Biochemical Pharmacology, Vol. 21, pp. 2489–2496. Pergamon Press, 1972. Printed in Great Britain.

# COMPARATIVE STUDY OF THE EFFECT OF AFLATOXIN AND SOME ANTITUMOUR ANTIBIOTICS ON RAT LIVER LYSOSOMES *IN VIVO* AND *IN VITRO*

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(*Received* 1 *February* 1972; *accepted* 4 *April* 1972)

**Abstract**—The effect of aflatoxin, one of the most potent hepatotropic carcinogens and antitumour antibiotics—mitomycin C and rubomycin C (identical daunomycin), on the activities of five rat liver lysosomal enzymes (acid deoxyribonuclease, arylsulphatases A and B, β-glucuronidase, β-glucosidase and β-galactosidase) has been investigated *in vivo* and *in vitro*.

Large differences are found in the behaviour of lysosomal enzymes in response to the administration of aflatoxin and antibiotics. In the first hours after treatment with aflatoxin there is a marked activation of all the acid hydrolases. At the same time, the administration of mitomycin C and rubomycin C, cause a decrease in activity of most of the lysosomal enzymes.

It is noted that only aflatoxin, both *in vivo* and *in vitro*, had a labilizing effect on lysosomal membranes, causing the release of the enzymes into the supernatant.

The data are discussed in connection with the possible role of lysosomes in biochemical changes, accompanying carcinogenesis.

THE mechanism of action of aflatoxins—metabolites of certain species of *Aspergillus*, and some cytostatic antibiotics (mitomycin C, actinomycin D, daunomycin) is considered at present to be connected with their interaction with DNA and with their effects on the biosynthesis of nucleic acids and proteins.[1–4] However, these ideas are in contradiction with the difference in their biological effects: the aflatoxins are the strongest hepatotropic carcinogens,[5,6] while the antibiotics have an antitumour effect.[7–9]

The reasons for these differences are unknown. Perhaps the study of the direct action of these drugs on subcellular membranes may be useful as an approach to the identification of these differences.

In particular, the study of lysosomes may be of interest as the cytostatic effects of a number of substances are thought to be associated with the activation of lysosomal enzymes, and these subcellular particles are thought to take part in the carcinogenesis.[12–14]

The purpose of the work was to report the comparative study of the effect of aflatoxin and some antitumour antibiotics, (mitomycin C and rubomycin C–identical with the daunomycin) on the stability of lysosomal membranes and on the activity of their enzymes.

## MATERIALS AND METHODS

*Experimental animals.* 320, young male Wistar rats weighing approximately 40 g were used. They were maintained on a nutritionally complete diet and water *ad lib.*

*Aflatoxin.* Aflatoxin was obtained by extraction from *Aspergillus flavus* using the

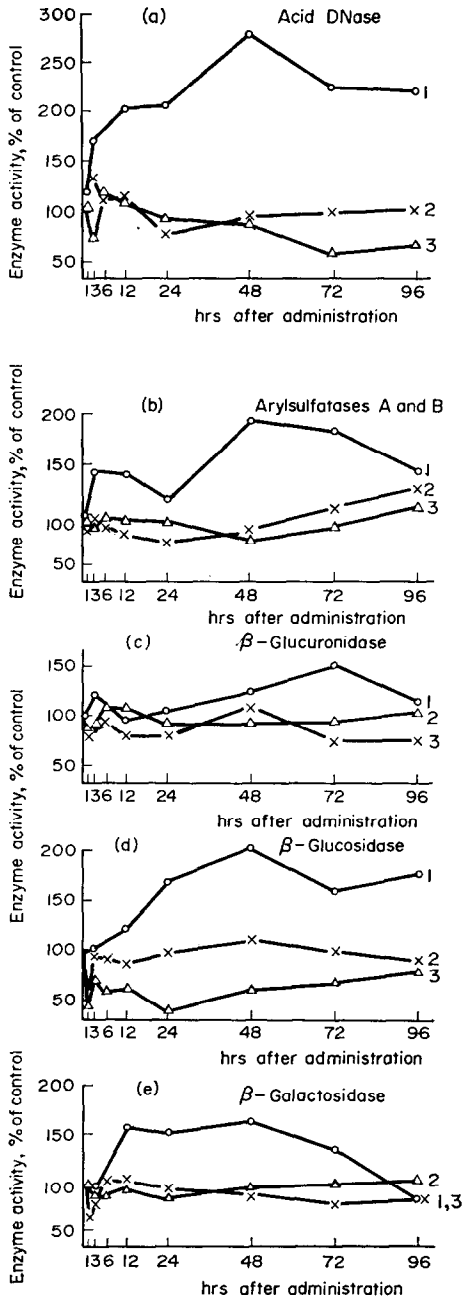


FIG. 1. The time-course of changes induced by aflatoxin (○——○ 1), mitomycin C (△——△ 2)

used for acid deoxyribonuclease; the methods of Roy,[23] for arylsulphatases A and B; of Nimmo-Smith,[24] for β-glucuronidase; of Sellinger *et al.*,[25] for β-galactosidase and of Beck and Tappel,[26] for β-glucosidase. The following reagents were used as substrates for these enzyme determinations: highly polymerized DNA; Koch–Light, Great Britain; nitro-catechol sulphate dipotassium salt, Sigma, U.S.A.; *p*-nitrophenyl-β-D-glucuronide, Serva, Austria; *o*-nitrophenyl-β-D-galactopyranoside, Chemapol, Czechoslovakia. The specific enzyme activities were expressed as μmoles/min per g of fresh tissue or per gram of protein.

The *protein content* was determined by means of a modification of the method of Lowry *et al.*[27] with the previous dissolution of homogenates in 0·2 N NaOH.

Tissues for electron microscopy were fixed in formaldehyde–glutaraldehyde solution.[28] They were then post-fixed in osmium tetroxide, dehydrated and embedded in araldite.

All quantitative data were analysed statistically with Student's *t*-test.

## RESULTS

Fourty-eight hr after administration of aflatoxin, we observed that the poisoned animals had symptoms of intoxication, including decrease of food intake, weight loss and reduced mobility. By the end of the observation period (96 hr) the symptoms of toxicity increased and several animals died. The animals which had been treated with mitomycin C and rubomycin C displayed symptoms of a lower toxicity than those which appeared later. At 72 and 96 hr after the administration of the antibiotics, depression and a considerable weight loss (about 40 per cent) were observed.

### *Effect of aflatoxin and antitumour antibiotics on the total activity of lysosomal enzymes of rat liver*

The time-course of changes in total activity of lysosomal enzymes in the liver of rats treated with aflatoxin and antibiotics, is shown in Fig. 1 (A–E).

Clearly the activity of most of the enzymes studied was already increased 3 hr after administration of aflatoxin. By that time the activity of acid DNase reached 168 per cent of the control level, and arylsulphatases A and B and β-glucuronidase, 141 and 121 per cent respectively. At 12 and 24 hr the activity of the enzymes continued to increase and reached maximal level at 48 hr. The activity of acid DNase increased the most (276 per cent of the control level), while the activity of arylsulphatases A and B and β-glucosidase increased 2-fold. The activity of β-glucuronidase and β-galactosidase increased moderately (123 and 163 per cent respectively). Seventy-two hr after aflatoxin treatment the activity of the enzymes began to decrease. However, even at the end of the experiment acid DNase activity exceeded the control level more than twice; the activity of β-glucosidase remained increased (160 per cent).

Thus, the administration of aflatoxin was characterized by lysosome enzyme activation which became apparent at the earliest time of the observation (3 hr).

It should be noted that the parallel electron microscopic studies showed a considerable increase of the quantity of lysosomes in the liver cells of the rats poisoned with aflatoxin.[29] These results coincide with the data of Theron,[30] who also observed an increase of the quantity of lysosomes after the administration of aflatoxin.

The time-course of changes in total activity of lysosomal enzymes in the liver of

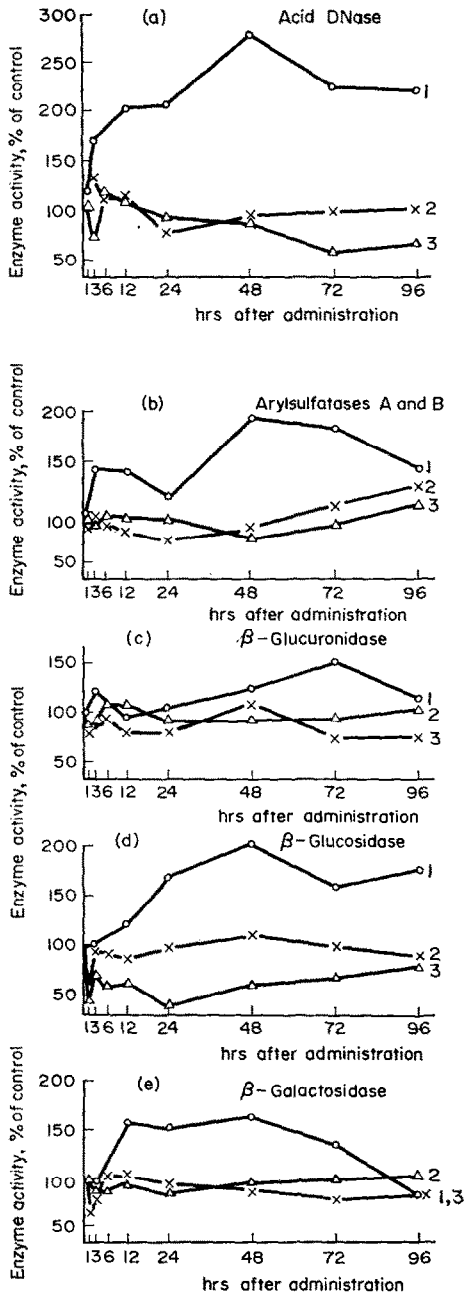


FIG. 1. The time-course of changes induced by aflatoxin (○——○ 1), mitomycin C (△——△ 2) and rubomycin C (×——× 3) in total activity of lysosomal enzymes of rat liver. Each value is the mean of eight experiments.

rats treated with mitomycin C and rubomycin C differed considerably from that observed in the experiments with aflatoxin. From the first hour after the administration of both antibiotics the activity of the most of the enzymes decreased considerably in comparison with control level. Thus, in case of mitomycin C the activity of β-glucosidase decreased more than 50 per cent while the activity of β-glucuronidase changed comparatively slightly, to 86 per cent of the control level. Three hr after the administration of these antibiotics the activities of the other enzymes (acid DNase, arylsulphatases A and B, β-galactosidase) decreased significantly. In the following periods (6–96 hr) some differences in the behaviour of lysosomal enzymes were found. Thus, the activity of β-glucosidase was decreased considerably to 62–64 per cent of the control level and after 96 hr it had only increased to a certain extent, (to 83 per cent). Beginning at 48 hr the activity of acid DNase fell appreciably, being 64 per cent of the control level at 96 hr. The activity of arylsulphatases A and B decreased slightly by the end of the experimental period, but the activity of β-galactosidase, and β-glucuronidase did not change significantly.

During the first hr after the administration of rubomycin C the differences in lysosomal enzyme activity were observed. Thus, the activity of acid DNase increased moderately (128 per cent of the control level), at the same time the group of enzymes participating in glycoprotein and mucopolysacharide metabolism showed a marked decrease in activity: the activity of arylsulphatases A and B was 83·5 per cent, β-glucuronidase, 81 per cent, β-glucosidase, 52·5 per cent and β-galactosidase, 64 per cent of the control level. This tendency remained during the whole period of observation. By the end of the first day of the experiment the activity of acid DNase was also decreased (77 per cent of the control level).

Thus, the lysosome reaction following the administration of mytomycin C and rubomycin C was expressed by a lowering of the activity of most of the acid hydrolases. A considerable lowering of the quantity of RNA in the liver was observed both in the mitomycin C and in the rubomycin C experiments during the whole period of observation (40–50 per cent of the control level).

*Effect of aflatoxin and antitumour antibiotics on the non-sedimentable activity of lysosomal enzymes of rat liver*

In the same experiments the simultaneous study of the nonsedimentable activity of lysosomal enzymes (for example, arylsulphatases A and B and β-glucosidase) showed that neither mitomycin C nor rubomycin C had any influence upon the membrane permeability of lysosomes. At the same time, aflatoxin caused significant enzyme solubilization (more than twice the increase of the enzymes activity in the supernatant: see Table 1).

*Effect of aflatoxin and antitumour antibiotics on rat liver lysosomes* in vitro

An attempt to explain the above results was made by a study of the direct influence of aflatoxin and the antibiotics on isolated lysosomes. The data obtained *in vitro* showed that in contrast to mitomycin C and rubomycin C only aflatoxin in concentrations similar to those used *in vivo* had a labilizing effect on lysosomal membranes, causing the release of lysosomal enzymes into the supernatant (Fig. 2).

TABLE 1. EFFECT OF AFLATOXIN AND ANTITUMOUR ANTIBIOTICS ON NON-SEDIMENTABLE ACTIVITY OF LYSOSOMAL ENZYMES OF RAT LIVER

| Group | Time after administration (hr) | Aflatoxin | | | | Mitomycin C | | | | Rubomycin C | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Arylsulphatases A + B | | β-glucosidase | | Arylsulphatases A + B | | β-glucosidase | | Arylsulphatases A + B | | β-glucosidase | |
| | | $\bar{X} \pm$ S.E. | P | $\bar{X} \pm$ S.E. | P | $\bar{X} \pm$ S.E. | P | $\bar{X} \pm$ S.E. | P | $\bar{X} \pm$ S.E. | P | $\bar{X} \pm$ S.E. | P |
| Control | 12 | 1·3 ± 0·04 | | 4·8 ± 0·11 | | 1·3 ± 0·01 | | 4·9 ± 0·12 | | 1·5 ± 0·08 | | 4·8 ± 0·10 | |
| | 48 | 1·4 ± 0·06 | | 4·6 ± 0·09 | | 1·2 ± 0·07 | | 4·8 ± 0·31 | | 1·6 ± 0·07 | | 3·2 ± 0·37 | |
| | 96 | 1·5 ± 0·17 | | 4·8 ± 0·21 | | 1·2 ± 0·08 | | 4·2 ± 0·09 | | 1·5 ± 0·02 | | 4·2 ± 0·86 | |
| Experimental | 3 | 1·3 ± 0·05 | > 0·05 | 4·3 ± 0·17 | > 0·05 | 1·2 ± 0·04 | > 0·05 | 5·0 ± 0·60 | > 0·05 | 1·5 ± 0·02 | > 0·05 | 4·3 ± 0·37 | > 0·05 |
| | 12 | 1·0 ± 0·06 | < 0·001 | 4·6 ± 0·24 | > 0·05 | 1·2 ± 0·02 | > 0·05 | 4·8 ± 0·23 | > 0·05 | 1·5 ± 0·04 | > 0·05 | 3·2 ± 0·20 | > 0·05 |
| | 24 | 2·4 ± 0·30 | < 0·01 | 5·0 ± 0·15 | < 0·05 | 1·2 ± 0·03 | > 0·05 | 4·5 ± 0·09 | > 0·05 | 1·6 ± 0·09 | > 0·05 | 3·0 ± 0·28 | > 0·05 |
| | 48 | 2·6 ± 0·06 | < 0·001 | 6·8 ± 0·25 | < 0·001 | 1·3 ± 0·02 | > 0·05 | 4·0 ± 0·17 | > 0·05 | 1·7 ± 0·08 | > 0·05 | 3·1 ± 0·18 | > 0·05 |
| | 72 | 2·1 ± 0·20 | < 0·05 | 7·2 ± 0·92 | < 0·05 | 1·2 ± 0·06 | > 0·05 | 3·9 ± 0·11 | > 0·05 | 1·6 ± 0·04 | > 0·05 | 4·5 ± 0·49 | > 0·05 |
| | 96 | 2·5 ± 0·22 | < 0·01 | 4·3 ± 0·25 | < 0·05 | 1·2 ± 0·02 | > 0·05 | 4·3 ± 0·48 | > 0·05 | 1·6 ± 0·03 | > 0·05 | 3·5 ± 0·60 | > 0·05 |

Each value is the mean of eight experiments.
Specific enzyme activities are expressed as per cent of total activity of whole homogenates ±S.E.

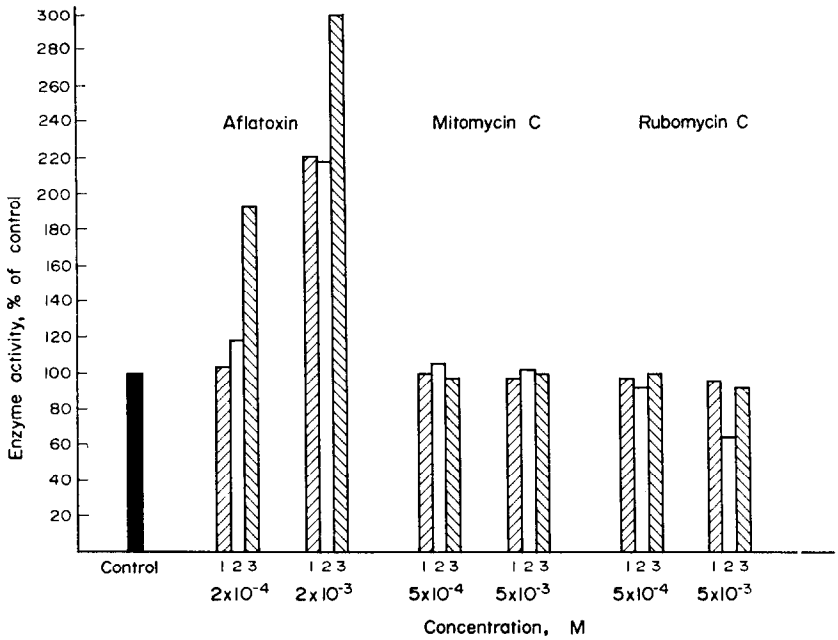


FIG. 2. Effect of aflatoxin, mitomycin C and rubomycin C *in vitro* on non-sedimentable activity of enzymes of isolated rat liver lysosomes. (1) Arylsulphatases A and B, (2) β-glucuronidase, (3) β-glucosidase. Each value is the mean of seven experiments.

## DISCUSSION

The results of these experiments, both *in vivo* and *in vitro*, showed clear differences in the behaviour of lysosomal enzymes in response to the administration of aflatoxin and the antitumour antibiotics, mitomycin C and rubomycin C (daunomycin).

Aflatoxin caused a sharp activation of all acid hydrolases within the first hours after its administration, evidently this could be related to the increase of the total quantity of lysosomes, shown by electron microscopic observations. Acid DNase activity remained at a high level throughout our experiments.

At the same time, the administration of mitomycin C and rubomycin C caused a decrease in the activity of most of the lysosomal enzymes.

It is interesting that the enzyme activation induced by aflatoxin was accompanied by their release into the supernatant, indicating an increase in the permeability of lysosomal membranes, the experiments *in vitro* confirming that aflatoxin had a labilizing effect on the lysosomal membranes.

We emphasize these differences in connection with Allison's hypothesis[12,13] on the important role of lysosomes in carcinogenesis. This hypothesis is based on the ability of lysosomes to accumulate a number of substances including carcinogens that promote the release of lysosomal enzymes into the cytoplasm by increasing the permeability of their membranes, and on the ability of the released lysosomal DNase to induce mutagenic changes in chromosomes.

Our findings do not contradict Allison's hypothesis. In our experiments aflatoxin, possessing carcinogenic action, led to the activation of lysosomal acid DNase and had a definite labilizing effect on lysosomal membranes both *in vivo* and *in vitro*.

On the other hand a number of authors have connected the cytostatic effect of mitomycin C with the activation of lysosomal enzymes of neoplastic tissue.[10,11] In our experiments, however, the reaction of the lysosomes of normal tissue of the liver was expressed as a decrease in enzyme activity. The selective activation of the acid hydrolases of malignant tumours by mitomycin C and rubomycin C suggests that lysosomes may have a special role in the antitmuour effects of these antibiotics.

*Acknowledgements*—The authors express their thanks to Dr. G. N. Wogan of the Massachusetts Institute of Technology, (Cambridge, U.S.A.) for a gift of crystalline aflatoxin $B_1$, and also Prof. V. A. Shorin of the Institute for New Antibiotics, Academy of Medical Sciences, Moscow, U.S.S.R. for a gift of crystalline rubomycin C.

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