Reprint from

RECENT ADVANCES IN DOPING ANALYSIS (3)

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Sport und Buch Strauß, Köln, 1996

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In: M. Donike, H. Geyer, A. Gotzmann, U. Mareck-Engelke (eds.) Recent advances in doping analysis (3). Sport und Buch Strauß, Köln, (1996) 13-54

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HUMAN CYTOCHROME P450 (CYP) ENZYMES IN DOPING CONTROL: METABOLISM, INTERACTIONS, ADVERSE EFFECTS

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Abstract

Catalytic properties of human cytochrome P450 (CYP) enzymes are reviewed regarding their involvement in metabolism of both the clinically used drugs and the drugs that are banned or restricted in sport. Of about 36 families of CYP enzymes identified until now, only few of them (families CYP1,2, and 3) are involved in metabolism of drugs in humans. Similarly to other drugs, also metabolism of doping agents by CYP enzymes is influenced by a number of factors including genetic polymorphism. Regarding the latter variability, the most important enzyme for metabolism of doping agents is CYP2D6. The most abundant enzyme subfamily, however, involved in metabolism of important doping agents such as steroid hormones, stimulants, and corticosteroids is CYP3A (in particular CYP3A4 enzyme). CYP3A4 enzyme might be inhibited and/or induced by a number of drugs, and was suggested to be responsible for major clinically important drug-drug interactions. Other enzymes that are also involved in metabolism od doping agents are CYP1A2 (substrates are caffeine and β-blockers), 2C subfamily (substrates are diuretic drug tienilic acid and β-blocker propranolol), and 2E6 (catalyzes ethanol oxidation and low affinity caffeine demethylations). Each enzyme involved in metabolism of doping agents is characterized by its substrates, inhibitors and inducers.

Introduction

Cytochrome P450 (CYP) enzymes are responsible for oxidative, as well as for reductive metabolic transformations of drugs and other exogenous chemicals. In its long history (since more than 3.5 billion years ago), cytochrome P450 superfamily of enzymes (CYP enzymes) developed to the most versatile enzymes. CYP enzymes were proposed to be used by early organisms for metabolism and/or synthesis of steroid molecules required for membrane integrity (for example sterols, ref. 1).

CYP enzymes are involved in metabolic detoxification, and in metabolic activation, of number of environmental chemicals and drugs (1-3). The activity of these enzymes might be influenced by other drugs or chemicals (for example by induction or inhibition of the activity) and this might occur in the liver, as well as in other tissues (4,5). Changed catalytic activity of CYP enzymes by coadministered drugs may result in changed therapeutic and/or toxic properties of a drug resulting in side effects known as drug-drug or drug-chemical interactions (6). Besides, as a consequence od such interactions, unusual or changed blood and urine metabolic profiles of a drug might occur. However, similarly to other drugs, also drugs misused in sport are substrates of only few CYP enzymes. In the present paper catalytic properties of the enzymes and influence of various factors on metabolic reactions regarding both the biological activity of the drugs-substrates and the metabolic profile will be presented.

At the present there are more than 200 CYP enzymes identified and characterized in the living organisms and classified to 36 families. These CYP families constitute the **CYP** (cytochrome P450) **SUPERFAMILY** of enzymes. Of 36 enzyme families so far described, 12 families exist in all mammals. In humans, more than 40 enzymes have been identified and characterized so far. These enzymes are classified into 12 families and 22 subfamilies of enzymes. C**YP enzymes** are classified in families and subfamilies according to homology of amino acid sequence in their structure. A P450 protein sequence from one gene family is defined as usually having less than 40% amino acid sequence identity to a P450 protein from any other family (7,8). Some families posses only a single subfamily with more than one enzyme (for example, in CYP1A subfamily there are CYP1A1 and CYP1A2 enzymes). Other families, however, contain only a single enzyme as a member of the family (for example, CYP2D6 is a single enzyme of the CYP2D subfamily).

These examples illustrate the present classification and naming of the enzymes. Arabic numbers are used for designation of families (for example, CYP1), a letter indicates subfamily (for example, CYP1A), and an Arabic numeral represents the single enzyme (for example, enzymes CYP1A1 and CYP1A2)(8).

CYP superfamily of enzymes and drug metabolism

Only few CYP families are involved in metabolism of drugs in humans (2). These enzymes are located in the liver and/or in other tissues. Some of them are predominately extrahepatic (for

instance, 1A1) and others are characterized by their predominant location in the liver, e.g., 1A2 (Tables 1 and 2). At each location, the enzymes are characterized by the level and the activity, and by the extent of variability. The extent of variability of an enzyme depends on its inducibility by environmental chemicals (environmental pollutants, smoking), drugs, food constituents, food additives, alcohol consumption, and on the genetic variability (2).

Similarly to metabolism of other drugs, also doping agents are predominately substrates of the CYP3A subfamily and of the 2D6 enzyme (Tables 4-8). In addition, 2D6 enzyme is responsible for genetic polymorphism in metabolism of important doping agents. Because of this, the 2D6 enzyme possess the highest variability in both the content and the activity (up to 1000 fold, Table 1, ref. 2).

Genetic polymorphism in metabolism of drugs and doping agents

Genetically determined biochemical variations (or genetic polymorphism) might be defined as **"inborn errors which are responsible for changes in the response to drugs caused by alteration in the functional activity of an enzyme "**(9).

Such inborn errors could be either very rare (when 1 in 10000 to 1 in 100000 people are involved) or they can take the form of genetic polymorphism. In the latter case abnormal gene has frequency of more than 1% in the general population (9).

Genetic polymorphism is related to changes in metabolism of drugs in persons that are described either as **poor** (**slow**) or as **extensive** (**fast**) metabolizers. The poor metabolizers define persons possessing no particular microsomal protein (*i.e.*, the particular enzyme). The extensive metabolizers are persons possessing a high level of the protein (*i.e.*, the high activity of the enzyme) which is involved in the metabolic reaction.

There are two major types of genetic polymorphism of drugs connected with the activity of the CYP enzymes:

1) The sparteine/debrisoquine type which reflects the activity of the CYP2D6 enzyme.

2) The (S)-mephenytoine type reflecting the activity of the CYP2C19 enzyme.

Table 1. Human CYP enzymes located in the liver
(according to ref. 2)

CYP	LEVEL OF THE ENZYME (% of total)	EXTENT OF VARIABILITY
1A1	< 1	ca. 100 fold
1A2	**ca. 13**	**ca. 40 "**
2A6	ca. 4	ca. 30 "
2B6	ca. 0.2	ca. 50 "
2C8,9,19	**ca. 18**	**25 to 100 fold (depending on the enzyme)**
2D6	**up to 2.5**	**>1000 fold**
2E1	**up to 7**	**ca. 20 "**
3A3,4,5	**up to 70 %**	**ca. 20 "**

Bold-marked are enzymes involved in metabolism of drugs restricted or banned in sport.

Table 2. Human CYP enzymes located in extrahepatic tissues (ref. 2).

CYP ENZYME	TISSUE
1A1	Lung, kidney, GI-tract, skin, placenta and others
1B1	Skin
2A6	Lung, nasal membrane and possibly others
2B6	(?)
2B7	Lung
2C	**GI-tract (small intestine mucosa), larynx**
2D6	**GI-tract,**
2E1	**Lung and others**
2F1	Lung
3A	**GI-tract, placenta, fetus, uterus**
4B1	Lung
4A11	Kidney

Bold-marked are enzymes involved in metabolism of drugs restricted or banned in sport.

For doping control considerations, genetic polymorphism of 2C9 and 2C19 enzymes is unimportant while only limited number of doping agents are metabolized by these enzymes, *i.e.*, diuretic drug tienilic acid and propranolol, respectively (Table 5). With exemption of 2D6 enzyme, polymorphism of other enzymes involved in metabolism of doping agents has not been determined yet. For example, polymorphism for the enzyme CYP1A2 and for the CYP3A subfamily has been only suggested (Tables 4 and 8) . It has been reported that some of the enzymes involved in Phase II metabolic reactions are polymorphic as well. Such enzymes are, for instance, N-acetylation, S- and O-methylation , and conjugation with glutathione (9). Stimulant drug caffeine is now widely used as a probe-drug for determination of acetylation phenotypes. For phenotyping, the molar ratio for two caffeine metabolites (*i.e.*, the ratio of 5-acetylamino-6-formylamino-3-methyluracyl and 1-methylxanthine) is determined in the urine sample collected between 4-8 hours after single oral dose. The ratio <0.3 signifies a slow acetylator, and the ratio >0.4 defines a fast acetylator (9).

Besides using the urine ratios, genotyping tests for polymorphism can be performed by taking DNA probes from blood leukocytes, hair roots, buccal epithelial cells, blood spots, urinary sediments *etc.* Probe-drugs (or so called marker-drugs) that might be administered *in vivo* for clinical testing of the enzyme activity are bold-marked (Tables 4-8). In some cases also doping agents are used as marker-drugs. For instance, caffeine is a marker-drug for metabolic activity of the CYP1A2, bufuralol-1´-hydroxylation for CYP2D6 polymorphism, and testosterone 6ß-hydroxylation for the CYP3A4 activity (Tables 4,6, and 8, respectively).

In doping analysis genetic polymorphism might influence results in a way of unexpected changes of both the metabolic profile and the ratios of a drug to metabolite(s) excreted in urine. Besides, therapeutic and toxic effects might be either enhanced or lowered depending the type of polymorphism. The end effect will be dependent on the enzyme involved and on the biological properties of a drug and metabolites formed (10).

To summarize, genetic variations in drug's (doping agent's) metabolism might result in individual variation in:

-therapeutic efficacy
-metabolic profile
-adverse drug effects
-pharmacokinetic parameters

CYP enzymes involved in metabolism of doping agents

Involvement of CYP enzymes in metabolism of clinically used drugs is illustrated in Table 3. The majority of clinically used drugs and doping agents are metabolized by the catalytic activity of the CYP3A subfamily of enzymes (ca. 55%), followed by the polymorphic CYP2D6 enzyme (ca. 30%). When considering significance of CYP enzymes for metabolism of a drug following characteristics should be taken into account:

-the activity and the level of an enzyme in a tissue

-induction and/or inhibition of drug's (doping agent's) metabolism by other drugs and/or environmental chemicals

-clinical importance and a number of drug classes metabolized by the enzyme

-variability of the enzyme activity due to interindividual variations or genetic polymorphism

For instance, the greatest variability due to drug-drug, and drug-environmental chemicals interactions might be expected if the major metabolic reaction of a drug is catalyzed by 3A subfamily of enzymes. This includes also potentiality that substrates could be made active to toxic metabolites and intermediates. The drug-substrate belonging to this subfamily of enzymes might exercise races and significant gender differences in both the therapeutic effect and the metabolic profile (11).

We might expect that, because of the polymorphism, the catalytic activity of 2D6 enzyme will be involved in the major differences caused by this type of variability. Referring to 2D6 polymorphism, for instance, 5-10% of Caucasian population are slow metabolizers while only <1 of Orientals posses the same characteristics (for example Chinese or Japanese population). Besides, the activity of the enzyme will be influenced by inhibition following coadministered drugs such us quinidine, cimetidine, ajmalicine and some others (Table 6).

Of particular interest for drug metabolism studies are inhibitions elicited by antiulcer drug cimetidine. Cimetifdine inhibits several human CYP enzymes with different degrees of

specificity (12). In early studies (13-15), cimetidine was reported to bind to the hem iron of cytochrome P450 enzymes due to both the imidazole and the ciano group in the structure. By this mechanism cimetidine inhibits metabolism of a number of drugs-substrates of CYP enzymes *in vitro* and *in vivo*. Such inhibitions were connected with a number of clinically important drug-drug interactions (16,17). Experiments using human CYP enzymes confirmed that cimetidine inhibits 1A, 2C, 2D6 and 3A4 enzymes, and all of them are involved in metabolism of doping agents (Tables 4-8). For instance, cimetidine caused 80% inhibition of bufuralol hydroxylation *in vitro*, the reaction catalyzed by the 2D6 enzyme (12). Besides drug metabolism reactions, cimetidine inhibits also biosynthetic reactions of endogenous steroids catalyzed by CYP enzymes as well (12).

Elimination of caffeine, as well as the ratio of the drug to metabolite in urine, processes which are completely controlled by the CYP1A2 enzyme activity (Table 4), were changed following coadministration with cimetidine or enoxacin. Total clearance of caffeine was reduced by ca. 50-80%, and the $t_{1/2}$-value was enhanced by ca. 2-3 times following administration of cimetidine *in vivo* (18, 19). Besides, elimination of caffeine in smokers is characterized by the higher clearance and the shorter $t_{1/2}$ when compared with the values obtained when nonsmokers were tested (18).

Table 3. Participation of the CYP enzymes in metabolism of clinically used drugs.

CYP enzyme	Drugs metabolized (%) of total	Catalytic activity influenced by:	
1A1, 1A2, 2C19	5	**Induction by:** cigarette and marihuana smoking etc. **Polymorphism**	**Inhibition by:** cimetidine enoxacin etc.
2C9	10	**Polymorphism**	
2D6	30	**Polymorphism**	
3A4,5	55	**Drug-drug interactions**	

Examples of CYP enzymes involved in metabolism of doping agents

CYP1A2 enzyme: CYP1A2 is a liver specific enzyme that is inducible by different chemicals such as polychlorinated biphenyls, polycyclic aromatic hydrocarbons and a number of aromatic and heterocyclic amines. The latter compounds include also those which are components of cooked food, food products and of charbroiled meet (20, 21). Some other natural constituents (for instance flavonoids) could behave as both the inhibitors and/or the inducers of the CYP1A2 activity. The type of the effect will depend on both the structure and the physicochemical properties of the compound (22,23,24). The only drug reported to induce activity of CYP1A2 enzyme is antiulcer drug omeprazole (Table 4, ref. 25).

CYP1A2 enzyme is toxicologically important as it converts a number of chemicals to active, toxic intermediates (for example aromatic and heterocyclic amines). Metabolic activation of this type is the most frequent mechanism of transformation of an procarcinogen to carcinogenic intermediate (26). Wide variations in urinary bladder and colo-rectal cancer incidence in humans have been attributed in part with differences in metabolic activation of the procarcinogenic chemicals by CYP1A2 (27,28). Drugs and chemicals, inducers and inhibitors of the enzyme, which might influence catalytic activity and therefore metabolic clearance, toxicity, and the metabolic profile of drugs-substrates appears in Table 4.

Caffeine, phenacetin, and theophylline might be used as acceptable probe-drugs for testing the CYP1A2 activity *in vivo* (2,29, 30, 31). Furafylline was reported to be a specific inhibitor of the 1A2 activity (32).

The N3-demethylation of caffeine accounts about 90% of the total caffeine metabolism in humans, and is completely controlled by the CYP1A2 enzyme (25). Being rather specific, this reaction is used as the most appropriate to measure the activity of the enzyme *in vivo* (25,33,34). Thus, the ratios of caffeine urinary metabolites are used for testing the metabolic capacity of CYP1A2 enzyme. By using this method, carcinogenic risks could be judged if the person is exposed to procarcinogenic chemicals. About 40-fold variability among population of both the level of CYP1A2 and the rate of caffeine oxidation was observed (Table 1, and ref. 2). Along

with the CYP1A2, also other enzymes are involved in caffeine metabolism in humans. For instance, the low affinity N1- and N7- demethylations of caffeine are catalyzed by the enzyme 2E1, and C8-hydroxylation by CYP3A subfamily of enzymes (33).

Metabolism of caffeine might be induced by environmental chemicals and by antiulcer drug omeprazole. Accelerated N3-demethylation and urinary elimination of caffeine by coadministration of omeprazole was followed by the urinary caffeine metabolite ratios (25, 34).

β-Blocker drugs bufuralol and propranolol are, at least in part, metabolized by 1A2 enzyme. It was reported that irreversible binding of the propranolol oxidation product to human liver microcosmes was inhibited by phenacetin. The binding to microsomal proteins was associated with 4'-hydroxylation of propranolol. Phenacetin, the probe-drug for 1A2 enzyme activity, was reported to competitively inhibit the reaction. In addition, cigarette smoke induces metabolism of both the phenacetin and the propranolol. These results indicate that both compounds are substrates of the same enzyme (35). Also bufuralol, another β-blocker, is hydroxylated by catalytic activity of this enzyme in C1', C4, and in other positions as well. However,additional enzymes such as 2C19 and 2D6 are involved in metabolism β-blockers as well (Table 4 and 6, ref. 2).

CYP2C subfamily: Enzymes belonging to 2C subfamily are involved in metabolism of a number of clinically important drugs (Table 5, and ref. 2). These enzymes are inducible by drugs and chemicals, and exercise variability caused by genetic polymorphism (for example enzymes 2C9 and 2C19). The 2C enzymes are all more than 80% mutually structurally identical. Important drug-substrates are tolbutamide, phenytoin, and warfarin. Polymorphism of (S)-mephenytion 4'-hydroxylation is associated with polymorphism of CYP2C19 enzyme (36,37,38,). Frequency of genetic polymorphism of this type is ca. 3% in Caucasians and nearly 20% in Orientals (2). Thus, if a drug's metabolism is primarily dependent on CYP2C19 enzyme (Table 2), than significant differences in both the metabolic clearance and the metabolite profile could be expected (2).

Inhibitors of the 2C enzymes are sulfa-drugs, disulfiram, cimetidine and others. Compounds acting as inducers are for instance, barbiturates and rifampicin. We might expect that

all these drugs will significantly influence metabolic properties of the drug-substrates listed in Table 5 (2, 39).

Diuretic drug tienilic acid is a substrate of 2C9, 2C10, and 2C18 enzymes (Table 5). By C5-hydroxylation tienilic acid is metabolically made active to intermediate which is a suicidal substrate of the enzyme. This reaction leads to both the catalysis-dependent loss of activity of CYP enzymes and the covalent binding of tienilic acid metabolites to microsomal proteins (40).

Beta-blocker drug propranolol has been reported as a substrate of the 2C19 enzyme as well(2).

Coadministration of drugs that are substrates of the same enzyme might cause mutual competitive inhibition of metabolism similarly as discussed with CYP1A2 enzyme. For example, reduced clearance of diazepam was observed when this drug was coadministered with omeprazole (9, 10). The reduced clearance of diazepam was connected with inhibition of its metabolism by omeprazole that is cosubstrate of 2C19 enzyme.

Clinically significant metabolic polymorphism associated with the 2C19 enzyme was reported for β-blockers (propranolol), antidepressants (imipramine, amitriptyline and clomipramine), and for antimalaric drug proguanil. However, also other drugs which are substrates of 2C19 enzyme are expected to exert genetic polymorphism when administered either to poor or to extensive metabolizers (9,10).

CYP2D6: This is the only enzyme from CYP2D subfamily expressed in humans, and was the first one shown to be polymorphic. Polymorphism connected with this enzyme is called sparteine/debrisoquine polymorphism. About 7-10% of total population of Caucasians, and <1% of Orientals or Africans are deficient in the enzyme possessing characteristics of "slow" metabolizers, (2, 9, 10, 26).

CYP 2D6 enzyme is involved in biotransformation of more than 40 drugs including also drugs which are banned or restricted in sport. Examples are stimulants (amphetamine and derivatives) (2,41-43), β-blockers (2,35,44), and narcotic drugs (10,45), Table 6. Individuals who are poor metabolizers possess very limited metabolism of the drugs listed as substrates. Large differences between extensive and poor metabolizers in metabolism of some of the drugs suggests that major clinical implications might occur because of their

polymorphic oxidation. Besides, administration of known inhibitors such as quinidine, cimetidine, and others might change persons with characteristics of extensive metabolizers to persons behaving as poor metabolizers. This might cause additional unexpected drug-drug interactions. However, if some potent inhibitors such is quinidine are administered to poor metabolizers, their effect will be negligible as the enzyme is not expressed in these persons (2,4,9,10).

Narcotic agents are predominately O-demethylated, and β-blockers are hydroxylated and O-dealkylated by the activity of 2D6 enzyme (Table 6).

O-demethylation of antitussive drug dextromethorphan, as well as C1'-hydroxylation of bufuralol are used as "probe" reactions for testing the enzyme activity *in vivo* (8, 46, 47). Quinidine was reported to be effective inhibitor of α-hydroxylation and O-demethylation of metoprolol, as well as of propranolol hydroxylation(44).

Metabolic conversion of hydrocodone to hydromorphone by O-demethylation is highly correlated with the O-demethylation of dextromethorphan, and was inhibited by quinidine. This indicates involvement of 2D6 enzyme in the reaction. Other O-substituted 17-methylmorphinans (for instance codeine, ethylmorphine and oxycodone) are substrates of the enzyme as well. Also these drugs are expected to exert metabolic polymorphism (10, 45). Administration of codeine to extensive metabolizers (for instance, Oriental and Black population) may produce more pronounced analgesic response when compared with the same effect elicited by poor metabolizers (for instance, Caucasian population). The difference was connected with different rate of formation of morphine by O-demethylation of codeine(10).

Hallucinogenic and stimulant drug methoxyphenamine is metabolized by N-demethylation, O-demethylation, and C5-aromatic hydroxylation. Only O-demethylation and C5-aromatic hydroxylation reactions have been reported as polymorphic in debrisoqiune-type poor metabolizers. These persons were shown defective in CYP2D6 enzyme. As a consequence, changed levels of the metabolites in plasma were reported (42). It was concluded that N-demethylation of the drug is catalyzed by different enzyme(s) when compared to both the O-demethylation and the aromatic hydroxylation.

Another hallucinogenic drug, methylenedioxymethamphetamine ("ECSTASY"), is converted to dihydroxymethylmphetamine by O-demethylenation reaction catalyzed by the 2D6 enzyme. This reaction produces protoxic catechol metabolite, and was connected with genetically-determined differences in toxicity of the drug (43).

Similarly to other CYP enzymes, also 2D6 enzyme is inhibited by substrates that forms catalytically inactive complexes with the enzyme (such a substrate is, for instance, methylamphetamine). Such inhibitions might lead to significant drug-drug interactions following coadministration of phenylakylamine drugs (44). Interactions by inhibitions might be expected to occure also following coadministration of other drugs-substrates of the enzyme. For example, classic substrates of 2D6 enzyme such us debrisoquine, sparteine and bufuralol are all inhibitors of O-demethylation of dexthromethorphan. Another example is drug flecainide that inhibits both the hydroxylation of bufuralol and the O-demethylation of debrisoqiune (10).

Polymorphism associated with genetic variations of the 2D6 enzyme was reported for β-blockers (propranolol), antiarrhytmics (encainide, flecainide, nifedipine, propafenone), tricyclic antidepressants (imipramine, desipramine, nortryptiline, clomipramine, amitriptyline), and neuroleptics (perphenazine, haloperidol, thioridazine), Table 6. Also other drugs substrates of the enzyme are expected to be polymorphic as well (9,10).

CYP2E1: The main property of CYP2E1 enzyme is its inducibility by organic solvents (ethanol, benzene and others), drugs (isoniazid), and by the pathophysiological state of diabetes. This enzyme is constitutively expressed in human liver. Along with its capability to metabolize and make organic compounds active to toxic metabolites, this enzyme catalyze metabolism of only a limited number of drugs (Table 7, ref. 2). Activity of CYP2E1 is strongly influenced by organic laboratory chemicals, as well as by other environmental chemicals. Metabolic polymorphism was suggested aas well (48, 49).

Ethanol is, for instance, a substrate of the enzyme, it can act as an inducer (at lower concentrations), and as an inhibitor (at higher concentrations) of the activity of the enzyme (49). CYP2E1 enzyme appears to be the main enzyme involved in N1- and N3- low-affinity demethylations of

caffeine. The extent of the latter reactions is unimportant for overall metabolism of caffeine in humans and therefore should not be considered as significant for metabolism of the drug (25,33).

2E1 enzyme metabolically makes a number of chemicals active to carcinogenic intermediates (26), Table 7.

A number of chemicals (such as benzene, aniline, polyhalogenated chemicals and others), as well as drugs (for instance, volatile anesthetics such as halothane, enflurane and others, and analgesic and antipyretic drug acetaminophen) can be activated to toxic species by the enzyme activity. Toxicity of these drugs and chemicals can be enhanced by organic solvents (for example ethanol, isopropanol, acetone, benzene), or by drug isoniazid (Table 7 and refs. 8,50,51,52).

Because of its involvement in toxic responses of chemicals, the activity of the enzyme should be taken into account when considering toxicity in chemical laboratories.

In vivo probe-reaction used for testing the activity of the enzyme is C6-hydroxylation of chlorzoxazone (53,54).

CYP3A subfamily: CYP3A subfamily is, besides 2D6 enzyme, of utmost importance for metabolism of doping agents. The CYP3A enzymes are most abundantly expressed enzymes in both the human liver and the human bowel (Table 8). Four enzymes that are characterized in humans are involved in metabolic reactions of a great number of drugs belonging to different therapeutic classes. These classes include also important doping agents as substrates of the enzymes such as androgen and anabolic hormones (testosterone and others), narcotics (for example N-demethylations of ethylmorphine, hydrocodone and others), corticosteroids and stimulants, Table 8. The list of substrates of CYP 3A subfamily, in particular of the 3A4 enzyme, which is the major contributor in oxidation of a great number of drugs, includes a variety of different structures. Having testosterone as an example, the enzyme catalyzes hydroxylation at positions which are the major pathways of testosterone metabolism in humans (for example, 6β-, 15β-, 15α-, and 2β-hydroxylation's), ref. 55 and Table 5. In addition, 6β-hydroxylation of testosterone is used as a probe-reaction for measurement of the activity of the enzyme *in vivo*. This reaction may be used to study the effects of

inducers and inhibitors on the *in vivo* activity of the enzyme (2, 11, 26).

Activity of the CYP3A4 enzyme is characterized by:

1. A high degree of stereoselectivity (such a case is, for instance, with testosterone and another steroid hormones as substrates, ref. 56).

2. Drugs-substrates of the enzyme may be involved in significant drug-drug interactions. The activity of the enzyme can be induced and/or inhibit by, for instance, macrolide antibiotics, imidazole drugs (ketoconazole), contraceptives (Gestodene), midazolam, nifedipine and others (2,11,57), Table 8.

3. Large interindividual variations in both the content and the activity of the enzyme have been reported (2).

4. The enzyme make some important pro-carcinogens (drugs and dietary compounds) toxic by formation of reactive metabolites (2, 26).

5. Along with androgen and anabolic hormones, 3A4 enzyme catalyzes metabolism of other endogenous steroids as well (for example, cortisol, estradiol and progesterone) (2, 58).

6. Some dietary compounds might stimulate or inhibit catalytic activity of 3A4 enzyme. For instance, administration of grape fruit juice containing flavonoid naringenin can influence both the nifedipine and the cyclosporine metabolism acting as an inhibitor of oxidation of the drugs (2, 59). We might expect that by similar mechanism naringenin can influence also metabolism of some doping agents. As the level of the enzyme is high in GI-tract, such interactions will be important if majority of a drug is metabolized already in gut (such a case is with orally administered cyclosporine).

7. Another doping agent that is, at least in part, metabolized by 3A4 enzyme is cocaine. Enzyme 3A4 catalyzes N-demethylation of cocaine, the pathway that accounts about 10% of total metabolism of the drug. By this reaction pharmacologically active N-demethylated metabolite is formed. Formation of this metabolite was associated with metabolism-related cocaine hepatotoxicity (60).

8. Metabolism of steroid hormones might be inhibited also by cytostatic agents cyclophosphamide and ifosphamide (63).

9. Additional probe-substrates used for testing the activity of the enzyme *in vivo* are midazolam (11, 61, 62), dapson, erythromycin, nifedipine (11), cortisol (11,58), and dextromethorphan (46,47).

Conclusions:

1. Good knowledge of metabolic properties and factors which influence metabolic reactions is necessary for assessment of therapeutic and toxic properties of a drug, as well as for interpretation of unexpected results obtained in doping analysis. Important factors that should be considered are: induction and inhibition of the enzyme activity by drugs and chemicals, and metabolic variation resulting from genetic polymorphism. This is of particular importance when ratio(s) of a drug to metabolite(s) are taken as criteria for administration of an doping agent by athletes.

2. For future consideration there is a need for continuos collection and scientifically based assessment of data related to each human CYP enzyme. Those enzymes are of particular interest that are responsible for major metabolic reactions involved in elimination of drugs. Besides, enzymes which controls formation of toxic intermediates and metabolites are important as well.

3. The list of substrates, inductors and inhibitors of the enzymes involved in metabolism of different drug classes which include also doping agents appears in Tables 4-8. The data summarize results from both the *in vitro* (using human microsomes, purified human enzymes, human cell cultures, and enzymes produced by recombinant DNA methods) and the *in vivo* clinically controlled experiments.

4. Additional differences in metabolism of a drug might appear from inhibition and/or induction of the enzymes by food constituents as illustrated in Tables 4-8.

It has to be emphasized that research in the field of biological role of human CYP enzymes is growing up extensively following introduction of recombinant DNA technology for

production of the enzymes. Unlimited source of human enzymes enables extensive drug metabolism studies producing a number of new data which has to be carefully considered. Already in the past year several reviews were published on human CYP enzymes related to drug metabolism and interactions. Each of these reports brought a new approach and/or information on additional classes of drugs as metabolites of the CYP enzymes so far characterized in humans. Additional data related to doping agents from both clinical and *in vitro* studies are necessary to enable scientifically based consideration of influence of metabolic reactions on the results of doping analysis.

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SUMMARY OF INFORMATION ON HUMAN CYTOCHROME P450 (CYP) ENZYMES

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Superfamily of cytochrome P450 enzymes (CYP enzymes):

Families 36
Subfamilies 22
Genes ca.200
Human enzymes characterized >40 (including enzymes involved in biosynthesis of steroids)

Abbreviations:

PAH=Polycyclic Aromatic Hydrocarbons
7,8-BF=7,8-Benzoflavone
5,6-BF=5,6-Benzoflavone
PCN=Pregnenolone, 16α-carbonitrile
TCDD=2,3,7,8-tetrachlorodibenzo-*p*-dioxin
BP=Benzo[a]pyrene
PCB=Polychlorinated biphenyls

Legend:

Compounds which might be used as probe-drugs for *in vivo* testing are bold-marked

Table 1. Human CYP enzymes located in the liver
(according to ref. 2)

CYP	LEVEL OF THE ENZYME (% of total)	EXTENT OF VARIABILITY
1A1	< 1	ca. 100 fold
1A2	**ca. 13**	**ca. 40 "**
2A6	ca. 4	ca. 30 "
2B6	ca. 0.2	ca. 50 "
2C8,9,19	**ca. 18**	**25 to 100 fold (depending on the enzyme)**
2D6	**up to 2.5**	**>1000 fold**
2E1	**up to 7**	**ca. 20 "**
3A3,4,5	**up to 70 %**	**ca. 20 "**

The enzymes involved in metabolism of drugs restricted or banned in sport are bold-marked.

TABLE 4. Summary of Information on Human CYP1A2

Substrates	Reaction	Inducer(s)	Inhibitor(s)
Drugs banned or restricted in sport:			
Stimulants			
CAFFEINE	**N3**-,N1-,N7-demethylation, C8-OH		
ß-Blockers			
Bufuralol	C1'-,C4-OH (and in other positions)		
Propranolol	C4'-OH N-delakyl.		
Other drug classes			
Analeptic respiratory		Omeprazole	Cimetidine
Theophylline	N1-, N3-demethylations C8-OH		Diethyldithio-carbamate (metabolite of disulfiram)
Analgetics antipyretics			
(a) Acetaminophen	ring oxid.		Enoxacin
Antipyrine	C4-OH 3-methyl-OH		Fluvoxamine **Furafylline**
Phenacetin	O-deethyla-tion		

Table 4/2.

Chemotherapeutics			
Trimethoprim	N3-oxide		
Sex hormones			
Estrogen			
17ß-estradiol	C2-OH		
Chemicals:			
7-ethoxyre-resorufin	O-deethy-lation	PCB (AroclorR) 7,8-BF 5,6-BF PAH (3MC, cigarette smoke) TCDD	**7,8-BF** 5,6-BF**
(a) Aromatic and hetero-cyclic amines	N-OH	Aromatic and heterocyclic amines (2-acetyl-aminofluorene) 2-aminoanthracene	

Table 4/3.

Natural products			
(a) Food pyrolysis products (aromatic and heterocyclic amines)	N-OH	Food pyrolysis products, Charbroiled meat	Psoralens: ** methoxsalen bergapten psoralen
		Flavones: flavone tangeritin nobiletin	Flavonoids: flavone tangeritin** quercetin apigenin ** fisetin ** myrcetin ** naringenin** galangin ** kaemferol ** morin ** chrysin **
		Curciferous vegetables (indole 3-carbinol)*,**	

Table 4/4

Characteristics:
- -variation caused by environmental impact, polymorphism suggested;
- -activated procarcinogens (marked with "a")
- -extent of variability in level and activity of the enzyme ca. 40-fold;
- - <12% of total hepatic CYP.

Location of the enzyme: Predominately liver

Legend:
*The effect described in rats but might be expected to occur with the human enzyme as well.

**Described as inhibition of human ethoxyresorufin-deethylase, BP-hydroxylase, and/or aflatoxinB1 activation.

TABLE 5. Summary of Information on Human CYP2C Subfamily of Enzymes

CYP Substrates	Reaction	Inducer(s)	Inhibitor(s)
2C8			
Drugs banned or restricted in sport			
Diuretic			
Tienilic acid	C5-OH		
Other drug classes:			
Analgesic			
antipyretic		**Rifampicin**	Diethyldithio-
		Barbiturates	carbamate
Antipyrine	N-demethyl.		(metabolite of disulfiram)
Anticoagulants			
S-Warfarin	C7-OH		**Sulfaphe-nazole**
Antidiabetic			
Tolbutamide	CH3-OH		Sulfapyra-zone
Antiepileptics			
Phenytoin	C4'-OH		
Antineoplastics			
Antimytotic			
Taxol	C6α-OH		
Table 5/2.			
Antiulcer			
Omeprazole	5-OH		
Neuroleptic			
Phenotiazines	S-oxid.		
Chemicals:			
Retinol	C4-OH		7,8-NF
Retinoic acid	C4-OH, side chain oxid		
2C9			
Drugs banned or restricted in sport			
Diuretic			
Tienilic acid	C5-OH		
Other drug classes			
Analgetics			
Antiinflamatory			
Diclofenac	C4'-OH	Barbitu-	7,8-BF
Ibuprofen	C2-, CH3-OH	rates	Cimetidine
Mefanamic acid	3-methyl-OH	Rifampicin	Fluconazole
Piroxicam	C5'-OH		Dicumarols
Tenoxicam	C5'-OH		**Sulfaphe-nazole**

Table 5/3.

Anticoagulants	
(S)-Warfarin	C6-,C7-OH
Phenprocoumon	C6-,C7-, C4'-OH
Antidiabetic	
Tolbutamide	CH_3-OH
Antiepileptics	
Phenytoin	C4'-OH
Trimethadone	N-demethyl.
(R)-Mephenytoin	C4'-OH
Hypnotic, sedative	
Hexobarbital	C3'-OH

Genaral characteristics of the 2C9 enzyme:
-polymorphic, extent of variability in level of the enzyme ca. 25-fold;
-ca. 20% of total hepatic CYP (total 2C).

2C18 (minor member of the family))

Drugs banned or restricted in sport

Diuretic	
Tienilic acid	C5 -OH

Table 5/4.

Other drug classes

Antidiabetic	
Tolbutamide	CH_3-OH
Antiepileptic	
S-Mephenytion	C4'-OH
Antiulcer	
Omeprazole	C5-OH

2C19

Drugs banned or restricted in sport

ß-Blocker	
Propranolol	side chain oxid., C4'-OH

Other drug classes

Antidepressants		Barbiturates	Ketoconazole
Amytriptiline	N-demethyl.	Rifampicin	Tranylcyp-romine
Clomipramine	N-demethyl.		
Imipramine	N-demethyl.		
Antiepileptic			
(S)-Mephenytoin	C4'-OH		
Antimalaric			
Proguanil	oxid. & cycliz.		

Table 5/5.

Antiulcer	
Omeprazole	C5-OH sulphone
Hypnotic, sedatives	
Hexobarbital	C2'-OH
Barbiturates	
Tranquilizer	
Diazepam	N-demethyl.
Other drugs	
Nirvanol	C4'-OH

Characteristics:
-polymorphic, extent of variability ca. 100-fold;
-CYP2C is the most complex CYP subfamily in humans (six genes present);
-involved in oxidation of some important drugs;
-all enzymes are >than 80% structurally similar.

Location of the enzymes: Liver, GI-tract (small intestine)

Table 6. Summary of Information on Human CYP2D6 Enzyme

Substrates	Reaction	Inducer(s)	Inhibitor(s
Drugs banned or restricted in sport:			
Stimulants			
Amphetamine	C4-OH (arom.)		
4-Methoxyamphetamine	O-demethyl.		
Methoxyphenamine	C5-OH (arom.), O-demethyl.		
Methylamphetamine	C4-OH (arom.)		
Methylenedioxymethamphetamine ("ECSTASY")	O-demethylen.		
Phentermine			
Phenylethylamine			
Narcotics			
(Codeine)	O-demethyl.		
Dextropropoxyphene	O-demethyl.		
Ethylmorphine	O-deethyl.		
Hydrocodon	O-demethyl.		
Oxycodon	O-demethyl.		

Table 6/2.

ß-Blockers	
Alprenolol	O-dealkyl.,
Bufuralol	C4'-,C6'-, **C1'-OH**
Bupranolol	
Metoprolol	αC-OH, O-demethyl.
Propranolol	C4'-OH
Timolol	
etc.	

Other drug classes:

Antiarrhytmics	
Ajmaline	
N-Propylajamaline	C-OH
Encainide	O-dealkyl.
Flecainide	
Mexiletine	
Propaphenone	C5-OH
Sparteine	oxidation (2- and 5-dehydrogenation)

Ajmalicine
Cimetidine
Flecainide
Quinidine
Yohimbine and deriv.

Table 6/3.

Antidepressants	
Amitryptyline	C-OH
Desipramine	C-=H
Clomipramine	C2-OH, C10-OH
Imipramine	C2-OH
Minaprine	
Nortriptyline	C-OH
Opipramol	
Tamoxifene	
Antidiabetic	
Phenformin	C-OH (arom.)
Antihipertensives	
Captopril	
Debrisoquine	4-OH
Guanoxan	
Indoramine	
Antitussive	
Dextromethorphan	O-demethyl.
Chemotherapeutics	
Trimetoprim	C4'-,3'--O-demethyl.

Table 6/4.

Neuroleptics	
Clozapine	
Fluphenazine	S-oxid.
Perphenazine	S-oxid.
Thioridazine	S-oxid.
Haloperidol	oxid.
Thioridazine	S-oxid.
Trifluperidol	
Vasodilatators	
Coronary	
Perhexiline	
Chemicals:	
(a) Tobacco smoke derived pro-carcinogens	

Characteristics:
- -responsible for a common human genetic defect in oxidation of several drugs, **polymorphic** (debrisoquine/sparteine polymorphism);
- -very limited metabolism of the listed drugs by "poor metabolizers", in some cases serious implication in therapy expected;
- -a number of inhibitors effective *in vitro* and *in vivo*, drug-drug interactions expected;
- -extent of variability in level of the enzyme of >1000-fold;
- -ca. 4% of total hepatic CYP;
- -inducer not known.

Predominant location: Liver, GI-tract

TABLE 7. Summary of Information on Human CYP2E1 Enzyme

CYP Substrates	Reaction	Inducer(s)	Inhibitor(s)
Drugs banned or restricted in sport			
Alcohols			
Ethanol	oxid.	Ethanol	Ethanol
Stimulants			
Caffeine	N1-,N7--demethyl.		
Other drug classes:			
Analgesics & antipyretic		Isoniazid	Diethyldithio-carbamate (metabolite of disulfiram)
(a) Acetaminophen	ring oxid.		
Antileprotic			
Dapsone	N-OH		
Muscle relaxant			
Chlorzoxazone	C6-OH		

Table 7/2.

Table 7/2.

(a) Volatile halogenated anaesthetics		
Halothane	defluorin.	
Enflurane	defluorin.	
Isoflurane	defluorin.	
Methoxyflurane	defluorin.	
Sevoflurane	defluorin.	
Chemicals:		
(a) Short chain nitrosamines		**4-Methyl-pyrazole**
(a) Small halogenated hydrocarbons	oxidative dehalogen.	3-Amino-1,2,4-triazole
(a) Organic solvents and other chemicals		
Benzene	arom.-OH &	Benzene
Phenol	hydroquinone	Isopropanol
etc.	format.	

Table 7/3.

NATURAL PRODUCTS:
Garlic oil* (diallyl sulfide-sulfone)
Red paper* (dihydrocapsaicin)
Cruciferous veg.* (phenethyliso-thiocianate)

Characteristics:
- -environmentally caused variation and suggested polymorphism;
- -procarcinogens activated are marked with "a";
- -relevance to alcoholism, chemical carcinogenesis, diabetes, tobacco- and alcohol-associated cancers of the head and neck area;
- -some polymorphism observed;
- -extent of variation in level of the enzyme ca. 20-fold;
- -the level elevated at alcoholics;
- -ca 6% of total hepatic CYP.

Location of the enzyme: Liver and other organs (minor in lung).

Legend:
*Inhibition described in rodents and expected to occur also in humans.

TABLE 8. Summary of Information on Human CYP 3A Subfamily of Enzymes

CYP Substrate	Reaction	Inducers	Inhibitors
3A4			
Drugs banned or restricted in sport:			
Stimulants			
Benzphetamine	N-demethyl. (minor)		
Caffeine	C8-OH		
Narcotics			
(Codeine)	N-demethyl.		
Ethylmorphine	N-demethyl.		
Anabolic & Androgen hormones:			
Testosterone	**C6ß-**, C15ß-OH, C2ß-OH, C15α-OH		
Androstenedione	C6ß-OH		
Dehydroepiandrosterone sulphate	C16α-OH		

Table 8/2.

Cocorticosteroids			
Budesonide	C6ß-OH	PCN	
	C16α-prednisolone	Dexamethasone	
Cortisol	C6ß-OH		
Local anesthetics			
(a) Cocaine	N-demethyl.		Cannabidiol
Other drug classes:			
(a) Anesthetics polyhalogenated		Barbiturates	
Methoxyflurane	defluor.		
Sevoflurane	defluor.		
Analgesics,			
Alfentanil	N-dealkyl. (piperidine and amide N)		
Noralfentanil	arom. ring-OH		
Analgesics and Antiinflamatoric			
Antipyrine	C3-, C4-OH		
(a) Acetaminophen	quinone form.		

Table 8/3.

Antiarrhytmics			
Amiodarone	N-deethyl.		
Quinidine	C3-,N-oxid.		
Verapamil			
Antibiotics cytostatic			
(a)Morpholino-doxorubicin			
Antibiotics macrolid.			
Erythromycin	N-demethyl.	**Rifampicin**	Erythromycin
Troleandomycin	N-oxid.	Troleando-mycin	Triacetoyl-oleando-mycin (Troleando-mycin)
Rapamycin	C41-OH & other positions		
Anticoagulants			
(S)-Warfarin	C10-,C14-OH		
(R)-Warfarin	C10-OH		
Antidepressant			
Imipramine	N-demethyl.		
Antiepileptic			
Trimethadone	N-demethyl.	Phenytoin	

Table 8/4.

Antifungials			
Ketoconazole		Clotrimazole	Clotrimazole Fluconazole Itraconazole Ketoconazcle Miconazole
Antihistaminic			
Terfenadine	t-butyl-OH, N-dealkyl.		
Antihypercho-lesterol.			
Lovastatin	C3'- C5'-OH & others		
Antihypertensive			
Losartan	OH-oxid.		
Antileprotic			
Dapsone	N-OH		
Antineoplastics			
Antimytotic			
Taxol	C3'-OH (in 2-phenyl-OH)		

Table 8/5.

Antipsychotic			
Sertindole	N-dealkyl.		
Antitussive			
Dextrome-thorphan	N-demethyl.		
Antiulcer			
Omeprazole	Sulphone format. Sulfide format.		Cimetidine (not inhibiting cyclosporin metab.)
Lansoprazole	Sulphone format.		
Chemotherapeutics			
Sulfametho-xazole	NH_2-OH		
Trimethoprim	N1-oxide		
Hypnotics			
Triazolam			
Midazolam	C1'-,C4-OH		
Immunosuppresants			
CyclosporinA	N-demethyl.		
CyclosporinG	& CH_3-OH		

Table 8/6

Local anesthetics			
Lidocaine (Lignocain)	N-deethyl.		
Muscle relaxant			
Chlorzoxazone	C6-OH		
Neuroleptics			
Phenothiazines	S-oxid.		
Chlorproma-zine	S-oxid.		
Steroid hormones			
Estrogen:	16α-OH	Steroids	17α-ethy-nylestradiol
17α-ethinyl-estradiol (contracept.)	C2-OH		
17ß-estradiol	C2-,C4-OH		
Progestagens:			
Gestodene			**Gestodene** (19-nortesto-sterone derivs., contraceptives)
Progesterone	C6ß-,C6α-OH		

Table 8/7

Sympatholytics	
Dihydroergo-tamine	proline-OH
Tranquilizers	
Alpidem	N-dealkyl.
	Cα-,Cß-OH (propyl)
Benzodiaze-pines (diazepam)	C3-OH
Vasodilators coronary	
Diltiazem	
Felodipine	oxid.
Nifedipine	oxid.
Niludipine	oxid.
Nimodipine	oxid.
Nisoldipine	oxid.
Nitrendipine	oxid.

Chemicals:

(a) Sterigmato-cystin	
(a) 1-Nitropyrene	

Table 8/8.

(a) 6-Nitrochry-sene	
(a) Arylamines	
(a) Heterocyclic amines	
(a) PAH-diols	epoxid.
7,8-BF	epoxid.

Natural products:

(a) Pyrizolidine alkaloids		Flavones	Flavonoids
Senecionine		Tangeritin	Naringenin
		Nobiletin	(grape fruit
		Flavone	juice)
			Myrcetin
			Quercetin
			Kaemferol
Mycotoxins			
(a) Aflatoxins			
AflatoxinB1	(a) 8,9-epoxid., (toxic prod.)	7,8-BF	
	C3α-OH (detoxicat.)		7,8-BF
AflatoxinG1			

Table 8/9

3A5

Drugs banned or restricted in sport:

Stimulants	
Caffeine	C8-OH
Anabolic & androgen hormones:	
Testosterone	C6ß-OH C2ß-OH
Glucocorticiods	
Cortisol	C6ß-OH

Other drug classes:

Antileprotic	
Dapsone	N-OH
Hypnotics	
Midazolam	C1'-,C4-OH
Immunosuppresants	
CyclosporinA	N-demethyl.

Troleandomycin
Gestodene (contracept.)

Table 8/10

Vasodilators coronary	
Nifedipine	oxidation (aromatiz.)

Characteristics of the 3A5 enzyme:
-polymorphism suggested;
-partially reflects 3A4 activity;
-probably not inducible.

Location of the enzyme: GI-tract, adult liver

3A7

Drugs banned or restricted in sport:

Narcotics	
Ethylmorphine	N-demethyl.
(Codeine)	N-demethyl.
Anabolic & androgen hormones	
Dehydroepi-androsterone 3-sulfate	16α-OH
Testosterone	