INDUCTION OF HEPATOMA IN SEVEN-DAY-OLD C57BL/6 MICE WITH A SINGLE DOSE OF AFLATOXIN AND THE INFLUENCE OF DIETHYLDITHIOCARBAMATE

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SUMMARY

The frequency of hepatomas was investigated in mice of both sexes, strain C57BL/6, after a single dose of 10 mg AFB$_1$.kg$^{-1}$, which was administered i.p. on day 7 after birth. In males, tumors were found beginning in the 12th month in 66% of animals, and in the 20th month in 80% of animals. In females, no tumors were found within 20 months. In the course of the 20 month hepatocarcinogenesis study the activities of microsomal cytochrome P-450 dependent enzymes (cyt P-450 and b$_5$, NADPH- and NADH- cyt c reductase, aniline hydroxylase and aminopyrine demethylase) decreased in the liver by 25-33%. The hepatic detoxifying enzymes (epoxide hydrase and glutathion S-transferase) had various activities. The activity of epoxide hydrase in the initiating stage of carcinogenesis decreased, whereas in the early stage of the progression of the hepatoma it achieved the fetal levels. The activity of glutathion-S-transferase increased in the preneoplastic stage, i.e. during the promotion of hyperplastic nodules, and then decreased.

In the hepatoma, 1/2 to 1/3 of the normal liver activities of activating enzymes were found; the activities further decreasing with intensifying dedifferentiation of the hepatoma. Glutathion-S-transferase behaved in a similar way, whereas the activity of epoxide hydrase increased in the early stage of the progression of the hepatoma.

Diethyldithiocarbamate administered continually in drinking water commencing with the 9th month of carcinogenesis did not decrease the number of hepatoma, but it produced a slight increase in the activities of cyt P-450 dependent enzymes and the activity of glutathion-S-transferase too. On the other hand, it decreased the activity of epoxide hydrase in the livers of both sexes and in the hepatoma.

Abbreviations

AFB$_1$ - aflatoxin B$_1$, DMSO - dimethyl sulfoxide, MFO - mixed function oxygenase system, P-450 - cytochrome P-450, b$_5$ - cytochrome b$_5$, NAD/P/H - NAD/P/H cytochrome c reductase, ADM - aminopyrine demethylase, AOH - aniline hydroxylase, EH - epoxide hydrase, GST - glutathion-S-transferase, DTC - diethyldithiocarbamate.
INTRODUCTION

The hepatocarcinogenicity of aflatoxin B₁ in rats, ducks and rainbow trout has been convincingly demonstrated (1). In contrast, adult mice appear to be resistant to the toxic and carcinogenic effects. Newborn and infant mice are more sensitive to the effects of different hepatocarcinogens (2,3) and also to AFB₁, which induced hepatomas in newborn C57BL/6 x C3H F₁ mice (4).

A single dose model of aflatoxin hepatocarcinogenesis was tested in infant C57BL/6 mice. AFB₁ was administered i.p. on day 7 after birth and the following data were investigated:

1) Changes in the activities of microsomal cytochrome P-450 dependant enzymes and conjugating enzymes in the course of the 20-month-long aflatoxin hepatocarcinogenesis study. At intervals of 6, 8, 12, 18 and 20 months after AFB₁ administration, the concentrations of cyt P-450 and b5 and the activities of NADPH-, NADH-cyt c reductase, AOH, ADM, EH and GST were determined.

2) Effect of continually administered diethyldithiocarbamate on the course of aflatoxin carcinogenesis.

MATERIALS AND METHODS

Aflatoxin B₁ was prepared biosynthetically in our laboratory and was checked for purity chromatographically, spectrophotometrically and by NMR. It was administered to seven-day-old mice i.p. in a dose of 10 mg.kg⁻¹, dissolved in DMSO. The administered volume represented 0.5 ug per g of body weight. Diethyldithiocarbamate (DTC) was administered to experimental animals from the 9th month of age until the termination of the experiment in the 20th month as a 0.05% solution in drinking water. The daily dose corresponded to 100 mg of DTC.kg⁻¹ of body weight.

Mice C57BL/6 came from the breeding station, Sunice and the standard Larsen diet and water were given ad libitum. At time intervals the animals were killed and the liver, or the tumorous tissue, was removed into a cooled solution of 0.25 M sucrose in 0.1 M sodium phosphate buffer, pH 7.4. Microsomes were isolated from the homogenate by differential centrifugation and the concentrations of cyt P-450 and b5 (5) and the activities of NADPH- and NADH-cyt c reductases (6), aminopyrine demethylase (7), aniline hydroxylase (8), and epoxide hydrase (9) were determined in them. The activity of glutathion-S-transferase was determined in cytosol (10). The results were calculated as mean and standard deviation.

RESULTS

Induction Of Hepatomas

It follows from Table 1 that hepatomas after one dose of AFB₁ to newborn mice were induced in males between the 8th and 12th months. They were determined histologically as hepatomas composed of large light or basophilic hepatocytes. In the 12th month after AFB₁ administration the incidence of tumors was 66.6%, and in the 20th month, 88.8%. DTC alone as well as DMSO did not influence the survival of animals. In combination with AFB₁ the percentage of surviving animals was the same as after AFB₁ as was the frequency of tumors.

In females, no macroscopic changes were found in the livers at any time.