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Effects of dietary fat and aflatoxin B_1 on microsomal monooxygenase activity*

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Abstract. An acute experiment was conducted to determine the short-term effect of an LD_{50} dose of AFB_1 on rats fed a diet containing either 30% corn oil (unsaturated) or 28% beef fat (saturated) for 3 weeks. The male weanling Wistar rats weighing 50–65 g were fed the respective dietary fats for 2 weeks and then given a single dose of AFB_1 (7 mg/kg body weight) dissolved in dimethyl sulfoxide by gastric intubation. One week later they were sacrificed and assays for *p*-nitroanisole demethylase and benzpyrene hydroxylase were performed on liver microsomes to determine the activity associated with the two types of dietary fat. The rats fed corn oil or unsaturated fat had lower total liver fat and a lower mortality rate than those fed beef fat. The basal levels of liver microsomal oxidase activity were higher in rats fed the corn oil diet than in those given the beef fat diet.

Key words: Diet – Aflatoxin – *p*-Nitroanisole demethylase – Benzo(a)pyrene hydroxylase

Introduction

Aflatoxin B_1 (AFB_1) is a mold metabolite produced by certain strains of *Aspergillus flavus* on stored foodstuffs. It is known to be very toxic and hepatocarcinogenic in a wide variety of animals (Newberne and Butler 1969). A positive correlation has been demonstrated between AFB_1 levels in the food eaten and the frequency of human liver cancer (Alpert et al. 1971).

This compound is not toxic or carcinogenic per se but is metabolically converted to highly reactive derivatives that react with various cell constituents in many different cell organelles. The enzymes responsible for its activation are located, predominantly, in the endoplasmic reticulum and belong to a general group known as the "mixed function oxidase system". This enzyme system has the capacity to both detoxify many chemical carcinogens, including aflatoxins, and to activate them to proximate or ultimate carcinogenic forms (Miller 1970).

Since the activity of this enzyme system is subject to major positive and negative influences such as diet, the possibility exists that diet may be an important factor in tumorigenesis (Carroll and Khor 1975). There has been considerable interest in studying the dietary factors that influence tumor development (Carroll 1975; Wattenberg 1975). The results reported here were derived from experiments conducted to determine the effects of low-melting and high-melting dietary fats and AFB_1 on liver microsomal mixed-function oxidase activity in rats.

Materials and methods

Treatment of animals. Male Wistar rats weighing an average of 60 g were fed either unsaturated dietary fat containing 30% corn oil or saturated dietary fat containing 28% beef fat for 3 weeks. Beef fat was a refined tallow and corn oil was a corn product of Elianto, Kenya Ltd. Corn oil (2%) was added to beef fat to prevent essential fatty acid deficiency. At the end of the 2nd week under these dietary conditions, they were given a single dose of AFB_1. AFB_1 was dissolved in dimethyl sulfoxide at a concentration of 1.25 mg/ml and administered by gastric tube at a dose of 7 mg/kg, which is equivalent to the LD_{50} (Newberne et al. 1979). The diets used in this study were adequate in all known respects, with equal fat content but different saturation.

Preparation of microsomes. On day 7 after AFB_1 treatment the surviving rats were killed by decapitation and the livers were homogenized in the cold in four volumes of a buffer consisting of 0.25 M sucrose, 0.02 M Tris-HCl, 5.5 mM EDTA, pH 7.5. Cell debris were eliminated by differential centrifugation at 10 000 rpm in 10 min. The microsomal fraction was pelleted by centrifugation for 60 min at 100 000 *g*. Microsomal pellets were washed once with a buffer consisting of 0.12 M KCl and 0.05 M Tris-HCl pH 7.5, and then resedimented by centrifugation at 100 000 *g* and resuspended in the same buffer.

Assays. The microsomal protein content was determined according to Lowry et al. (1951), while the fat extracted from the liver was determined gravimetrically. Assays for hepatic microsomal system (*p*-nitroanisole demethylase and benzpyrene hydroxylase) were determined as described in the method of Kinoshita et al. (1966) and McLean (1966), respectively.

* Dedicated to Professor Dr. med. Herbert Remmer on the occasion of his 65th birthday

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Table 1. Mortality rate and total liver fat associated with dietary fat and AFB_1 treatment

Dietary fats	Body wt (g)	Mortality (%)	Liver fat (% of wet wt)
Beef fat	126±3	48.7	8.3±0.5
Corn oil	128±4	20.3	6.7±0.3

Thirty rats per group were fed either beef fat or corn oil diets for 2 weeks and then given a single dose of AFB_1 (7 mg/kg body wt). The diets contained either 28% beef fat with 2% corn oil or 30% corn oil only and had the same basal composition described by Newberne et al. (1979). Total liver fat on wet weight was determined gravimetrically. Values are mean ± SD

Results

Table 1 shows the mortality rate and total liver fat associated with the two types of dietary fat and AFB_1 treatment. The beef fat diet led to a higher liver fat content and to a higher mortality rate than did the corn oil diet. About 49% of rats fed beef fat died, compared to the corn oil-fed group in which only 20% died.

Table 2 contains data on hepatic microsomal oxidase activity associated with the two types of dietary fat before and after AFB_1 treatment. Two groups of 20 rats each were fed the respective diets for 2 weeks and then given a single dose of AFB_1 (7 mg/kg body weight) without discontinuation of food. Enzyme assays were performed on ten rats per dietary group both before and after AFB_1 treatment. Basal microsomal metabolic activities were higher following corn oil diet than following beef fat diet. AFB_1 treatment led to a significant increase in *p*-nitroanisole demethylase activity in both dietary groups and to a moderate increase in benzpyrene hydroxylase in the group fed corn oil.

Discussion

A number of in vitro experiments have shown that chemical carcinogenesis can be inhibited or induced by altering mixed function oxidase enzyme activity by dietary constituents (Wattenberg 1975; Newberne et al. 1979). This report also supports the findings of these workers that beef fat induces fatty livers and enhances the toxicity of AFB_1 in rats more frequently than does the corn oil diet.

Table 2. Effects of dietary fat and AFB_1 on liver microsomal oxidase activity

	Enzymes	Beef fat	Corn oil
Diet alone	PNA	211±21	283±19[a]
	BPOH	14± 4	20± 5
Diet + AFB_1	PNA	292±21	403±17[a]
	BPOH	16± 4	24± 5

Ten rats per group that survived the trial period were used, i.e., ten for beef fat diet and ten for corn oil diet. Values are from dry, fat-free liver tissues and are mean ± SD PNA: *p*-nitroanisole demethylase (μg *p*-nitrophenol per g liver per h)
BPOH = benzyprene hydroxylase (quinine units of McLean and McLean 1966)
[a] Significantly different from beef fat group at 1% level

The observed increases in basal microsomal metabolic activities in corn oil-fed group before and after AFB_1 treatment are most probably due to the quantity and quality of unsaturated fatty acids found in corn oil compared to those found in beef fat. The low-melting long chain fatty acids predominating in vegetable oils, such as corn oil, could interfere with the mechanism of AFB_1 metabolism through the alteration of the lipid-rich membrane structure and lead to the induction of enzyme activity.

Although the interpretation of the results is difficult, the induction of increased mixed function oxidase activity might increase the carcinogenic response to a chemical carcinogen such as AFB_1. The induction of increased mixed function oxidase activity is associated with the induction of liver tumors by dietary fat (Newberne et al. 1979). In a longterm study initiated to investigate the effects of dietary fat on the induction of liver tumors by AFB_1, Newberne et al. (1979) observed that AFB_1-treated rats fed corn oil diet developed more liver tumors than did rats fed beef fat. This report also indicates that both metabolic changes for AFB_1, namely, *O*-demethylation by PNA and ring hydroxylation by BPOH, were clearly affected by dietary corn oil and beef fat and that corn oil was most effective.

Dietary corn oil may thus be considered to exert a promoting effect on liver tumors through the alteration of basal microsomal metabolic activities. These observations may support the view that in some population groups of East Africa and South East Asia, where primary liver cancer is common, the livers of hepatic cancer patients are usually fatty and cirrhotic, presumably due to excessive consumption of unsaturated fatty acids of vegetable origin and fish.

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