Aflatoxin Metabolism by Liver Microsomal Preparations of Two Different Species

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Received October 2, 1968

Aflatoxin, the well known carcinogen produced by the mold Aspergillus flavus, has been shown to relatively ineffective in producing characteristic toxicity (Platonow, 1964) and liver cancer symptoms (Newberne et al., 1965) in mice. Studies were undertaken to evaluate the reasons for this resistance shown by mice as opposed to rats, since subtle differences between these species may be suggestive of the toxicologic and/or carcinogenic mechanism. Previous work done in this lab and reported elsewhere (Portman et al., 1968) showed that rat liver slices promoted a faster aflatoxin B_1 disappearance from the incubating medium than did mouse liver slices. However, it was not determined whether this rate of disappearance was a reflection of uptake by the cells or metabolic rate, per se. Therefore, further experiments were designed to evaluate the relative rates of metabolism in these two species using liver microsomal preparations.

The metabolism of aflatoxin B_1 proceeds through a hydroxylated derivative similar to many other polycyclic hydrocarbons (de Iongh et al., 1964; Holzapfel et al., 1966). In these experiments using microsomal preparations, we measured the kinetic properties of the hydroxylation reaction shown in Figure 1. These results show a much faster rate of metabolism with the mouse microsomal preparation, which may contribute to the greater resistance of mice. These data would therefore implicate aflatoxin B_1 as the more toxic component.

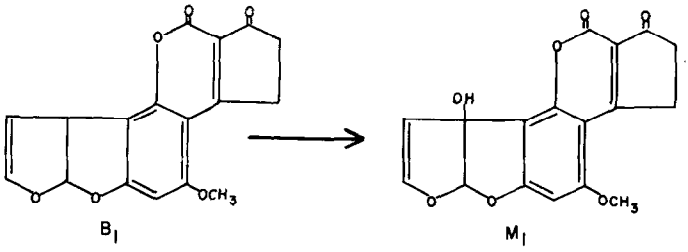

Figure 1. Hydroxylation of Aflatoxin B_1.

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^{14}C-methyl-labeled acetate (specific activity = 57.6 C/ mole) was purchased from Tracerlab and used to synthesize ^{14}C-uniformly-labeled aflatoxin B_1 according to the method of Adye and Mateles (1964). Nicotinamide adenine dinucleotide phosphate (disodium salt) and yeast glucose-6-phosphate dehydrogenase were obtained from Calbiochem and glucose-6-phosphate was purchased from Sigma Chemicals. Unlabeled aflatoxin B_1 was prepared and purified according to the method of Hanna and Campbell (1968).

Two-month old Sprague-Dawley derived rats and ICR derived mice were used in these studies. All animals were maintained on commercial lab chow from weaning until sacrifice. Livers of both species were excised, weighed, chilled, pooled and homogenized in 2 volumes of phosphate buffer (pH 7.4) with 0.25 M sucrose. Homogenates were centrifuged at 20,000 x g for 20 minutes to remove the mitochondrial and nuclear fractions. The microsomes were subsequently collected as a pellet of the 105,000 x g centrifugation of the above supernatant. The microsomal pellet was resuspended and washed 3 times in phosphate buffer (pH 7.4) and finally resuspended to give a concentration of microsomes equivalent to 1 gm of liver/ml.

Each incubation flask contained 1.2 ml phosphate buffer with 0.25 M sucrose (pH 7.4), and 0.3 ml of the microsomal preparation. Concentrations of 5.9 M glucose 6-P, 0.53 mM NADP and 3 units of

glucose-6-P dehydrogenase together with the appropriate amounts of ^{14}C-labeled aflatoxin B_1 were also included. The labeled aflatoxin preparation was diluted with cold aflatoxin to give a specific activity of 6.24 μC/μmole and from this stock solution, appropriate hot to cold dilutions were made to give adequate counting statistics of approximately 15,000 cpm per flask. Each aflatoxin concentration was added to the flask in 0.01 ml of ethanol. All incubations were carried out at 37° in a Dubnoff metabolic shaker. Reactions were stopped with 5 ml of chloroform which was subsequently used to extract the unmetabolized B_1 and M_1 product. A second extraction with 5 ml of chloroform was repeated and combined with the first extract. These extracts were then spotted on thin layer chromatography plates coated with 750 μ Adsorbosil-1 (Applied Science Labs) and developed in the benzene rich phase of benzene:ethanol:water (46:35:19). The appropriate B_1 and M_1 adsorbosil spots were removed from the plates, added to 15 ml of counting solution containing 1 ml of methanol and counted in a scintillation counter.

For the rat, velocities were found to be linear for 20 minutes; and for the mouse, velocities were found to be linear for 10 minutes. The data were analyzed using the computer program of Cleland, $V = V_m A/K + A$ (1963).

Results and Discussion

The apparent Michaelis constants and maximal velocities for these two species are shown in Table 1. It should be emphasized that these kinetic parameters represent 'apparent' values since pure enzymes are not involved and only aflatoxin was varied.

For the mouse both the V_{max} and K_m are significantly different from the corresponding values for the rat. Both parameters for the mouse preparation are such that a much greater rate of metabolism is

Table 1. Comparative Kinetic Data of Aflatoxin B_1 Metabolism by Two-month Old Rat and Mouse Liver Microsomes.

	Rat	Mouse
K_m [a]	358 ± 55	130 ± 37
V_{max} [b]	21.2 ± 2.2	44.7 ± 8.4
$K/_V$	16.9 ± 0.8	2.9 ± 0.3

[a] Substrate concentration in mM.

[b] Appearance of M_1 (hydroxylated derivative) in mμmoles/300 mg liver/hr (these data are not expressed per unit weight of protein since equivalent concentrations of crude microsomal protein would not necessarily imply equivalent levels of active enzyme).

achieved, whatever the concentration of aflatoxin used.

Previously we had shown (Portman et al., 1968) that the rate of disappearance of aflatoxin B_1 from media incubated with liver slices was relatively faster with rat preparations. From the data reported in these experiments, however, it is apparent that those aflatoxin B_1 disappearance rates are not equivalent to rates of metabolism and therefore imply faster rates of transport across the rat liver cell membrane. Taken together, these two observations show that the mouse enjoys the dual advantage of not only transporting aflatoxin into the liver cell more slowly but als, once the aflatoxin is within the cell, of metabolizing the aflatoxin more rapidly.

These data also suggest that the parent aflatoxin B_1 is the molecular species of greatest toxicity, as opposed to the metabolites. Whether the rate of B_1 uptake by the cells and the rate of subsequent B_1 metabolism are totally responsible for the difference in resistance between these species is not yet known, although it would be interesting to compare such activities with acute in vivo toxicities.

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