Experimental and Molecular Pathology 96 (2014) 27–35

Contents lists available at ScienceDirect

Experimental and Molecular Pathology

journal homepage: www.elsevier.com/locate/yexmp

Nonselective inhibition of prostaglandin-endoperoxide synthases by naproxen ameliorates acute or chronic liver injury in animals

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ARTICLE INFO

Article history:
Received 20 August 2013
Available online 10 November 2013

Keywords:
Drug
Injury
Liver
Protection
Soluble factors

ABSTRACT

The rising prevalence of hepatic injury due to toxins, metabolites, viruses, etc., necessitates development of further mechanisms for protecting the liver and for treating acute or chronic liver diseases. To examine whether inhibition of inflammation is directed by cyclo-oxygenase pathways, we performed animal studies with naproxen, which inhibits prostaglandin-endoperoxide synthases 1 and 2 and is in extensive clinical use. We administered carbon tetrachloride to induce acute liver injury and ligated the common bile duct to induce chronic liver injury in adult rats. These experimental manipulations produced abnormalities in liver tests, tissue necrosis, compensatory hepatocyte or biliary proliferation, and onset of fibrosis, particularly after bile duct ligation. After carbon tetrachloride-induced acute injury, naproxen decreased liver test abnormalities, tissue necrosis and compensatory hepatocellular proliferation. After bile duct ligation-induced chronic injury, naproxen decreased liver test abnormalities, tissue injury and compensatory biliary hyperplasia. Moreover, after bile duct ligation, naproxen-treated rats showed more periductular oval liver cells, which have been classified as hepatic progenitor cells. In naproxen-treated rats, we found greater expression in hepatic stellate cells and mononuclear cells of cytoprotective factors, such as vascular endothelial growth factor. The ability of naproxen to induce expression of vascular endothelial growth factor was verified in cell culture studies with CFSC-8B clone of rat hepatic stellate cells. Whereas assays for carbon tetrachloride toxicity using cultured primary hepatocytes established that naproxen was not directly cytoprotective, we found conditioned medium containing vascular endothelial growth factor from naproxen-treated CFSC-8B cells protected hepatocytes from carbon tetrachloride toxicity. Therefore, naproxen was capable of ameliorating toxic liver injury, which involved naproxen-induced release of physiological cytoprotective factors in nonparenchymal liver cells. Such drug-induced release of endogenous cytoprotectants will advance therapeutic development for hepatic injury.

Introduction

The burden of liver diseases due to chronic viral hepatitis, metabolic diseases, e.g., diabetes and obesity, drugs, alcohol, environmental toxins, etc., has been rising throughout the world. Hepatic inflammation is a major component in the pathophysiology of these liver conditions although the role of anti-inflammatory drugs has not been well studied for therapeutic development. Whereas inflammation is transduced by multiple cell types and various molecular pathways driving inflammation are complex, the cyclooxygenase (COX) pathways are incriminated in many situations, including chronic liver disease in people (Cheng et al., 2002; Mohammed et al., 2004; Núñez et al., 2004). In experimental settings, COX pathways serve roles in liver injury, e.g., after exposure to alcohol, bacterial endotoxin, carbon tetrachloride (CCl_4), chloroform, concanavalin A, or D-galactosamine (Albrecht et al., 1997; Begay and Gandolfi, 2003; Mayoral et al., 2008; Nanji et al., 1997). Similarly, COX pathways were found in transgenic mice to serve roles in hepatic injury (Yu et al., 2007). Also, disease-relevant synergisms were observed in COX pathways and other inflammatory mediators, i.e., 5-lipoxygenase pathway of arachidonic acid metabolism (Horrillo et al., 2007).

The conversion of arachidonic acid into prostaglandins (PG) by prostaglandin-endoperoxide synthases (PTGS) 1 and 2, the former constitutive and the latter inducible, leads to multiple substrates for inflammatory mediators. Among these, major PG-derived inflammatory mediators include PGE2, thromboxane A2, prostacyclins, e.g., PGI_2, and other prostanoids (Khanapure et al., 2007). The ability to interfere with COX pathways by widely used drugs, such as naproxen, a nonselective PTGS blocker, or celecoxib, a selective PTGS2 blocker, raised interest for their uses in hepatic inflammation and/or fibrosis (Enami et al., 2009; Yu et al., 2009). However, despite presumed anti-inflammatory mechanisms of their action, studies in a rat model of acute liver inflammation also showed that in some instances naproxen

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0014-4800/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.yexmp.2013.10.017

or celecoxib improved outcomes not by altering activation of inflammatory cells or expression of inflammatory cytokines and chemokines (Enami et al., 2009), but by stimulating release of cytoprotective factors, such as vascular endothelial growth factor (VEGF) from hepatic stellate cells (HSC). This observation suggested an altogether different paradigm by which COX pathways could be manipulated for altering liver injury.

To develop this concept, we examined whether inhibition of PTGS1 and 2 by naproxen could be hepatoprotective in well-characterized models of CCl_4-induced acute liver injury and bile duct ligation (BDL)-induced chronic liver injury (Glaser et al., 2010; Joseph et al., 2005). Our consideration was that use of naproxen in suitable inhibitory doses will protect liver from acute as well as chronic injury by either direct anti-inflammatory effects on liver cells or by secondary release of cytoprotective molecules, e.g., VEGF. To demonstrate these potential hepatoprotective effects of naproxen, we performed studies at the levels of liver tests, tissue morphology, gene expression, and changes in various liver cell types. We used doses of naproxen that were previously established to inhibit PTGS1 and PTGS2 activity sufficient for 50% reduction in inflammation (ID50 doses) under clinical settings (Huang et al., 2011). Moreover, cell culture assays were performed with primary rat hepatocytes and the CFSC-8 clone of HSC, which was isolated from rats with chronic liver injury (Ohayon et al., 2008).

Materials and methods

Drugs and chemicals

Naproxen was purchased commercially (Sigma Chemical Co, St. Louis, MO) and was dissolved in 20% ethanol to 2 mg/ml with dilution as needed in normal saline. For cell culture studies, naproxen was diluted in Dulbecco's Minimum Essential Medium (DMEM).

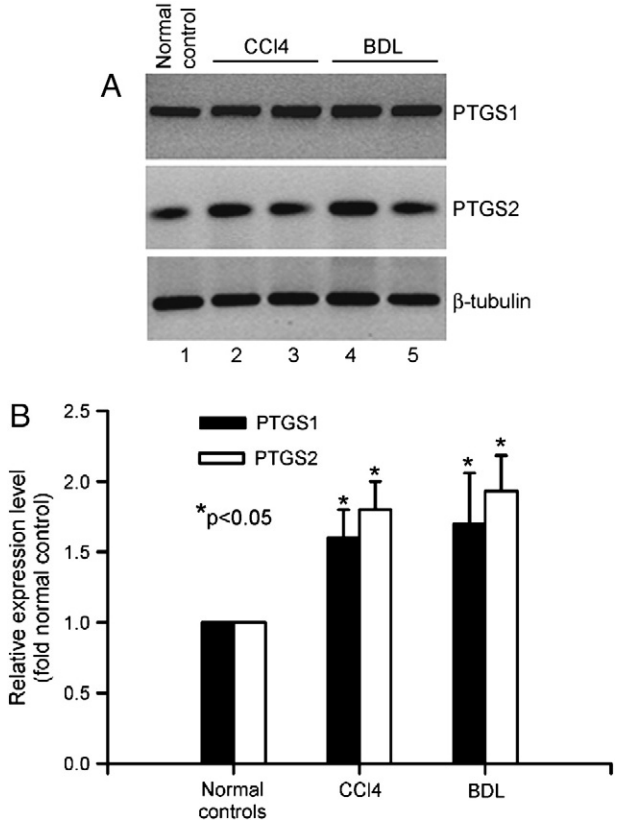

Fig. 1. Expression of PTGS1 and PTGS2 by western blot in rats. (A) Expression of PTGS1 and PTGS2 shown in each lane in liver from individual animals. (B) Densitometric quantitation of PTGS1 and PTGS2 levels ($n = 3$ each). Asterisks indicate $p < 0.05$.

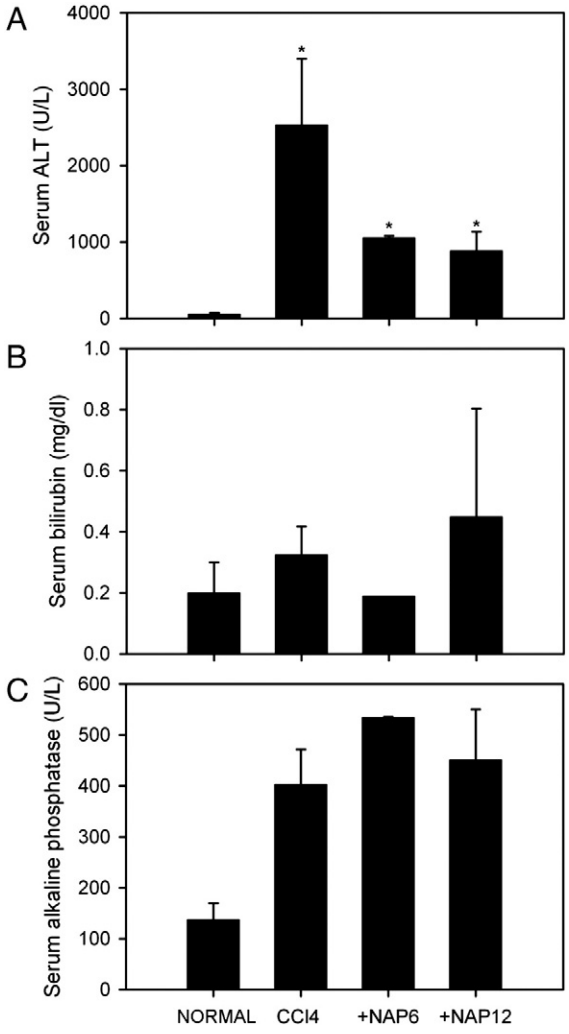

Fig. 2. Effect of naproxen on CCl_4-induced acute liver injury. (A–C) Serum ALT, bilirubin and alkaline phosphatase levels in healthy animals, animals treated once with CCl_4, and animals treated with CCl_4 followed by 6 mg/kg or 12 mg/kg naproxen. Asterisks in panel A indicate naproxen improved serum ALT levels.

Cells and cell culture

Primary hepatocytes were isolated from F344 rats by two-step collagenase perfusion, as described (Enami et al., 2009). CFSC-8B cells were originally from late Dr. M. Rojkind. Cells were cultured at 1×10^4 cells/cm^2 culture plastic in DMEM with 10% fetal bovine serum and antibiotics. For conditioned medium (CM), CFSC-8B cells were cultured without serum for 24 h or 48 h under hypoxia (5% O_2, 5% CO_2, 11% N_2). Cell viability was examined by thiazolyl blue (MTT) assays, as described previously (Gagandeep et al., 1999). Primary rat hepatocytes were attached to culture dishes in serum-containing medium under normoxia (21% O_2, 5% CO_2). For cytotoxicity assays, hepatocytes were cultured for 24 h with 12 μM CCl_4 (Sigma), followed by MTT assays.

Animal studies

Male F344 rats of 8–10 week age weighing 150–180 g were obtained from the National Cancer Institute (Bethesda, MD). The Animal Care and Use Committee at Albert Einstein College of Medicine approved protocols, according to institutional regulations and guidelines from the National Institutes of Health (Bethesda, MD).

Naproxen was given i.p. in 6 or 12 mg/kg doses. For acute liver injury, rats were given 1 ml/kg CCl_4 in mineral oil (1:1, v/v) i.p. once (Joseph