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COMMENTARY

THE ROLE OF LIPID PEROXIDATION IN THE TOXICITY OF FOREIGN COMPOUNDS TO LIVER CELLS

MARTYN T. SMITH,* HJÖRDIS THOR and STEN ORRENIUS
Department of Forensic Medicine, Karolinska Institutet, Box 60400, S-104 01 Stockholm, Sweden

The peroxidation of polyunsaturated fatty acids present in the membrane lipids has been proposed as the mechanism by which a number of foreign compounds produce structural tissue injury [1–3]. For certain chemicals, such as bromotrichloroethane and carbon tetrachloride, it is clear that lipid peroxidation may have a critical role in bringing about the tissue damage [3–5]. However, in respect to others, such as paracetamol, bromobenzene, adriamycin and paraquat, the importance of peroxidative damage is a matter of continued debate and controversy [6–12]. In a critical review of the importance of lipid peroxidation in the hepatotoxic effects of a number of toxins, Mitchell *et al.* [13] concluded that the lipid peroxidation which occurred during paracetamol and bromobenzene hepatocellular necrosis was due to the depletion of glutathione (GSH) from dying cells and had little to do with the initiation of liver damage. In short, the measured lipid peroxidation was a consequence rather than a cause of cell death. Recently, however, a report [14] has appeared in which the authors conclude that lipid peroxidation is an early important event in the toxicity of bromobenzene to monolayer cultures of rat hepatocytes. The major piece of evidence which supports this conclusion is the finding that the antioxidant *N,N'*-diphenyl-*p*-phenylenediamine (DPPD) delays, but does not prevent, the toxicity of bromobenzene to the cultured hepatocytes [14]. Once again, therefore, the importance of peroxidation vs arylation in bromobenzene hepatotoxicity has become a matter of debate.

During the past few years we, along with other colleagues in our laboratory, have performed detailed studies on the mechanism of bromobenzene-induced cytotoxicity, using suspensions of freshly isolated rat hepatocytes as the experimental model [15–21]. These studies show that freshly isolated hepatocytes metabolize bromobenzene to reactive intermediate(s) which are inactivated by conjugation with GSH. Increased cell damage is observed with GSH-depleted cells which, in turn, are protected by facilitated GSH resynthesis [15–20]. More recently, we [21] have tried to further evaluate the importance of lipid peroxidation in bromobenzene-induced cytotoxicity. By utilizing two very sensitive assays of lipid peroxidation, namely ethane production and chemiluminescence measurement, we were able to investigate the relationship between the time of onset of lipid peroxidation and cell viability in hepatocytes exposed to either carbon tetrachloride or bromobenzene. The results of this study showed that lipid peroxidation occurred at an early stage and prior to cell death in carbon tetrachloride toxicity, but at a much later stage and only after cell death in bromobenzene cytotoxicity. We therefore concluded, in agreement with Mitchell *et al.* [13], that the lipid peroxidation which occurred during bromobenzene toxicity was merely a consequence of GSH depletion [22] and cell death.

Further support for the latter conclusion comes from the fact that there is no probable mechanism by which bromobenzene could initiate lipid peroxidation in liver cells. Casini *et al.* [14] propose that bromobenzene could create an oxidative stress by uncoupling microsomal electron transport and gen-

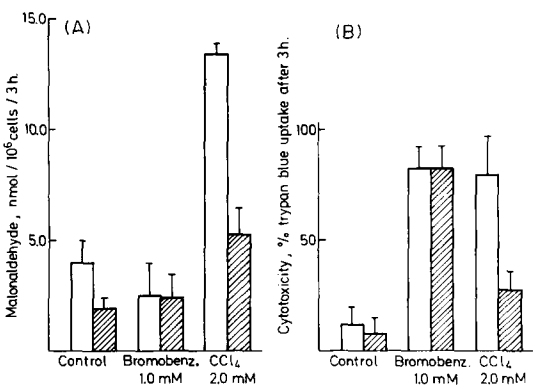

Fig. 1. Effect of α-tocopherol on bromobenzene- and carbon tetrachloride induced malonaldehyde production and cytotoxicity in isolated hepatocytes. Freshly isolated hepatocytes were prepared from phenobarbital- and diethylmaleate-pretreated rats as described previously [15–18] and incubated in Krebs–Henseleit buffer, pH 7.4, at 37° in the presence and absence of bromobenzene (1 mM) and carbon tetrachloride (2 mM) (*open bars*); α-tocopherol (0.5 mM) was included in some of the incubations (*striped bars*). After 3 hr, total malonaldehyde production (A) was measured as described previously [21] and the level of cytotoxicity (B) assessed using trypan blue uptake as being indicative of cell death [21, 25]. The means and ranges of at least three experiments are shown.

*Present address: Department of Biomedical and Environmental Health Sciences, School of Public Health, University of California, Berkeley, CA 94720, U.S.A.

erating active oxygen species, such as O_2^- and H_2O_2, thus initiating lipid peroxidation following the overwhelming of the cellular defence mechanisms. If this were so, bromobenzene should cause: (a) an increased rate of formation of catalase compound I in GSH-depleted hepatocytes (a measure of H_2O_2 production) [23], and (b) an increased rate of efflux of oxidized GSH (GSSG) from the cells. Our studies with freshly isolated rat hepatocytes show that bromobenzene does, in fact, cause neither of these effects [24]. The rate of GSSG efflux is actually decreased to less than control values when bromobenzene is present in the incubation [24]. Furthermore, as shown in Fig. 1, α-tocopherol has no protective effect against bromobenzene toxicity to freshly isolated hepatocytes, whereas the toxicity of carbon tetrachloride is markedly reduced by the presence of this antioxidant. Bromobenzene does, in fact, have mild antioxidant properties itself (Fig. 1). In light of this evidence we consider the fact that the antioxidant DPPD delays the toxicity of bromobenzene to hepatocytes in a monolayer culture to be a weak argument for a critical role for lipid peroxidation in bromobenzene hepatotoxicity, especially since DPPD may have other effects, such as delaying the loss of GSH or altering the pattern of bromobenzene metabolites. These possible effects require further investigation.

Our data therefore suggest that the arylation of critical cellular low- and macromolecular nucleophiles is far more important than lipid peroxidation in bromobenzene hepatotoxicity. This is not to say that we support the hypothesis that the total amount of covalent binding should correlate closely with the amount of bromobenzene-induced cell death. We presently favour the notion that critical target sites are damaged by arylation due to the reactive metabolite(s) of bromobenzene and that this, in turn, brings about a disturbance of cellular homeostatic mechanisms, most notably those controlling intracellular thiol and calcium ion homeostasis [25, 26]. Furthermore, we have little doubt that lipid peroxidation plays an important role in the toxicity of a wide variety of other compounds, including many other halogenated hydrocarbons, but once again is it the gross peroxidation of membrane lipids which is important or the peroxidation of certain lipids associated with critical membrane proteins?

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