Effect of antidepressant drugs on cytochrome P450 2C11 (CYP2C11) in rat liver

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Abstract:
Background: Rat CYP2C11 (besides CYP2C6) can be regarded as a functional counterpart of human CYP2C9. The aim of the present study was to investigate the influence of classic and novel antidepressant drugs on the activity of CYP2C11, measured as a rate of testosterone 2α- and 16α-hydroxylation.

Methods: The reaction was studied in control liver microsomes in the presence of antidepressants, as well as in microsomes from rats treated intraperitoneally (ip) with pharmacological doses of the tested drugs (imipramine, amitriptyline, clomipramine, nefazodone – 10 mg/kg ip; desipramine, fluoxetine, sertraline - 5 mg/kg ip; mirtazapine - 3 mg/kg ip) for one day or two weeks (twice a day), in the absence of antidepressants in vitro.

Results: The investigated antidepressant drugs added to control liver microsomes produced certain inhibitory effects on CYP2C11 activity, which were moderate (sertraline, nefazodone and clomipramine: Ki = 39, 56 and 66 µM, respectively), modest (fluoxetine and amitriptyline: Ki = 98 and 108 µM, respectively) or weak (imipramine and desipramine: Ki = 191 and 212 µM, respectively). Mirtazapine had no inhibitory effect on CYP2C11 activity. One-day exposure of rats to the antidepressant drugs did not significantly change the activity of CYP2C11 in liver microsomes; however, imipramine, desipramine and fluoxetine showed a tendency to diminish the activity of CYP2C11. Of the antidepressants studied, only desipramine and fluoxetine administered chronically elevated CYP2C11 activity; those effects were positively correlated with the observed increases in the enzyme protein level.

Conclusion: Three different mechanisms of the antidepressants-CYP2C11 interaction are postulated: 1) a direct inhibition of CYP2C11 shown in vitro by nefazodone, SSRIs and TADs; 2) in vivo inhibition of CYP2C11 produced by one-day treatment with imipramine, desipramine and fluoxetine, which suggests inactivation of the enzyme by reactive metabolites; 3) in vivo induction of CYP2C11 produced by chronic treatment with desipramine and fluoxetine, which suggests their influence on enzyme regulation.

Key words: antidepressant drugs, rat, CYP2C11, direct effect, one-day treatment, chronic treatment

Introduction

Cytochrome P450 2C11 (CYP2C11) is the most abundant male-specific isoform of CYP, which comprises approximately 50% of the total hepatic CYP in the adult male rat [40]. CYP2C11 is involved in the metabolism of benzphetamine, aminopyrine, benzo(a)pyrene, antipyrine, aflatoxin B1, R-mephenytoin and S-warfarin [21, 32]. Moreover, CYP2C11 mediates the hydroxylation of some endogenous steroids such as, e.g., testosterone and androstenedione, the
epoxyxygenation of arachidonic acid and the hydroxylation of vitamin D [4, 37, 39]. The 2α- and 16α-hydroxylation of testosterone is used as a marker reaction for studying CYP2C11 activities in rats [40, 46]. The CYP2C11 isoform is inhibited by cimetidine, diclofenac, ethanol and inflammatory mediators [3, 24, 26]. The expression of CYP2C11 depends on the proper frequency, duration and amplitude of the pulse [1, 34]. At a molecular level, CYP2C11 is regulated by pulsatile growth hormone (GH) secretion of the pituitary gland, which in turn affects the expression of other CYP isoforms (CYP2C6, CYP2D, CYP3A, 2A) and inhibits a few CYP isoforms (CYP3A4, CYP1A2, CYP2C9). It was shown that antidepressants exert an inhibitory effect on the production of pro-inflammatory cytokines, the latter suppressing the expression of CYP isoforms [25, 36].

The aim of the present study was to investigate the effect of antidepressants with different chemical structures and mechanisms of action, which affect noradrenergic and/or serotonergic transmission (TADs, SSRIs, as well as the novel antidepressants nefazodone and mirtazapine) on the activity and protein level of CYP2C11 in the liver.

**Materials and Methods**

**Drugs and chemicals**

Imipramine was provided by Polfa (Jelenia Góra, Poland), amitriptyline by H. Lundbeck A/S (Copenhagen, Denmark), while clomipramine was from RBI (Natick, MA, USA) and desipramine from Ciba-Geigy (Wehr, Germany). Fluoxetine was purchased from Eli Lilly (Indianapolis, USA) and sertraline from Pfizer Corp. (Brussels, Belgium). Mirtazapine was donated by Organon (The Netherlands) and nefazodone by Bristol-Myers Squibb International, Ltd. (Uxbridge, UK). Testosterone and its metabolites, 2α- and 16α-OH-testosterone were from Steraloids (Newport, USA). NADP, glucose-6-phosphate and glucose-6-phosphate dehydrogenase were purchased from Sigma (St. Louis, MO, USA). Polyclonal antibody, anti-rat CYP2C11 goat serum and Supersomes CYP2C11 (cDNA-expressed rat isoform) were ob-
Animal procedures

All the experiments with animals were performed in accordance with the Polish governmental regulations (Animals Protection Act, DZ.U. 97.111.724, 1997). The experiments were carried out on male Wistar rats (230–260 g) kept under standard laboratory conditions. The investigated antidepressant drugs were administered intraperitoneally, in 0.5 ml of physiological saline, twice a day for one day or two weeks at the following pharmacological doses (mg/kg ip): imipramine, amitriptyline, clomipramine and nefazodone 10, desipramine, fluoxetine and sertraline 5, mirtazapine 3. The doses used were of pharmacological magnitude which produced a therapeutic plasma concentration of the drugs [7, 13, 16]. The rats were sacrificed at 12 h (one-day treatment) or 24 h (two-week treatment) after the drug withdrawal, and liver microsomes were prepared by differential centrifugation in 20 mM Tris/KCl buffer (pH = 7.4), including washing with 0.15 M KCl according to a conventional method. The above procedure deprives microsomes of the presence of parent drugs administered in vivo, which was confirmed in our experiment by using the HPLC method [13].

In vitro studies into CYP2C11 activity – measurement of the rate of 2α- and 16α-hydroxylation of testosterone in liver microsomes

The activity of the CYP2C11 was studied by measurement of the rate of CYP2C11-specific reactions, i.e., 2α- and 16α-hydroxylation of testosterone in liver microsomes. After optimizing of in vitro conditions of the reactions, the drug effects were investigated at linear dependence of the product formation on time and protein and substrate concentrations.

To distinguish between a direct effect of antidepressants on the activity of CYP2C11 and the changes produced by their one-day or two-week administration, three experimental models were used:

Model I

The experiment was conducted on pooled liver microsomes from three control rats. The rate of 2α- and 16α-hydroxylation of testosterone (testosterone concentration between 50–300 µM) was assessed in the absence and presence of one of the antidepressants added in vitro (antidepressant concentration between 50–200 µM). Each sample was prepared in duplicate.

Model II

The experiment was carried out on liver microsomes from rats treated with an antidepressant for one day. Testosterone was added to the incubation mixture in vitro at a concentration of 100 µM. The 2α- and 16α-hydroxylation of testosterone was studied in the absence of antidepressants.

Model III

The experiment investigated liver microsomes from rats subjected to two-week antidepressant treatment. Testosterone was added to the incubation mixture in vitro at a concentration of 100 µM. The reaction was studied in the absence of antidepressants.

Incubations (Models I, II and III) were carried out in a system containing liver microsomes (1 mg of protein in 1 ml), Tris/KCl buffer (50 mM, pH = 7.4), MgCl₂ (3.0 mM), EDTA (1 mM), NADP (1.0 mM), glucose 6-phosphate (5 mM) and glucose-6-phosphate dehydrogenase (1.7 U in 1 ml). The final incubation volume was 1 ml. After a 15-min incubation, the reaction was stopped by adding 200 µl of methanol and then by cooling down in ice.

Determination of the concentration of testosterone and its metabolites (2α- and 16α-hydroxytestosterone) in liver microsomes

Testosterone and its metabolites, 2α- and 16α-hydroxytestosterone, were extracted from the microsomal suspension with dichloromethane (1 ml of microsomal suspension + 6 ml of the organic phase). Concentrations of testosterone, 2α- and 16α-hydroxytestosterone formed in liver microsomes were assessed by the high performance liquid chromatography (HPLC) method based on Sonderfan et al. [44]. The residue obtained after evaporation of the extracts was dissolved in 100 µl of 50% methanol. An aliquot (20 µl) was injected into the HPLC system (LaChrom, Merck-Hitachi), equipped with UV detector, L-7100 pump and D-7000 System Manager. The analytical column (Supelcosil LC-18, 5 µm, 4.6 × 150 mm) was
purchased from Supelco (Bellefonte PA, USA). A linear gradient of solvents A (methanol : water : acetonitrile, 39 : 60 : 1, v/v/v) and B (methanol : water : acetonitrile, 80 : 18 : 2 v/v/v) was applied during analysis from 100% A, 0% B in minute 0 to 30% A and 70% B in minute 22. The flow rate was 1.5 ml/min. The column temperature was 40°C. The absorbance was measured at a wavelength of 254 nm. The compounds were eluted in the following order: 16α-hydroxytestosterone 8.7 min, 2α-hydroxytestosterone 11.1 min and testosterone 15.6 min. The sensitivity of the method allowed for quantification of 16α-hydroxytestosterone as low as 0.005 nmol and 2α-hydroxytestosterone as low as 0.004 nmol in one sample. The lower limit of quantification (LLOQ) was 0.01 nmol/ml for 16α-hydroxytestosterone and 0.008 nmol/ml for 2α-hydroxytestosterone. The accuracy of the method amounted to 1.3% (16α-hydroxytestosterone) and 1.2% (2α-hydroxytestosterone). The inter- and intra-assay coefficients of variance were about 7% for both metabolites.

**Western blot analysis**

The level of CYP2C11 protein in liver microsomes of rats treated chronically with antidepressants (Model III) was estimated by western analysis. SDS-PAGE and immunoblot assay were performed using a methodology provided by Gentest, USA. Briefly, 5 µg of microsomal protein was separated on a 0.75 mm-thick sodium dodecyl sulfate-polyacrylamide 4% (w/v) stacking gel and a 12% (w/v) resolving gel employing a MINIPROTEAN II electrophoresis system (Bio-Rad, Hemmel Hempstead, UK; 130 V, 65 min). Protein was electrobotted onto a nitrocellulose membrane (100 V, 100 min) and blocked overnight with 5% dried nonfat milk in PBS (phosphate-buffered saline, pH = 7). After incubation with primary antibody (polyclonal goat anti-rat antibody raised against CYP2C11), the blots were incubated with secondary antibody, i.e., the appropriate species-specific horse-radish peroxidase-conjugated anti-IgG. Supersomes CYP2C11 (cDNA-expressed rat isoform) were used as a standard. Immunoreactivity was assessed using an enhanced Lumiglo chemiluminescent substrate. The intensities of the bands corresponding to the enzyme protein on the nitrocellulose membrane were measured with Luminescent Image analyzer LAS-1000 using Image Reader LAS-1000 and Image Gauge 3.11 programs (Fuji Film, Japan).

**Calculations and statistics**

The presented inhibition constants (K_i) for the inhibition of a specific metabolic pathway were obtained using a non-linear regression analysis (Program Sigma Plot 8.0, Enzyme Kinetics). Statistical significance (Model II and Model III) was assessed using an analysis of variance followed by Dunnett’s test. All values are the means ± SEM from 5–8 animals.

**Results**

The obtained results showed that the investigated antidepressants directly inhibited CYP2C11 activity in rats, shown as inhibition of the rate of CYP2C11-specific reactions, i.e., the 2α- and the 16α-hydroxylation of testosterone by the drug added to control liver microsomes in vitro (Model I). The inhibitory effects

<table>
<thead>
<tr>
<th>Antidepressants (inhibitors)</th>
<th>Inhibition of CYP2C11 activity</th>
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<td></td>
<td>2α-OH-T</td>
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<td>I. Tricyclic antidepressants (TADs)</td>
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<td>Mirtazapine</td>
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The presented inhibition constants (K_i) for the inhibition of a specific metabolic pathway were obtained using a non-linear regression analysis (Program Sigma Plot 8.0, Enzyme Kinetics)

**Tab. 1.** The influence of antidepressants given in vitro to rat liver microsomes on CYP2C11 activity measured as the rate of 2α- and 16α-hydroxylation of testosterone (Model I)
of antidepressants on CYP2C11 activity were moderate (sertraline, nefazodone and clomipramine: $K_{i} = 39, 56$ and $66 \mu M$, respectively), modest (fluoxetine and amitriptyline: $K_{i} = 98$ and $108 \mu M$, respectively) or weak (imipramine and desipramine: $K_{i} = 191$ and $212 \mu M$, respectively) (Tab. 1). Mirtazapine had no inhibitory effect on CYP2C11 activity (Tab. 1).

Our study demonstrated that the investigated antidepressant drugs exerted no statistically significant effect on CYP2C11 activity when they were given to rats for one day (i.e., for 24 h; Model II) (Fig. 1). However, imipramine, desipramine and fluoxetine showed a tendency to diminish the activity of CYP2C11 (down to 70, 66 and 68% of the control, respectively) after a one-day treatment of rats with those antidepressants (Fig. 1).

After a two-week treatment with the tested antidepressants (Model III), desipramine and fluoxetine significantly increased the activity of CYP2C11 (up to 186 and 143% of the control, respectively) (Fig. 2). The other antidepressants studied did not produce any statistically significant effect when administered in vivo for two weeks. As shown in Figs. 3A and B, the changes observed in CYP2C11 protein level after chronic treatment with antidepressant drugs corresponded well with those related to the enzyme activity. Desipramine and fluoxetine substantially elevated CYP2C11 protein level up to 196 and 158% of the control, respectively.
Discussion

We have searched for possible mechanisms of CYP2C11-antidepressants interactions by applying drugs in vitro (to investigate the direct effect of drugs, i.e., their binding to the cytochrome) or in vivo – for one day – to look for CYP2C11 inactivation after a 24-h exposure to antidepressants [11, 12, 18]. We have searched for possible mechanisms of CYP2C11-antidepressants interactions by applying drugs in vitro, i.e., their binding to the cytochrome) or in vivo – for one day – to look for CYP2C11 inactivation after a 24-h exposure to antidepressants [11, 12, 18]. The obtained results indicate that antidepressants may evoke both direct (binding to the enzyme) and indirect effects (inhibition produced by a one-day treatment and induction evoked by a two-week treatment) on CYP2C11.

The tested antidepressants (except for mirtazapine) directly decrease CYP2C11 activity in rats, shown as inhibition of the rate of a CYP2C11-specific reaction (the 2α- and the 16α-hydroxylation of testosterone) by antidepressants added to control liver microsomes in vitro (Model I). However, their inhibitory effects are moderate or weak (Ki = 39–212 µM). Hence, the direct inhibitory effect of antidepressant drugs observed in vitro should be of minor importance in vivo, since these drugs rarely reach liver concentrations that approximate their relatively high Ki values, as is indicated by their plasma concentrations [10, 16] and plasma/tissue distribution patterns [7, 14, 15, 47, 49]. Like in rats, the investigated antidepressants are not regarded as potent inhibitors of human CYP2C9 [19, 33, 43].

As has been mentioned elsewhere, tricyclic antidepressants and fluoxetine can form reactive/intermediate metabolites, which irreversibly inactivate a few CYP isoforms (CYP2B, CYP2C6, CYP3A) [11, 12, 18], as well as when used at therapeutic concentrations in vivo after a 24-h exposure to antidepressants [11, 12, 18]. The present results suggest that CYP2C11 inactivation via this mechanism by imipramine, desipramine and fluoxetine (used in therapeutic concentrations) via this mechanism is also possible, since a one-day treatment with pharmacological doses of the above-mentioned antidepressants diminishes (down to 66–70% of the control values) the enzyme activity in rats (Model II).

On the other hand, prolonged administration of fluoxetine (a selective serotonin reuptake inhibitor) and desipramine (a selective noradrenaline reuptake inhibitor) to rats in vivo increases the activity of the enzyme (Model III), these effects correlating positively with the observed enhancement of CYP2C11 protein level. TADs, which – apart from the inhibition of noradrenaline and serotonin reuptake – also display either α1-adrenergic receptor antagonistic activities (imipramine, amitriptyline, clomipramine) or 5-HT2- serotonin receptor ones (amitriptyline) [41], do not exert such an effect. Moreover, some newer antidepressants, e.g., nefazodone (a 5-HT2-receptor antagonist and a moderate inhibitor of serotonin and noradrenaline reuptake) and mirtazapine (an α2-adrenergic receptor antagonist at presynaptic sites of noradrenergic and serotoninergic neurons and a 5-HT2- and 5-HT3-serotonegic receptor antagonist), are not active in this respect [20, 41]. The above-discussed findings may imply that the observed effects of the tested antidepressants on CYP2C11 regulation in vivo depend on differences in the action of these drugs on the enzyme neuroendocrine regulation. As shown previously, the antidepressant drugs that increase noradrenergic and serotonegic transmission in the brain [41] may affect the secretion of anterior pituitary hormones by regulating the hypothalamic secretion of the respective releasing and inhibiting factors [42]; this, in turn, may stimulate the expression of some CYP isoforms (CYP2B, CYP2C6, CYP3A) [11, 17, 18, 48].

Some earlier studies suggested a negative regulatory effect of central noradrenaline on the benzo(a)pyrene-induced up regulation of CYP1A1 expression. Stimulation of α2-adrenoceptors with dexmedetomidine and blockade of α1- or β-adrenoceptors with prazosin or propranolol, respectively, resulted in a further increase of CYP1A1 inducibility. Both, reduced noradrenaline release in central nervous system induced with dexmedetomidine and central catecholamine depletion, as well as blockade of central α1-adrenoceptors induced with prazosin, all were associated with up-regulation of CYP1A1 expression. In contrast, stimulation of central β-adrenoceptors with isoprenaline resulted in a down-regulation of CYP1A1 expression. The above observations indicate that drugs, which stimulate or block adrenoceptors and catecholamine release may lead to complications in drug therapy and modulate the toxicity or carcinogenicity of drugs that are substrates for the CYP1A1 [22].

The available data on the effects of prolonged treatment with antidepressant drugs on the activity of cytochrome P450 in humans are scarce and are based mainly on the in vivo application of CYP substrates. The results of such in vivo metabolic tests do not ex-
plain what exactly happens to individual liver CYP isoforms. Moreover, in in vivo studies, different mechanisms may overlap, e.g., the binding of a parent drug to an enzyme and the formation of CYP-reactive metabolite complexes, as well as changes in enzyme regulation, produced by prolonged drug administration. The biochemical studies carried out on human liver microsomes have shown that TADs administered to depressive patients for a long time accelerate the demethylation of benzphetamine and ketotifen, which corresponds well with the increase in the CYP fraction responsible for the metabolism of these substrates [8]. These results indicate that chronic treatment with antidepressants may induce some CYP isoforms in humans, but the specificity of CYP regulation by antidepressants in humans, but the specificity of CYP regulation by antidepressants requires further studies.

In summary, our study conducted on rats shows that the investigated antidepressants produce a direct, weak-to-moderate effect on rat CYP2C11 (by binding to the enzyme protein) and weak in vivo inhibition of CYP2C11 activity after a one-day treatment (which suggests inactivation of the enzyme by reactive metabolites). On the other hand, the significant in vivo induction of CYP2C11 by desipramine and fluoxetine, produced by chronic treatment with the above mentioned antidepressants, indicates their influence on the enzyme regulation.

The present data may be useful for interpreting the results of pharmacological experiments, obtained after administration of antidepressants to rats. However, we do not know yet to what extent the obtained results may be related to humans. Although rat CYP2C11 and human CYP2C9 exhibit high identity of the amino acid sequence and are involved in the metabolism of clinically important drugs (e.g., S-warfarin) and endogenous substrates (steroids, retinoic and arachidonic acid) [2, 4, 32, 38], they differ in their regulation [1, 6, 35]. Therefore, it seems of primary importance to ascertain whether during long-term therapy human CYP isoforms are subject to analogous induction by antidepressants as are rat CYP2C11 or CYP2B and CYP3A, previously investigated by us.

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