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Effects of Psoralen and Angelicin on Hepatic Drug-Metabolizing Enzyme Activities*

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Abstract □ The effects of psoralen and angelicin on hepatic microsomal drug-metabolizing enzyme (DME) activities were investigated to elucidate the mode of the interaction of furanocoumarins with DME system. A single administration (30mg/kg, i.p.) of both coumarins to mice caused a significant prolongation of hexobarbital-induced hypnosis as well as an increase in strychnine toxicity. The inhibitory potencies of both coumarins as measured by rat hepatic microsomal aminopyrine N-demethylase and hexobarbital hydroxylase activities *in vitro* were considerably weaker than those of other furanocoumarins which possess a side chain moiety. Both coumarins were found to have significant inducing effects on DME system, with repeated treatments of them. The activities of an angular coumarin were stronger than those of a linear coumarin.

Keywords □ Angelicin, psoralen, enzyme inhibition, enzyme induction, aminopyrine N-demethylase, hexobarbital hydroxylase.

Furanocoumarins, alternatively known as furocoumarins, are a group of plant constituents which occur predominantly in two families, the Umbelliferae and the Rutaceae[2]. Furanocoumarins produce interesting photobiological effects[3] and some of them have been used in the cure of vitiligo[4,5] and for the therapeutic treatments of psoriasis[5]. Besides their photosensitizing action, it is also known that furanocoumarins exhibited light-independent effects[5]. A number of linear furanocoumarins were found to alter drug-metabolizing enzyme (DME) activities[7,8], and it was noted that furanocoumarins having prenyl side chain inhibited strongly DME system and the inhibitory potency increased as the polarity of the side chain moiety decreased[8]. The present report deals with a comparison of the effects of a linear-furanocoumarin (psoralen) and an angular furanocoumarin (angelicin) which have no side chain on hepatic microsomal DME system in order to elucidate more clearly the mode of the interaction of furanocoumarins with DME system.

MATERIALS AND METHODS

Materials and animals

Psoralen and angelicin were purchased from HRI Associates Inc., Emeryville, California, U.S.A. NADP, glucose-6-phosphate were obtained from Sigma Chem. Co. All other chemicals were of the special grade commercially available. Male mice (ICR) weighing 20-30g were used for *in vivo* experiments and male Sprague-Dawley rats weighing 200-250g were used for *in vitro* enzyme assay.

Evaluation procedure of actions on DME system

The effect of the coumarins on hepatic DME system was investigated by two different procedures: In a first series of procedure (the acute phase), mice were administered i.p. with each compound suspended in 0.5% CMC 30min prior to the estimation of hexobarbital (HB)-induced hypnosis (i.p.) or to perform enzyme assays. In one animal group, the HB-induced sleeping time was measured as the time interval between loss and recovery, of the righting reflex after administration of HB-Na (50mg/kg, i.p.). In the other animal groups, aminopyrine (AP) N-demethylase and HB Hydroxylase activities were measured in 10,000g supernatant fraction of the liver homogenate. In the second series of procedure (the chronic phase), mice were pretreated with 3 daily consecutive administrations of each compound and forty eight hr. after the last treatment, HB-Na (100mg/kg, i.p.) was injected to observe the duration of sleeping time as well as to perform

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hepatic enzyme assay. For conformation of the fact that the prolongation of HB-induced sleeping time is due to enzyme inhibitory activity, the effect on strychnine (ST) mortality was investigated: ST nitrate was injected i.p. at a dose of 1.2mg/kg to mice 30min after a single administration of each compound. The convulsive death of 20% of the control animals was induced at this dose of ST nitrate. Mice were observed for 30min and the mortality was recorded.

Preparation of microsomal enzyme fraction

Livers of rats or mice were excised and immediately weighed. They were washed twice in ice-cold isotonic phosphate buffer (1.15% KCl, pH 7.4) and then homogenized in 5 volume of the buffer. The homogenate was centrifuged for 20min at 10,000g and the supernatant fraction was used in the enzyme assays. The protein concentration in the supernatant was determined by the method of Lowry *et al.*[9] using bovine serum albumin as a standard.

Enzyme assays

AP N-demethylase activity was measured as described by Mazel[10]. The formaldehyde formed was estimated spectrophotometrically as described by Nash[11]. The oxidative metabolism of HB was assayed by measuring HB concentration in the incubation mixture remaining unmetabolized, as described by Cooper and Brodie[12].

RESULTS AND DISCUSSION

Table I shows the results of the *in vivo* effect of a single dose of psoralen and angelicin on hepatic microsomal DME system as measured by HB-induced sleeping time and ST-mortality in mice. Thirty min after the i.p. administration of 0.5% CMC in the control mice, the mean duration of sleeping time was 29.7min, whereas a single i.p. dose of 30mg/kg of both coumarins caused significant prolongation of sleeping time compared to that of the control. The effect of angelicin (193% increase) was approximately 2 fold potent than that of psoralen (95% increase). A single administration of the same dose of both coumarins also, caused a significant increase in ST-toxicity; 40% increase in case of psoralen and 60% increase in angelicin.

This enhancement of toxicity of ST, therefore, implies that the prolongation of the duration of HB-induced sleeping time caused by both compounds did not result from the simple potentiation of a CNS depressant action of HB by the coumarins but from the retardation of drug metabolism.

We previously reported that various linear furanocoumarins with a side chain moiety at C-5 or C-8 position showed remarkable prolongation of HB-induced hypnosis[8]. However, distinct fall in the efficacy for the prolongation of HB-hypnosis was observed in cases of psoralen and angelicin which do not possess any side chain moiety (Table IV). From these results, it can be readily postulated that various side chain moieties attached on the furanocoumarin skeleton play an important role in their interaction with microsomal enzyme system. These tendencies were also observed in *in vitro* assays of rat hepatic microsomal DME activities. The inhibitory potency of the two coumarins on AP N-demethylase and HB hydroxylase activities of rat liver microsomes was evaluated in the presence of various concentrations of the compounds. As indicated in Fig. 1 and 2, the IC_{50} values of angelicin and psoralen, the half-inhibition of AP N-demethylase were calculated to be 0.045mM and 0.05mM, respectively. And IC_{50} for HB hydroxylase were

Table I. Effect of a single treatment of psoralen and angelicin on hexobarbital-induced hypnosis and strychnine mortality in Mice

Treatment	Dose (mg/kg, i.p.)	Hexobarbital (50mg/kg, i.p.) hypnosis[a] (min ± S.E.)	Strychnine (1.2mg/kg, i.p.) mortality (No. died/No. dosed)
Control	—	29.7 ± 4.8(5)	2/10
Psoralen	30	57.8 ± 4.5(5)* (194.6)	6/10
Angelicin	30	87.0 ± 0.6(5)* (292.7)	8/10

Animals were treated 30min prior to the experiment.
[a] Five mice were used for each group.
Significantly different from the control, *$p < 0.01$

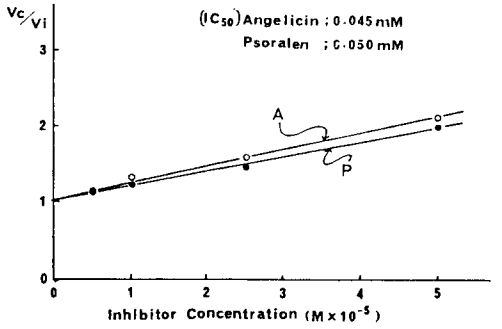


Fig. 1. Inhibitory potencies of coumarins on AP N-demethylase *in vitro*.

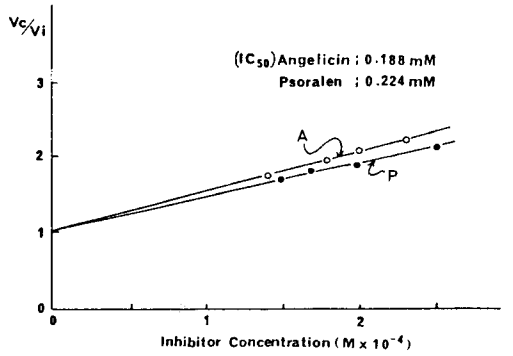


Fig. 2. Inhibitory potencies of coumarins on HB hydroxylase *in vitro*.

0.188 and 0.224mM, respectively. These inhibitory potencies appear to be considerably less (less than 10 times) than those of other furanocoumarins with side chain moiety, which were previously evaluated[8] (Table IV). *In vitro* enzyme assays, the angular furanocoumarin (angelicin) was found to show somewhat more stronger effect than the linear furanocoumarin (psoralen), suggesting that the inhibitory potency might be related to the stereo configuration of the inhibitor to the enzyme binding site.

The effect of both coumarins on DME activities *in vivo* was shown in Table II. At a single dose of the coumarins as low as 30mg/kg, i.p. neither psoralen nor angelicin had statistically significant inhibitory effect on AP N-demethylase and HB hydroxylase. These results suggest that a relatively large concentration of the coumarins are required to be enough to saturate DME system *in vivo*. Strong support for this assumption was also attained from our experimental evidence of relatively large IC_{50} values for AP N-demethylase and HB hydroxylase *in vitro* (Fig. 1, 2).

It is well known that a variety of DME inhibitors produce biphasic alteration in the metabolism of drugs.[13]. And our recent study on imperatorin demonstrated that it had biphasic response on DME system and caused significant increase in hepatic enzyme activities with repeated treatments of it[14]. In order to know whether this property is common to all furanocoumarins, effect of repeated treatments of both coumarins on DME activities was evaluated and the results were summarized in Table III.

It was shown that both coumarins, with 3 daily consecutive pretreatments to mice, caused not only a significant shortening of the duration of HB-induced hypnosis but a significant increase in activities of AP N-demethylase and HB hydroxylase, which gave an evidence for an induction of hepatic microsomal enzymes upon their repeated treatments.

In conclusion, it is postulated that angular furanocoumarins have more stronger effect on DME system than linear furanocoumarins and introduction of a side chain to the C-5 or C-8 increases the activity.

Table II. Effect of a single treatment of psoralen and angelicin on mixed function oxidase in mice

Treatment	Dose (mg/kg, i.p.)	Aminopyrine N-demethylase (μ moles/g prot./min)	Hexobarbital hydroxylase (μmoles/g prot./min)
Control		0.604 ± 0.135	0.659 ± 0.126
Psoralen	30	0.560 ± 0.037 (92.7)	0.586 ± 0.059 (88.9)
Angelicin	30	0.483 ± 0.037 (80.0)	0.479 ± 0.108 (72.7)

Animals were treated 30min before sacrifice.
Livers from two mice were pooled for an enzyme assay.
Data represent mean ± S.E. of 3 determinations.
Figures in parentheses indicate % of control.

Table III. Effect of three-day treatments of psoralen and angelicin on hexobarbital-induced hypnosis and mixed function oxidases in Mice

Treatment	Dose (mg/kg, i.p.)	Hexobarbital hypnosis[a] (min ± S.E.)	Aminopyrine N-demethylase[b] (μ moles/g prot./min)	Hexobarbital hydroxylase[b] (μ moles/g prot./min)
Control	—	42.8 ± 2.9	0.566 ± 0.048	0.585 ± 0.088
Psoralen	30	35.0 ± 2.8* (81.8)	0.745 ± 0.083* (131.6)	1.014 ± 0.110** (173.3)
Angelicin	30	31.0 ± 3.8** (72.4)	0.848 ± 0.027*** (149.8)	0.864 ± 0.089* (147.7)

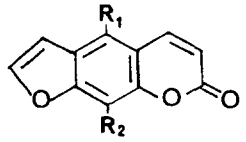
Forty eight hr after the last treatment:
[a] HB (100mg/kg, i.p.). was injected and the duration of sleeping time was estimated. Five mice were used for each group.
[b] Livers from two mice were pooled for an enzyme assay.
Data represent mean ± S.E. of 3 determinations.
Figures in parentheses indicate % of control.
Significantly different from the control; *$p < 0.1$, **$p < 0.05$, ***$p < 0.01$

Table IV. Comparison of inhibitory potency of coumarins

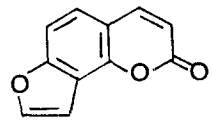
Inhibitor	*In vivo*[a] HB hypnosis (% of control)	*In vivo*[a] ST mortality[b] No. died/No. dosed	*In vitro* IC_{50}(mM)[c] AP N-demethylase	*In vitro* IC_{50}(mM)[c] HB hydroxylase
SKF-525A	749.8	10/10	0.011	0.058
Imperatiorin (I)	981.9	9/10	0.017	0.046
Isoimperatorin (II)	1012.8	9/10	0.015	0.059
Oxypeucedanin (III)	884.0	9/10	0.020	0.081
Isooxypeucedanin (IV)	457.6	8/10	0.037	0.098
Oxypeucedanin (V) methanolate	342.4	7/10	0.092	0.107
Psoralen (VI)	194.6	6/10	0.050	0.224
Angelicin (VII)	292.7	8/10	0.045	0.188

[a] Dose of coumarins; 30mg/kg, i.p.
[b] Mortality of the control; 20%
[c] Calculated values from regression equations.

	R_1	R_2
(I)	H	$OCH_2CH=C(CH_3)_2$
(II)	$OCH_2CH=C(CH_3)_2$	H
(III)	$OCH_2CH(O)C(CH_3)_2$	H
(IV)	$OCH_2C(=O)CH(CH_3)_2$	H
(V)	$OCH_2CH(OH)C(CH_3)_2OCH_3$	H
(VI)	H	H



(VII)



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