EFFECTS OF SOLUBLE FRACTIONS OF LIVERS FROM NEONATAL, MATURE AND HEPATOMA-BEARING RATS ON MICROSOMAL LIPID PEROXIDATION

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1. Introduction

Recent studies have indicated the presence of a lipid peroxidation inhibitor (LPI) in the soluble fraction from rat liver. Increases in the level of LPI resulting from the administration of a fat-free, high carbohydrate diet [1], or dosage with carbon tetrachloride [2] have been reported. A marked increase in activity of the NADPH-dependent lipid peroxidation system of rat liver microsomes has been shown to accompany development from birth to maturity [3] but activity was found to be extremely low in microsomes of hepatoma D30 [4].

This paper reports on investigations of the effects on enzymic microsomal lipid peroxidation of supernatant fractions (105 000 × *g*; 60 min) from livers of rats of various ages, as well as from hepatomas and livers of hepatoma-bearing animals. These experiments indicated that increasing the concentration of the soluble fraction from mature rat liver resulted in increased inhibition of lipid peroxidation, while increasing the concentration from neonatal rat liver stimulated the reaction. The soluble fractions from hepatomas investigated had no effect on liver microsomal lipid peroxidation, but the fraction taken from the livers of animals with subcutaneous hepatomas caused a noticeable inhibitory lag to the reaction.

2. Materials and methods

Neonatal (1–2 days) and mature (6 months) Wistar rats were bred in this department. Wistar rats from the Cancer Research Campaign Laboratories, University of Nottingham, were used for experiments with hepatomas. Primary hepatomas were induced by feeding 4-dimethylaminoazobenzene [5] and hepatomas D30 and D192A were maintained by subcutaneous passage. Chemicals were purchased from Sigma (London), Kingston-upon-Thames, Surrey.

Liver microsomal fractions from mature rats were prepared as in [3] and stored at −20°C until required. Soluble fractions were prepared by homogenization of the liver or hepatoma tissue in 0.15 M Tris–HCl (pH 7.4) (2–3 ml buffer/g tissue). Liver tissue from animals with primary tumours was selected from lobes in which hepatoma growth was not visible. The homogenates were centrifuged at 105 000 × *g* for 60 min. The supernatants were removed and subjected to a similar centrifugation.

Malonaldehyde (MDA) production estimated by reaction with thiobarbituric acid [6] was used as an indication of the extent of lipid peroxidation. Superoxide dismutase (SOD) was measured as in [7]. For SOD determination, the tissue was homogenized in 50 mM phosphate buffer (pH 7.8) and 0.1 mM EDTA. Data shown in the text are the average values for 3 determinations in each case. Protein estimation was by the biuret method [8].

The incubation system used to investigate the effect of the soluble fractions on microsomal lipid peroxidation consisted of 0.15 M Tris–HCl (pH 7.4), 1 mM KH_2PO_4, 4 mM ADP, 0.4 mM NADPH and 0.05 mM $FeCl_3$. Samples of the soluble fractions (1.0 ml) were added to the incubation system (2.0 ml) giving final concentrations of soluble protein and microsomal protein as indicated in the figure legends. Incubation was at 30°C in a shaking water bath. Samples (0.5 ml) were taken periodically for MDA determination.

3. Results and discussion

Figure 1 demonstrates that the soluble fraction from mature rat liver was stimulatory to microsomal NADPH-dependent lipid peroxidation compared to the system incubated in the presence of buffer alone. However, as the protein concentration of this fraction was increased, the reaction became increasingly inhibited. The soluble fraction from neonatal rat liver was also shown to stimulate lipid peroxidation (fig.2). In this case, increasing the concentration of the fraction in the incubation system produced increased stimulation.

SOD has been reported to be inhibitory to lipid peroxidation [9], although other workers could not show this effect [10]. An increase in SOD activity concomitant with a decrease in lipid peroxidation of rat liver homogenates has been shown to occur with increase in age from birth [11]. An increase in SOD activity of liver cytosol from birth (10.8 units/mg protein) to maturity (22.6 units/mg protein) has been observed in this laboratory. Such an increase may partly explain the increased inhibition of the control supernatant with increased protein concentration. The increasing catalytic ability of the neonatal fraction with increased concentration may be explained by a decrease in the iron content of the liver from birth (25.2 mg/100 g) to 8 months (9.9 mg/100 g) [12]. Iron acts as a catalyst in lipid peroxidation [6,13] and this higher concentration may be responsible for the stimulation observed.

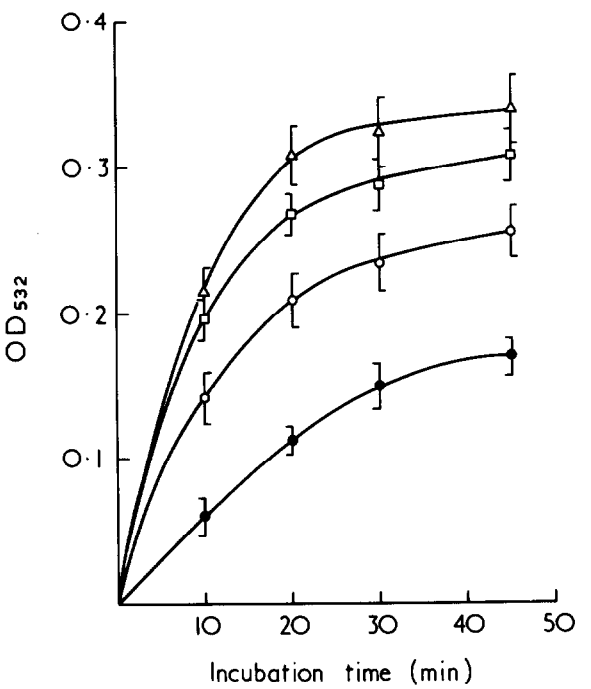

Fig.1. Effect of the soluble fraction from mature rat liver on microsomal lipid peroxidation. The incubation systems contained 0.25 mg microsomal protein/ml and soluble protein concentrations of 0 (•), 2.7 (△), 5.5 (□) and 8.2 (○) mg/ml. The results are the mean ± SD of 3 expt.

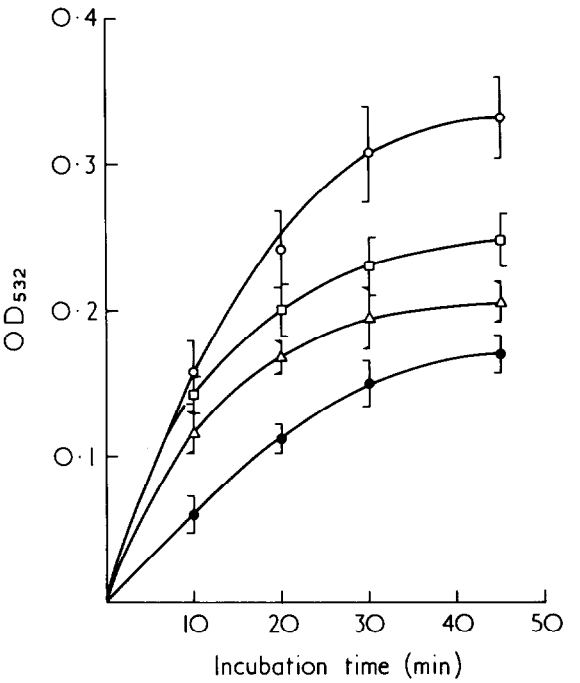

Fig.2. Effect of the soluble fraction from neonatal rat liver on microsomal lipid peroxidation. The incubation systems contained 0.25 mg microsomal protein/ml and soluble protein concentrations of 0 (•), 6 (△), 9 (□) and 18 (○) mg/ml. The results are the mean ± SD of 3 expt.

The soluble fraction from the hepatomas investigated had no effect on microsomal lipid peroxidation (fig.3), and no microsomal lipid peroxidation system was detected [4]. Hepatoma D30 displayed a low SOD activity (5.1 units/mg protein). If the only role of SOD is to protect the cell against enzymic lipid peroxidation where the involvement of O_2^- radicals (and subsequently $OH^{\cdot}$ radicals) has been postulated [14], then a low level of activity in hepatoma tissue

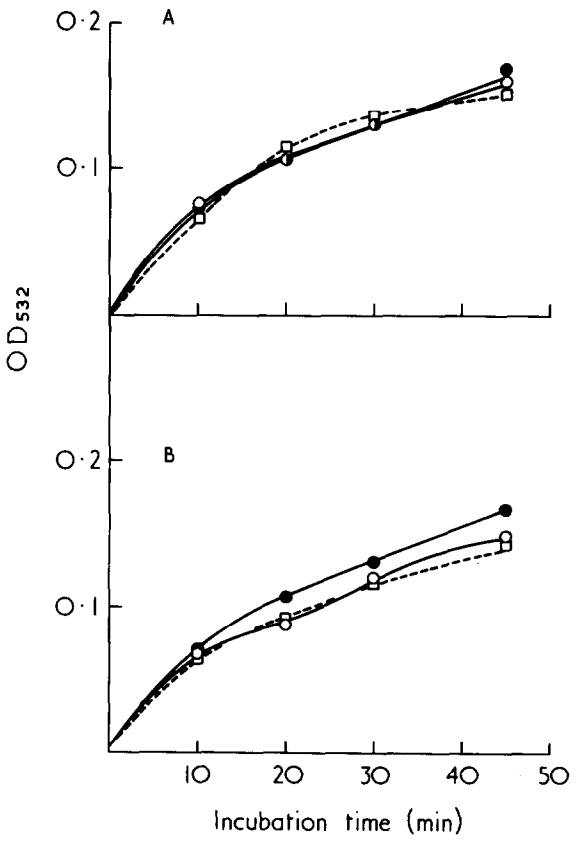

Fig.3. Effect of the soluble fraction from (A) hepatoma D192A and (B) hepatoma D30 on microsomal lipid peroxidation. Each system contained 0.25 mg microsomal protein/ml and soluble protein concentrations of 0 (•), 4.2 (○) and 8.3 (□) mg/ml. The average values for 2 expt in each case are shown.

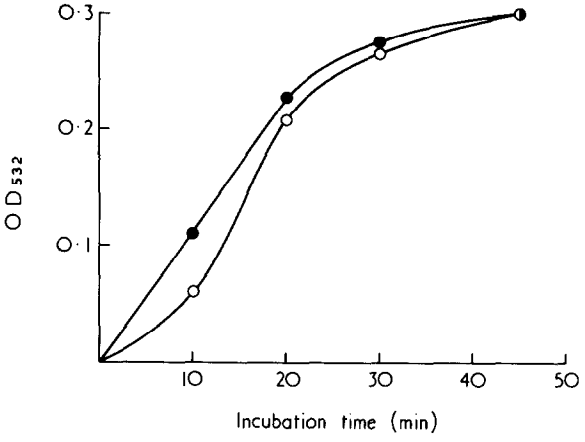

Fig.4. Effect of the soluble fraction of rat liver from animals with primary hepatomas on microsomal lipid peroxidation. The incubation systems contained 0.20 mg microsomal protein/ml and soluble protein concentrations of 2.5 mg/ml. Hepatoma-bearing rats (○), control rats (•). The results show the mean values of 2 expt performed in duplicate.

may be expected. Similarly, increasing SOD levels in liver with increasing age corresponds to an increase in microsomal and mitochondrial NADPH-dependent lipid peroxidation [3].

The soluble fraction from liver with no obvious tumour tissue isolated from animals bearing primary tumours caused a lag in the peroxidation of lipids (fig.4). Heat treatment (100°C/5 min) destroyed this effect. Inhibition in this system was apparent in the early stages of incubation. After 45 min, peroxidation reached similar levels to those observed with control systems. It is noteworthy that this lag effect is different from the inhibition found with increased concentrations of control supernatants which resulted in inhibition over the complete time course of the incubation. Similar observations were made using supernatants from livers of rats 3–4 weeks after subcutaneous implantation with hepatomas D30 and D192A (fig.5). Livers taken from animals 10 days after implantation did not differ from control animals in this respect. The lipid peroxidation activities of microsomal samples taken from the livers of animals bearing hepatomas D30 or D192A did not differ from control animals. Thus, it appears that the inhibitor responsible for the lag period is not incorporated into the microsomal membrane. However, apparently the inhibitor was incorporated into the microsomal membrane when induced by a low fat, high carbohydrate diet [1].

The induction of an inhibitor in the soluble fraction of liver by hepatomas, whether they are primary or passaged tumours, is of considerable interest. The levels of inhibition observed do not appear to be as great as those in experiments with a low fat, high carbohydrate diet and this may account for the fact that at lower levels, incorporation into the liver microsomal fraction with resultant reduced activity could not be detected.

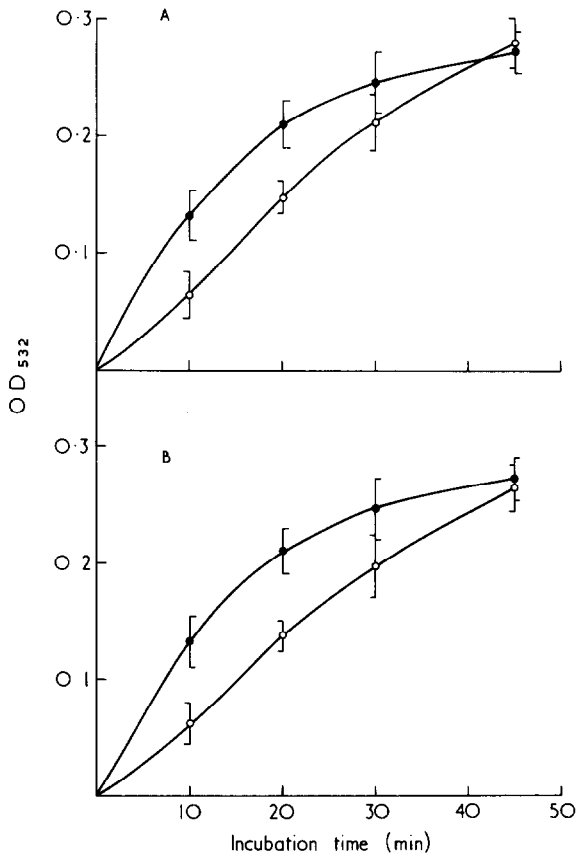

Fig.5. Effect of the soluble fraction of rat livers from animals bearing (A) hepatoma D192A and (B) hepatoma D30 on microsomal lipid peroxidation. The incubation systems contained 0.25 mg microsomal protein/ml and soluble protein concentrations of 7.5 mg/ml. Hepatoma-bearing animals (○), control animals (●). The results are the mean ± SD of 3 expt.

Acknowledgements

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