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ORIGINAL ARTICLE

Role of dihydromyricetin in cytochrome P450-mediated metabolism and carcinogen activation

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Abstract

OBJECTIVES: Dihydromyricetin (DHM) is a flavonoid, which has been shown to antagonize effects of ethanol intoxication. As a potential pharmacological agent, its biological interactions with enzymes metabolizing foreign compounds should be tested. Thus, the aim of this study was to analyze the influence of DHM on the induction and metabolic activity of selected cytochromes P450 (CYPs).
METHODS: After flavonoid administration by oral gavage to stomach the CYP expression at protein and mRNA levels was determined in rat liver and small intestine. The effects of flavonoids on CYP1A1/2, CYP1A2 or CYP2B1/2 enzyme activities in microsomes were measured using marker activities of these enzymes. Flavonoid-mediated inhibition of recombinant CYP1A2 was also assayed with luciferin-ME substrate. The flavonoid interaction with aryl hydrocarbon receptor (AhR) was assayed by reporter luciferase activity in Hep2G cells.
RESULTS: The value of half maximal inhibitory concentration of DHM for CYP1A1/2, CYP1A2, and CYP2B1 were determined to be 4.1, 14.2, and 98.5 mmol.L^{-1}, respectively. With the exception of a weak induction of CYP2B1 and CYP1A2 in the middle part of small intestine and in the liver, respectively, DHM did not affect the CYP expression at protein levels. On the contrary, real-time PCR revealed elevated expression of CYP1A1 and CYP1A2 mRNA in proximal part of the small intestine while decreased in the middle part. In the study utilizing the HepG2 cells, DHM showed only an additive effect on the benzo[*a*]pyrene-mediated activation of Ah receptor.
CONCLUSIONS: Dihydromyricetin doesn't significantly interfere with metabolic activity of CYP1A1/2 and CYP2B1 enzymes.

Abbreviations

AhR	- aryl hydrocarbon receptor
BaP	- benzo[a]pyren
BCIP/NBT	- 5-bromo-4-chloro-3'-indolyphosphate/nitro-blue tetrazolium
CYP	- cytochrome P450
d	- distal
DHM	- dihydromyricetin
DMSO	- dimethyl sulfoxide
EROD	- 7-ethoxyresorufin-O-deethylase
IC_{50}	- half maximal inhibitory concentration
IgG	- immunoglobulin G
LDR	- luciferin detection reagent
m	- middle
mRNA	- messenger ribonucleotid acid
MROD	- 7-methoxyresorufin-O-demethylase
MYR	- myricetin
NADPH	- nicotinamide adenine dinucleotide phosphate
p	- proximal
PAH	- polycyclic aromatic hydrocarbons
PCR	- polymerase chain reaction
PROD	- 7-penthoxyresorufin-O-depenthylase
RLU	- relative lights units
SDS	- sodium dodecyl sulphate
si	- small intestine
XRE	- xenobiotic responsive element

INTRODUCTION

Flavonoids are chemicals produced by plants as secondary metabolites. They are indispensable in plant protection against microbial infection or herbivores and serve also as pigments and olfactants (Hodek *et al.* 2002). Their possible biological impacts on human body are still questionable (Hodek *et al.* 2009). The most investigated properties of flavonoids are associated with the reduced risk of cardiovascular diseases, osteoporosis, other age-related degenerative diseases and cancer (Morris & Zhang 2006). Dihydromyricetin (DHM) is a flavonoid found in high concentrations in *Ampelopsis grossedentata*. Besides anti-cancer activity (Lu *et al.* 2015), DHM has been shown to provide a unique antagonistic effect against an ethanol intoxication and addiction (Shen *et al.* 2012). Having this therapeutic potential, DHM is presumed to be supplied in high doses into the organism. On account to this, there is a need to reveal DHM biological impacts, especially on enzymes involved in the metabolism of xenobiotics.

The main organ involved in processing of foreign compounds absorbed in gastrointestinal tract is the liver. By the first-pass elimination process, hepatic enzymes modify hydrophobic molecules by the cascade of two biotransformation pathways into more polar and easily eliminated compounds. Final products show usually a lower biological toxicity, but some intermediate metabolites can be reactive and cause cytotoxicity, genotoxicity or mutagenicity. Cytochromes P450 (CYPs) are the key enzymes participating in the so called phase I of xenobiotic biotransformation. The activity of this hemoprotein could be modulated by diverse mechanisms, from which the most common one is the induction by xenobiotics, including flavonoids (Hodek *et al.* 2002). They influence the level of transcription, which is controlled *via* ligand-activated nuclear receptors or transcriptional factors. For instance, the gene expression of a CYP1 family is mediated by aryl hydrocarbon receptor (AhR), which is a ligand-activated transcriptional factor dimerizing with AhR nuclear translocater. As a heterodimer, AhR is binding to specific DNA target sequences, called xenobiotic-response elements (XRE) and by this mechanism AhR is able to initiate the transcription (Pavek & Dvorak 2008). This molecular pathway explains the induction effect of polycyclic aromatic hydrocarbons (PAH), which are specific AhR ligands. PAH represent not only the example of CYP1 inducers, but simultaneously, they are frequently also the substrates of CYP1A1, which might be metabolically activated to reactive intermediates (metabolites) possessing the (geno)toxic effects. Benzo[*a*]pyrene (BaP) is a PAH found in smoke originated from incomplete combustion of organic material, such as compounds present in the cigarette smoke or in the charred food. By successive oxidation *via* CYP1A1 BaP may form reactive intermediates forming covalent adducts with DNA (Phillips 1999; Stiborova *et al.* 2013).

The modulation of CYP activities results in changes in their substrate metabolism. Flavonoids are compounds, which may affect the CYP system as inducers, inhibitors or activators. For example, the transcription of CYP1A1/2 can be induced by tangeretin while CYP2B family has flavanone as a specific inducer (Hodek *et al.* 2002). Myricetin was found to be an inhibitor of the CYP3A4-mediated metabolism of several substrates (Ho *et al.* 2001). In this study, we investigated the influence of DHM on induction of CYP1A1/2 and CYP2B1 in rats *in vivo*, in hepatocellular carcinoma cell line, Hep2G, and in the microsomal systems containing these CYPs.

MATERIAL AND METHODS

Chemicals

High-Capacity cDNA Reverse Transcription Kits and Gene Expression Master Mix (Applied Biosystems, USA), λ DNA EcoRI + Hind III (Fermentas, USA), Trizol® Reagent (Invitrogen, USA.), Prime & Probes β-actin and Prime & probes CYP1A1 (Life Technologies, USA), blue Gel loading Dye (New England BioLabs, USA), ethanol, isopropanol, EDTA, chloroform, NADPH, bicinchoninic acid, resorufin, 7-ethoxyresorufin, 7-methoxyresorufin, 7-pentoxyresorufin, 5-bromo-4-chloro-3'-indolyphosphate/nitro-blue tetrazolium (BCIP/NBT), anti-chicken IgG alkaline phosphatase conjugate (Sigma Chemical Co., USA) were used in the experiments. Dihydromyricetin was provided from APIchem (Shanghai, PRC) and MYR from TCI (Tokyo Chemical Industry Co., Japan). Specific chicken anti-CYP1A1/2 and anti-CYP1A2 antibodies were prepared by Prof. Hodek as described (Hodek *et al.* 2013). The hepatocellular carcinoma (HepG2) cells were purchased from the European Collection of Cell

Cultures (ECACC, UK). The HepG2 cells were cultivated in antibiotic-free DMEM medium supplemented with 10% fetal bovine serum and 1% non-essential amino acids at 37 °C in humidified 5% CO_2 incubator. All chemicals used in the experiments were of analytical grade purity or better.

Animal experiments and preparation of microsomes

The study was conducted in accordance with the Regulations for the Care and Use of Laboratory Animals (311/1997, Ministry of Agriculture, Czech Republic). Male Wistar rats (150–200 g, AnLab, Czech Republic), were housed in groups of 3 in wire cages at 22 °C with a 12 hrs light/dark period and *ad libitum* diet (ST-1 diet from Velaz, Czech Republic) and water access. Tested flavonoids, DHM and MYR, were dissolved in sunflower oil and administered *per os* by gastric gavages in four doses (0.5 mL, 60 mg.kg^{-1} body weight) in four consequent days. Animals of all experimental groups were sacrificed 24 hrs after the last treatment. The control group was treated with sunflower oil only. Microsomes were prepared from 3 sections of dissected small intestine (proximal, middle, and distal parts) and the whole liver, as described elsewhere (Krizkova *et al.* 2008, Hodek *et al.* 2011; Stiborova *et al.* 2013). Microsomal fractions were stored at –80 °C before use. Protein concentrations in the microsomal fractions were determined using the bicinchoninic acid protein assay with bovine serum albumin as the standard (Weichelman *et al.* 1988). The concentration of CYP was estimated according to Omura & Sato (1964) based on the absorption of the of reduced CYP complex with carbon monoxide.

Western blot analysis

The CYP1A1, 1A2 and 2B1 proteins were detected by Western blotting on the PVDF, Immobilon-P membrane (Millipore, USA) using specific chicken anti-CYP1A1/2, anti-CYP1A2 and anti-CYP2B1 antibodies (20 μg.mL^{-1}). For sodium dodecyl sulfate (SDS)-electrophoresis (10% polyacrylamide gel), 60 μg protein/well of small intestine and 15 μg protein/well of liver microsomes was applied. Western blotting was carried out as described earlier (Krizkova *et al.* 2008). Visualization was performed using an anti-chicken IgG alkaline phosphatase-conjugated antibody (diluted 1:2000) and BCIP/NBT substrate tablets (10 mg) for alkaline phosphatase.

Enzyme assays

Microsomal samples, in which the CYP1A1/2 were induced with BaP and CYP2B1 by phenobarbital were analyzed for specific activity of CYP1A1/2, CYP1A2 or CYP2B1, by monitoring dealkylation of 7-ethoxyresorufin (EROD), 7-methoxyresorufin (MROD) or 7-pentoxyresorufin (PROD), respectively, according to the method described by Burke & Mayer (1974). Final mixtures (175 μL) in 100 mmol.L^{-1} potassium phosphate buffer (pH 7.4) contained: 0.5 mmol.L^{-1} NADPH, microsomal protein in quantities 0.7 μg (EROD), 8.5 μg (MROD) or 7.2 μg (PROD), 2.0 μmol.L^{-1} 7-alkoxyresorufin (from 0.2 mmol.L^{-1} stock in dimethyl sulfoxide, DMSO) and the flavonoid (methanol solution of a given concentration). The reaction was initiated by addition of NADPH. The formation of resorufin was monitored by its fluorescence (excitation and emission wavelengths of 530 and 585 nm, respectively) in 1 minute intervals for 10 minutes at room temperature on luminescence spectrometer (PerkinElmer LS-55 equipped with 96-well plate reader). The data were determined from doublets of two independent experiments. The reaction mixture without flavonoid containing methanol only was used as a control (100% activity). Further, we used commercially available P450-Glo™ Screening System (Promega, USA) to assess the effects of MYR and DHM on the enzyme activity of human CYP1A2. Briefly, we added tested compounds in different concentration to the wells of white opaque 96-well plate and further mixed with CYP1A2 reaction mixture including luciferin-ME substrate and a preparation containing human CYP1A2 enzymes. After 10 min NADPH regeneration system was added to start the conversion of luciferin-ME substrate to luciferin product followed by adding 50 μL of Luciferin Detection Reagent (LDR) 30 min later. After additional 20 min the luminescent signal was measured by a plate reader reflecting activity of CYP1A2. The luminescence from vehicle (DMSO)-treated CYP1A2 reactions constitutes total CYP1A2 activity. The average signal gained from non-CYP1A2 reactions were subtracted from the signals of CYP1A2 mediated reactions. The data are shown as mean of three independent experiments measured in triplicate and presented in relative lights units (RLUs).

Real-time PCR

Samples of mRNA were isolated from liver and small intestine tissues using Trizol® Reagent. The integrity of isolated mRNA was proved by gel agarose electrophoresis and its purity and concentrations were measured spectrophotometrically. Reverse transcription to cDNA was followed by the real-time polymerase chain reaction in the presence of a TaqMan probe in 50 cycles (95 °C/15 sec + 60 °C/60 sec). Data were analyzed by the program RotorGene v6 (Corbett Research, Australia) and evaluated by comparative cycle threshold method for relative quantization of gene expression as described earlier (Stiborova *et al.* 2008).

Luciferase gene reporter assay

HepG2 cells were seeded onto 48-well plates (30,000 cells per well). After 24h, they were transiently transfected with AhR-responsive gene reporter construct (pXRE-luc, 100 ng per well) together with pRL-TK (Promega, USA) transfection control plasmid (30 ng per well) using Lipofectamine 3000 (Life Technologies, USA) reagent following manufacturer's protocol. Treat-

ment of cells by tested compounds (0.1–100 μmol.L^{-1}) was performed 24 hrs later. As a model ligand of AhR receptor BaP (10 μmol.L^{-1}) was used. After additional 24 hrs, cells were lysed and activity of luciferase was measured using luciferase detection system (Dual Luciferase Reporter Assay System, Promega, USA). The data are presented as the activation fold of firefly luciferase activities of flavonoids-treated cells relative to vehicle-treated cells (DMSO, 0.1%, set to be 1). Each sample was normalized to luciferase activity of transfection control. The data were measured in triplicates and showed as mean of two independent experiments. The concentration of DMSO used in the culture medium was maintained at 0.1% (v/v) in all performed experiments. The experimental data were analysed using standard statistical methods ANOVA an Student's *t*-test.

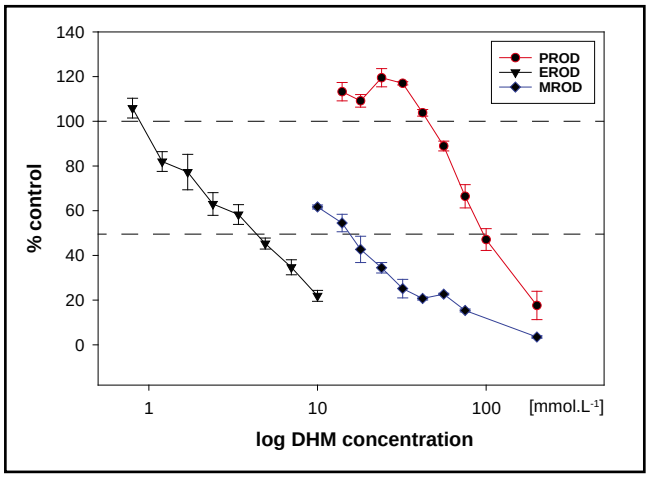

Fig. 1. Inhibition of CYP activities by dihydromyricetin. In microsomal samples, in which CYP1A1/2 were induced by benzo[*a*]pyrene and CYP2B1 by phenobarbital, the specific activity EROD (A), MROD (B) and PROD (C) of CYP1A1/2, CYP1A2 and CYP2B1, respectively, were determined. Control activity was measured in samples with methanol added only. The plotted data are means of doublets ± standard deviation of two independent experiments.

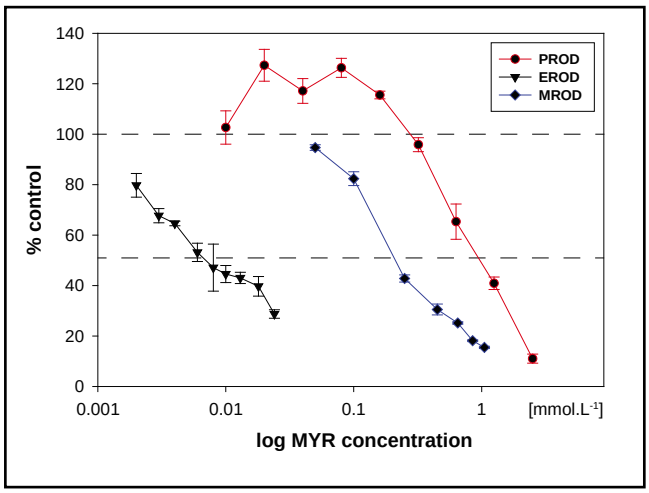

Fig. 2. Inhibition of CYP activities by myricetin. In microsomal samples, in which CYP1A1/2 were induced by benzo[*a*]pyrene and CYP2B1 by phenobarbital, the specific activity EROD (A), MROD (B) and PROD (C) of CYP1A1/2, CYP1A2 and CYP2B1, respectively, were determined. Control activity was measured in samples with methanol added only. The plotted data are means of doublets ± standard deviation of two independent experiments.

RESULTS

The impact of DHM on the CYP induction was analyzed in microsomal fractions of small intestine and liver after the oral premedication of rats with flavonoid. The expression of CYP1A and 2B families in microsomal samples at the protein levels was determined by Western blotting, and at mRNA levels by real-time PCR method in rat tissues. Also HepG2 cells were employed to determine the influence of DHM on the activation of AhR receptor. The DHM mediated inhibition of specific catalytic activities (EROD, MROD and PROD) of CYP1A and 2B families. The inhibitory effect of DHM on CYP1A2 was also confirmed with the recombinant human enzyme instead of microsomal samples.

Enzyme inhibition assays

MYR and DHM mediated inhibition was assayed using microsomal fractions containing elevated levels of CYP1A1/2 and CYP2B1 by their induction with BaP and phenobarbital. Figures 1 and 2 show the inhibition of microsomal CYP activities by increasing concentrations of DHM or MYR relative to activity of flavonoid un-affected controls (100%). By plotting the data into semi-logarithmic graphs, half maximal inhibitory concentration (IC_{50}) values for each flavonoid were determined. For inhibition of CYP1A1/2, CYP1A2 and CYP2B1/2 by DHM, the IC_{50} values of 4.1, 14.2 and 98.5 mmol.L^{-1} were determined, respectively. Interestingly, a low but significant increase ($p<0.001$) in CYP2B1/2 activity was found at 20–30 mmol.L^{-1} DHM concentrations. The stimulation of activity of NADPH:CYP oxidoreductase by DHM was not detected in this microsomal sample (data not shown). Values of IC_{50} for MYR were 0.1, 0.3, and 0.9 mmol.L^{-1} for CYP1A1/2, CYP1A2 and CYP 2B1, respectively. Thus, MYR is a moderate inhibitor while DHM causes only a weak inhibition of CYP activities. To confirm these results obtained with microsomal samples, MYR and DHM were examined as potential inhibitors of human recombinant CYP1A2 in microsomes isolated from baculovirus-infected insect cells expressing also NADPH:CYP oxidoreductase. The activity of this CYP1A2 was much more sensitive to MYR than DHM under conditions used (see Figure 3). The IC_{50} value for MYR was in a micromolar range while that for dihydromyricetin was not reached under conditions used.

Induction studies

At first an *ex vivo* gene reporter assay based on the HepG2 cell line was used to analyze the influence of DHM and MYR on AhR receptor (see Figure 4). This

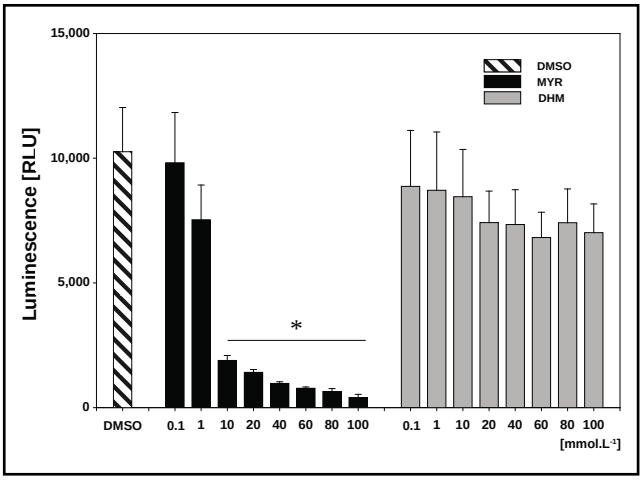

Fig. 3. The effect of DHM on human recombinant CYP1A2. The impact of MYR (in black) and DHM (in gray) on CYP1A2 activity was determined using the commercial set P450-Glo™. The luminescence of DMSO treated CYP1A2 reaction (hatched box) represents the total CYP1A2 activity. The data are showed as means and standard deviations of three independent experiments measured in triplicate and ploted in relative lights units (RLUs). Values significantly different from DMSO control (*p<0.001, Student's t-test).

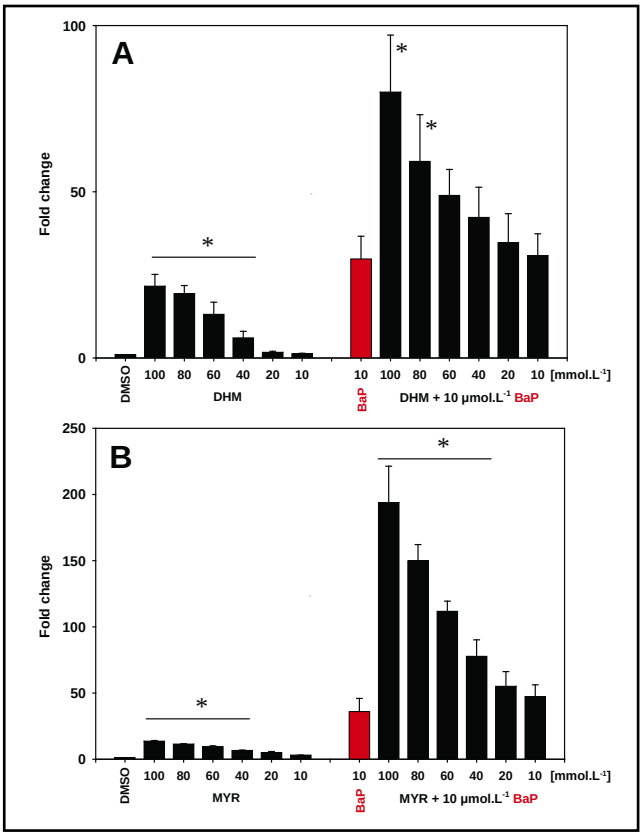

Fig. 4. Gene reporter assay with pXRE-luc in HepG2 cells. By this method the effect of DHM (A) and MYR (B) on the activation of AhR receptor was examined. The simultaneous application of flavonoid and carcinogen BaP (10 µmol.L^{-1}) was studied, too. Samples were normalized to luciferase activity of transfection control. The data represent the fold of the firefly luciferase activity activation. The plotted values are means and standard deviations of triplicates of two independent experiments. Values significantly different from DMSO control (*p<0.001, Student's t-test).

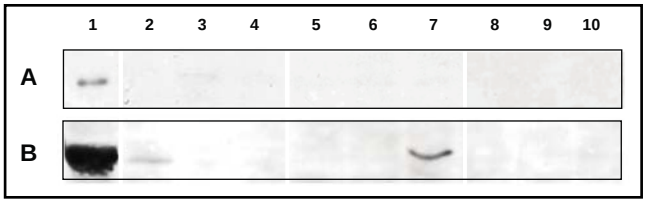

Fig. 5. Western blots of CYP1A1 and 2B1 in small intestine microsomes of rats pre-treated with flavonoids. Microsomal samples were isolated from small intestine of experimental animals pre-treated with sunflower oil (lines 2, 5, 8), MYR (lines 3, 6, 9) or DHM (lines 4, 7, 10). The small intestines were divided into 3 parts: proximal (lines 2–4), middle (lines 5–7) and distal (lines 8–10). In panel A recombinant CYP1A1 was used as a standard (1) and the blot was developed with chicken anti-CYP1A1/2 antibody. In panel B phenobarbital-induced liver microsomes were used as a CYP2B1 standard (1) and the blot was developed with chicken anti-CYP2B1 antibody.

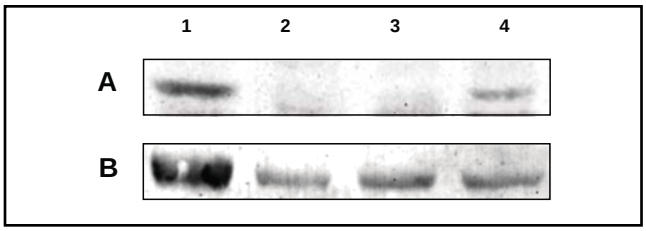

Fig. 6. Western blots of CYP1A1/2 and 2B1 in liver microsomes of rats pre-treated with flavonoids. Microsomal samples were isolated from liver of experimental animals pre-treated with sunflower oil (line 2), DHM (line 3) or MYR (line 4). In panel A recombinant CYP1A1 was used as a standard (1) and the blot was developed with chicken anti-CYP1A1/2 antibody. In panel B phenobarbital-induced liver microsomes were used as a CYP2B1 standard (1) and the blot was developed with chicken anti-CYP2B1 antibody.

study showed that neither DHM nor MYR presence in the AhR receptor reporter assay significantly activated XRE. Then flavonoids were applied simultaneously with BaP, the compound known as AhR ligand. Under this setting, DHM showed only an additive effect on BaP. Addition of MYR produced only a moderate synergistic effect on the BaP activation of AhR receptor. Results in Figure 4 show the dependence on the flavonoid concentration.

Next experiments were carried out in *in vivo* with rats treated with DHM or MYR. The induction of CYP1A1/2 and CYP2B1 at their protein levels was investigated in rat microsomal fractions using the Western blot method (see Figures 5 and 6). Dihydromyricetin increased the expression of CYP2B1 in the middle part of the small intestine, only. Myricetin did not induce CYP2B1 in any organ examined, but noticeable induction of CYP1A1 was detected in the liver. In the case of DHM animal treatment, the flavonoid effect on expression levels of CYP1A1 and CYP1A2 mRNAs was determined. Results in Table 1 show an increased mRNA expression of CYPs of both families in the proximal part of small intestine. In any other organ examined no influence of DHM on CYP1A1 and CYP1A2 mRNA expression was detected.

Tab. 1. Expression of CYP1A1/2 mRNA in the liver and the small intestine (si) after treatment of rats with DHM.

SAMPLE	CYP1A1	CYP1A2
control	1.00	1.00
liver	1.10	0.88
si - p	5.69 **	16.12 ***
si - m	0.13 *	ND
si - d	0.26	ND

Experimental animals (rats) were exposed to DHM and then mRNA was isolated from their liver and the small intestine divided in three parts – proximal (p), middle (m) and distal (d). Presented results from a real-time PCR method were measured in triplets and then evaluated in the program REST2009. Statistical significance of noticeable results is *p<0.05, **p<0.01, ***p<0.005. Values bellow the detection limit are marked ND – non-detectable. Data are expressed as fold increase over the control normalized on the internal standard (β-actin).

DISCUSSION

DHM, also called ampelopsin, is a natural flavonoid known for several health promoting properties; it provides e.g. anti-oxidant, anti-bacterial and hepatoprotective effects (Zhang *et al.* 2007). In addition, DHM counteracts the alcohol intoxication and addiction as shown in studies with experimental animals (Shen *et al.* 2012). Because of that activity, DHM is getting to be popular and available on the market as a dietary supplement. Moreover, it is likely that DHM will be used in a clinical practice. This flavonoid is considered as non-toxic compound but the knowledge on its impact on biochemical pathways in the living organism is rather limited. Thus, the present study was undertaken to investigate the influence of DHM on CYP1A1/2 and CYP2B1, activity changes of which may impact e.g. carcinogen activation or drug metabolism.

To get the complex insight into the interactions of DMH with CYPs, the *in vivo* and *in vitro* experiments were carried out. First, the direct influence of DHM on the CYP enzyme activity was analyzed with a recombinantly expressed CYP or with CYPs induced in microsomal samples. Second, studies focused on the ability of DHM to induce expression of the selected CYPs were conducted. HEPG2 cells were used to find possible interactions of DHM with AhR. Then after, the CYP mRNA and protein levels were determined in samples isolated from animals pretreated with the flavonoid. MYR was included in the study because of its similar structure to DHM.

In addition to flavonoid inhibition studies with rat microsomal CYPs, human recombinant CYP1A2, which is involved in drug metabolism and activation of ingested carcinogens in liver, was used, too. The comparison of results shown in Figures 1, 2 and 3, indicates that DHM and MYR inhibit human recombinant CYP1A2 at much lower concentrations than in the microsomal samples. This discrepancy was probable caused by the differences between both experimental setups, namely in the CYP concentrations and substrates used. Moreover, the microsomal fraction is much more complex than the CYP1A2 membrane system in the respect of protein constituents of the sample. Regardless the conditions used, in both systems, MYR was a significantly more potent inhibitor than DHM. Evaluating the results of the flavonoid inhibition of CYP specific substrate metabolism (EROD, MROD and PROD) it is clear that CYP1A1/2 enzyme activities were efficiently inhibited only by MYR. This finding is in accordance with results of related studies (Master *et al.* 2012; Schwarz *et al.* 2011).

Additional experiments were performed to assess the induction effect of DHM and MYR on the expression of proteins, which are regulated *via* AhR. The reporter system based on cell line HepG2 was employed. None of flavonoids used notably stimulated the activity of AhR. Moreover, in this study BaP was used as a known inducer of CYP1A enzymes. BaP is a carcinogenic agent, which induces the enzymes of a CYP1 family and stimulates CYP-mediated metabolism of BaP. The biotransformation of BaP results into less toxic compounds as well as dangerous reactive intermediates. When MYR or DHM were applied simultaneously with BaP a marked synergistic effect was developed by MYR, while DHM produced an additive effect, only. However, this stimulatory effect of DHM on BaP-mediated activation of AhR was seen neither *in vivo* by Western blots nor *in vitro* by CYP activities determined in microsomal samples of the liver and small intestines in our previous studies (Hodek *et al.* 2014). In a distal part of small intestines, the ^{32}P-postlabeling analysis of BaP-DNA adduct formation revealed increased levels when BaP was administered simultaneously or after DHM (Hodek *et al.* 2014). However, it should be noted that cell culture systems are hardly comparable with *in vivo* conditions. Diverse mechanisms such as chemical absorption, distribution and metabolism in other sites than the liver or small intestines might be involved in the entire biotransformation fate of the tested compounds *in vivo*.

The finding that DHM does not induce transcription of a *CYP1A* gene in the liver was confirmed also by mRNA expression analysis. On the contrary, the pre-treatment of rats with DHM increased levels of CYP1A1 and CYP1A2 mRNA in a proximal part of the small intestine. In the rest of small intestine the induction by DHM was not observed. These results may reflect the major absorption of DHM in the proximal part of small intestines. In contrast to mRNA analysis, results of Western blots revealed a slight induction of CYP2B1 in the liver (Figure 6) and in the middle part of the small intestine (Figure 5) of DHM pre-treated animals. But no induction was determined in the proximal or distal parts of the small intestine. The other flavonoid, MYR, slightly induced CYP1A2 (Figure 6) in the

liver, that is, however, in contradiction to results of the HepG2-based study. In general, none of the Western blots show an extensive induction of CYPs tested.

Taking together, flavonoids DHM and MYR were not found to be strong inducers of certain CYPs of 1A and 2B families. From the point of view of the CYP inhibition, DHM is only a weak inhibitor of CYPs tested in comparison to a higher inhibitory potency of MYR. Otherwise, MYR and DHM exhibited comparable low induction effects, especially in XRE activation assay. However, when MYR or DHM were used together with BaP, higher levels of XRE stimulation were detected in the presence of MYR. Even though both flavonoids do not significantly interfere with examined CYP activities, the MYR synergy with BaP should be reconsidered in future studies.

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