



Glucocorticoid pretreatment suppresses chemokine expression and inflammatory cell infiltration in cholestatic rats receiving biliary intervention

Chih-Sung Hsieh^a, Pei-Wen Wang^b, Shin-Ye Lee^a, Chao-Cheng Huang^c,
Nyuk-Kong Chang^d, Ching-Mei Chen^b, Chia-Ling Wu^d,
Hsiu-Chuan Wang^d, Jiin-Haur Chuang^{a,d,*}

^aDepartment of Surgery, Chang Gung Memorial Hospital, Chang Gung University, Kaohsiung 833, Taiwan

^bDepartment of Internal Medicine, Chang Gung Memorial Hospital, Chang Gung University, Kaohsiung 833, Taiwan

^cDepartment of Pathology, Chang Gung Memorial Hospital, Chang Gung University, Kaohsiung 833, Taiwan

^dGraduate Institute of Clinical Medical Sciences, Chang Gung University, Kaohsiung 833, Taiwan

Index words:

Glucocorticoid;
Chemokine;
Cholestasis;
Biliary intervention

Abstract

Aim: Biliary intervention may augment chemokine expression and inflammatory cell infiltration and aggravates liver injury in cholestatic rats. We tested the efficacy of glucocorticoid pretreatment to prevent the complications.

Methods: A model of biliary intervention was established in rats without (sham) or with bile duct ligation (BDL). Before biliary intervention, rats were randomly assigned to receiving intravenous injection of dexamethasone (DX group) or normal saline (NS group). Plasma levels of monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-2 (MIP-2) were measured with enzyme-linked immunosorbent assay, and liver messenger RNA of these chemokines was quantified with real-time quantitative reverse transcriptase-polymerase chain reaction. Monocytes, Kupffer cells, and neutrophils in the rat liver were characterized with antibodies to ectodermal dysplasia 1 (ED1), ED2, and myeloperoxidase, respectively.

Results: By 3 hours after biliary intervention, plasma MCP-1 and MIP-2 proteins in BDL-NS rats were significantly higher than in BDL-DX. At 3 hours, liver MCP-1 and MIP-2 messenger RNA levels were significantly upregulated in BDL-NS than in BDL-DX. The amount of ED1-, ED2- and myeloperoxidase-staining cells were significantly greater in BDL-NS than in BDL-DX. Most of the changes returned to baseline levels by 24 hours.

Conclusion: Glucocorticoid pretreatment suppresses chemokine expression and inflammatory cell infiltration, which may consequently alleviate liver injury in cholestatic rats receiving biliary intervention.

© 2006 Elsevier Inc. All rights reserved.

* Corresponding author. Department of Surgery, Chang Gung Memorial Hospital, Niao-Sung Hsiang, Kaohsiung Hsieh, 833 Taiwan. Tel.: +886 7 7317123; fax: +886 7 7338009.

E-mail address: jhchuang@adm.cgmh.org.tw (J.-H. Chuang).

Patients with obstructive jaundice frequently have associated severe liver dysfunction, leading to significant complications even when short, minor procedures are performed [1-3]. Inflammatory cell infiltration, accumulation of

hydrophobic bile acids, endotoxemia, and changes of the mitochondrial permeability transition are possible factors responsible for cholestatic liver injury [4-7], which may interplay to cause morbidity and mortality during intervention of the biliary system. Mitochondrial dysfunction can be ablated with glucocorticoid administration [8]. Little is known about the strategy of decreasing inflammatory cell infiltration in obstructive jaundice.

A family of proinflammatory proteins, called chemokines, is able to specifically affect the migration of leukocytes. Two classes of chemokines have been defined. One is referred to as C-X-C chemokines, which have 1 amino acid residue separating the first 2 conserved cysteine residues, and the other subclass, C-C chemokines, wherein the first 2 cysteine residues are adjacent. The C-X-C subfamily includes granulocyte chemoattractant interleukin-8 (IL-8) and macrophage inflammatory protein (MIP)-2. The C-C subfamily includes monocyte chemoattractant protein-1 (MCP-1). Inflammatory cells are recruited to the liver during cholestasis by C-X-C chemokines such as IL-8 or MIP-2 and C-C chemokines such as MCP-1 [9]. Both MIP-2 and MCP-1 can also exert deleterious effects on the liver by stimulating the release of reactive oxygen species from Kupffer cells and neutrophils [10,11]. Glucocorticoids have been shown to modulate chemokines MIP-1 α , MIP-1 β , IL-8, and MCP-1 production in patients with liver transplantation, and in rats with bacterial cholangitis or CCl₄-induced liver cirrhosis [12-14]. It may reduce reactive oxygen species formation, but accelerate fibrogenesis in the rat liver after bile duct ligation (BDL) [15]. The benefits and risks of glucocorticoid administration in many life-threatening conditions are still a matter of controversy and are unknown in cholestatic patients undertaking biliary intervention.

We have established a rat model of obstructive jaundice, in which intervention of the biliary system augments both MIP-2 and MCP-1 expression as well as inflammatory cell infiltration [16]. In this study, we tested the efficacy of dexamethasone pretreatment in ablating liver injury of a rat model of biliary intervention.

1. Materials and methods

1.1. Animal model and experimental protocol

Male Sprague-Dawley rats were purchased from the National Animal Center, the Academia Sinica, Taipei, Taiwan, after weanling. The animals were maintained on standard laboratory rat chow in a 12-hour light-dark cycle. The care and use of the laboratory animal strictly followed the protocol, which was approved by the animal ethics committee of the Chang Gung University, Kaohsiung, Taiwan.

After anesthesia by intraperitoneal injection of 50 mg/kg of thiopentone sodium (Pentothal, Abbott Laboratories, North Chicago, Ill), the rat received a median laparotomy

and the extrahepatic bile duct was identified. A silicone catheter (Silicone Elastomer, Helix Medical, Carpinteria, Calif) with the ID of 0.508 mm and the OD of 0.930 mm was inserted into the proximal bile duct. The tube was advanced into the bile duct for about 0.5 cm and was then secured in the lumen by double ligation with 4-0 silk sutures. The other end of the catheter was carried out through a small puncture hole to the subcutaneous space and again brought back to the abdominal cavity through another puncture hole, to leave a subcutaneous segment of about 1 cm. The distal end was then inserted into the distal bile duct immediately above the pancreatic tissue and the tube was secured with 4-0 silk ligature. The subcutaneous segment of the silicone tube was ligated to create obstructive jaundice and was designated as BDL, similar to BDL in commonly used model of obstructive jaundice.

Two weeks after bile duct cannulation, the animals without ligation of the catheter were designated as sham rats, whereas the rats with ligation of the catheter were designated as BDL rats. A small incision along the previous abdominal wound, immediately above the subcutaneous segment of the indwelling catheter, was done. The rats received injection of 1 mL of sterile normal saline into the indwelling catheter through a 30F Short Needle (ULTRA-FINE II, Becton Dickinson, Franklin Lakes, NJ) to simulate biliary intervention. Ten minutes before biliary intervention, both groups of the rats were randomly assigned to receive intravenous infusion of 5 mg/kg dexamethasone (DX, Veterans Pharmaceuticals, Taipei, Taiwan) in 1 mL of normal saline (sham-DX or BDL-DX) or 1 mL of sterile normal saline (NS) alone (sham-NS or BDL-NS). The rats were further divided into those killed at 3 hours and those killed at 24 hours after the second operation.

Blood was withdrawn from the indwelling femoral vein, at the time of biliary intervention (0 hour), and hourly by 3 hours after biliary intervention. Liver was removed at 3 or 24 hours after biliary intervention. Some of the liver tissues were snap-frozen for messenger RNA (mRNA) and protein determinations and the remains fixed in 4% paraformaldehyde and embedded in paraffin for histology and for immunohistochemical analysis.

1.2. Plasma MIP-2 and MCP-1 assay

Plasma MIP-2 and MCP-1 concentrations were determined by enzyme-linked immunosorbent assay using a commercially available kit (rat MIP-2, Biosource, Calif; rat MCP-1, Biosource, Camarillo, CA). The procedures strictly followed the manufacturer's guidelines.

1.3. Liver MIP-2 and MCP-1 mRNA expression

To quantify mRNA levels of MIP-2 and MCP-1 in the liver, we used an ABI7700 Sequence Detection System (Perkin-Elmer Applied Biosystems, Tokyo, Japan), which measured mRNAs by real-time quantitative reverse transcriptase-polymerase chain reaction (QRT-PCR). Total RNA

Download English Version:

<https://daneshyari.com/en/article/4160720>

Download Persian Version:

<https://daneshyari.com/article/4160720>

[Daneshyari.com](https://daneshyari.com)