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ACUTE INHIBITION OF OXIDATIVE DRUG METABOLISM BY PROPOXYPHENE (DARVON)

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Abstract—Propoxyphene is a potent inhibitor of the hepatic, microsomal mixed-function oxidases, acting in a manner similar to the prototype inhibitor of drug metabolism, SKF 525-A (β-diethylaminoethyl-2,2-diphenylvalerate), which it resembles chemically. Propoxyphene ($K_i = 4.6 \pm 0.9 \times 10^{-5}$ M) and SKF 525-A ($K_i = 4.0 \pm 1.1 \times 10^{-6}$ M) inhibited competitively the activity of aminopyrine *N*-demethylase in washed microsomes from mouse liver. Both drugs were considerably weaker, noncompetitive inhibitors of the hydroxylation of aniline by microsomes. They manifested typical type I binding spectra to cytochrome P-450, propoxyphene binding approximately 15 per cent as avidly as SKF 525-A ($K_s = 6.9 \pm 0.5 \times 10^{-5}$ M for propoxyphene; $9.1 \pm 1.8 \times 10^{-6}$ M for SKF 525-A). When propoxyphene and SKF 525-A were injected i.p. in equimolar (0.26 m-mole/kg) doses, both depressed the *N*-demethylation of aminopyrine and the hydroxylation of aniline by the 10,000 *g* supernatant fraction of mouse liver removed 0.5 hr post-administration. Propoxyphene was 30–40 per cent as potent as SKF 525-A as an inhibitor of these activities. When potassium ferricyanide (50 μM) was added to suspensions of hepatic microsomes from animals pretreated with propoxyphene or SKF 525-A, the complexes formed between the drugs and cytochrome P-450 were destroyed. This same equimolar dose of propoxyphene and SKF 525-A prolonged hexobarbital sleeping time and zoxazolamine paralysis time, propoxyphene again being 30–40 per cent as potent as SKF 525-A in these tests. Chronically, propoxyphene increased the rate of its own metabolism as well as that of aminopyrine and aniline, and levels of cytochrome P-450 and microsomal protein. Propoxyphene thus seems to act in a manner very similar to that of SKF 525-A, acting as a potent inhibitor of microsomal drug metabolism when given acutely and as an inducer when given chronically.

Propoxyphene (Darvon) is one of the most widely prescribed drugs in the world, accounting yearly for some 18 million prescriptions in the United States alone [1]. Propoxyphene is a relatively safe drug when given under proper medical supervision, especially in relation to its extensive use. However, recent reports have indicated that propoxyphene has considerable abuse potential [1, 2]. It can also be extremely toxic, especially in conjunction with other depressant drugs [3–6]. Propoxyphene was implicated in some 1300 deaths over a 3-year period, according to a Drug Enforcement Administration survey of drug crisis centers and hospital emergency rooms [1]. McBay and Hudson [4, 5] estimate that plasma concentrations of propoxyphene together with other depressants that exceed 0.1 mg/100 ml and of propoxyphene alone that exceed 0.2 mg/100 ml can cause death. As a result of these reports of the involvement of propoxyphene in over-dose deaths, the United States Food and Drug Administration has recommended recently that propoxyphene be controlled under Schedule IV of the Controlled Substances Act [1].

To prevent misuse and increase safety, the water insoluble salt, propoxyphene napsylate (PN), was introduced in 1971 [7]. Presumably, the slower rate of gastric absorption resulting in lower blood levels of propoxyphene, and the slow rate at which these levels are attained account for the increased safety of PN. The availability of the more slowly absorbed napsylate salt also allowed its introduction as a chemotherapeutic alternative to methadone in narcotic detoxification and maintenance programs [8–10]. Previous work involving the administration of the HCl salt to narcotic-dependent patients indicated that the high doses (approximately 1200 mg/day, p.o.) needed to suppress withdrawal sickness were extremely toxic [11]. In recent years, clinicians using the napsylate salt reported that these high doses seemed to be without any untoward effects and still suppressed withdrawal signs [8–10]. Rossiter [12] reported that many clinicians may be prescribing PN in high doses for heroin detoxification, perhaps because no federal licensure is required.

We observed recently that orally administered PN would successfully block the narcotic withdrawal signs normally seen upon removal of subcutaneously implanted morphine pellets from mice [13, 14]. Surprisingly, while a dose of 5–600 mg/kg of PN would suppress withdrawal signs with no obviously discernible toxic effects, subsequent administration of PN, 12–18 hr later at the same dose, would cause severe respiratory depression, convulsions and death in approximately 30–40 per cent of the mice. This observation suggested to us that propoxyphene and/or its metabolite(s) could be acting as a long-term inhibitor of its own metabolism by the hepatic, microsomal mixed-function oxidases (MFO) that biotransform propoxyphene [15] and other drugs [16]. We further suggested [13, 14] that, given the close chemical similarity of propoxyphene to SKF 525-A (β-diethylaminoethyl-2,2-diphenylvalerate), a widely used model inhibitor of the MFO, such a finding would not be surprising (Fig. 1).

In this report, we present evidence that propoxy-

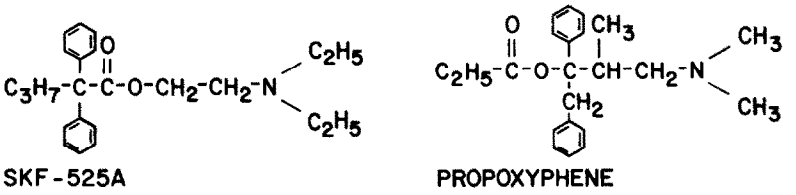

Fig. 1. Structural formulae of SKF 525-A and propoxyphene.

phene is indeed a potent inhibitor of the MFO, approximately 25–40 per cent as potent as the prototypical inhibitor of drug-metabolizing enzymes, SKF 525-A. A large percentage of propoxyphene-related deaths have occurred in conjunction with other drugs [2–5], and our observations provide evidence that deaths related to propoxyphene abuse could be, in part, a consequence of pharmacokinetic drug interactions in which propoxyphene (or metabolites), by inhibiting the metabolism of other drugs, may produce more profound pharmacological effects than those produced by the sum of the pharmacodynamic effects of the drugs.

MATERIALS AND METHODS

Animals. Male NIH/Swiss mice, 22–28 g, were used in all experiments and obtained from the Laboratory Supply Co., Indianapolis, IN. Animals had free access to food and water until 24 hr before death, after which time they were allowed access to water only. They were kept on a 24-hr light–dark cycle (12 hr of each). Cages were cleaned every 2 days to avoid accumulation of urine and feces which is known to depress drug-metabolizing activity [17].

Chemicals. *d*-Propoxyphene HCl and *d*-propoxyphene napsylate were gifts of Eli Lilly & Co., Indianapolis, IN. SKF 525-A HCl was generously donated by Smith, Kline & French Laboratories, Philadelphia, PA. Hexobarbital and zoxazolamine were supplied by Sterling–Winthrop Research Institute, Rensselaer, NY, and McNeil Laboratories, Fort Washington, PA, respectively. Aminopyrine was purchased from the Aldrich Chemical Co., Milwaukee, WI. Econofluor and 2-[^{14}C]pentobarbital were supplied by the New England Nuclear Co., Boston, MA. All other reagents and drugs were purchased from the Sigma Chemical Co.. St. Louis, MO.

Tissue preparations. Livers were homogenized in 3 vol. of 0.05 M Tris–HCl–0.15 M KCl, pH 7.4. Microsomes were prepared from the 10,000 *g* supernatant fraction by centrifugation at 105,000 *g* for 1 hr. The microsomal pellet was resuspended in a volume equal to that of the 10,000 *g* supernatant fraction and washed once with the same homogenization buffer by centrifugation at 105,000 *g* for 45 min. Enzyme assays and cytochrome P-450 determinations were made on the day of sacrifice.

Enzyme assays. The activities of aminopyrine *N*-demethylase, aniline hydroxylase and propoxyphene *N*-demethylase were determined using the basic procedure described by Fouts [18], except for the omission of nicotinamide, which we found would inhibit activity by 10–20 per cent. Tris–HCl (0.1 M, pH 7.4) was the buffer. Optimal substrate concentrations were systematically determined to be 1.4 mM for aminopyrine, 2 mM for aniline and 2.4 mM for propoxyphene HCl. All reactions were found to be linear with time for at least 20 min. Washed microsomes from 250 mg liver were used in all assays involving kinetic constants. All values for kinetic constants represent the mean ± S.E.M. of at least three determinations. Other assays (see text) were carried out with aliquots of the 10,000 *g* supernatant fraction prepared from 250 mg liver as the enzyme source. Formaldehyde (HCHO) was the product determined when either aminopyrine or propoxyphene was the substrate of the mixed-function oxidation [19]. In assays involving the use of an added inhibitor that could generate HCHO, aminoantipyrine concentration was assessed as the product of aminopyrine *N*-demethylation [20]. The rate of aniline hydroxylation was determined by analysis of *p*-aminophenol, using method 2 described by Mazel [20].

Protein and cytochrome P-450. Protein concentrations were determined according to the procedure of Lowry *et al.* [21]. Cytochrome P-450 was determined according to the method in [20], except that 0.05 M Tris–HCl–0.15 M KCl served as the buffer for homogenization and for washing and making suspensions. Microsomal suspensions were diluted to 1–2 mg/ml of protein for determinations of cytochrome P-450 and the spectral dissociation constant (K_s).

Formation of cytochrome P-450–drug complexes. Oxidation by potassium ferricyanide [$K_3Fe(CN)_6$] was used to examine the cytochrome P-450–drug complexes formed after administration of SKF 525-A or propoxyphene [22, 23]. Microsomal suspensions (3.0 ml, 1–2 mg protein/ml) from control and drug-treated animals were incubated with 50 μM $K_3Fe(CN)_6$ for 3 min. Determinations of cytochrome P-450 content were then carried out as described above.

Clearance of [^{14}C] *pentobarbital.* The method of Shuster and Hannan [24] was used to estimate blood levels of 2-[^{14}C] pentobarbital. Fifteen-μl blood samples were collected from the excised tops of the animals' tails. The unmetabolized [^{14}C] pentobarbital was extracted from the blood into 1 ml of 0.2 M NaH_2PO_4 and back extracted directly into the scintillation fluid (toluene-based Econofluor) for counting. Shuster and Hannam [24] reported that this procedure compared favorably to the analytical technique of Brodie *et al.* [25] used to assess blood concentrations of pentobarbital. Statistical differences between groups were determined by Student's *t*-test. Lines were drawn and intercepts determined according to the method of least squares linear regression.

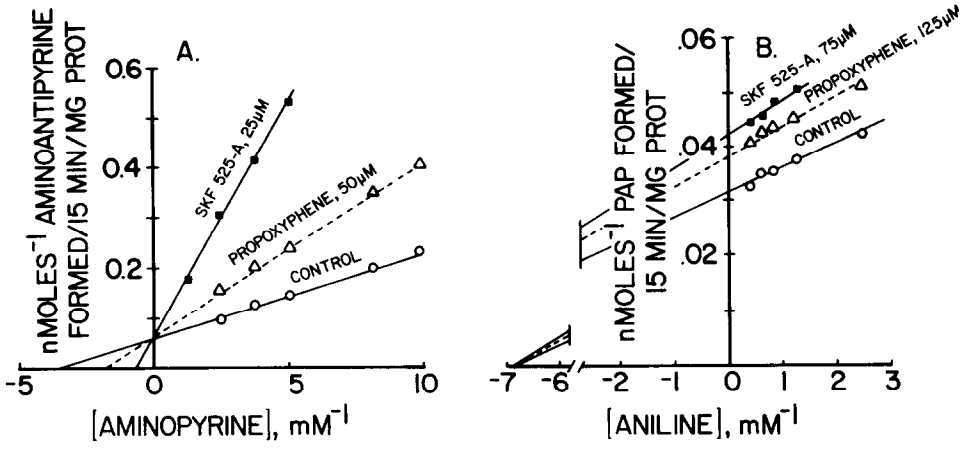

Fig. 2. Washed microsomes from ten pooled livers of untreated mice were assayed for *N*-demethylase activity using graded concentrations of aminopyrine as substrate (A) or for hydroxylase activity using graded concentrations of aniline as substrate (B). Assays were run with and without the inhibitors SKF-A HCl and propoxyphene HCl at the indicated concentrations. Each point represents the mean of triplicate determinations.

RESULTS

In vitro *experiments*. The capability of propoxyphene to inhibit the *in vitro* biotransformation of representative substrates of the microsomal mixed-function oxidases (MFO) was compared to that of SKF 525-A, the prototype of inhibitors of microsomal drug metabolism [26]. Propoxyphene proved to be a potent inhibitor of the *n*-demethylation of the type I substrate, aminopyrine (Fig. 2A). The inhibition was competitive in nature, the K_i for propoxyphene being approximately one order of magnitude greater than that determined for SKF-A, which also competitively inhibited the oxidation of aminopyrine (Table 1). Both compounds were much less potent inhibitors of the hydroxylation of the type II substrate, aniline (Fig. 2B). The inhibitory constant for propoxyphene was approximately three times greater than the one measured for SKF 525-A (Table 1). The inhibition of aniline hydroxylase was noncompetitive for both compounds. For purposes of comparison, phenobarbital, an MFO substrate that we have previously shown not to be a strong inhibitor of drug metabolism [14], was also incubated with microsomes at concentrations up to 200 μM, and no inhibition of either aminopyrine *N*-demethylase activity or aniline hydroxylase activity was seen.

Another kinetic parameter, the spectral dissociation constant (K_s), a measure of binding to cytochrome P-450 [27], was obtained for both propoxyphene and SKF 525-A (Table 1). Both compounds bind strongly to cytochrome P-450, propoxyphene binding approximately 15 per cent as avidly as SKF 525-A, and manifest typical type I spectra with absorption maxima at 386–388 nm and minima at 422–424 nm. The data obtained in these experiments for SKF 525-A compare favorably to inhibitory constants and spectral dissociation constants reported in the literature [26].

In vivo *experiments*. The relatively low inhibitory constants and K_s obtained for propoxyphene suggested that this drug would bind tightly to enzymic sites and that inhibition could be demonstrated by the acute administration of the drug to a living animal and assessment of MFO activities [28]. The results of this experiment are in Table 2. Equimolar doses of SKF-A, phenobarbital and propoxyphene HCl were administered in an identical manner, i.p. Both SKF 525-A and propoxyphene depressed the two activities. Inhibition of the metabolism of the type I substrate was more pronounced than inhibition of the metabolism of the type II compound. *In vivo*, propoxyphene was seen to be approximately 30–40 per cent as potent an inhibitor as SKF 525-A. Equimolar phenobarbital gave no inhibition of either activity.

Propoxyphene was also compared to SKF 525-A (and phenobarbital) with respect to its capability to prolong the pharmacological action of selected agents. Hexobarbital was used as a type I and zoxazolamine as a type II drug [29]. SKF 525-A prolonged hexobarbital sleeping time and zoxazolamine paralysis time 8- and 6-fold, respectively, compared to 5- and 4-fold

Table 1. Inhibitory (K_i) and spectral dissociation (K_s) constants for SKF 525-A and propoxyphene*

Treatment	K_i vs aminopyrine (M)	Type of inhibition	K_i vs aniline (M)	Type of inhibition	K_s (M)	Type of spectrum
SKF 525-A	$4.0 \pm 1.1 \times 10^{-6}$	Competitive	$2.0 \pm 0.4 \times 10^{-4}$	Noncompetitive	$9.1 \pm 1.8 \times 10^{-6}$	I
Propoxyphene	$4.6 \pm 0.9 \times 10^{-5}$	Competitive	$6.1 \pm 1.2 \times 10^{-4}$	Noncompetitive	$6.9 \pm 0.5 \times 10^{-5}$	I

* See Fig. 1. for details of enzyme assays. See Materials and Methods for procedure used to assess the K_s. Each value represents the mean ± S.E.M. for three individual determinations.

Table 2. Effects of SKF 525-A, phenobarbital and propoxyphene on mixed-function oxidases*

Treatment	Aminopyrine N-demethylase Activity†	% Inhibition	Aniline hydroxylase Activity‡	% Inhibition
Control	0.102 ± 0.015		0.417 ± 0.020	
SKF 525-A HCl (100 mg/kg)	0.029 ± 0.002§	73	0.133 ± 0.023§	68
Phenobarbital Na (68 mg/kg)	0.106 ± 0.012	0	0.433 ± 0.022	0
Propoxyphene HCl (100 mg/kg)	0.070 ± 0.005§	31	0.325 ± 0.025§	22

* Mice were administered water (i.p.) or drug (0.26 m-moles/kg, i.p.) 30 min before death. Assays were done on the 10,000 *g* supernatant fraction of liver homogenates. Each value is the mean ± S.E.M. for five to six animals.

† Expressed as μmoles formaldehyde formed/g of liver/15 min.

‡ Expressed as μmoles *p*-aminophenol formed/g of liver/20 min.

§ Significantly different from water control ($P < 0.05$).

increases for equimolar propoxyphene HCl (Table 3). Phenobarbital produced much smaller but significant prolongation of the effects of hexobarbital and zoxazolamine. This last observation may seem a bit surprising in view of the fact that phenobarbital did not inhibit the activities of the MFO enzymes tested either *in vivo* or *in vitro*. However, phenobarbital is a sedative-hypnotic, and its enhancement of the pharmacological effects of the depressants, hexobarbital and zoxazolamine, may have been the results of a pharmacodynamic rather than a pharmacokinetic interaction.

The experiment described in Fig. 3, on the other hand, indicates that the interaction of propoxyphene with pentobarbital is pharmacokinetic rather than pharmacodynamic in nature. Mice dosed with propoxyphene cleared [^{14}C]pentobarbital from the blood at a less rapid rate than vehicle-dosed animals. Propoxyphene prolonged pentobarbital-induced sleeping time nearly 3-fold. Since blood levels of [^{14}C]pentobarbital were identical for both groups of animals at the time of awakening (Fig. 3), it would seen that the ability of propoxyphene to inhibit the metabolism of pentobarbital would account for the potentiated effect rather than some pharmacodynamic interaction.

Cytochrome P-450–propoxyphene complex. The procedure described by Buening and Franklin [22] was used to examine microsomes from propoxyphene-treated mice to determine whether a portion of cytochrome P-450 was made unavailable for detection by the dithionite-reduced difference spectrum. Some metabolic complexes of cytochrome P-450 are destroyed by ferricyanide [30]. The effect of treating microsomal suspensions from SKF 525-A-treated and propoxyphene-treated mice with ferricyanide before spectral determinations of cytochrome P-450 concentration is shown in Table 4. Buening and Franklin [22] have shown that SKF 525-A treatment of rats can result in the formation of a cytochrome P-450 metabolic inter-

Table 3. Effects of SKF 525-A, phenobarbital and propoxyphene on hexobarbital sleeping time and zoxazolamine paralysis time*

Treatment	Sleeping time (min)	Paralysis time (min)
Control (H_2O)	12 ± 2	57 ± 7
SKF 525-A HCl (100 mg/kg)	98 ± 10†	328 ± 45†
Phenobarbital Na (68 mg/kg)	22 ± 2†	172 ± 16†
Propoxyphene HCl (100 mg/kg)	60 ± 7†	242 ± 17†

* Mice were injected i.p. with water or 0.26 m-mole/kg of drug. Either hexobarbital Na, 80 mg/kg, or zoxazolamine, 175 mg/kg, suspended in 0.25% agar, was injected i.p., 30 min later. Each value is the mean ± S.E.M. for five to six animals.

† Significantly different from water controls ($P < 0.05$).

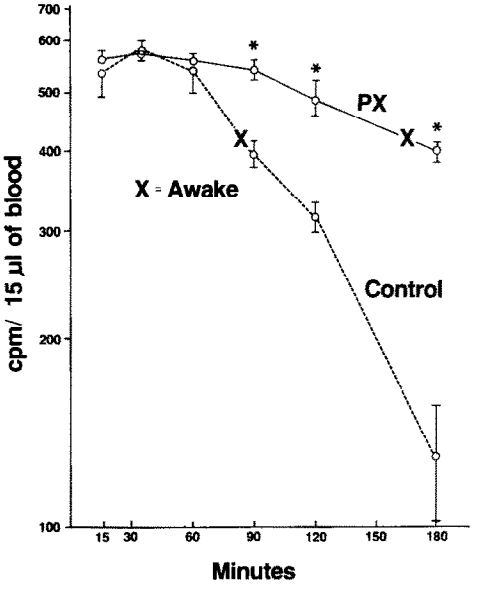

Fig. 3. Animals were administered either propoxyphene HCl (PX), 100 mg/kg, i.p., or water (controls). Thirty min later, 2-[^{14}C]pentobarbital Na, 55 mg/kg, 30 μCi/kg, was administered i.p. Blood samples (15 μl) were collected at the indicated intervals after pentobarbital administration. Sleeping times for individual animals were noted. Unmetabolized [^{14}C]pentobarbital was extracted and counted (see Materials and Methods). Each point is the mean ± S.E.M. for five to six animals. The asterisk (*) indicates significantly different from controls, $P < 0.05$.

Table 4. Effect of potassium ferricyanide treatment of drug–cytochrome P-450 complexes*

Treatment	Cytochrome P-450(nmoles/mg protein)	
	No ferricyanide	+ Ferricyanide
Controls (H_2O)	0.630 ± 0.013	0.613 ± 0.014
SKF 525-A HCl	0.436 ± 0.022†	0.580 ± 0.014
Propoxyphene HCl	0.512 ± 0.012†	0.591 ± 0.013

* Animals were injected (i.p.) with water, 100 mg/kg of propoxyphene HCl or 100 mg/kg of SKF 525-A HCl. Livers were removed 2 hr later. The washed pellet was diluted to 1 mg/ml of protein for determination of cytochrome P-450 with and without 50 μM potassium ferricyanide. Each value is the mean ± S.E.M. of five mice.

† Significantly different from controls ($P < 0.05$).

mediate complex, the peak effect occurring 1–6 hr post-administration of a 50 mg/kg dose. Roberts and Franklin [23] reported that 30 mg/kg of propoxyphene administered to rats would also sequester cytochrome P-450 as a ferricyanide-dissociable metabolic intermediate complex. The results here, comparing equimolar SKF 525-A and propoxyphene, suggest that propoxyphene has less capability to form such complexes, sequestering approximately 20 per cent of the cytochrome P-450 compared to approximately 30 per cent for SKF 525-A when determinations were done 2 hr post-administration (Table 4).

Propoxyphene induces MFO activities. Drugs that initially inhibit the activity of the microsomal MFO when given acutely often stimulate the biotransformation of other drugs when administered chronically. SKF 525-A is one of several acute inhibitors producing such an effect [26]. Table 5 shows that propoxyphene, given as the napsylate salt, will also act as an inducer when given chronically at 300 mg/kg, once daily p.o. for 3 days. We have shown previously that this dose of PN will block narcotic withdrawal signs in morphine tolerant/dependent mice with no readily discernible toxic effects [13, 14]. The oxidative metabolism of propoxyphene, as well as that of other MFO substrate, is enhanced, as evidenced by increments in the activities of propoxyphene *N*-demethylase, aminopyrine *N*-demethylase and aniline hydroxylase. Increases in microsomal protein and cytochrome P-450 were also seen, although the former did not quite reach the level of statistical significance.

DISCUSSION

Inhibition, competitive, noncompetitive or otherwise, of the metabolism of one substrate in the presence of another is often seen *in vitro*. These drug interactions seldom have practical (clinical) significance, however, because drugs are usually present in the body below concentrations required to saturate the MFO. However, enzymic sites start to become saturated at concentrations of an inhibitor greater than the K_i. Therefore, in the presence of drugs with low inhibitory constants, metabolism of other drug substrates will be decreased because enzymic sites rather than drug concentration become limiting [28].

Bioavailability data on various formulations containing propoxyphene show that, after ingestion of commonly prescribed daily doses of 195 mg of the HCl or 300 mg of the napsylate salt [31, 32], the concentration of propoxyphene in the blood often exceeds 0.2 mg/liter (approximately 6×10^{-7} M). However, blood concentrations of propoxyphene are not indicative of concentrations elsewhere in the body. The liver and other organs tend to concentrate the drug. Emmerson *et al.* [33] observed liver concentrations 20–30

Table 5. Effects of chronic administration of propoxyphene napsylate on mixed-function oxidases*

Treatment	Propoxyphene *N*-demethylase†	Aminopyrine *N*-demethylase‡	Aniline hydroxylase§	Microsomal protein‖	Cytochrome P-450¶
Control (0.25% Agar)	0.785 ± 0.14	0.232 ± 0.027	0.496 ± 0.029	16.4 ± 1.23	1.38 ± 0.20
Propoxyphene napsylate	1.45 ± 0.10**	0.390 ± 0.016**	0.694 ± 0.043**	19.8 ± 2.19	2.92 ± 0.40**

* Mice were given vehicle or propoxyphene napsylate (300 mg/kg, p.o.) daily for 3 days. Enzyme activities were determined in the 10,000 *g* supernatant fractions of livers homogenized 24 hr after the last dose. Protein and cytochrome P-450 determinations were done on microsomal fractions. Each value is the mean ± S.E.M. for five to six animals.

† Expressed as μmoles formaldehyde formed/g of liver/20 min.

‡ Expressed as μmoles formaldehyde formed/g of liver/15 min.

§ Expressed as μmoles *p*-aminophenol formed/g of liver/20 min.

‖ Expressed as mg/g of liver.

¶ Expressed as nmoles/mg of protein.

** Significantly different from controls ($P < 0.05$).

times those of blood concentrations 1–2 hr following oral administration to rats. Robinson *et al.* [34] summarized findings from several groups showing that the level of propoxyphene in the livers of humans who had taken fatal doses of the drug were 5–100 times greater than blood concentrations. McBay [35] published data showing that the concentration of propoxyphene in livers of individuals who had ingested fatal doses of the drug were 5–50 times greater than blood concentrations (30-fold being the average of six reported cases). If a 30-fold concentration of propoxyphene by the liver is assumed, then even ordinary doses can achieve hepatic concentrations in the 10^{-5} M range, very close to the K_i value we report here for propoxyphene against a representative type I substrate. Very much higher concentrations of propoxyphene have occurred in the livers and blood of individuals who had taken fatal and nonfatal doses [3, 34–36].

Although extrapolation from one species to another in the field of drug metabolism is not always warranted [37], it is nevertheless conceivable that propoxyphene will inhibit MFO activities in man, and that combinations of otherwise non-lethal concentrations of propoxyphene and other depressant drugs could result in clinically significant inhibition of drug metabolism (pharmacokinetic interactions) which could culminate in mortalities. The high incidence of propoxyphene deaths seen in combination with other drugs supports this possibility. For example, Sturner and Garriott [3] report that, of 41 deaths involving propoxyphene in the Dallas area in 1970–71, the drug had been taken concomitantly with other depressants, especially alcohol and barbiturates, in 31 of the cases. Gustafson and Gustafson [2] point out that, in fatal poisonings with propoxyphene, other drugs, usually barbiturates and alcohol, have often been ingested. The role of propoxyphene and its metabolities in these deaths has been difficult to assess. Since barbiturates [16] and alcohol [38] are MFO substrates, our observations are consistent with the possibility that the increase in deaths related to propoxyphene abuse in conjunction with other drugs may be, in part, the consequence of a pharmacokinetic drug interaction in which propoxyphene and/or metabolites (see below), by inhibiting the metabolism of other drugs, may produce a more profound pharmacological effect than that produced by the sum of the pharmacodynamic effects of the drugs. Indeed, data presented here (Fig. 3) indicate that the interaction between inhibitory doses of propoxyphene and pentobarbital is pharmacokinetic in nature.

We have also evaluated the role of norpropoxyphene, the only major metabolite of propoxyphene in humans [39], with regard to the observed inhibition of the MFO [40,*]. Our observations suggest that the *N*-demethylated metabolite, although a weaker inhibitor than propoxyphene of the metabolism of type I substrates, is a more potent inhibitor of the MFO-mediated oxidation of type II substrates. The half-life, 36.6 hr, of norpropoxyphene, is three times that of the parent compound [32]. In addition, the plasma concentration of the metabolite is as high as, and sometimes higher than that of propoxyphene in even the earliest samples taken from victims of propoxyphene poisoning [34, 35]. Thus, one might suspect that both propoxyphene and its metabolite play a role in the observed inhibition of the MFO, and that the biotransformation of a wide variety of substrates could be affected.

In the experiments described here, SKF 525-A and propoxyphene exhibited type I binding spectra in the presence of cytochrome P-450 and strongly inhibited the biotransformation of a representative type I substrate, aminopyrine, competitively. The metabolism of a type II substrate, aniline, was weakly inhibited in a noncompetitive manner by these compounds. In each case, SKF 525-A was the stronger inhibitor. The heterogeneity of microsomal preparations and the presence of multiple forms of cytochrome P-450 [41] make interpretations and between-laboratory comparisons difficult. For example, Schenkman *et al.* [42] found that the inhibition of aniline hydroxylase activity by SKF 525-A could be either competitive or noncompetitive depending upon whether NADPH was present when SKF 525-A was pre-incubated with the microsomes. Gillette [43] points out that SKF 525-A inhibited aminopyrine *N*-demethylase competitively in rats and noncompetitively in rabbits. Despite these complexities, our *in vivo* findings were strikingly consistent with our *in vitro* observations. Propoxyphene inhibited the activity of aminopyrine *N*-demethylase in the 10,000 *g* supernatant fraction of livers from propoxyphene-injected animals less than SKF 525-A and more than phenobarbital, a result that could have been predicted, given our data concerning inhibitory constants. The observation that propoxyphene administered to a living animal inhibited the activity of aniline hydroxlase less than that of aminopyrine *N*-demethylase is also consonant with these *in vitro* kinetic constants. The potentiation by propoxyphene of the pharmacological actions of hexobarbital and zoxazolamine likewise reflects the *in vitro* data. There is greater enhancement of the action of the type I drug, hexobarbital, than that of the type II compound, zoxazolamine. Also, the compound with the lower K_i, SKF 525-A, gave greater potentiation of the effects of each drug than did propoxyphene.

Other drugs used in narcotic treatment programs show structural similarities to propoxyphene. Methadone and *l*-α-acetylmethadol (LAAM), for example, are congeners of propoxyphene. We have found that methadone shares all of the above described attributes of propoxyphene, methadone exhibiting a slightly higher K_s and higher inhibitory constants [14]. Studies on LAAM are currently in progress. The primary structural requirement for inhibitory activity of the MFO would seem to be the presence of a diphenylmethane or a closely related grouping. Cooper *et al.* [44] demonstrated that, when SKF 525-A was hydrolyzed to diethylaminoethanol and diphenylpropylacetic acid, the latter compound was as potent an inhibitor of MFO activities as the parent ester. Diethylaminoethanol was inactive as an inhibitor. It seems unlikely, however, that clinically important pharmacokinetic drug interactions would result from the simultaneous administration of methadone or LAAM with other MFO drug substrates because of the relatively low doses of methadone and LAAM employed in treatment programs [45]. Propoxyphene itself, given at its usual doses, seems relatively safe, especially in light of its extensive use [1]. However, the clinical implications of adminis-

* G. R. Peterson and T. Lehman, manuscript in preparation.

tering a chronic inhibitor of drug metabolism as a detoxification/maintenance drug [6, 8–10, 12] to heroin addicts are considerable, especially since: (1) the napsylate salt, designed to achieve prolonged blood levels, guarantees a long, continuous inhibition of the MFO, (2) individuals to whom it will be administered (maintenance patients) have histories of polydrug abuse [46]; thus, PN may enhance the toxicity of many drugs these individuals may abuse, and (3) individuals to whom it will be given have a high incidence of hepatic disease [47], thus raising the possibility of further impairment of an already compromised hepatic capability to metabolize drugs.

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