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Effect of allyl alcohol on hepatic transporter expression: Zonal patterns of expression and role of Kupffer cell function

Sarah N. Campion [a], Cristina Tatis-Rios [a], Lisa M. Augustine [b], Michael J. Goedken [c], Nico van Rooijen [d], Nathan J. Cherrington [b], José E. Manautou [a,*]

[a] Toxicology Program, Department of Pharmaceutical Sciences, School of Pharmacy, University of Connecticut, 69 North Eagleville Road, Unit 3092, Storrs, CT 06269-3092, USA
[b] Department of Pharmacology and Toxicology, University of Arizona, Tucson, AZ, USA
[c] Department of Pathology, Schering Plough Research Institute, Lafayette, NJ, USA
[d] Department of Molecular Cell Biology, VU Medical Center, Amsterdam, The Netherlands

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ABSTRACT

During APAP toxicity, activation of Kupffer cells is critical for protection from hepatotoxicity and up-regulation of multidrug resistance-associated protein 4 (Mrp4) in centrilobular hepatocytes. The present study was performed to determine the expression profile of uptake and efflux transporters in mouse liver following treatment with allyl alcohol (AlOH), a periportal hepatotoxicant. This study also investigated the role of Kupffer cells in AlOH hepatotoxicity, and whether changes in transport protein expression by AlOH are dependent on the presence of Kupffer cells. C57BL/6J mice received 0.1 ml clodronate liposomes to deplete Kupffer cells or empty liposomes 48 h prior to dosing with 60 mg/kg AlOH, i.p. Hepatotoxicity was assessed by plasma ALT and histopathology. Hepatic transporter mRNA and protein expression were determined by branched DNA signal amplification assay and Western blotting, respectively. Depletion of Kupffer cells by liposomal clodronate treatment resulted in heightened susceptibility to AlOH toxicity. Exposure to AlOH increased mRNA levels of several Mrp genes, while decreasing organic anion transporting polypeptides (Oatps) mRNA expression. Protein analysis mirrored many of these mRNA changes. The presence of Kupffer cells was not required for the observed changes in uptake and efflux transporters induced by AlOH. Immunofluorescent analysis revealed enhanced Mrp4 staining exclusively in centrilobular hepatocytes of AlOH treated mice. These findings demonstrate that Kupffer cells are protective from AlOH toxicity and that induction of Mrp4 occurs in liver regions away from areas of AlOH damage independent of Kupffer cell function. These results suggest that Kupffer cell mediators do not play a role in mediating centrilobular Mrp4 induction in response to periportal damage by AlOH.

Introduction

The expression profile of hepatic uptake and efflux transport proteins in mice in response to centrilobular hepatotoxicant exposure (acetaminophen, APAP and carbon tetrachloride, CCl_4) has been well-characterized (Aleksunes et al., 2005, 2006; Campion et al., 2008). We have demonstrated that mice exposed to toxic doses of these hepatotoxicants exhibit increased levels of Mrp3 and Mrp4 localized to centrilobular hepatocytes in regions with damage (Aleksunes et al., 2005, 2006). Mrp4, which is the most significantly up-regulated efflux transporter in mouse liver after APAP administration, was also found to be increased in livers of patients following ingestion of toxic APAP doses (Barnes et al., 2007). The up-regulation of efflux transporters and decreased expression of uptake transporters in response to chemical injury appears to be a compensatory response to limit the intracellular accumulation of potentially toxic chemicals in the liver as the organ attempts to repair the damage.

Preliminary studies have begun to provide clues on the regulatory mechanisms behind these alterations in hepatic transporter expression. In addition to the transcription factor NFE2-related factor 2, activation of Kupffer cells has been implicated in the regulation of hepatic transporters during drug-induced liver injury (Aleksunes et al., 2008b; Campion et al., 2008). Recent studies in our laboratory utilizing liposomal clodronate to eliminate Kupffer cells revealed that these cells participate in the up-regulation of hepatic Mrp4 protein during APAP-induced liver injury (Campion et al., 2008). These studies have not only shown that Kupffer cells play a significant role in the regulation of efflux transporter expression in response to APAP, but that they also confer protection against APAP hepatotoxicity. Of note, APAP toxicity targets hepatocytes in centrilobular

* Corresponding author. Fax: +1 860 486 5792.
E-mail addresses: jose.manautou@uconn.edu, manautou2001@yahoo.com (J.E. Manautou).

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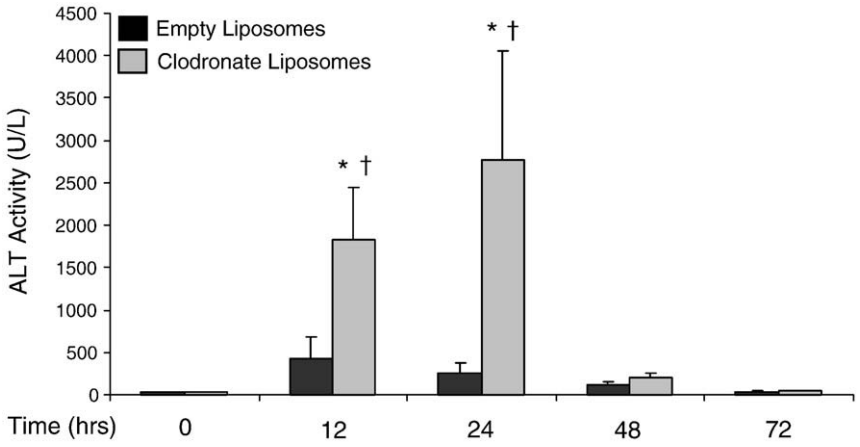

Fig. 1. Plasma ALT activity after AlOH treatment. Plasma was isolated from empty or clodronate liposome pretreated mice 12, 24, 48, and 72 h following dosing with AlOH (60 mg/kg) or vehicle. The data are presented as mean plasma ALT (U/L)±SE (n=4–14 animals). Asterisks (*) represent a statistical difference (p<0.05) from pooled control mice of the same liposome treatment (0 h) and daggers (†) represent a statistical difference (p<0.05) from empty liposome AlOH treated mice.

regions of the liver, where Kupffer cells are functionally different from periportal Kupffer cells.

Morphological and functional heterogeneity in Kupffer cell populations within the liver lobule are well known. Kupffer cells are more abundant in periportal regions where they are larger in size and exhibit greater phagocytic activity as compared to those in centrilobular regions (Armbrust and Ramadori, 1996; Kono et al., 2002). Periportal Kupffer cells also produce more superoxide and TNF-α in comparison to Kupffer cells in centrilobular regions. The latter have a greater capacity for IL-6 production (Armbrust and Ramadori, 1996). It is unknown if toxicants that target different regions of the liver lobule may differentially activate distinct Kupffer cell populations, thus affecting the ultimate outcome of toxicity. Additionally, selective activation of functionally different Kupffer cell populations may also influence the expression profile of hepatobiliary transporters.

To date, the effect of periportal hepatotoxicants on liver transport protein expression and zonal localization has not been characterized. The goal of this study was to determine: a) how hepatic transporter expression may change in response to periportal hepatocyte damage, and b) the role of Kupffer cells in periportal hepatotoxicity and in regulation of hepatobiliary transporters. In the present study, Kupffer cells were depleted by treatment of C57BL/6J mice with liposome encapsulated clodronate prior to administration of the periportal hepatotoxicant allyl alcohol (AlOH) (Badr et al., 1986; Belinsky et al., 1986; Badr, 1991). These experiments revealed that AlOH treatment resulted in up-regulation of efflux transport proteins with a concomitant down-regulation of uptake transport proteins. These changes were independent of the presence of Kupffer cells. Interestingly, the same selective centrilobular induction of Mrp4 that we previously reported with APAP treatment is also observed with AlOH despite the fact that periportal hepatocytes are the preferential targets for injury.

Methods

Animal care and treatment. Male 10–12 week old C57BL/6J mice were purchased from Jackson Laboratories (Bar Harbor, ME). Upon arrival, mice were acclimated for 1 week and maintained in a 12-h dark/light cycle, temperature- and humidity-controlled environment. Clodronate was a gift from Roche Diagnostics GmbH (Mannheim, Germany) and clodronate liposomes were prepared as previously described (Van Rooijen and Sanders, 1994). Clodronate or empty liposomes (100 μl, i.v.) were administered to mice 48 h prior to AlOH treatment. Animals were fasted for 16–18 h prior to challenge with AlOH. Groups of mice (n=4–14) were administered AlOH (60 mg/kg; 10 ml/kg, i.p.) or vehicle control (saline). Livers were collected 12, 24, 48, or 72 h after AlOH treatment. A portion of the liver was fixed in 10% zinc formalin and the remaining tissue was snap-frozen in liquid nitrogen and stored at −80 °C until assayed. All animal studies were conducted in accordance with National Institutes of Health standards and the *Guide for the Care and Use of Laboratory Animals*.

ALT activity assay. Plasma ALT activity was measured as a biochemical indicator of hepatocellular necrosis. Infinity ALT Liquid Stable Reagent (Thermotrace, Melbourne, Australia) was used to determine ALT activity according to the manufacturer's protocol.

Histopathology. Liver samples were fixed in 10% zinc formalin followed by paraffin embedding. Liver sections (5 μm) were stained with hematoxylin and eosin. Sections were examined by light microscopy for the presence and severity of hepatocellular degeneration and necrosis. A grading system similar to one described previously for APAP-induced histopathology (Manautou et al., 1994) was used. Liver injury was scored as follows: no injury=grade 0; minimal injury involving less than 10% injury/death=grade 1; mild injury affecting 10–25% of hepatocytes=grade 2; moderate injury affecting 25–40% of hepatocytes=grade 3; marked injury affecting 40–50% of hepatocytes=grade 4; or severe injury affecting more than 50% of hepatocytes=grade 5. Images of liver sections were captured using a Zeiss Axioplan 2 microscope equipped with a Sony DXS-S500 digital camera.

RNA isolation and branched DNA (bDNA) signal amplification assay. RNA Bee reagent (Tel-test Inc., Friendswood, TX) was used to extract total liver RNA according to the manufacturer's protocol. Mouse Oatp1a1 (*Slco1a1*), Oatp1a4 (*Slco1a4*), Oatp1b2 (*Slco1b2*), Ntcp (*Slc10a1*), and Mrp1-6 (*Abcc1-6*) mRNA were measured using the bDNA signal amplification assay (Quantigene® Screen Assay Kit, Panomics, Fremont, CA) as previously described (Hartley and Klaassen, 2000; Aleksunes et al., 2005). The data are reported as relative light units (RLU) per 10 μg total RNA.

Preparation of crude membrane fractions. Liver plasma membrane fractions were prepared as previously described (Aleksunes et al., 2006). The resulting pellets were resuspended in sucrose-Tris buffer and protein concentrations were determined using Bio-Rad protein assay reagents (Bio-Rad Laboratories, Hercules, CA).