Effect of Aflatoxin B_1 on the Incorporation of ^{14}C-Acetate into Cholesterol by Rat Liver

Aflatoxins are a group of highly toxic and hepatocarcinogenic metabolites produced by certain strains of *Aspergillus flavus*. Of these metabolites aflatoxin B_1 is the most toxic as well as the most potent hepatocarcinogen in a variety of animal species[1].

CLIFFORD and REES[2,3] proposed that the biochemical changes underlying the development of liver necrosis in rats after the administration of aflatoxin B_1 were initiated by the toxin interacting with the DNA. The interaction prevented the RNA polymerase transcribing the DNA and inhibited the formation of messenger RNA. A failure in messenger-RNA formation resulted in an inhibition in protein synthesis which they considered to be the cause of the liver-cell necrosis. Thus, they demonstrated that in rats a single high dose of aflatoxin B_1 did not produce significant change in the incorporation of leucine into liver protein up to 18 h after administration[3].

In the present communication, we wish to report an early and marked inhibition of the 1-^{14}C-acetate incorporation into liver cholesterol by aflatoxin treatment.

Male rats of Wistar strain weighing 200–250 g were used. Aflatoxin B_1 was dissolved in dimethylsulfoxide and given i.p. at dose of 3 or 5 mg per ml/kg. The rats were killed 24 h after the treatment. The liver slices (500 mg) were incubated for 60 min at 37 °C with 5 ml of Krebs phosphate buffer containing 2 μc 1-^{14}C-acetate. Cholesterol was extracted with hexan and precipitated and purified as digitonide according to the method of Gould with a little modification[4]. The radioactivity of the cholesterol was counted in a liquid scintillation counter. Liver and serum cholesterol was determined by the method of ZACK et al.[5]. The determinations of aminopyrine N-demethylation and pentobarbital oxidation by liver microsomes were carried out as described in a previous paper by using female rats weighing about 160–180 g[6]. The incorporation of 1-^{14}C-acetate into cholesterol was decreased to 10% of control level at 24 h after the aflatoxin treatment (Table I).

Two mg/kg of aflatoxin B_1 resulted in similar results, but 0.5 mg/kg did not produce significant decrease. The content of liver cholesterol was slightly increased by aflatoxin B_1, in contrast the serum cholesterol was slightly decreased.

In other experiments, the incorporation of 1-^{14}C-acetate was decreased to a similar extent in both the incubation of 30 min and 60 min.

On the other hand, the administration of aflatoxin B_1 did not significantly alter the activity of aminopyrine N-demethylation and pentobarbital oxidation (Table II).

Table I. Effect of aflatoxin B_1 on the incorporation of 1-^{14}C-acetate into cholesterol by liver slices.

Treatment	Cholesterol content (mg/g liver)	Specific activity (dpm × 10^{-4}/mg cholesterol)	Total activity (dpm × 10^{-4}/g liver)
Control	1.90 ± 0.07	5.57 ± 0.84	10.68 ± 1.75
Aflatoxin B_1 (5 mg/kg)	2.71 ± 0.11 (+43%)	0.66 ± 0.22 (−88%)	1.77 ± 0.59 (−84%)
Aflatoxin B_1 (2 mg/kg)	2.36 ± 0.09 (+24%)	0.54 ± 0.06 (−90%)	1.28 ± 0.11 (−88%)

Aflatoxin was given i.p. 24 h before sacrifice. The results are expressed as average ± S.E. from 5 rats. The figures in the parentheses indicate the difference in percentage from control.

Table II. Effect of aflatoxin B_1 on the activity of drug-metabolizing enzymes of liver microsomes

Treatment	Aminopyrine N-demethylation (nmole/g per 30 min)	Pentobarbital oxidation (nmole/g per 30 min)
Control	134 ± 15	245 ± 21
Aflatoxin B_1 (5 mg/kg)	129 ± 17 (−4%)	223 ± 19 (−9%)

Aflatoxin was given intraperitoneally 24 h before sacrifice. The results are expressed as average ± S.E. from 6 rats. The figures in the parentheses indicate the difference in percentage from control.

Since the activity of drug-metabolizing enzymes was easily decreased by hepatotoxic agents[7], these results suggested that the oxidizing activities of liver microsomes are still intact by 24 h after aflatoxin injection. These results are in accordance with the report of CLIFFORD and REES that aflatoxin did not inhibit hepatic isocitrate dehydrogenase, malate dehydrogenase and glutamate dehydrogenase, and did not increase the activities of these enzymes and alkaline phosphatase in the serum and serum bilirubin concentration up to 48 h after administration.

Moreover, recent works have revealed that the drug-oxidation and a part of cholesterol synthesis by liver microsomes are mediated by similar mechanism(s)[8]. Therefore these results suggested that the inhibitory action of aflatoxin B_1 on the incorporation of 1-^{14}C-acetate into cholesterol is specific and it is not due to result of general hepatic injury.

Further studies on the mechanism and specificity of aflatoxin B_1 induced inhibition of cholesterol biosynthesis are now in progress[9].

Résumé. L'administration d'aflatoxine B_1 (2 et 5 mg/kg, i.p., 24 h avant le sacrifice) a résulté en un abaissement de 80% de l'incorporation du 1-^{14}C-acétate en cholestérol par des coupes de tissu de foie. Le même traitement n'a pas altéré l'activité des 'drug metabolizing enzymes' au niveau des microsomes du foie.

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[1] B. F. NESBITT, J. O'KELLEY, K. SARGEANT and A. SHERIDAN, Nature *195*, 1062 (1962).
[2] J. I. CLIFFORD and K. R. REES, Nature *209*, 312 (1966).
[3] J. I. CLIFFORD and K. R. REES, Biochem. J. *102*, 65 (1967).
[4] R. G. GOULD, C. B. TAYLOR, J. S. HAGERMAN, I. WARNER and D. J. CAMPBELL, J. biol. Chem. *201*, 519 (1953).
[5] B. ZACK, R. C. DICKENMAN, E. G. WHITE, H. BURNETT and P. J. CHERNEY, Am. J. clin. Path. *24*, 1307 (1954).
[6] R. KATO, T. OSHIMA and S. TOMIZAWA, Jap. J. Pharmac. *18*, 356 (1958).
[7] H. A. SASAME, J. A. CASTRO and J. R. GILLETTE, Biochem. Pharmac. *17*, 1759 (1968).
[8] F. WADA, K. HIRATA, H. SHIBATA, K. HIGASHI and Y. SAKAMOTO, J. Biochem. *62*, 134 (1967).
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