GENERAL ARTICLE

AFLATOXINS : TOXICITY AND CARCINOGENICITY

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Aflatoxins (AFs) (see Figure 1), a group of mycotoxins produced by the fungi *Aspergillus parasiticus* and their animal biotransformation products, have been considered as responsible for the deaths of large numbers of domestic animals and possibly for the high incidence of liver cancer among exposed human populations (1). These aflatoxin producing mold strains can grow at temperatures of 40-45°c with an optimum at 30°c, and a relative humidity of 75% or greater. These naturally occured toxins had a characteristic fluorescence pattern on thin layer chromatograms at four components designed as aflatoxins B_1, B_2,G_1 and G_2 ; the four components were distinguished by their blue or green fluorescence and by their Rf values on thin layer chromatograms. Twelve of structurally related components have been isolated and chemically characterized, ie. M_1, M_2, B_2, G_{2a},etc. The M_1 and M_2 fractions were first isolated from the milk of cows feeding on AFs contaminated food.

The chemical structure of the AFs contains a difurofuran or bisfuran ring system fused to a substituted coumarin moiety, with a methoxy group attach at the corresponding benzene ring. These functional groups all have definite effects on the toxicity of AFs (2-4).

The toxin that has been studied most extensively and constitutes by far the major components of most naturally produced mixtures of AFs is AFB_1. It is the most toxic member of the group and has also been shown to be the most potent hepatocarcinogen yet found for the rat and rainbow trout (3,4).

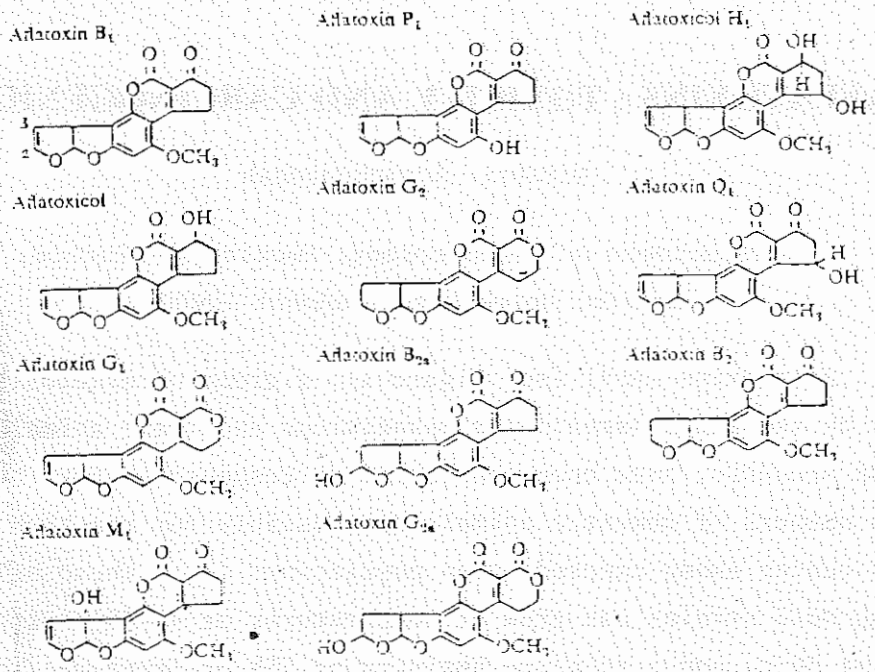

Figure 1. Structures of the naturally occuring aflatoxins and metabolites.

TOXICITY

The toxic actions of aflatoxins have been investigated in many kinds of biologic test systems. The aflatoxins are lethal to many animal species thus far studied, when administered acutely at suitable dose levels. Typical single dose LD_{50} values of aflatoxin B_1, is in the range of 0.5-10 mg/kg body weight (Table 1).

In all species, AFs toxicity main target organ is the liver ; other can be kidney, stomach, lung, salivary and lacrimal glands, colon and skin. In AFB_1 acute and subacute poisoning, liver is the main target organ. It cause centrilobular necrosis and/or bile duct proliferation. Toxicity depends on age, sex, species, strain and diet. Repeated dose or chronic feed AFB_1 can induce hepatocellular carcinoma and cholangioma in animal at least 3 species (rat, rainbow trout, duck). Sheep is resistant to chronic exposure to AFB_1. And in all

Table 1. Single dose lethality of AFB_1 in various animal species (5)

Animal	Age or Weight	Sex	LD50(mg/kg)
Duckling	1 day	male & female	0.40
Rabbit	weanling		0.50
Dog	adult	male & female	0.50
Trout*	100 g	male & female	0.50
Guinea pig	adult	male & female	1.40
Rat	1 day	male & female	0.56
	21 days	male	5.50
	21 days	female	7.40
Hamster	21 days	male	5.50
	30 days	male	10.20
Mouse	21 days	male	63.00
Sheep	adult	male & female	500.00

* Intraperitoneal; other values refer to the oral route.

species, young animals are more susceptible than adult, male are more susceptible than female to acute and subacute toxicity and develop tumor more quickly than female. Toxic and carcinogenic effects are enhanced by low protein diets or cirrhogenic diets prior to or during exposure to toxic compound.

In human, number of cases and evidence involving AFs in acute poisonings were reported in Thailand (6) Uganda (7) and Taiwan (8). Reye's syndrome is epidemic in northeastern Thailand and is limited to children up to adolescene; it is characterized by a short prodrome of several hours followed by vomiting, hypoglycemia, convulsions, hyperammoniemia, and coma usually ending in death within 24 to 48 hours after onset. Histopathologic examination revealed severe cerebral edema and extensive fatty accumulation in hepatocytes, renal tubular epithelium, and myocardial fibers. Autopsy specimens from 23 Thai children who died with Rey's syndrome were chemically assayed for aflatoxins; AFB_1 and AFB_2 were found in the liver specimens from 22 cases, usually at the levels

between 1 and 4 µg/kg tissue wet weight. In two cases, the concentrations of AFB_1 were of the same magnitude as the levels in the livers from monkeys in the acute toxicity study. At this level, AFB_1 intoxication in the monkey was remarkably similar to Rey's syndrome. However, other factors may be involved in this syndrome such as malnutrition status, viral infection and seasonal changes.

CARCINOGENICITY

In vitro stuies of the mode of action of AFs revealed that AFB_1 requires metabolic activation for toxic activity (3). Since distinct differences in drug metabolizing activity exist among various animal species, the differences in species susceptibility to carcinogenic aflatoxicosis have been considered with these metabolic differences. Correlations between the in vitro hepatic metabolism of AFB_1 and species susceptibility to aflatoxicosis have been shown (8-10). Under *in vitro* conditions as reviewed by Campbell and Hayes (3), AFB_1 is known to be oxidized by the mixed-function oxygenases of the microsomal fraction of homogenized liver to from the products of hydroxylation, AFM and AFQ_1; of o-demethylation, AFP_1; of hydration, AFB_{2a} ; and of epoxidation, the 2,3-epoxide of AFB_1. AFB_1 is also reduced by the reductase. In addition, the cytosol fraction contains numerous enzymes and other molecules involved in the conjugation of certain AF metabolite to render them water-soluble.

To determine the relative contributions of these metabolites toward the carcinogenic effect of AFB_1 and to seek the active form of AFB_1, Wong and Hsieh (4) performed the Ames mutagen assay on all of these metabolites except for the 2,3-epoxide of AFB_1, which has not yet been isolated. The results are summarized in Table 2. The 2,3-epoxide was postulate to be an ultimate carcinogenic metabolite of AFB_1 that can bind covalently to the DNA at the N^7 -position guanine base yielding N^7 -guanine AFB_1 adduct of DNA or RNA molecules (11).

Table 2. Correlation of *S.typhimurium* mutagenicity of aflatoxins to their animal carcinogenicity

Aflatoxin	Relative mutagenicity (%)[a]	Carcinogenicity
AFB_1	100	most potent hepatocarcinogen in rat and rainbow trout
AFL	22.8	possessed half the tumor activity of AFB_1 in rainbow trout
AFG_1	3.3	less tumorigenic in rat than AFB_1 less hepatocarcinogenic in rainbow trout than AFB_1
AFM_1	3.2	possessed one-third the tumor activity of AFB_1 in rainbow trout less tumorigenic than AFB_1 in rat
$AFLH_1$	2.0	
AFQ_1	1.1	nontumorigenic in rainbow trout
AFB_2	0.2	inactive in rat, weak activity in rainbow trout
AFP_1	0.1	
AFG_2	0.1	found inactive in rainbow trout
AFB_{2a}	0.00	
AFG_{2a}	0.00	

Data from Wong and Hsieh (4)
a = Slope value percent : compound slope divided by AFB_1 slope x 100.

The postmitochondrial supernatant (S-9) of the rat liver was used as the activation system for the AFs to cause reverse mutation to a sensitized frameshift auxotroph of *Salmonella typhimurium*, strain TA 98. The hepatic S-9 preparation was definitely required for the mutagenic activity of all the AFs tested, indicating that none of them is the

active from but that they are indeed *procarcinogens*(precarcinogens). Of all the AFs tested AFB_1 had the highest mutagenic activity, followed by AFL, AFG_1, and other derivatives Therefore,it appears that AFB_1 has the molecular structure optimal for mutagenic activity. Any alteration of either the 2, 3-vinyl ether double bond, the methoxy, or the cyclopentenone groups invariably results in a marked reduction in activity.

When these mutagenicity data were compared with the carcinogenicity data for various AFs obtained from animal-feeding experiments a remarkable correlation was found between the *in vitro* bacterial mutagenicity of these compounds and their *in vivo* animal "carcinogenicity, as shown in Table 3.

The difference in the carcinogenic potential of various AFB_1 metabolites suggests that the susceptibility of an animal to carcinogenic aflatoxicosis may be a reflection of the net bioactivation efficiency of the simultaneous action of various metabolic pathways in the liver. In other words, the probability that a biochemical lesion caused by AFB_1 may be a function of the concentration of the active form of AFB_1 formed at the target site. This critical concentration of AFB_1 in a particular liver can be estimated as Salmonella mutagenicity by the Ames assays using the hepatic S-9 preparation derived from the liver in question. Hsieh et al. (12) used the hepatic S-9 preparations derived from different animals for the Salmonella mutagenicity test to compare the net bioactivation efficiency for AFB_1 in their livers. Because of their distinct differences in susceptibility to the *in vivo* carcinogenic effect of AFB_1, four species, i.e., ducks, rats, monkey and mice were used as source of hepatic S-9 system for *in vitro* asssays of AFB_1 bioactivation. This data,including human liver S-9 preparation activity were shown in Table 3. The *in vivo* responses of each species to the carcinogenic effect of AFB_1, as available from the literature, are also tabulated for comparison.

From the limited data complied, the ranking of the relative

Table 3. Aflatoxin-B_1-induced in vitro *Salmonella* mutagenicity and in vivo animal carcinogenicity (12).

		AFB_1 exposure		
Species (no., sex)	*Mutagenic activity (rev./μg AFB_1/mg prot.)*	*level*	*time (y-v)*	*Hepatoma incidence (%)*
Duck (3, male)	8493 (6508–10,489)	35 ppb	1.2	72
Rat (9, male)	2005 (1262–2643)	15 ppb	1.3–1.5	100
		100 ppb	0.8–1.2	86
		100 ppb	2.0	48
Monkey (5, male)	814 (119–2056)	360 ppb	3.0	0
		1800 ppb	3.0	0
		99–842 mg	4–6.0	7
Mouse (9, male)	491 (420–605)	1000 ppb	1.3	15
		1000 ppb	1.7	0
		100 μg/day	1.0	0
Human (5, female)	286 (100–755)	?	?	?

net bioactivation efficiencies for AFB_1 of the livers of different animal species appears to be consistent with the ranking of their susceptibilities to the carcinogenic effect of AFB_1.

Based on the observations of long-term feeding experiments, mice and monkeys are shown to be relatively resistant to the carcinogenic effects of AFB_1, compared to the rat (the most wildly used animal for AF carcinogenicity determinations), which developed a 48% incidence of liver carcinoma after 2 years of continous feeding of 100 ppb of AFB_1 in the diet (13). Only 7% of monkeys developed liver tumors after 2 years of multiple subacute doses and multiple routes of exposure to AFB_1 (14), and no tumor was detectable in mice fed 1,000 ppb of AFB_1 for 80 weeks (2). Hsieh et. al. (12) concluded that the low in *in vitro* bioactivation activity of human S-9 preparations relative to that of the other species indicate that the human were less sensitive or more resistant than mice to the carcinogenic aflatoxicosis. So far, correlations have been presented between the levels of aflatoxins found in diets consumed in certain areas of Thailand (1) East Africa (15,16) and

the high frequency of occurence in these areas of primary liver cancer. However, liver tumors in these areas may arise from more than one etiological factor, i.e., alcohol consumption, low protein diet and liver fluke infection.

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