ORIGINAL ARTICLE

Intersexual differences in inhibitory influence of trans-resveratrol on activity of cytochrome P450 2D2 in rats

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Abstract

OBJECTIVES: Differences in the metabolism between males and females have been seen over time. Hormonal regulation of cytochrome P450 activity is understood to be involved. *Trans*-resveratrol (RES) is an estrogenically active plant polyphenol with many protective biological activities including neuroprotection. The present report studied the influence of sex and RES on variances in rat's cytochrome P450 2D2 hepatic metabolic activity.

METHODS AND DESIGN: Isolated perfused rat liver was used for determination of cytochrome P450 2D2 activity. Wistar albino rats of both sexes were treated with RES at the dose of 5 mg/kg/day for 10 days prior to liver isolation. Levels of marker substance dextromethorphan (DEM) and its 2D2 specific metabolite dextrorphan (DEX) were measured during perfusion. The metabolic ratios (DEM/DEX) and the levels of DEM and DEX in perfusate were compared.

RESULTS: In the controls, the activity of CYP2D2 was found to be higher in male rats compared to females. RES produced inhibition of CYP2D2, expressed by significant changes of both DEM and DEX levels in males and significant increase of only DEM levels in females. There were no gender changes in DEX levels in RES treated animals whilst DEM levels were significantly increased during the whole perfusion in females.

CONCLUSION: The results confirmed gender differences in the metabolic activity of CYP450 2D2 with a higher rate in male rats. *RES* acted as an inhibitor, however again with greater impact in males than in females. This metabolic divergence could be a cause for different sensitivity or even toxicity of drugs metabolized by the CYP450 2D2.

INTRODUCTION

Intersexual metabolic differences in humans are known in general, but specific divergences are usually not described in details. Such variance can influence biotransformation of xenobiotics including drugs. Appropriate data describing particular differences can improve drug dosing optimization or prevent adverse effects. It can also clarify higher sensitivity to diseases caused by different xenobiotic polutants metabolized in human body. Enzymatic transformation of xenobiotics is usually involved in detoxification Phase I and II processes. One of the major systems of Phase I is cytochrome P450. Its activity is not rigid and can be regulated by many endogenous systems and

Abbreviations & units
CYP450 – cytochrome P450
DEM – dextrometorphan
DEX – dextrorphan
DMSO – dimethylsulfoxide
MR – metabolic ratio
RES – *trans*-resveratrol

influenced by various exogenous factors. Beyond age, genetic polymorphism and xenobiotic influence, the hormonal regulation also takes a part, including sexual hormons what may be a reason for gender differences in CYP450 metabolism. Estrogens are proved to influence the amounts of some CYP450 isoenzymes in liver (Williams et al, 2004). Phytoestrogens are natural substances with estrogenic activity found in plants. Their ability to activate the estrogenic receptors can also be manifested in changes of CYP activity. *Trans*-resveratrol (RES) is a plant polyphenol with many protective effects on human organism including neuroprotection (Athar *et al.* 2007). RES is structurally similar to estrogens with phytoestrogenic activity. It binds to estrogen receptors probably in partial agonistic manner (Bowers *et al.* 2000). Influence of RES on some CYP450 isoenzymes has been also described (Piver *et al.* 2001). The use of RES in different food supplements declared as a neuroprotective agents can probably lead to changes in CYP450 activity in clinical practice. Among many of CYP450 isoenzymes CYP2D6 metabolizes a variety of drugs with psychotropic effects (antidepressant, antipsychotic). Some of them (fluoxetine or paroxetine) are also inhibitors of this isoenzyme and can even cause a switch from an extensive to poor metabolizer phenotype (Zourkova *et al.* 2008). There is a strong probability of changes in the metabolism of drugs which are CYP2D6 substrates when combined with RES treatment. In this study the activity of rat CYP2D2 (an orthologue of human CYP2D6) enzyme (Zahradnikova *et al.* 2007) was studied. The aims were to investigate possible intersexual differences in CYP2D2 activity and the influence of estrogenically active polyphenol RES in rats.

MATERIAL AND METHODS

Experiment was carried out on male and female Wistar albino rats weighing 200 ± 20 g (Biotest, Czech Republic). Animals were housed in standard plastic cages (540 x 320 x 200 mm) with wood shavings and with free access to water and commercial pelleted diet. Controlled conditions were kept in the animal room: temperature 21-22 °C; humidity 50-60%; light regime – 12h light/12h dark (lights on from 6:00 to 18:00). Male and female rats were randomly subdivided into 2 groups per 8 animals and underwent a 7 day acclimatization before experiment. RES (Sigma-Aldrich, Czech Republic) was dissolved in 30% DMSO/saline solution and administered intraperitoneally at the dose of 5 mg/kg/day for 10 days prior to liver isolation. All experimental procedures were approved by the Czech Central Commission for Animal Welfare.

The model of isolated perfused rat liver was used for CYP450 2D2 activity assessment as described elsewhere (Zendulka *et al.* 2008). The animal was anesthetized (ketamine+xylazine), vena portae was cannulated and the liver was isolated from the abdominal cavity. Liver was perfused in modified recirculating apparatus described by Miller (Miller *et al.* 1951) with tempered and oxygenated William's medium E. Levels of 2D2 isoenzyme specific marker dextromethorphan (DEM) and its 2D2 specific metabolite dextrorphan (DEX) (***Fig.1***) were measured in withdrawn samples in the 30th, 60th and 120th minute of perfusion. Analyses of samples were performed after incubation with β-glucuronidase and liquid/liquid extraction using HPLC methods described by Zimova (Zimova *et al.* 2001). Metabolic ratios (MR) were calculated using the formula MR = conc. DEM / conc. DEX.

F-test and Student's t-test (Microsoft Excel 2000) were used for statistical calculations. *p* values lower than 0.05 were considered to be a statistically significant difference.

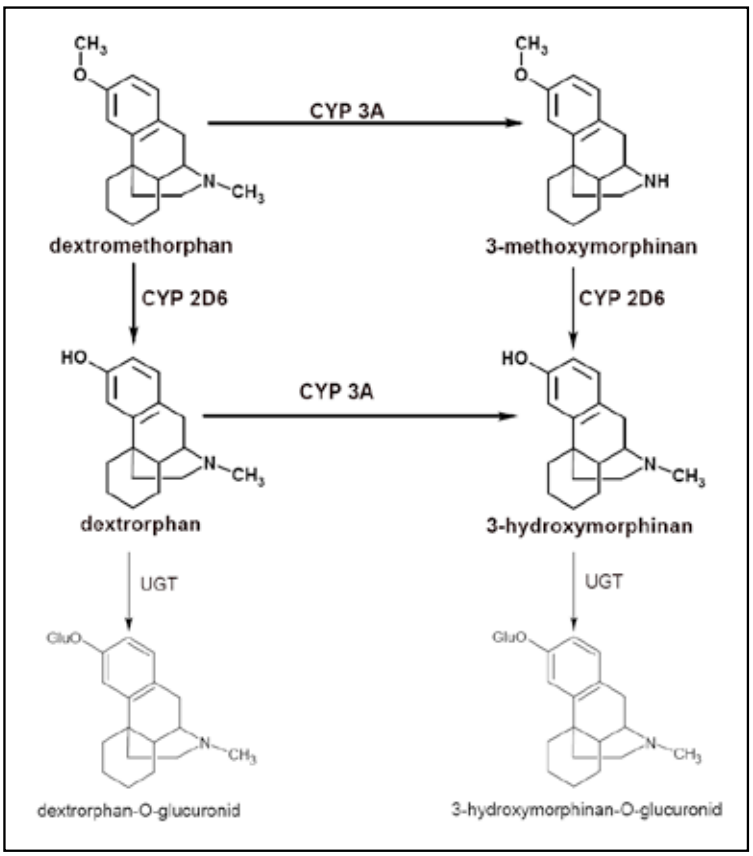

Figure 1. Dextromethorphan's metabolic pathways

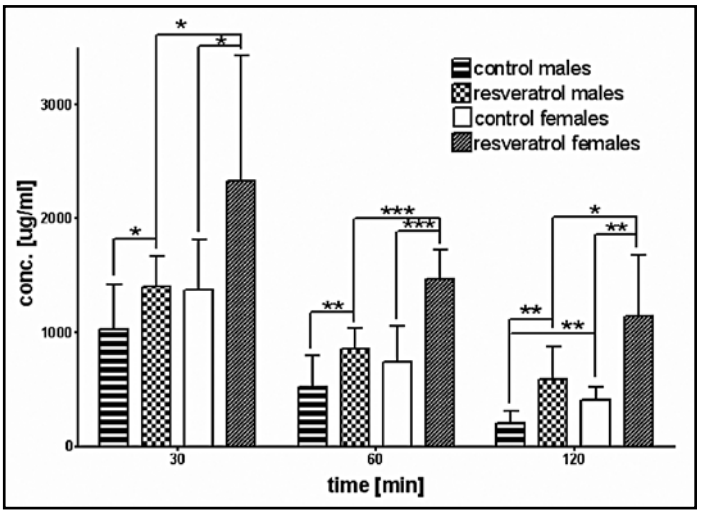

Figure 2. Levels of DEM in perfusion medium in male and female controls and trans-resveratrol pretreated animals (5 mg/kg/day, 10 days). Data represent mean ± S.E.M. * p≤0.05; ** p≤0.01; *** p≤0.001.

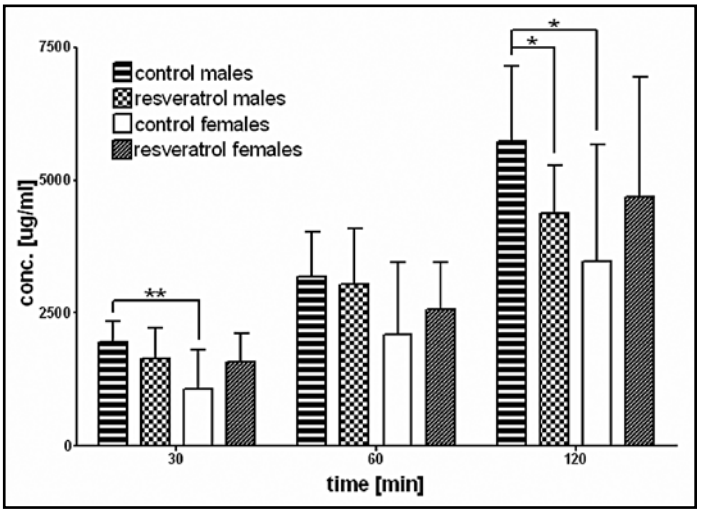

Figure 3. Levels of DEX in perfusion medium in male and female controls and trans-resveratrol pretreated animals (5 mg/kg/day, 10 days). Data represent mean ± S.E.M. * p≤0.05; ** p≤0.01.

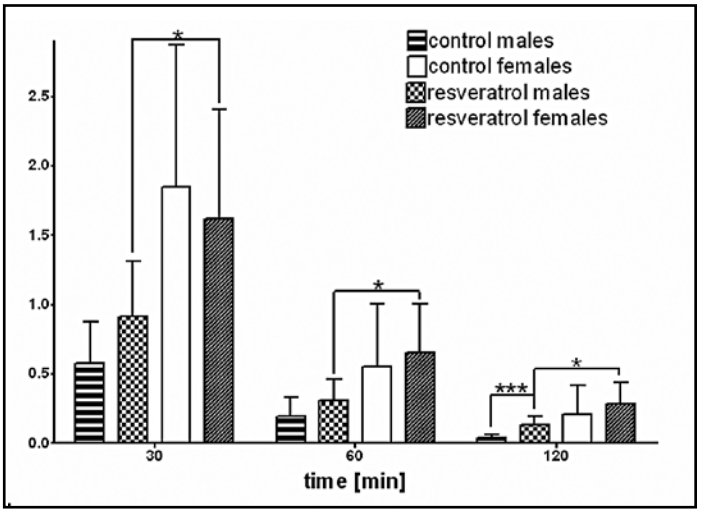

Figure 4. DEM/DEX metabolic ratios in male and female controls and trans-resveratrol pretreated animals (5 mg/kg/day, 10 days). Data represent mean ± S.E.M. * p≤0.05; ** p≤0.01.

RESULTS

The intersexual differences in CYP2D2 activity. Measured values of DEM in samples were different between male and female animals (***Fig. 2***), but statistical significance was found only in the 120th minute of perfusion ($p \leq 0.01$). The concentration of DEM was increased by 53% in females. On the other hand amounts of DEX were significantly increased in males (***Fig. 3***). Levels were changed in the 30th ($p \leq 0.01$) and 120th minute ($p \leq 0.05$). These results corresponded with DEM levels and declare a higher metabolic activity of CYP2D2 in male rats than in females and a faster conversion of DEM to DEX. Furthermore MR values confirmed this finding (***Fig. 4***). MR was significantly higher ($p \leq 0.01$) in females in the 30th minute and in other two intervals (60th and 120th min) the p values were close to significance ($p=0.06$ and 0.08 respectively).

The influence of RES on CYP2D2 activity The administration of RES caused an inhibition of CYP2D2 activity. This was demonstrated by an increase in levels of DEM and a decrease in amounts of DEX in both sexes. Compared to controls in RES treated males the concentrations of DEM were significantly raised (Fig. 2) by 26% in the 30th min up to 65% in the end of perfusion and associated with lower levels of DEX (Fig. 3), which were significantly changed only in the 120th min. These results correlate with lower values of MR in the control males (Fig. 4), specifically in the 120th min ($p \leq 0.001$). The data obtained from females administered with RES resemble DEM levels changes in males with significant due time increase from 41% to 62%.

*The intersexual differences in influence of RES on CYP2D2.*Differences between RES administered males and females correlate with controls only in the 120 min.

As shown in the Fig. 2, DEM levels were significantly elevated in females and there were registered no sex differences in changes of DEX levels (Fig. 3). Metabolic ratios were significantly higher in female rats (Fig. 4) during the whole perfusion ($p \leq 0.05$).

DISCUSSION

The working hypothesis that there could exist sex differences in the activity of CYP2D2 isoenzyme postulated in the view of our earlier results describing sex dependent activity of CYP1A2 in rats (Zendulka *et al.* 2008) was confirmed in the present study. Conversely to 1A2 isoenzyme, male rats metabolized substrates of 2D2 faster than females. Approximation of our results to humans is difficult, while there are some interspecies (Langsch *et al.* 2009) and even interstrain (Schulz-Untermoehl

et al. 1999) differences in CYP450 activities and data describing intersexual differences on CYP2D6 are inconsistent (Scandlyn *et al.* 2008; Zahradnikova *et al.* 2007). Some studies on humans resulted in no CYP2D6 sex specific difference (Aichhorn *et al.* 2005), however others found either higher metabolic rate in males (Gexfabry *et al.* 1990, Pritchard *et al.* 1992) or faster metabolization via CYP2D6 by females (Tamminga *et al.* 1999). We suggest that results of human studies focused on gender differences in CYP2D6 activity are probably highly dependent on the marker substance used and methodology of CYP450 activity assessment.

The second outcome of the present study is that the phytoestrogen RES produced an inhibition of CYP2D2 activity with at least some sex differences suggesting greater susceptibility of males. However, this effect was weaker comparing to some other CYP2D2 inhibitors we have studied previously, e.g. fluoxetine (Zendulka *et al.* 2009) as the significant changes were measured only at the end of perfusion.

Although the influence of polyphenolic compounds on the CYP450 metabolic system is extensively studied, the influence of RES on human 2D6 or rat 2D2 isoenzyme was not according to the literature available described yet. RES is reported to be an inhibitor of 1A2 and 3A4 isoenzymes (Piver *et al.* 2001). Mechanism of 1A subfamily inhibition by RES is believed to be present due to RES biotransformation via this metabolic pathway (Piver *et al.* 2004), which cannot be the case of 3A4 and 2D6 isoenzymes. RES estrogenic activity might be involved in sex differential regulation of 2D2 isoenzyme. RES perhaps can enhance intersexual difference by influencing the activity of 2D2 more in males than in females, similarly as described with 1A2 isoenzyme (Zendulka *et al.* 2008).

It can be concluded that metabolic activity of 2D2 isoenzyme is higher in male rats and is inhibited by RES administration. The effect of RES is sex dependent with greater impact on male rats.

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