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DRUG INTERACTIONS IN BIOSYNTHESIS AND METABOLISM OF STEROID HORMONES: The Role of Human Cytochrome P450s

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This paper is designed to provide a framework for interpreting data on drug-steroid and steroid-steroid **metabolic interactions**. It is an effort to summarize the functions of known human cytochrome P450s which catalyze **biosynthesis** and **biotransformations** of steroid hormones in terms of their specific catalytic activities, substrates, inducers, and inhibitors. The subject of the paper updates information on the metabolism of testosterone presented at the 11th Colgone Workshop on Dope Analysis as well as those published elsewhere (1,2).

Superfamily of CYP enzymes and metabolism of steroid hormones

Synthetic chemicals and some natural compounds are substrates for about 24 of the human CYP enzymes classified in the four families. The following human CYP enzymes which belong to three human CYP families of enzymes are involved in the **biotransformations** of endogenous steroids (3,4; Table 1):

Family of enzymes	*Enzymes*
CYP1	CYP1A1 and CYP 1A2
CYP2	CYP2A6 and CYP 2B6
CYP3	CYP 3A4, CYP 3A5, and CYP 3A7

These enzymes are located in endoplasmic reticulum of liver and also in extrahepatic tissues. Properties of these enzymes have been discussed elsewhere (3,4). Drugs know to behave as inhibitors and/or inducers of the activity of these enzymes are presented in Table 2.

Enzymes which catalyze the **biosynthesis** of steroid hormones are located either in the endoplasmic reticulum or in mitochondria of the adrenals and other steroidogenic tissues (4,5,6). Four families of CYP enzymes catalyze biosynthesis of steroid hormones from cholesterol:

Family of enzymes	*Enzymes*
CYP11	CYP11A1 , CYP 11B1, and CYP 11B2 (named also P450scc; $P450_{11\beta}$, and $P450_{aldo}$)
CYP17 (P450c17)	
CYP19 (P450arom)	
CYP21 (P450c21)	

Reactions and enzymes are listed in Table 3, and inhibitors of the activity of these enzymes are presented in Table 4.

Some metabolic reactions of steroid hormones in humans are so well characterized that they are used as specific markers to measure the catalytic activity of the specific CYP enzyme. Such reaction is, for instance, 6β-hydroxylation of testosterone which is used for testing the metabolic activity of CYP3A4 enzyme both *in vivo* and *in vitro* (Table 1). This reaction has been frequently used to study the effects of inducers and inhibitors on the activity of CYP3A4 enzyme (3,4). Some drugs which are listed as potent inhibitors of biosynthesis of testosterone (for instance imidazole drug ketoconazole, Table 4) are also specific inhibitors of the biotransformation reactions of the steroid (Table 2). Interestingly, ketoconazole, as a specific inhibitor of CYP3A4 enzyme, has been reported to elicit a number of clinically significant drug-drug interactions when coadministered also with other drugs which are specific substrates of CYP3A4 enzyme (3,4; Table 2).

For illustration, the catalytic activity of P450s purified from human and rat hepatic microsomes is presented in Fig. 1. The numbers in the figure indicate hydroxylation sites of testosterone. Depending on sex, rat may give metabolic profile of testosterone which differs from that of the human. For instance, only human 2B6 and 3A4 possess testosterone hydroxylating activity. In rat, however, the high activity was observed also with CYP2A and 2C enzymes (7).

Tabular presentation

The data are presented in tables for the ready access of information (Tables 1-4) and contain only those CYP enzymes which participate to a significant extent in the metabolism or catalyze the biosynthesis of steroid hormones in humans.

Data presented in Tables 1-4 refer to both *in vivo* and *in vitro* experiments. The *in vitro* experiments were performed using human tissue preparations, purified enzymes, and/or enzymes obtained by recombinant technology.

Substrates: drugs are listed in accordance with their therapeutic uses as presented in **Martindale, The Extra Pharmacopoeia, 38th ed., James E.F. Reynolds (ed.), The Pharmaceutical Press, London 1993**.

Inducers: these substances, which are frequently substrates of the induced enzyme, enhance the quantity or activity of the enzymes.

Inhibitors: these substances inhibit or suppress enzyme activity either reversibly or irreversibly.

The data presented in tables should be considered together with those presented at the 13th Cologne Workshop (3). The cited reviews should be consulted for the original literature (e.g. 1-6).

References:

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(7) S. Imaoka, T. Yamada, T. Hiroi, K. Hayashi, T. Sakaki, Y. Yabusaki, and Y. Funae, Multiple forms of human P450 expressed in *Saccharomyces cerevisiae*, Systemic characterization and comparison with those in rat, Biochem. Pharmacol. **51**, 1041-1050 (1996).

TABLE 1. METABOLIC REACTIONS OF STEROID HORMONES CATALYSED BY HUMAN CYTOCHROME P450s (for details see refs. 3-5)

Hormone	Enzyme	Reaction
ANABOLIC AND ANDROGEN		
Androstenedione	CYP3A4	6ß-hydroxylation
Dehydroepian-drosterone sulphate	CYP3A4	16α-hydroxylation
Dehydroepi-androsterone 3-sulfate	CYP3A7	16α-hydroxylation
Dehydroepi-drosterone	CYP?	7α-hydroxylation
Methandrostenolone	CYP3A4	6ß-hydroxylation
Testosterone	CYP2B6	16α-hydroxylation 16β-hydroxylation
Testosterone	CYP3A4	6ß-hydroxylation 2α-hydroxylation 2ß-hydroxylation 15α- and 15ß-hydroxylation 16α-hydroxylation
Testosterone	CYP3A5	6ß-hydroxylation 2ß-hydroxylation
Testosterone	CYP 3A7	6ß-hydroxylation 2ß-hydroxylation 2α-hydroxylation
PROGESTAGEN		
Gestodene	CYP3A4	Oxidation
Progesterone		6ß-hydroxylation 16α-hydroxylation

CORTICOSTEROIDS

Budesonide	CYP3A4	6ß-hydroxylation 16α-hydroxypredni- solone formation
Prednisone	CYP3A4	
Corticosterone		
Cortisol	CYP3A4	6ß-hydroxylation
Hydrocortisone	CYP3A4	6ß-hydroxylation
Cortisol	CYP3A5	6ß-hydroxylation

ESTROGENS

17ß-Estradiol	CYP1A1	2-hydroxylation 6α-hydroxylation 15α-hydroxylation
17ß-Estradiol	CYP1A2	2-hydroxylation
17ß-Estradiol	CYP1B1	4-hydroxylation
17ß-Estradiol	CYP3A4	2-,4-hydroxylations (main reactions)
17α-Ethynyl- estradiol (contraceptives)	CYP3A4	2-hydroxylation (and other positions?)

TABLE 2. EXAMPLES OF INHIBITORS AND INDUCERS OF METABOLIC REACTIONS OF STEROID HORMONES CATALYSED BY CYP ENZYMES

(for details see refs. 3,4)

Inhibitors	*Inducers*	*CYP Enzymes*	
<u>Antibiotics macrolide</u>			
Erythromycin	Erythromycin[1]	3A4	
Triacetoyl-oleandomycin[1]		3A4	
	Rifampicin[1]	3A4	
<u>Corticosteroids</u>			
	Dexamethasone[1]	2B6	
	Pregnenolone[1]	3A4	
	Pregnenolone-16α-carbonitrile[1]	3A4	
<u>Antineoplastic</u>			
Alkylating			
Cyclophosphamide		3A4	
Ifosfamide		3A4	
Anti-oestrogen			
Tamoxifen		3A4	
<u>Imidazole drugs</u>			
Antimicotics			
Ketoconazole[1]		3A4	
Gastrointestinal drugs:			
Cimetidine		3A4	
<u>Sex hormones</u>			
Anabolic and androgen			
Dehydeoepi-androsterone[1]			3A7
	Testosterone	3A4	
Estrogen			
	17β-Estradiol	3A4	
7α-Ethynylestradiol		3A4	

Progestagen				
Gestodene				
Progesterone[1]			3A4	
Progesterone	Progesterone		3A4	3A7
<u>Other drugs</u>				
Caffeine		1A2		
Cannabidiol			3A4	
	Clofibrate[1]			
Cocaine			3A4	
Cyclosporine			3A4	
	Carbamazepine		3A4	
Diltiazem			3A4	
Midazolam[1]			3A4	
Nifedipine			3A4	
Phenacetin		1A2		
	Phenobarbital[1]	2B6		
Phenytion			3A4	
Pilocarpine[1]			3A4	
<u>Other compounds:</u>				
Metyrapone[1]			3A4	
	α-Naphtoflavone		3A4	

[1]Drugs reported to influence metabolism of testosterone.

TABLE 3. BIOSYNTHETIC REACTIONS OF STEROID HORMONES CATALYSED BY CYP ENZYMES (for details see refs. 3-6)

Substrates	Enzymes	Reactions
Cholesterol	CYP 11A1	C22-hydroxylation C20,22-bond
11-Deoxycortico sterone	CYP 11B1	C11ß-hydroxylation
11-Deoxycortisol		C11ß-hydroxylation
11-Deoxycortico-sterone	CYP 11B2	C11ß-hydroxylation
11-Deoxycortisol		C11ß-hydroxylation
Corticosterone		C18- hydroxylation
Cortisol		C18- hydroxylation
18-OH corticoste-rone		C18-oxidation
Pregnenolone	CYP17	C17α-hydroxylation
Progesterone		C17α-hydroxylation
		C16α-hydroxylation
C17α-hydroxy--pregnenolone		
Testosterone	CYP19	Oxidation
Androstene-dione		(aromatization)
16α-OH Testosterone		
Progesterone	CYP21	C21-hydroxylation
17-OH-Progeste-rone		C21-hydroxylation
17-OH Progesterone		C21α-hydroxylation

TABLE 4. EXAMPLES OF INHIBITORS OF BIOSYNTHETIC REACTIONS OF STEROID HORMONES CATALYSED BY CYP ENZYMES

(for details see ref. 4)

Inhibitors:	*CYP Enzymes Inhibited*					
Aminoglutethimide	11A1,	11B1,	11B2,	17,	19,	21,
Imidazole drugs						
Antimicotics						
Econazole					19	
Fadrozole		11B1,	11B2,		19	
Ketoconazole	11A1,	11B1,		17,	19,	21
Liarazole				17,	19	
Miconazole					19,	
Gastrointestinal drugs:						
Cimetidine		11B1,				
Omeprazole	11A1,					21
Steroidal:						
Epitestosterone				17		
Atamestane					19	
Formestane					19	
4-Hydroxy-androstene-diene					19	
Testolactone					19	
Other drugs						
Etomidate		11B1,	11B2			
Other compounds:						
4-Cyclohexylaniline					19	
7,8-Benzoflavone					19	
Grapefruit juice (Flavonoids)		11B1				
Metyrapone		11B1				
Pyridyl- and imidazoyl-benzocykloalkanes				17		
4-Pyridineacetic acid derivatives				17,	19	
Triazoles					19	

FIG. 1. HYDROXYLATIONS OF TESTOSTERONE BY RAT AND HUMAN CYP ENZYMES

(for details see refs. 1-4,7)