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AFLATOXIN B1 AND ITS INFLUENCE IN THE LIVER CANCER IN THE SMALL MAMMALS

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Abstract

Hepatocarcinogen, aflatoxin B1 (AFB1), undergoes metabolic activation via epoxidation and the interaction of this epoxide with DNA is believed to be responsible for its initiations of carcinogenesis. Several in vitro and in vivo studies suggest that epoxidation alone cannot account for differences in AFB1-DNA binding and carcinogenicity observed in rats (susceptible species) and hamsters (resistant). Recent work on AFB1 metabolism with hepatocellular fractions and isolated hepatocytes from rats has been reviewed. These studies indicate that cytosolic glutathione (GSH) S-transferases play an important role in the modulation of hepatic AFB1-DNA binding. Inhibition of hepatocarcinogenesis and AFB1-DNA bindingby pretreatment of rats with various inducers are discussed. Even though Phenobarbital (PB) is an inducer of both cytochrome P-450 and GSH S-transfer-ases, the subcellular, hepatocyte and in vivo data suggest that induced levels of hepatic AFB1-DNA binding in PB-treated rats. In contrast, the mechanism of inhibition of AFB1-DNA binding and hepatocarcinogenesis by B-naphthoflavone pretreatment of rats is mediated by stimulation of an inactivation pathway involving cytochrome P-450 dependant oxiadive reaction. Finally, data on chemoprevention of AFB1 hepatocarcinogenesis by pretreatment of rats with various antioxidants suggesting a major role of GSH S-transferases are also reviewed

Key words: initiation of carcinogenesis, aflatoxin B1, cytochrome P-450 enzyme.

INTRODUCTION

Epidemiological evidence suggest that aflatoxin B1 (AFB1) may be an etiological agent in addition to hepatitis virus responsible for the induction of human liver cancer in several parts of Africa and Asia. It is known that whereas epoxidation of AFB1 via cytochrome P-450 enzyme system is an activation pathway, hydroxylation of AFB1 to aflatoxin M1 (AFM1), P1 (AFP1), and Q1 (AFQ1) is an inactivation pathway. Among the interaction of AFB1-epoxide with various cellular macromolecules, covalent interaction of AFB1-epoxide with cellular DNA is believed to be responsible for initiation of carcinogenesis. The major product of AFB1-DNA interaction formed in vitro and in vivo has been characterized as 8,9-dihydro-8-(guan-7-y1)-9-hydroxy-AFB1. the presence of AFB1-glutathione (AFB1-SG) conjugate was not only demonstrated in the rat bile, but its formation was also shown during the hepatic microsomal oxidative metabolism of AFB1 in the presence of cytosol. The GSH conjugate of AFB1 formed in vitro and in vivo has been characterized as 8-(S-glutathionyl)-9-hydroxy-8,9-dihydro-AFB1.

In this report, the metabolic basis for susceptibility and resistance of rodents to AFB1 hepatocarcinogenesis is discussed. Data on the epoxidation of AFB1 and its interaction with DNA and conjugation with GSH in the rat and hamster liver are examined. In addition, the effects of pretreatment of rats with various antioxidants, Phenobarbital (PB) and B-naphthoflavone (BNF) on AFB1 hepatocarcinogenesis, hepatic AFB1-DNA binding, AFB1-SG conjugation and AFB1-hydroxylation are also reviewed.

MATERIALS AND METHODS

SPECIES DIFFERENCES IN AFB1 METABOLISM

A wide difference exists in hepatocarcinogenesis of AFB1 and AFB1DNA binding in rats and hamsters both in vivo and in vitro studies (Table 1). The rat is highly susceptible whereas the hamster is less susceptible to AFB1 carcinogenesis. The carcinogenicity of AFB1 in these two species parallels with AFB1-DNA binding both in vivo and in liver slice experiments. However, in vitro studies, hamster liver microsomes or nuclei activate AFB1 as indicated by AFB1-DNA binding four fold more than respective liver fractions from the rat. Thus, it appeared that epoxidation alone cannot account for the differences in AFB1-DNA binding observed in vivo and in tissue slice experiments in these two species.

TABLE 1

Parameter examined	Rat	Hamster
Carcinogenicity	++++	+
DNA binding in vivo	++++	+
DNA binding in liver slices	+++	+
DNA binding in vitro	+	++++
(microsomes with exogenous		
DNA)		
DNA binding in vitro (nuclei)	+	++++

Effects of Aflatoxin B1 on Rat and Hamster Liver

Role of cytosolic GSH S-transferases in modulating AFB1-DNA binding: Since AFB1-SG conjugation in vitro was enzymatic, it appeared that microsome-mediated AFB1-DNA binding could be modulated by cytosolic GSH S-transferases. A laboratory showed that microsomemediated AFB1 binding to exogenous DNA could be inhibited by hepatic cytosolic GSH S-transferases. Comparison of liver cytosolic GSH Stransferase activities with various substrates demonstrated several fold more activity with the hamster compared to the rat. In addition, hamster liver cytosol was severalfold more effective than the rat cytosol in inhibiting AFB1-DNA binding mediated by microsomes from either rat or hamster. Subsequently, it was shown that concomitant with cytosolic inhibition of AFB1-DNA binding, AFB1-DNA conjugation occurred. In reconstitution studies with rat or hamster nuclei as a source of endogenous DNA, AFB1DNA binding was much less in the presence of microsomes and cytosol from the hamster than those from the rat. AFB1-SG conjugation, however, was severalfold higher with microsomes and cytosol from the hamster than those from the rat. The ratio of AFB1-SG conjugation to AFB1-DNA binding was an order of magnitude higher with the hamster in comparison with the rat indicating preponderance of inactivation of the AFB1-epoxide in the former species. These sub cellular data suggest that in addition to AFB1 epoxidation, cytosolic GSH S-transferases play an important role in modulating hepatic AFB1-DNA binding and AFB1 carcinogenicity insusceptible and resistant species. Other investigators have also correlated differences in susceptibility of various other species with their hepatic cytosol capability in vitro to inactivate the AFB1-epoxide by conjugation with GSH.

The subcellular studies were extended to freshly isolated intact hepatocytes from both rats and hamsters. AFB1-DNA binding and AFB1-SG conjugation durin the metabolism of AFB1 and the effects of styrene oxide on these reactions have been examined (Table 2). AFB1-DNA binding was fivefold higher in the rat whereas AFB1-SG conjugation was an order of magnitude higher in the hamster. The ratio of AFB1-SG to AFB1-DNA binding was fiftyfold higher in the hamster than in the rat. Presence of styrene oxide (SO) gave dramatic results with the hamster hepatocytes compared to the rat hepatocytes. Like subcellular studies, these intact hepatocytes data provide additional evidence that GSH S-transferases play a more significant role in modulating AFB1-DNA binding in the hamster than that in the rat.

TABLE 2

AFB1-DNA Binding and AFB1-SG Conjugation during

AFB1 Metabolism with Isolated Hepatocytes from Rats and Hamsters*

Species	Addition (1mM)	AFB1-DNA AFB1-SG pmo1	AFB1-SG AFB1-DNA					
Rat		209 ± 19 405 ± 50	1.9					
	SO	367 ± 5 105 ± 15	0.3					
Hamster		43 ± 10 $4,890 \pm 90$	114					
	SO	978 ± 12 1,425 ± 1051.5						

*Male Fischer rats and Syrian hamsters used. Hepatocytes isolated by the collagenase method.

[³H]AFB1 concentration during incubation was 2 μ M. Metabolites calculated for 10^8 cells corresponding to 1 mg DNA-hr.

RESULTS AND DISCUSSION

PRETREATMENTS OF RATS WITH ANTIOXIDANTS, PHENOBARBITAL OR β-NAPHTHOFLAVONE

Data on the effects of aflatoxin B1 on rat liver after various pretreatments are summarized in Table 3. pretreatment of rats with either 2, (3) tert-butyl-4hydroxyanisole (BHA), 3,5-di-tert-butyl-4-hydroxytoluene (BHT), ethoxyquin (EQ), BNF or PB has been shown to inhibit AFB1 induced hepatocarcinogenesis. Such data are not available with 5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione (oltipraz) even though recent studies have shown inhibitory effect of oltipraz on AFB1-induced γ -glutamyl transpeptidase foci in the rat liver. All such pretreatments hewever, inhibited hepatic AFB1-DNA binding in vivo. Concomitant with the inhibition of hepatic AFB1-DNA binding, pretreatment of rats with either BHA, ethoxyquin or PB increased biliary excretion of AFB1-SG conjugate. In isolated hepatocyte system, a laboratory has observed that pretreatment with either BHA, PB, or BNF inhibited AFB1-DNA binding; however concomitant with inhibition of AFB1-DNA binding, increase in AFB1-SG conjugate formation was seen only with BHA and PB treatment.

TABLE 3

Parameters examined	Pretreatment with											
				BHA	BHT	EQ	Oltipraz	BNF	PB			
Carcinogenecity				\downarrow	\downarrow	\downarrow		\downarrow	\downarrow			
AFB1 -DNA (in vivo)				\downarrow	\downarrow	↓	\downarrow	↓	↓			
AFB1-DNA (hepatocytes)				\downarrow				\downarrow	\downarrow			
AFB1-SG (in vivo)				↑		1			1			
AFB1-SG (hepatocytes)				ſ				\rightarrow	1			
Subcellular												
AFB1-SG				1		ſ			1			
P-450 Content	\rightarrow	1	↑	1	1	1						
AFB1-epoxidation				\rightarrow		Î		\downarrow	ſ			
AFM1, AFQ1, AFP1 formation					1	1	Î	1				
GSH S-transferases				Î	Î	↑	Î	\rightarrow	1			

Effects of Aflatoxin B1 on Rat Liver after Various Pretreatments*

*Symbols: \uparrow , increase; \downarrow , decrease; \rightarrow , no effect.

CONCLUSIONS

Subcellular investigations have demonstrated that pretreatment of rats with either BHT, ethoxyquin, oltipraz, BNF or PB increased hepatic microsomal cytochrome P-450 content; BHA pretreatment however did not have any significant effect on cytochrome P-450 content. Microsomemediated epoxidation as measured by either AFB1-DNA binding or dihydrodiol formation was increased with ethoxyquin or PB pretreatment, was decreased with BNF and unaffected with BHA pretreatment. Inactivation via hydrowylation of AFB1 to AFM1, AFQ1, and AFP1 metabolites was elevated with ethoxyquin, oltipraz, BNF or PB pretreatment. Hepatic cytosolic GSH S-transferases activities were increased with various pretreatments except BNF, whereas AFB1-SG conjugation was increased with BHA, ethowyquin or PB pretreatment of rats.

Thus, except BHA and BNF, other antioxidants examined such as BHT, ethozyquin, oltipraz and related compounds, and PB appear to induce both phase I and phase !! metabolizing enzymes in the rat. BHA pretreatment induces phase II enzymes whereas BNF pretreatment induces phase I enzymes.

On the basis of careful examinations of several parameters, it appears that induced cytosolic GSH S-transferases play a major role in the modulating hepatic AFB1-DNA binding and carcinogenesis in the rat by pretreatment with either BHA, BHT, ethoxyquin, oltipraz and related compounds or PB. In contrast, inhibition of AFB1 hepatocarcinogenesis in the rat by BFN pretreatment is suggested to be due to induced cytochrome P-450 mediated inactivation to AFM1.

Since cytochrome P-450 enzyme system is involved in both activation and inactivation processes of many carcinogens and xenobiotics, it may not be prudent to manipulate the phase I enzymes for chemoprevention for cancer in man. However, this goal can be better achieved by inducing phase II enzymes including GSH S-transferases.

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