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COMMENTARY

INTERRELATION BETWEEN LIPID PEROXIDATION AND OTHER HEPATOTOXIC EVENTS

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Lipid peroxidation reflects the interaction between molecular oxygen and polyunsaturated fatty acids and results in the oxidative deterioration of the latter [1]. The natural targets for such reactions are the biological membranes; therefore one must assume that oxidative damage to them should result in an impairment of cellular and subcellular functions. In fact, lipid peroxidation has been associated with several physiological, pathophysiological and toxic processes including prostaglandin biosynthesis [2, 3] ageing [4, 5] and, mainly, toxic reactions to various organs (for reviews see Refs. 6–8).

Dissociation of lipid peroxidation from cellular toxicity

Recent publications have indicated that lipid peroxidation might take place without inducing cellular damage. This proved true in the case of ADP/Fe^{3+} [9] and several heavy metals [10, 11]. On the other hand, Stacey and Priestly [9] have shown that cellular damage to isolated hepatocytes in the presence of CCl_4 became evident *before* lipid peroxidation was measurable. Similarly, controversies exist with respect to the role played by lipid peroxidation in cellular toxicity induced by paracetamol, bromobenzene, adriamycin and paraquat [7, 12–16]. It was suggested that lipid peroxidation may also be a consequence of cellular damage [8].

The discrepancies described above might be due to the presence of several defense mechanisms in the cell which are capable of reversing primary lesions induced by lipid peroxidation. The initiating free radicals, such as $\cdot O^{-2}$ and H_2O_2, will be scavenged by superoxide dismutase or catalase and glutathione peroxidase respectively [17–19]. These enzyme activities will thereby inhibit the production of secondary, more damaging free radicals, such as $\cdot OH$ [20]. Once lipid peroxidation has started, endogenous antioxidants will interfere with the chain propagation, as they are more easily oxidized than polyunsaturated fatty acids [21]. Of these, the tocopherols seem to play a major role [22], possibly in connection with ascorbic acid [23]. Finally, lipid hydroperoxides already formed will be reduced by the glutathione peroxidases [19, 24], resulting in a reversal of lipid peroxidation.

Thus, impairment of these defense systems should result in an augmentation of lipid peroxidation. Vitamin E deficiency was shown to result in an enhancement of lipid peroxidation both in the presence and in the absence of hepatotoxic chemicals [25–28]. In some cases, selenium deficiency was shown to augment lipid peroxidation [25], although selenium deficiency does not necessarily mean the lack of glutathione peroxidase activity [27].

Glutathione deficiency will impair several lines of defense against oxidative damage. Apart from its direct free-radical scavenging properties and its ability to conjugate with several electrophilic intermediates that are capable of initiating lipid peroxidation [29], GSH is the physiological co-substrate of the GSH peroxidases [19]. Furthermore, GSH-dependent cytosolic [30] and microsomal factors [31] were reported which protect against lipid peroxidation. Thus, lipid peroxidation following acetaminophen treatment could only be seen when hepatic glutathione was depleted in mice [32, 33]. We have shown that depletion of hepatic glutathione below a minimum threshold concentration results in a strong enhancement of endogenous NADPH-dependent lipid peroxidation both *in vitro* [34–37] and *in vivo* [38]. The initiating species is thought to be oxygen bound to a physiological, iron-containing complex [38]. These findings have been confirmed by others both in liver homogenates [39] and in isolated hepatocytes [40].

Thus, impairment of the cellular antioxidative systems will render cells more susceptible to peroxidative damage. The presence of these systems may account for the discrepancies seen with agents that initiate lipid peroxidation but are unable to induce measurable cytotoxicity, as primary lesions are more or less effectively repaired.

Lipid peroxidation and lysosomal enzyme release

Early reports on the interrelation between lipid peroxidation and the release of lysosomal enzymes came from the field of radiation biology. γ-Irradiation of various tissues was found to result in an enhanced release of hydrolytic enzymes from lysosomes [41–44], which correlated well with the malondialdehyde content [43]. The same is true, when isolated lysosomes are subjected to irradiation [45]. But also, chemically-induced lipid peroxidation was shown to result in an accelerated liberation of lysosomal enzymes: NADPH-dependent microsomal lipid peroxidation in the presence of ADP/Fe^{3+} was shown to produce a factor, probably $\cdot OH$, which

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promotes the lysis of lysosomes [46, 47]. *In vitro* experiments performed in our laboratory indicate that agents which promote lipid peroxidation will give rise to a release of lysosomal β-glucoronidase *in vitro* [48]. Agents which inhibited lipid peroxidation prevented lysosomal enzyme release in parallel, suggesting that both events are closely related. *In vivo*, however, lysosomal enzyme release was only seen with those agents which evoked cellular damage also (own unpublished observations). Thus, damage to lysosomal membranes with the consequent liberation of hydrolytic enzymes seems to be a consecutive step to lipid peroxidation, leading finally to cellular damage.

Lipid peroxidation and microsomal enzyme activities

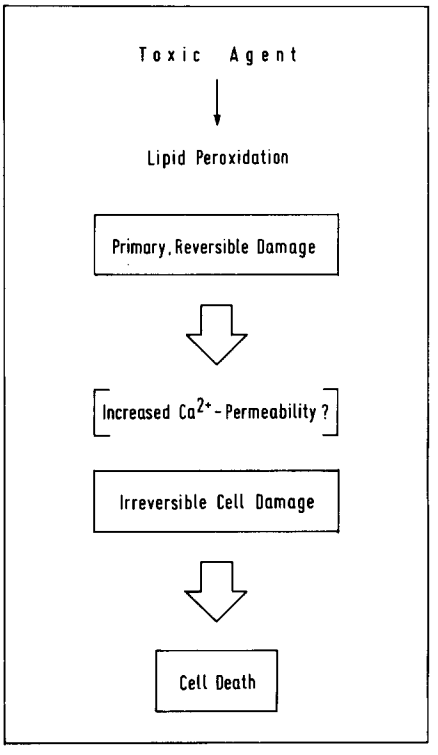
Early reports have indicated that microsomal lipid peroxidation results in the loss of some enzyme activities including glucose-6-phosphatase [49, 50], NADPH-cytochrome *c* reductase [49], cytochrome P-450 [51] and UDP-glucuronyltransferase [51]. Loss of enzyme activities was also reported when lipid peroxidation was induced in isolated hepatocytes [52]. Apart from the decrease in cytochrome content, aminopyrine demethylase, glucose-6-phosphatase and ornithine decarboxylase were decreased following lipid peroxidation. Thus, lipid peroxidation seems to affect a variety of enzyme activities.

The release of aldehydes

Benedetti *et al.* isolated some low molecular weight, diffusible products of peroxidation of rat liver microsomal lipids [53], which evoked lysis of erythrocytes and a decrease in microsomal enzyme activities. They were shown to bind covalently to non-lipid constituents of microsomes [54]. These products were isolated and found to contain carbonyl groups [55]. Upon further purification, these factors were composed in particular of 4-hydroxyalkenals, the main damaging species being 4-hydroxynonenal [56]. 4-Hydroxynonenal proved capable of inhibiting microsomal enzyme activities [57] and protein synthesis [58], and it increased the osmotic fragility of lysosomes [59]. Upon treatment of rats with CCl_4 or $BrCCl_3$, Benedetti *et al.* found that aldehydes were bound to microsomal protein, and they presented evidence that the same protein-bound product is obtained upon reaction of 4-hydroxynonenal with microsomes [60]. Thus, aldehydic products seem to be produced following lipid peroxidation *in vivo*, also. The release of these aldehydes is also reported as a consecutive step after lipid peroxidation has occurred [59].

Ca^{2+} *influx*

Recently, Ca^{2+} influx through membranes has been suggested as a final common pathway leading to cellular death [61, 62]. It was argued that the accumulation of Ca^{2+} is the feature which converts initially non-lethal into irreversible cell injury by hepatotoxic agents [62]. In this respect, a vital role has been assigned to the microsomal ATP-dependent Ca^{2+}-sequestration activity [63]. Several hepatotoxic substances, including carbon tetrachloride [64], bromotrichloromethane [65] and 1,1-dichloroethylene [66, 67], were shown to inhibit microsomal calcium pump activity both *in vivo* [64, 66] and *in vitro* [65, 67]. In a recent publication we presented evidence that lipid peroxidation resulted in an inhibition of microsomal Ca^{2+}-sequestration activities only when hepatocellular damage occurred [68]. Thioacetamide, which evoked cellular damage without inducing lipid peroxidation, suppressed the microsomal calcium pump. Thus, it seems that lipid peroxidation reflects primary reversible damage which will initiate a series of subsequent, more or less reversible events like those listed above. A crucial step must be undergone to turn primary, reversible cellular injury into an irreversible process. This seems necessary to sustain cellular damage and for cellular death to occur. Destruction of the microsomal Ca^{2+} pump leading to intracellular calcium accumulation [62] seems to be a suitable candidate (Scheme 1).



Scheme 1. Proposed sequence of events leading to cellular death after lipid peroxidation.

Conclusions

In all, three general cases may be distinguished: (1) lipid peroxidation that will lead to tissue injury by subsequent events, e.g. lysosomal enzyme release, and intracellular Ca^{2+} accumulation; (2) lipid peroxidation that is reversed by the action of several protective cellular systems; and (3) lipid peroxidation as a result of tissue injury.

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