Effect of deoxycholic acid and ursodeoxycholic acid on lipid peroxidation in cultured macrophages

P Ljubuncic, B Fuhrman, J Oiknine, M Aviram, A Bomzon

Abstract

Background—Kupffer cells are essential for normal hepatic homeostasis and when stimulated, they secrete reactive oxygen species, nitric oxide, eicosanoids, and cytokines. Some of these products are cytotoxic and attack nucleic acids, thiol proteins, or membrane lipids causing lipid peroxidation. Hydrophobic bile acids, such as deoxycholic acid (DCA), can damage hepatocytes by solubilising membranes and impairing mitochondrial function, as well as increasing the generation of reactive oxygen species.

Objectives—The hypothesis that hydrophobic bile acids could stimulate Kupffer cells to increase their capacity to generate reactive oxygen species by measuring cellular lipid peroxidation was tested. Because the hydrophilic bile acid, ursodeoxycholic acid (UDCA) can block hydrophobic bile acid induced cellular phenomena, it was also hypothesised that UDCA could antagonise macrophage activation by hydrophobic bile acids to blunt their capacity to generate reactive oxygen species.

Methods—J-774A.1 murine macrophages were incubated for 24 hours with either 10^3 M and 10^4 M (final concentration) DCA alone, or 10^3 M UDCA alone, or a mixture of 10^4 M 1:1 molar ratio of DCA and UDCA. At the end of the incubation period, the culture medium was collected for determination of cellular lipid peroxidation by measuring thiobarbituric acid (MDA) content in the medium with the thiobarbituric acid reactive substances assay.

Results—10^3 M and 10^4 M DCA increased MDA generation by cultured macrophages. 10^4 M UDCA alone did not increase MDA generation but blocked the peroxidative actions of DCA.

Conclusions—Hydrophobic bile acids, after their hepatic retention, can oxidatively activate Kupffer cells to generate reactive oxygen species. Because UDCA can block this action, the beneficial effect of UDCA is, in part, related to its ability to act as an antioxidant.

Keywords: macrophages, deoxycholic acid, ursodeoxycholic acid, antioxidant.

Hepatic macrophages or Kupffer cells are essential for normal hepatic homeostasis.
on cultured macrophages by measuring their cell lipid peroxidation.

Methods

Materials
Culture medium RPMI-1640, streptomycin, penicillin, phosphate buffered saline (PBS), and fetal calf serum (FCS) were all purchased from Biological Industries (Beth Haemek, Israel). Bovine serum albumin (BSA), thiobarbituric acid (TBA), and DCA were all purchased from Sigma Chemical Co (St Louis, MO, USA). UDCA was purchased from Calbiochem (La Jolla, CA, USA).

Cells
J-774A.1 murine macrophage-like cell line was obtained from the American Tissue Culture Collection (Rockville, MD, USA). The cells were maintained in culture in RPMI-164 medium without phenol red (to eliminate the interference of the medium color with the production of the pink chromophore during the determination of cellular lipid peroxidation (see later for details)) supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, and 5% heat inactivated (56°C for 30 minutes) FCS. This medium did not contain added iron ions or other transition metals. The cells were plated at 5 x 10^5 cells per 35 mm multiwell dishes, and were fed every three days.

Measurement of cellular lipid peroxidation and the effect of bile acids
The determination of cellular lipid peroxidation was similar to the method previously described by us.25 Briefly, cells were incubated for 24 hours in RPMI-1640 medium containing 0-2% BSA in the absence (control) and presence of either 1 x 10^{-5} M and 1 x 10^{-4} M (final concentration) DCA alone, or 1 x 10^{-4} M UDCA alone, or a mixture of 1 x 10^{-4} M 1:1 molar ratio of DCA and UDCA. At the end of the incubation period, the culture medium was collected for analysis of the malondialdehyde (MDA) content by the thiobarbituric acid reactive substances (TBARS) assay.26 The TBARS assay measures the amount of MDA, an end product of peroxidative decomposition of polyene fatty acids, and is widely used as a screening assay to quantify the extent of lipid peroxidation in vitro.27 We have previously shown that MDA produced by macrophages is almost completely secreted into the medium.25 To exclude possible bile acid-thiobarbituric acid reactions, the TBARS assay was performed with bile acids alone; TBARS were not generated. The cell protein content was determined by the method of Lowry et al after the cells were lysed with 0-1 N NaOH.

Statistical analysis of the data
Each experiment was repeated three times and analysed by one way ANOVA. A p<0.05 (two tailed) was considered significant.

Results
Incubation of J-774.1 macrophages in subconfluent culture with 1 x 10^{-5} M DCA and 1 x 10^{-4} M DCA, alone for 24 hours at 37°C significantly increased the MDA content in the medium from a control value (mean (SD)), 0.74 (0.12) nmol MDA/mg cell protein, to 1.70 (0.07) nmol MDA/mg cell protein (p<0.01) and 1.29 (0.08) nmol MDA/mg cell protein, respectively, (p<0.05; Figure). Incubation with 1 x 10^{-4} M UDCA alone did not increase MDA content (0.77 (SD 0.07) nmol MDA/mg cell protein), a value almost identical to the control (Figure). When the cells were incubated with 1 x 10^{-5} M DCA and 1 x 10^{-4} M UDCA, at a 1:1 molar ratio, the MDA content (0.70 (SD 0.09) nmol MDA/mg cell protein) was also not significantly different from the control or from the value when UDCA alone was added (Figure).

The cell protein content of the cultured macrophages exposed to bile acids was not different from the control indicating that cell viability was preserved after 24 hours of exposure to bile acids.

Discussion
Methodological considerations
Before discussing the data, some comments on the concentrations and the possible toxicity of bile acids used in these experiments are necessary. In liver tissue of patients with cholestatic liver disease, such as primary biliary cirrhosis, the intrahepatic concentration of total bile acids can rise as high as 600 nmol/g liver tissue29 and is largely due to the accumulation of the hydrophobic bile acids, chenodeoxycholic acid, and DCA.30 In our experiments, the cultured macrophages were incubated with 1 x 10^{-5} M and 1 x 10^{-4} M DCA. Assuming that 1 g liver is equivalent to 1 ml, these concentrations approximate to the hydrophobic bile acid concentrations found in cholestatic livers.

The total fasting serum bile acid concentration (normally <10 µM), rises to reach.
values as high as 200 μM in patients with cholestatic liver disease. When these patients are treated with UDCA at the usual dose of about 10 mg/kg/day (750–1000 mg/day), liver function improves. This beneficial effect is associated with further increases in the total fasting bile acid concentration and changes in the serum bile acid profile with the plasma UDCA concentration reaching around 90 μM. This concentration of UDCA determined the choice of the concentration of UDCA in our experiment.

For these experiments, we relied on total cell protein as a measure of cell viability and function. Previously, we have shown that the correlation between this index and other indices of cell viability is high indicating that the concentrations of DCA were not toxic to the cultured macrophages.

**Effect of deoxycholic acid on lipid peroxidation in macrophages**

This study has shown that DCA at a concentration below its critical micelle concentration can increase the level of cellular lipid peroxidation, one of the consequences of increased production of reactive oxygen species. We have interpreted this result to suggest that hydrophobic bile acids, after their hepatic retention, can oxidatively stimulate Kupffer cells to generate reactive oxygen species in vivo. This suggests that bile acids may also damage hepatocytes by an indirect pathway mediated by increased generation of reactive oxygen species by hydrophobic bile acid stimulation of Kupffer cells. Sokol et al have shown that hydrophobic bile acids can also stimulate the generation of reactive oxygen species in hepatocytes. Given this additional information, our data also indicate that hydrophobic bile acids can affect both the hepatocyte and Kupffer cells to generate reactive oxygen species and raise the level of oxidative stress in cholestatic liver disease.

**Effect of ursodeoxycholic acid on lipid peroxidation in macrophages**

UDCA has been used with considerable success in the treatment of chronic liver disease. The manner in which UDCA improves liver function is apparently diverse and several mechanisms have been proposed. Therapeutic dosing concentrations of UDCA enrich the bile acid pool with UDCA and, in doing so, shift the pool profile from one of hydrophobicity to hydrophilicity. Consequently, toxic hydrophobic bile acids are displaced by UDCA to the extent that UDCA becomes the major circulating bile acid. As much of the hepatic damage is caused by the endogenous hydrophobic bile acids, it has been suggested that UDCA may prevent or reduce hydrophobic bile acid damage itself and by virtue of displacement of the hydrophobic bile acids with UDCA, this may be the operative mechanism by providing cytoprotection of the hepatocyte. Secondly, UDCA can increase transcellular and canalicular transport of the bile acids thus reducing the hepatic retention of hydrophobic bile acids in patients, as well as inducing a biconarotate rich hypercholerosis in rats and hamsters.

Thirdly, UDCA can reduce the level of hepatic expression of human leucocyte antigen, lower serum IgM concentrations, and reduce mononuclear cell production of IgG, IgA, IgM, and cytokines interfering with the surface receptors or signalling systems of immunologically active cells. Consequently, these immunomodulatory properties of UDCA are also considered an integral mechanism of its ameliorative actions in patients with primary biliary cirrhosis. The beneficial action of UDCA has also been attributed to its ability to change the physicochemical properties of cell membranes by creating a membrane ‘barrier’ and thus stabilising membrane structure and preventing its disruption by toxic bile acids.

UDCA can also block the metabolic depressive effects of hydrophilic bile acids on hepatocyte mitochondrial function.

This study has shown that the peroxidative effect of hydrophilic bile acids, such as DCA, on cultured macrophages can be blocked by UDCA, which itself has no oxidative properties on cultured macrophages when used at therapeutic plasma concentrations. We have interpreted this result to suggest that the beneficial effect of UDCA may be related to its ability to prevent hydrophilic bile acid induced macrophage oxidative stimulation. Thus we suggest that UDCA has antioxidant properties that can also contribute to its advantageous effects in patients with cholestatic liver disease. Moreover, it is also tempting to speculate that UDCA may suppress Kupffer cell peroxidation, irrespective of the activating factor. This ‘dampening’ effect on macrophages may also explain the beneficial immunomodulatory properties of UDCA.

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