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Reduction of Covalent Binding of Aflatoxin B_1 to Rabbit Liver DNA After Immunization Against this Carcinogen

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Abstract. The covalent binding of [^{3}H]aflatoxin B_1 (AF) to liver DNA was determined, 6 h after oral administration to male rabbits. A Covalent Binding Index, CBI (μmol AF/mol DNA-P)/(mmol AF/kg b. w.) = 8,500 was found. Pretreatment of rabbits with AF coupled to bovine serum albumin in Freund's adjuvant led to the production of AF-directed antibodies. Administration of [^{3}H]AF to such immunized rabbits resulted in a CBI of only 2,500, i.e., the immunization provided a protection by a factor of more than 3. Although this is encouraging evidence for the potential of active immunization against genotoxic carcinogens, a number of points will have to be clarified, such as the time course for the DNA binding and the question of a possible shift to other target cells.

Key words: Aflatoxin – Cancer prevention – Carcinogen – Covalent binding – DNA – Immunization

Introduction

Most genotoxic carcinogens are believed to exert their activity via covalent interaction with the DNA of the target cell (Lutz 1979). In addition to this reaction with nucleic acids, reaction with protein always occurs, in most cases to an even higher degree. Since protein-carcinogen adducts are potentially immunogenic, an immune response can result with the bound carcinogen acting as a hapten (Moolten et al. 1981). It was the aim of this study to determine whether immunization of rabbits against the hepatocarcinogen aflatoxin B_1 (AF) can result in a reduction of liver DNA binding exerted by a subsequent AF dose, due to a reduction by antibodies of carcinogen available for the uptake by liver cells.

Materials and Methods

Chemicals. Generally tritiated aflatoxin B_1 (AF), specific activity 16 Ci/mmol, was obtained from Moravek Biochemical (Brea CA, USA). The radiochemical

purity was > 96% as checked by thin layer chromatography. All other chemicals were obtained from Merck (Darmstadt, F.R.G.) or Fluka AG (Buchs, Switzerland) and were of analytical grade.

Animals and Treatments. Adult male rabbits (Swiss rabbit, 3–3.5 kg, Hoffmann-La Roche, Basel) were immunized according to Nieschlag et al. (1975) with bovine serum albumin-AF conjugate prepared by the method described by Chu and Ueno (1977). [^{3}H]AF was administered orally in a gelatine capsule, filled by dropping 100 µl of an ethanol solution of AF onto lactose. After 6 h, the rabbits were killed by an overdose of Nembutal and the livers were excised.

Antibody Titer. Diluted serum taken from immunized rabbits and incubated with 2 nM [^{3}H]AF resulted in a 79% AF-binding at a titer of 1 : 500. Control serum used 1 : 10 gave rise to a background value of 1.7% [^{3}H]AF precipitation with anti-rabbit antiserum.

DNA Isolation. DNA was isolated immediately from 10 g of the left posterior lobe of the liver. A crude chromatin fraction was prepared at 4° C by a slightly modified method described by Yaneva and Dessev (1976). The further purification of the DNA from chromatin followed the standard method used in this laboratory which is based on a deproteination with phenol : chloroform : isoamyl alcohol and a hydroxyapatite column chromatography (Markov and Ivanov 1974). After dialysis against 0.2 M NaCl, DNA was precipitated with ethanol and dissolved in 10 mM Tris buffer pH 7.0. Repetitive redissolving of the DNA and precipitation with ethanol did not result in a decrease of the specific activity. Acid degradation of DNA and HPLC analysis of the purines revealed no measurable radioactivity co-eluting with the natural bases. It can therefore be assumed that no radiolabel was incorporated into DNA via biosynthesis. The amount of DNA was determined assuming an absorbance of 20 at 260 nm for a solution of 1 mg/ml (Viviani and Lutz 1978). An aliquot of the highly purified DNA (< 0.1% protein) was used for radioactivity counting in a Packard 460 CD after addition of 10 ml Insta-Gel (Packard).

Enzyme Assay. Cytochrome P-450 dependent mono-oxygenase in liver microsomes was determined with the gas chromatography (GC) assay of aldrin epoxidation as described by Wolff et al. (1979).

Results

Six hours after the oral administration of [^{3}H]AF to immunized and non-immunized rabbits, the concentration of the radiolabel was slightly lower in the livers of the immunized animals, but two to four times higher in the blood (Table 1). This difference could be due to antibodies keeping the AF molecules in the circulation for a longer period of time, thereby reducing the concentration of AF diffusible into liver cells.

Table 1. Covalent binding of aflatoxin B_1 to rabbit liver DNA, 6 h after oral administration of [^{3}H]labelled AF to AF-immunized and control animals

	Immunized		Control	
Body weight (kg)	2.90	3.40	3.40	3.08
AF Dose administered (ng/kg)	110	94	94	104
(10^7 dpm/kg)	1.25	1.07	1.07	1.18
[^{3}H] Activity in				
Blood[a] (% of dose)	7.4	4.2	2.0	1.9
Liver (% of dose)	0.5	0.5	0.9	0.9
Kidney (% of dose)	0.05	0.05	0.07	0.07
Liver DNA				
Total isolated (mg)	5.0	4.6	5.7	4.5
Amount in scint. vial (mg)	1.06	1.07	1.17	0.99
Total activity (cpm)	51.3	59.9	98.7	161.0
Specific activity[b] (dpm/mg)	71	113	204	428
Covalent binding index[c]	1,755	3,263	5,891	11,208
Mean	2,509		8,550	

[a] The amount of whole blood was assumed to represent 7% of the body weight

[b] The calculation of net radioactivity is based on background activities of 19.6 ± 1.54 cpm determined with DNA samples isolated from a control rabbit which had not been given radiolabels

[c] Conversion of the experimentally available data, CBI′ = (dpm/mg DNA)/(dpm/kg b.w.) to the CBI is done by multiplication with $3.09 \cdot 10^8$. CBI = (μmol chemical bound/mol DNA-P)/(mmol chemical applied/kg b.w.) (Lutz 1979)

This hypothesis is supported by the data on liver DNA binding (Table 1). On average, the immunization reduced the CBI by a factor of 3.4, i.e., provided a substantial protection from the genotoxicity exerted by AF, 6 h after oral administration.

A determination of the aldrin epoxidase activity in liver microsomes revealed no difference between the two groups of animals. The specific enzyme activities were 2.52 and 2.43 nmol dieldrin formed per min and per mg microsomal protein isolated from the immunized rabbits, whereas the control values were 2.14 and 2.22. This assay has been shown to measure cytochrome P-450-type activity (Wolff et al. 1979) which also generates the aflatoxin-2,3-oxide (Kawajiri et al. 1980) thought to be the most important electrophilic metabolite responsible for DNA binding (Lin et al. 1977). It is therefore unlikely that the immunization gave rise to changes in enzyme levels which, in turn, could have resulted in the observed difference in DNA binding.

Discussion

Covalent binding of aflatoxin B_1 to liver DNA of various animal species has been shown to reflect their susceptibility for liver tumor formation (Lutz et al. 1980). Since non-immunized rabbits gave rise to a CBI of almost 10,000 they could be about as susceptible as rats for which CBI of 10,000 has been reported (Lutz et

al. 1980). The decrease of the CBI to 2,500 after immunization indicates some type of protection, but it would be premature to postulate this as a general phenomenon.

Firstly, the concentration of circulating antibody must be large enough to bind most of the dose administered. This condition was met in the present experiment by choosing a very low dose of AF.

Secondly, the scavenging of the AF molecules by antibodies obeys the laws of chemical complex formation equilibria and it is conceivable that AF is slowly released from the antibody binding site in parallel with the metabolism of the free fraction in the liver. It cannot, therefore, be excluded at present, that the damage to liver DNA is lowered only in the first hours and that the important damage × time integral will not be reduced by immunization.

Thirdly, it is possible that the DNA damage in the liver is indeed lower but that other cells take up more AF so that other tissue might become a target for AF-induced tumor formation.

Future work will have to elucidate whether the protection observed under the present experimental conditions will be real or whether it will be diminished by one or the other drawbacks mentioned above.

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