Studies on Drug-metabolizing Enzymes

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Drugs mean not only medicines but also poisons, pesticides, food additives, cosmetics, cleaning agents, environmental pollutants and so on, which are normally considered foreign to the body. It is important to know what happens to these drugs when they get into the body. In the past the metabolic changes of drugs had been referred to as "detoxication mechanism", but since there are many instances in which drugs are converted in the body to more active substances. Thus, metabolism of drugs is responsible for activation and inactivation of the drugs in the body.

The major reactions in drug metabolism are oxidation, reduction, hydrolysis and conjugation. Of these four areas, most of the attention had been focused on the oxidation. Therefore, in contract of ample literatures on drug-oxidizing enzymes, there were relatively few reports on drug-reducing enzymes. In recent years, however, the reduction has received an increasing interest due to its pharmacological or toxicological significance. The present lecture is organized keeping with a focus on drug-reducing enzymes which have been explored by us and by other groups.

Metabolic reactions of drugs

The majority of drugs undergo definite chemical changes in the animal body resulting in the excretion, usually by the kidney, of specific metabolites. There are, however, some drugs which are not metabolized in the body and are excreted unchanged. Excretion by other channels such as the expired air, the bile, the feces, the saliva and the skin may also occur.

Metabolic reactions of drugs can be divided into four main types, i.e., oxidation, reduction, hydrolysis and conjugation (synthesis). The type of change which occurs depends primarily upon the structure of the drug, but other factors such as species, route of administration and diet may also be involved.

I. Oxidation

Oxidation is one of the most general reactions of drugs in the body. It includes the following reactions: Hydroxylation of aromatic, alicyclic and heterocyclic rings; oxidation of alkyl groups; *O*-, *N*-, and *S*-Dealkylation; epoxidation of arenes and olefins; oxidation of amino and carbamoyl groups; oxidation of sulfides and sulfoxides; oxidation of alcohols and aldehydes; deamination; desulfuration.

II. Reduction

Reduction is usually less common than oxidation, but for several types of drugs, it is a general and important reaction. Reductions which have been observed in the body are the following : Reduction of nitro groups to hydroxylamino and amino groups; split of azo linkages to amino groups; reduction of *N*-oxides to amines; reduction of sulfoxides to sulfides; reduction of *N*-nitroso groups to hydrazino groups; reduction of oximes to ketimines; reduction of *N*-hydroxycarbamoyl groups to carbamoyl groups; reduction of carbon-carbon double bonds; reduction of carbonyl groups to hydroxyl groups; split of disulfide linkages to sulfhydryl groups; reductive dehalogenation.

III. Hydrolysis

A typical example of drug hydrolysis is conversion of esters to carboxylic acids and alcohols or phenols. Hydrolytic reactions observed in the body are the following: Hydrolysis of esters, amides, epoxides, carbamates, hydrazides, sulfates, glucuronides, phosphate and so on.

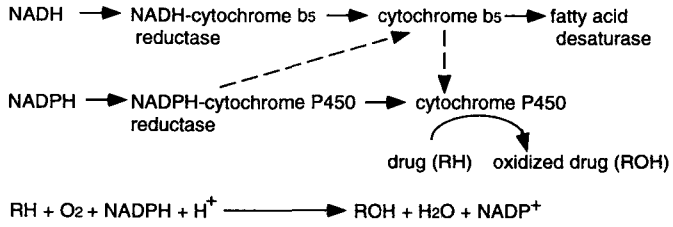
IV. Conjugation

Conjugation processes are mainly reactions involving carbohydrates and amino acids. Whether or not a given drug undergoes any of conjugation depends upon its possessing

particular chemical groups or centers for conjugation. If the drug does not carry such a group, it may acquire one by oxidation or reduction or some other processes. Conjugation processes observed in the body are the following: Conjugation with glucuronic acid, glucose, sulfuric acid, glycine, glutamine, ornithine and glutathione; methylation of amino, hydroxyl and sulfhydryl groups; formation of thiocyanide from cyanide; acylation of amino groups.

Mechanism for biotransformation of drugs

I. Oxidizing enzymes
1) Microsomal mixed-function oxidase (cytochrome P450)
An electron transfer system containing cytochrome P450 in microsomal fraction plays an important role in the oxidation of aromatic carbocyclic rings, alicyclic rings, alkyl chains and carbon-carbon double bonds of drugs.



2) Microsomal FAD-containing monooxygenase
The flavoenzymes in microsomal fraction catalyzes oxidation of tertiary amine *N*-oxides and secondary hydroxylamines, respectively.

3) Aldehyde oxidase and xanthine oxidase
The cytosolic enzymes, which are known as "molybdenum hydroxylases", form a closely related group with similar molecular properties but differ somewhat in substrate specificity. The presence of one or more nitrogen atoms in the aromatic ring makes heterocyclic compounds susceptible to oxidation by aldehyde oxidase and/or xanthine oxidase. As the number of nitrogen atoms in the molecule increases, the molybdenum hydroxylases play a more dominant role in the oxidative biotransformation of these heterocyclic compounds. The enzymes can also catalyze oxidation of aldehydes to the corresponding carboxylic acids.

Microsomal mixed-function oxidase described above catalyzes electrophilic attack involving molecular oxygen. In contract, the oxidation by the molybdenum hydroxylases occurs at the carbon atom adjacent to a ring nitrogen, which is generally the most electropositive carbon. The oxygen atom incorporated into the substrate is ultimately derived from water.

II. Conjugating enzymes
1) UDP glucuronosyltransferase
The enzyme catalyzes conjugation of drugs with glucuronic acid using uridine diphosphate glucuronic acid (UDPGA) as a glucuronic acid donor. This is to be one of the most common of conjugation processes. Drugs which usually form such glucuronides are those which possess hydroxyl, carboxyl, amino and sulfhydryl groups or can form them in the body by oxidation, reduction or some other process.

2) Sulfotransferase
The enzyme catalyzes conjugation of drugs with sulfuric acid using 3'-phosphoadenosine-5'-phosphosulfate(PAPS) as a sulfuric acid donor. This conjugation is a common reaction of phenol in most species of animals and consists of the combination of a phenol with sulfuric acid to form an acid ester of sulfuric acid. Sterols,

alcohols, bile acids, hydroxamic acids and amines in addition to phenols are the substrates of this enzyme.

3) Glutathione transferase
The enzyme catalyzes conjugation of drugs with glutathione. This conjugation is a common reactions of aromatic nitro compounds, halogen-containing organic compounds, alkenes, aromatic hydrocarbons, arenes and olefin epoxides. Glutathione conjugates formed are further metabolized to mercapturic acids (*N*-acetylcysteinyl derivatives)

4) Acetyltransferase
The enzyme catalyzes acetylation of amino groups, which is usually regarded as a general reaction of aromatic amines and hydrazine derivatives.

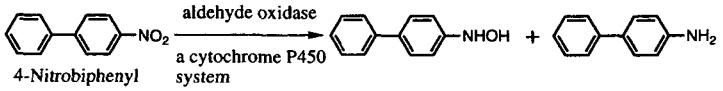
5) Methyltransferase
The enzyme catalyzes methylation of amino, hydroxyl and sulfhydryl groups using *S*-adenosyl-*L*-methionine as a methyl donor.

6) Formamidase
The enzyme catalyzes formylation of aromatic amines using *N*-formyl-*L*-kynurenine as a formyl donor. Formylation as well as acetylation is a common reaction of polycyclic aromatic amines.

III. Hydrolyzing enzymes
Hydrolysis of esters and amides is catalyzed by esterase. Epoxides, glucuronides and sulfates are hydrolyzed by epoxide hydrolase, β-glucuronidase and sulfatase, respectively.

IV. Reducing enzymes
1) Enzymes involved in reduction of aromatic nitro groups
Certain aromatic nitro compounds have been proved to be carcinogenic, some of which have been noticed as important environmental pollutants. The mechanism for carcinogenicity of aromatic nitrocompounds is thought to be mediated by the metabolic reduction of the nitro group to reactive intermediates. The reduction of aromatic nitro compounds occurs with microsomal and cytosolic fractions of mammalian livers. Previous studies shows that a cytochrome P450 system is involved in the reduction of *p*-nitrobenzoic acid catalyzed by rat liver mirosomes. Recently, we showed the first example of enzymatic conversion of 2-nitrofluorene, 4-nitrobiphenyl and 1-nitronaphthalene to the corresponding hydroxylamines together with amines using rabbit liver preparations (1). In addition, the study provides the first evidence that aldehyde oxidase, which is a cytosolic enzyme, functions as a major liver enzyme responsible for the reduction of the aromatic nitro compounds.

[Example]

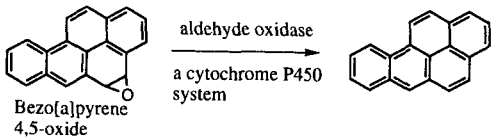


4-Nitrobiphenyl

2) Enzymes involved in reduction of arene oxides
Aromatic hydrocarbons undergo metabolic oxidation to their epoxides, some of which are proximate or ultimate carcinogens. The arene oxides formed are known to be further metabolized to the corresponding dihydrodiols, glutathione conjugates and parent hydrocarbons. Among these metabolic reactions of arene oxides, the reduction back to the parent hydrocarbons was first reported by Booth et al. (2) They demonstrated the NADPH-dependent reduction of several arene oxides by rat liver microsomes. The participation of cytochrome P450 in the microsomal reduction of benzo[a]pyrene 4,5-oxide was indicated by inhibitory effect of carbon monoxide and by reconstitution studies

which showed a requirement for cytochrome P450 and NADPH-cytochrome P450 reductase (3). However, reduction of arene oxides by liver cytosols had not so far been reported. Recently, we demonstrated that mammalian liver cytosols exhibited arene oxide reductase activity originating from aldehyde oxidase, when supplemented with an electron donor of the enzyme using benzo[a]pyrene 4,5-oxide and naphthalene 1,2-oxide as model substrates (4).

[Example]

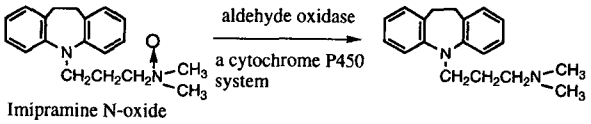


Bezo[a]pyrene 4,5-oxide

3)Enzymes involved in reduction of tertiary amine *N*-oxides

The reduction of tertiary amine *N*-oxides to the corresponding amines is a well known metabolic reaction. Kato and his coworkers (5) reported that the liver *N*-oxide reductase activity toward imipramine *N*-oxide, tiaramide *N*-oxide and *N,N*-dimethylaniline *N*-oxide is mainly located in the microsomes, and that the reduced form of cytochrome P450 is involved in the microsomal *N*-oxide reduction. However, little *N*-oxide reductase activity was found in the liver cytosol. Recently, we examined the reduction of tertiary amine *N*-oxides to the corresponding amines by mammalian liver preparations using imipramine *N*-oxide and cyclobenzaprine *N*-oxide as substrates (6). Rabbit liver cytosol in the presence of an electron donor of aldehyde oxidase exhibited a significant *N*-oxide reductase activity which is comparable to the activity of the liver microsomes supplemented with NADPH. Rabbit liver aldehyde oxidase also exhibited the *N*-oxide reductase activity in the presence of its electron donor, indicating that the activity observed in the liver cytosol is due to this cytosolic enzyme.

[Example]

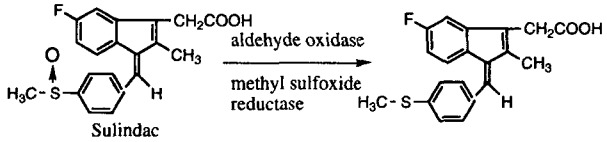


Imipramine N-oxide

4)Enzymes involved in reduction of sulfoxides

Sulfoxides are easily reduced to the corresponding sulfides in animal body. Such sulfoxide reduction is responsible for activation, inactivation and alteration of action of sulfoxides; e.g. the pharmacological activities of sulindac, one of the nonsteroidal antiinflammatory agents, are attributable to the reduction product, sulindac sulfide. However, little had been known about the nature of mammalian sulfoxide reductase. Previously, Anders *et al.* (7) suggested the involvement of thioredoxin in the sulfoxide reduction of sulindac by cytosolic enzyme(s) of rat liver and kidney. Recently, we demonstrated that guinea pig and rabbit liver aldehyde oxidase in the presence of its electron donors functions as a sulfoxide reductase toward sulindac and other sulfoxides (8). More recently, Fukazawa *et al.* (9) reported the purification and properties of methyl sulfoxide reductases from rat kidney, which are different from aldehyde oxidase and responsible for specific reduction of methyl sulfoxides.

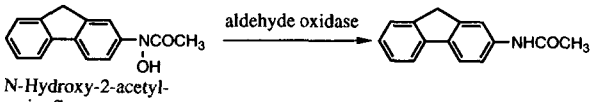
[Example]



Sulindac

5)Enzymes involved in reduction of hydroxamic acids
Hydroxamic acids have been shown to possess a variety of important biological activities, some of which have been used clinically as therapeutic agents. As regards biotransformation, these compounds are known to undergo the enzymatic reduction to the corresponding amides. Early works showed that salicylhydroxamic acids was reduced to salicylamide by rat liver homogenate, nicotinohydroxamic acid to nicotinamide by mouse liver mitochondria, anthranilhydroxamic acid to anthranilamide by rat and mouse liver slices, and certain arylhydroxamic acids to the corresponding amides by liver mitochondria from mammals and birds. However, no report was available describing the nature of the enzyme(s) responsible for the reduction of such hydroxamic acids. Recently, we found that liver aldehyde oxidase can catalyze the reduction of aromatic, heterocyclic and aliphatic hydroxamic acids (10,11).

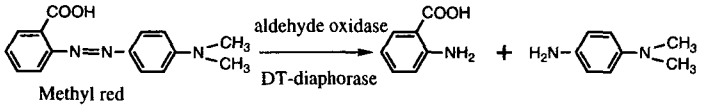
[Example]



N-Hydroxy-2-acetyl-aminofluorene

6)Enzymes involved in reduction of azo linkages
A main metabolic pathway of azo compounds is reductive cleavage of the azo compounds, which is thought to be a detoxication reaction for carcinogenic azo compounds. However, the activation of a recently proposed antineoplastic drug depends on the azo reduction by a reductase so as to produce a short-lived , potent alkylating agent. Studies concerning azoreductase activity in mammalian livers have shown that several different enzymes, depending on substrates used, are responsible for the cleavage of the azo linkage. The reduction of *p*-dimethylaminoazobenzene could be mainly catalyzed by a liver microsomal flavoprotein, NADPH-cytochrome P450 reductase, while that of amaranth was completely dependent on cytochrome P450 (12,13). In addition, neoprontosil was reduced by NADPH-cytochrome P450 reductase or cytochrome P450. On the other hand, the azo reduction of methyl red could be exclusively catalyzed by a liver cytosolic enzyme, DT-diaphorase (14). Our studied provides the first example of aldehyde oxidase functioning as an azoreductase toward some azo compounds (15).

[Example]



Methyl red

7)Aldehyde oxidase-catalyzed other reduction
Oxime and *N*-nitroso groups are also reduced by the enzyme (16,17).

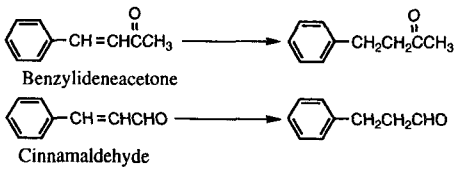
[Example]

Camphor oxime

N-Nitrosodiphenylamine

8)Enzymes involved in reduction of carbon-carbon double bonds
Metabolic reduction of the carbon-carbon double bond *in vivo* or *in vitro* has been known with α,β-ketoalkene, 2-alkenals, stilbene, substituted cinnamates, 7-dehydrocholesterol, 2-enoyl-CoAs, Δ^4-3-ketosteroids, synthetic oral contraceptives, biliverdin, 15-ketoprostaglandins, and so on. However, reports on purification of mammalian enzymes responsible for such double bond reduction had been limited to 2-enoyl-CoA reductase, Δ^4-3-ketosteroid-5- β-reductase, biliverdin reductase, and 15-ketoprostaglandin Δ^{13}-reductase. Recently, we purified an enzyme responsible for reduction of α,β-ketoalkenes to the corresponding ketoalkanes from rat liver cytosol and named the enzyme α,β-ketoalkene double bond reductase (18). The enzyme requires NADPH as an electron donor and can also reduce the double bond of 2-alkenals.

[Example]



9)Properties of aldehyde oxidase (19)
Aldehyde oxidase (EC 1.2.3.1) is one of two molybdenum-containing flavoproteins known to be present in mammalian cells. Like the other mammalian molybdoflavoprotein, xanthine oxidase (EC 1.2.3.2), aldehyde oxidase can catalyze the oxidation of a variety of both aldehyde and *N*-heterocyclic substrates and can utilize a wide range of electron acceptors, including molecular oxygen and inorganic nitrate. Although the ability to oxidize aldehydes was among the first properties of the enzyme to be described, its ability to oxidize nonaldehydic *N*-heterocyclic substrates (purines, pteridines and pyrimidines) is quantitatively more significant.

Aldehyde oxidase and xanthine oxidase have similar internal electron transport systems which are capable of functioning with a wide variety of electron acceptors. Analyses of highly purified preparations indicate that both enzymes contain iron, FAD and molybdenum in the ratios of 4:1:1. Both enzymes have molecular weights near 300,000.

References - (1)Tatsumi,K.et al. (1986) Cancer Res., **46**, 1089. (2)Booth,J. et al. (1975) Xenobiotica, **5**, 197. (3)Sugiura,M. et al. (1980) Cancer Res., **40**, 2910. (4)Hirao,Y. et al.(1994) Carcinogenesis, **15**, 739. (5)Sugiura,M. et al.(1976) Mol.Pharmacol., **12**, 322. (6)Kitamura,S. & Tatsumi,K.(1984) Biochem.Biophys.Res.Commun., **121**, 749. (7)Anders,M.W. et al.(1980) Biochem.Biophys.Res.Commun., **97**, 846. (8)Tatsumi, K. et al.(1983) Biochim.Biophys.Acta, **747**, 86. (9)Fukazawa, H. et al.,(1987) Arch.Biochem.Biophys., **256**, 480. (10)Sugihara,K. & Tatsumi,K. (1986) Arch.Biochem.Biophys., **247**, 289. (11)Kitamura,S. et al. (1994) Biochem.Mol.Inter., **34**, 1197. (12)Mueller,G.C. & Miller,J.A. (1950) J.Biol.Chem., **185**, 145. (13)Fujita,S. & Peisach,J. (1978) J.Biol.Chem., **253**, 4512. (14)Huang,M.T. et al. (1979) J.Biol.Chem., **254**, 11223. (15)Kitamura,S. & Tatsumi,K. (1983) Chem.Pharm.Bull., **31**, 3334. (16)Tatsumi,K. & Ishigai,M. (1987) Arch.Biochem.Biophys., **253**, 413. (17)Tatsumi,K. et al. (1983) Arch.Biochem.Biophys., **226**, 174. (18)Kitamura,S. & Tatsumi,K. (1990) Arch.Biochem.Biophys., **282**, 183. (19)Beedham,C. (1985) Drug Metab.Rev., **16**, 119.