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Role of Drug Metabolism in Drug Research and Development: Factors Affecting Metabolism of Drugs and Their Pharmacological and Toxicological Activity

BITTEN STRIPP▲ and JAMES R. GILLETTE

Abstract □ Drug effects are influenced by binding to target sites, by inhibition or induction of hepatic drug-metabolizing enzymes by various agents, and by the formation of physiologically active metabolites in the liver as well as in extrahepatic tissue. Knowledge of the principles involved provides a better understanding of the pharmacological action of a drug and helps in the design of less toxic drugs.

Keyphrases □ Drug metabolism—role in drug research and development, symposium □ Pharmacological, toxicological activity—effects of binding, hepatic drug-metabolizing enzymes, formation of active metabolites □ Toxicological activity of drugs—effects of binding, hepatic drug-metabolizing enzymes, formation of active metabolites □ Metabolizing enzymes, drug—inhibition, induction, effect on drug metabolism □ Metabolite formation—effect on drug metabolism

Although the pharmacological effect of a drug is determined by a number of factors, such as absorption, distribution, excretion, and metabolism, it is generally terminated by the conversion of the therapeutic agent into nonactive metabolites. Sometimes a change in metabolism may affect the pharmacological activity in a predictable way, and plasma levels of a drug can be directly related to the effect of the drug. But when a compound has to be converted to an active metabolite to exert its activity, as is seen with imipramine and a number of cholinesterase inhibitors of the phosphorothionate type, a correlation between plasma levels of the drug and its activity is usually not observed. Moreover, some drugs during their metabolism can be irreversibly or pseudoirreversibly bound to the target organ and exert their activity long after they are undetected in plasma. This occurs with cholinesterase inhibitors and reserpine. In these cases the complex interplay of the above-mentioned factors that determine the effect of a drug will quite often make it impossible to predict the effects of inhibitors or inducers of drug metabolism on the pharmacological effect of the drug. There are general principles, however, regarding the effects of inhibitors or inducers of drug-metabolizing enzymes on drug action; these principles can be helpful to the pharmacologist who is evaluating a new drug.

Table I—Effect of Phenobarbital on Diazepam Metabolism by Liver Microsomes in Different Animal Species[a]

Species	Increase by Phenobarbital Induction— Hydroxylation Formation of *N*-Methyloxazepam, %	*N*-Demethylation Formation of *N*-Demethyldiazepam, %
Mouse	109	0
Rat	233	323
Guinea pig	0	426

[a] From F. Marcucci, R. Fanelli, E. Mussini, and S. Garrattini, *Biochem. Pharmacol.*, **19**, 1771(1970) (with permission from the authors).

ILLUSTRATION OF GENERAL PRINCIPLES

When a drug is eliminated largely unchanged by the kidneys, inducers or inhibitors of drug metabolism play a negligible role in altering the action of the drug. Thus, inducers and inhibitors do not affect the duration of barbital anesthesia in rats (1). On the other hand, if a drug is rapidly metabolized in the liver, the blood flow rate through the liver may become rate limiting (2); thus, the inhibitors or inducers of drug metabolism may or may not play a significant role in changing the effect of the drug. For example, tremorine and oxotremorine are more rapidly metabolized in rats after intraperitoneal administration than after intravenous administration (3). Thus, if the drug is rapidly absorbed as well as rapidly metabolized, inhibitors have a greater effect when the drug is administered orally or intraperitoneally than when it is administered intravenously. The reason for this is that the initial ratio of total amount of drug to its concentration in the portal vein can be smaller after oral than after intravenous application. This is presumably the reason that desipramine intensifies the action of oxotremorine to rats after intraperitoneal but not after intravenous doses (4).

There are other instances where the action of a drug may not be profoundly altered by inducers or inhibitors. For example, when a highly lipid-soluble drug with a slow metabolism and excretion is given intravenously, the concentration rises rapidly in the highly perfused tissues such as brain and then in the more slowly perfused tissues such as muscle and fat. If during the redistribution phase the plasma level drops below that required for the drug to exert its pharmacological action, one would not expect that the action would be affected by inducers or inhibitors. Thus, the inhibitor proadifen hydrochloride [SK&F 525-A, 2-(diethylamino)ethyl 2,2-diphenylvalerate hydrochloride] does not appreciably prolong the duration of action of thiopental (5).

Therefore, inducers shorten and inhibitors prolong the effect of drugs only when the dominant factor in determining drug action is the hepatic metabolizing enzyme system. Thus, the sleeping time of animals receiving hexobarbital or pentobarbital can be profoundly altered by prior treatment with inducers, such as phenobarbital (6), or by inhibitors, such as proadifen hydrochloride (7).

If only the parent drugs but none of their metabolites exerted pharmacological responses, the pharmacologist would need to study only the rate of metabolism of the drugs and not the formation of metabolites. But metabolites frequently can exert pharmacological responses, so it becomes important to elucidate the pathways of drug metabolism and the relative rates at which the various metabolites are formed and eliminated.

A number of studies have revealed that the relative importance of different pathways of drug metabolism may differ among various animal species. Active metabolites may accumulate in some species but not in others. For example, the antireserpine effects of imipramine persist for a longer time in rats than in mice because desipramine, an active metabolite, accumulates in rats receiving imipramine but not in mice (8). This occurs because desipramine is formed more slowly and metabolized more rapidly in mice than in rats. On the other hand, the antimetrazol effects of diazepam persist for a longer time in mice than in rats because *N*-demethyldiazepam, an active metabolite, accumulates in mice but not in rats (9, 10) (Fig. 1). Studies (11) with the metabolites of diazepam revealed that diazepam is rapidly converted in mice to *N*-demethyldiazepam which, in turn, is slowly converted to oxazepam and then to other metabolites. By contrast, diazepam is slowly converted in rats to *N*-demethyldiazepam which, in turn, is converted to oxazepam slightly more rapidly in rats than it is in mice.

In considering the possible effects of an inducer on the accumulation of active metabolites, it should be kept in mind that inducers can stimulate one pathway of drug metabolism without affecting others. For example, prior treatment of rats with 3-methylcholanthrene preferentially increases the hydroxylation of biphenyl in the *ortho*-position, whereas prior treatment with phenobarbital preferentially increases the hydroxylation of biphenyl in the *para*-position (12). Moreover, inducers can exert diverse effects in different species. For example, prior administration of phenobarbital increases the formation of hydroxylated metabolites of diazepam by liver microsomes from mice; it increases the formation of both hydroxylated and *N*-demethylated metabolites of diazepam by liver microsomes of rats; but it increases the formation of only *N*-demethylated metabolites by liver microsomes of guinea pigs (13) (Table I). Furthermore, inducers are seldom specific and can increase the activity of different kinds of drug-metabolizing en-

Table II—Effect of Spironolactone Pretreatment of Female Rats on Hexobarbital Sleeping Time; on Ethylmorphine, Hexobarbital, and Benzo[*a*]pyrene Metabolism; and on Cytochrome P-450 and Cytochrome *c* Reductase in Female Rat Liver Microsomes

	Sleeping Time[a]	Protein, mg./g. Liver	Ethylmorphine Metabolism, nmoles HCHO/mg. Protein/min.	Hexobarbital Metabolism, nmoles Disappeared/mg. Protein/min.	Benzo[*a*]pyrene Metabolism, nmoles/mg. Protein/min.	Cytochrome P-450, $A_{450-490\ nm.}$/mg. Protein	Cytochrome *c* Reductase, nmoles/mg. Protein/min.
Control	60.0 ± 3.3[b]	20.1 ± 0.57	2.41 ± 0.045	1.19 ± 0.17	0.087 ± 0.0081	0.062 ± 0.0039	105 ± 2
Treated	18.9[c] ± 3.0	19.1 ± 0.86	9.26[c] ± 0.37	2.53[c] ± 0.41	0.161[c] ± 0.013	0.048[c] ± 0.0025	189[c] ± 2

[a] Sleeping time measured after intraperitoneal injection of 80 mg./kg. of hexobarbital as the time difference between the disappearance and reappearance of righting reflex. [b] Values are the mean of five animals ± *SE*. [c] $p < 0.01$ with respect to control.

Table III—Effect of Spironolactone Pretreatment of Male Rats on Hexobarbital Sleeping Time; on Ethylmorphine, Hexobarbital, and Benzo[*a*]pyrene Metabolism; and on Cytochrome P-450 and Cytochrome *c* Reductase in Male Rat Liver Microsomes

	Sleeping Time[a]	Protein, mg./g. Liver	Ethylmorphine Metabolism, nmoles HCHO/mg. Protein/min.	Hexobarbital Metabolism, nmoles Disappeared/mg. Protein/min.	Benzo[*a*]pyrene Metabolism, nmoles Metabolized/mg. Protein/min.	Cytochrome P-450, $A_{450-490\ nm.}$/mg. Protein	Cytochrome *c* Reductase, nmoles/mg. Protein/min.
Control	17.1 ± 4.9[b]	19.4 ± 1.8	11.06 ± 0.80	6.07 ± 0.87	0.298 ± 0.028	0.060 ± 0.003	156 ± 4.7
Treated	19.1 ± 1.8	21.6 ± 0.63	16.3[c] ± 0.5	4.11[c] ± 0.73	0.210[c] ± 0.013	0.055 ± 0.003	268[c] ± 15

[a] Sleeping time measured after intraperitoneal injection of 80 mg./kg. of hexobarbital as the time difference between the disappearance and reappearance of the righting reflex. [b] Values are the mean of five animals ± *SE*. [c] $p < 0.01$.

Table IV—Effect of Steroid Pretreatments of Female Rats on Cytochrome P-450 Content and Metabolism of Hexobarbital and Ethylmorphine

Pretreatment[a]	Cytochrome P-450, $A_{450-490\ nm.}$/ mg. Protein	Hexobarbital Metabolism, nmoles/mg. Protein/min., V_{max}	Ethylmorphine Metabolism, nmoles/mg. Protein/min., V_{max}
Control	0.057 ± 0.001	1.39 ± 0.087	3.53 ± 0.18
Spironolactone	0.050 ± 0.002	3.25 ± 0.33[b]	12.4 ± 0.81[b]
Methyltestosterone	0.046 ± 0.003[b]	3.75 ± 0.57[b]	5.79 ± 0.29[b]
Cortisone acetate	0.048 ± 0.001[b]	2.52 ± 0.34[c]	6.79 ± 0.36[b]

[a] Values in acute studies are the mean of four animals ± *SE;* dose: 100 mg./kg. twice daily for 4 days. [b] $p < 0.03$ with respect to control. [c] $p < 0.05$ with respect to control.

zymes such as the cytochrome P-450 enzymes, glucuronyl transferase, and epoxide hydrase. For these reasons, it is sometimes difficult to predict whether an inducer will increase or decrease the accumulation of active metabolites.

Moreover, inducers can exert diverse effects in male and female rats, as is the case with spironolactone (14) which decreases the hexobarbital sleeping time in females but is without effect in males (Tables II and III). Accordingly, the *in vitro* hydroxylation of hexobarbital and benzo[*a*]pyrene (3,4-benzpyrene) is increased in liver microsomes from female rats but is decreased in microsomes from male rats. The *in vitro* *N*-demethylation of ethylmorphine, however, is increased in both males and females, suggesting that the rate-limiting step in the oxidation by microsomal enzymes may not be the same for all type I substrates.

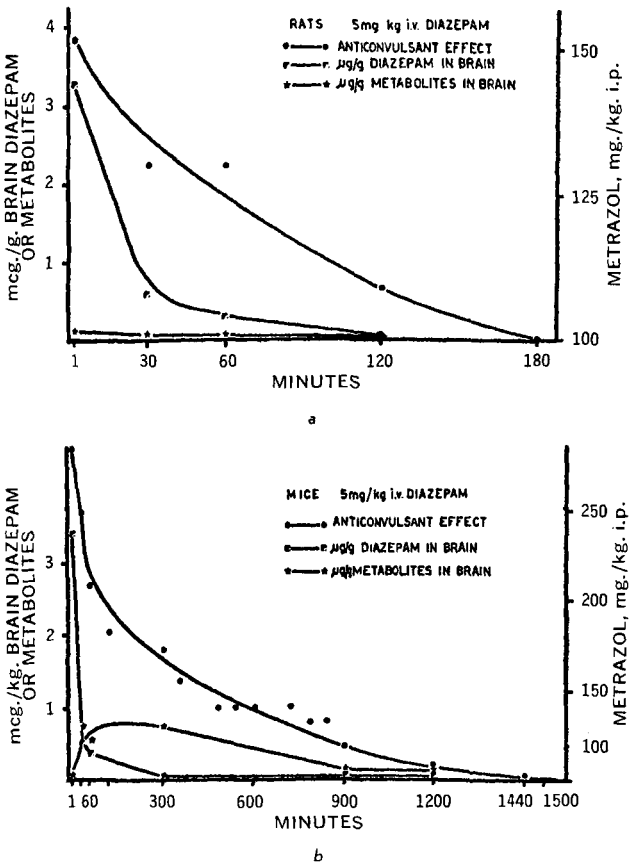

Figure 1—*The points represent the dose of metrazol which was protected by at least 50%. The parameter used for measuring antimetrazol activity was mortality. From S. Garattini, "Metabolism of Diazepam in Animals and Man," CINP Meeting, Tarragona, Spain, 1968 (with the author's permission).*

Table V—Effect of Steroid Pretreatments of Male Rats on Cytochrome P-450 Content and Metabolism of Hexobarbital and Ethylmorphine

Pretreatment[a]	Cytochrome P-450, $A_{450-490\ nm.}$/ mg. Protein	Hexobarbital Metabolism, nmoles/mg. Protein/min., V_{max}	Ethylmorphine Metabolism, nmoles/mg. Protein/min., V_{max}
Control	0.061 ± 0.006	8.79 ± 0.59	8.50 ± 0.95
Spironolactone	0.054 ± 0.004	3.33 ± 1.11[b]	12.6 ± 0.63[c]
Methyltestosterone	0.064 ± 0.006	6.18 ± 0.61[c]	11.8 ± 1.08
Cortisone acetate	0.031 ± 0.002	6.08 ± 0.21[b]	8.32 ± 0.95

[a] Values in acute studies are the mean of four animals ± *SE;* dose: 100 mg./kg. i.p. twice daily for 4 days. [b] $p < 0.01$ with respect to control. [c] $p < 0.05$ with respect to control.

Spironolactone is also an example of an inducer that can exert its effect without changing the level of cytochrome P-450 (14), which is often used as an indicator for enzyme induction. Similarly, pretreatment with other steroids such as methyltestosterone or cortisone acetate can increase the metabolic activity as measured *in vitro* in liver microsomes from female rats but decrease the activity in microsomes from males; in both sexes, however, decreased or unchanged levels of cytochrome P-450 are observed (unpublished results) (Tables IV and V).

Besides exerting different effects in different species and sexes, the inducers can also be selective in their choice of tissue. The benzo[*a*]pyrene hydroxylase activity, which is characteristically induced by polycyclic hydrocarbons, seems more readily induced by 7,12-dimethylbenzanthracene in lung than in liver of rats with respect to time and dose[1]. Moreover, induction in male rats with 3-methylcholanthrene makes the benzo[*a*]pyrene hydroxylase activity in liver inhibitable by 7,8-benzoflavone, a compound that in uninduced rats inhibits the hydroxylase activity only in lungs and kidneys but not in liver (15). Thus, the metabolism in extrahepatic tissues, which usually is not thought to be of importance compared to that in the liver, might be of vital significance if highly reactive alkylating agents are formed by extrahepatic enzymes. For example, the bone marrow damage caused by 7,12-dimethylbenzanthracene may be caused by a covalent bond formed in this tissue. In accord with this view, Suria *et al.* (16) found that proadifen hydrochloride completely prevented the toxicity of 7,12-dimethylbenzanthracene in the bone marrow and partially prevented the formation of the covalent bonding. In working with inhibitors like proadifen hydrochloride or 7,8-benzoflavone, however, one must keep in mind that they both exert inducing effects on drug metabolism at a later stage after their administration (6, 17, 18), but the time between the inhibitory and inducing stages may not necessarily be the same in hepatic and extrahepatic tissue.

The effects of inhibitors and inducers of drug metabolism on tissue damage by certain drugs may be difficult to predict. Indeed, one inducer may potentiate while another may ameliorate the effect of a toxicant. For example, the damaging effects of carbon tetrachloride on the microsomal enzymes are enhanced by phenobarbital induction (19) but partially prevented by 3-methylcholanthrene induction (20, 21). The former may act by enhancing the metabolism and thereby the formation of free radicals of carbon tetrachloride, which are believed to be responsible for the damaging effects (22), but the protective effect of 3-methylcholanthrene still remains obscure. Similarly, phenobarbital increases and 3-methylcholanthrene decreases the centrolobular necrosis seen after bromobenzene administration to rats. It now seems likely that bromobenzene causes necrosis by being converted to its epoxide, which then becomes covalently bound to macromolecules in the liver (23). After phenobarbital induction, bromobenzene metabolism and covalent bonding to the necrotic areas increased. Most of the covalent bonding occurred at 12–24 hr. after administration (24), a time when glutathione was decreased in the liver[2]. It seems likely,

[1] Unpublished observation, Blaszkowski, Stripp, and Bogdanski, Fifth International Congress on Pharmacology, San Francisco, Calif, 1972.

[2] N. G. Zampagalione, D. J. Jollow, J. R. Mitchell, and J. R. Gillette, in preparation.

Table VI—Effects of Phenobarbital or 3-Methylcholanthrene Pretreatment on the Urinary Metabolites of Bromobenzene[a]

Pretreatment	Dose, nmoles/kg. Body Weight	Bromophenylmercapturic Acid	*p*-Bromophenol	*o*-Bromophenol	Bromocatechol + Bromobenzene Dihydrodiol
			Percent of Total 48-hr. Excretion		
None	10.0	46 ± 4	38 ± 4	5 ± 1	10 ± 3
3-Methylcholanthrene	10.0	30 ± 4	20 ± 3	22 ± 4	26 ± 3
Phenobarbital	1.50	46 ± 4	36 ± 4	2 ± 1	16 ± 1

[a] Values are the mean of 12 animals ± *SE*.

therefore, that glutathione becomes completely depleted only in the centrolobular region where necrosis takes place. It is also in this region that the proliferation of the endoplasmic reticulum is observed after phenobarbital induction (25). After 3-methylcholanthrene induction, the hepatotoxicity of bromobenzene is completely prevented (26), even though the 3-methylcholanthrene slightly increases bromobenzene metabolism[3]. But analysis of the metabolites in urine showed that this induction also increases the formation of bromobenzene dihydrodiol (27) (Table VI). These results suggested that after the centrolobular region is depleted of glutathione, bromobenzene epoxide can be inactivated by epoxide hydrase, thereby preventing covalent bonding and necrosis. This relatively different enhancement seen after phenobarbital or 3-methylcholanthrene induction of the activity of the glutathione-conjugating system, the epoxide hydrase, and the activity of the cytochrome P-450 system that forms the epoxide thus determines the different effects of the two inducers.

In conclusion, it is difficult to predict whether inducers of drug metabolism will shorten and inhibitors will prolong the action of a drug. The knowledge of the metabolic pattern and the pharmacological and/or toxicological effects of the metabolites of the drug becomes of great importance in helping to understand and sometimes to predict the interaction of different drugs.

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