the rise of the alkaloid plasma concentrations, therefore this might have been an effect of engagement in the study and not due to a pharmacological action of the alkaloids. An impairment of other psychophysical functions of the participants could not be objectified. The negative results of our other tests might be due to insensitivity or the small number of participants, but it may also be concluded that the ingestion of a khat dose as used in our study, which is approximately one-quarter of the dose used in a traditional khat session, has no drastic effect on psychophysical properties in man. However, as the participants reported personal feelings of stimulation, it can be assumed that the ingested khat in the study has had an effect on the central nervous system.

In three forensic cases of suspected driving under the influence of drugs, unaccompanied khat use was proved and cathinone, cathine and norephedrine were present in the blood samples. The policemen and the medical staff did not report signs of an actual impairment (data on file, Centre of Legal Medicine, Frankfurt/Main, Germany). The higher cathine concentrations in these blood samples (109, 229 and 154 µg L^{-1}) in comparison with the highest value obtained in our study (87.6 µg L^{-1}) indicates a two- to threefold higher khat dosage which agrees with the usually higher amounts chewed during authentic khat sessions. The fact, that no impairment was observed in forensic cases may be attributed to habituation due to chronic use [17]

Various studies have shown that khat has psychostimulating properties, which are comparable to those of amphetamine but less intense. It has a moderate potential for psychic dependence and chronic use may lead to a certain degree of tolerance. From our observations it can be concluded that the effects of khat are rather moderate and may not be noticeable after the ingestion of a low dose or in habituated users.

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