SHORT REPORT

The influence of methanandamide on the activity of hepatic CYP1A2 and 2C6 isoenzymes in preclinical experiment

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Introduction

The effects of psychoactive substances from marihuana (*Cannabis sativa/indica, Lam.*) are known for a long time, but the pharmacological mechanisms of them are not uncovered in details yet. Most of cannabinoid actions are evoked by interaction with specific CB1 or CB2 cannabinoid receptors (CB). CB1 receptors are localized predominantly in the central and peripheral nervous systems while CB2 receptors are found in the highest density in non neuronal tissue (Fride 2004). Many synthetic as well as naturally occuring ligands for cannabinoid receptors are known. Anandamide is endogenous agonist for both CB1 and CB2 receptors. One from the synthetic group of selective CB1 ligands is methanandamide (MA). It has metabolically more rigid molecule than anandamide.

Some of the CB receptor ligands, including MA, are often discussed in relation to their probable clinical use. Indications for such substances are wide. CB1 receptor ligands are suggested to be used in the therapy of e.g. asthma bronchiale, glaucoma, sclerosis multiplex, parkinsonism, schizophrenia (Pertwee 1999).

Endogenous as well as some of the exogenous cannabinoids are substrates for CYP450 (Pertwee 2005; Snider *et al* 2007). Cytochrome P450 (CYP450) is one of the major drug metabolizing systems. It is localized predominantly in hepatocytes and consists of many isoenzymes, which have different substrate specificity. The activity of this system is not rigid at all. It is influenced by many factors (genotype, sex, age).

For clinical use of a new potential pharmacotherapeutical agent it is important to know how it is metabolized and if it influences metabolism of endogenous substrates or co-administered drugs. Serious side effects and interactions between drugs can be prevented using this information. The present study was designed to explore impact of cannabinoid CB1 receptor agonist methanandamide on the activity of CYP1A2 and 2C6 isoenzyme.

Methods and materials

Animals. The experiment was carried out on male Wistar albino rats (Biotest s.r.o., Konárovice, CZ) weighing 200 ± 20 g. Animals were housed under the controlled conditions (24°C, 50% humidity, light/ dark regime 12h/12h) in the groups of four with free access to food and water.

Methanandamide (Tocris Bioscience, Ellisville, USA) was administered intraperitoneally at the dose of 1mg/kg daily for 7 days and was dissolved in Tocrisolve™ (Tocris Bioscience, Ellisville, USA). Control group animals were treated with adequate volume of pure Tocrisolve™. MA and control group consisted of 8 animals. All procedures were approved by the Czech Central Commission for animal welfare.

Model of perfused rat liver. Model of perfused rat liver was used for the determination of CYP1A2 and 2C6 isoenzyme activity. Rats were anesthetized with the combination of xylasine (Rometar, Spofa, Praha, CZ) and ketamine (Narkamon 5%, Spofa, Praha, CZ). Isolation was performed using standard surgeon technique. Abdominal cavity was opened using wide laparotomy. Cannula was inserted into *vena cava* and liver was perfused with tempered saline (37°C) during isolation. Liver were placed into perfusion apparatus

Tab. 1: The influence of methanandamide on selected pharmacokinetic parameters of CYP1A2 activity. (* p≤0.05)

	AUC_{PHE} [mg/L.h]	Cl_{PHE} [L/h]	$T_{1/2PHE}$ [h]	C_{maxPAR} [mg/L]	AUC_{PAR} [mg/L.h]
control	3.443 ± 0.734	3.099 ± 1.051	0.843 ± 0.490	0.160 ± 0.193	0.179 ± 0.108
methanandamide	4.249 ± 0.886*	2.499 ± 0.798	0.967 ± 0.346	0.190 ± 0.213	0.187 ± 0.152

Tab. 2: The influence of methanandamide on selected pharmacokinetic parameters of CYP2C6 activity.

	AUC_{TOL} [mg/L.h]	Cl_{TOL} [L/h]	$T_{1/2TOL}$ [h]	C_{maxHTL} [mg/L]	AUC_{HTL} [mg/L.h]
control	10.041 ± 2.928	1.089 ± 0.232	2.029 ± 1.006	0.425 ± 0.189	0.347 ± 0.096
methanandamide	9.239 ± 1.990	1.163 ± 0.431	2.043 ± 0.370	0.555 ± 0.298	0.470 ± 0.376

after extraction from abdomen and saline was substituted with perfusion medium.

The modified Miller's (Miller *et al* 1951) recirculating apparatus was used with Williams medium E for perfusion. Medium was pumped from medium reservoir into oxygenator, where is saturated with the mixture of oxygen and carbon dioxide (Carbogen, Linde Gas, Praha, CZ). Medium then flows into cannulated *vena portae* and through the liver into the reservoir. Whole system was tempered onto 37°C.

CYP1A2 marker substance phenacetine (PHE) (Sigma Chemical Co., St. Louis, USA) and CYP2C6 marker tolbutamide (TOL) (Sigma Chemical Co., St. Louis, USA) were added as a bolus into perfusion medium after 20 minutes of pre-perfusion. As a time for marker addition was considered a beginning of the perfusion. Samples of perfusate were withdrawn on the 30th; 60th and 120th minutes of perfusion and stored frozen (-20°C) until extraction.

Quantitative analysis of markers and metabolites. Concentrations of marker substances and their isoenzyme specific metabolites paracetamol- PAR (1A2) and hydroxytolbutamide-HTL (2C6) in perfusion medium were measured after extraction from perfusion medium using HPLC method.

Simultaneous determination of both markers and metabolites was performed on Shimadzu chromatograph, using 10.0 mM KH_2PO_4 and acetonitrile mixture in the rate 60:40 as a mobile phase and chlorpropamide as an internal standard. Analyzed substances were separated on LUNA C18 (150 × 4.6, 5 μm) column, mobile phase flow was 1.5 ml/min and UV-VIS detection: 229 nm (chlorpropamide, TOL, HTL), 245 nm (PAR) and 247 nm (PHE).

Selected pharmacokinetic parameters were computed using non-compartmental intravascular bolus model in Kinetica 4.0 software (Thermo Fisher Scientific Inc., Waltham, USA). Statistical analysis was performed using F test and Student's t test. Value p≤0.05 was considered as statistically significant.

Results

Influence of methanandamide on CYP1A2 activity. Levels of PAR in perfusion medium were not affected by MA administration (***Fig.1***), while concentrations of PHE were significantly higher (p≤0.001) in MA group animals than in control animals (see ***Fig.2***) at the end of the perfusion. Pharmacokinetic parameters computed from detected concentrations of PAR and PHE did not differ among animals except AUC_{FEN}, which was higher in MA group (***Tab.1***).

Influence of methanandamide on CYP2C6 activity. In comparison to values of control animals the levels of TOL were significantly decreased only in the 60th minute of perfusion (***Fig.3***) what indicates that the metabolic activity of CYP1A2 in MA group animals was induced. This fact was manifested as significant increase of HTL levels in MA animals in the 60th and 120th minute of perfusion (p≤0.05) (***Fig.4***). The induction did not influence pharmacokinetic parameters of TOL or HTL (***Tab.2***).

Conclusions

Our results confirmed the induction of CYP2C6 activity and on the other hand the inhibition of CYP1A2 activity caused by methanandamide application. Our recent results demonstrate heterogenous effect of MA on rodent CYP450 hepatal isoenzymes. MA significantly increased levels of PHE (p≤0,001). The elevation of marker levels is a sign of inhibiting action and is usually followed by decrease of metabolite amounts. We did not prove lower levels of PAR in MA group and the increase of PHE was manifested only in the 120th minute of perfusion, while in the 60th and 30th min. were levels similar in both groups. The inhibition was confirmed by the difference in values of AUC_{PAR}. The elevation of AUC_{PAR} in MA demonstrates slower metabolism of PHE. The inhibition is slight and may depend on substrate availability. This would make clear why we found elevation of PHE levels only in the end of the perfusion. The influence of MA on the activity

of CYP2C6 isoenzyme was totally opposite to those we determined in CYP1A2. MA caused increase of HTL levels and decrease TOL level in the 60th min. With regard to these results we interpret the influence of MA on CYP2C6 activity as induction. As well as in the case of CYP1A2 modulation of CYP activity was not strong.

Few available works documented inhibition of CYP450 mediated metabolism after cannabis (hashish) administration (Sheweita 2003). Induction of CYP450 activity after chronic treatment with endogenous cannabinoid anandamide is described (Costa *et al* 2002) and as the mechanism of such action the increase of the total liver CYP450 content is approved. Specific isoenzymes CYP2B, 3A and 2C can be induced by chronic administration of another cannabinoid cannabidiol in mice (Bornheim *et al* 1993). Literature on the effect of specific CB1 receptor ligands on CYP450 activity is scarce. Our previous results documented inhibition of MA on CYP2D6 isoenzyme (Zahradnikova 2007).

In conclusion, changes of isoenzymes CYP1A2 and CYP2C6 activities under the influence of cannabinoid CB1 receptor agonist methanadamide found in the present experimental study indicate possibilities of pharmacokinetic interactions with drugs biotransformed through this pathway. At the same time we also conclude, that exogenously given CB1 receptor agonists can perhaps alter the endocannabinoid system activity because of their impact on metabolism of endogenous cannabinoids (e.g. anandamide) mediated by CYP450.

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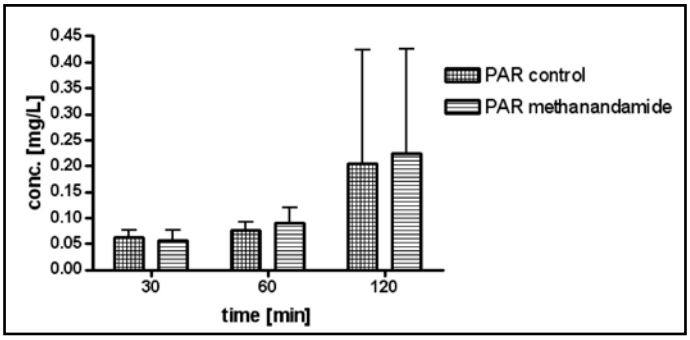

Fig. 1: The influence of methanandamide on levels of PAR in perfusion medium.

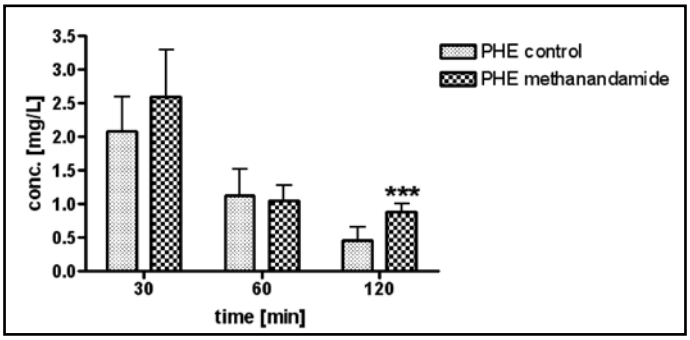

Fig. 2: The influence of methanandamide on levels of PHE in perfusion medium. (*** p≤0.001)

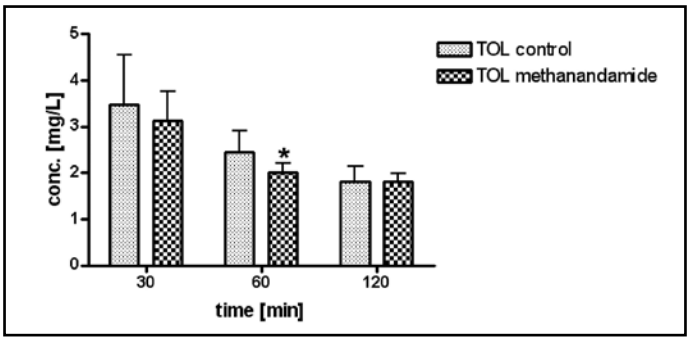

Fig. 3: The influence of methanandamide on levels of TOL in perfusion medium. (*p≤0.05)

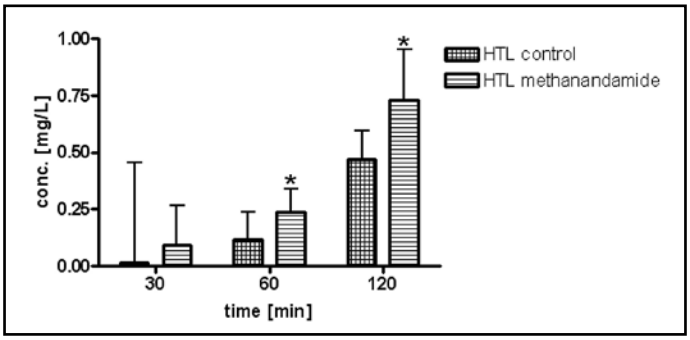

Fig. 4: The influence of methanandamide on levels of HTL in perfusion medium. (*p≤0.05)

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