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THE EFFECT OF ENDOTOXIN ON THE LIVER LIPID PEROXIDE LEVEL AND ON THE LIVER AND PLASMA ZINC CONCENTRATIONS IN RATS AS RELATED TO TIME AFTER ADMINISTRATION

By

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SANKARI, SATU and TIMO PEKKANEN: *The effect of endotoxin on the liver lipid peroxide level and on the liver and plasma zinc concentrations in rats as related to time after administration.* Acta vet. scand. 1982, *23*, 24—29. — The lipid peroxide level of rat liver increased due to i.p. endotoxin injection, reaching its maximum value 24 h after administration. After that the level declined, reaching approximately the initial value at 48 h. The liver Zn concentration increased and the plasma Zn concentration rapidly decreased after the injection. The liver Zn concentration then gradually declined and the plasma Zn respectively increased near the initial level 48 h after the injection.

The highest lipid peroxide level occurred at 24 h when the mean Zn concentration of the plasma and liver were already relatively near the initial level, suggesting a regulative role of Zn in lipid peroxidation.

endotoxin; lipid peroxide level.

The hepatotoxic effect of a number of substances has been shown to result from their ability to initiate the peroxidative degradation of polyunsaturated fatty acids of the lipid bilayer of the endoplasmic reticulum (*Plaa & Witchi* 1976). Lipid peroxide increases in rat liver, e.g. in carbon tetrachloride poisoning, following chloroacetamide administration, and with increasing age of the animal (*Uchiyama & Mihara* 1978, *Ohkawa et al.* 1979, *Anundi et al.* 1980).

Endotoxins of intestinal bacteria are known to play a significant role in the pathogenesis of liver injury (*Nolan* 1975, *Nolan & Leibowitz* 1978), and injections of endotoxin into experimental

animals affect the lipid metabolism (*Cook et al.* 1979) and give rise to changes in various basic biochemical parameters e.g. in liver (*Hejemanova et al.* 1979). The present investigation was undertaken in order to study the effect of intraperitoneally (i.p.) administered endotoxin on lipid peroxide levels in rat liver determined by the thiobarbituric acid (TBA) reaction (*Kohn & Liversedge* 1944), modified by *Uchiyama & Mihara,* in relation to time after the injection. Since serum and liver zinc (Zn) concentrations are influenced in a predictable manner by endotoxin injection (*Pekarek et al.* 1972, *Suzuki & Yamamura* 1980) their Zn determinations were included in the study as a control of endotoxin influence.

MATERIAL AND METHODS

Thirty-six male Sprague Dawley rats weighing about 100 g each were included in the study. The rats were randomly divided into groups of 6; they were then kept in cages of 3 animals each and given commercial rat feed pellets and distilled water ad libitum. At the beginning of the experiments the animals were weighed and given 0.5 mg per 100 g E. coli endoxin (O26:B6, Difco Lab., Mich., USA) in about 0.5 ml pyrogen-free distilled water (Orion Pharmaceutica, Espoo, Finland) i.p. Then 0, 4, 8, 12, 24 and 48 h after the injection all animals of 1 group were sacrificed by decapitation in light ether anaesthesia. The blood was collected in heparinised test tubes and the plasma separated. The livers were rapidly removed and an approximately 1 g piece of the left lobe immediately frozen in liquid nitrogen and stored in carbon dioxide ice until the next day, when the lipid peroxide levels were analysed. The rest of the livers were kept at —20°C until analysed for their Zn concentration.

The lipid peroxide levels of the livers were determined by the TBA reaction (*Kohn & Liversedge*), as modified by *Uchiyama & Mihara.* Absorbances of the butanol layer were measured at 535 and 520 nm. The lipid peroxide level (the TBA value) given per g protein was obtained by calculating the difference between the 2 absorbances and dividing it by the protein concentration of the liver homogenate, determined by the method of *Lowry et al.* (1951). The Zn concentrations of the livers and plasmas were determined by atomic absorption.

Before the analyses the liver samples were dried to a constant weight and ashed twice overnight at 425°C.

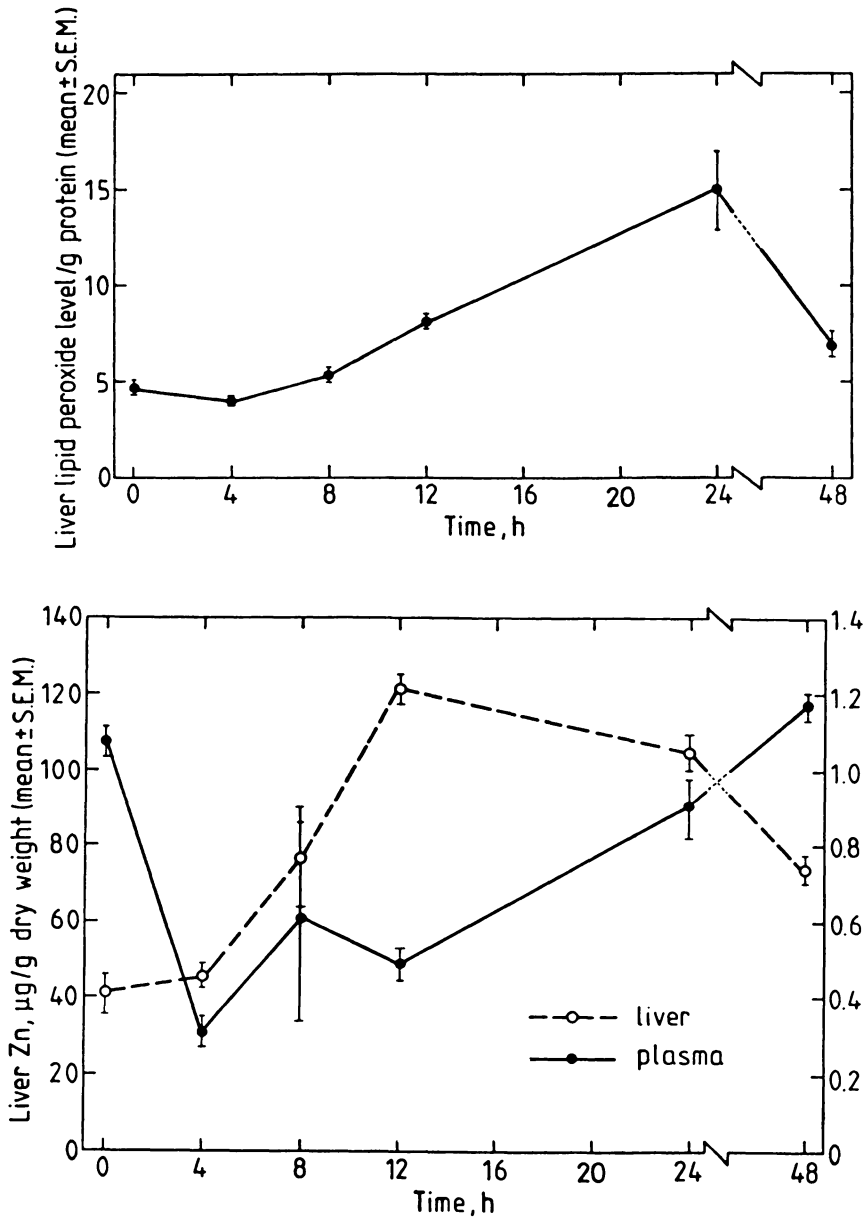

Figure 1. The effect of i.p. administered endotoxin on the lipid peroxide level (top) and on the liver and plasma Zn concentrations (bottom) in rats as related to time after administration.

RESULTS

Four of the remaining rats, 1 in each group of 6, were found dead 8 h after the endotoxin injection. The results of the lipid peroxide level determinations of the livers and the results of the Zn determinations of the livers and plasmas are presented in Fig. 1. The mean lipid peroxide level (TBA value) was found to be somewhat increased at 8 h and was apparently highest 24 h after the injection. After 48 h the level had declined to near the starting level. The mean liver Zn concentration increased upto 12 h after the injection, the began to fall. The mean plasma Zn level decreased steeply during the first 4 h after the injection and was at about the initial level at 48 h.

DISCUSSION

Changes in basic biochemical indices are known to occur in rat liver after the i.p. application of endotoxin (*Hejemanova et al.* 1979). The present work shows that the i.p. administration of endotoxin increases the lipid peroxide level of the liver as measured by the TBA value, being highest at 24 h after the injection (Fig. 1).

Increased lipid peroxidation is associated with liver injury, although it does not necessarily cause permanent damage of the liver cells (*Anundi et al.* 1980). Since in the present work determinations were not taken between 12 and 24 h after the injection, the highest lipid peroxide level may equally well have been found during that interval. At 48 h after the endotoxin administration the lipid peroxide level of the liver was almost the same as at the beginning of the experiment.

The mean plasma Zn level decreased and then increased and the mean liver Zn level increased and then decreased after the endotoxin injection, as was to be expected according to ealier work (*Suzuki & Yamamura* 1980). The increase in the lipid-peroxide of the liver seems to occur, according to Fig. 1, somewhat later than the initial changes in the Zn levels especially with regard to blood plasma. This could indicate some kind of regulative role for Zn in lipid peroxidation due to endotoxin, as has been observed in connection with carbon tetrachloride poison (*Chapril et al.* 1973, *Gagen & Klaassen* 1980).

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SAMMANDRAG

Effekten av endotoxin på leverns lipidperoxidnivå och på koncentrationen av zink i lever och plasma hos råtta som en funktion av tiden efter endotoxinbehandlingen.

Leverns lipidperoxidnivå på råtta steg efter intraperitoneal endotoxin-injektion ocr nådde maximalvärdet 24 timmar efter injektionen. Därefter sjönk värdet och var ungefär på ursprungsnivån efter 48 timmar. Leverns Zn koncentration steg och plasmans Zn koncentration minskade hastigt efter injektionen. Senare sjönk leverns Zn koncentration gradvis och plasmans Zn koncentration steg på motsvarande sätt. Båda koncentrationerna var vid ursprungsnivån ca 48 timmar efter injektkionen. Den högsta lipidperoxidnivån nåddes när den genomsnittliga koncentrationen av Zn i plasma och lever redan hade stigit resp. sjunkit till närheten av initialvärdet före behandlingen. Detta antyder att Zn möjligen har en regulerande roll i lipidperoxidationen.

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