DLPC ATTENUATES ALCOHOL-INDUCED CYTOTOXICITY IN HEPG2 CELLS EXPRESSING CYP2E1

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Abstract — Aims: Alcoholic liver injury was shown to result largely from oxidative stress generated by ethanol metabolism via cytochrome P4502E1 (CYP2E1). Our aim was to determine whether this could be overcome by using dilinoleoylphosphatidylcholine (DLPC), an innocuous antioxidant extracted from soybeans. Methods: To address this question, we determined whether DLPC protects against alcohol-induced cytotoxicity in HepG2 cells expressing CYP2E1. A HepG2 subclone (2E1) expressing CYP2E1 and a control subclone (Neo) were exposed for 2 h to DLPC (10 µM), and then 100 mM ethanol was added for 5 days. Results: Ethanol significantly decreased cell viability in the 2E1 cells and increased apoptosis. These alterations were attenuated by DLPC with the most significant effects in the 2E1 cells. This was accompanied by a reduction of the ethanol-induced oxidative stress, including diminished hydrogen peroxide production in the 2E1, but not in the Neo cells. The mitochondrial membrane potential was significantly diminished by ethanol in both cells. It was also improved after adding DLPC, but only in the 2E1 cells. In these cells, mitochondrial glutathione (GSH) was also partially restored by DLPC, which significantly inhibited the CYP2E1 induction by ethanol. Conclusion: DLPC opposes the cytotoxicity induced by alcohol in HepG2 cells expressing CYP2E1, a protective action due, at least in part, to an attenuation of the alcohol-induced oxidative stress and the alteration in the mitochondrial membrane potential. On account of these beneficial effects of DLPC and its innocuity, it is now germane to assess its therapeutic action in alcoholics.

INTRODUCTION

Alcoholic liver injury results largely from oxidative stress generated by ethanol metabolism via cytochrome P4502E1 (CYP2E1) (Lieber, 1997, 1999). This oxidative stress is caused by the generation of reactive oxygen species during alcohol metabolism. The consequences of the oxidative stress, such as lipid peroxidation (Lieber, 1997, 1999) and decreased glutathione (GSH), particularly mitochondrial GSH (Hirano, 1992), play an important role in alcohol-induced liver damage. CYP2E1 inhibitors were found to oppose this effect of ethanol but the compounds tested so far have insufficient inhibitory activity or intrinsic toxicity, which precludes their chronic use in humans (Lieber, 1997, 1999). In contrast, dilinoleoylphosphatidylcholine (DLPC), the main and active component of polyenylphosphatidylcholine extracted from soybeans, attenuated the apoptosis of hepatocytes induced by ethanol feeding in rats (Mak et al., 2003). It also exhibited a remarkable specificity in its antioxidant and cytoprotective properties (Aleynik et al., 1999). In the present study, our aim was to determine to what extent DLPC has a protective effect on the alcohol-induced CYP2E1-mediated cytotoxicity by assessing its action in the HepG2 cells expressing CYP2E1.

MATERIALS AND METHODS

Chemicals

Ethanol (95%) was purchased from Pharmco Products Inc. (Brookfield, CT) and 2′,7′-dichlorofluorescein diacetate (DCF-DA) from Molecular Probes, Inc. (Eugene, OR). DLPC was obtained from Avanti Polar Lipid Inc. (Alabaster, AL).

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In preliminary experiments, the Neo and 2E1 cells were cultured for 5 days in a medium containing 10 µM DLPC or an equal volume of vehicle (control). At the end of the treatment, cell viability was determined by the MTT assay. As expected, there was no significant difference among the various groups, and DLPC was not toxic in either the Neo or 2E1 cells. However, ethanol significantly decreased cell viability in both cells, but more in the 2E1 ones, and DLPC attenuated the effect of ethanol (Fig. 1).

The decreased viability was paralleled by increased apoptosis, again with a greater effect in 2E1 cells, and DLPC attenuated this toxic effect of ethanol (Fig. 2). Since one of the mechanisms of apoptosis is an alteration of the mitochondria, with a specific involvement of mitochondrial membranes, this

Inhibitory effect of DLPC on ethanol-induced apoptosis is shown in Fig. 2. Ethanol significantly increased apoptosis in both Neo (***P < 0.01) and 2E1 cells (**P < 0.001), but more in the 2E1 cells (P < 0.05); DLPC attenuated this ethanol-induced effect in the 2E1 cells (*P < 0.05).
parameter was measured in the two cell lines and documented by the retention of the DiOC6 dye. Ethanol decreased the membrane potential, particularly in the 2E1 cells, and DLPC opposed this effect (Fig. 3).

One of the mechanisms of ethanol toxicity is also its increase in the production of hydrogen peroxide. This action was greatest in the 2E1 cells and DLPC reduced this ethanol effect (Fig. 4). When GSH was measured in the homogenate of the two cell lines, no difference was found, but in the mitochondrial fraction, GSH was significantly reduced by alcohol in both cell types, with a more prominent effect in those transfected with CYP2E1. DLPC opposed this alcohol effect but only in the 2E1 cells (Fig. 5).

Finally, as expected, ethanol increased CYP2E1 protein (determined by western blot), whereas DLPC abolished this induction (Fig. 6).

**DISCUSSION**

This study revealed that DLPC is protective against the toxicity of the oxidative stress generated by ethanol in HepG2 cells transfected with CYP2E1. The mechanism of the DLPC effect involves specifically the CYP2E1 induction by ethanol, which is suggested by the fact that DLPC was mostly active in the 2E1 cells. The beneficial effects of DLPC reported in the

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**Fig. 3.** Effect of DLPC on mitochondrial membrane potential. This parameter was significantly affected by ethanol in Neo and, particularly, in the 2E1 cells (**p < 0.001**), with a significant difference between the two cell types (**p < 0.05**); DLPC corrected the ethanol-induced effect in the 2E1 cells (**p < 0.01**).

**Fig. 4.** Effect of DLPC on hydrogen peroxide (measured by DCF-DA). Compared with controls, ethanol favored the production of hydrogen peroxide in both Neo (**p < 0.01**) and 2E1 cells (**p < 0.001**), with a significantly greater effect in the 2E1 cells (**p < 0.05**); DLPC corrected, in part, the production due to ethanol in the 2E1 cells (**p < 0.01**).

**Fig. 5.** Effect of DLPC on GSH (glutathione). No significant effect was observed in the hGSH (homogenate GSH), whereas in the mGSH (mitochondrial GSH) fraction, ethanol significantly reduced GSH in both cells (**p < 0.001**); this decrease was greater in the 2E1 cells (**p < 0.01**), and DLPC corrected the ethanol-induced reduction in these cells (**p < 0.01**).

**Fig. 6.** Effect of DLPC on CYP2E1 in 2E1 Cells. Ethanol significantly increased CYP2E1, as shown by western blot (**p < 0.05**), and DLPC prevented this induction (**p < 0.05**).
present study extend other favorable effects of DLPC against the toxicity of ethanol, revealed in several prior studies both in vivo (Navder and Lieber, 2002) and also in vitro in other hepatic cells or cell lines (Cao et al., 2002a,b,c,d), as well as in human lipoproteins (Navder et al., 2000). These favorable effects of DLPC were secondary, at least in part, to its antioxidant properties and its attenuation of the ethanol-induced oxidative stress resulting from CYP2E1 induction, a major mechanism of liver injury (Lieber, 2004). However, the mechanism of prevention by DLPC of the ethanol-associated CYP2E1 induction has not yet been elucidated. In any event, DLPC is also a physiological phospholipid and it is a major constituent of membranes and other tissue components (Lieber et al., 1994). Its depletion was observed in the livers of baboons fed with alcohol chronically and its repletion was beneficial (Lieber et al., 1994).

The combination of innocuity and high efficacy against several toxic manifestations of ethanol makes DLPC an attractive candidate for therapeutic trials in humans. Furthermore, the preparation used in the present study is now available for human consumption. For these reasons, it would be of interest to assess the beneficial effects of DLPC in patients with alcoholic liver disease.

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