SHORT REPORT

Can methanandamide and gender influence pharmacokinetics of psychoactive drugs?

Preclinical study on activity of cytochrome P450

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Introduction

Effects of substances from Cannabis indica in human are well known. The primary active constituent of the hemp plant is Δ^9-tetrahydrocannabinol (THC) (Justinova *et al* 2005). Experiments with natural molecules and their analogues led to discovery of endogenous cannabinoid system. Researchers in the 1970s, 80s, and 90s primarily assessed cannabis ability to temporarily alleviate various disease symptoms, such as the nausea associated with cancer chemotherapy. Of particular interest, scientists are investigating cannabinoid capacity to moderate autoimmune disorders, such as multiple sclerosis, rheumatoid arthritis and inflammatory bowel disease, as well as their role in the treatment of neurological disorders such as Alzheimer's disease and amyotrophic lateral sclerosis. By now, there are many ligands of cannabinoid receptors (CB) known. Anandamide, an endogenous ligand for brain cannabinoid CB_1 receptor, produces many behavioural effects similar to those of THC, the main psychoactive ingredient in marijuana (Bornheim *et al* 1993). Anandamide is one of the natural agonists of CB1 and CB2 receptors. (*R*)-(+)-methanandamide is metabolically more stable and is a selective ligand of CB1 receptors (Justinova *et al* 2005). In addition to studying the receptor activity of cannabinoids, a possible role of non-receptor mechanism in their effects should also be considered. It is required to determine the potential for interactions with other drugs with regard to their possible clinical use.

The enzymatic system of cytochrome P450 (CYP450) is a part of phase I of enzymatic biotransformation. It consists of many isoenzymes characterized by specific substrates and organ localization. These isoenzymes are most predominant in the liver, but can also be found in the intestine, lungs and other organs. Interindividual variability of activity of drug metabolizing enzymes, especially the system of CYP450, can be based on many exogenous as well as endogenous influences e.g. sex, age, genetic factors or interactions between simultaneously applied drugs (Dostálek 2006). The effort to adapt the dosage of drug for the particular patient and to individualize and optimize the therapy to prevent adverse effects, to decrease the duration and costs of therapy is often seen in modern pharmacotherapy. Action of methanandamide on the activity of hepatic cytochrome P450 (CYP450) can be an important factor influencing its therapeutical use.

Methods

The work was carried out on male and female Wistar Albino rats (weighing 250 ± 40 g, BioTest, Konarovice, Czech Republic). Control group (CG) animals were treated with Tocrisolve™ 100 (Tocris Cookson Ltd.) (1mg/kg/day intraperitoneally) and (*R*)-(+)-methanandamide group (R-MG) animals were treated with the drug dissolved in Tocrisolve™ 100 (Tocris Cookson Ltd.) at the dose of 1mg/kg/day intraperitoneally for 7 days.

The rat liver was isolated from donors using a standard surgical technique. Cannula was introduced into the portal vein and inferior cava vein, the liver was shortly washed out by a tempered (38°C) saline which was changed for the perfusion medium (120

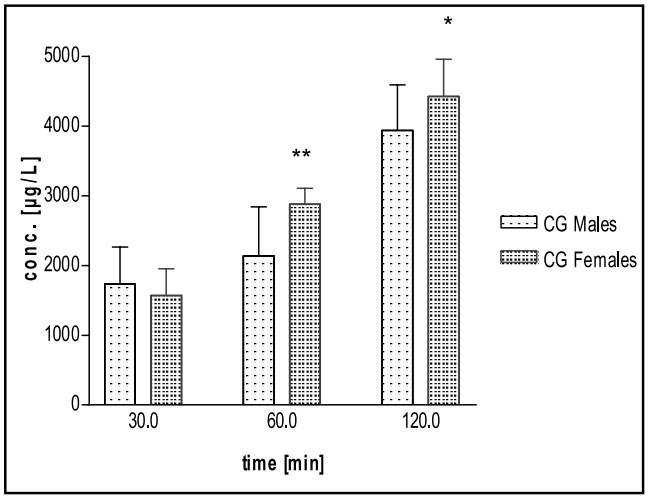

Fig.1: Influence of sex in control group (CGs). Columns represent concentrations of DOR in perfusate in the 30th, 60th and 120th minute of perfusion in males and females (*significant difference between male and female animals p≤0.05, **p≤0.01).

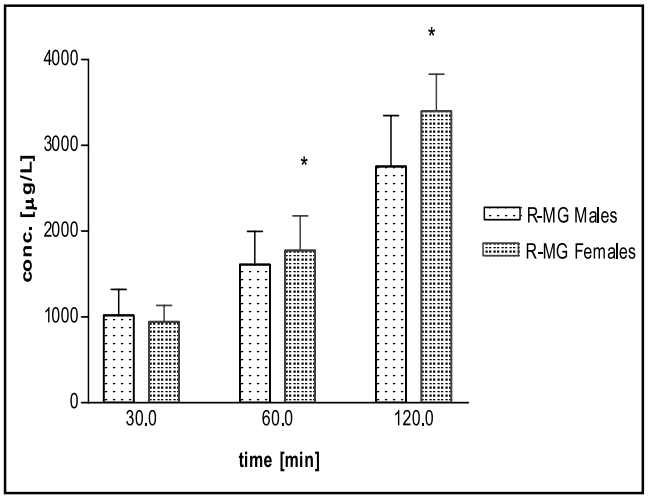

Fig.2: Influence of sex in (*R*)-(+)-methanandamide group (R-MGs). Columns represent concentrations of DOR in perfusate in the 30th, 60th and 120th minute of perfusion in males and females (*significant difference between male and female animals p≤0.05).

ml of Williams medium E) equilibrated with a mixture of 95% O_2 and 5% CO_2 in a short time. The recirculating perfusion apparatus was constructed according to the principles originated by Hugo Sachs GmbH (Germany). After 20 min pre-perfusion, a specific marker – dextromethorphan (DEM) (10.0 mg/l) was added as a bolus into the perfusion medium. Samples of perfusate (1.0 ml) were collected at the 30th, 60th and 120th min of perfusion and were stored at -75°C until analysis. Quantitative analysis detecting DEM and its metabolite dextrorphan (DOR) was performed by HPLC method (Shimadzu, Japan). Method by Zimová et al. (2000) was used to asses the levels of specific metabolite DEM and it's metabolite DOR in the perfusion medium (Zimová *et al* 2000).

For statistical calculations F-test and Student's t-test (Microsoft Excel 2000) were used, $p < 0.05$ considered to be statistically significant difference.

Results

In the CG, the levels of CYP2D2 – dependent metabolite DOR were significantly higher compared to the R-MG in both sexes. The levels of DOR in males were not increased in the 30th, 60th and 120th minute in R-MG than in CG. In males /R-MG/ level of the parent drug DEM was significantly higher in 30th and 120th min and in females /R-MG/ level of DEM was significantly higher during the whole perfusion. The sex specific changes of the CYP2D2-dependent metabolite DOR concentrations in the perfusate evoked by a 7 day methanandamide treatment are documented in the ***Figure 1*** and ***2***.

Conclusions

The model of isolated perfused rat liver is suitable for investigation of the activity of hepatic CYPs 450 and biotransformation processes is represented by the. The main advantage of this model, in comparison with other methods used for measuring the CYP activity, is that conditions resemble physiological situation in the organism.

As we expected, the activity of CYP2D2 differed due to pretreatment with methanandamide. In our experiments the influence of this compound on rat CYP2D2 was inhibitive and metabolic activity of studied isoenzyme CYP2D2 in male and female rats was significantly lower in (*R*)-(+)-methanandamide group than in the control group.

CYP2D6 (human orthologue of the rat CYP2D2) represents the second most frequent enzyme implicated in the biotransformation of therapeutic drugs (codeine, amitriptyline, clomipramine, imipramine and β-blockers such as propranolol and metoprolol) (Tanaka 1998). The literature data focused on the influence of gender on CYP450 is scarce and articles describe variable results. A more recent study on CYP2D6 mediated metabolism of metoprolol in extensive metabolizers showed greater clearance in men compared to women (Labbe *et al* 2000). The present study demonstrated that CYP2D2 activity in the rat liver was higher in females than in males. The gender difference was distinguished in the methanandamide-pretreated rats as well as in the control group; formation of a CYP 2D2-controlled metabolite DOR in both groups was significantly higher in females than in males.

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