Studies on the Interactions between Drug and Estrogen. II.¹⁾ On the Inhibitory Effect of 29 Drugs Reported to Induce Gynecomastia on the Oxidation of Estradiol at C-2 or C-17

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Received December 24, 2002; accepted February 12, 2003

A study was investigated on the inhibitory effect of 29 drugs that have been reported to induce gynecomastia on the 2-hydroxylation of estradiol (E2) by recombinant P450 CYP3A4 and on the 17-oxidation of E2 by hepatic microsomal type II 17 β -hydroxysteroid dehydrogenase (17 β -HSD) of human male. The IC₅₀ values were determined for each drug relative to the 2-hydroxylation of E2 (catalytic activity: 1.54 nmol/nmol P450/min), and the inhibition constants (*K_i*) were determined for 13 drugs of which IC₅₀ values were 100 μ M or less. Ketoconazole exhibited the lowest inhibitory concentration, and IC₅₀ and *K_i* values of 0.007 and 0.01 μ M, respectively, were obtained. The IC₅₀ and *K_i* values for each of the 12 remaining drugs were as follows: cyclosporin A (IC₅₀: 0.064, *K_i*: 0.30), nicardipine hydrochloride (0.55, 0.29), tacrolimus (0.64, 0.88), mandipine hydrochloride (3.9, 2.6), nisoldipine (10, 3.3), verapamil hydrochloride (10, 20), domperidone (13, 7.2), haloperidol (14, 55), nitrendipine (14, 2.5), chlormadinone acetate (16, 10), flutamide (30, 39) and omeprazole (49, 47). With the exception of cyclosporin A that exhibited a competitive inhibition, the inhibition mechanisms of these drugs were all non-competitive. Next, the percentage inhibition of the above 29 drugs relative to the 17-oxidation of E2 (catalytic activity: 0.47 nmol/mg protein/min) was investigated at the approximate therapeutic concentration (1 μ M) and at the non-clinical overdose concentration (100 μ M). Although none of the drugs investigated exhibited inhibitory effects at a concentration of 1 μ M, spironolactone and ketoconazole at 100 μ M demonstrated percentage inhibitions of 96% and 77%, respectively. When the *K_i* values were determined for these two drugs, the former had a *K_i* value of 2.4 μ M and the latter, 41 μ M, and both of their inhibition mechanisms were non-competitive. On the basis of the above results, a total of 14 drugs consisting of the above 13 drugs plus spironolactone were found to inhibit the 2-hydroxylation or 17-oxidation of E2 in the liver, and this is presumed to act as a trigger that causes an increase in the estradiol pool, followed by induction of gynecomastia.

Key words drug-induced gynecomastia; recombinant CYP3A4; estradiol; type II 17 β -hydroxysteroid dehydrogenase

Previously, we proposed a model unit reaction to meet the demands of the clinical fields to establish a numerical system for predicting drug-induced gynecomastia.^{5,6)} The prediction is possible by using such indexes as the IC₅₀ of drugs on the *in vitro* 2-hydroxylation of estradiol (E2).¹⁾ The principle is based on the assumption that the onset of drug-induced gynecomastia is caused by the accumulation of E2 as a result of the inhibitory effect of the drugs on the 2-hydroxylation of E2, its major catabolic pathway. We used *Escherichia coli* (*E. coli*) co-expressing human P450 CYP3A4 with human NADPH-P450 reductase⁷⁾ (hereafter, recombinant CYP3A4) as the enzyme source of this reaction, and obtained IC₅₀ values of representative CYP3A4 inhibitors such as cyclosporin A, cimetidine and ketoconazole.¹⁾ The prediction system thus constructed for use as a routine laboratory technique promises the ability to test the inhibitory effect of drugs. The accumulation of E2, however, is not caused by only the inhibition of the 2-hydroxylation. In addition to this catabolic reaction, we also have to consider the effect of drugs on the biosynthesis of E2.

In the preceding study,⁸⁾ we investigated the effect of 29 drugs reported to induce gynecomastia on such estrogen production pathways as the “aromatase pathway” and the “sulfatase pathway”⁹⁾ (Fig. 1) by using human placental microsomes. The results indicated no inhibition of the drugs on both pathways. Interestingly, however, a few drugs such as

spironolactone were found to increase the production ratio of estradiol to estrone (E2/E1), in spite of no significant change of the total amount of both estrogens (E2+E1) produced, thereby indicating the increase of the estradiol pool by the drugs.

This paper comprises of two parts. In the first part, the IC₅₀ values of 29 drugs reported to induce gynecomastia *via* the 2-hydroxylation of E2 were determined using the unit reaction for the prediction system,¹⁾ and their inhibition mechanisms were demonstrated. In the second part, the effects of these drugs on the conversion of E2 to E1 in hepatic microsomes of human male were investigated.

MATERIALS AND METHODS

Reagents E1, E2, 2-hydroxyestradiol (2-OH-E2) and 2-methoxyestradiol (2-MeO-E2) were obtained from Steraloids Inc. (Newport, RI, U.S.A.). Prior to use, the purity of these steroids was checked by HPLC. The same lot numbers as those used in a previous report⁸⁾ were employed for the 29 drugs reported to induce gynecomastia. The substrate E2 and the drugs were suitably diluted by dissolving in methanol, the final methanol concentration being 1 v/v% in the incubation mixture. Glucose-6-phosphate (G6P), glucose-6-phosphate dehydrogenase (G6P DH), NADP⁺ and NAD⁺ were obtained from Oriental Yeast (Osaka, Japan). The same recombinant

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Fig. 1. Relationships among Aromatase, Sulfatase and Catabolic Pathways Related to the Estradiol Pool in Human Liver

CYP3A4 as that used in a previous report⁷⁾ was employed in this study. Human male liver microsomes pooled from 10 individuals were purchased from Gentest Corp. (Woburn, MA, U.S.A.). Protein concentrations were suitably diluted as necessary based on the instructions in the package insert. Commercially available, highest-grade products were used for all other reagents, solvents and so forth.

HPLC Analysis of the reaction products of 2-OH-E2 and E1 was performed in accordance with the previously reported methods.¹⁾ HPLC was carried out using a Model L-7000 pump (Hitachi, Tokyo, Japan) equipped with an EC-8020 electrochemical detector (Hitachi) having an Hg/Hg₂Cl₂ reference electrode (0.8 V). A stainless steel column (250 mm×4.6 mm, i.d.) packed with TSK gel ODS-80T_M (Tosoh, Tokyo, Japan) was used for the stationary phase, and held at a temperature of 40 °C. A mixed solution (60/40 v/v) of methanol and 50 mM NH₄H₂PO₄ (pH 2.5) was used as the mobile phase, and the flow rate was 0.7 ml/min. The relative retention times of 2-OH-E2, E1, E2 and 2-MeO-E2 under these conditions were 0.530, 0.788, 0.821 and 1.000 (33.00 min), respectively, and the resolution between E1 and E2 was 1.90.

Assay for 2-Hydroxylation of E2 The assay for the 2-hydroxylation of E2 was carried out using a method that was slightly modified from that of a previous report.¹⁾ The incubation mixture was composed of 100 mM sodium-potassium phosphate buffer (pH 7.4) containing 0.1 mM ascorbic acid, 50 μM EDTA, E2 and 10 pmol recombinant CYP3A4. The concentration of E2 was set at 20 μM in the experiment for determining IC₅₀, because the production rate of 2-OH-E2 was linear from zero to 20 μM, and at 5, 10, 20 and 40 μM in the experiment for determining the inhibition constant (*K_i*) with a Lineweaver-Burk plot. The concentration of drugs was from 1×10⁻³ to 3×10³ μM (5–7 points) for IC₅₀, and from 1 to 100 μM (3 points) for *K_i*. Following the addition of a drug, the incubation mixture was incubated for 5 min at

37 °C, followed by the addition of an NADPH-generating system (0.5 mM NADP⁺, 5 mM MgCl₂, 5 mM G6P and 1 U/ml of G6P DH) to make a final volume of 1 ml, and incubation for 10 min at 37 °C. The reaction was terminated by adding 3 ml ethyl acetate to the reaction mixture, followed by addition of 2-MeO-E2 (internal standard), and the resulting mixture was shaken for 10 min and then separated by centrifuging for 10 min at 3000 rpm. The organic layer was evaporated under reduced pressure to give the residue, which was dissolved in 100 μl of 60% aqueous methanol containing 0.1 mM ascorbic acid, followed by injecting 10 μl of the solution into an HPLC.

Assay for 17-Oxidation of E2 The assay for the activity of type II 17β-hydroxysteroid dehydrogenase (type II 17β-HSD) was carried out using a method that was slightly modified from that of a previous report.⁸⁾ The incubation mixture was composed of 50 mM potassium phosphate buffer (pH 7.4) containing 50 μM EDTA, 0.1 mg protein/ml human male liver microsomes and E2. The concentration of E2 was set at 2.5 μM in the experiment for determining percentage inhibition, because of the formation rate of E1 from E2 from zero to 2.5 μM, and at 2.5 or 10 μM in the experiment for determining *K_i*. In the experiment for determining percentage inhibition, drug concentration was set at 1 or 100 μM. In the experiment for determining *K_i* using a Dixon plot, the concentration of spironolactone was from 0 to 10 μM, and that of ketoconazole from 0 to 100 μM. Next, 0.5 mM NAD⁺ was added to the incubation mixture to make a final volume of 250 μl and to start the reaction, followed by incubation for 10 min at 37 °C. The reaction was terminated by adding 3 ml ethyl acetate to the reaction mixture, the resulting solution was shaken for 10 min and separated by centrifuging for 10 min at 3000 rpm. The organic layer was evaporated under reduced pressure to give the residue, which was dissolved in 250 μl of 60% aqueous methanol. The solution was filtered through a membrane filter (0.45 μm), and 100 μl of the filtrate was in-

Table 1. Inhibitory Concentration (IC_{50}) of Drugs on the 2-Hydroxylation of Estradiol Catalyzed by Recombinant CYP3A4^{a)}

	IC_{50} (μM)		IC_{50} (μM)
Ketoconazole	0.007 ^{b)}	Allylestrenol	>100
Cyclosporin A	0.064 ^{b)}	Cimetidine	>100 ^{b)}
Nicardipine hydrochloride	0.55	Delapril hydrochloride	>100
Tacrolimus	0.64	Digoxin	>100
Manidipine hydrochloride	3.9	Famotidine	>100
Nisoldipine	10	Griseofulvin	>100
Verapamil hydrochloride	10	Ipriflavone	>100
Domperidone	13	Isoniazid	>100
Haloperidol	14	Lansoprazole	>100
Nitrendipine	14	Metoclopramide hydrochloride	>100
Chlormadinone acetate	16	Nifedipine	>100
Flutamide	30	Nizatidine	>100
Omeprazole	49	Pilsicainide hydrochloride	>100
		Ranitidine hydrochloride	>100
		Spironolactone	>100
		Sulpiride	>100

a) The catalytic activity in the absence of drug was 1.54 nmol/nmol P450/min. All experiments were performed in duplicate. b) Data from the previous report (ref. 1).

Table 2. Apparent K_i Concentrations of 2-Hydroxylation of Estradiol Catalyzed by Recombinant CYP3A4^{a)}

	K_i (μM)	Inhibition mechanism
Ketoconazole	0.01	Non-competitive
Nicardipine hydrochloride	0.29	Non-competitive
Cyclosporin A	0.30	Competitive
Tacrolimus	0.88	Non-competitive
Nitrendipine	2.54	Non-competitive
Manidipine hydrochloride	2.64	Non-competitive
Nisoldipine	3.30	Non-competitive
Domperidone	7.21	Non-competitive
Chlormadinone acetate	10.4	Non-competitive
Verapamil hydrochloride	20.2	Non-competitive
Flutamide	38.9	Non-competitive
Omeprazole	47.0	Non-competitive
Haloperidol	54.6	Non-competitive

a) All experiments were performed in duplicate.

jected into the HPLC.

RESULTS

Effect of Drugs on 2-Hydroxylation of E2 The inhibitory effects of 29 drugs on the 2-hydroxylation of E2 were studied using recombinant CYP3A4. The catalytic activity of E2 to 2-OH-E2 under the absence of drug was 1.54 nmol/nmol P450/min, and the IC_{50} values obtained at each drug concentration relative to this are shown in Table 1. Ketoconazole exhibited the lowest value at 0.007 μM , followed by the other drugs in the given order: cyclosporin A (0.064), nicardipine hydrochloride (0.55), tacrolimus (0.64), manidipine hydrochloride (3.9), nisoldipine (10), verapamil hydrochloride (10), domperidone (13), haloperidol (14), nitrendipine (14), chlormadinone acetate (16), flutamide (30) and omeprazole (49). The IC_{50} values of the other 16 drugs, including allylestrenol, all exceeded 100 μM , and their effects were either extremely weak or not observed at all.

A more detailed study was carried out on the drugs that exhibited IC_{50} values of 100 μM or less. The apparent K_i values and inhibition mechanisms of the 13 drugs as obtained from a Lineweaver–Burk plot are shown in Table 2. Keto-

Fig. 2. Comparison of Inhibitory Effect of 29 Drugs on the 17-Oxidation of E2 by Type II 17 β -Hydroxysteroid Dehydrogenase (Type II 17 β -HSD) in Human Liver Microsomes

conazole exhibited the lowest K_i value of 0.01 μM , followed by the other drugs in the given order: nicardipine hydrochloride (0.29), cyclosporin A (0.30), tacrolimus (0.88), nitrendipine (2.5), manidipine hydrochloride (2.6), nisoldipine (3.3), domperidone (7.2), chlormadinone acetate (10), verapamil hydrochloride (20), flutamide (39), omeprazole (47)

Fig. 3. Dixon Plots of the Effect of Spironolactone and Ketoconazole on the Oxidation of Estradiol to Estrone in Human Liver Microsomes

Data are mean values obtained by triplicate determinations.

and haloperidol (55). The inhibition mechanisms of these drugs were all non-competitive with the exception of cyclosporin A that exhibited a competitive inhibition.

Effects of Drugs on 17-Oxidation of E2 The effects of the drugs on type II 17 β -HSD of human male liver microsomes were investigated. The catalytic activity of E2 to E1 under the absence of drug added was 0.47 nmol/mg protein/min, and the percentage inhibition of each drug relative to this was determined at a concentration of 1 or 100 μ M. The results are shown in Fig. 2. None of the drugs exhibited significant inhibitory effects at 1 μ M. When the drug concentration was increased to 100 μ M, however, spironolactone exhibited a marked percentage inhibition of 95%, followed by ketoconazole at 77%. The other drugs exhibited percentage inhibitions of 50% or less. When the K_i values of spironolactone and ketoconazole were determined using a Dixon plot, they were found to be 2.4 μ M and 41 μ M, respectively, and both demonstrated a pattern of non-competitive inhibition (Fig. 3).

DISCUSSION

Six types of P450 enzymes, namely, CYP1A2, 2C9/10, 2C19, 2D6, 2E1 and 3A4, are predicted to be responsible for at least 90% of human drug oxidation, and nearly all of this can be attributed to the four types of P450 enzymes, CYP1A2, 2C9/10, 2D6 and 3A4, with nearly half of this oxidation being predicted to be due to the action of CYP3A4.¹⁰ Extensive research has been conducted on P450 enzymes because drug-drug interactions in which they are involved frequently cause serious adverse reactions.¹⁰

Certain types of P450 catalyze the oxidation of endogenous compounds including estrogen. For example, the hydroxylation of several sites including position 2 of E2, resulting in the deactivation of estrogenic action.^{11,12} We have previously reported that CYP1A1 has the highest metabolic clearance ($V_{\max}/K_m$) in the 2-hydroxylation of E2 using nine recombinant CYP1A1, 1A2, 2A6, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4.¹¹ However, when the resulting metabolic clearance was compared with the expression level of each CYP in the liver, CYP3A4 was found to have the highest contribution ratio of approximately 71% in the 2-hydroxylation of E2. This finding indicates that drugs in which there is CYP3A4 involvement promote an interaction with E2.

Moreover, E2 is converted to E1, which has lower estrogenic activity, by being subjected to 17-oxidation by type II 17 β -HSD. Since E1 is reconverted to E2 by type I 17 β -

HSD,¹³ a type II isoenzyme, the redox reactions by both enzymes mutually regulate the production ratio of the two estrogens (E2/E1). Thus, when a drug that inhibits type II 17 β -HSD is taken, a drug-estradiol interaction¹⁴ may ensue, and as a result, this may disrupt the physiological equilibrium between E1 and E2.

Effects of Drugs on 2-Hydroxylation of E2 When the IC_{50} values of 29 drugs on the 2-hydroxylation of E2 by recombinant CYP3A4 were determined, the imidazole-based antifungal agent, ketoconazole, exhibited the strongest inhibitory effect (0.007 μ M) (Table 1). Ketoconazole is a potent inhibitor of P450 enzymes, and although it strongly inhibits CYP3A4 in particular,¹⁵ its inhibition mechanism was found to be non-competitive (Table 2). Since ketoconazole inhibits not only the 2-hydroxylation of E2, but also the 6 β -hydroxylation of testosterone (T), with which CYP3A4 is involved,¹⁶ the level of E2 in plasma is expected to further increase due to the quantitative increase in T level as a result of taking ketoconazole, followed by accompanying acceleration of aromatization and the interruption of the catabolism (2-hydroxylation) of E2.¹⁷ There is concern regarding drug-estrogen interaction in the liver.

The drug that exhibited the second strongest inhibitory effect after ketoconazole was the immunosuppressant, cyclosporin A (IC_{50} : 0.064 μ M), and was the sole drug that exhibited competitive inhibition among all the drugs studied. Since immunosuppressants, including tacrolimus (0.64 μ M), are used over a long period of time in the same manner as antifungal agents, they result in the chronic inhibition of the 2-hydroxylation of E2, and are predicted to lead to an increase in the estradiol pool. Since cyclosporin A in particular has a whole blood trough level (approx. 0.15 μ M)^{18,19} that approximates the IC_{50} and K_i values (0.30 μ M) for the 2-hydroxylation of E2, the interaction with E2 cannot be ignored clinically.

Although numerous cases of gynecomastia caused by dihydropyridine calcium antagonists have been reported, the mechanism of its occurrence is unclear.²⁰ According to the results obtained in this study, since the K_i values of nifedipine, manidipine, nisoldipine and nitrendipine (0.29–3.3 μ M) are lower than the K_m value for the 2-hydroxylation of E2 by CYP3A4 (16 μ M),¹¹ the ingestion of these drugs is expected to cause an increase in the estradiol pool. However, these inhibition mechanisms were shown to be non-competitive. Dihydropyridine calcium antagonists including nifedipine²¹ are known to become substrate for CYP3A4. The inhibitions by nifedipine on the oxidation of cyclosporin A by human hepatic microsomes²² and on the 6 β -hydroxylation of testosterone by recombinant CYP3A4²³ were competitive. Divergence between these results and the present ones remained as the problem to be investigated. Since the K_i value of a similar calcium antagonist, verapamil, is also comparatively low at 20 μ M, the correlation between the inhibition of E2 metabolism of these depressors and gynecomastia is an area of potential interest.

According to Estabrook and co-workers, the prostate cancer therapeutic drug, flutamide, is metabolized by not only CYP1A2, but also CYP3A4.²⁴ In the results obtained in this study, the IC_{50} and K_i values of flutamide relative to the 2-hydroxylation of E2 by CYP3A4 were 30 and 39 μ M, respectively, indicating comparatively potent inhibition. These *in*

vitro results are supported by the report of Zumoff *et al.* that states that urine 2-hydroxyestrone concentrations are low in prostate cancer patients administered flutamide as compared with a control group,²⁵⁾ and a report on a clinical research conducted in Japan indicating that the incidence of gynecomastia caused by flutamide is comparatively high at 15.4%.²⁶⁾

Effects of Drugs on 17-Oxidation of E2 Although the optimum pH of type II 17 β -HSD is 8–10,²⁷⁾ since this research was conducted based upon the presumption of clinical studies, incubation was carried out at physiological conditions of pH 7.4.

The assessment results of the inhibitory effects of 29 drugs on the 17-oxidation of E2 by type II 17 β -HSD of human male liver microsomes are shown in Fig. 2. Although none of the drugs inhibited oxidation at the approximate therapeutic concentration of 1 μ M, at the non-clinical overdose concentration of 100 μ M, the percentage inhibition of spironolactone was the highest at 95%, and its inhibition mechanism was non-competitive (K_i : 2.4 μ M). These findings agree with the results of Tremblay *et al.* who reported that spironolactone strongly inhibits type II 17 β -HSD.²⁸⁾ They reported that the spiro- γ -lactone group at position 17 is important as a pharmacophore in the inhibition of type II 17 β -HSD, and that the 7 α -thioacetyl group further enhances the inhibitory potency.²⁸⁾ Although it was previously reported that spironolactone exhibits the highest percentage inhibition in terms of estrogen production ratio (E1/E2) in the aromatase pathway (4.5 compared to 0.78 of the control),⁸⁾ on the basis of the results of this study, this drug was clearly found to inhibit type II 17 β -HSD in the aromatase pathway as well. Thus, in the case of long-term administration of spironolactone, the following types of quantitative increases in the estradiol pool can be expected, as shown in Fig. 1; namely, interruption of the metabolic pathway from E2 to E1 (type II 17 β -HSD), supply of E1 from E1S (E1S-sulfatase), the male plasma concentration of which is comparatively high at 1.80–1.97 nmol/l,²⁹⁾ the pathway leading from E1 to E2 (type I 17 β -HSD), and the pathway for the generation of E2 from T (aromatase). Pitt *et al.* reported that 61 in 603 men who were treated with spironolactone became gynecomastia, about 10% frequency, as compared with 9 in 641 men in the placebo group (1%).³⁰⁾ It may be considered, therefore, that there is a correlation between the clinical research of gynecomastia and the present results.

In the conversion of E2 to E1, ketoconazole exhibited the second strongest percentage inhibition after spironolactone at 77% (K_i : 41 μ M, non-competitive inhibition). As previously mentioned, since this drug not only increases T level by inhibiting the 6 β -hydroxylation by CYP3A4, forms E2 by the aromatization, and increases E2 level by inhibiting the 2-hydroxylation of E2 by CYP3A4, but also inhibits the conversion of E2 to E1, the long-term administration is likely to induce an increase in the estradiol pool. The percentage inhibitions of the other drugs were all less than 50% even on addition of the non-clinical overdose concentration of 100 μ M.

CONCLUSION

In conclusion, among the 29 drugs reported to induce gynecomastia, 13 drugs, including ketoconazole and cy-

closporin A, inhibited the 2-hydroxylation of E2 strongly or relatively strongly. In addition, ketoconazole and spironolactone also relatively strongly inhibited the 17-oxidation of E2. These 14 drugs are presumed to cause a clinical upward imbalance in the estrogen/androgen ratio due to drug-estradiol interaction, thereby leading to an increase in the estradiol pool, which in turn serves as a trigger to the induction of gynecomastia. Since ketoconazole in particular inhibits both oxidations at positions 2 and 17 of E2, adequate precautions must be taken when using this drug.

Rodriguez and Jick³¹⁾ reported the relative risk of gynecomastia with drugs in the clinical setting in their open cohort study. Verapamil exhibited the highest relative risk at 9.7, followed by spironolactone at 9.3, and a trend was observed that indicated a comparatively strong correlation with the results obtained in this study. These drugs suggested that there is a correlation between the oxidation of E2 at position 2 or position 17 and drug-induced gynecomastia. However, questions remain as to how to explain the complete lack of inhibition of the oxidation of E2, based on our results, of a drug like cimetidine despite having a high relative risk of 7.2. This can probably be explained by taking into consideration the duration of its administration. Furthermore, endocrine factors other than the inhibition of the phase I reaction of estrogen, clinical pharmacokinetic interactions as well as hepatic or renal insufficiency and so forth must also be considered as potential causes of gynecomastia induced by drugs other than the 13 drugs mentioned above. It is particularly imperative to examine additional studies on the effects of drugs affecting the catabolic pathway in the manner of the glucuronidation of E2, as shown in Fig. 1.

REFERENCES AND NOTES

- 1) Part 1: Satoh T., Fujita K., Munakata H., Itoh Shi., Nakamura K., Kamataki T., Itoh Shu., Yoshizawa I., *Anal. Biochem.*, **286**, 179–186 (2000).
- 2) Present addresses: *Yakuhan Pharmaceutical Co., Ltd., Kitahiroshima, Hokkaido 061-1111, Japan.*
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- 5) Wilson J. D., Braunwald E., Isselbacher K. J., Petersdorf R. G., Martin J. B., Fauci A. S., Root R. K. (eds.), "Principles of Internal Medicine," 12th ed., Vol. 2, McGraw-Hill, New York, 1996, pp. 1796–1798.
- 6) Thompson D. F., Carter J. R., *Pharmacotherapy*, **13**, 37–45 (1993).
- 7) Iwata H., Fujita K., Suzuki A., Konno Y., Nakamura K., Fujino A., Kamataki T., *Biochem. Pharmacol.*, **55**, 1315–1325 (1998).
- 8) Satoh T., Itoh Shi., Seki T., Itoh Shu., Nomura N., Yoshizawa I., *J. Steroid Biochem. Molec. Biol.*, **82**, 209–216 (2002).
- 9) Chetrite G. S., Cortes-Prieto J., Philippe J. C., Wright F., Pasqualini J. R., *J. Steroid Biochem. Molec. Biol.*, **72**, 23–37 (2000).
- 10) Guengerich F. P., *Adv. Pharmacol.*, **43**, 7–35 (1997).
- 11) Martucci C. P., Fishman J., *Pharmac. Ther.*, **57**, 237–257 (1993).
- 12) Zhu B. T., Conney A. H., *Carcinogenesis*, **19**, 1–27 (1998).
- 13) Labrie F., Luu-The V., Lin S. X., Simard J., Labrie C., El-Alfy M., Pelletier G., Bélanger A., *J. Molec. Endocrinol.*, **25**, 1–16 (2000).
- 14) Yamazaki H., Shimada T., *Xenobiotica*, **29**, 231–241 (1999).
- 15) Maurice M., Pichard L., Daujat M., Fabre I., Joyeux H., Domergue J., Maurel P., *FASEB J.*, **6**, 752–758 (1992).
- 16) Gillam E. M. J., Baba T., Kim B.-R., Ohmori S., Guengerich F. P., *Arch. Biochem. Biophys.*, **305**, 123–131 (1993).
- 17) Fishman J., Guzik H., Dixon D., *Biochemistry*, **8**, 4304–4309 (1969).
- 18) Kohan D. E., *New Engl. J. Med.*, **314**, 448 (1986).

- 19) Ferguson R. M., Sutherland D. E. R., Simmons R. L., Najarian J. S., *Lancet*, **16**, 882—883 (1982).
- 20) Boyd I. W., *Med. J. Aust.*, **161**, 328 (1994).
- 21) Guengerich F. P., Martin M. V., Beaune P. H., Kremers P., Wolff T., Waxman D. J., *J. Biol. Chem.*, **261**, 5051—5060 (1986).
- 22) Pichard L., Fabre I., Fabre G., Domergue J., Aubert B. S., Mourad G., Maurel P., *Drug Metab. Disp.*, **18**, 595—606 (1990).
- 23) Kato M., Nakajima M., Shimada N., Yamazaki H., Yokoi T., *Eur. J. Clin. Pharmacol.*, **55**, 843—852 (2000).
- 24) Shet M. S., Mcphaul M., Fisher C. W., Stallings N. R., Estabrook R. W., *Drug Metab. Disp.*, **25**, 1298—1303 (1997).
- 25) Zumoff B., Fishman J., Freed S., Levin J., Whitmore W. F., Hellman L., Fukushima K., *J. Clin., Endocrinol. Metab.*, **49**, 467—468 (1978).
- 26) Akaza H., Usami M., Kotake T., Koiso K., Aso Y., *Jpn. J. Clin. Oncol.*, **23**, 178—185 (1993).
- 27) Luu-The V., Zhang Y., Poirier D., Labrie F., *J. Steroid Biochem. Molec. Biol.*, **55**, 581—587 (1995).
- 28) Tremblay M. R., Luu-The V., Leblanc G., Noël P., Breton E., Labrie F., Poirier D., *Biorg. Med. Chem.*, **7**, 1013—1023 (1999).
- 29) Giton F., Valleix A., Boudou P., Villette J. M., Bélanger A., Galons H., Fiet J., *J. Steroid Biochem. Molec. Biol.*, **81**, 85—94 (2002).
- 30) Pitt B., Zannad F., Remme W. J., Cody R., Castaigne A., Perez A., Palensky J., Wittes J., *New Engl. J. Med.*, **341**, 709—717 (1999).
- 31) Rodríguez L. A. G., Jick H., *Br. Med. J.*, **308**, 503—506 (1994).