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Dietary factors affecting aflatoxin Bi carcinogenicity

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ABSTRACT

This review paper describes briefly on the history of aflatoxins, the metabolism of aflatoxin B_1 (AFB_1) that leads to the activation and detoxification of AFB_1, and the findings of some of the studies relating to food nutrients and additives, and drugs on AFBJ carcinogenicity and detoxification. Aflatoxins have been linked to many public health problems, especially to liver cancer incidences, in different parts of the world. Many studies have shown the potential of dietary factors modulating the formation of AFB_1 - DNA adduct, the initial and important step of AFB_1 carcinogenesis process. Among the food nutrients that have been shown to reduce the binding of AFB_1 to DNA are vitamin A, vitamin C and riboflavin. On the contrary, vitamin E and β-carotene increase the DNA binding. Choline-deficient animals when subjected to multiple doses of AFB_1 had higher amount of the DNA adduct being formed than the choline-sufficient animals. Carnitine supplement, feed restriction, and some vegetables and their extracts can also decrease the AFB_1 -DNA adduct formation. The observed and proposed mechanisms for the reduction include the inhibition of bioactivation of AFB_1 and induction of glutathione S-transferase activity that detoxify the activated AFB_1. However, more research is needed before nutritional recommendations could be given to the public to control AFB_1 toxicity and carcinogenicity.

INTRODUCTION

History of Aflatoxins

Aflatoxins are a group of mycotoxins produced by the molds *Aspergillus flavus* and *A. parasiticus.* They are commonly found to contaminate food and feeds, such as milk, corn, peanuts, cottonseed, rice, and barley, grain-fermented beverages and edible animal tissues (Park & Pohland, 1986).

Aflatoxins were first discovered in 1960 when a series of outbreaks in poultry and fish occurred in different parts of the world. One of the worst outbreaks was the "Turkey-X" disease that caused the deaths of many turkies, ducklings, and chicks in Britain (Blount, 1961). Consumption of aflatoxin-contaminated Brazilian groundnut meal was implicated in the disease. At the same time, feeding of contaminated corn, peas and cottonseed to farm animals and fish were reported to cause outbreaks analogous to the "Turkey-X" disease elsewhere (Palmgren & Ceigler, 1983).

Experiments conducted on the contaminated Brazilian peanut meal resulted in the isolation of A. flavus, and when the fungus was inoculated into untainted peanut meal, the fungus produced toxins similar to those found in the contaminated meal (Sargeant *et al.*, 1961). The isolated toxins were named "aflatoxin."

The current technology and knowledge can neither totally inhibit aflatoxins synthesis by the molds nor completely eliminate them once they are produced. As a result, the significance of aflatoxins contamination has long been recognized, and limits in agricultural commodities have been set since 1965. In 1993, the International Agency for Research on Cancer upgraded AFB_1 from a Group II to a Group I human carcinogen classification (IARC, 1993).

Structure and Toxicity

Aflatoxin B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1), and G_2 (AFG_2) are the four main naturally-occurring aflatoxins. The letters B and G refer to the aflatoxins' color under UV light (B for Blue; G for Green), and the subscripts 1 and 2 refer to their relative positions on a developed thin-layer chromatography plate. The structure of aflatoxins consists of a coumarin nucleus attached to a bifuran and either pentanone (AFB_1 and AFB_2) or a six-membered lactone (AFG_1 and AFG_2) (Figure 1). AFB_1 and AFG_1 are more toxic to rats and ducklings as compared to AFB_2 and AFG_2 (Wogan, Edwards & Newberne, 1971). As for carcinogenicity, AFB_1 is more carcinogenic than AFG_1, while AFG_1 is more carcinogenic than AFB_2 (Shoenhard *et al.,* 1981). The main target organs for AFB 1 toxic and carcinogenic effects are the liver and kidney.

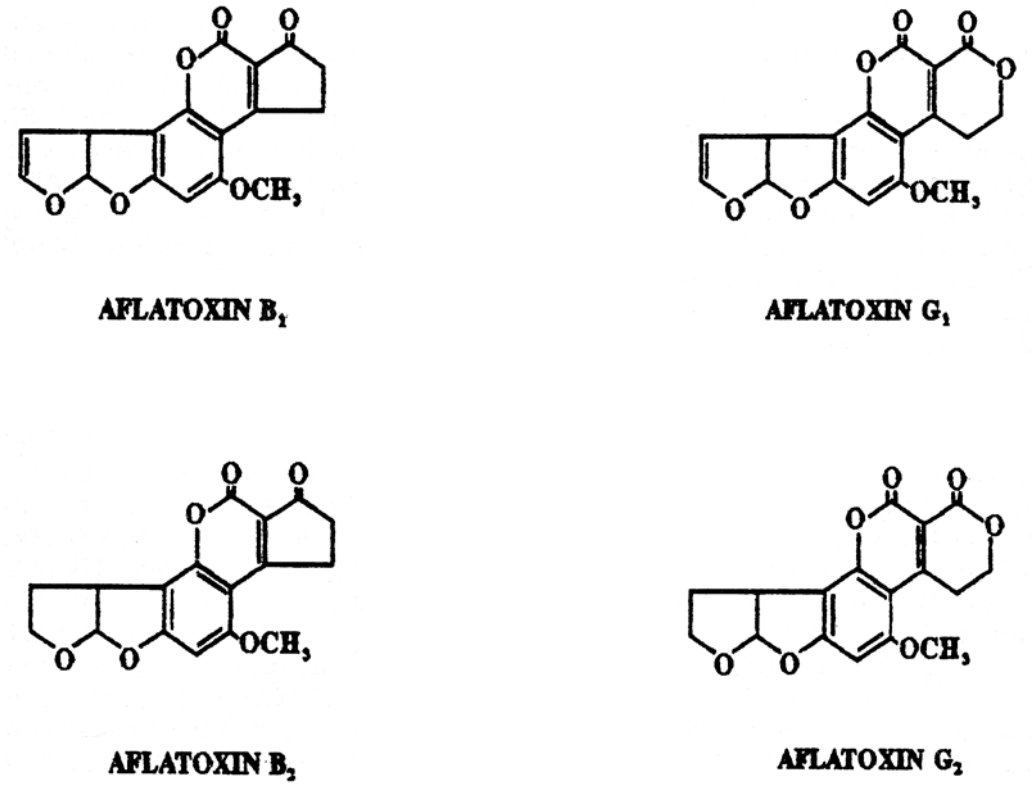

Figure 1: Structures of naturally occurring aflatoxins

AFB_1 Metabolism and Carcinogenicity

AFB_1 is the most abundant and toxic form of all naturally occurring aflatoxins. AFB_1 represents 75% of all aflatoxins found in contaminated food and feeds. It is hepatotoxic (O'Brien *et al.,* 1983), hepa-tocarcinogenic (Adamson *et al.,* 1979), and teratogenic (Bassir & Adekunle, 1970) to various animal species. AFB_1 is first metabolized (Phase I metabolism) mainly by the cytochrome P-450 enzyme (CYP450) system found in the microsome. This metabolism will produce a variety of metabolites such as AFB_1-epoxide and hydroxylated metabolites (AFM_1, AFP_1, AFQ_1, and aflatoxicol).

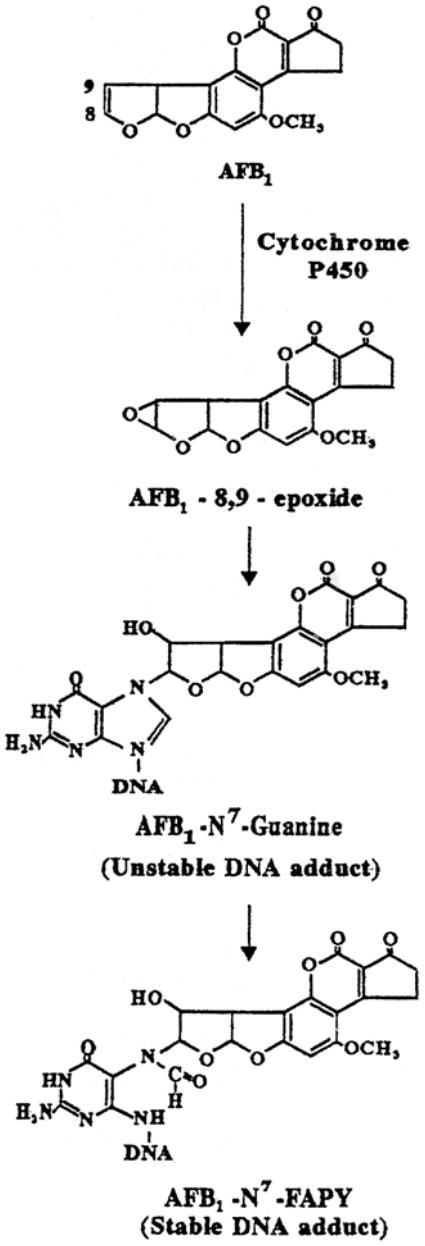

Figure 2: AFB_1-DNA adduct formation

AFB_1-epoxide is a very reactive and unstable metabolite of AFB_1 that will bind to cellular DNA, RNA, and protein. The formation of AFB_1-DNA adduct is highly correlated to the carcinogenic effect of AFB_1 in both animal and human cancer cases (Thabrew & Bababumi, 1980; Wogan *et al.,* 1971; Alpert *et al.,* 1971; Groopman, Cain, & Kensler, 1988). The "Virtually Safe Dose" of AFB_1 is estimated at 0.016 ng/kg/day (reviewed by Eaton & Gallagher, 1994).

The major AFB_1-DNA adduct formed with liver DNA is aflatoxin B_1-N^7-guanine (AFB_1-N^7-gua) (Essigmann *et al.,* 1977). This adduct is unstable and subjected to decomposition. The major decomposed derivatives of AFB_1-N^7-gua in rat liver are the imadozole ring-opened AFB_1 formamido-pyrimidine adducts, namely AFB_1-N^7-FAPY major and minor (Croy & Wogan, 1981) (Figure 2). AFB_1-N^7-FAPY adducts are more stable, and their accumulation in liver DNA is related to the subsequent reduction of AFB_1 -N^7-gua adduct level.

Hepatocellular carcinoma (HCC) is a major health problem in China where each year approximately 110,000 patients are diagnosed with it. The HCC cases in China account for almost 45% of HCC incidences in the world (Parkin, Sternward & Muir, 1984). The mortality rate for HCC is more than 95%. Excluding other risk factors, the consumption of AFB_1-contaminated food such as corn, soya-based products, and peanut oil was correlated

(r = 0.55) to the HCC fatality rates in people living in ten Chinese villages that were studied. See Yu (1995) for a current review of HCC in China.

The phase I AFB_1 metabolites may undergo phase II biotransformation involving the enzymes glutathione S-transferase (GST), β-glucuronidase, and/or sulfate transferase which produce conjugates of AFB_1-glutathione, AFB_1-glucuronide, and AFB_1-sulfate, respectively. The major conjugate of AFB_1-epoxide identified is the AFB_1-glutathione conjugate (Monroe & Eaton, 1987; O'Brien *et al.,* 1983). This conjugation is the principal detoxification pathway of activated AFB_1 in many mammals. It has been accepted that cytosolic GST activity is inversely correlated to susceptibility of the several animal species to AFB_1 carcinogenicity (Eaton & Gallager, 1994; Neal, 1987).

The hydoxylated (AFM_1 and AFQ_1) and O-demethylated (AFP_1) metabolites of AFB_1 can undergo glucuronidation and sulfation. Glucuronidation, catalyzed by liver microsomal UDP-glucuronyl transferase (UDPGT), has been reported for a variety of endogenous and foreign compounds (Burchell & Coughtrie, 1989). These conjugations results in formations of water-soluble aflatoxin esters that are excreted in the urine or bile (Hseih & Wong, 1982).

AFB_1 and Diet Interactions

Species differences, nutritional manipulations, health status, drugs, and chemical treatments affect AFB_1 biotransformations, and thus, its potency. There are many reports on the effects of various foods or nutrients and xenobiotics on AFB_1-macromolecule adducts formation. Obviously, the major objectives of these studies were to determine if and how those nutrients or xenobiotics could affect adducts formation, especially DNA adduct. Tables 1 and 2 summarize some of the effects of nutritional factors and drugs or xenobiotics on the formation of AFB_1-DNA adduct.

Table 1: Influences of dietary nutrients on AFB_1-DNA adducts formation.

Compound*	Test System	Increase (↑) / Decrease (↓)	Reference
Low Protein	Rat liver	↑	Mandell et al. (1992)
Low fat (high carbohydrate)	Rat liver	↑	Nyathi et al. (1993)
Fat (saturated or unsaturated)	Rat liver	NSE*	Marzuki & Norred (1984)
Essential oils	Rat liver microsome	↓	Hashim et al. (1994)
Vitamin A	Rat liver	↓	Bhattacharya et al. (1989)
	Rat liver microsome	↓	Aboobaker et al. (1997)
	Rat liver	NSE	Chen et al. (1982)
	Woodchuck hepatocyte	↓	Yu et al. (1994)
β-carotene	Woodchuck hepatocyte	↑	Yu et al. (1994)
Carotenoids	Rat liver	↓	Gradelet et al. (1998)
Vitamine E	Woodchuck hepatocyte	↑	Yu et al. (1994)
Riboflavin	Rat liver	↓	Webster et al. (1996)
Vitamin B_6	Rat liver microsome	NSE	Bhattacharya et al. (1984)
Thiamin	Rat liver microsome	NSE	
Vitamin C	Woodchuck hepatocyte	↓	Yu et al. (1994)
Lipotropes (deficient)	Rat liver	↓	Campbell, Hayes &

			Newbeme (1978)
Carnitine	Rat liver	↓	Sachan & Ayub (1992)
Choline (deficient)			Bhattacharya et al. (1984)
Rat liver			
- Single AFB_1 dose		NSE	Schrager et al. (1990)
- Multiple AFB_1 doses		↑	Schrager et al. (1990)
Copper	Rat liver microsome	↓	Bhattacharya et al. (1984)
Selenium	Rat liver	↓	Chen et al. (1982)
(Excess or deficient)			
Selenium	Chick liver	NSE	Chen et al. (1982)
Selenium	Hamster ovary cells	NSE	Shi et al. (1995)
Feed restriction	Rat liver	↓	Pegram et al. (1989)
			Gao & Chou (1992)
			Chen et al. (1995)
			Chou et al. (1997)
Indole-3-carbinol	Trout liver	↓	Dashwood et al. (1989)
Trout liver microsome		↓	Takahasi et al. (1995)
R-goitrin	Rat liver	↓	Chang & Bjeldanes (1987)
Curcumin	Rat liver microsome	↓	Firozi et al. (1996)
Garlic compounds	Rat liver S-9 fraction	↓	Tadi, Teel & Lau (1991)
Green tea	Rat liver	↓	Guozhong et al. (1997)
Coffee extracts	Rat liver fractions		Cavin et al. (1998)

*NSE, no significant effect

Table 2: Influences of food additives and drugs on AFB_1 -DNA adducts formation.

Compound*	Test System	Increase (↑) / Decrease (↓)	Reference
BHA	Rat liver microsome	↓	Bhattacharya et al. (1984)
	Rat liver	↓	Chang & Bjeldanes (1987)
	Trout liver	NSE**	Goeger et al. (1988)
	Rat and mouse livers	↓	Monroe & Eaton (1987)
BHT	Rat liver microsome	↓	Bhattacharya et al. (1984)
Cortisol	Rat liver	↑	Chentanez et al. (1984)
Crocetin	Fibroblast cell	↓	Wang et al. (1991a)
DDB	Rat liver	↓	Liu et al. (1995)
Ethoxyquin	Rat liver	↓	Kensler et al. (1986)
Ethanol	Rat liver	↓	Toskulkoa & Glinsukon (1986)
Geniposide	Rat liver	↓	Wang et al. (1991b)
	Rat liver microsome	↓	Wang et al. (1992)
Phenobarbital	Rat liver	↓	Lotlikar et al. (1989)
Dithio- Carbamate	Rat liver	↓	Gopalaswamy et al. (1998)

* BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; DDB; dimethyl-4-4' dimethoxy-5, 6, 5'6'-dimethylenedioxy biphenyl-2,2' dicarboxylate.
**NSE no significant effect

Fat-soluble vitamins

A number of vitamins and vitamin analogs have been tested on AFB_1-macromolecule adduct formation. Bhattacharya and coworkers (1984; 1987) reported comprehensive studies on the effects of various vitamins on in vitro adducts formation.

Vitamin A supplemention in rats inhibited AFB_1-DNA binding (Bhattacharya, Prabhu & Aboobaker, 1989). The protective effects of retinoids such as retinol, retinal, retinoic acid, and retinal esters on AFB_1 carcinogenicity were due to inhibition of AFB_1-DNA adduct formation by affecting the CYP45O systems resulting in less epoxide being formed (Bhattacharya *et al.,* 1984; Aboobaker *et al.,* 1997). Retinal had the same inhibitory effect on the formation of AFB_1 -protein adducts (Bhattacharya *et al.,* 1989). Vitamin A has been shown to induce the activity of glutathione S-transferase, thereby enhancing the detoxification of AFB_1-epoxide. On the other hand, vitamin A deficiency decreased glutathione S-transferase activity.

A combined deficiency of vitamin E and selenium decreased AFB_1 binding to DNA, RNA, and protein (Chen *et al.,* 1982). Vitamin E and menadione (a water-soluble synthetic vitamin K) have been found to prevent AFB_1 -induced mutagenesis in the Ames bacterial system (Raina & Gurto, 1985). β-carotene and vitamin E increased DNA adduct formation in woodchuck hepatocytes (Yu *et al.,* 1994).

Water-soluble vitamins

Riboflavin, riboflavin-5'-phos-phate (FMN), and flavin adenine dinucleotide (FAD) inhibited AFB_1-DNA adduct formation in vitro. Riboflavin was reported as the most effective of the three vitamins (Bhattacharya *et al.,* 1984). It has been recently suggested that the mechanism for the riboflavin effect is its ability to induce the enzymes involved in repairing damaged DNA (Webster, Gaude & Bhattacharya, 1996).

Vitamin C, vitamin B_6 and thiamin had no significant effect on DNA adduct production (Bhattacharya et al., 1984). However, vitamin C, B_6, and folic acid inhibited mutagenesis in bacterial systems (Bhattacharya et al., 1984; Bhattacharya *et al.,* 1987). The inhibition by vitamin C was not as great as with the fat-soluble vitamins.

In a study employing woodchuck hepatocytes to find the role of vitamins A, C, and E, and β-carotene on the initiation of AFB_1-induced carcinogenesis, the workers found that vitamin A was more effective than vitamin C in inhibiting DNA adduct formation. In contrast, vitamin E and β-carotene enhanced the binding (Yu *et al.,* 1994). However, a current study reported that carotenoids were effective in lowering AFB_1-DNA adduction in rats. This reduction was due to the enhancement of the detoxification of the activated AFB_1 (Gradelet *et al,* 1998). Therefore, these results suggest that different antioxidant vitamins may effect AFB_1-DNA binding differently.

Amino acids

There are conflicting reports on the effects of different amino acids on AFB_1 carcinogenesis. A diet marginally deficient in methionine (which was also deficient in choline and lacking in folacin) depressed DNA and RNA adducts formation in rat liver. Protein adduct formation was

not affected by the diet. The inhibition of AFB_1-nucleic acid adducts in the marginally lipotrope-deficient diet was due to the decrease in the activation of AFB_1 and not due to an increase in glutathione levels (Campbell, Hayes & Newberne, 1978). A choline and methionine-deficient diet fed rats showed no significant AFB_1 dose-response changes in serum biochemical parameters or liver pathology compared to the complete amino acid diet (Mehta *et al.*, 1993). These two studies treated the rats with a single dose of AFB_1.

Schrager *et al* (1990) found that a single dose of AFB_1 did not affect the DNA adduct concentration in both choline-deficient and control animals. However, when multiple doses of AFB_1 were administered, the AFB_1-DNA adduct levels were significantly higher in the rats fed a choline-deficient diet than in the rats fed with a control diet. An earlier report also found that marginally deficient lipotrope diets induced AFB_1 tumorigenesis in rats (Rogers & Newberne, 1969).

L-carnitine supplementation in rats has been found to decrease AFB_1-DNA binding in rats (Sachan & Ayub, 1992). Carnitine, a quarternary amine whose structure is quite similar to choline, can also affect AFB_1 binding to hepatic RNA and protein (Table 3). The total amount of AFB_1 present in the liver and kidney were not significantly different between the carnitine supplemented and control animals. However, the concentrations of AFB_1 were higher in the plasma of carnitine supplemented rats than the non-supplemented rats. Recently, we found that carnitine inhibited the microsomal activation of AFB_1 and on the binding of activated AFB_1 to exogenous (calf thymus) DNA (Ayub *&* Sachan, unpublished data). This maybe the mechanisms for carnitine reducing the AFB_1-DNA adducts formation. We had also shown that carnitine ameliorated the earlier signs of acute toxicity of AFB_1, such as the elevation of total lipid concentration in the liver and the decrease in total lipids and triacylglycerol concentrations in the plasma (Sachan & Ayub, 1991; Sachan & Ayub, 1992).

Sulfur-containing amino-acids such as cysteine, N-acetylcysteine, cystine, methionine, and glutathione inhibited AFB_1 mutagenicity in microbial systems. Cysteine and Nacetylcysteine were more potent inhibitors than glutathione. The investigators suggested that the inhibition was due to amino acids affecting the synthesis of AFB_1-epoxide (Shetty Francis & Bhattacharya, 1989).

Table 3: Effects of L-carnitine supplement on aflatoxin B_1-macromolecules adducts formation in rat liver 6-h post-aflatoxin B_1 administration[1]

	Group		
Parameter	Control	L-Carnitine[2]	Difference (%)
	pmol/mg macromolecules[3]		
AFB_1-DNA	6.8 ± 28[a]	4.7 ± 2.9[b]	30.9
AFB_1-RNA	21.6 ± 0.3[a]	14.3 ± 0.5[b]	33.8
AFB_1-Protein	1.2 ± 0.1[a]	1.2 ± 0.1[a]	0

1 Values are mean ± SEM, n = 5.
2 Diet contained 0.4% L-carnitine (w/w) and given to animals for 6 weeks.
3 Different letters indicate significant difference between groups ($p < 0.05$).

(Source: Sachan & Ayub 1992)

Protein

Weanling rats fed a low protein diet (5% casein) had fewer AFB_1-induced preneoplastic foci in their livers than rats fed a high protein diet (20% casein) (Youngman & Campbell, 1992). The enhanced development of the preneoplastic foci (g-glutamyl transpeptidase-positive foci) by the high protein diet was reversed when the animals were put on a low protein diet. Thus, it was concluded that the low protein diet prevented lesions caused by AFB_1.

Mandell *et al.,* (1992) reported that AFB_1-induced hepatocar-cinogenicity can occur in both low- and high-protein fed weanling animals. Moreover, the low protein diet (5% lactalbumin) also caused severe liver histopathological changes or sub-acute toxicity symptoms such as necrosis and bile duct proliferation due to AFB_1. Also, the protein-deficient animals had a more rapid decrease in glutathione S-transferase activity than the protein-sufficient (20% lactalbumin) animals. High protein fed animals did not show the sub-acute toxicity responses induced by AFB_1. Therefore, the authors suggested that protein deficiency is more likely to enhance, rather than protect the liver against, AFB_1 toxicity and carcinogenicity.

Fat and Essential Oils

Different types and amounts of fat may have different effects on the carcinogenesis of AFB_1. High polyunsaturated oil (corn oil) increased the incidence of liver cancer in rats caused by AFB_1 as compared to rats fed with saturated oil (beef fat). The cancer incidence was higher when the corn oil was fed with or after the exposure to AFB_1 than when the oil was fed before the AFB_1 dose. An increase in induction of AFB_1 activation by corn oil was suggested for the high cancer incidence (Newberne, Weiger & Kula, 1979). However, in a similar study, dietary saturated (coconut oil) and unsaturated (corn oil) fats were found to have no significant effect on the adduct formation and the production of AFB_1-epoxide in rat livers (Marzuki & Norred, 1984).

In another report, the metabolism and mutagenicity of AFB_1 were not significantly different between mice fed with beef fat and olive oil diets (Brennan-Craddock *et al,* 1990). With respect to the levels of dietary fat, a low-fat (high carbohydrate) diet increased the AFB_1-DNA binding more than a high fat diet (Nyathi *et al.,* 1993). The protective effect of a high fat diet may be due to a decrease in the uptake of AFB_1 into hepatocytes or a reduction of AFB_1-epoxide production.

Hashim *et al.* (1994) investigated the capability of essential oils extracted from nutmeg, ginger, cardamom, celery, xanthoxylum, coriander, cumin, and black pepper to inhibit AFB_1-DNA adducts formation mediated by liver microsomal enzymes. All the essential oils tested were suppressive to the adducts formation, and the inhibition was dose-dependent. The modulating effect of these oils was through their ability to inhibit the activation of AFB_1.

Trace Elements

Copper inhibited AFB_1-DNA binding in vitro (Bhattacharya *et al.,* 1984). A deficiency and an excess of selenium decreased the adduct formation in rats. In chicks, however, excess of selenium did not change the concentration of adducts formed (Chen *et al.,* 1982). Recently,

selenium was demonstrated to have no effect on DNA adduct formation in ovary cells and did not effect AFB_1 mutagenesis (Shi, How & Ong, 1995).

Copper, manganese, zinc, and selenium were effective in preventing in vitro AFB_1-induced mutagenesis. Copper was the most potent among the elements tested. To a lesser extent, iodine, molybdenum, cobalt, and iron were antimutagenic. The investigators suggested that the inhibition was due to interaction of trace elements with the microsomal enzymes (Francis, Shetty & Bhattacharya, 1988).

Feed Restriction

The potentially protective effects of caloric restriction on cancer-causing compounds has promoted considerable interest and investigation. It has been reported that rats fed with 60% of the food consumed by ad libitum animals had lower AFB_1 microsomal activation, lower AFB_1-adducts, faster plasma clearance, and increased urinary excretion of AFB_1 than the ad libitum fed animals. The authors concluded that 40% feed restriction may decrease AFB_1 carcinogenicity (Pegram, Allaben & Chow, 1989).

Similarly, about more than 50% reduction in AFB_1-DNA binding was found when rats were fed 40% caloric restricted diet (Gao & Chou, 1992; Chou *et al.,* 1997). The restriction also decreased the hepatic DNA double strand damage induced by AFB_1. Induction in glutathione S-transferase activity in feed restriction will enhance AFB_1-epoxide conjugation to GSH and thus reduce AFB_1-DNA adducts formation (Chen *et al,* 1995).

Cruciferous Vegetables

Cruciferous vegetables have been shown to enhance detoxification of xenobiotics by inducing xenobiotic-metabolizing enzymes in animals and humans (Salbe & Bjeldanes, 1989). High consumption of vegetables such as broccoli, cabbage, cauliflower, and Brussels sprouts has been related to a reduced risk of bladder, colon, and rectum cancers (Grahams, 1983).

Brussels sprouts significantly decreased AFB_1-DNA binding and increased the GST activity in rats. Indole-3-carbinol, a compound found in cruciferous vegetables, did not have much effect on the DNA binding and GST activity (Salbe & Bjeldanes, 1989). The same investigators also found that the route of administration, intragastric or intraperitoneal, did not have a different effect on AFB_1-DNA binding. Thus, they concluded that the small intestine did not play an important role in AFB_1 metabolism. However, in another study that utilized trout, 1000 and 2000 ppm of indole-3-carbinol were shown to strongly depress AFB_1-DNA adducts formation (Dashwood *et al,* 1989). R-goitrin, another compound found in cruciferous vegetables, also exhibited anticarcinogenic properties such as inhibition of AFB_1-DNA binding, induction of GST activity, and enhancement of biliary excretion of AFB_1 in rats (Chang & Bjeldanes, 1987).

Plant Flavonoids and Phenolic Compounds

Five major derivatives of plant flavonoids, namely flavone, flavonol, isoflavone, and flavanol, have been tested on activation of AFB_1 and AFB_1-DNA adducts formation (Bhattacharya &

Firozi, 1988). Most of the flavonold derivatives significantly inhibited adduct formation, and flavonols being the most potent. Flavonols also showed greater inhibition of AFB_1 mutagenicity in bacterial system (Goeger *et al,* 1988).

Phenolic compounds may have protective effects against AFB_1-induced mutagenicity. Gallic acid, chlorogenic acid, caffeic acid, dopamine, p-hydroxybenzoic acid, and salicyclic acid decreased mutation caused by AFB_1 in bacterial system containing rat-liver microsomes. The inhibition occurred when the compounds and AFB_1 were administered concurrently (San & Chan, 1987). Using the S9 liver fraction that contains the metabolic enzymes, ellagic acid (a compound found in strawberries, grapes, and walnuts) has been shown to be antimutagenic against AFB_1 in bacterial assay (Loarca-Pina et al., 1996). The inhibition was greatest when the acid was incubated together with AFB_1.

Curcumin, a phenolic compound extracted from tumeric, was recently reported to inhibit the production of AFB_1-epoxide by affecting CYP450 enzyme function (Firozi, Aboobaker, Bhatacharya, 1996). The inhibition became higher as the curcumin concentration was increased in the incubation mixture. However, the suppression was reversed when the CYP level in the mixture was higher.

Diterpenes cafestol and kahweol extracted from coffee were shown to inhibit AFB_1-DNA covalent binding in rat liver fractions. The decrease in activation of AFB_1 and induction of GST expression were the suggested mechanisms of the inhibition (Cavin *et al.,* 1998). Green tea drink fed to rats also inhibit AFB_1-DNA binding by affecting AFB_1 metabolism (Guozhong *et al.,* 1997).

AFB_1 and Food Additives/Drugs Interactions

Food Additives

Rats fed a butylated hydroxyanisole (BHA)-containing diet had lower AFB_1-DNA binding, higher GST activity, and higher biliary excretion of AFB_1 (Chang & Bjeldanes, 1987). Animals treated with butylated hydroxytoluene (BHT) before or together with AFB_1 had lower cancer incidences than the animals administered AFB_1 alone (Dragon and Pitot, 1994). However, in trout, BHA did not effect liver tumor incidence, AFB_1-DNA binding, or AFB_1-glutathione conjugation (Goeger *et al.,* 1988). In vitro system, both BHA and BHT inhibited AFB_1-DNA binding (Bhattacharya *et al.,* 1984). Another antioxidant, ethoxyquin, also suppressed AFB_1 carcinogenesis by inducing the activity of glutathione S-transferase activity (Kensler *et al.,* 1986).

Drugs

The activities of CYP, GST, and UDPGT enzymes can be induced by several drugs or xenobiotics. Enzyme inducing drugs such as pheno-barbital (anti-seizure drug) and Aroclor 1254, given before or together with AFB_1, reduced the number of neoplasms as compared to animals given AFB_1 only (Dragan and Pitot, 1994). Although phenobarbital enhanced AFB_1 activation, it also induced GST activity and thus increased AFB_1-glutathione conjugation.

Therefore, the overall hepatic binding of AFB_1 to DNA is reduced (Loury, Hseih & Brard, 1984; Lotlikar *et al,* 1989). Other inducers such as ethoxyquin (Kensler et al., 1986), and oltipraz (Primiano *et al.,* 1995), have also been shown to inhibit AFB_1-induced carcinogenesis by inducing GST activity. In a study employing human hepatocytes, oltipraz was also reported to lower the production of AFB_1-epoxide by inhibiting the CYP1A2 and CYP3A4 activities (Longouet *et al.,* 1995).

A compound isolated from a Chinese herb, dimethyl-4,4' dimethoxy-5,6,5',6'-dimethylenedioxy biphenyl-2,2'-dicarboxylate (DDB), is a drug used for its liver protective effects. Pretreatment of rats with DDB inhibited liver damage caused by AFB_1. DDB also induced the activity of glutathione S-transferase and therefore enhanced detoxification of AFB_1-epoxide (Liu *et al.,* 1995). Crocetin, a carotenoid isolated from the seeds of Cape jasmine, has been reported to elevate the cytosolic glutathione S-transferase activity and glutathione concentration in a fibroblast cell line treated with AFB_1 (Wang, Shiah & Lin, 1991a). Another Chinese herbal drug, geniposide, isolated from a fruit of a species of gardenia, can also inhibit AFB_1-induced DNA binding. Induction of glutathione S-transferase and gamma glutamyl cysteine synthase (involved in glutathione synthesis) activities, and suppression of AFB_1-induced unscheduled DNA synthesis were the suggested mechanisms of action of geniposide (Wang, Wang & Lin, 1991b; Wang, Lai & Wang, 1992).

Cortisol pretreatment in rats markedly increases the acute hepatoxicity of AFB_1 (Chentanez *et al.,* 1988). The toxicity effects, such as higher mortality rates, increased in liver triacylglycerol, and elevated AFB_1 binding to DNA and protein, were dose-dependent. These cortisol effects may by due to increased metabolism of AFB_1 to its epoxide derivative.

Ethanol, when given to animals together with or prior to aflatoxin, increased the aflatoxin hepatotoxicity and DNA binding (Toskulkoa & Glinsukon, 1986; Toskulkoa, Lohokachonpan & Glinsukon, 1991; Sahaphong,Toskulkoa & Glinsukon, 1992). The alcohol pretreatment increased the activation of AFB_1 but not the GST activity. This explains the increased binding of AFB_1 to DNA. On the other hand, when given after AFB_1 administration, ethanol showed no influence on AFB_1-DNA binding (Messlbeck Campbell & Roe, 1984).

CONCLUSION

Aflatoxins are a real public health problem and research should be continued to prevent their presence in food, and to inhibit their harmful effects. Much progress has been achieved in showing the importance of dietary factors in modulation of AFB_1 toxicity and carcinogenicity. Obviously, there are still a wide range of dietary components that can be explored and investigated. Additionally, the more important research areas would be in explaining the protective mechanisms and formulating the effective "dose" before any true public health measures could be recommended.

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