Biochemical Pharmacology, Vol. 22, pp. 1801–1805. Pergamon Press, 1973. Printed in Great Britain.

INFLUENCE OF AFLATOXIN B_1 AND AFLATOXIN B_2 ON RAT LIVER LYSOSOMAL ACID DEOXYRIBONUCLEASE

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(*Received* 10 *October* 1972; *accepted* 19 *January* 1973)

Abstract—The *in vitro* effects of aflatoxins B_1 and B_2 were studied on the permeability of isolated rat liver lysosomes. Only aflatoxin B_1 altered the lysosomal membrane with the consequent release of lysosomal enzymes. The results of the *in vivo* experiment with aflatoxin B_1 show that the specific activity of acid DNase in liver lysosomes was markedly decreased in the rats dosed aflatoxin B_1 while the specific activity of the cytoplasmic acid DNase, or nonsedimentable acid DNase, was dramatically increased. The results are discussed in relation to a hypothesis concerning the role of lysosomes in carcinogenesis.

ACID DEOXYRIBONUCLEASE (DNase II; deoxyribonucleate 3′-nucleotidohydrolase, EC 3.1.4.6) is an ubiquitous enzyme that occurs mainly in the lysosomes together with a variety of acid hydrolases,[1] but it also occurs in nuclei.[2–4] It has been suggested by several authors that the biological role of this enzyme might be involved in some essential biological mechanism such as DNA replication and DNA recombination.[5,6]

Aflatoxin B_1 is one of the most potent hepatocarcinogens known and it has been shown by Pitout *et al.*[7] that this toxin markedly increases the activity of the acid DNase of rat liver nuclei. This increase can possibly be attributed to a variety of causes, one of which is the possible deleterious effect of the toxin on lysosomal membranes with resultant leakage of the contained enzymes. Recently it has been suggested that the released lysosomal DNase might be able to penetrate the nuclear membrane and attack the chromosomes.[8–10]

In order to elucidate the possible cause of this phenomenon, we decided to investigate whether aflatoxin B_1 does indeed affect hepatic lysosomes when administered to rats. In addition, the effect of aflatoxin B_2 on rat liver lysosomes was also studied. The latter toxin has a similar molecular structure to aflatoxin B_1, but it is a relatively weak toxin and carcinogen in rats when compared to aflatoxin B_1.[7]

MATERIALS AND METHODS

Animals. Random-bred male Wistar derived rats from our own colony with an initial average body weight of 150 g were used. The animals were fed our standard laboratory diet and water *ad lib.*

Chemicals. Coalfish DNA and hog spleen acid DNase were obtained from Sigma Chemical Co. All other chemicals used were of analytical reagent grade. Sephadex

CM C-25 was purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. Triton X-100 was purchased from Triton Chemical Co.,Natal, South Africa. Dimethyl sulfoxide [DMSO] was bought from FLUKA, Switzerland.

Aflatoxin B_1 was isolated and purified according to the method of Steyn.[11] Aflatoxin B_2 was prepared from aflatoxin B_1 by means of hydrogenation according to van Dorp *et al.*[12] The chemical structures of aflatoxins B_1 and B_2 are given in Fig. 1.

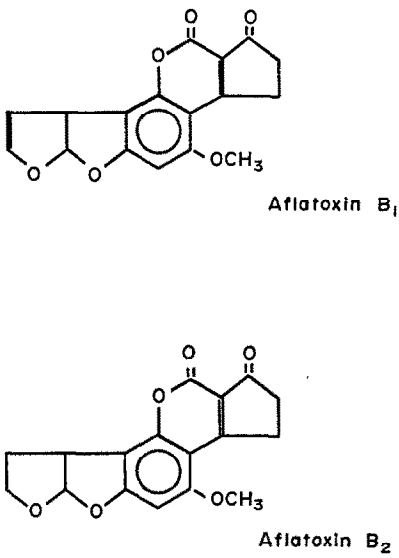

FIG. 1. The chemical structures of aflatoxins B_1 and B_2, respectively.

Acid DNase activity was measured essentially as described by Hodes *et al.*[13] and one unit (U) of enzyme is that amount of enzyme which produces one A^{260} unit/hr. Specific activity is expressed as U/mg of protein and U/g of wet liver. Protein was determined according to the method of Lowry *et al.*[14]

Extraction and isolation of lysosomes from rat livers. All operations were done in the cold (2–5°). The lysosomes were processed according to the method of Sawant *et al.*[15] The purity of all lysosome preparations was assessed by electron microscopy.

The isolation and partial purification of cytoplasmic or nonsedimentable acid DNase. The supernatant fluid, obtained after the centrifugation of the liver extract at 16,300 *g* for 20 min to remove the lysomes, was again centrifuged at 150,000 *g* for 40 min to yield a clear, red supernatant. After the pH of this supernatant was carefully adjusted to 4·7 with concentrated acetic acid, centrifugation at 20,000 *g* for 10 min was resorted to in order to remove the heavy precipitate which formed. This step was done to remove interfering proteins which precipitated during the assay of the activity. The pH of the supernatant was adjusted to 6·5 with 5 N NaOH and the resultant cloudy suspension was clarified by centrifugation at 20,000 *g* for 10 min. The clear, red supernatant was filtered through a 1 × 5 cm CM C-25 Sephadex column which was equilibrated with 0·05 M sodium phosphate buffer, pH 6·5. After the column was washed with 30 ml of the same buffer, the enzyme was eluted with a 1·0 M sodium chloride–0·05 M sodium phosphate buffer, pH 6·5. The final volume of the eluate was 25 ml. Appropriate dilutions were done with 0·05 M sodium phosphate buffer, pH 6·5.

It is interesting to note that the enzyme extract of the toxin-treated animals contained much more haemoglobin when compared with that of the control group.

Effects of aflatoxin B_1 and aflatoxin B_2 on rat liver lysosomes in vitro. The lysosomal fraction, isolated from the livers of ten rats, was suspended in 0·05 M sodium acetate buffer, pH 5·0 in the ratio 1:4 (w/v). Two ml aliquots of the lysosomal fraction were incubated with 0·1 ml quantities of Triton X-100 (4%, v/v), DMSO (control), and three different concentrations of aflatoxin B_1 (0·2, 2 and 20 mM) and aflatoxin B_2 (20 mM), respectively, at 37° for 30 min. The toxins were dissolved in DMSO. After centrifugation at 40,000 *g* for 5 min the activity of acid DNase was determined as described. Appropriate dilutions were done with the acetate buffer.

Effects of aflatoxin B_1 on rat liver lysosomes in vivo. For this experiment, thirty rats were divided into two equal groups. To the first group aflatoxin B_1, dissolved in DMSO, was administered intragastrically every day at a dosage rate equivalent to one-fifth of the LD_{50} of the toxin (the LD_{50} is 7·2 mg/kg body wt). The other group received only DMSO. Three animals from each group were killed by decapitation on days 1, 3, 4, 5 and 7 after dosage, respectively. The livers were excised and immediately processed for the isolation of lysosomes and cytoplasmic acid DNase. Lysosomal acid DNase was prepared by treating the lysosomes with 1·0 ml of a 0·2% Triton X-100 solution (v/v).

RESULTS

Effects of aflatoxins B_1 and B_2 on the permeability of rat liver lysosomes in vitro. The effects of aflatoxins B_1 and B_2 on the release of acid DNase from rat liver lysosomes *in vitro* is illustrated in Fig. 2. It can be seen that only aflatoxin B_1 at the two higher levels released lysosomal acid DNase (except at the very low concentration). On the other hand, Triton X-100, a neutral detergent, was responsible for the largest release of lysosomal acid DNase. It is known that this detergent is capable of disintegrating membranes,[10] and it has no effect on hog and rat DNase II activity.

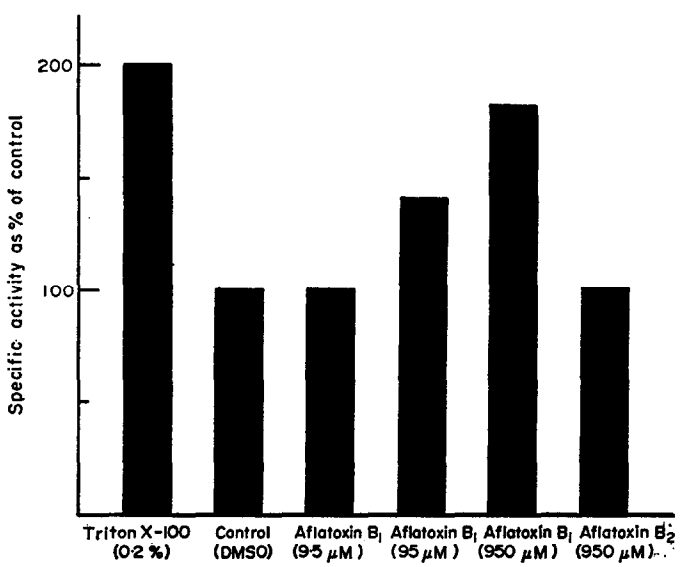

FIG. 2. The effects of aflatoxins B_1 and B_2 on the release of acid DNase from rat liver lysosomes.

Effect of aflatoxin B_1 on rat liver lysosomes in vivo. The results of the *in vivo* experiments clearly show that the specific activity of acid DNase from liver lysosomes was markedly decreased in the rats dosed aflatoxin B_1 (Fig. 3). In addition, the specific activity of the cytoplasmic acid DNase was dramatically increased as shown in Fig. 3.

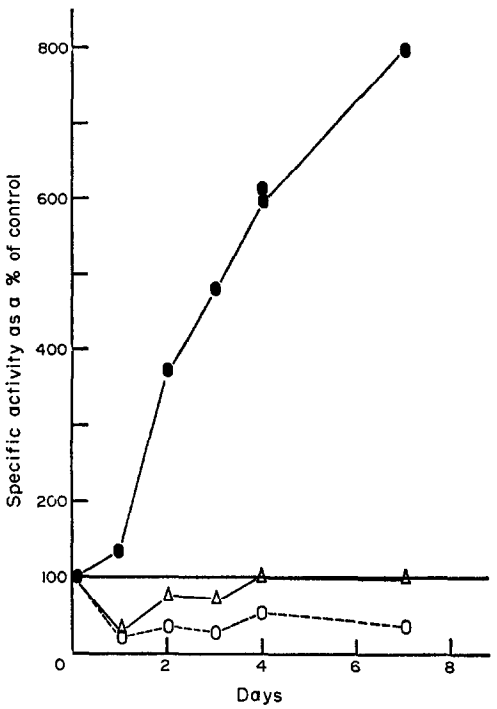

FIG. 3. The influence of aflatoxin B_1 on lysosomal acid DNase *in vivo*. Cytoplasmic DNase, ● —— ● (U/g of liver); lysosomal DNase, ○ —— ○, (U/mg of prot); △ —— △ (U/g of liver).

DISCUSSION

In short-term experiments with ducklings, Theron[16] found that aflatoxin B_1 caused an increase of acid phosphatase which was released from the lysosomes. It was concluded that the cytotoxic effects of the toxin, or a metabolite of the toxin, was due to a direct action on the liver cell membrane and the membranes of the various intracytoplasmic structures. Yung *et al.*[17] observed that the carcasses of chickens that died as a result of acute aflatoxicosis decomposed more rapidly than those of control animals. These authors also observed a significant increase in liver acid phosphatase when chickens were fed a diet containing aflatoxin B_1 and they concluded that lysosomal enzymes are released in the cytoplasm during aflatoxicosis. It is well established that post-mortem decomposition of tissues is a result of the activity of lysosomal enzymes.[18]

The enzyme complex of the lysosomes and the diversity of their functions predetermines the participation of lysosomes in the development of a number of pathological processes. In recent years special attention has been devoted to the study of the role of lysosomes in carcinogenesis.[19] The results of Pokrovskii *et al.*[10] showed that aflatoxin B_1 caused a distinct activation of lysosomal acid hydrolases which may be

associated with the increase in the total number of lysosomes observed. Cytoplasmic (nonsedimentable) acid DNase was maintained at a very high level throughout the whole duration of their experiment (96 hr).

The dramatic increase of cytoplasmic acid DNase (see Fig. 3) is probably due to:

(1) the release of enzyme into the supernatant liquid which indicates an increase in the permeability of the lysosomal membrane;

(2) the induction of the *de novo* synthesis of acid DNase. The experiments *in vitro* (see Fig. 2) confirm that aflatoxin B_1 has a labilizing effect on lysosomal membranes. The inability of aflatoxin B_2 to alter the permeability of the lysosomes, is probably due to the saturation of the terminal furane ring (Fig. 1) which is a less active chemical group.

The data obtained in this study confirms the labilizing action of aflatoxin B_1 on the lysosomal membranes as observed by Pokrovskii *et al.*[10] This fact is of undoubted interest in regard to the assessment of the possible role of lysosomes in the biochemical changes accompanying carcinogenesis.[8,9,19]

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