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High molecular weight protein aggregates formed in the liver of the rat following large doses of paracetamol

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Abstract—Paracetamol (200 and 500 mg kg^{-1}) was given intraperitoneally to rats pretreated with 3-methylcholanthrene for 3 days. Glutamic oxalacetic acid transaminase (GOT) activity in plasma increased in rats receiving 500 mg kg^{-1} paracetamol. Plasma GOT activity was low at the dose of 200 mg kg^{-1}, but the same dose to diethyl maleate pretreated rats increased the GOT activity. High mol. wt protein aggregates were found to be formed in liver homogenates and microsomes of rats which showed high plasma GOT activity, accompanied by depletion of hepatic glutathione. The formation of protein aggregates in the liver of rats following large doses of paracetamol suggests a contribution of lipid peroxidation to paracetamol-induced hepatotoxicity.

Paracetamol is a widely used analgesic-antipyretic drug. Large doses of this drug have been known to cause hepatotoxicity in man (Davidson & Eastham 1966; Rose 1969) and laboratory animals (Boyd & Bereczky 1966; Dixon et al 1971). This hepatotoxicity has been reported to be induced by covalent binding of *N*-acetyl-*p*-benzoquinone imine, an oxidation product of paracetamol, to sulphydryl groups of proteins resulting in cell necrosis (Jollow et al 1973; Mitchell et al 1973; Dahlin et al 1984). On the other hand, lipid peroxidation was found to be markedly increased in-vivo in mouse acutely intoxicated with paracetamol as determined by ethane expiration or by thiobarbituric acid reactive substance in liver homogenates (Wendel et al 1979). Paracetamol was also reported to stimulate lipid peroxidation in rat and mouse isolated hepatocytes (Albano et al 1983). Further, we have reported that paracetamol affected the physical structure of the membranes of liver microsomes in-vivo and in-vitro (Araya et al 1987a, b).

We also reported that the occurrence of lipid peroxidation in biological membranes produced high mol. wt protein aggregates (Itoh et al 1990). This protein aggregation is a useful indicator to characterize lipid peroxidation, since the protein aggregates are retained in the membranes. The direct detection of the protein aggregates in paracetamol-induced hepatotoxicity could indicate the occurrence of lipid peroxidation in the liver. In this study, we have investigated the in-vivo effect of large doses of paracetamol on the rat liver proteins by separating the liver proteins with sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE).

Materials and methods

Materials. Paracetamol, tragacanth gum (powder), diethyl maleate (DEM), *o*-phthalaldehyde and glutamic oxalacetic acid transaminase (GOT)-UV Test Wako were purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan). An electrophoresis calibration kit (phosphorylase b, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor and α-lactalbumin) was purchased from Sigma Chemical Co. (St Louis, MO, USA). All other reagents were of analytical grade.

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Treatment of animals. Male Wistar rats, 170–250 g, were pretreated with 3-methylcholanthrene (20 mg kg^{-1}, i.p., in olive oil) for 3 days according to Albano et al (1983). Paracetamol (1·8 g) was mixed in 4 mL 0·9% NaCl (saline) containing tragacanth gum (0·12 g) and then 2 mL saline was added to the suspension. The rats underwent intraperitoneal treatment according to the following six groups: (a) 0·5 mL saline, (b) DEM (0·2 mL kg^{-1}), (c) paracetamol suspension (200 mg kg^{-1}), (d) paracetamol suspension (500 mg kg^{-1}), (e) paracetamol suspension (200 mg kg^{-1}) 30 min after DEM (0·2 mL kg^{-1}) was given to the rats, (f) paracetamol suspension (500 mg kg^{-1}) given 30 min after DEM (0·2 mL kg^{-1}) was given to the rats. The rats were killed 6 h after the start of the treatment. Just before killing, a 4 mL blood sample was withdrawn from the jugular artery and was used for the determination of GOT. The liver homogenates and microsomes of the treated rats were prepared as described elsewhere (Itoh et al 1988).

Determination of GOT and glutathione. Plasma GOT was determined according to Karmen et al (1955). Glutathione in the liver was determined by HPLC according to Keller & Menzel (1985) with a slight modification of the mobile phase (74 mM sodium formate, pH 4·0, instead of 0·1% trifluoroacetic acid).

SDS-PAGE. SDS-PAGE of the liver homogenates and microsomes was carried out as described by Itoh et al (1990). The gel

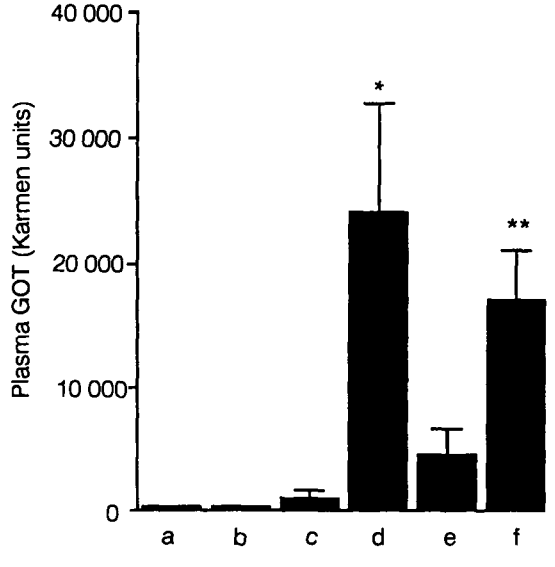

FIG. 1. Activity of GOT in plasma of rats given a large dose of paracetamol. The following intraperitoneal treatments were carried out following pretreatment with 3-methylcholanthrene : a, saline; b, DEM alone; c, 200 mg kg^{-1} paracetamol; d, 500 mg kg^{-1} paracetamol; e, DEM and 200 mg kg^{-1} paracetamol; f, DEM and 500 mg kg^{-1} paracetamol. Columns and vertical bars represent means and s.e. of 3–6 rats. $*P<0.05$; $**P<0.01$ compared with the control.

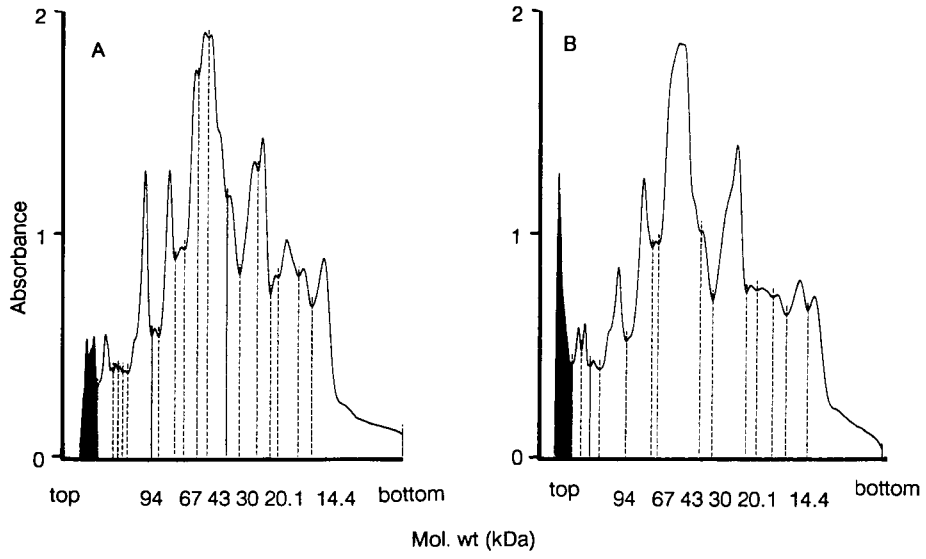

FIG. 2. SDS–PAGE of liver microsomes. A. Liver microsomes of the control rats. B. Liver microsomes of DEM and 500 mg kg^{-1} paracetamol-treated rats. The shaded portion in the scanning profile was designated as high mol. wt proteins in this study. Standard proteins: phosphorylase b (mol. wt 94 kDa); bovine serum albumin (mol. wt 67 kDa); ovalbumin (mol. wt 43 kDa); carbonic anhydrase (mol. wt 30 kDa); soybean trypsin inhibitor (mol. wt 20·1 kDa); α-lactoalbumin (mol. wt 14·4 kDa).

bands stained with 0·1% Coomassie brilliant blue were detected using a Shimazu chromatoscanner CS-9000 (550 nm as a sample and 490 nm as a reference). Protein was estimated by integrating the scanning curve with the built-in program in the chromatoscanner.

Results and discussion

Large doses of paracetamol were given intraperitoneally to the 3-methylcholanthrene-pretreated rats. The in-vivo toxicity induced by paracetamol was evaluated by plasma GOT activity (Fig. 1). Plasma GOT activity of 200 mg kg^{-1} paracetamol-treated rats was low, but it increased in rats pretreated with DEM. At the dose of 500 mg kg^{-1} paracetamol, plasma GOT activity increased, irrespective of DEM pretreatment. Plasma GOT was not affected by DEM itself, but the in-vivo toxicity of rats following large doses of paracetamol was found to be pronounced on DEM pretreatment.

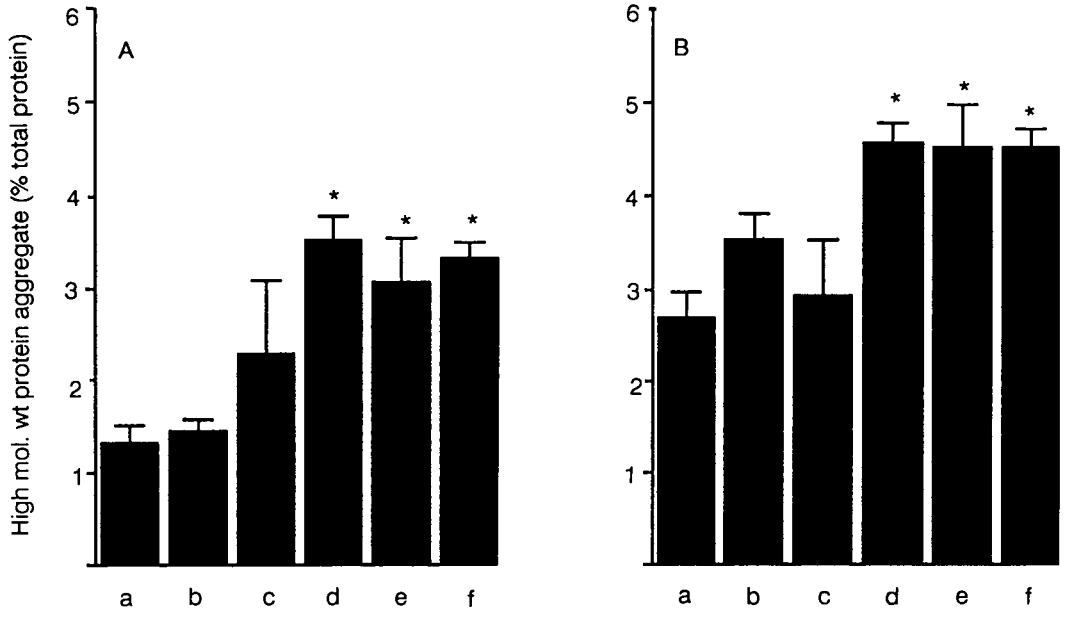

FIG. 3. High mol. wt protein aggregates formed in the liver homogenates and microsomes of the treated rats. Liver homogenates (A) and microsomes (B) of the treated rats were separated by SDS-PAGE. High mol. wt proteins at the top of the gel rods were expressed as percentage of the amount of total proteins. Columns and vertical bars represent means and s.e. of 3–7 rats. $*P < 0.01$ compared with the control.

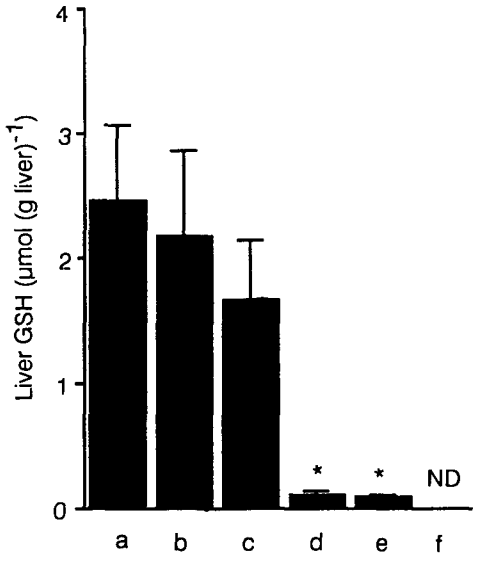

FIG. 4. Glutathione (GSH) in the liver of treated rats. The groups of rats, (a–f) were treated in the same way as described in the legend of Fig. 1. Columns and vertical bars represent means and s.e. of 3 rats. *$P<0.05$ compared with the control. ND: not detected.

The livers were isolated from the treated rats and the liver homogenates and microsomes were separated by SDS-PAGE. The scanning patterns of SDS-PAGE for the liver microsomes of the control rats and the DEM and 500 mg kg^{-1} paracetamol-treated rats are shown as typical examples in Fig. 2. A large absorbance in the paracetamol-treated rats was observed at the top of the gel rods, although in the control rats the absorbance at the top was small. This increase at the top of the gel rods indicates the formation of high mol. wt protein aggregates, accompanied by a decrease in the amount of lower mol. wt proteins separated towards the bottom of the gel rods. This distribution pattern of proteins in the in-vivo paracetamol-induced hepatotoxicity was the same as that seen in the in-vitro lipid peroxidation in rat liver microsomes reported previously (Itoh et al 1990).

To compare the formation of high mol. wt protein aggregates among the six groups of treated rats (a–f), the extent of high mol. wt protein formation was expressed as the amount of protein at the top of the gel rods as a percentage of the amount of total protein. The formation of aggregates in the liver homogenates and microsomes is shown in Fig. 3. The 200 mg kg^{-1} paracetamol-treated rats (group c) showed no significant difference in the formation of high mol. wt proteins, compared with the control rats (group a), for either liver homogenates or microsomes. Groups d (500 mg kg^{-1} paracetamol), e (DEM and 200 mg kg^{-1} paracetamol) and f (DEM and 500 mg kg^{-1} paracetamol) showed a significant increase in the formation of high mol. wt proteins, compared with the control rats (group a), for both liver homogenates and microsomes. DEM alone (group b) did not affect the formation. It should be noted that the groups with a significant increase in the formation of high mol. wt proteins also indicated a significant increase in plasma GOT activity (Fig. 1).

Glutathione is probably related to the paracetamol-induced hepatotoxicity (Mitchell et al 1973). The glutathione in the liver homogenates of the six groups of rats was determined (Fig. 4). Rats treated with 200 mg kg^{-1} paracetamol (group c) indicated a slight decrease of hepatic glutathione, although the decrease was not significant compared with the control rats (group a). On the other hand, the hepatic glutathione levels of groups d (500 mg kg^{-1} paracetamol), e (DEM and 200 mg kg^{-1} paracetamol) and f (DEM and 500 mg kg^{-1} paracetamol) decreased markedly.

In conclusion, a large dose of paracetamol to rats induced the toxicity as shown by increased plasma GOT and high mol. wt protein aggregates formed in the liver, accompanied by the depletion of hepatic glutathione. The significant changes did not occur at a dose of 200 mg kg^{-1} paracetamol, but this dose of paracetamol to DEM-pretreated rats increased the plasma GOT and the formation of the protein aggregates in the liver. The occurrence of lipid peroxidation in the liver of rats following large doses of paracetamol is suggested by the formation of the protein aggregates.

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