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Effect of 7,8-benzoflavone on the duration of zoxazolamine paralysis and hexobarbital hypnosis in rats

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Flavones and flavonoids are potent inducers of microsomal drug-metabolizing enzymes *in vivo* [1–5] and modulate the activity of these enzymes *in vitro* [6–8]. The synthetic flavone, 7,8-benzoflavone, not only induces aryl hydrocarbon hydroxylase, a typical mixed-function oxygenase, in hepatic and extrahepatic tissues [2, 9], but also strongly inhibits the metabolism of polycyclic hydrocarbons by these enzymes in cultured cells and in isolated microsomes from mammalian tissues [6–10]. Inhibition of the aryl hydrocarbon hydroxylases by 7,8-benzoflavone was instrumental in demonstrating the involvement of these enzymes in the conversion of polycyclic hydrocarbons to cytotoxic and carcinogenic intermediates [2, 11, 12]. 7,8-Benzoflavone markedly reduced the binding of carcinogenic polycyclic hydrocarbons to macromolecules of skin, suggesting that 7,8-benzoflavone inhibits carcinogen activation *in vivo* [11, 12]. 7,8-Benzoflavone has also served as a probe for the existence of different forms of polycyclic hydrocarbon-metabolizing enzymes [6, 8, 13]: *in vitro*, this compound shows greater inhibition toward the methylcholanthrene-induced aryl hydrocarbon hydroxylase than the constitutive or phenobarbital-induced enzyme from rat liver [6, 13]. In view of the differential effects *in vitro* of 7,8-benzoflavone on the aryl hydrocarbon hydroxylases, it was of interest to determine the properties of this compound as an inhibitor of drug-metabolizing enzymes *in vivo*.

In this communication, we report the effect of 7,8-benzoflavone on the duration of zoxazolamine paralysis and hexobarbital hypnosis times, both of which are functions of microsomal oxygenases [14, 15]. Hexobarbital (a) and zoxazolamine (b) were chosen as model substrates for two types of drug-metabolizing enzymes: (a) oxygenases which are inducible by phenobarbital and not by polycyclic hydrocarbons and contain cytochrome P-450, and (b) oxygenases which are induced by polycyclic hydrocarbons and contain cytochrome P-448.

Materials and methods. Male Sprague Dawley rats weighing 100–150 g were used throughout the experiment. They were kept on a Wayne Blox diet and water *ad lib.* and housed in metal cages. All compounds were given by intraperitoneal injection. Animals were pretreated with phenobarbital (80 mg/kg), injected in 0.2 ml of 0.9% NaCl on 3 consecutive days, or with 3-methyl-cholanthrene (40 mg/kg) injected once in 0.5 ml corn oil. The effects of zoxazolamine or hexobarbital were tested 20 hr after the last injection of phenobarbital or methylcholanthrene.

Zoxazolamine solutions were prepared by dissolving the compound in 1 N HCl (500 mg/0.6 ml) and diluting with 0.9% NaCl to give a final concentration of 20 mg/ml. 7,8-Benzoflavone was dissolved in corn oil with slight heating. Animals were injected with 100 mg/kg of 7,8-benzoflavone in 0.75 ml corn oil or with corn oil alone. After 15 min, they received 100 mg/kg of zoxazolamine injected in 0.75 ml solvent or 100 mg/kg of hexobarbital injected in 0.65 ml of 0.9% NaCl if not stated otherwise.

The duration of hexobarbital sleeping and zoxazolamine paralysis times were measured from the time of injection to the time the rats regained the ability to "right" themselves three times in 30 sec.

Chemicals were obtained from the following sources: sodium phenobarbital from Merck & Co., Inc., sodium hexobarbital from Winthrop Laboratories, and 3-methylcholanthrene from Eastman Organic Chemicals. 7,8-Benzoflavone (= α-naphthoflavone) was purchased from Aldrich Chemical Co., and recrystallized from ethanol. Zoxazolamine was a generous gift from McNeil Laboratories, Inc.

Results and discussion. Pretreatment of rats with phenobarbital or methylcholanthrene decreases zoxazolamine paralysis time to $\frac{1}{2}$ and $\frac{1}{10}$, respectively, of that in untreated animals (Table 1) in agreement with observations of others [14, 16]. Injection of 7,8-benzoflavone shortly before the application of zoxazolamine has no significant effect on the paralysis time in control animals or on the change in paralysis time caused by phenobarbital pretreatment. However, 7,8-benzoflavone nearly doubles the duration of paralysis time in methylcholanthrene-pretreated animals. This suggests a strong inhibitory effect of the methylcholanthrene-induced enzyme activity. The preferential inhibition of methyl-cholanthrene-inducible metabolism of zoxazolamine *in vivo* corresponds to the inhibition of hydroxylation *in vitro* of benzo(a)pyrene by the methylcholanthrene-induced enzyme [6–8] and *O*-demethylation of *p*-nitroanisole, *O*-deethylation of 7-ethoxycoumarin, and *N*-demethylation of 3-methyl-4-methylaminoazobenzene by the 5,6-benzoflavone-induced* enzyme [3]. These inhibitory effects of 7,8-benzoflavone were not observed in the livers of untreated or phenobarbital-pretreated (male) rats.

As shown earlier [14, 16], phenobarbital pretreatment strongly decreases hexobarbital sleeping time (Table 2). The dose of hexobarbital was doubled to 200 mg/kg to achieve a measurable hypnotic effect. In contrast, pretreatment with methylcholanthrene causes a prolongation of

*5,6-Benzoflavone belongs to the group of inducers of mixed-function oxygenases typified by polycyclic hydrocarbons [1–5].

Table 1. Effect of 7,8-benzoflavone on the duration of zoxazolamine paralysis time in rats

Pretreatment	Zoxazolamine (mg/kg)	Sleeping time (min) Zoxazolamine + corn oil	Zoxazolamine + 7,8-benzoflavone	Corn oil/ 7,8-benzoflavone
None	100	228 ± 74* (12)	236 ± 105 (11)	0.97
None	65	108 ± 40 (14)	140 ± 57 (14)	0.77
Phenobarbital	100	119 ± 12 (15)	115 ± 19 (15)	1.03
Methylcholanthrene	100	22 ± 7 (13)	40 ± 6 (13)	0.55

* Mean ± standard deviation. The number of rats/group is given in parentheses. Other conditions are as described in Materials and Methods.

Table 2. Effect of 7,8-benzoflavone on the duration of hexobarbital sleeping in rats

Pretreatment	Hexobarbital (mg/kg)	Sleeping time (min) Hexobarbital + corn oil	Hexobarbital + 7,8-benzoflavone	Corn oil: 7,8-benzoflavone
None	100	49 ± 11* (10)	46 ± 18 (8)	1.06
Phenobarbital	100	0 ± (15)	0 ± (15)	
Phenobarbital	200	20 ± 4 (13)	22 ± 5 (13)	0.91
Methylcholanthrene	100	92 ± 39 (9)	78 ± 28 (9)	1.18

* Mean ± standard deviation. Number of rats/group is given in parentheses. Other conditions are as described in Materials and Methods.

hexobarbital sleeping time (Table 2, also Ref. 14). This finding is in agreement with the decrease in metabolism *in vitro* of hexobarbital in microsomes from methylcholanthrene-treated rabbits [17].

As shown in Table 2, 7,8-benzoflavone did not significantly affect the duration of hexobarbital sleeping time in untreated animals or after treatment with either phenobarbital or methylcholanthrene.

The molecular mechanism by which 7,8-benzoflavone inhibits hepatic drug metabolism has not been determined. Previous observations indicate that at lower concentrations (3 μM) 7,8-benzoflavone acts as a competitive inhibitor for microsomal benzo(a)pyrene hydroxylase from methylcholanthrene-treated animals [13].

It has not been excluded that 7,8-benzoflavone has to be metabolized to become an inhibitor of the oxygenase. Pretreatment with methylcholanthrene might induce the metabolism of 7,8-benzoflavone similarly to that of polycyclic hydrocarbons or of zoxazolamine. An increased metabolism and activation of the benzoflavone could then account for the prolongation of the paralysis time observed in the methylcholanthrene-treated but not the untreated animals. It should be pointed out that 7,8-benzoflavone apparently does not interfere with the metabolism *in vivo* of hexobarbital even after pretreatment with methylcholanthrene when the presumed activation of the inhibitor may occur (Table 2).

Livers of mammals contain several forms of microsomal mixed-function oxygenases with widely differing substrate specificities [15]. The development of specific inhibitors is important for the exploration of these various oxygenases and ultimately their therapeutic manipulation. Preferential inhibitors of the enzyme group typified by hexobarbital hydroxylases or the constitutive aryl hydrocarbon hydroxylase are already available in compounds such as SKF-525A or metyrapone [5, 18–21]. The present results indicate that 7,8-benzoflavone may provide the means to selectively inhibit *in vivo* those oxygenases which are characterized by their induction by polycyclic hydrocarbons.

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