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ORIGINAL PAPER

Influence of ethanol on cannabinoid pharmacokinetic parameters in chronic users

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Abstract Cannabis is not only the most widely used illicit drug worldwide but is also regularly consumed along with ethanol. In previous studies, it was assumed that cannabis users develop cross-tolerance to ethanol effects. The present study was designed to compare the effects of ethanol in comparison to and in combination with a cannabis joint and investigate changes in pharmacokinetics. In this study, 19 heavy cannabis users participated and received three alcohol dosing conditions that were calculated to achieve steady blood alcohol concentrations (BAC) of about 0, 0.5 and 0.7 g/l during a 5-h time window. Subjects smoked a Δ^9-tetrahydrocannabinol (THC) cigarette (400 μg/kg) 3 h post-onset of alcohol dosing. Blood samples were taken between 0 and 4 h after smoking. During the first hour, samples were collected every 15 min and every 30 min thereafter. Mean steady-state BACs reached 0, 0.36 and 0.5 g/l. The apparent elimination half-life of THC was slightly prolonged (1.59 vs. 1.93 h, $p<0.05$) and the concentration 1 h after smoking was slightly lower (24 vs. 17 ng/ml, $p<0.05$) with the higher ethanol dose. The prolonged THC elimination might be explained by a small ethanol-mediated change in distribution to and from deep compartments. Concentrations and pharmacokinetics of 11-hydroxy-THC and 11-nor-9-carboxy-THC (THCA) were not significantly influenced by ethanol. However, THCA concentrations appeared lower in both ethanol conditions, which might also be attributable to changes in distribution. Though not significant in the present study, this might be relevant in the interpretation of cannabinoid concentrations in blood.

Keywords Forensics/toxicology · Drug monitoring/drug screening · Kinetics · Cannabis · Ethanol · Interaction

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Introduction

Cannabis is still the most widely used illegal drug in Europe [1]. In a 2006 and 2007 national study of the Substance Abuse and Mental Health Services Administration, it was found that among alcohol users in the past month, 4.8% had a concurrent marijuana use and prevalence decreased with age [2]. In police blood samples obtained during 2004–2009 in the German federal state Hessen and analysed in the Institute of Legal Medicine in Frankfurt/Main, the ingestion of ethanol was detected in 13.5–21.6% (median 16.3%) of cannabis users involved in traffic offences, indicating that this is a rather frequent drug combination.

Previous studies have shown that ethanol might have an influence on the pharmacokinetics of drugs, e.g. on absorption, metabolism and distribution [3–8]. In view of the frequency of ethanol and cannabis co-use, it is surprising that there are only two studies on the effects of

ethanol on cannabinoid pharmacokinetics. In the study by Perez-Reyes et al. [9], no significant effect on Δ^9-tetrahydrocannabinol (THC) concentrations measured in plasma using radioimmunoassay was observed, but with increasing ethanol doses, a trend to higher concentrations shortly after smoking was noted. In the study of Lukas and Orozco [10], placebo and two moderate ethanol doses were given in combination with two doses of THC, and a significant increase in THC concentrations in the absorption phase was observed.

On the other hand, also effects of cannabis use on ethanol pharmacokinetics have been found. It appears that cannabis use may delay and/or decrease ethanol absorption [9, 11] and reduce ethanol metabolism [12].

The aim of the present study was to investigate possible changes in cannabinoid concentrations or pharmacokinetic properties after smoking a standardized cannabis dose following ingestion of one of two moderate ethanol doses or placebo.

Experimental

Chemicals, reference standards and apparatus

THC (1 mg/ml), 11-hydroxy-THC (THCOH, 1 mg/ml), 11-nor-9-carboxy-THC (THCA, 1 mg/ml) and the deuterated analogs THC-d_3, THCOH-d_3 and THCA-d_3 (each 0.1 mg/ml) were purchased from Cerilliant (LGC Promochem, Wesel, Germany). The derivatization reagent *N*-methyl-*N*-trimethylsilyltrifluoroacetamide was from Macherey &Nagel (Düren, Germany). All other reagents and organic solvents were of analytical grade and from Merck (Darmstadt, Germany).

Gas chromatographic–mass spectrometric (GC-MS) analyses were performed on an Agilent (Waldbronn, Germany) GC-MS (6890N GC, 7683 series injector, 5973 MSD) with Varian (Darmstadt) factorFour VF-1MS capillary column (30 m×0.25 mm I.D., 0.25-μm film thickness) and carrier gas helium with a flow rate of 1.0 ml/min. The MS conditions were: 280 °C transferline temperature and 70-eV ionization energy. Data analysis was performed using Agilent ChemStation software.

Study design

The results presented here supplement data from the study by Ramaekers et al. [13] on the influence of cannabis in combination with or without ethanol on cognition, impulse control and psychomotor function in a group of 19 heavy cannabis users (14 men, 5 women) aged 19–38 years (median 23). Specific inclusion criteria were frequent use of cannabis (smoking on more than 4 days per week) during the previous year and presence of THC in serum on the day of screening. The study was conducted according to a double-blind, placebo-controlled, three-way crossover design and was approved by the local ethics committee. Written informed consent was obtained from each participant.

Subjects received three alcohol dosing conditions that were designed to achieve steady-state blood alcohol concentration (BACs) of about 0, 0.5 and 0.7 g/l during a 5-h time window. Three hours post-onset of each alcohol dosing, subjects smoked cigarettes containing body weight (BW)-normalized THC (400 μg/kg BW), resulting in effective doses of 19.6–32.8 mg of THC (median 25.6 mg). Initial alcohol doses in the morning were placebo, 0.5 g/kg (low alcohol dose) or 0.7 g/kg alcohol (high alcohol dose). Additional alcohol doses of about 0.1 g/kg or alcohol placebo were given on an as-needed basis at approximately every half hour till 4.5 h after onset of alcohol dosing in order to maintain BACs at the desired levels. Alcohol was administered as 'pure' ethanol (96%) mixed with orange juice to a volume of 300 ml for the initial dose. Total volumes of maintenance doses mixed with orange juice were approximately 80 ml. Marijuana cigarettes were prepared for each individual from a stock provided by the Dutch Bureau for Medicinal Cannabis (containing 11% THC) and were weight-adjusted for each subject. The marijuana was mixed with tobacco to achieve a standard cigarette size and weight. Subjects were instructed to smoke the cigarette according to a standardized procedure [14] which lasted for about 15 min. Blood samples were taken prior to smoking, every 15 min during the first hour and every 30 min up to 4 h after smoking. The separated serum was stored at −20 °C and shipped to the laboratory in Frankfurt/Main.

Blood alcohol concentrations

In the first 2 h after smoking, when the last maintenance doses were given, the mean ± SD BACs measured using gas chromatography were 0.36±0.08 and 0.50±0.12 g/l in the low- and high-dose conditions, respectively. The breath alcohol monitoring during the study yielded slightly higher estimates (c.f. Fig. 1 in [13]), which may be due to residual mouth alcohol from the maintenance doses. However, the BACs as determined in the blood samples were significantly different between the two alcohol conditions ($p<$ 0.01) during the 4 h after smoking cannabis and are representative of moderate alcohol use.

Determination of cannabinoids in serum by GC-MS

The determination of THC, THCOH and of free THCA in serum was performed according to the routine procedure

for the determination of cannabinoids in forensic samples which has been described previously [15]. In the analysis series, precision was assessed by measuring three control levels in each series with concentrations of THC and THCOH in the range of 1–25 μg/l and of THCA in the range of 3–75 μg/l, which yielded variations below 13% (n=6). Accuracy was assessed using external serum reference material (BTMF S-plus 2/09-B, MEDICHEM Diagnostica GmbH & Co. KG, Steinenbronn, Germany), yielding −11.1% at 0.9 ng/ml for THC, −1.7% at 1.0 ng/ml for THCOH and 8.0% at 10 ng/ml for THCA.

Evaluation of the data

The quantitative data were evaluated in the same way as in previous studies [15, 16] with model-independent methods using Microsoft Excel 2010. The highest concentrations observed (C_{max*}) in this study are probably not the maximal concentrations achieved because the first blood samples after dosing were taken 15 min after smoking. From the concentration–time curves, it was assumed that the marked decrease of THC concentrations during the initial distribution process (α-phase) lasted 1 h [17]. The apparent elimination half-lives ($t_{1/2}$) were calculated from the result of exponential regression of the data where only samples taken 1 h or more up to 4 h after smoking were considered. Results were discarded in the case of less than six valid data points, e.g. due to missing samples, concentrations lower than the limit of quantification, or insufficient regression quality (34 out of 171 data sets). The areas under the curves (AUC) were estimated using the trapezoidal rule. As large inter-individual variations in the data from the chronic users were observed, the non-parametric Mann–Whitney U test (SPSS version 16, SPSS Inc., Chicago, IL, USA) was used to test for significant differences.

Results and discussion

In previous studies, the impact of different THC doses (250 vs. 500 μg/kg BW) on the pharmacokinetic properties of THC and its metabolites [16] and the differences between occasional and chronic users [15] were examined. In the present study, the impact of moderate alcohol doses on cannabinoid pharmacokinetics after a THC dose of 400 μg/kg BW was investigated.

Pharmacokinetic properties of THC

Subjects were allowed to continue their usual cannabis smoking routine between the study days. All participants exhibited THC in their blood samples obtained prior to smoking ($C_{0\,h}$, Table 1), with a median of 4.7 μg/l (range 1.0–39.8 μg/l), and one individual with 70 μg/l who obviously smoked at least one additional joint on his own during the study. The THC dose in the present study was a bit lower than in the previous studies (400 vs. 500 μg/kg BW), but maximum THC concentrations were observed in the first samples after smoking and were with 106.6 (58.0–224.0) μg/l, 88.3 (24.7–201.7) μg/l and 89.3 (32.3–172.7) μg/l in the usually observed ranges in the placebo, low- and high-dose ethanol conditions, respectively (median and range). Four hours after smoking, all THC concentrations were again in the baseline range (median 6.5, range 1.0–27.0 μg/l). The THC concentrations and the AUCs were not significantly different between the three alcohol conditions, except that the values 1 h after the end of smoking were lower in the high alcohol dose group than in the placebo group (median, range 17.0, 5.2–38.0 μg/l vs. 23.6, 8.0–41.2 μg/l, respectively, p=0.027). This might be due to ethanol-mediated changes in blood perfusion and dependent distribution or redistribution of exogenous substances, which has been discussed previously (e.g. [7, 18]). Concurrently, the apparent elimination half-lives in the high alcohol dose group were longer than in the placebo group (1.93 h, 0.95–7.36 h vs. 1.59 h, 0.93–2.11 h, respectively, p=0.046), though still being in the ranges previously reported [15]. However, all differences appear of negligible magnitude and are considered not relevant for routine forensic interpretation.

Lukas and Orozco [10] also observed a significant impact of ethanol on THC concentrations in their study. Their alcohol dosing scheme (0.35 and 0.7 g/kg BW) is comparable to that used in the present study (0.5 and 0.7 g/kg BW), except that cannabis smoking started 3 h after the first drink and that small ethanol doses were given in order to maintain the blood alcohol concentration. Lukas and Orozco observed that peak THC concentrations appeared sooner and, in contrast to our results, that the THC concentrations were higher in their high-dose alcohol condition (0.7 g/kg BW). Their study focused on the short time effects during the smoking itself and only up to 1.5 h afterwards. As they employed occasional users, the THC concentrations exhibited a rather moderate variation. However, significant differences were only observed during the time of the actual smoking, and it was concluded that ethanol has an influence on the absorption only and not on the descending limb of the THC plasma/time curve. This was thought to be due to ethanol-mediated dilation of the pulmonary microcirculation.

In evaluating the different findings in the present investigation, it must be considered that the alcohol ingestion in our study preceded the cannabis smoking by 3 h (<30 min in [10]), allowing the subjects to achieve an ethanol steady state. Previous studies have shown that

Table 1 Pharmacokinetic properties of THC, THCOH and THCA in 19 heavy users after smoking cannabis in combination without or with two different alcohol dosing regimens

Subject	THC					THCOH					THCA				
	$C_{0\ h}$ (µg/l)	C_{max}* (µg/l) (t_{max}, h)	$C_{4\ h}$ (µg/l)	$AUC_{0\rightarrow 4\ h}$ (µg/l h)	$t_{1/2}$ (h)	$C_{0\ h}$ (µg/l)	C_{max}* (µg/l) (t_{max}, h)	$C_{4\ h}$ (µg/l)	$AUC_{0\rightarrow 4\ h}$ (µg/l h)	$t_{1/2}$ (h)	$C_{0\ h}$ (µg/l)	C_{max}* (µg/l) (t_{max}, h)	$C_{4\ h}$ (µg/l)	$AUC_{0\rightarrow 4\ h}$ (µg/l h)	$t_{1/2}$ (h)
Placebo alcohol condition															
01	5.8	78.7 (0.25)	7.9	68.1	2.11	2.3	8.3 (0.25)	2.1	16.8	1.84	42.2	57.0 (0.50)	27.1	157.6	3.24
02	1.6	145.3 (0.25)	3.7	78.7	1.17	1.1	13.0 (0.25)	2.8	23.8	1.69	25.5	48.2 (0.50)	31.9	152.2	
03	2.0	86.3 (0.25)		49.6		1.0	18.0 (0.25)		16.5		28.4	103.3 (0.25)		121.8	
04	1.1	58.0 (0.25)		21.8		0.5	12.7 (0.25)		6.4		9.1	48.7 (0.25)		33.3	
05	2.2	101.3 (0.25)	4.3	81.2	1.15	1.7	18.8 (0.50)	3.3	29.7	1.78	34.4	106.0 (0.50)	49.9	250.3	6.36
06		75.0 (0.25)	6.4	59.5	1.77		9.2 (0.50)	3.1	20.3	2.29		81.0 (0.50)	57.5	251.7	7.74
07	1.2	114.7 (0.25)	3.5	79.3	0.93	0.4	4.4 (0.50)	1.2	10.9	1.42	27.3	51.0 (0.75)	34.1	170.2	6.13
08	5.1	58.3 (0.25)	5.1	58.5	1.59	2.7	9.3 (0.25)	3.4	23.8	2.32	83.5	115.0 (0.25)	70.8	394.0	5.30
09	6.7	224.0 (0.25)	10.2	142.9	1.58	3.6	26.3 (0.25)	6.0	42.4	2.20	78.2	193.3 (0.25)	107.4	485.8	
10	29.2	106.6 (0.25)	14.2	109.5	1.79	13.0	23.2 (0.25)	11.0	45.1		139.0	213.4 (0.50)	84.3	438.6	
11	17.6	174.3 (0.25)	27.0	153.8		7.6	20.3 (0.25)	7.0	41.6	2.72	92.4	131.0 (0.25)	83.0	390.6	6.95
12	38.3	212.3 (0.25)	13.4	165.5	1.70	29.5	49.2 (0.50)	16.3	107.9	2.94	292.8	468.0 (0.50)	258.9	1383.1	4.65
15	4.9	125.0 (0.25)	6.5	91.5	1.49	2.1	14.2 (0.50)	3.6	29.9	1.73	76.2	118.6 (0.50)	53.2	316.4	3.12
16	14.4	115.3 (0.25)	12.3	118.0	2.05	9.9	25.0 (0.25)	6.5	51.7	2.17	183.1	236.7 (0.25)	169.6	775.1	5.05
17	5.8	113.7 (0.25)	8.9	98.6	2.10	2.3	16.0 (0.25)	3.7	27.5	2.42	77.7	133.0 (0.25)	72.9	371.7	6.52
19	8.9	108.3 (0.25)	12.2	107.1	2.01	2.9	11.3 (0.25)	2.8	19.6	2.39	45.7	63.7 (0.25)	33.6	168.9	5.87
20	1.4	67.0 (0.25)	2.7	48.7	1.04	1.1	14.7 (0.25)	1.8	20.1	1.36	23.3	55.7 (0.25)	22.9	129.9	3.61
21	1.7	78.0 (0.25)	4.0	58.4	1.43	0.8	9.2 (0.50)	2.3	19.0	1.90	11.3	49.8 (0.50)	20.8	109.8	4.80
23	7.0	87.0 (0.25)	6.0	82.4	1.47	5.6	18.4 (0.50)	4.4	38.0	1.89	181.8	254.0 (0.50)	191.9	883.4	7.74
Median	5.5	106.6	6.5	81.2	1.6	2.3	14.7	3.4	23.8	2.0	61.0	106.0	57.5	251.7	5.6
Mean ± SD	8.6±10.3	112.1±47.5	8.7±6.0	88.1±37.9	1.6±0.4	4.9±7.1	16.9±9.8	4.8±3.8	31.1±22.2	2.1±0.4	80.7±75.1	133.0±104.6	80.6±67.0	367.6±331.5	5.5±1.5
Low alcohol dose condition															
01	8.5	77.6 (0.25)	7.3	72.8	2.13	2.9	14.4 (0.25)	2.4	22.4	1.78	32.4	73.0 (0.25)	29.9	156.0	
02	3.0	55.0 (0.25)	6.8	64.0	1.84	1.9	11.4 (0.75)	3.7	27.5	2.09	36.1	57.8 (0.75)	31.2	167.4	3.89
03	1.5	41.3 (0.25)	2.4	31.4		1.2	12.0 (0.25)	2.2	17.6	2.03	28.7	49.4 (0.50)	31.0	151.0	6.16
04	1.2	24.7 (0.25)	2.3	27.0	1.62	0.7	9.7 (0.25)	1.6	14.9	1.88	10.4	32.7 (0.25)	17.7	92.6	6.39
05	2.0	98.0 (0.25)	3.5	69.6	1.10	1.1	15.3 (0.25)	2.5	25.5	1.32	22.9	39.8 (0.75)	21.0	114.8	3.30
06	3.4	114.7 (0.25)	7.3	83.7	1.89	2.2	11.7 (0.25)	4.2	26.4	2.81	56.2	84.6 (0.50)	61.2	263.7	11.73
07	1.2	88.3 (0.25)	4.5	86.2	1.07	0.3	12.3 (0.25)	2.3	22.7	1.48	7.8	51.0 (0.25)	24.9	131.9	4.07
08	3.1	46.7 (0.25)		21.8		2.2	11.0 (0.25)		5.9		50.0	82.7 (0.25)		54.4	
09	4.9	90.3 (0.25)	9.6	82.0	1.87	2.3	14.3 (0.25)	5.3	30.6	2.22	78.4	112.0 (0.50)	74.8	323.1	5.45
10	32.4	175.4 (0.25)	16.2	191.8	1.85	11.8	25.4 (0.50)	8.0	60.7	2.63	158.0	198.0 (0.75)	121.0	630.2	6.71

11	26.9	149.7 (0.25)		138.0		10.8	26.0 (0.50)		40.5		108.2	146.0 (0.50)		296.6	
12	39.8	119.7 (0.25)	13.1	111.0	3.20	29.7	62.3 (0.25)	13.7	98.5	3.27	199.6	277.3 (0.25)	181.7	854.9	6.16
13	1.9	84.7 (0.25)		46.9		0.6	11.7 (0.25)		12.4		11.4	46.7 (0.25)		53.4	
15	2.6	122.0 (0.25)	5.5	69.0	2.03	1.4	17.0 (0.25)	3.3	25.4	2.16	41.3	60.0 (0.25)	36.8	179.5	5.45
16	18.5	180.3 (0.25)	17.1	160.4	2.13	9.7	43.3 (0.25)	8.2	69.1	2.05	112.6	141.6 (1.00)	95.0	500.9	5.00
17	4.7	85.7 (0.25)	9.4	73.6	2.67	2.9	27.7 (0.25)	5.7	38.0	2.72	83.0	142.3 (0.25)	86.4	349.5	
19	13.1	201.7 (0.25)	17.7	158.1	2.59	3.2	22.0 (0.25)	4.3	32.0	2.53	52.6	72.6 (0.75)	48.9	223.3	
20	1.0	60.7 (0.25)	1.5	37.2	1.16	0.6	8.3 (0.25)	1.1	12.6	1.48	14.4	29.8 (0.50)	16.0	83.0	4.54
21	1.6	51.3 (0.25)	2.6	30.1	2.00	0.8	6.0 (0.25)	1.2	9.1	2.38	9.8	14.7 (0.25)	7.2	38.3	4.53
Median	3.1	88.3	7.1	72.8	1.9	2.2	14.3	3.5	25.5	2.1	41.3	72.6	34.0	167.4	5.5
Mean ± SD	9.0±11.8	98.3±50.0	7.9±5.5	81.8±49.4	1.9±0.6	4.5±7.0	19.0±13.7	4.4±3.3	31.1±23.0	2.2±0.5	58.6±53.4	90.1±66.1	55.3±46.8	245.5±214.5	5.6±2.1
High alcohol dose condition															
01	7.6	83.3 (0.25)	5.8	58.9	3.42	3.2	15.7 (0.25)	3.2	22.8	3.00	31.0	58.8 (0.75)	36.7	163.3	
02	2.9	82.7 (0.25)	4.1	59.6	1.50	1.8	16.7 (0.25)	3.0	28.2	1.84	25.6	41.4 (0.50)	25.6	131.7	5.77
03	1.2	43.0 (0.25)	3.0	38.3	1.94	0.8	16.0 (0.25)	2.0	18.6	2.09	28.0	142.8 (0.75)	33.6	209.5	6.01
04	1.0	58.3 (0.25)	2.0	31.5	1.35	0.8	22.3 (0.25)	2.0	19.0	1.62	10.0	55.0 (0.25)	16.0	82.5	4.18
05	2.1	114.0 (0.25)	5.2	79.9	1.59	1.1	14.3 (0.25)	2.5	23.7	1.74	25.4	40.0 (0.50)	24.4	129.6	4.79
06	3.5	67.7 (0.25)	5.3	59.1	1.92	4.9	14.7 (0.25)	4.1	28.1	3.07	105.6	121.0 (0.25)	72.0	364.0	6.88
07	1.1	148.7 (0.25)	3.6	83.5	1.14	0.4	8.4 (0.50)	1.5	15.4	1.42	11.1	29.0 (0.50)	16.3	83.2	4.00
08	6.3	43.0 (0.25)	5.8	44.2	2.21	3.7	7.7 (0.25)	3.7	21.3	2.94	114.5	150.7 (0.25)	109.5	424.7	
09	7.8	88.0 (0.25)	7.1	84.6	1.78	8.8	31.7 (0.25)	7.9	65.6	2.13	132.8	186.4 (0.50)	134.5	606.1	8.44
10	18.5	91.8 (0.50)	17.0	109.8	7.36	15.6	32.2 (0.25)	7.9	54.8		181.0	221.6 (0.75)	67.5	413.4	
11	19.2	157.7 (0.25)	20.7	151.3	2.89	7.8	26.7 (0.25)	6.9	45.9	2.61	111.2	119.4 (0.50)	78.8	367.4	5.27
12	70.0	119.0 (0.25)	12.2	110.7	2.73	41.4	41.4 (0.00)	15.8	97.0	3.33	219.0	299.6 (0.50)	178.6	930.4	5.94
13	4.7	107.7 (0.25)	7.4	87.4	2.28	2.1	13.2 (0.50)	3.3	25.3	2.84	27.5	59.4 (0.50)	35.3	162.2	
15	1.1	89.3 (0.25)		44.1		0.5	10.0 (0.25)		10.4		23.9	44.0 (0.50)		59.0	
16	13.4	172.7 (0.25)	19.1	142.5	3.80	7.9	36.0 (0.25)	10.3	66.0	2.92	119.4	213.0 (0.50)	137.4	675.6	5.28
17	4.8	122.7 (0.25)	8.1	97.5	1.75	2.2	18.0 (0.50)	4.0	33.3	1.88	60.3	79.6 (0.50)	50.3	239.8	5.17
19	13.3	118.0 (0.25)	11.3	125.4	2.21	4.9	22.2 (0.50)	3.6	31.7	2.22	56.0	93.0 (0.50)	36.4	193.6	
20	2.6	53.0 (0.25)	1.6	39.1	0.95	1.3	9.0 (0.25)	1.1	15.4	1.09	41.3	46.4 (0.50)	18.5	117.4	2.40
21	1.2	32.3 (0.25)	1.0	24.7	1.53	1.0	5.8 (0.50)	0.6	10.5	1.56	12.5	19.6 (0.50)	3.8	52.8	
Median	4.7	89.3	5.8	79.9	1.9	2.2	16.0	3.5	25.3	2.1	41.3	79.6	36.6	193.6	5.3
Mean ± SD	9.6±15.7	94.4±40.0	7.8±6.0	77.5±37.9	2.4±1.5	5.8±9.5	19.0±10.3	4.6±3.8	33.3±22.8	2.3±0.7	70.3±61.7	106.4±78.1	59.7±49.9	284.5±239.2	5.3±1.5

Below the data for each alcohol condition, the median and mean ± SD are given

The concentrations prior to smoking ($C_{0\ h}$), the maximum observed concentration (C_{max*}) with the corresponding time (t_{max}) and the last concentration measured 4 h after smoking ($C_{4\ h}$) are given The areas under the curves were calculated for the time of measured concentrations ($AUC_{0\rightarrow 4\ h}$) without further extrapolation. Apparent elimination half-lives ($t_{1/2}$) are calculated from exponential regression of valid concentration–time data (1–4 h)

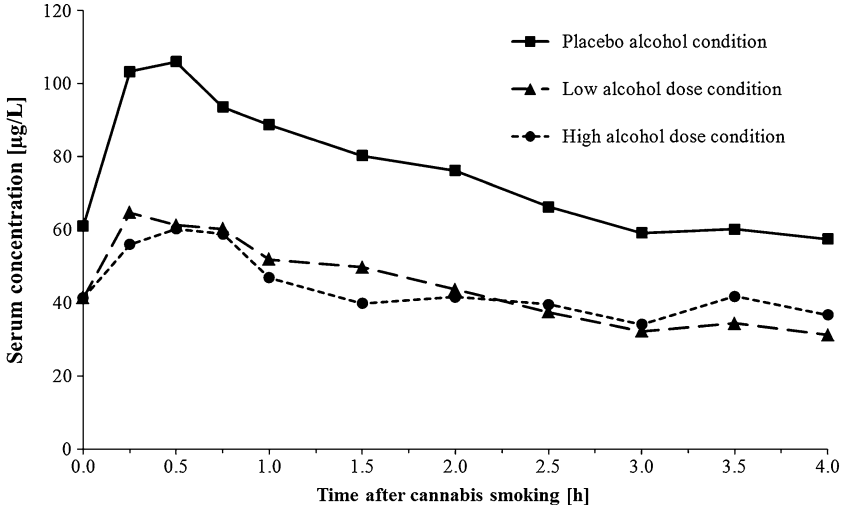

Fig. 1 Overlay of the median THCA plasma concentrations at baseline (0 h) and up to 4 h after cannabis smoking in 19 chronic users in three alcohol conditions (c.f. $C_{0\ h}$ and C_{max*} in Table 1 for individual values). Blood alcohol concentrations (mean ± SD) during the first 2 h after smoking were 0±0 g/l (placebo alcohol condition), 0.36±0.08 g/l (low alcohol dose condition) and 0.50±0.12 g/l (high alcohol dose condition)

timing and strength of alcoholic beverages in a study design may have an important effect on the outcome [8]. Furthermore, the pre-study cannabis use of our subjects has led to an accumulation of THC, as demonstrated by their baseline THC values, whilst the subjects in the study by Lukas and Orozco [10] did not exhibit residual cannabinoids. The redistribution of THC from deep compartments of chronic users has a marked impact on THC concentrations in blood [15, 19]; therefore, the lowered THC concentrations in the present study may be a result of other pharmacokinetic phenomena than assessed by Lukas and Orozco [10].

Table 2 Comparison of statements on cannabis use prior to the three study days and the respective measured serum THCA concentrations

Subject	Subject recrution day			Placebo alcohol condition			Low alcohol dose condition			High alcohol dose condition		
	Joints since yesterday	Joints in the past 5 days	THCA (µg/l)	Joints since yesterday	Joints in the past 5 days	THCA (µg/l)	Joints since yesterday	Joints in the past 5 days	THCA (µg/l)	Joints since yesterday	Joints in the past 5 days	THCA (µg/l)
01	4	18	54.7	5	22	42.2	2	22	32.4	3	21	31.0
02	2	10	42.2	4	14	25.5	3	13	36.1	1	10	25.6
03	6	25	39.7	1	20	28.4	2	13	28.7	3	18	28.0
04	4	7	11.2	3	13	9.1	1	8	10.4	2	8	10.0
05	2	12	6.6	2	17	34.4	3	19	22.9	1	19	25.4
06	3	11	88.5				2	12	56.2	3	14	105.6
07	3	14	15.9	3	17	27.3	1	4	7.8	2	7	11.1
08	3	11	59.7	3	10	83.5	4	10	50.0	2	20	114.5
09	3	11	80.4	2	15	78.2	2	16	78.4	4	18	132.8
10	12	62	95.7	2	2	139.0	3	20	158.0	1	62	181.0
11	12	62	70.0	3	20	92.4	1	62	108.2	2	2	111.2
12	6	24	115.5	2	73	292.8	3	52	199.6	3	34	219.0
13	2	16	38.4				1	19	11.4	3	23	27.5
15	4	16	33.6							3	12	23.9
16	12	34	141.1	7	32	183.1	1	43	112.6	2	42	119.4
17	6	18	24.2	2	19	77.7	3	19	83.0	1	15	60.3
19	8	20	117.8	4	15	45.7	1	1	52.6	3	23	56.0
20	3	19	46.2	2	6	23.3	3	19	14.4	1	1	41.3
21	3	7	21.8	1	12	11.3	2	2	9.8	3	3	12.5
23	6	27	173.9	2	22	181.8						

Also the statements and concentrations observed in the blood samples obtained during subject recruitment are given. Cannabis use is given as number of joints smoked since the day before or as number of joints smoked during the last 5 days

Pharmacokinetic properties of THCOH and THCA

The pharmacokinetic characteristics of THCOH are in agreement with the results of our previous study [15] and are given in Table 1. The concentrations and the derived parameters did not exhibit any differences between the three alcohol conditions. This is in agreement with the results for THC and suggests that THCOH concentrations are not affected by ethanol-mediated changes in blood flow or by distribution to or redistribution from deep compartments. On the other hand, the closely overlapping values confirm the validity and reproducibility of the analytical data in the present study.

The concentrations and the derived pharmacokinetic parameters of THCA did not exhibit significant differences. However, a tendency to markedly lower concentrations in both alcohol conditions was noted for each sampling time (Fig. 1, c.f. medians of $C_{0\ h}$, C_{max*} and $C_{4\ h}$ in Table 1). The failure in the significance tests is very probably attributable to the large inter-individual variation in THCA concentrations from the pre-study cannabis use (Table 2). As the aim of the study was to assess a cross-tolerance to effects of alcohol in cannabis-tolerant subjects, such residual cannabinoid concentrations were inherent to the study design, but concealed significant differences in the evaluation. Furthermore, the study design did not allow the detection of the onset of ethanol effects because at the time of the pre-dose blood sample ($C_{0\ h}$), which was 3 h after the start of alcohol ingestion, all effects of ethanol have been manifest. As not only the concentrations after smoking the cannabis joint were affected but also the baseline values ($C_{0\ h}$ in Table 1), such an effect appears to be not related to metabolism but rather to distribution. The distribution of THCA is different from that of THC [20]; therefore, it is conceivable that ethanol-mediated changes in distribution volume affected THCA much more than THC or THCOH.

If moderate alcohol doses as used in the present study lead to lower concentrations of THCA, this might have relevance for forensic interpretation. At least in Germany, the differentiation of chronic and occasional cannabis use is a prerequisite in deciding on the renewal of a driving licence after confiscation due to driving under the influence of cannabis. High concentrations of THCA in blood samples are supposed to be an indicator for chronic use [21]; therefore, rather low concentrations should be evaluated with care if ethanol is also present. In the same way, this affects the evaluation of THC concentrations in blood as a sign of acute cannabis use. Though elevated concentrations in occasional users are only observed during 8 h after smoking [15], this is different in chronic users [15, 19]. Therefore, in a thorough evaluation of cannabinoid concentrations in a blood sample, the assessment of the drug use frequency is necessary. If THCA concentrations are used for such a differentiation, a potential bias due to ethanol ingestion should be included in the considerations.

Furthermore, some other data from the present study can add to the uncertainty of the correlation of THCA concentrations with the extent of cannabis use. During subject recruitment and in the morning of each study day, the individual drug use during the past week was assessed via a questionnaire. These statements are given together with the measured THCA concentrations in Table 2, and it is obvious that there is no reliable correlation. Therefore, the aim of future studies should be the search for other markers of chronic cannabis use.

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