The Influence of *Trans*-resveratrol and Quercetin on the Activity of CYP1A2 in Rat

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Abstract: Polyphenolic compounds are widely distributed in plants and are a common part of human diet. Polyphenols are known to be potent bioactive molecules, predominantly with protective effects. Many of xenobiotics, including polyphenols, influence the activities of various enzymatic systems. Such interactions can modulate the activities of co-administered drugs. The identification of polyphenols' potential for the interactions based on metabolic changes is thus necessary. Cytochrome P450, which takes part in the metabolism of more than 90% of used drugs, is an important enzymatic system which can be influenced. We therefore determined the influence of quercetin and *trans*-resveratrol on the activity of cytochrome P450 1A2 in rats. A perfused rat liver model and phenacetin as a marker of 1A2 activity were used. Moreover, we studied the dependence of *trans*-resveratrol's activity on sex in both sexes. *Trans*-resveratrol did not influence the 1A2 activity, but it enhanced sexual differences in the metabolic activity. Our results also confirmed different metabolic activities between sexes. Female rats metabolised faster through 1A2. Based on our results, we suggest that quercetin is an inhibitor of cytochrome P450 1A2 isoenzyme.

Keywords: *trans*-resveratrol; quercetin; CYP 1A2; rat

Human health has been influenced by many factors. One of the most important ones, which cannot be excluded from our lives, is a diet. The amount and composition of food can prevent human body from many diseases. On the other hand, improper diet can be a risk factor or a promotor of many serious diseases including obesity (Terra *et al.* 2008), diabetes mellitus (Sabu *et al.* 2002), and illnesses of cardiovascular system (Sesso *et al.* 2003). From this point of view, the modulation of diet composition is desirable for the prevention of these illnesses.

Positive effects of diet on human health are caused by many biologically active substances. However, the characterisation of such molecules is problematic because of the variability in their chemical structures and differences in biological activity. Generally, these compounds are mainly of non-nutritive character and often act as antioxidants. Many such molecules belong to the group of polyphenolic compounds. Polyphenols are widely distributed in plants (Bravo 1998) and their protective effects were proved in many *in vitro* and *in vivo* studies (Rechner *et al.* 2002). Their good availability from natural sources as well as high biological activity determine them to be a perfect highly valuable diet component. High amounts of fruits and vegetables in diet guarantee a high polyphenolic intake. Recent lifestyle and people's reluctance to change their diet habits lead to the formulation of many dietary supplements containing polyphenols, and to the production of polyphenol-enriched foods. Such preparations contain either polyphenolic extracts from various

Supported by the Czech Science Foundation (Grant No. GA 525/06/1757) and by the Ministry of Education, Youth and Sports of the Czech Republic (Project No. 0021622404).

plants or single polyphenolic compounds. *Trans*-resveratrol (*t*-res) and quercetin (Q) are often used in dietary supplements.

Biological activities of polyphenolic compounds are presented at different levels of human organism including changes in metabolic activity of specific enzymatic systems (He *et al.* 2007). This action can be crucial for the metabolism of other substrates of the goal enzyme. These interactions can have either a positive or negative influence. The inhibition of the enzyme action can decrease the conversion of xenobiotics to their toxic forms (e.g. procarcinogens to carcinogens) or, vice versa, the induction can stimulate the same reaction. A strong probability also exists of combining polyphenols (dietary supplements, high diet intake) and medication. The interactions between polyphenols and drugs at the enzymatic level can play a crucial role in the drug effectiveness and toxicity.

One of the major metabolic systems in organism is cytochrome P450 (CYP450). It consists of many substrate and reaction specific isoenzymes, which are localised at different sites of the organism, but predominantly in liver (Seliskar & Rozman 2007). The majority of the intaken xenobiotics, as well as some endogenous molecules, are substrates for this enzyme and many of them are able to change its activity. Clinically the most important isoenzymes of CYP450 are: 1A2, 2C9, 2D6 and 3A4. More than 95% of drugs are metabolised by these four isoenzymes (Anzenbacher & Anzenbacherova 2001). Isoenzyme 1A2, which metabolic activity was determined in this study, plays a significant role in the conversion of promutagens and procarcinogens into their active forms (Hammons *et al.* 2001). Flavonoids are usually described as inhibitors of CYP450 (Piver *et al.* 2003) and inhibition of CYP1A2 is believed to be a part of their cancer preventive action. On the other hand, some authors showed data indicating the inductive activity of polyphenols on CYP450 (Rahden-Staron *et al.* 2001).

The aim of our recent work was to determine the influence of polyphenols *t*-res and Q on the activity of hepatic CYP450 1A2 (CYP1A2) isoenzyme in rats. *Trans*-resveratrol has a phytoestrogenic activity (Bowers *et al.* 2000). That is why we tested the activity of *t*-res in male and female rats together. The differences in the metabolic activity of CYP1A2 between sexes and the influence of sex on *t*-res activity have not been reported either until now.

MATERIAL AND METHODS

Animals. The experiments were carried out on male and female Wistar albino rats (Biotest, Czech Republic) weighing 200 ± 40 g. The rats were randomly divided into groups of 10 animals and were housed in a room with controlled standard conditions. The rats were acclimatised for 5 days before the start of the experiment. *Trans*-resveratrol and Q (both Sigma-Aldrich, USA) were dissolved in 30% DMSO/saline solution. The solutions were administered to the animals by intraperitoneal injections. One group of male and female rats was treated with *t*-res in the dose of 5 mg/kg/day. Quercetin in the same dose was administered to one group of male rats. To the control groups, the male and the female ones, 30% DMSO/saline solution in an adequate volume (1.0 ml/kg/day) was administered. Animals were used for liver perfusion after 10-day premedication. All experimental procedures were approved by the Czech Central Commission for Animal Welfare.

Model of isolated perfused rat liver. The rats were anesthetised with intraperitoneal injection of a mixture of ketamin – 2 ml/kg and xylazine – 0.8 ml/kg live weight (both Spofa, Czech Republic). The liver was isolated from the donors using a standard surgical technique. A plastic cannula was inserted into the portal vein after opening the abdominal cavity by wide laparotomy. The liver was shortly washed out by tempered (38°C) saline equilibrated with Carbogen – 95% O_2 and 5% CO_2 (Linde Technoplyn, Czech Republic). The saline solution was changed for tempered and Carbogen equilibrated William's medium (Sigma-Aldrich, USA) in a short time. A recirculating apparatus was constructed according to the principles described by Miller (1951). The marker substance phenacetin (Sigma-Aldrich, USA), in the final concentration of 10 mg/l was added as a bolus into the perfusion medium after 20 min of preperfusion. The samples of perfusate (1.0 ml) were collected in the 30th, 60th and 120th min of perfusion and were stored at –75°C until the analysis.

Extraction and determination of phenacetin and paracetamol. Quantitative analysis of phenacetin (PHE) and its CYP1A2 specific metabolite paracetamol (PAR) levels in the perfusate samples was performed after liquid-liquid extraction. Briefly, 500 µl of sample was mixed with 100 µl of internal standard – chlorpropamide (40 mg/l) (Sigma-Aldrich, USA). After the addition

of diethyl ether, the samples were shaken for 10 min and then centrifuged. The organic phase was separated, evaporated, and reconstituted in 250 µl of mobile phase for HPLC analysis. The levels of PHE and PAR were analysed using Shimadzu LC10 series (Japan) HPLC system. The mixture of 10mM KH_2PO_4 and acetonitrile in the 60:40 (v:v) ratio was used as the mobile phase. The separation was performed on Luna C18 (150 × 4.6 mm, 5 µm) column and the detection of the analysed molecules was performed on UV-VIS detector.

Statistical analysis. For statistical calculations, *F*-test and Student's *t*-test (Microsoft Excel 2000) were used, $P \leq 0.05$ was considered to be a statistically significant difference.

RESULTS AND DISCUSSION

The influence of sex on the activity of CYP1A2

The concentration curve courses of the markers and metabolites in the control groups did not differ from the expected ones. The levels of PHE decreased, while PAR levels increased with the ongoing perfusion time. The difference between the male and female groups was observed in the speed rate of PHE-PAR metabolic transformation. The concentrations of PHE were significantly increasing ($P \leq 0.01$) in females only in the 30th min (Figure 1), while PAR levels were increased ($P \leq 0.05$) during the whole perfusion as compared to the male control rats (Figure 2). The higher metabolic activity of CYP1A2 in the female rats confirms the results obtained with humans (Scandlyn *et al.* 2008).

The clinical relevance of different metabolic activities between sexes is usually insignificant due to the safety of the majority of the administered drugs. Higher metabolic activity of CYP1A2 in females should be taken into account when drugs with narrow therapeutic index are used.

The influence of trans-resveratrol on the activity of CYP1A2

The speed of conversion between *t*-res treated male and female rats was different, as well as in the control animals. The concentrations of PHE did not differ between sexes (Figure 1), but PAR was elevated ($P \leq 0.001$) in females for the whole perfusion time again (Figure 2). The data obtained with *t*-res pre-treated animals resembled the data obtained with the controls and no statistical difference was found. In our opinion, *t*-res at the dose of 5 mg/kg l.w. for 10 days did not influenced the activity of CYP1A2 in rats. On the other hand, the literature sources describe its diverse influence on various CYP 450 isoenzymes. Chan and Delucchi (2000) and other authors (Piver *et al.* 2003; Regev-Shoshani *et al.* 2004) described the inhibition of CYP3A4 and 2E1, while Kluth *et al.* (2007) defined *t*-res as an inducer of hepatal CYP450. None of the experiments cited was carried out using the methods with whole animals or organs, so their conclusions may be incongruous with reality.

The comparison between the sexes of *t*-res administered animals was again similar to that in the

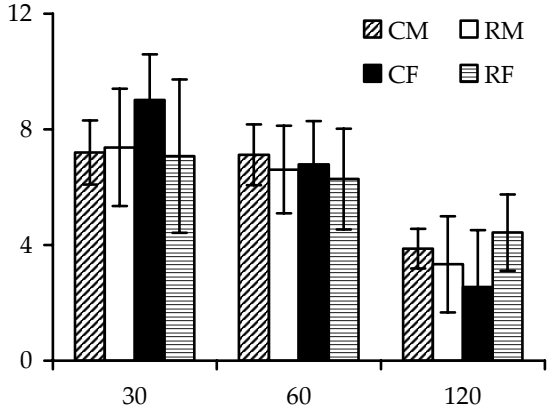

Figure 1. Levels of phenacetin in control male rats (CM), control female rats (CF), *trans*-resveratrol male rats (RM) and *trans*-resveratrol female rats (RF)

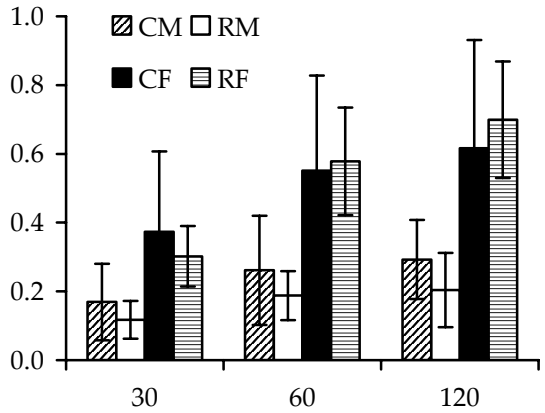

Figure 2. Levels of paracetamol in control male rats (CM), control female rats (CF), *trans*-resveratrol male rats (RM) and *trans*-resveratrol female rats (RF)

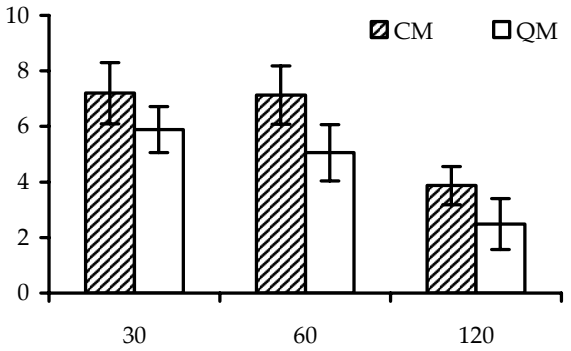

Figure 3. Levels of phenacetin in quercetin male rats (QM) and male control rats (CM)

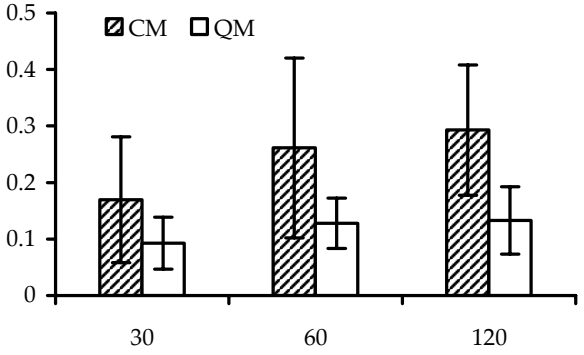

Figure 4. Levels of paracetamol in quercetin male rats (QM) and male control rats (CM)

controls. The females metabolised via CYP1A2 faster, which was confirmed by the elevation of PAR levels in them. This difference was statistically more significant ($P \leq 0.001$) than that in the controls ($P \leq 0.05$). In our opinion, *t*-res at the dose of 5 mg/kg l. w. enhances intersexual differences in rats. The possible explanation consists in the estrogenic effect of *t*-res which can bind to estrogenic receptors. Its agonistic effect on β-estrogen receptor was described (Bowers *et al.* 2000). Estrogens influence the activity of some CYP450 isoenzymes by various mechanisms (van Lipzig *et al.* 2005).

The influence of quercetin on the activity of CYP1A2

The administration of Q led to a decrease of both PHE and PAR levels. The concentrations of PHE as well as those of PAR in Q treated males were lower in the 60th ($P \leq 0.001$) and 120th min ($P \leq 0.01$) of perfusion as compared to the male control (Figures 3 and 4). The decrease of metabolite levels is a signal for CYP1A2 activity inhibition. Oppositely, the marker levels should be increased in the case of a slower biotransformation. Our explanation is that Q can change the activity of other pathways for the metabolic conversion of PHE, like CYP 2A enzymes (Devore *et al.* 2008). The induction of this enzymatic subfamily can cause a decrease in the levels of PHE in the perfusate, and consequently a lack of the substrate for CYP1A2, followed by the reduction of PAR production. We conclude that Q probably acts as an inhibitor of isoenzyme 1A2 and simultaneously induces other CYP isoenzymes, predominantly of 2A family. Ciolino *et al.* (1999) referred on the bindings of Q to arylhydrocarbon receptor and the induction of CYP1A2. However, a majority of works (Tsyrlov *et al.* 1994; Obach 2000) resulted in the inhibition of CYP1A2 activity by Q. The inhibition of CYP1A2 by polyphenols is frequently mentioned in relation to their antimutagenic and tumour protective effects.

CONCLUSION

It can be concluded from our results that the metabolic activity of cytochrome P450 in rats is sex dependant. Female rats metabolise phenacetin faster than male rats do. This sexual difference is enhanced by the administration of *trans*-resveratrol. Resveratrol alone does not influence the activity of CYP1A2. Quercetin is probably an inhibitor of CYP1A2 and the induction of 2A subfamily is possible.

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