Effects of Aflatoxin B[1] on Human Skin Lipids Metabolism – a Site of Action on 1-^{14}C-Acetate Incorporation

Aflatoxin B_1 is the most potent carcinogen among the 4 toxins isolated from *Aspergillus flavus*[1,2]. Its role to the target organ, liver, has been well studied[3–5]. Recently, JOFFE and UNGAR[6] demonstrated an epidermal lesion obtained by the local application of aflatoxins onto rabbit skin and postulated a possible cause of the human occupational dermatoses in the handler of groundnuts or other grain contaminated with toxin-producing *Aspergillus flavus*. There was evidence that aflatoxin B_1, indeed, has significant effect on the skin lipid synthesis[7]. The incorporation of 1-^{14}C-acetate into human skin lipids was partially inhibited by aflatoxin B_1 while those of the specific activities were somewhat significantly enhanced. This similar phenomenon has also been reported in numerous cases[8,9]. The present note intends to elucidate the role of aflatoxin B_1 on this inhibition so as to locate the possible biochemical site(s) of its action.

Materials and methods. Fresh human skin specimens were obtained from the lower abdomen of male cacasians. The area was anesthetized with 1% lidocaine and the skin removed with a 6 mm dermal punch. The specimens were trimmed and further treated as previously described[10]. The prepared biopsies were placed in 15 ml manometric reaction flasks containing 50 µg aflatoxin B_1, 2.0 ml Krebs-Ringer phosphate buffer, pH 7.4. The aflatoxin B_1 was previously dissolved in ethanol-propylene glycol (1:1 v/v) and freshly prepared for each use. The control contained equal volume of ethanol-propylene glycol and the amount did not exceed 1% of the total incubation volume. The flasks were incubated at 37 °C for 1 h after which 0.5 ml buffer containing either 1 µCi 1-^{14}C-acetate (61.4 mCi/mmole) or 1 µCi ^{3}H-acetyl CoA (1.2 Ci/mmole) was added to the incubation medium from the side-arm of the reaction flask. Incubation was continued for an additional 5 h. Respiratory rates were determined manometrically for the total incubation period. After incubation, the total lipids of the specimens were extracted. Radioactivity from the total lipids was determined with duplicate samples (10% of the total extract) by liquid scintillation spectrometry. The mean counting efficiency for ^{14}C and ^{3}H were 78% and 30%, respectively. Quenching was determined by the automatic external standardization method.

The remaining sample was reduced to 50 µl under a stream of N_2. Polar lipids, fatty acids, sterols, triglycerides and sterol esters of the total lipid extract were separated and the radioactivity of each fraction of lipids were measured by the method previously described[11].

Results and discussion. The incorporation of 1-^{14}C-acetate into skin total lipids was markly inhibited by aflatoxin B_1. This inhibition was non-specific in regard to a similar level of the inhibitory responses of the different classes of lipids to its action (Table I). Although there were more pronounced inhibition in both triglycerides and sterol ester synthesis, the data, at least 72.1% of the inhibition for all of the classes of lipid synthesis, suggested that the major site of the inhibitory effect of aflatoxin B_1 on the incorporation of 1-^{14}C-acetate may act upon the formation of a common precursor of these synthesis, i.e. acetyl CoA. A possible reduced level of acetyl CoA formation caused by the presence of aflatoxin B_1 in skin may, thus, result in a general inhibition of lipid synthesis. This assumption is deduced by the results shown in Table II. Instead of 1-^{14}C-acetate, ^{3}H-acetyl CoA was used as precursor of lipid synthesis. The diffusion of ^{3}H-acetyl CoA into skin slices and thus its utilization to synthesize skin lipids is demonstrated (control of Table II). In contrast to 1-^{14}C-acetate, the incorporation of ^{3}H-acetyl CoA into total lipids as well as different classes of lipids are not significantly affected by aflatoxin B_1 (Table II). A marked inhibition of ^{3}H-acetyl CoA incorporation which may parallel to that of 1-^{14}C-acetate would have been expected if the major site of the effect of aflatoxin B_1 was located somewhere after acetyl CoA synthesis. These great differences between 1-^{14}C-acetate and ^{3}H-acetyl CoA incorporation strongly suggested the site of aflatoxin B_1 inhibitory effect may be prior to the synthesis of this precursor-acetyl CoA.

The effect of aflatoxin B_1 might be due to a general cellular injury was eliminated by the examining of the respiratory rates of toxin-treated tissues. There were only 7% less than controls at the end of the experiments.

Lately, KATO et al.[8] demonstrated a similar inhibitory effect of the same toxin to rat liver cholesterol synthesis. Although there is no evidence for the same site of action of toxin on lipid synthesis in rat liver, the results presented here as well as the light effect on the inhibition of lipid synthesis[10] also acting on the site prior to the formation of acetyl CoA (unpublished data) suggested that the action of toxin on lipid synthesis in different tissues and

Table I. Effect of aflatoxin B_1 on the incorporation 1-^{14}C-acetate into human skin lipids[a]

Fraction	Experiment		
	Control	Aflatoxin (dpm/100 mg wet wt.)	Change (%)
Total lipids	7,000	1,820	— 74.0
Polar lipids[b]	3,961	1,107	— 72.1
Free fatty acids	444	91	— 79.5
Free sterols	2,250	384	— 82.9
Glycerides	282	0	—100
Sterol esters	374	0	—100

[a] Total lipids were determined by the direct measurements of the radioactivity of the samples obtained from the chloroform: methanol (2:1, v/v) extract of human skin. Different classes of lipids were measured by the scrapping off the correspondent spots from the thin-layer chromatogram (TLC) and counted directly by mixing them with the scintillation cocktails. Details were described in ref.[11].
[b] Polar lipids represent phospholipids and other polar materials of total lipid extract.

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the different agents, namely light and aflatoxin B_1, acting on the same tissue served a similar mode of reaction. Although a possible limitation of the availability of the co-factors, either ATP or CoA to retain the precursor synthesis caused by the presence of aflatoxin B_1 in skin is not eliminated, the binding of aflatoxin with some proteins[12,13] and its inhibition of some enzyme activity[14] may suggest its interaction with acetate thiokinase (EC. 6.2.1.1.) and thus, results in the observed inhibitory effect[15].

Table II. Effect of aflatoxin B_1 on the incorporation of ^{3}H-acetyl CoA into human skin lipids[a]

Fraction	Experiment		
	Control	Aflatoxin (dpm/100 mg wet wt.)	Change (%)
Total lipids	5,570	5,240	−5.4
Polar lipids	1,767	1,800	+1.9
Free fatty acid	845	799	−5.4
Free sterols	871	954	+9.5
Glycerides	771	774	+0.3
Sterols esters	771	745	−3.4

[a] Details were described in ref.[11]

Zusammenfassung. Aflatoxin B_1 hemmt den Einbau von 1-^{14}C-Azetat in die Lipide der menschlichen Haut. Wird aber ^{3}H-Azetyl-CoA als Vorstufe für den Lipideinbau verwendet, so kommt es zu keiner merklichen Hemmung. Die Azetataktivierung scheint somit die wichtigste, vom Toxin beeinflussbare Stufe der Lipidsynthese zu sein.

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Antrectomy Prevents Nicotine from Activating Rat Stomach Histidine Decarboxylase

The activity of rat stomach histidine decarboxylase seems to vary with the serum gastrin level[1]. Nicotine has been reported to cause activation of the enzyme[2]. This effect may be a direct one, elicited in the enzyme-containing cell itself, or an indirect one, mediated by nicotine-induced release of gastrin from its storage site in the antrum[3]. If the enzyme activation seen after administration of nicotine is mediated by gastrin, antrectomized rats should not respond.

Adult male albino rats (Wistar strain, 150–200 g body weight) were used. Antrectomy was performed by resection of the distal half of the glandular stomach (the pyloric gland area together with the adjacent portion of the oxyntic gland area) and the duodenal bulb[4]. Gastrointestinal continuity was re-established by gastro-duodenostomy end-to-end. Operated rats were allowed to recover for at least 3 weeks before they were used in experiments. Nicotine (0.2–5 mg/kg) was given s.c. in a single dose. If not otherwise stated the rats were killed 2 h after injection. Controls received saline. All rats were fasted for 48 h (free access to water) before sacrifice. They were killed by decapitation under light diethyl ether anaesthesia. The stomachs were taken out, cut open along the major curvature and washed with ice-cold 0.9% saline. The mucosa was scraped off the oxyntic gland area, homogenized in 0.1 *M* phosphate buffer, pH 6.9 (final tissue concentration 100 mg wet weight per ml) and centrifuged at $10{,}000 \times g$ for 15 min at 0 °C. Enzyme activity was measured as $^{14}CO_2$ produced from 1-^{14}C-L-histidine[4,5]. The reaction mixture (0.5 ml) contained 0.4 ml of the supernatant, 10^{-5} *M* pyridoxal-5′-phosphate,

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Table I. Effect of nicotine on the activity of rat stomach histidine decarboxylase (dose-response relationship)[a]

Nicotine (mg/kg)	Histidine decarboxylase activity (nmoles $CO_2 \times 10^{-3}$/mg/h) mean ± S.E.M. (*n*)	
0	6.3 ± 0.7 (23)	
0.2	13.7 ± 1.5 (17)	$P < 0.001$
1.0	12.8 ± 1.6 (20)	$P < 0.001$
5.0	5.8 ± 1.2 (16)	

[a] The rats were killed 2 h after injection.

Table II. Histidine decarboxylase activity at various times after administration of 0.2 mg/kg nicotine

Time (h)	Histidine decarboxylase activity (nmoles $CO_2 \times 10^{-3}$/mg/h) mean ± SEM (*n*)	
0	6.3 ± 0.7 (23)	
1/2	4.0 ± 1.1 (5)	
1	8.1 ± 2.5 (5)	
1 1/2	13.8 ± 3.6 (5)	
2	13.7 ± 1.5 (17)	$P < 0.001$