Effect of Sterculic Acid upon Aflatoxicosis in Rats Fed Diets Containing Saturated and Unsaturated Fat

P. WELLS, L. AFTERGOOD, and R.B. ALFIN-SLATER, School of Public Health, University of California, Los Angeles, California 90024, and R. STRAUS, Providence Hospital, Portland, Oregon 97213

ABSTRACT

Investigations on trout have shown that the cyclopropenoid fatty acids, which occur naturally in small amounts in unrefined cottonseed oil, may act as powerful cocarcinogens when fed in conjunction with aflatoxin. Attempts at confirming these findings in mammals, i.e. rats, have been inconclusive. In this study, the effects of sterculic acid and aflatoxin upon lipid metabolism and tumor formation in male rats have been examined using basal diets containing either saturated or unsaturated fat to which the following additions were made: (A) basal diet (no supplements); (B) aflatoxin B_1 at 1.7 ppm; (C) sterculic acid at 210 ppm; and (D) aflatoxin B_1 at 1.7 ppm, plus sterculic acid at 210 ppm. The rats consumed these diets for 3 months and, thereafter, were fed the unsupplemented basal diet until sacrifice 9 months later. Growth was depressed in rats in groups B, C, and D, but no synergistic inhibition was observed, regardless of the fat source. Liver wt doubled in response to aflatoxin; however, only when the diet contained unsaturated fat did sterculic acid, in combination with aflatoxin, exaggerate the increase in liver wt (a reflection of the more severe liver pathology observed in these rats). In the animals fed the saturated fat diet, aflatoxin administration to animals fed the control or sterculic acid supplemented diets resulted in marked increases in plasma cholesterol levels; the unsaturated fat diets, supplemented with aflatoxin, evoked a slight increase in plasma cholesterol content which was nullified by sterculic acid supplementation.

INTRODUCTION

Aflatoxin contaminated diets have been shown to cause liver damage in most species of animals (1), and aflatoxin is a potent hepatocarcinogen for at least three (rat, rainbow trout, and duckling). Not only does contamination of animal feed with this toxin have an adverse effect upon domestic animal production, but this fungal metabolite also has been isolated from human food supplies (2).

Although the evidence is primarily circumstantial in nature, there is the possibility that aflatoxin may be related to the high incidence of liver disease in those areas of the world where aflatoxin contamination of the food supply is common. At the present time, it is not practical to decontaminate affected foodstuffs on a large scale. Consequently, the only way to avoid ingestion of aflatoxin is to eliminate contaminated foodstuffs from the diet. In many populations where the food supply is limited, this promotes a serious problem. As a result, it is of interest and importance to identify possible factors in the diet which may either alter the toxicity of this substance or improve the resistance of the host.

Previous studies in our laboratory (3) have suggested that specific interactions may occur between some as yet unknown component of the diet and a particular type of fat, resulting in modifications in the toxicity of aflatoxin. It is, therefore, possible that the type of dietary fat may influence the response of an animal to aflatoxin administration.

Concurrent with the outbreak of Turkey X disease in England in 1961 a trout epidemic was observed in many fish hatcheries in the U.S., particularly in California (4) where the causative agent was found to be the cottonseed meal component of the diet (5). This cottonseed meal later was found to have been contaminated with small amounts of aflatoxin (6-8). Sinnhuber, et al., (7-11) then showed that feeding contaminated cottonseed meal to trout resulted in more rapid development and greater incidence of hepatoma than did control diets containing comparable amounts of purified aflatoxin B_1. Further work with trout has shown that the cyclopropenoid fatty acids, which normally occur in small amounts in unprocessed cottonseed oil, are powerful cocarcinogens when fed in conjunction with aflatoxin (12).

While the exact mechanism by which sterculic acid functions in biological systems has not been elucidated clearly, it is known that sterculic acid inhibits the fatty acid desaturase system responsible for the formation of monoenoic fatty acids, resulting in increased levels of saturated fatty acids, particularly stearic acid, and decreased levels of their monounsaturated derivatives (13-16). It is known that these changes further result in marked alterations in normal membrane permeability (17-20). It is quite possible that increased permeability of cellular membranes may permit an increased rate of entry of aflatoxin into cells, thus increasing its toxic effects.

Although the synergistic action of sterculic acid and aflatoxin is well documented in trout, there are conflicting reports in the literature as to whether this effect is observed in mammals. Lee, et al., (21) reported finding slightly more liver tumors in rats when sterculic acid was fed together with aflatoxin. Friedman and Mohr (22), on the other hand, observed no interaction between aflatoxin and sterculic acid in short term experiments on rats. There are no reports on the effect of modifications in dietary fat on this synergism, if, in fact, it does exist.

EXPERIMENTAL PROCEDURES

The plan of the experiment is shown in Table I. Male weanling rats of the former USC strain were placed on basal diets containing either saturated (beef fat) or unsaturated (corn oil) fat to which was added either: no supplement (control groups B-C and CO-C); 1.7 ppm pure aflatoxin B_1, (groups B-A and CO-A); 210 ppm sterculic acid, (groups B-S and CO-S), and both 1.7 ppm aflatoxin B_1 and 210 ppm sterculic acid (groups B-AS and CO-AS). The rats continued on these diets for 3 months and were, thereafter, placed on aflatoxin-free diets for an additional 9 months to allow time for tumor development.

The basal diets which have been used in our laboratory for many years are shown in Table II.

During the experimental period, growth, morbidity, and mortality were recorded. At the time of sacrifice, gross pathology, as well as histopathological examinations of tissues, was conducted, and organ wt was measured. Cholesterol analyses, thin layer separations of lipid fractions, and gas liquid chromatographic analyses were performed on lipid extracts of plasma, liver, and tumor tissue, as described previously (24,25).

TABLE I

Plan of Experiment

Group	Dietary fat	Aflatoxin B_1 (ppm)	Sterculic acid (ppm)
B-C	Beef fat	0	0
B-A	Beef fat	1.7	0
B-S	Beef fat	0	210
B-AS	Beef fat	1.7	210
CO-C	Corn oil	0	0
CO-A	Corn oil	1.7	0
CO-S	Corn oil	0	210
CO-AS	Corn oil	1.7	210

RESULTS AND DISCUSSION

Wt gains achieved by these animals are shown in Figure 1. When the basal diet contained saturated fat, growth was inhibited by either aflatoxin or sterculic acid, administered separately. However, when aflatoxin and sterculic acid were administered together, the inhibition of growth was less marked than when aflatoxin was given alone, but was the same as when sterculic acid was administered alone. In other words, the effect on growth was neither additive nor synergistic. When the basal diet contained polyunsaturated fat, sterculic acid administration did not result in any growth inhibition. Aflatoxin produced the same amount of growth inhibition, regardless of whether or not sterculic acid was included in the diet. It would, therefore, appear that only on the saturated fat diet does sterculic acid modify the growth response to aflatoxin in some still not explained fashion.

At the time of sacrifice various organs, i.e. liver, heart, spleen, kidneys, adrenals, and testes were weighed. Of these, only the wt of the liver was changed in response to aflatoxin (Table III). The addition of aflatoxin to the basal diets resulted in an approximate doubling of the liver wt, regardless of the fat source. When sterculic acid also was included in the diet, the size of the liver doubled in the animals fed beef fat and quadrupled in those fed corn oil. Gross examination of these livers revealed that tumor formation was more severe in the sterculic acid fed animals.

The results of the pathological examination of the livers is shown in Table IV. The severity of liver involvement was categorized in the following way. Minimal involvement included livers with a few small nodules, usually less than .5 cm in diameter, and mild fatty degeneration. Moderately involved livers were those which contained one or more large fatty nodules, usually 1 cm or more in diameter, but with a significant amount of unaffected liver tissue surrounding the nodules. Maximally involved livers were those which contained large, multiple nodules and tumor masses in all lobes with little, if any, unaffected liver tissue remaining. Maximal involvement of the liver occurred most often in the corn oil, aflatoxin fed animals, and this was most prevalent among those rats fed the aflatoxin together with sterculic acid. In addition to the liver pathology, one kidney tumor was found in an animal fed beef fat with aflatoxin.

TABLE II

Basal Diets

Nutrient	Saturated fat Beef (%)	Unsaturated fat Corn oil (%)
Sucrose	52.75	52.75
Casein	23.72	23.72
Salts mix[a]	4.00	4.00
Solka floc	4.00	4.00
Vitamin mix[b]	.29	.29
Choline Cl	.24	.24
Beef fat	15.00	-
Corn oil	-	15.00

[a]Wesson modification of the Osborne-Mendel formula (23).
[b]Vitamin mix contained (in mg/100 g diet); p-aminobenzoic acid, 50; inositol, 50; α-tocopherol, 40 (1 mg = 0.25 international units [IU] as α-tocopheryl acetate); ascorbic acid, 100; thiamin, 5; Ca-pantothenate, 8; niacin, 10; vitamin B_{12} triturate, 6; riboflavin, 3; pyridoxine, 3; folic acid, 1; menadione, 5; crystalets (500 IU vitamin A and 50 IU vitamin D/mg), 5; and biotin, 0.2.

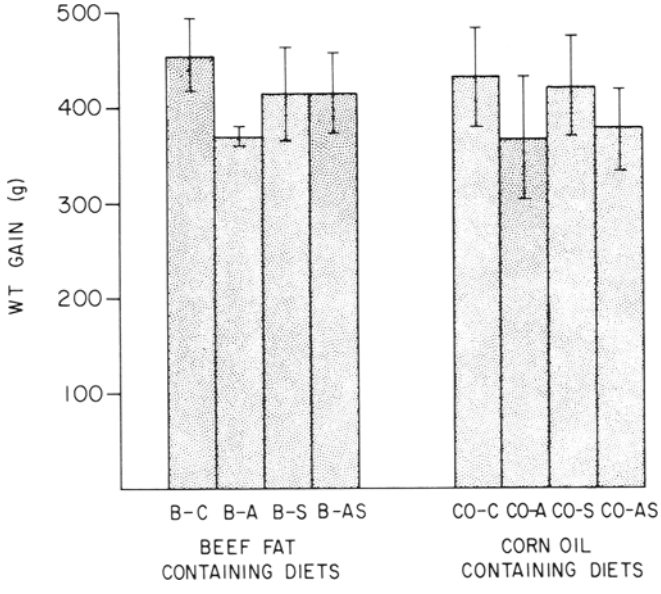

FIG. 1. Wt gains of rats fed beef fat or corn oil containing diets with and without aflatoxin and sterculic acid.

Lipid extracts of liver and plasma were analyzed for free and total cholesterol (Table V). When saturated fat was fed, aflatoxin administration resulted in a marked increase in liver cholesterol. When sterculic acid also was included in the diet, the aflatoxin induced increase in cholesterol was not as marked, although it was still highly significant. When the diet contained polyunsaturated fat, the previously observed increase in liver cholesterol, resulting from aflatoxin administration, while apparent, was not statistically significant (by Student's t-test) and did not occur at all when sterculic acid also was included in the diets. A similar phenomenon was observed with plasma cholesterol values. The

TABLE III

Liver Wt

Group	Saturated fat Beef		Unsaturated fat Corn oil	
	g	Percent body wt	g	Percent body wt
–C	13.0 ± 1.3[a]	2.5	13.0 ± 1.0	2.6
–A	27.2 ± 6.1[a]	6.8	22.2 ± 12.3	5.4
–S	12.1 ± 0.9[b]	2.6	12.1 ± 1.1[c]	2.5
–AS	20.5 ± 5.3[b]	4.3	44.9 ± 16.7[c]	10.6

[a-c]Matched superscripts indicate $p<.05$.

TABLE IV

Gross Liver Pathology

Group	Number of animals	Liver involvement			
		Normal	Minimal	Moderate	Maximal
B-C	6	6 (100)[a]	-	-	-
B-A	6	-	2 (34)[b]	3 (50)	1 (17)
B-S	6	6 (100)	-	-	-
B-AS	6	1 (17)	4 (66)	1 (17)	-
CO-C	5	5 (100)	-	-	-
CO-A	3	-	1 (33)	1 (33)	1 (33)
CO-S	6	6 (100)	-	-	-
CO-AS	5	-	1 (20)	1 (20)	3 (60)

[a]Number in parenthesis is percent of group.
[b]Number of animals (percent of animals) with pathology.

TABLE V

Liver and Plasma Cholesterol

Group	Liver				Plasma			
	Beef		Corn oil		Beef		Corn oil	
	mg/g	Percent free	mg/g	Percent free	mg percent	Percent free	mg percent	Percent free
–C	2.30 ± 0.40[a]	70	2.36 ± 0.43	73	77.1 ± 5.1[c]	29	90.8 ± 5.6	28
–A	4.00 ± 0.14[a]	58	4.20 ± 2.00	75	125.4 ± 6.3[c]	31	105.3 ± 18.3	32
–S	2.40 ± 0.20[b]	78	2.30 ± 0.40	81	75.8 ± 10.4[d]	28	99.1 ± 12.9	26
–AS	3.50 ± 0.40[b]	69	2.40 ± 0.70	68	101.2 ± 20.0[d]	29	98.4 ± 6.5	35

[a-d]Matched superscripts indicate $p<.05$.

TABLE VI

Major Fatty Acids of Pooled Plasma Sterol Esters

Group	Percent			
	18:2	20:3	20:4	18:2/20:4
B-C	10.4	9.6	23.4	.44
B-A	10.6	9.2	19.5	.54
B-S	11.9	9.1	30.3	.39
B-AS	12.4	9.9	19.4	.64
CO-C	18.8	0	45.1	.42
CO-A	20.8	0	36.8	.56
CO-S	20.9	0	54.2	.38
CO-AS	23.6	0	45.1	.52

marked increase produced by aflatoxin among the saturated fat fed rats was slightly diminished when sterculic acid also was present. When the fat source was corn oil, however, the elevation produced by aflatoxin was not statistically significant and again did not occur at all when sterculic acid also was included in the diet.

Gravimetric determinations of total lipid were conducted on extracts taken from portions of liver which were free of tumors. There were no significant differences between any of the groups.

Fatty acid analyses were conducted on the sterol ester and triglyceride and phospholipid fractions of pooled samples of plasma. In most cases, pooling resulted in less than four samples/group and, therefore, the data were not subjected to statistical analysis.

The major fatty acids of the plasma sterol ester fraction are shown in Table VI. As was expected, the animals consuming the beef fat containing diet had lower levels of C18:2 and C20:4 than did rats fed corn oil containing diets. In addition, C20:3, the eicosatrienoic acid which also has been observed in essential fatty acid deficiency, was present in the beef fat fed rats. Ratios of C18:2 to C20:4 were increased somewhat with aflatoxin administration. This suggests that aflatoxin in the diet causes either an impairment of the mechanism responsible for the conversion of C18:2 to C20:4 or an increased utilization or destruction of C20:4.

There were no changes in the fatty acid composition of either the triglyceride or phospholipid fractions of plasma which were related to aflatoxin administration.

In the sterol ester fraction of liver (Table VII), rats fed saturated fat were found to have decreased levels of monoenes (particularly 18:1) and increased levels of their saturated counterparts (particularly 18:0) in response to sterculic acid administration. At the same time, aflatoxin administration was found to promote increases in the levels of monoenes and decreases in the levels of the saturated counterparts. The combined response when aflatoxin and sterculic acid were fed together was an increase in the level of C18:1 and a decrease in the level of C18:0, the magnitude of which was less than when sterculic acid was fed alone. These changes were not seen in the rats fed the corn oil containing diets.

In the liver triglyceride fraction, the animals fed beef fat containing diets (Table VIII) also had decreased levels of C18:1 and increased levels of C18:0 as a result of sterculic acid administration; however, in this lipid fraction, there was no additional effect of the aflatoxin administration. Among the animals fed corn oil containing diets, there were decreased amounts of C16:0 and increased amounts of C18:2 as a result of aflatoxin administration, which became statistically significant only when sterculic acid was administered along with the aflatoxin.

There were no changes in the fatty acid composition of the phospholipids of liver of any of the animals which could be attributed to aflatoxin administration.

Fatty acid analyses also were conducted on the fatty tumors which occurred in the livers of the aflatoxin supplemented rats; these were compared with the fatty acid composition of the unaffected surrounding liver tissue.

Among the animals fed corn oil containing diets, there were no statistically significant differences between the fatty acid composition of the tumors and that of the surrounding liver in any of the various lipid fractions. In the animals fed the beef fat containing diet, however, in the sterol ester fraction (Table IX), tumors had less C16:0 and slightly more C16:1 than did the surrounding liver. In addition, when the levels of C18:1 were decreased and C18:0 increased in liver as a result of sterculic acid administration,

TABLE VII

Major Fatty Acids of Liver Sterol Esters

Group	Percent					
	16:0	16:1	18:0	18:1	18:2	20:4
B-C	27.9 ± 3.8[a]	12.2 ± 3.1[c]	6.2 ± 2.4[e]	42.3 ± 7.8[i]	5.3 ± 1.7	3.0 ± 2.4
B-A	16.4 ± 1.4[a]	12.1 ± 1.3[d]	4.7 ± 1.2[f]	60.8 ± 3.7[ij]	3.1 ± 2.2	1.0 ± 0.7
B-S	27.9 ± 6.2	7.3 ± 1.9[c]	11.4 ± 2.3[e]	37.7 ± 3.3[k]	5.1 ± 2.9	2.1 ± 1.9
B-AS	21.0 ± 2.5	8.0 ± 3.8[d]	7.7 ± 3.8[d]	50.6 ± 8.4[jk]	5.3 ± 3.4	2.6 ± 1.1
CO-C	22.2 ± 7.3	5.5 ± 2.5	8.5 ± 0.8[g]	27.9 ± 6.1	19.3 ± 2.2	7.0 ± 1.9
CO-A	25.8 ± 4.6	4.3 ± 1.2	7.8 ± 1.7	24.1 ± 1.8	19.0 ± 0.6	11.1 ± 3.7
CO-S	25.9 ± 5.3[b]	4.1 ± 1.3	11.0 ± 1.3[gh]	25.2 ± 1.9	15.8 ± 5.7	9.1 ± 4.6
CO-AS	19.2 ± 4.1[b]	3.1 ± 0.3	6.0 ± 1.6[h]	28.6 ± 6.8	24.3 ± 6.9	11.1 ± 3.7

[a-k]Matched superscripts indicate $p<.05$.

TABLE VIII

Major Fatty Acids of Liver Triglycerides in Rats Fed Diets Containing Different Fats

Group	Percent					
	16:0	16:0	18:0	18:1	18:2	20:4
B-C	25.1 ± 5.1	10.1 ± 2.8	2.6 ± 0.7[c]	56.2 ± 7.6	3.2 ± 1.0	0
B-A	25.4 ± 4.6[a]	8.0 ± 1.4	2.8 ± 1.4	58.8 ± 5.3[d]	2.3 ± 0.5	0
B-S	32.3 ± 7.4	7.6 ± 1.3	4.7 ± 1.4[c]	49.7 ± 8.1	2.8 ± 1.0	0
B-AS	31.6 ± 4.2[a]	9.9 ± 3.1	4.4 ± 2.1	49.1 ± 6.1[d]	2.3 ± 0.6	0
CO-C	27.2 ± 7.0	5.0 ± 1.7	3.7 ± 1.8	28.1 ± 4.2	27.5 ± 5.9	4.3 ± 4.0
CO-A	21.2 ± 6.9	3.0 ± 1.0	3.0 ± 1.9	31.3 ± 4.4	33.7 ± 6.6	2.8 ± 1.9
CO-S	30.8 ± 6.1[b]	2.6 ± 2.0	4.4 ± 2.2	27.5 ± 4.0	27.4 ± 7.0[e]	3.1 ± 1.9
CO-AS	21.6 ± 1.9[h]	2.0 ± 1.3	2.1 ± 0.7	28.5 ± 2.9	37.8 ± 4.4[e]	4.0 ± 1.2

[a-e]Matched superscripts indicate $p<.05$.

TABLE IX

Major Fatty Acids of Tumor as Compared to Liver in Rats Fed Beef Fat Containing Diets. Sterol Esters

Group	Percent					
	16:0	16:1	18:0	18:1	18:2	20:4
B-A (tumor)	9.4 ± 1.7[a]	14.3 ± 2.1	5.9 ± 1.3	57.1 ± 3.0[f]	3.7 ± 0.9	2.6 ± 0.9
B-A (liver)	16.4 ± 1.4[a]	12.1 ± 1.3[c]	4.7 ± 1.2[e]	60.8 ± 3.7[g]	3.1 ± 2.2	1.0 ± 0.7
B-AS (tumor)	12.8 ± 2.5[b]	14.3 ± 3.3[d]	7.8 ± 1.6	49.5 ± 6.7[f]	5.0 ± 3.4	3.3 ± 1.1
B-AS (liver)	21.0 ± 2.5[b]	8.0 ± 3.8[cd]	7.7 ± 2.8[e]	50.6 ± 8.4[g]	5.3 ± 3.4	2.6 ± 1.1

[a-g]Matched superscripts indicate $p<.05$.

TABLE X

Major Fatty Acids of Tumor as Compared to Liver in Rats Fed Beef Fat Containing Diets. Triglycerides

Group	Percent					
	16:0	16:1	18:0	18:1	18:2	20:4
B-A (tumor)	12.5 ± 0.8[a]	6.9 ± 4.0	5.0 ± 1.8	59.1 ± 4.3	4.6 ± 1.5[c]	1.6 ± 0.2
B-A (liver)	25.4 ± 4.6[a]	8.0 ± 1.4	2.8 ± 1.4	58.8 ± 5.3	2.3 ± 0.5[c]	0
B-AS (tumor)	13.4 ± 2.6[b]	5.9 ± 1.8	6.3 ± 1.7	63.0 ± 10.1	2.8 ± 0.8	0
B-AS (liver)	31.6 ± 4.2[b]	9.9 ± 3.1	4.4 ± 2.4	49.1 ± 6.1	2.3 ± 0.6	0

[a-c]Matched superscripts indicate $p<.05$.

TABLE XI

Major Fatty Acids of Tumor as Compared to Liver in Rats Fed Beef Fat Containing Diets. Phospholipids

Group	Percent						
	16:0	16:1	18:0	18:1	18:2	20:3	20:4
B-A (tumor)	13.2 ± 2.2	5.2 ± 1.0[a]	18.0 ± 1.7[c]	31.4 ± 2.3[e]	5.6 ± 1.1	6.8 ± 0.9[g]	14.2 ± 2.3
B-A (liver)	13.8 ± 1.3	3.5 ± 0.9[a]	23.0 ± 1.7[c]	22.1 ± 1.7[e]	5.8 ± 0.9	9.4 ± 1.2[g]	14.7 ± 2.9
B-AS (tumor)	15.9 ± 2.4	5.5 ± 1.4[b]	19.9 ± 2.8[d]	31.4 ± 2.9[f]	5.6 ± 1.2	5.4 ± 1.6[h]	10.5 ± 4.9
B-AS (liver)	15.1 ± 3.3	3.4 ± 0.8[b]	24.8 ± 1.8[d]	19.8 ± 3.6[f]	5.6 ± 1.4	9.4 ± 1.2[h]	13.9 ± 3.5

[a-h]Matched superscripts indicate $p<.05$.

this also was reflected in the tumor fatty acids.

Tumor triglycerides (Table X) also contained smaller amounts of C16:0 than did the surrounding unaffected liver tissue. There was also a slight tendency for the tumor to accumulate C18:2 and C20:4 when the diets did not contain sterculic acid.

In the phospholipid fraction (Table XI), tumor tissue contained more C16:1 and C18:1 and smaller amounts of C18:0 and C20:3 than did the surrounding liver. The fact that levels of C18:1 were increased while C18:0 decreased in the tumors suggests that tumor tissue of saturated fat fed rats either may be synthesizing C18:1 from C18:0 at an increased rate or may be utilizing less C18:1 for other processes.

We have not yet been able to establish whether or not the changes in fatty acid metabolism which produced changes in the composition of tumor fatty acids only among the rats fed saturated fat containing diets were directly related to the decreased pathology of these animals or were merely coincidental to it.

These findings suggest that the fat source in the diet may be of prime importance in determining whether or not certain dietary contaminants, for example sterculic acid, will either enhance or alleviate the toxic effect of aflatoxin. While the rat does not react to aflatoxin and sterculic acid in the same manner as does the rainbow trout, there do seem to be differences in the response of the animal to aflatoxin with regard to growth, pathology, and biochemistry which result from feeding a diet containing sterculic acid.

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