Jap. J. Pharmac. **19**, 438–450 (1969)

SPECIES DIFFERENCE IN THE INHIBITION OF DRUG METABOLISM BY LIVER MICROSOMES BY DIFFERENT INHIBITORS

RYUICHI KATO, KINICHI ONODA AND MICHIKO TAKAYANAGI

Department of Pharmacology, National Institute of Hygienic Sciences, Setagaya-ku, Tokyo

Received for publication March 17, 1969

It has been reported that the activities of drug-metabolizing enzymes of liver microsomes are inhibited by different kind of drugs (1). SKF 525-A (β-diethylaminoethyl-diphenylpropylacetate HCl) was discovered for the first time and it has been known as the most popular and typical inhibitor of microsomal drug-metabolizing enzymes (2–4), while DPEA (2, 4-dichlorophenylphenoxyethylamine HCl) has been known as the most potent inhibitor (5–6). Moreover, Kato *et al.* (1, 7, 8) reported that the inhibitors of the microsomal enzymes, such as SKF 525-A, DPEA and MG 3062 (phenyl-(4-chlorophenyl)-4-piridylmethanol) increase the activities of drug-metabolizing enzymes, on the other hand, the inducers of the microsomal enzymes, such as chlorcyclizine, glutethimide and phenaglycodol inhibit the activities of drug-metabolizing enzymes.

It has been established that a number of commonly used drugs of high lipid-solubility inhibit the activities of drug-metabolizing enzymes and the *in vivo* metabolism of various drugs (1, 9–11). The inhibition in the metabolism of drugs seems to be popular phenomenon in combined administration of more than two drugs and sometimes even mutual inhibition may be occurred, therefore, these phenomena are important for the evaluation of drug activity and for the determination of dosage schedule in drug therapy (1). However, the mechanism by which many drugs produce the inhibition of the activities of drug-metabolizing enzymes has not yet been elucidated (12, 13). Netter (14) observed that SKF 525-A noncompetitively inhibited the O-demethylation of *o*-nitroanisole. In contrast, McMahone (5) reported that SKF 525-A and DPEA competitively inhibited the N-demethylation of butynamine. Moreover, Rubin *et al.* (11) reported that SKF 525-A competitively inhibited the N-demethylations of morphine and ethylmorphine. In a previous work (15), we briefly reported that N-demethylation of aminopyrine and hydroxylation of hexobarbital in rat liver microsomes were competitively inhibited by SKF 525-A, while they were noncompetitively inhibited by SKF 525-A in rabbit liver microsomes.

The present communication is concerned with the species difference in the inhibition of various pathways of drug metabolisms in liver microsomes by different inhibitors. The

加藤　隆一・小野田　欽一・高柳　美智子

differences in the type and potency of the inhibition between rats, rabbits and mice may offer some insight for the mechanism of the inhibition of drug-metabolizing enzymes.

MATERIALS AND METHODS

Male rats of Wistar strain, weighing about 200 g, *dd* strain male mice weighing about 25 g and male rabbits weighing about 2.5 kg were used. The animals were killed by decapitation and the livers were removed, chopped into small pieces, washed well, and homogenized with 4 volumes of 1.15% KCl solution in a Teflon-glass homogenizer. The homogenate was centrifuged at $9,000 \times g$ for 20 minutes. The supernatant solution was then centrifuged at $105,000 \times g$ for 1 hour, and the microsomes were suspended in 1.15% KCl solution. All procedures were carried out under 0–4°C.

Assays of activities of drug-metabolizing enzymes: Typical incubation mixture consisted of $9,000 \times g$ supernatant (2.0 ml) equivalent to 500 mg of liver, 20 μmoles of glucose-6-phosphate, 0.8 μmoles of NADP, 50 μmoles of nicotinamide, 50 μmoles of $MgCl_2$, 1.4 ml of 0.2 M sodium phosphate buffer (pH 7.4), various substrate (5 μmoles of aminopyrine, aniline, *p*-nitroanisole, phenacetin, morphine, meperidine, cocaine, diphenhydramine, N-methylaniline; 3 μmoles of hexobarbital; 1.5 μmoles of pentobarbital; 2 μmoles of zoxazolamine), and water to a final volume of 5.0 ml. In some experiments, microsomal fraction was used instead of $9,000 \times g$ supernatant, in this case, 40 μmoles glucose-6-phosphate, 1.2 μmoles of NADP and 1.5 U of glucose-6-phosphate dehydrogenase were added. The mixtures were incubated at 37°C for 30 minutes under air.

The hydroxylation of hexobarbital was determined by measuring the disappearance of the substrate according to the method of Cooper and Brodie (16). The hydroxylation of zoxazolamine was determined by measuring the disappearance of the substrate according to the method of Conney *et al.* (17). The oxidation of pentobarbital was determined by measuring the disappearance of the substrate according to the method of Brodie *et al.* (18). The N-demethylation of aminopyrine was determined by the formation of 4-aminoantipyrine according to the method of La Du *et al.* (19). The N-demethylation of morphine, meperidine, cocaine and diphenhydramine was determined by the formation of formaldehyde according to the method of Nash (20). Semicarbazide (50 μmoles) was added for trapping of formaldehyde formed. The hydroxylation of aniline was determined by the formation of *p*-aminophenol as described by Kato and Gillette (21). The O-deethylation of phenacetin was determined by the formation of N-acetyl-*p*-aminophenol by the method of Axelrod *et al.* (22).

The metabolic activities were expressed as millimicromole of the substrates metabolized or metabolites formed per gram wet weight of liver per 30 minutes. The inhibitory action was expressed as the concentration of 50 percent inhibition. For the analysis of enzyme kinetic data, reciprocal velocities were plotted against reciprocal substrate concentration according to Lineweaver and Burk (23) described by Dixon and Webb (24).

RESULTS

1. Kinetic study on the inhibition of aminopyrine N-demethylation in liver microsomes of rats, rabbits and mice by SKF 525-A, DPEA, chlorcyclizine and U-16392-A

The N-demethylation of aminopyrine in liver microsomes of rats was competitively inhibited by SKF 525-A, DPEA, chlorcyclizine and U-16392-A (*o*-chlorisopropylphenylhydrazine) (Fig. 1). However, in strict means the inhibitory types of SKF 525-A, DPEA,

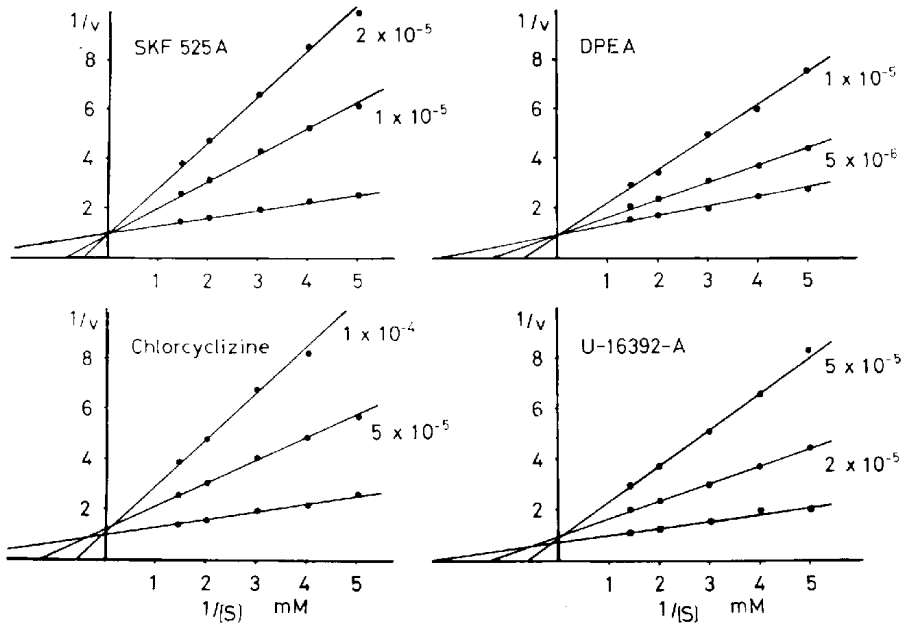

FIG. 1. Inhibition of N-demethylation of aminopyrine in liver microsomes of rats by SKF 525-A, DPEA, chlorcyclizine and U-16392-A.

The results was expressed by plotting of the N-demethylation according to Lineweaver-Burk.

v = velocity of N-demethylation (μmole/g/30 min)

S = concentration of aminopyrine

chlorcyclizine and U-16392-A were not exactly competitive; in some experiments, SKF 525-A caused quasi-competitive mixed-type inhibition. These mixed-type inhibitions were observed more frequently by chlorcyclizine and U-16392-A. For example, the averages of the cross-point between the inhibitor line and noninhibitor line from 3 to 5 experiments were not exactly on the 1/v axis (1/s=0), but they are on −0.07 mM, −0.05 mM, −0.04 mM and −0.5 mM, respectively, for SKF 525-A, DPEA, chlorcyclizine and U-16392-A. In contrast, as shown in Fig. 2 the N-demethylation of aminopyrine in liver microsomes of rabbits was noncompetitively inhibited by SKF 525-A, DPEA, chlorcyclizine and U-16392-A. Moreover, the N-demethylation of aminopyrine in liver microsomes of mice was inhibited quasi-competitively by SKF 525-A, DPEA, chlorcyclizine and U-16392-A (Fig. 3). When the high concentration of the inhibitors was used the inhibitory types had a tendency to move for more competitive. The Km for the N-demethylation

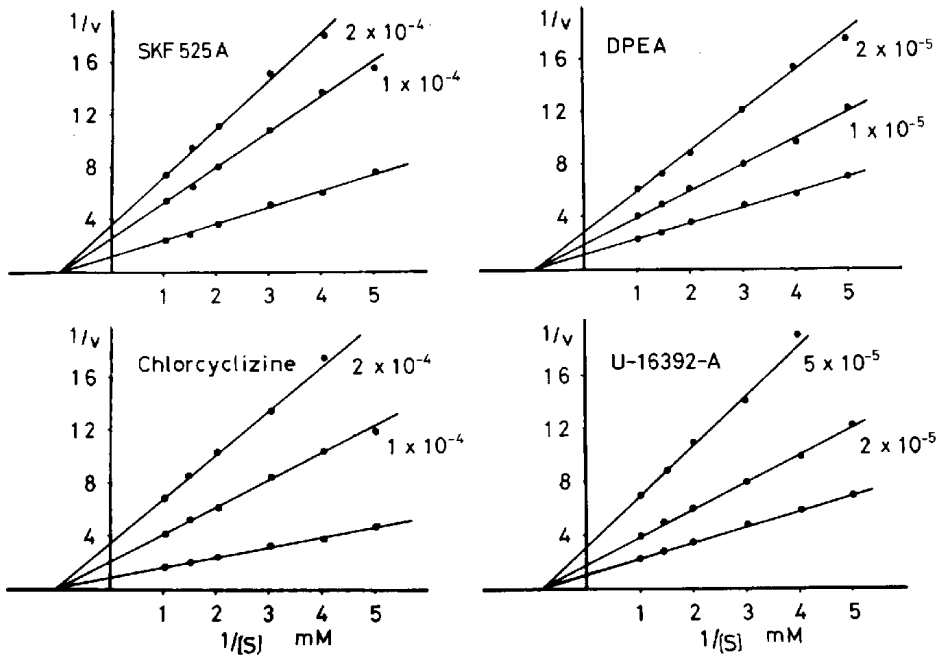

FIG. 2. Inhibition of N-demethylation of aminopyrine in liver microsomes of rabbits by SKF 525-A, DPEA, chlorcyclizine and U-16392-A.
See the legends for Fig. 1.

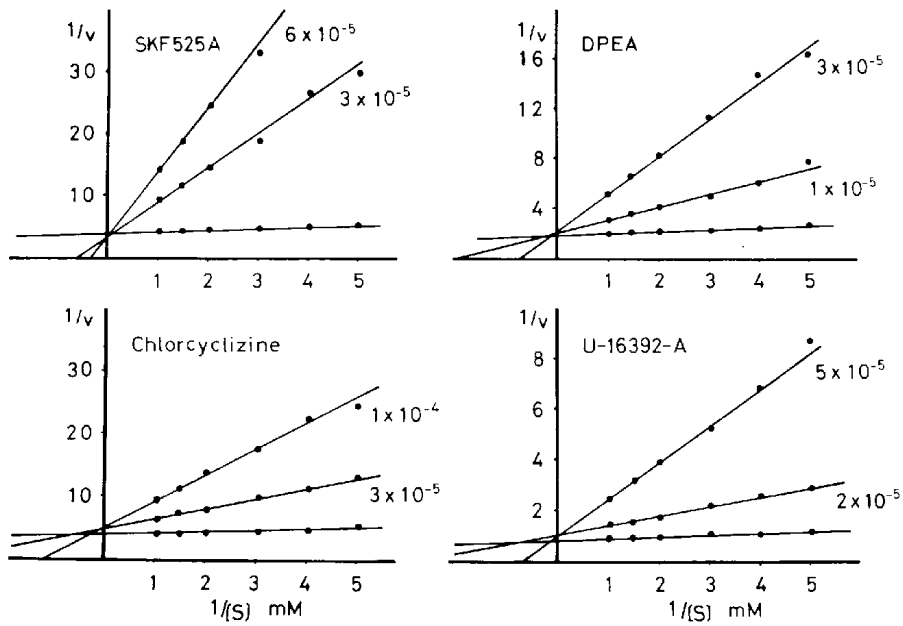

FIG. 3. Inhibition of N-demethylation of aminopyrine in liver microsomes of mice by SKF 525-A, DPEA, chlorcyclizine and U-16392-A.
See the legends for Fig. 1.

of aminopyrine in liver microsomes of rats was 3.6×10^{-4}M, while in liver microsomes of rabbits and mice the Km were 1.1×10^{-3}M and 7.1×10^{-5}M, respectively. Moreover, 2×10^{-5}M of SKF 525-A produced 65% inhibition of N-demethylation of aminopyrine (1×10^{-3}M) in rats, while it produced inhibition of 21% and 38%, respectively, in mice and rabbits. Similar results were obtained by employing microsomal fraction instead of $9{,}000\times g$ supernatant, but the inhibitory activities with microsomal fraction were 20 to 30% higher than those with $9{,}000\times g$ supernatant.

2. Kinetic study on the inhibition of hexobarbital hydroxylation in liver microsomes of rats, rabbits and mice by SKF 525-A, DPEA, chlorcyclizine and U-16392-A

The hydroxylation of hexobarbital in liver microsomes of rats was competitively inhibited by SKF 525-A, DPEA, chlorcyclizine and U-16392-A (Fig. 4). However, if strictly speaking, the inhibitory types of SKF 525-A, DPEA, chlorcyclizine and U-16392-A were not exactly competitive; in some experiments, these inhibitors caused inhibition of quasi-competitive "mixed-type". As previously stated on the N-demethylation of aminopyrine, the inhibitions of hexobarbital hydroxylation by chlorcyclizine and U-16392-A were more similar to mixed-type than those by SKF 525-A and DPEA. In contrast, as shown in Fig. 5 the hydroxylation of hexobartibal in liver microsomes of rabbits was noncompetitively inhibited by SKF 525-A, DPEA, chlorcyclizine and U-16392-A. Whereas, the hydroxylation of hexobarbital in liver microsomes of mice was inhibited quasi-competitively by SKF 525-A, DPEA, chlorcyclizine and U-16392-A. The Km for the hydroxylation of hexobarbital in liver microsomes of rats was 6.2×10^{-4}M, while in liver

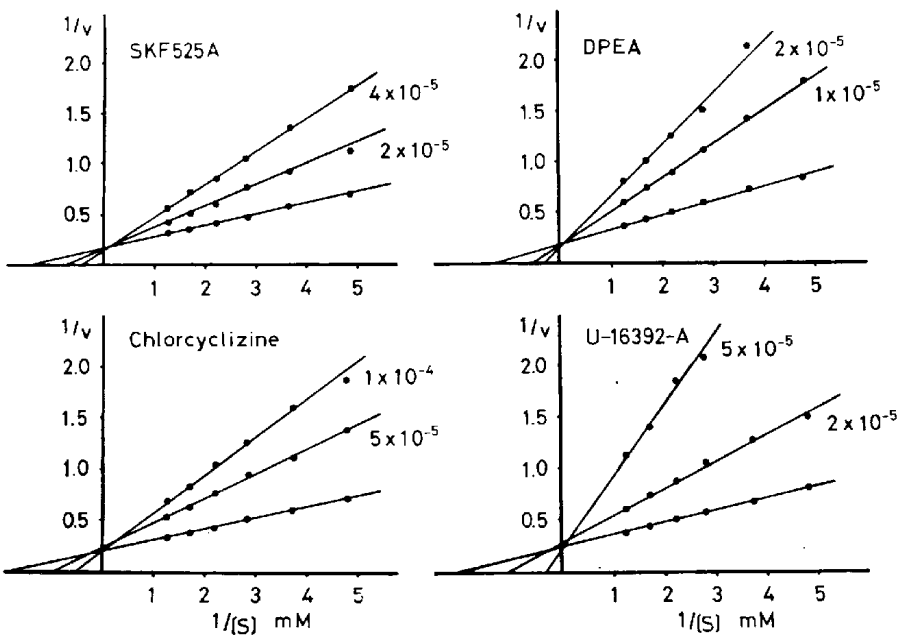

FIG. 4. Inhibition of hydroxylation of hexobarbital in liver microsomes of rats by SKF 525-A, DPEA, chlorcyclizine and U-16392-A.
See the legends for Fig. 1.

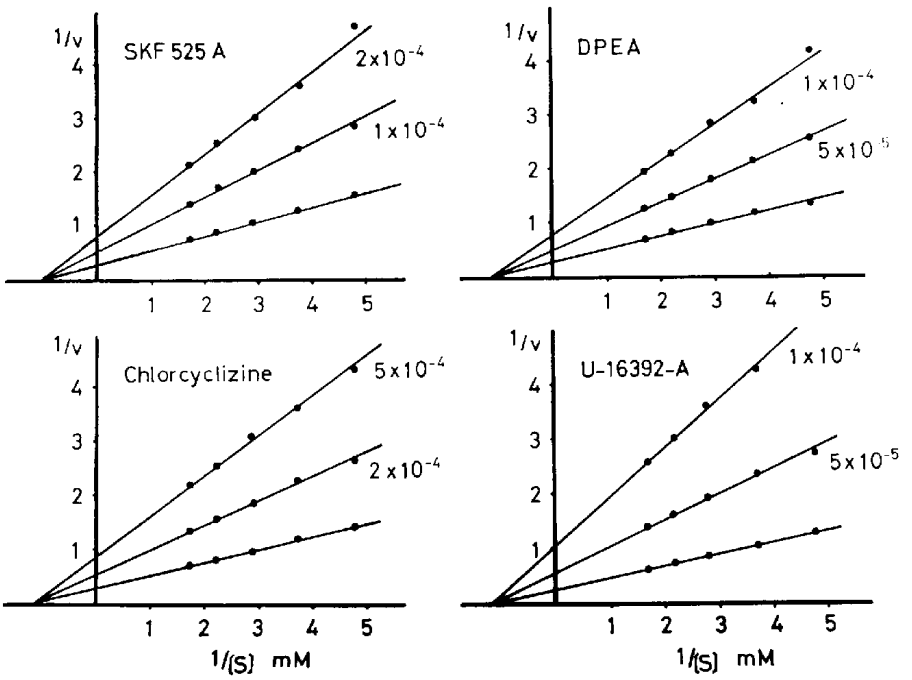

FIG. 5. Inhibition of hydroxylation of hexobarbital in liver microsomes of rabbits by SKF 525-A, DPEA, chlorcyclizine and U-16392-A.
See the legends for Fig. 1.

microsomes of rabbits and mice the Km were 8.5×10^{-4}M and 2.5×10^{-4}M, respectively. Moreover, 1×10^{-4}M of SKF 525-A produced 82% inhibition of the hydroxylation of hexobarbital in rats, while it produced inhibitions of 38% and 42%, respectively, in rabbits and mice.

3. Kinetic study on the inhibition of p-nitroanisole O-demethylation in liver microsomes of rats, rabbits and mice by SKF 525-A, DPEA, chlorcyclizine and U-16392-A

Netter (14) reported that SKF 525-A noncompetitively inhibited the O-demethylation of *o*-nitroanisole in liver microsomes of rats. Thus, it was of interest to investigate whether O-demethylation of *o*-nitroanisole in rat liver microsomes is inhibited competitively or noncompetitively by SKF 525-A. As shown in Fig. 6, the inhibitory types of SKF 525-A, DPEA, chlorcyclizine and U-16392-A in the O-demethylation of *p*-nitroanisole by rat liver microsomes were mixed-type. The inhibitory type of SKF 525-A was similar to competitive, whereas that of U-16392-A was similar to noncompetitive. DPEA and chlorcyclizine showed intermediate type. The higher concentrations of SKF 525-A, DPEA, chlorcyclizine and U-16392-A had a tendency to show more competitive type inhibition than did the lower concentrations. A similar results were also obtained by using *o*-nitroanisole as the substrate. Therefore, we could not conclude from the present investigations whether the inhibitory action of SKF 525-A on the O-demethylations of *o*-nitroanisole and *p*-nitroanisole is competitive or noncompetitive. In further experiments, noncom-

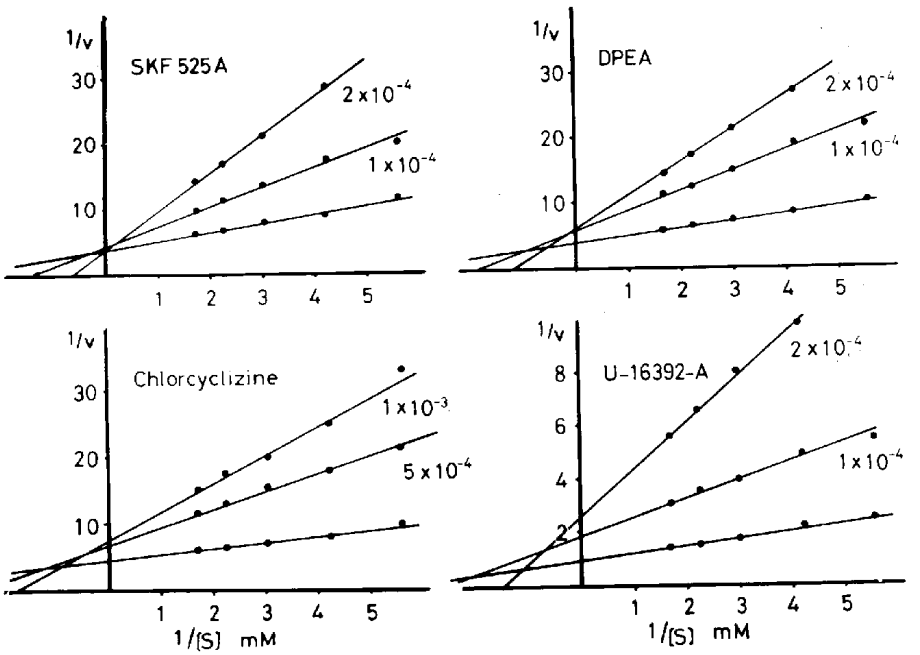

FIG. 6. Inhibition of O-demethylation of *p*-nitroanisole in liver microsomes of rats by SKF 525-A, DPEA, chlorcyclizine and U-16392-A.
See the legends for Fig. 1.

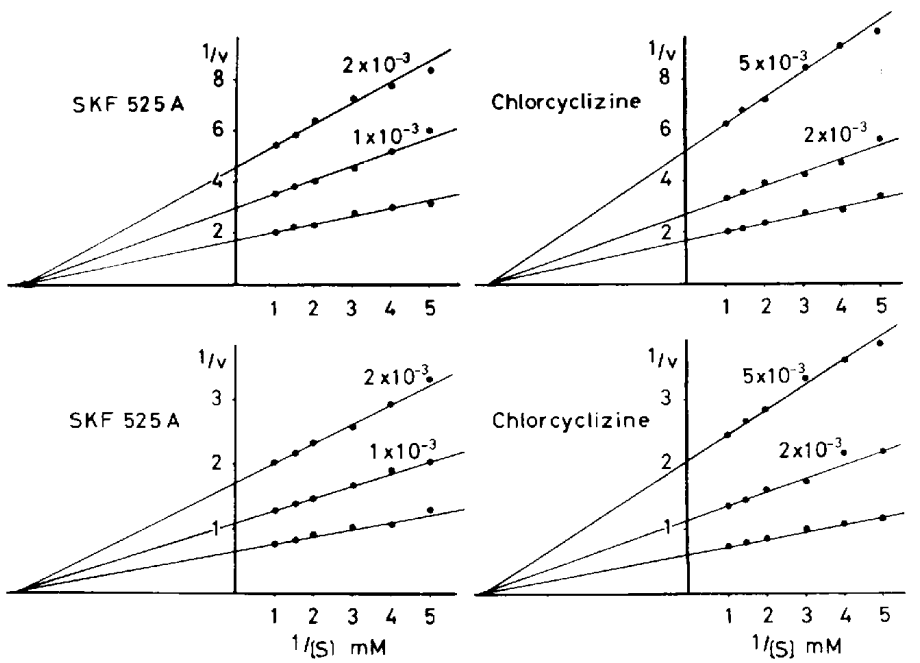

FIG. 7. Inhibition of hydroxylation of aniline by liver microsomes of rats and mice by SKF 525-A and chlorcyclizine.
The upper parts of the figure are rat liver microsomes and the lower parts are mouse liver microsomes.

petitive type inhibitions were obtained with mouse and rabbit liver microsomes by SKF 525-A and DPEA.

4. Kinetic study on the inhibition of aniline hydroxylation in liver microsomes of rats, rabbits and mice by SKF 525-A and chlorcyclizine

The hydroxylation of aniline by liver microsomes was noncompetitively inhibited by SKF 525-A and chlorcyclizine in rats as well as in mice (Fig. 7). The Km for aniline hydroxylation in rats and mice were 2.0×10^{-4}M and 1.8×10^{-4}M, respectively. Similarly, the hydroxylation of aniline by rabbit liver microsomes was noncompetitively inhibited by SKF 525-A and chlorcyclizine. The Km for aniline hydroxylation in rabbits was 4.0×10^{-4}M. These results are in accordance with recent observation of Ikeda *et al.* (25). They reported that the hydroxylation of aniline by guinea-pig liver microsomes was noncompetitively inhibited by SKF 525-A.

5. Difference among rats, mice and rabbits in the inhibitory effect of SKF 525-A on the drug-metabolizing enzymes

The inhibitory effect of SKF 525-A on the metabolism of various drugs in rats, mice and rabbits was given in Table 1. There were marked species differences in the 50% inhibitory concentration of SKF 525-A. The 50% inhibitory concentrations of SKF 525-A seemed to be lowest in rats and highest in rabbits, but the magnitudes of the species difference were depended on the substrate and metabolic pathway. For example, the

TABLE 1. Difference among rats, mice and rabbits in the inhibition of drug-metabolizing enzymes in liver microsomes by SKF 525-A.

Substrate	Metabolic pathway	50% Inhibitory concentration of SKF 525-A (M)		
		in rats	in mice	in rabbits
Hexobarbital	Aliphatic hydroxylation	5.5×10^{-5}	1.3×10^{-4}	1.4×10^{-4}
Pentobarbital	Aliphatic hydroxylation	2.1×10^{-5}	4.5×10^{-5}	9.5×10^{-5}
Aminopyrine	N-Demethylation	1.5×10^{-5}	5.1×10^{-5}	9.5×10^{-5}
Morphine	N-Demethylation	4.8×10^{-5}	6.5×10^{-5}	1.1×10^{-4}
Meperidine	N-Demethylation	4.2×10^{-5}	6.8×10^{-5}	1.0×10^{-4}
Cocaine	N-Demethylation	8.8×10^{-5}	5.0×10^{-4}	8.3×10^{-4}
Diphenhydramin	N-Demethylation	4.5×10^{-5}	6.7×10^{-5}	2.2×10^{-4}
p-Nitroanisole	O-Demethylation	2.8×10^{-4}	7.7×10^{-4}	2.1×10^{-3}
Phenacetin	O-Deethylation	1.7×10^{-4}	4.5×10^{-4}	5.1×10^{-4}
Aniline	Aromatic hydroxylation	8.1×10^{-4}	9.5×10^{-4}	1.7×10^{-3}
Zoxazolamine	Aromatic hydroxylation	2.0×10^{-4}	5.1×10^{-4}	8.3×10^{-4}

The results are given as the average concentrations of SKF 525-A which produce 50% inhibition obtained from 3–6 experiments. The average values (mμmole/g liver/30 min) of normal animals as follows : Hexobarbital hydroxylation : rats 3528, mice 1429, rabbits 1966 ; pentobarbital hydroxylation ; rats 625, mice 294, rabbits 513 ; aminopyrine N-demethylation ; rats 489, mice 225, rabbits 438 ; morphine N-demethylation ; rats 873, mice 293, rabbits 158 ; meperidine N-demethylation ; rats 2453, mice 1358, rabbits 1423 ; cocaine N-demethylation ; rats 720, mice 1025, rabbits 285 ; diphenhydramine N-demethylation ; rats 1877, mice 723, rabbits 1428 ; *p*-nitroanisole O-demethylation ; rats 208, mice 484, rabbits 643 ; phenacetin O-deethylation ; rats 548, mice 455, rabbits 1052 ; aniline hydroxylation ; rats 525, mice 1258, rabbits 878 ; zoxazolamine hydroxylation ; rats 414, mice 985, rabbits 781.

magnitudes of species differences between rats and rabbits in the 50% inhibitory concentrations of SKF 525-A for aminopyrine N-demethylation and pentobarbital hydroxylation were 6.3 and 4.5 times, respectively, whereas that of the inhibitory concentration for the aniline hydroxylation was only 1.5 times.

6. Difference among rats, mice and rabbits in the inhibitory effect of DPEA on the drug-metabolizing enzymes

The inhibitory effect of DPEA on the metabolism of various drugs in rats, mice and rabbits was given in Table 2. There were clear species differences as observed with SKF 525-A, in the 50% inhibitory concentration of DPEA. In agreement with previous report (6) the inhibitory effects of DPEA were higher than those of SKF 525-A in rats. Moreover, it was observed in the present experiments that the inhibitory effects of DPEA in mice and rabbits were also higher than those of SKF 525-A. Similar results as obtained with SKF 525-A were observed in the magnitude of species differences in the inhibitory effect of DPEA in relation to the substrate and metabolic pathway.

TABLE 2. Difference among rats, mice and rabbits in the inhibition of drug-metabolizing enzymes in liver microsomes by DPEA.

Substrate	Metabolic pathway	50% Inhibitory concentration of DPEA (M)		
		in rats	in mice	in rabbits
Hexobarbital	Aliphatic hydroxylation	1.9×10^{-5}	2.5×10^{-5}	5.6×10^{-5}
Pentobarbital	Aliphatic hydroxylation	6.9×10^{-6}	1.9×10^{-5}	4.7×10^{-5}
Aminopyrine	N-Demethylation	7.0×10^{-6}	1.4×10^{-5}	1.2×10^{-5}
Morphine	N-Demethylation	1.5×10^{-5}	2.2×10^{-5}	5.1×10^{-5}
Meperidine	N-Demethylation	1.0×10^{-5}	1.7×10^{-5}	4.2×10^{-5}
Cocaine	N-Demethylation	5.3×10^{-5}	8.9×10^{-5}	2.0×10^{-4}
Diphenhydramine	N-Demethylation	9.2×10^{-6}	1.9×10^{-5}	8.8×10^{-5}
p-Nitroanisole	O-Demethylation	1.2×10^{-4}	2.9×10^{-4}	3.2×10^{-4}
Phenacetin	O-Deethylation	8.5×10^{-5}	1.1×10^{-4}	3.1×10^{-4}
Aniline	Aromatic hydroxylation	2.1×10^{-4}	3.1×10^{-3}	5.0×10^{-4}

See the legends for Table 1.

TABLE 3. Difference among rats, mice and rabbits in the inhibition of drug-metabolizing enzymes in liver microsomes by chlorcyclizine.

Substrate	Metabolic pathway	50% Inhibitory concentration of chlorcyclizine (M)		
		in rats	in mice	in rabbits
Hexobarbital	Aliphatic hydroxylation	8.5×10^{-5}	1.6×10^{-4}	1.5×10^{-4}
Pentobarbital	Aliphatic hydroxylation	5.0×10^{-5}	2.2×10^{-4}	1.4×10^{-4}
Aminopyrine	N-Demethylation	5.4×10^{-5}	1.3×10^{-4}	1.2×10^{-4}
p-Nitroanisole	O-Demethylation	6.1×10^{-4}	1.5×10^{-3}	7.7×10^{-4}
Phenacetin	O-Deethylation	1.4×10^{-4}	5.9×10^{-4}	4.7×10^{-4}
Aniline	Aromatic hydroxylation	2.8×10^{-3}	3.1×10^{-3}	3.5×10^{-3}
Zoxazolamine	Aromatic hydroxylation	5.5×10^{-4}	6.2×10^{-4}	7.1×10^{-4}

See the legends for Table 1.

7. Difference among rats, mice and rabbits in the inhibitory effect of chlorcyclizine on the drug-metabolizing enzymes

The inhibitory effect of chlorcyclizine on the metabolisms of various drugs in rats, mice and rabbits was given in Table 3. The species differences in the inhibitory action of chlorcyclizine were not clear and the inhibitory action seemed to be somewhat higher in rabbits than in mice. The reason for these differences between the inhibitory action SKF 525-A and chlorcyclizine is not clear.

8. Difference among rats, mice and rabbits in the inhibitory effect of U-16392-A on the drug-metabolizing enzymes

The inhibitory effect of U-16392-A on the metabolisms of various drugs in rats, mice and rabbits was given in Table 4. In contrast to SKF 525-A, only small species differences were observed in the inhibitory action of U-16392-A. Since the mechanism of inhibitory action of U-16392-A has been assumed to be differ from that of SKF 525-A (26), the difference between SKF 525-A and U-16392-A may be attributed to the difference in the inhibitory mechanisms.

TABLE 4. Difference among rats, mice and rabbits in the inhibition of drug-metabolizing enzymes in liver microsomes by U-16392-A.

Substrate	Metabolic pathway	50% Inhitory concentration of U-16392-A		
		in rats	in mice	in rabbits
Hexobarbital	Aliphatic hydroxylation	5.9×10^{-5}	7.1×10^{-5}	8.9×10^{-5}
Pentobarbital	Aliphatic hydroxylation	2.1×10^{-6}	3.2×10^{-5}	2.8×10^{-5}
Aminopyrine	N-Demethylation	1.8×10^{-5}	2.2×10^{-5}	2.8×10^{-5}
Morphine	N-Demethylation	2.8×10^{-5}	2.9×10^{-5}	3.1×10^{-5}
Meperidine	N-Demethylation	4.1×10^{-5}	4.5×10^{-5}	7.5×10^{-5}
Cocaine	N-Demethylation	8.1×10^{-5}	1.0×10^{-5}	2.1×10^{-4}
Diphenhydramine	N-Demethylation	5.9×10^{-5}	6.7×10^{-5}	8.8×10^{-5}
p-Nitroanisole	O-Demethylation	1.4×10^{-4}	1.3×10^{-4}	3.1×10^{-4}
Phenacetin	O-Deethylation	1.4×10^{-4}	1.2×10^{-4}	2.3×10^{-4}
Aniline	Aromatic hydroxylation	2.3×10^{-4}	3.1×10^{-4}	4.0×10^{-4}
Zoxazolamine	Aromatic hydroxylation	1.2×10^{-4}	1.6×10^{-4}	3.0×10^{-4}

See the legends for Table 1.

TABLE 5. Amount of tissue on the inhibitory effect of SKF 525-A on the metabolism of aminopyrine N-dimethylation and hexobarbital hydroxylation.

Amount of tissue (g)	50% Inhibitory concentration (M)	
	Aminopyrine N-demethylation	Hexobarbital hydroxylation
0.1	3.4×10^{-6}	9.8×10^{-6}
0.2	7.1×10^{-6}	2.0×10^{-5}
0.4	1.4×10^{-5}	4.2×10^{-5}
0.6	2.1×10^{-5}	6.5×10^{-5}

The results given as the average concentrations of SKF 525-A which produce 50% inhibition obtained from 3-4 experiments.

9. Inhibitory effect of SKF 525-A in relation to the tissue concentration

The inhibitory effects of SKF 525-A on the N-demethylation of aminopyrine and the

hydroxylation of hexobarbital clearly depended on the amount of 9,000×*g* supernatant in the incubation mixture (Table 5). The 50% inhibitory concentration of SKF 525-A for aminopyrine N-demethylation was decreased to about one-sixth as the amount of 9,000×*g* supernatant was decreased to one-sixth. Similar results were obtained with microsomes. Moreover, similar differences related to the tissue concentration were observed with mice and rabbits. The difference in the inhibitory effects of SKF 525-A is probably related to the nonspecific binding of SKF 525-A with liver microsomes (27).

DISCUSSION

Lipid soluble foreign compounds are metabolized by the mechanism of mixed-function oxygenase in liver microsomes (13, 27). However, the detailed mechanism of this mixed-function oxygenase has not yet been elucidated and involvements of the reduction of NADPH-specific flavoprotein and the oxygenation of cytochrome P-450 only have been established (13, 28, 29). Therefore, the mechanism of inhibition of SKF 525-A and other drugs on the mixed-function oxygenation has not yet been completely unknown (13).

The inhibitors of the mixed-function oxygenation are also the substrate of this enzymic process (1) and mutual competitive inhibitions in rat liver microsomes have been reported (5, 11). Rubin *et al.* (11) have suggested that the inhibition of SKF 525-A and other drugs on the mixed-function oxygenation is attributed to the competition for "active oxygen" between two substrates.

However, the present investigation showed that the inhibitory type of SKF 525-A and other drugs was not only competitive, but also noncompetitive and mixed-type according to the substrate and metabolic pathway and to the species of animals used. In some case, the inhibitory types of SKF 525-A and other drugs seemed to be altered by the concentration of inhibitor. These results suggest that the mechanism of the inhibition of the mixed-function oxygenation seems to be complicated and not only due to the competition for "active oxygen" (oxygenated cytochrome P-450). In fact, SKF 525-A inhibits the biosynthesis of proteins (30) and cholesterol (31) and glucuronyltransferase (4, 25). Brodie (32) has suggested that SKF 525-A may have a physiochemical effect on the microsomal membrane to alter its permeability to drugs. The stabilization of red cell membrane by SKF 525-A was recently demonstrated by Lee *et al.* (33). These results suggest that SKF 525-A has multiple actions and may has nonspecific effects on the microsomal membrane.

The kinetic analysis of enzyme inhibition is usually carried out with crude or purified enzymes (24). On the other hand, the experiments of the present and all the other studies were performed on the microsomes or 9,000×*g* supernatant and moreover the process of the mixed-function oxygenation is complicated. It is, thus, reasonable that the inhibitory types of SKF 525-A and other drugs may be altered according to the experimental condition as shown in the present studies. Moreover, it has been reported that many drugs, including SKF 525-A binds nonspecifically to liver microsomal proteins or phospholipids and apparently effective concentration is dependent on the experimental condition

(13, 27, 34, 35). These all evidences thus, indicate that it contains many danger to drow the conclusion for the mechanism of inhibition of drug-metabolizing enzymes from the study on the inhibitory enzyme kinetics.

However, the enzyme kinetic study may have potential usefulness in the pharmacological meanings for the interpretation of the results on the inhibition of drug metabolism. On the other hand, the species difference was observed not only in the enzyme kinetic studies, but also in the potency of the inhibitors. Thus, the results of the present experiments, provided an important evidence for the evaluation and interpretation of the pharmacological activities and toxicities of combined drugs in relation to the rates of their metabolisms.

SUMMARY

The inhibitions of drug metabolisms by liver microsomes by SKF 525-A, DPEA, chlorcyclizine and *o*-chlorisopropylphenylhydrazine were studied in rats, mice and rabbits.

The inhibitory type of SKF 525-A was competitive in rats for aminopyrine N-demethylation and hexobarbital hydroxylation, but noncompetitive type of the inhibition was observed for aniline hydroxylation. The inhibition of *p*-nitroanisole seemed to be mixed-type. By contrast, the inhibitory type of SKF 525-A was noncompetitive in rabbits for aminopyrine N-demethylation, hexobarbital hydroxylation and aniline hydroxylation. Whereas, the inhibitory type of SKF 525-A was quasi-competitive in mice for aminopyrine N-demethylation and hexobarbital hydroxylation. Similar tendency was observed with DPEA, chlorcyclizine and *o*-chlorisopropylphenylhydrazine, but mixed-type inhibition was often observed depending on the substrates and inhibitors used.

In addition, there were marked species differences in the intesity of the inhibitory effects of SKF 525-A and other inhibitors. SKF 525-A usually more effective in rat liver microsomes than in mouse and rabbit microsomes. Similar results, but even to less extensive, were observed with the other inhibitors.

REFERENCES

1) KATO, R., CHIESARA, E. AND VASSANELLI, P.: *Biochem. Pharmac.* **13,** 69 (1964)
2) COOK, L., TONER, J.J. AND FELLOWS, E.J.: *J. Pharmac. exp. Ther.* **111,** 131 (1954)
3) AXELROD, J., REICHENTHAL, J. AND BRODIE, B.B.: *J. Pharmac. exp. Ther.* **112,** 49 (1954)
4) COOPER, J.R., AXELROD, J. AND BRODIE, B.B.: *J. Pharmac. exp. Ther.* **112,** 55 (1964)
5) MCMAHONE, R.E.: *J. Pharmac. exp. Ther.* **138,** 382 (1962)
6) KATO, R., VASSANELLI, P. AND CHIESARA, E.: *Biochem. Pharmac.* **12,** 353 (1963)
7) KATO, R., CHIESARA, B. AND VASSANELLI, P.: *Med. Exp.* **6,** 254 (1962)
8) KATO, R., VASSANELLI, P. AND CHIESARA, E.: *Experientia* **18,** 453 (1962)
9) DIXON, R.L. AND FOUTS, J.R.: *Biochem. Pharmac.* **11,** 715 (1962)
10) KATO, R., CHIESARA, E. AND VASSANELLI, P.: *Biochem. Pharmac.* **12,** 357 (1963)
11) RUBIN, A., TEPHLY, T.R. AND MENNARING, G.J.: *Biochem. Pharmac.* **13,** 1053 (1964)
12) NETTER, K.J.: *Proc. Ist Intern. Pharmac. Meeting*, Sotckholm, 1961, Vol. **6**, p. 213, Pergamon Press, Oxford (1962)

13) GILLETTE, J.R.: *Adv. Pharmac.* **4,** 219 (1966)
14) NETTER, K.J.: *Arch. exp. Path. Pharmak.* **238,** 292 (1960)
15) KATO, R. AND TAKAYANAGI, M.: *Jap. J. Pharmac.* **16,** 127 (1966)
16) COOPER, J.R. AND BRODIE, B.B.: *J. Pharmac. exp. Ther.* **114,** 409 (1955)
17) CONNEY, A.H., TROUSOF, N. AND BURNS, J.J.: *J. Pharmac. exp. Ther.* **128,** 333 (1960)
18) BRODIE, B.B., BURNS, J.J., MARK, L.C. AND LIEF, P.A.: *J. Pharmac. exp. Ther.* **109,** 26 (1953)
19) LA DU, B.N., GAUDETTE, L., TROSOF, N. AND BRODIE, B.B.: *J. biol. Chem.* **193,** 265 (1951)
20) NASH, I.: *Biochem. J.* **55,** 416 (1953)
21) KATO, R. AND GILLETTE, J.R.: *J. Pharmac. exp. Ther.* **150,** 279 (1965)
22) BRODIE, B.B. AND AXELROD, J.: *J. Pharmac. exp. Ther.* **94,** 22 (1948)
23) LINEWEAVER, H. AND BURK, D.: *J. Am. Chem. Soc.* **56,** 658 (1934)
24) DIXON, M. AND WEBB, E.C.: *Enzymes,* 2nd ed., Academic Press, New York (1964)
25) IKEDA, M., TANAKA, S. AND KATAYAMA, T.: *Mol. Pharmac.* **4,** 38 (1968)
26) KATO, R., TAKANAKA, A. AND SHOJI, H.: *Jap. J. Pharmac.* **19,** 315 (1969)
27) GILLETTE, J.R.: *Progress Drug Res.*, Edited by JUCKER, E., Vol. **6**, p. 13, Birkhauser, Basel (1963)
28) OMURA, T., SATO, R., COOPER, D.Y., ROSENTHAL, O. AND ESTABROOK, R.W.: *Fedn Proc.* **24,** 1181 (1965)
29) KATO, R.: *J. Biochem.* **59,** 574 (1966)
30) NEUBERT, D. AND TIMMLER, R.: *Arch. exp. Path. Pharmak.* **238,** 358 (1960)
31) HOLMES, W.L. AND BENTZ, J.D.: *J. biol. Chem.* **235,** 3118 (1960)
32) BRODIE, B.B.: *J. Pharm. Pharmac.* **8,** 1 (1956)
33) LEE, P.I., YAMAMURA, H.I. AND DIXON, L.R.: *Biochem. Pharmac.* **17,** 1671 (1968)
34) ERNSTER, L. AND ORRENIUS, S.: *Fedn Proc.* **24,** 1190 (1965)
35) SOYKA, L.F.: *Proc. Soc. exp. Biol. Med.* **128,** 322 (1968)