Dissertation zur forensischen Toxikologie - PhD Thesis in Forensic Toxicology

Analytical studies on the kavain metabolism in human specimen and liver cell lines

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About Kava-Kava

"Kava-Kava" is an intoxicating beverage made from the roots of Piper methysticum G. Forst. It is a widespread and since a long time well known drink on the islands of the South Pacific. For the effect of the kava drug certain ingredients of Piper methysticum, the so called kava-pyrones (kava-lactones) (Figure 1), are made responsible. Kavain is one of the main kava-lactones representing about 5 to 12 % of the total amount of kava-lactones. Kavain is supposed to have similar to benzodiazepines an allosteric influence on the $GABA_A$ receptor-complex, through which the anxiolytic and antidepressant effect is explained. The fact that its central effects can generally lead to abuse, makes kavain an interesting subject for questions from the field of forensic toxicology. Thus the aim of this study was to develop suitable methods to detect and identify kavain and its metabolites and on this basis to gain new knowledge regarding the metabolism and pharmacokinetics of kavain. The available information from the literature on this topic is very incomplete.

Methods development and validation

In an in-vitro study the kavain metabolism was first exemplary examined in Hep-G2 cells. After extraction (fluid-fluid extraction and SPE), kavain and its metabolites were identified and characterised by GC/MS (MSD HP 5970 (70 eV), GC HP 58790, column: HP-5 (i.D. 0,25 mm, 30 m); linearity for kavain (K) ranged between 20 to 5000 ng/ml (partly derivatised) and HPLC-DAD Waters Alliance with PDA 996, acetonitrile (31 % w/w) with phosphate-buffer pH = 2,3, flow 1,0 ml/min isocratic, column: LiChrospher 60 RP select B; linearity for kavain (K) from 5 to 100 ng/ml) and in some cases by LC/MS. To verify the suitability of the used methods, limits of detection, linearity and coefficients of variation were determined (GC/MS: for kavain (K), 12-hydroxykavain (III), 12-hydroxy-7,8-dihydrokavain (V) and 12-hydroxy-5,6-dehydrokavain (IX); HPLC-DAD: for kavain (K) and 12-hydroxykavain (III)). For the quantification HPLC-DAD proved to be suitable. For this, precision, reproducibility and repeatability were determined.

Kavain metabolism by Hep G2-cell cultures

Unchanged kavain as well as 14 metabolites were extracted from the cell cultures after incubation with kavain. 12 of these metabolites are already known to be excreted via urine (human, rat). 4-Oxy-cinnamyl-acetone (XIII) is exclusively available in liver cell cultures, but not in blood or urine.

The detected metabolite pathways in the liver cells are hereafter: a) hydroxylation (to I and III), hydroxylation with reduction of 7,8 double bonds (to give IV and V) and finally glucuronidation and sulfatation, b) dehydrogenation (to VI and after hydroxylation - to give

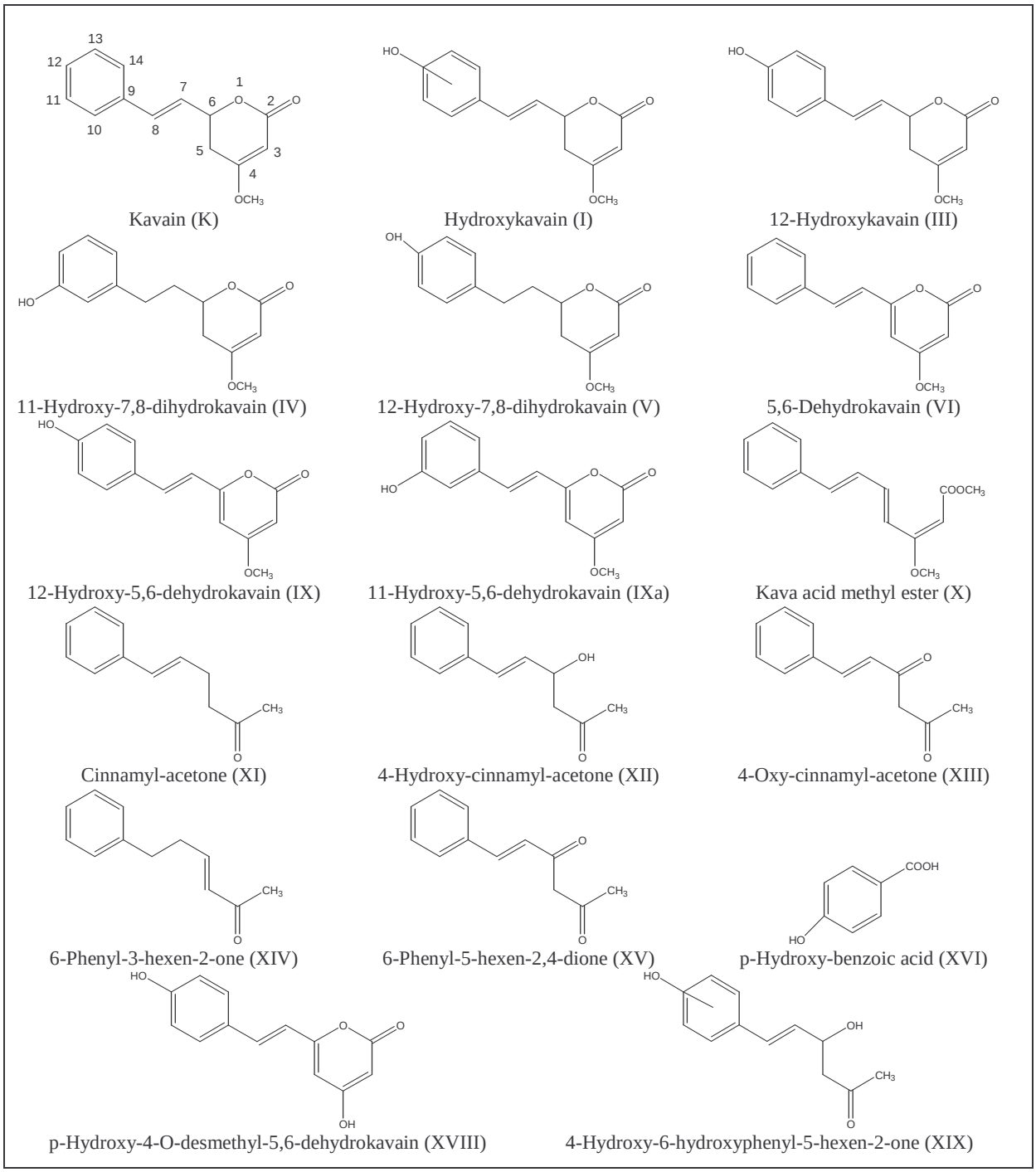

Figure 1: Chemical structures of kavain and its metabolites detected in this study

IX and IXa) and c) opening of the lactone ring, oxidation and decarboxylation at the side chain to give X, XI, XIII, XIV, XV and XIX. After enzymatic cleavage using ß-glucuronidase from E.coli a clear increase of IV is detectable, which is likely to be identical with 11-hydroxy-7,8-dihydrokavain according to the MS- and UV data.

Concrete hints regarding toxic effects could not be observed even after usage of high kavain amounts (up to 60 mg/ 200 ml culture media) and incubation times of up to 70 hours. Due to this, the metabolism studies were continued with the help of self-medication experiments. In a series of four experiments blood and urine samples were taken before and after oral uptake of 200 or 800 mg D,L-kavain and analysed.

Kavain metabolism by human

In a series of four experiments blood and urine samples were taken before and after oral uptake of 200 or 800 mg D,L-kavain and analysed. Unchanged kavain could not be detected in urine. The kavain concentrations in blood and serum were very low compared to the administered dose (10 to 40 ng/ml, detectable 30 min. to about 4 hours after kavain uptake). It is obvious that kavain is quickly metabolised via the first pass effect.

Of the possible metabolic pathways, the hydroxylation at position C-12- of the phenyl ring and thus the formation of 12-hydroxykavain (III) plays a major part. The experiments revealed that the maximum concentration of III in blood is reached after 45 minutes. In serum 50 % of III were available as glucuronide, 12 % as sulfate, and 38 % in its free form (ratio of serum to blood is about 2:1). In urine only 1.4 % of III were excreted in its free form (maximum concentration after 2 hours). 15.3 % were found as glucuronide, and 83.3 % as sulfate.

Another possibility of the phenyl ring hydroxylation was that it has taken place at the non established position of the phenyl ring, this can produce the metabolite hydroxykavain (I). It is an important metabolite which is detected in blood after oral uptake of 800 mg kavain at concentrations of 30 to 50 ng/ml. The maximum was reached after 2.5 hours. It was shown via enzymatic cleavage that this metabolite is not conjugated.

Dehydrogenation at the postion 5,6 of the lactone ring was the metabolism pathway to form 5,6-dehydrokavain (VI) which was detected in urine. It could be further metabolised to the unidentified metabolite (XVIII) by desmethylation at the C-4-position of the lactone ring. XVIII was detected in serum in concentrations between 38 and 84 ng/ml. The maximum concentrations were observed after 2 hours. In urine XVIII was still detectable after 24 hours. It was shown via enzymatic cleavage that this metabolite is partly conjugated as glucuronide or respectively sulfate.

In urine, though not in blood or serum, 12-hydroxy-7,8-dihydrokavain (V) could be detected as a representative of the 7,8-dihydro-derivates. It is remarkable that this metabolite is eliminated by the urine just after 5 hours from oral uptake and exclusively in its conjugated form (glucuronide:sulfate = 2:1)

The last degradation pathway which was observed in the liver cell experiment could be detected as well, the metabolite 6-phenyl-5-hexene-2,4-dione (XV) was detected in urine by GC/MS as well as LC/MS.

For the metabolite 12-hydroxykavain (III) and its conjugates (glucuronide, sulfate) additional kinetic parameters (rate constant of terminal slope, plasma elimination half life, AUC, volume of distribution as well as renal clearance) were determined.

Conclusion

The presented results revealed new insights regarding the kavain metabolism. The degradation pathways of kavain proved to be applicable from the in-vitro model to humans by evidence of the metabolites. The methods used for identification and quantification of kavain and its metabolites could be validated and thus comply with the requirements of forensic toxicology. In the field of forensic toxicology and traffic medicine, it was necessary to have a validated method for the determination of kavain and its metabolites in different materials to give an expert opinion in cases of misuse or abuse of this drug. Our experiences show that there is a big interest in such methods in countries with widespread kava consumption. Some aspects of the kavain metabolism are therefore resolved, though further research is necessary to fully comprehend the pharmacokinetics and -dynamics of the kava-lactones.

Parts of this Ph.D. Thesis have already been presented and/or published in:

1. Tarbah F. A., Mahler H., Temme O. and Daldrup Th. Mass spectral characterisation of hepatic cell metabolites of D,L-kavain using HPLC and GC/MS systems. Special issue: 37th TIAFT triennial meeting "Problems of Forensic Sciences" XLII: 173-180 (1999)
2. Tarbah F. A., Mahler H., Temme O. and Daldrup Th. Determination of d,l-kavain and its metabolites in blood, serum and urine. Rapid quantitative method using fluid/fluid extraction and gas chromatography/mass spectrometry (GC/MS). Poster in 79. Jahrestagung der Deutschen Gesellschaft für Rechtsmedizin, Medizinische Einrichtungen der Universität / Gesamthochschule Essen (2000)
3. Tarbah F., Mahler H., Kardel B., Weinmann W., Hafner D. and Daldrup Th. Kinetics of kavain and its metabolites after oral application. J. Chromatogr. B 789 (1): 115-130 (2003)
4. Cabalion P., Barguil Y., Duhet D., Mandeau A., Warter S., Russmann S., Tarbah F. and Daldrup Th. Kava in modern therapeutic uses: to a better evaluation of the benefit/risk relation. Researches in New Caledonia and in Futuna (Draft). 5th European Symposium of Ethnopharmacology, Valencia, Spain, 8th-10th May 2003

Literaturhinweis

Regulatorische Toxikologie - Gesundheitsschutz, Umweltschutz, Verbraucherschutz

Michael Schwenk und Franz-Xaver Reichl.

2004, XXII, 616 S. 99 Abb., 59 Tab., geb. ISBN: 3-540-00985-X Versandfertig innerhalb von 3 Tagen

Über dieses Buch: Führende Experten geben Einblick in die grundlegenden Prozesse der toxikologischen Regulierung. Die wichtigen Fragen zu Risikoanalyse, Risikobewertung und Risikomanagement werden jeweils in einem eigenen Kapitel beantwortet. Weitere Themen gelten den neuesten Arbeitsmethoden und Beurteilungsgrundlagen, dem Spannungsfeld zwischen naturwissenschaftlichen Argumenten und weltanschaulichen Aspekten sowie den Grundlagen von Gesundheits-, Verbraucher-, Umwelt- und Arbeitsschutz. Ein „Muss" für alle, die in Behörden, Industrie, Universitäten oder anderen Institutionen mit der toxikologischen Beurteilung und Beratung befasst sind.

Geschrieben für: Toxikologen, Pharmakologen, Umweltämter, Gesundheitsämter, Untersuchungsämter, alle im Arbeitsschutz tätigen Behörden.