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THE EFFECT OF DIETHYLDITHIOCARBAMATE ON THE LIPID PEROXIDATION OF RAT-LIVER MICROSOMES AND INTACT HEPATOCYTES

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Abstract—The role of the oxygen radicals in lipid peroxidation, induced by ADP/Fe^{3+} or cumene hydroperoxide was investigated by administering diethyldithiocarbamate, an inhibitor of superoxide dismutase, to hepatocytes or rats.

Intact rat-liver hepatocytes perform a delayed ADP/Fe^{3+}-induced lipid peroxidation after pretreatment with diethyldithiocarbamate. The cumene hydroperoxide-induced lipid peroxidation is unchanged. Hepatocytes, isolated from a rat administered with diethyldithiocarbamate *in vivo*, exhibit the same pattern, a delayed iron-induced lipid peroxidation and an unchanged cumene hydroperoxide-induced lipid peroxidation.

Liver microsomes isolated from liver of a rat administered with diethyldithiocarbamate do not perform lipid peroxidation with NADPH/ADP/Fe^{3+}, but do undergo lipid peroxidation with cumene hydroperoxide.

It can be concluded that besides the inhibition of superoxide dimutase, diethyldithiocarbamate inhibits directly the microsomal lipid peroxidation. Although this inhibition hampers the conclusion, evidence is obtained that superoxide dismutase is probably involved in the protection against lipid peroxidation of the mitochondria, but not of the microsomes.

Recently we showed that the formation of malondialdehyde by intact hepatocytes, induced by ADP/Fe^{3+} or cumene hydroperoxide can be inhibited by the addition of thiourea [1]. This indicates that hydroxyl-like radicals are involved in this process. Lipid peroxidation of intact hepatocytes leads to the formation of both malondialdehyde and chromolipids and this is associated with the genesis of high molecular weight protein. When ADP/Fe^{3+} and cumene hydroperoxide are evenly effective in producing malondialdehyde, the latter agents is manifold effective in producing chromolipids and high molecular weight protein. This prompted us to further experiments to investigate the nature of the molecular difference between ADP/Fe^{3+} and cumene hydroperoxide induced lipid peroxidation and the possible preventive role of superoxide dismutase.

Diethyldithiocarbamate (DDC) is a well-known inhibitor of Cu-superoxide dismutase (Cu-SOD) [2, 3]. The mechanism of the inhibition is the chelation of Cu from the SOD protein, which results in inactivation of the enzyme. Incubation with copper ions restores the activity. The drug has been used to show an eventual role of Cu-SOD in various processes. Frank *et al.* [4] claimed an increased oxygen toxicity on lung for rats treated with DDC. These authors also reported that the cytochrome *c* oxidase activity is diminished by treatment with DDC. Further studies have shown that the administration of DDC to mice potentiates the lethal effect of ozone and paraquat [5] and enhances the effect or radiations and hyperthermia on Chinese hamster cells [6, 7].

If Cu-SOD plays a role in the defence against oxygen radicals by capturing the superoxide anion, it is to be expected that in case of an inhibition Cu-SOD an enhanced lipid peroxidation will occur. However, it is reported that DDC inhibits the CCl_4-induced lipid peroxidation of rat-liver microsomes [8] and the NADPH- and cumene hydroperoxide-induced lipid peroxidation in mouse-liver microsomes [9]. In these studies diethyldithiocarbamate was present during the lipid peroxidation with isolated microsomes and because diethyldithiocarbamate can also bind Fe^{3+} a discrimination between the effect upon Fe^{3+} binding or superoxide dismutase activity is impossible. In the present study it was chosen to administer the drug to intact hepatocytes and *in vivo* adminsitration, after which the hepatocytes or microsomes were isolated. It was expected that this approach could elucidate the eventual involvement of superoxide dismutase in the prevention of lipid peroxidation.

METHODS AND MATERIALS

Hepatocytes were isolated from Wistar rats according to [10]. The purity and intactness of the cell preparations were both tested biochemically [10]. It was checked that the cell viability was not influenced by pretreatment of the rats or hepatocytes with the various reagents. Before incubation, the cells were washed two times with Krebs–Ringer bicarbonate buffer (pH 7.2)/20 mM Hepes/5 mM glucose. Lipid peroxidation was induced either by Fe^{3+} (chelated by ADP) or by cumene hydroperoxide at concentrations indicted in the legends of the figures. At indicated times samples were withdrawn and malondialdehyde and fluorescent chromolipids were measured.

Microsomes were isolated according to [11]. Lipid

peroxidation was either induced by NADPH/Fe^{3+} (chelated by ADP) or by cumene hydroperoxide at concentrations indicted in the legends. The same procedure was followed as for the hepatocytes.

Malondialdehyde was determined by the method described by Ottolenghi [12].

SOD activities were measured as described earlier [14], glutathione peroxidase according to [15], NADPH oxidation according to [16] and glucose-6-phosphatase according to [17]. Thiobarbiturate and diethyldithiocarbamate were obtained from Merck (Darmstadt, F.R.G.). Cumene hydroperoxide was a gift from AKZO (Amersfoort, The Netherlands). All other reagents were of the highest analytical purity available.

RESULTS

Preliminary experiments were performed with hepatocytes, preincubated with DDC (2.5 mM) for 15 min. After this preincubation the lipid peroxidation was induced by the addition of ADP/Fe^{3+} (10 mM, 0.37 mM). No malondialdehyde formation could be detected during 1 hr of incubation. The lack of lipid peroxidation could be due to the iron chelating action of DDC [8]. To eliminate this possibility the cells were first preincubated with 2.5 mM DDC for 15 min at 37° and after this preincubation the cells were centrifuged, washed two times and resuspended in Krebs bicarbonate buffer. The lipid peroxidation was then induced by either Fe^{3+}/ADP or cumene hydroperoxide. Fig. 1 shows the malondialdehyde production vs times of treated and untreated cells. The ADP/Fe^{3+} induced malondialdehyde production of the treated cells shows a marked delay, but finally reaches the same level of malondialdehyde as the untreated cells. The treated cells show a 30% decrease in total SOD activity and the glutathione peroxidase is unchanged. Furthermore, Fig. 1 exhibits that the cumene hydroperoxide induced lipid peroxidation is hardly affected by the pretreatment of DDC. The results shown in Fig. 1 are one experiment of four cell preparations, which show all the same phenomena.

To minimize the eventual residual presence of DDC during the cell incubation the rats received intraperitoneally DDC (1.0 g/kg body weight) after 1 hr the parenchymal cells were isolated. These liver cells contained 2.7 μg SOD activity/mg protein, which could be restored after Cu^{2+} (0.5 mM) for 10 min to 10 μg SOD/mg protein. The final Cu^{2+} concentration of 8 μM does not disturb the SOD activity assay. The glutathione peroxidase was unchanged. These *in vivo* pretreated cells showed a delayed formation of malondialdehyde (Fig. 2). The obtained malondialdehyde production for the ADP/Fe^{3+} induced lipid peroxidation at the indicated times are presented as means ± S.E.M. for control ($n = 7$) and treated hepatocytes ($n = 3$) (Fig. 2A). It shows clearly that the shape of the curve is drastically changed from a hyperbolic curve for control to an S-shaped curve for treated hepatocytes. The differences between the malondialdehyde production is significant at 5, 10 and 20 min ($P < 0.025$, $P < 0.025$ and $P < 0.05$ resp.). At longer incubation times no significant differences are found. For the cumene hydroperoxide induced lipid peroxidation no significant differences are found between the control and treated hepatocytes (Fig. 2B).

From Figs. 1 and 2 it can cautiously be concluded that in the treated cells the ADP/Fe^{3+} induced lipid peroxidation of a cell organelle is blocked and to explain the similar final malondialdehyde level another cell organelle is peroxidized. It is known that the lipid peroxidation of microsomes is not prevented by SOD [11, 13], while the mitochondria are protected by SOD [18]. Furthermore it is well known that liver microsomes are very susceptible for lipid peroxidation. In order to investigate if the microsomal lipid peroxidation is affected by the administration of DDC, the microsomes are isolated from the livers of DDC pretreated rats. The general properties of microsomal glucose-6-phosphatase activity and NADPH oxidation are not different for

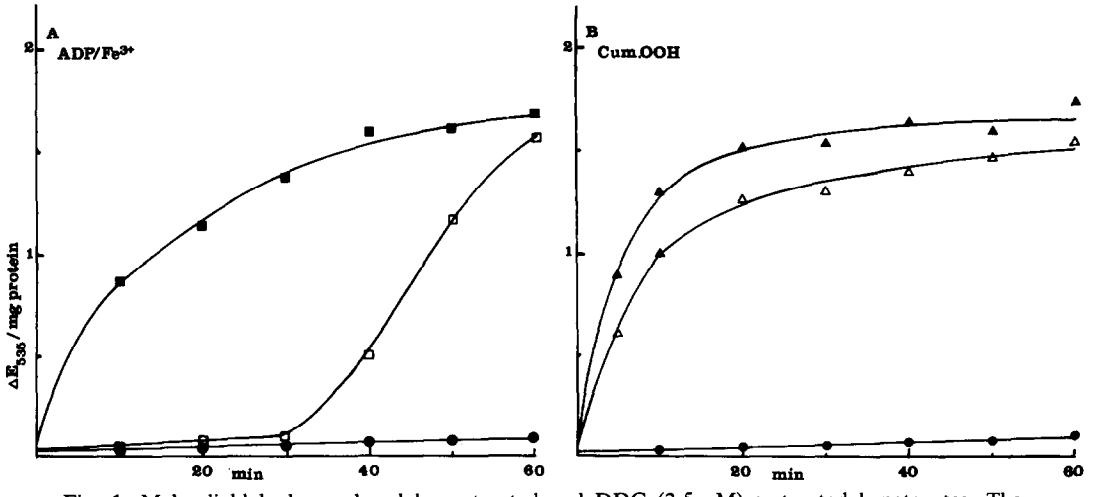

Fig. 1. Malondialdehyde produced by untreated and DDC (2.5 mM)-pretreated hepatocytes. The malondialdehyde is expressed as ΔE_{535}/mg protein. (A) ADP/Fe^{3+} (10 mM, 0.37 mM)-induced process and (B) cumene hydroperoxide (0.3 mM)-induced process. ●—●, control (without Fe^{3+} or cumene hydroperoxide) ■—■, ▲—▲ untreated cells and □—□, △—△ treated cells.

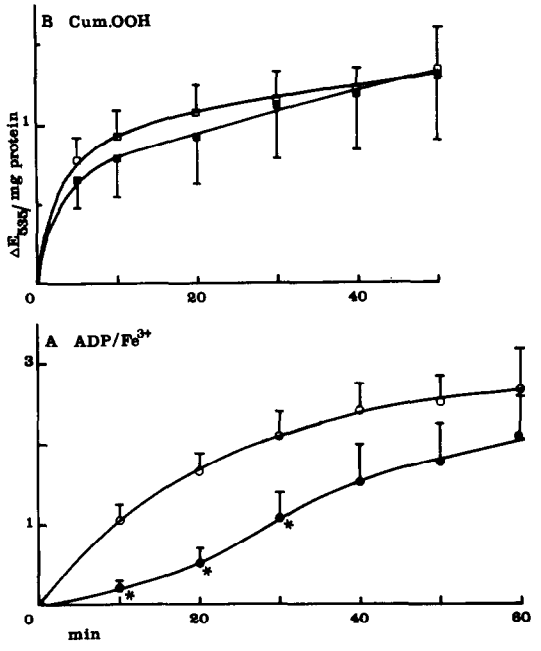

Fig. 2. The malondialdehyde production of hepatocytes isolated from DDC administered rats. (A) ADP/Fe^{3+} (10 mM, 0.37 mM) induced process. (B) Cumene hydroperoxide (0.3 mM) induced process. Open symbols are control hepatocytes ($n = 7$) and closed symbols are for hepatocytes isolated from DDC adminstered rats ($n = 3$). The data are given as means ± S.E.M. Malondialdehyde is expressed as ΔE/mg protein. ★ indicated points are significatly different from control (at 5 and 10 min $P < 0.025$ and at 20 min $P < 0.05$).

DDC treated and untreated rats. However, these microsomes do not produce any malondialdehyde in the NADPH/ADP/Fe^{3+} induced process (Fig. 3), while under the same conditions malondialdehyde is produced by the cumene hydroperoxide induced lipid peroxidation.

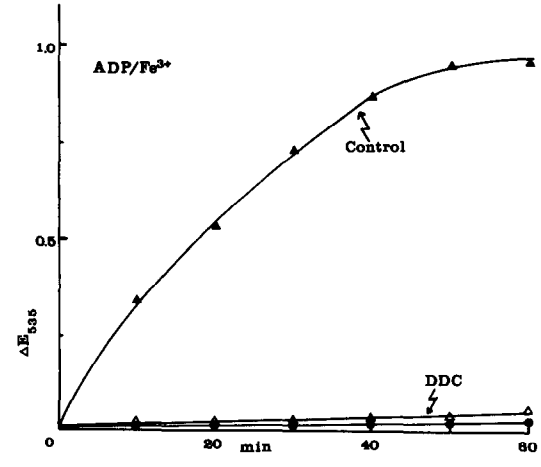

Fig. 3. Malondialdehyde production of control microsomes and microsomes isolated from a DDC-administered rat, provoked by Fe^{3+}/ADP (0.12 mM/2 mM) and NADPH (0.4 mM). Microsomes 1 mg/ml. ▲—▲, control microsomes; △—△, treated microsomes and ●—●, control microsomes (minus NADPH).

DISCUSSION

It is generally agreed that in the process of lipid peroxidation oxygen radicals are involved. Although there is still a dispute which oxygen radical is the actual aggressor, there is evidence that superoxide (O_2^-) is probably too inert [20], but in the presence of Fe^{3+} can be lead to the formation of other oxygen radicals. It can be rationalized that an inhibition of superoxide dismutase, the main defence enzyme against O_2^-, will enhance lipid peroxidation. Actually quite the opposite is observed and it is shown here that although DDC administration reduces markedly the SOD activity in the isolated hepatocytes no enhancement of lipid peroxidation is observed. The cumene hydroperoxide provoked malondialdehyde formation is hardly affected by this pretreatment while the ADP/Fe^{3+} induced process showed a delayed occurrence of the malondialdehyde production.

The ADP/Fe^{3+} induced lipid peroxidation of microsomes isolated from a DDC-treated rat is totally blocked. A general effect of DDC on the structure of the endoplasmic reticulum is unlikely as no change in glucose-6-P-ase activity of NADPH oxidation of the isolated microsomes is observed. The possibility that DDC is still present in the microsomes and chelates the iron cannot be ruled out. However, as an excess of iron is added, the eventual chelation of iron by DDC cannot be the reason for an impaired ADP/Fe^{3+} induced lipid peroxidation.

The eventual role of cytochrome P_{450} in the process of lipid peroxidation is challenged [20] and in the reconstituted system of NADPH-cyt.*c* reductase and microsomal lipids in the absence of cytochrome P_{450} lipid peroxidation is happening [21]. Probably cytochrome P_{450} functions as iron donor because inhibition of lipid peroxidation by inhibiting of cytochrome P_{450} can be overcome by the addition of iron [21]. According to Cadenas and Sies [22] we have to conclude from the unchanged cumene hydroperoxide induced lipid peroxidation that cytochrome P_{450} is not affected by DDC.

The inhibition of ADP/Fe^{3+} provoked lipid peroxidation of microsomes isolated from a DDC-treated rat and the unchanged cumene hydroperoxide lipid peroxidation of the microsomes might indicate that DDC blocks this process by preventing the formation of lipid peroxides. From which cell organelles the produced malondialdehyde originates is unknown, but besides the microsomes also the mitochondria can be peroxidized [18, 23]. The contribution of either to the total amount of malondialdehyde formed is unknown. It is possible that the malondialdehyde measured in hepatocytes from treated rats is from the mitochondrion and the reaching of the same final level as in hepatocytes from untreated rats can be an indication that SOD protects the mitochondrion against O_2^-. Earlier we have found that the addition of SOD to native microsomes does not hamper the ADP/Fe^{3+} induced lipid peroxidation [13] and for mitochondria it is reported that the lipid peroxidation is inhibited by the addition of SOD [18].

Further experiments under controlled conditions

of the hepatocytes seem promising in elucidating the complex interplay of the various oxygen radicals, the lipids of cell membranes and the protecting enzymes.

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