PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 164, 35–39 (1980)

Protective Effect of Cycloheximide on Acute Aflatoxin B_1 Intoxication (40820)

JOHN J. CH'IH,[1] SHIH-CHUNG WANG, AND HSIEN-WEN FENG

Kohlberg Medical Research Laboratory, Veterans General Hospital and Department of Biochemistry, National Defense Medical Center, Taipei, Taiwan 112, Republic of China

Studies reported by Farber's and Flaks' groups demonstrate that *in vivo* administration of a nonlethal dose of cycloheximide (CHI) to a rat protects the rat crypt cells against lethal chemical or radiation damage (1) and the rat liver against acute toxicity caused by hepatocarcinogens such as DL-ethionine, 2-acetylaminofluorene, 3'-methyl-4-dimethylaminozaobenzene, and carbon tetrachloride (2–4). Their studies are confined to the histological and fine structural aspects of the hepatocytes.

Aflatoxin B_1 (AFB), a potent hepatocarcinogen, is known to induce acute necrosis prior to the formation of hepatocarcinoma in experimental animals (5). Although the mechanism by which AFB produces liver cancer is not known, the *in vivo* administration of AFB can cause chemical modification of DNA (6–9), inhibition of RNA synthesis (10–13), and interference with the protein synthetic activity (14–16) before histopathological alterations.

Since earlier studies suggest that CHI has a protective effect against hepatocarcinogens (1–4), the present study was undertaken to determine whether CHI prevents AFB-induced acute liver injury. Serum enzymes, lactate dehydrogenase (LDH), alanine aminotransferase (GPT), aspartate aminotransferase (GOT), and alkaline phosphatase (ALP), were used as indicators for liver damage.

Materials and methods. Male Sprague–Dawley rats (190 ± 10 g) were fed chicken diet (a product of Taiwan Sugar Corporation, Taipei, Taiwan, with a similar composition to Purina chow) and water *ad libitum*. CHI (Sigma Chemical Co., St. Louis, Mo.) in 0.9% NaCl was injected ip at a dose of 2.0 mg/kg body wt 24 hr prior to the administration of AFB. AFB (Makor Chemical Co., Jerusalem, Israel), dissolved in dimethyl sulfoxide: 1,2-propylene glycol (1:1), was injected ip at doses of 1, 2, or 4 mg/kg body wt 2 or 16 hr before sacrifice. CHI-treated control animals were killed 24 hr after dosing. Untreated control animals were given an equivalent amount of solvent. There were four experimental groups (Group I, untreated control; Group II, AFB treated; Group III, CHI-treated control; Group IV, CHI-AFB treated). In each experiment, each group consisted of 2–3 animals. Data presented are means ± SEM of six separate experiments. P values less than 0.05 are considered significant. Collection of blood and preparation of liver homogenate were carried out as described previously (17). Serum enzyme activities of LDH, GOT, GPT, and ALP were determined according to the Automated Technicon Methodology for the SMA-12/60 autoanalyzer (Technician Instruments Corp., Tarrytown, N.Y.). Hemolyzed sera were discarded. Liver protein was determined by the method of Lowry *et al.* (18) with crystalline bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) as the standard. DNA and RNA were determined according to diphenylamine and orcinol reactions as described previously (19).

Preliminary light microscopic studies were performed with pieces of liver fixed in 10% neutral formalin sectioned at 5 μM and stained with hematoxylin and eosin.

Results and discussion. The sensitivity of experimental animals to AFB toxicity and carcinogenic effect varies not only with species but also with sex, strain, age, and nutritional states within the species. AFB doses of 1.0, 1.5, 2.0, 2.5, 3.0, and 4.0 mg/kg body wt killed 0/12, 0/12, 1/12, 2/12, 8/12, and 12/12 rats within 8 days. The esti-

[1] Author to whom all correspondence should be sent. A Senior International Fellow (FO6-TW00262-01) of the Fogarty International Center, National Institutes of Health. The author's current address is the Department of Biological Chemistry, Hahnemann Medical College and Hospital, Philadelphia, Pa. 19102.

0037-9727/80/050035-05$01.00/0

TABLE I. SERUM ENZYME ACTIVITIES OF RATS TREATED WITH AFLATOXIN B_1[a]

Condition of rats	Liver Wt (% of body wt)	Liver protein (mg/g liver)	Serum enzymes (mμ/ml)			
			ALP	LDH	GOT	GPT
Untreated	4.4 ± 0.2	217 ± 9	171 ± 12	726 ± 88	167 ± 27	32 ± 7
AFB treated						
1 mg/kg	4.1 ± 0.2	221 ± 12	183 ± 19	559 ± 83	169 ± 9	43 ± 2
4 mg/kg	4.2 ± 0.2	211 ± 11	175 ± 4	418 ± 54	190 ± 28	26 ± 3

[a] AFB at doses indicated was given 2 hr prior to animals being killed.

mated LD_{50} of AFB for male Sprague–Dawley rats used in this study was 2.8 mg/kg. CHI pretreatment did not alter AFB lethality. Therefore, AFB at doses of 1 or 2 mg/kg was considered nonlethal whereas 4.0 mg/kg was lethal. Administration of 1 or 4 mg AFB for 2 hr produced no significant changes in liver weight, protein concentration, and serum enzyme activities (Table I). However, 16 hr after the administration of 1, 2, or 4 mg AFB, significant elevation of serum LDH, GOT, and GPT activities were clearly seen (Table II, Group II). The ALP activity did not increase until 24 hr later. The histological examinations revealed occasional periportal necrosis in the livers of AFB-treated animals. Biliary proliferation was not noted. These results were similar to the study carried out by Clifford and Rees (20). The increase of liver enzymes in serum implies that immediate tissue trauma occurred with the release of intracellular enzymes into the blood circulation. This cellular damage leading to loss of these enzymes may be due to the inhibition by AFB of the synthesis of cellular protein(s) required to maintain the integrity of the cell membrane; it is also possible that AFB, a hydrophobic compound, interacts with membrane lipids leading to an alteration in the permeability of the cell. In the CHI pre-treated animals receiving AFB (Group IV), nonlethal doses of AFB did not cause any significant elevation of these enzymes in the serum. Statistically, they are not different from either the untreated animal (Group I) or CHI-treated control (Group III). When a lethal dose of AFB was given to the CHI pretreated rats, moderate elevations of LDH and GPT activities were observed (Table II, Group IV). These results suggest that the damages to the liver cells caused by nonlethal doses of AFB are reversible and can be prevented by CHI-treatment. However, disturbances caused by a lethal dose of AFB to the liver cells or

TABLE II. ACTIVITIES OF SERUM ALKALINE PHOSPHATASE, LACTATE DEHYDROGENASE, ASPARTATE, AND ALANINE AMINO TRANSFERASES IN RATS TREATED WITH AFLATOXIN B_1, CYCLOHEXIMIDE AND BOTH[a]

Conditions		AFB treatment (mg/kg body wt)	Alkaline phosphatase (mμ/ml)	Lactate dehydrogenase (mμ/ml)	Amino transferases (mμ/ml)	
Group	CHI pretreatment				Aspartate	Alanine
I	–	0	171 ± 12	726 ± 88	167 ± 27	32 ± 17
II	–	1.0	221 ± 24	2083 ± 182*	389 ± 39*	67 ± 5*
	–	2.0	190 ± 19	2334 ± 128*	598 ± 70*	133 ± 20*
	–	4.0	199 ± 10	2556 ± 318*	623 ± 43*	175 ± 11*
III	+	0	138 ± 12	740 ± 103	183 ± 22	36 ± 8
IV	+	1.0	166 ± 14	485 ± 26	248 ± 9	39 ± 2
	+	2.0	167 ± 9	858 ± 129	257 ± 11	48 ± 2
	+	4.0	157 ± 17	1460 ± 180*	551 ± 72*	74 ± 11*

[a] AFB at doses indicated was given 16 hr prior to animals being killed.
* $P < 0.05$ when compared to untreated group (Group I).

TABLE III. CHANGES OF LIVER WEIGHT, PROTEIN, DNA, AND RNA CONTENT OF RATS TREATED WITH AFLATOXIN B_1, CYCLOHEXIMIDE, AND BOTH[a]

Treatments	AFB_1 (mg/kg body wt)[b]				AFB_1 (mg/kg body wt)[c]			
	0[d]	1[e]	2	4	0[f]	1[g]	2	4
Liver weight (% of body wt)	4.4 ± 0.2	3.8 ± 0.2	3.8 ± 0.1	3.5 ± 0.2*	4.1 ± 0.2	3.8 ± 0.2	4.1 ± 0.1	4.1 ± 0.1
Protein content (mg/g liver)	217 ± 9	207 ± 12	212 ± 10	219 ± 7	226 ± 19	225 ± 4	262 ± 22	246 ± 5
DNA content (mg/g liver)	1.7 ± 0.08	1.9 ± 0.04	1.6 ± 0.08	1.8 ± 0.08	1.8 ± .008	2.0 ± 0.04	1.7 ± 0.08	1.9 ± 0.04
RNA content (mg/g liver)	8.1 ± 0.2	8.4 ± 0.1	7.4 ± 0.2	6.4 ± 0.1*	7.8 ± 0.1	7.4 ± 0.2	8.2 ± 0.1	7.9 ± 0.2

[a] CHI at a dose of 2 mg/kg body wt was given 24 hr prior to the injection of AFB_1; AFB_1 at doses of 1,2, or 4 mg/kg body wt was given 16 hr prior to the animals being killed.
[b] CHI pretreatment (−).
[c] CHI pretreatment (+).
[d] Group I.
[e] Group II.
[f] Group III.
[g] Group IV.
* Indicates P value <0.05.

the organism are irreversible and cannot be prevented. The histological changes such as occasional periportal necrosis caused by AFB were seen less frequently in CHI pretreated animals. Theoretically speaking an increase of cellular enzyme levels in the blood circulation due to altered cell membrane should correspond to a decrease of intracellular components. For this reason, cellular contents of protein, DNA, and RNA were determined. As shown in Table III, 16 hr after various doses of AFB, there were decreases in liver weight and RNA content. Significant changes were observed in animals receiving a lethal dose of AFB. The liver protein and DNA contents, however, were not affected by the hepatotoxin. In CHI pretreated animals (Group IV) AFB administration did not cause any significant change in either the liver weight or RNA content. Preliminary studies on the incorporation of [^{3}H]leucine and [^{3}H]orotic acid into cellular macromolecules (data not shown) demonstrated that CHI pretreatment did not prevent the inhibition on RNA synthesis caused by the AFB but the inhibition of protein synthesis was prevented by the CHI pretreatment. These results indicate that the CHI protection against liver necrosis caused by the carcinogen is not related to the transcriptional event but may involve the activation of the translational process. The protective effect of CHI against the decrease of liver RNA (Table III) may be due to the fact that CHI may prevent the cytoplasmic polysomal breakdown caused by AFB (12, 14) so that cytoplasmic RNAs were not further degraded by the nucleolytic enzymes.

This study demonstrates that CHI can protect the liver against acute toxicity caused by AFB. No explanation is known for the exact mechanism except that CHI's protection may also involve a specific inhibition of protein synthesis (21). It is known that in intact rats the inhibition of protein synthesis by CHI is transient (17, 19, 22–24) and associated with an increased rate of phospholipid synthesis (24). Since it is also known that protein synthesis is no longer inhibited by CHI 12 hr after dosing (19, 22–24), the proposed inhibition of the synthesis of a lethal protein (21) to explain the protective effect (3) requires further experimentation by demonstrating the existence of such a protein. Nevertheless, the protective effect of CHI against liver damage caused by AFB presented in this report as well as the earlier works of Flaks' and Farber's groups (1–4) offers an animal model to investigate the relationship between acute liver damage and neoplastic transformation.

Summary. The protective effect of CHI on the acute toxicity of AFB at nonlethal and lethal doses on rat liver was monitored by determining serum activities of LDH, GOT, GPT, and ALP and the content of protein, DNA, and RNA. At 2 hr after AFB administration, there were no indications of hepatic damage revealed by biochemical as well as histological studies. At 16 hr after AFB dosing, serum LDH, GOT, and GPT activities increased with increasing doses. Liver weight and RNA content showed significant decreases when a lethal dose of AFB was used. When CHI was given 24 hr prior to the administration of AFB, the elevated levels of serum LDH, GOT, and GPT caused by nonlethal doses of AFB were prevented. The decreases of liver weight and RNA content caused by the lethal dose were also prevented. These results indicate that since CHI protects against acute toxicity caused by nonlethal doses of AFB it may be used to study the relationship between acute liver injury and hepatocarcinogenesis.

The authors would like to express their appreciation to Dr. H. Chiang for the histopathological evaluations of the tissue preparations and Professor W. K. Ting and his staff for the use of the Technicon autoanalyzer. The authors also wish to express their gratitude to Dr. Thomas M. Devlin for his advice during the preparation of this manuscript.

1. Lieberman, M. W. Verbin, R. S., Landay, M., Liang, M., Farber, E., Lee, T. N., and Starr, R. Cancer Res. 30, 942 (1970).
2. Flaks, B., and Nicoll, J. W., Chem. Biol. Interact. 8, 135 (1974).
3. Flaks, B., and Nicoll, J. W., Toxicol. Appl. Pharmacol. 32, 603 (1975).
4. Popp, J. A., Sinozuka, H., and Farber, E., Toxicol. Appl. Pharmacol. 45, 549 (1978).

5. Miller, J. A., and Miller, E. C., Fed. Proc. **35**, 1316 (1976).
6. Clifford, J. I., and Rees, K. R., Nature (London) **209**, 312 (1966).
7. Swenson, D. H., Lin, J. K., Miller, E. C., and Miller, J. A. Cancer Res. **37**, 172 (1977).
8. Croy, R. G., Essigmann, J. M., Reinhold, V. N., and Wogan, G. N. Proc. Nat. Acad. Sci. USA **75**, 1745 (1978).
9. Garner, R. C., Martin, C. N., Lindsay Smith, J. R., Coles, B. F., Tolson, M. R., Chem. Biol. Interact. **26**, 57 (1979).
10. Sporn, M. B., Dingman, C. W., Phelps, H. L., and Wogan, G. N., Science **151**, 1539 (1966).
11. Gelboin, H. V., Wortham, J. S., Wilson, R. G., Friedman, M., and Wogan, G. N., Science **154**, 1205 (1966).
12. Roy, A. K., Biochem. Biophys. Acta. **169**, 206 (1968).
13. Yu, F. L., J. Biol. Chem. **252**, 3245 (1977).
14. Sarasin, A., and Moulé, Y. FEBS Lett. **29**, 329 (1973).
15. Sarasin, A., and Moulé, Y. FEBS Lett. **32**, 347 (1973).
16. Sarasin, A., and Moulé, Y. Eur. J. Biochem. **54**, 329 (1975).
17. Rothblum, L. I., Devlin, T. M., and Ch'ih, J. J., Biochem. J. **156**, 151 (1976).
18. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. J. Biol. Chem. **193**, 265 (1951).
19. Ch'ih, J. J., Procyk, R., and Devlin, T. M., Biochem. J. **162**, 501 (1977).
20. Clifford, J. I., and Rees, K. R., Biochem. J. **102**, 65 (1967).
21. Lieberman, M. W., *in* "The Pathology of Transcription and Translation" (E. Farber, ed.), p. 37. Dekker, New York (1972).
22. Ch'ih, J. J., Faulkner, L. S., and Devlin, T. M., Biochem. Pharmacol. **28**, 691 (1979).
23. Glaumann, H., Biochim. Biophys. Acta **224**, 206 (1972).
24. Hwang, K. M., Yang, L. C., Carrico, C. K., Schulz, R. A., Schenkman, J. B., and Sartarelli, A. C., J. Cell. Biol. **62**, 20 (1974).

Received December 3, 1979. P.S.E.B.M. 1980, Vol. 164.