CYCLOOXYGENASE-2 EXPRESSION IN HEPATOCYTES PROTECTS AGAINST HEPATIC ISCHEMIA-REPERFUSION INJURY IN MICE

Daniel 5Luis ♦ 3,7,8Marta ♦ Motiño, E. Francés, M. Fuertes Agudo, M. Bañares, M. Casado ‐ 1Instituto de Investigaciones Biomédicas “Alberto Sols”‐CSIC, 4, 28293 Madrid, Spain; 2Instituto de Fisiología Experimental (IFisé‐CONCEIT), Suipecha 570, 2500 Rosario, Argentina; 3Instituto de Biomedicina de Valencia, IBV‐CSIC, Jaume Roig 11, 46101 Valencia, Spain; 4Department of Animal Medicine and Surgery, Veterinary Faculty, Universidad Complutense de Madrid, Spain; 5Instituto de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Monforte de Lemos 3‐5, 28029 Madrid, Spain; 6Centro de Investigación Biomédica en Red de Enfermedades Cardiovasculares (CIBERCV), Monforte de Lemos 3‐5, 28029 Madrid, Spain; 7Istituto di Biologia Medicina della Reproduzione, Avogadro 1 20133 Milan, Italy

Liver ischemia and reperfusion injury (IRI) remains a serious clinical problem affecting liver transplantation outcomes. IRI causes up to 10% of early organ failure and predisposes to chronic rejection. Cyclooxygenase-2 (COX-2) is involved in different liver diseases but the significance of COX-2 in liver IRI is a matter of controversy. This study was designed to elucidate the role of COX-2 expression in hepatocytes in the pathogenesis of liver IRI. In the present work hepatocyte-specific COX-2 transgenic mice (hCOX-2-Tg) and their wild-type (Wt) littersmates were subjected to partial IRI and the results show that hCOX-2-Tg exhibited lower grades of necrosis and inflammation than Wt mice in part by reduced recruitment and infiltration of hepatic macrophages and neutrophils with a corresponding decrease in serum levels of pro-inflammatory cytokines. Moreover hCOX-2-Tg mice showed a significant attenuation of the IR-induced increase in oxidative stress and hepatic apoptosis an increase in autophagic flux and a decrease in endoplasmic reticulum (ER) stress comparing with that observed in Wt mice. Preconditioning of Wt mice resembles the beneficial effects of hCOX-2-Tg mice in IRI due to an increase in endogenous COX-2 expression. Furthermore measurement of PGE2 levels in plasma from patients who underwent orthotopic liver transplantation revealed a significantly negative correlation between PGE2 levels and graft function and time of ischemia. Overall the data support the view of the beneficial effects of hepatic COX-2 dependent prostaglandins after liver IRI.

EXPERIMENTAL PROCEDURES

Animal models: Transgenic mice (hCOX-2-Tg), constitutively express human COX-2 in hepatocytes under the control of the human APRT promoter and its specific hepatic control region (HCR), were divided randomly into four groups: 1) Sham operation (Sham); 2) hepatic ischemia (90 min); 4) 24h reperfusion (IR); 3) Preconditioning (PC); and 4) DFU treated group (I/R DFU). A model of segmental (70%) hepatic ischemia was used. If ischemic preconditioning experiments, Wt mice were subject to 20 min hepatic ischemia following 30 min of reperfusion prior to ischemia-reperfusion protocol. For DFU treatment, Wt and hCOX-2-Tg mice were injected intraperitoneally (i.p.) with 5 mg/kg DFU in DMSO for 4 days and 45 min before the hepatic ischemia.

Patients: Donor livers (Donor age was 54±18 years) were perfused through the aorta and portal vein with University of Wisconsin solution (ViaSpan; Barr Laboratories, Pomona, NY). The LT procedure was conducted in a standardized way with the usual surgical technique with the piggyback method and no venoconstrictive bypass (2). Serial blood samples from radial arterial catheter were collected from 63 patients (41 men and 22 women, with a mean age of 51 ± 11 years) 60 minutes after reperfusion (860). Ischemia time was 568±1611 minutes. Hospital stay was 38±43 days. Plasma was obtained by centrifugation for 10 minutes at 9000 × g at 4°C, and stored at −20°C. Graft function was evaluated during the first 3 days after LT; transplanted livers were grouped into four grades of function. In grade I, aspartate transaminase (AST) levels remained less than 1,000 U/L for the 1st 3 days post-IT, and there was good bile production (>40 ml/d) and improving coagulation. In grade II, serial AST levels were greater than 1,000 U/L, but decreased during the subsequent 48h, with improving coagulation and adequate bile flow. In grade III, AST levels were greater than 2,500 U/L, for the 1st 48h post-IT, bile production was reduced (<40 ml/d), and coagulopathy was more severe. In grade IV, there were rapidly increasing AST levels, with no bile production and severe coagulopathy (2-4). Liver function were distributed as follows: Grade I: 18 patients (44%), II: 15 (32.5), III: 17 (27%), and IV: 6 (4%).

Ischemic preconditioning induces COX-2 mRNA and PGE2 production attenuating ischemia/Reperfusion-induced liver damage

Necrotic areas are marked with ~ and vascular congestion with #. Necrosis grade assessed by histological examination. Scores were used: 0: none, 1: mild lesion (0–20% of necrosis), 2: moderate lesion (20–40% of necrosis), 3: severe lesion (more than 40% of necrosis). Data are expressed as means ± S.E. (n=6 per group). *P<0.05 vs. Wt-Sham; #P<0.05 vs. Wt-I/R.

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Specific nuclear staining is marked with *. Data are expressed as means ± S.E. (n=6 per group). *P<0.05 vs. Wt-Sham; #P<0.05 vs. Wt-I/R.

Prostaglandin E2 protects the patient receiving a liver transplant

NO, good graft function (grades I or II); YES, poor graft function (grades III or IV), (**P<0.05 vs. Sham; #P<0.05 vs. I/R).

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