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Inhibition by aflatoxin B_1 of rat liver zoxazolamine hydroxylase induction*

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AFLATOXIN B_1 is a potent hepatocarcinogen and hepatotoxin.[1] Among several biochemical effects observed in the rat liver, rapid and extensive inhibition of precursor incorporation into nuclear and cytoplasmic RNA follows injection of the toxin.[2] Dissociation of ribosomes from hepatic rough endoplasmic reticulum resulting from aflatoxin treatment of rats has been reported.[3,4] Polysomal disaggregation in liver within 3 hr after toxin treatment has been observed.[5]

It has also been found that aflatoxin B_1 affects rat liver protein synthesis, as indicated by its suppression of amino acid incorporation into liver slices, cell-free systems, and the isolated perfused rat liver.[6–9]

In view of these responses, it seemed important to determine whether the toxin would show analogous effects on specific syntheses *in vivo* of micromolecules in rat liver. Microsomal drug-metabolizing

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enzymes were regarded as convenient model systems for this purpose. Numerous investigations have demonstrated the inducibility of these enzymes, and *de novo* syntheses of enzyme proteins has been implicated in the induction processes.[10–14] Available evidence also indicates a dependency of the induction on concomitant synthesis of RNA.[15–17] Recently, it has been suggested that inducers of microsomal enzymes, in addition to stimulating the amount of enzymes, may also effect qualitative changes in the newly synthesized enzymes.[18]

We have conducted a series of experiments to determine the actions of aflatoxin B_1 on the induction of zoxazolamine hydroxylase by 3,4-benzpyrene. Our results indicate that aflatoxin B_1 is a potent inhibitor of microsomal enzyme induction in this system.

Male Fischer rats weighing about 100 g were used. They were fed an agar-gel diet[19] *ad lib.* Animals were sacrificed by decapitation after various treatments at 9 a.m. in all instances.

Aflatoxin B_1 used in these experiments was produced by submerged culture of *Aspergillus flavus* (ATCC 15517) as described by Mateles and Adye.[20] After purification, the aflatoxin B_1 was more than 99·5 per cent pure as demonstrated by thin-layer chromatography and molar extinction at 363 mμ.[21] It was dissolved in spectral grade dimethylsulfoxide (DMSO, Burdick and Jackson Laboratories, Inc., Muskegon, Mich.) for administration. Three mg aflatoxin B_1/kg of body weight was given intraperitoneally (i.p.) to rats in 0·05 ml DMSO.

The inducer, 3,4-benzpyrene (BP, Sigma), was dissolved in commercial Mazola corn oil and administered i.p. to rats at doses of 10 mg/rat in 0·5 ml corn oil.

Livers were homogenized in 1·15% KCl and microsomes were prepared from post-mitochondrial fractions of 25% homogenates by centrifugation at 105,000 *g* for 1 hr. Zoxazolamine hydroxylase activity was determined by the method of Conney *et al.*[22] Briefly summarized, the assay was carried out as follows. Microsomes from a weight equivalent of 165 mg liver were incubated with 100 μg zoxazolamine and other cofactors for 30 min in a 0·1 M phosphate buffer solution, pH 7·4, at 37°. Unmetabolized zoxazolamine was extracted with redistilled ethylene dichloride, which was then washed with 0·3 M sodium borate and extracted with 1 N HCl. The acidic aqueous phase was analyzed for unmetabolized zoxazolamine spectrophotometrically at 278 mμ.

The kinetics of the induction of zoxazolamine hydroxylase by administration of BP are shown in Fig. 1. A single injection of the inducer caused a 3-fold increase in zoxazolamine hydroxylase activity within 24 hr. Corn oil, the vehicle for the inducer, had no effect on the enzyme activity; throughout a 30-hr period after an injection of 0·5 ml corn oil, zoxazolamine hydroxylase activity remained essentially unchanged [Table 1 (A)].

Subsequent experiments also showed that simultaneous injection of the vehicle DMSO and corn oil or of DMSO alone was without effect on the basal level of enzyme activity [Table 1 (A)]. Furthermore, DMSO failed to influence the rate or other characteristics of zoxazolamine hydroxylase induction, when administered simultaneously with or at 6, 12 or 18 hr after BP, the enzyme inducer [Table 1 (B)].

A single dose of aflatoxin B_1, at a level of 3 mg per kg of body weight, completely inhibited the induction of zoxazolamine hydroxylase when the toxin was administered simultaneously with or 3 h after the injection of the inducer (Fig. 1, A and B). This inhibition persisted throughout the 24-hr study period. When the toxin was given 6 or 12 hr after the inducer (Fig. 1, C and D), it had no significant effect for 6 hr thereafter, but caused a marked decline in enzyme activity at succeeding time periods. The administration of toxin at 18 hr caused a small decline in enzyme activity during the succeeding 12-hr period.

Positive control experiments with actinomycin D (1 mg/kg) showed results similar to those caused by aflatoxin B_1, except that: (1) when actinomycin D and BP were administered simultaneously, the inhibition of rat liver zoxazolamine hydroxylase induction began to reverse after 6 hr, reaching induced control enzyme activity by 24 hr; and (2) when actinomycin D was given 6, 12 or 18 hr after BP, liver zoxazolamine hydroxylase activities were comparable to those of the untreated induced rats for the succeeding 12 hr.

These data show that aflatoxin B_1 is a potent inhibitor of microsomal enzyme induction. Zoxazolamine hydroxylase induction was completely inhibited by aflatoxin B_1 when it was injected simultaneously with or 3 hr after the inducer. However, when the toxin was administered 6 hr after induction or later, zoxazolamine hydroxylase activity was not altered for 6 hr after dosing, but was suppressed thereafter.

These responses to aflatoxin B_1 reflect several properties of the induction process and of the

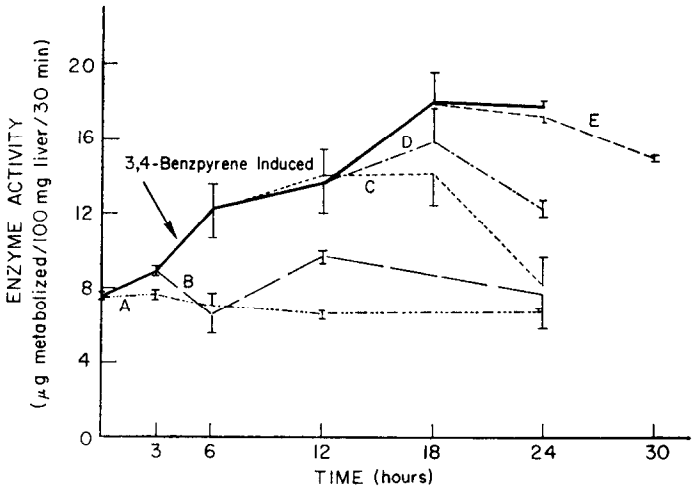

FIG. 1. Effects of aflatoxin B_1 on zoxazolamine hydroxylase induction. Rats received a single injection of 10 mg BP at zero hour. Aflatoxin B_1 (3 mg/kg) was administered at 0, 3, 6, 12 or 18 hr after inducer. The heavy line represents the induction curve from animals receiving only the inducer. A, rat receiving the toxin at zero hour; B, at 3 hr; C, at 6 hr; D, at 12 hr; and E, at 18 hr after inducer. Values are means ± standard errors from microsomes of pooled livers of four rats assayed in duplicate.

TABLE 1. ZOXAZOLAMINE HYDROXYLASE ACTIVITY AFTER VARIOUS TREATMENTS

Sacrifice time (hr after initial treatment)	Treatment*	Enzyme activity†
	(A) UNINDUCED	
0	None	6·46 ± 1·08
6, 12, 18, 24	Corn oil at 0 hr	6·23 ± 0·75
24	DMSO at 0 hr	7·21 ± 0·73
24	Corn oil + DMSO at 0 hr	5·95 ± 1·49
	(B) INDUCED	
0	BP at 0 hr	7·33 ± 0·20
6	BP at 0 hr	8·60 ± 0·43
12	(i) BP at 0 hr	12·28 ± 1·06
	(ii) BP + DMSO at 0 hr	14·36 ± 1·44
18	BP at 0 hr	15·59 ± 0·53
24	(i) BP at 0 hr	15·22 ± 0·76
	(ii) BP + DMSO at 0 hr	13·59 ± 0·15
	(iii) BP at 0 hr + DMSO at 6 hr	14·66 ± 1·75
	(iv) BP at 0 hr + DMSO at 12 hr	14·06 ± 0·67
30	BP at 0 hr + DMSO at 18 hr	15·98 ± 1·87

*All injections were given i.p. at the following dosages: corn oil, 0·5 ml; DMSO, 0·05 ml; and BP, 10 mg in 0·5 ml corn oil.
†Enzyme activities (μg zoxazolamine metabolized by microsomes equivalent to 165 mg rat liver in 30 min at 37°) are expressed as means ± standard errors. In each instance, assays were done in triplicate using microsomes from pooled livers of five rats.

inhibitor. Based on numerous previous reports of the ability of the toxin to block RNA synthesis,[25] the present findings are in agreement with earlier suggestions that induction of microsomal enzymes is dependent upon concomitant RNA synthesis. In the present experiments, the induction process became insensitive to aflatoxin B_1 by 6 hr after induction had started, as indicated by continued increases in enzyme activity despite injection of the toxin 6 hr or later after the inducer had been given. This pattern of early sensitivity to aflatoxin followed by insensitivity has been observed earlier in studies of tryptophan pyrrolase induction by hydrocortisone,[23] suggesting that similar mechanisms may be operative, but with a different time course. It is probable that RNA synthesis necessary for zoxazolamine hydroxlase induction is completed by 6 hr after BP administration.

When effects of aflatoxin on enzyme activity were determined during the later phases of the induction curve, toxin treatment resulted in decay of enzyme activity much more rapidly than in controls (Fig. 1, C and D). The mechanisms responsible for this decay are not known, but available information would suggest that the cytotoxicity of aflatoxin B_1 may be involved. The toxin has been implicated to interact with membranes of hepatocyte endoplasmic reticulum,[24] and this cytotoxic action of aflatoxin may account for the decay in enzyme activity.

It has been suggested that more than one mechanism may be involved in induction of drug-metabolizing enzyme.[25] Early postulates indicated that microsomal enzyme induction may involve *de novo* protein synthesis with concomitant RNA synthesis. More recent information concerning the induction process shows that phenobarbital and 3-methylcholanthrene have different microsomal enzyme-inducing properties.[18] Treatment with these inducers results in differences in the nature of microsomal hemoprotein and in the kinetics of benzpyrene hydroxylation. These observations have led to the postulation that barbiturates differ from polycyclic hydrocarbons in inducing microsomal enzymes not only in the amount of the enzymes newly synthesized but also in their quality with respect to the kinetics of the induced enzyme systems.[18] Furthermore, BP and aflatoxin B_1 (0·67 mg/kg) have been shown to stimulate benzpyrene hydroxylation primarily by activation of a microsomal hydroxylase system.[26]

Results from our present experiments show that aflatoxin B_1 at a toxic dose (3 mg/kg) is inhibitory to induction of zoxazolamine hydroxylase by BP. This inhibitory action may result from a suppression of *de novo* synthesis of new enzyme protein, a consequence of inhibition of RNA polymerase activity,[27] and/or from a suppression of activation of a microsomal hydroxylase system. In our earlier observations on soluble enzyme systems, inhibition of tryptophan pyrrolase induction by aflatoxin B_1 was interpreted to result from suppression of concurrent RNA synthesis.[29] Our present data do not exclude an action of the toxin on microsomal enzyme activation. However, the close resemblance of the kinetics of inhibition by aflatoxin B_1 to those of actinomycin D on zoxazolamine hydroxylase induction is interpreted to support the hypothesis that inhibition of enzyme induction by aflatoxin results primarily from suppression of concomitant RNA synthesis.

Recently, it has been reported that aflatoxin B_1 administered to rats 2 hr prior to phenobarbital not only failed to inhibit induction of liver microsomal enzymes, but enhanced aminopyrine *N*-demethylation and pentobarbital oxidation in animals killed 40 hr after the inducer was given.[28] These results are in disagreement with those reported in this paper. We feel, however, that this discrepancy can be explained mainly on the basis of the reversibility of aflatoxin effects. Several reports dealing with the suppression of RNA polymerase inhibition by aflatoxin B_1 at a dose comparable to that used by Kato *et al.*[28] indicate that the toxin's effects are fully reversible before 40 hr.[2,27] On this basis, therefore, no inhibition would be expected in rats killed 40 hr after inducer was administered. The apparent enhancement of microsomal enzyme activity by aflatoxin B_1[28] may also be a result of the long time interval involved in the experiments, particularly in view of the recent report[29] that aflatoxin B_1 stimulates its own metabolism by rat liver microsomes.

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Drug metabolism and pharmacologic action in mice exposed to reduced barometric pressure*

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THE ENHANCEMENT or depression of drug action or metabolism or of both, induced by a variety of drugs and chemicals has been extensively investigated.[1] Similar effects are also produced by a number of stress conditions such as starvation,[2] hind-limb ligation,[3] hypobaric conditions,[4] increased or decreased temperature, and dehydration.[5]

*The research reported in this paper was conducted by personnel of the Pharmacology–Biochemistry Branch, Biosciences Division, USAF School of Aerospace Medicine, Aerospace Medical Division, AFSC, Brooks Air Force Base, Texas. Further reproduction is authorized to satisfy the needs of the U.S. Government. Animals involved in this study were maintained in accordance with the *Guide for Laboratory Animal Facilities and Care* as published by the National Academy of Sciences-National Research Council.