Original Article

Effect of a Prostaglandin I₂ Analog on the Expression of Thrombomodulin in Liver and Spleen Endothelial Cells After an Extensive Hepatectomy

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Abstract

Purpose. Dysfunction of the remnant liver after a hepatectomy is caused by microthrombus formation due to endothelial cell (EC) damage. This study evaluated the effect of prostaglandin I₂ (PGI₂) on the expression of thrombomodulin (TM), a marker for the anticoagulant properties of ECs, using cultured human umbilical vein endothelial cells (HUVECs), and using a canine extensive hepatectomy model.

Methods. The presence of PGI₂ receptors was confirmed on HUVECs by reverse transcription–polymerase chain reaction, and the effect of the PGI₂ analog on TM expression on HUVECs was determined by an enzyme-linked immunosorbent assay. Twenty mongrel dogs were divided into four groups comprising a sham operation, 70% hepatectomy, 84% hepatectomy, and 84% hepatectomy, with the administration of the PGI₂ analog, respectively, and TM expression in the liver, spleen, pancreas, kidney, lung, portal vein, and intestine was determined immunohistochemically.

Results. The TM expression on HUVECs was upregulated by the PGI₂ analog. The TM expression on ECs in the hepatic sinusoids and splenic sinus were markedly decreased after the 84% hepatectomy, but such damage was markedly mitigated following an 84% hepatectomy with administration of the PGI₂ analog.

Conclusions. An extensive hepatectomy induced severe EC damage not only in the hepatic sinusoids but in the splenic sinuses as well. Prostaglandin I₂ prevented damage to these ECs, suggesting that PGI₂ improves the microcirculation in the remnant liver.

Key words Extensive hepatectomy · Prostaglandin I₂ · Thrombomodulin

Introduction

Cytokines secreted from activated Kupffer cells play a critical role in the dysfunction of the remnant liver after an extensive hepatectomy.¹² These cytokines impair not only hepatocytes but also hepatic sinusoidal endothelial cells (ECs),³ and it is hypothesized that the dysfunction of the remnant liver is caused by microthrombus formation derived from the damage of ECs. Endothelial cells have various anticoagulant properties, such as tissue-type plasminogen activator secretion,⁴ and expression of heparin-like glycosaminoglycan⁵ and thrombomodulin (TM).⁶ Thrombomodulin plays a role as a cofactor for thrombin-catalyzed activation of protein C.⁷ Activated protein C proteolytically inactivates blood coagulation cofactors, factors Va and VIIIa, on platelets and ECs.⁸⁹ Thrombomodulin also inhibits the procoagulant activity of thrombin, including platelet activation¹⁰ and fibrin formation, thereby preventing excessive thrombus formation. Cleavage of the TM on the ECs by certain proteinases secreted by neutrophils activated by inflammation and infection decreases the anticoagulant properties on the surface of the ECs, facilitating the microthrombus formation. Therefore, the levels of soluble TM in plasma and TM antigen on the cell surface may a useful molecular marker of EC damage after a hepatectomy.

The expression of TM on the surface of ECs is down-regulated by tumor necrosis factor (TNF),¹¹ interleukin (IL)-1,¹² endotoxin,¹³ and homocysteine,¹⁴ and upregulated by cyclic adenosine monophosphate (cAMP)¹⁵ and PGI₂ analog (beraprost¹⁶), and PGI₂ increases intracellular cAMP levels via PGI₂ receptors and increases cAMP-mediated expression of TM on ECs.¹⁶

The impairment of ECs in the remnant liver after an 84% hepatectomy is mainly caused by the formation of numerous microscopic thrombi, consisting primarily of platelets and erythrocytes. The thrombus formation is found in the sinusoids in the remnant liver, and their
formation is reduced by the administration of PGI₂ analog. However, it is unknown whether an extensive hepatectomy impairs ECs outside the liver and how PGI₂ protects ECs in the liver and/or other organs from the damage by cytokines or other humoral substances.

This study examines the effect of OP-2507, a PGI₂ analog, on the expression of TM in cultured human umbilical vein endothelial cells (HUVECs), and on the damage to ECs in the liver and other organs after an extensive hepatectomy in canine models.

**Materials and Methods**

**In Vitro Studies**

**Cell Culture Techniques**

Human umbilical vein endothelial cells were prepared and cultured in modified MCDB131 (Chlorella Industry, Tokyo, Japan), supplemented with 10% fetal calf serum (Gibco, Grand Island, NY, USA), 50 mg/ml gentamycin (Flow Laboratories, Irvine, UK), 10 mg/ml endothelial growth supplement (Collaborative Research, Bedford, MA, USA), and 10 mg/ml heparin (Sigma, St. Louis, MO, USA) under a 5% CO₂ atmosphere at 37°C using 90-mm diameter collagen-coated dishes (Sumitomo Bakelite, Tokyo, Japan), according to the method of Jaffe et al. Human umbilical vein endothelial cells were passage cultured using 0.025% trypsin–0.265 mM ethylenediamine tetra-acetic acid and were used for assay after three or four passages. The human leukemic cell line (CMK), provided by Dr. Takeyuki Sato, Department of Pediatrics, School of Medicine, Chiba University, Japan, was routinely maintained in RPMI 1640 medium (Nissui Seiyaku, Tokyo, Japan) supplemented with 10% fetal calf serum in a 5% CO₂ atmosphere at 37°C using a 75-cm² untreated culture flask.

**Detection of PGI₂ Receptor mRNA in HUVECs by Reverse Transcription–Polymerase Chain Reaction (RT-PCR)**

Total RNA was isolated from HUVECs and CMK cells that had been found to bear PGI₂ receptors (positive control) by using RNAzol (TEL-TEST, Friendswood, TX, USA). Equal aliquots (1 mg) of total RNA were used to synthesize first-strand cDNA by using reverse transcriptase and oligo-dT primers. Polymerase chain reaction amplification of this synthesized first-strand cDNA as a template was conducted for 40 cycles (at 94°C for 2 min, at 55°C for 2 min, and at 72°C for 2 min) using Taq DNA polymerase and a pair of oligonucleotide primers (5'-GCG AAT TCA TCT ACG CCT TCT GCG TCC T-3' and 5'-GGG AAA GGA T-3') with an inserted EcoRI site (underlined) targeted to the coding region of human PGI₂ receptor cDNA. The amplified fragments were separated by agarose gel electrophoresis, and the gel was stained with ethidium bromide to analyze the PCR product.

The PCR product was subcloned into pBluescript SKII that was digested with EcoRI. After amplification of pBluescript SKII including PCR band, the PCR band was cleaved by EcoRI. The amplified PCR bands were obtained from agarose gel. Both strands were completely sequenced by the dideoxy chain termination method.

**Treatment of HUVECs with PGI₂ Analog**

The PGI₂ analog, OP-2507 (Ono Pharmaceutical, Osaka, Japan), was dissolved in sterile water to a concentration of 1 mg/ml and added to the medium at the desired concentration (1–100 ng/ml). Media containing various concentrations of OP-2507 were added onto cell monolayers 1–2 days post confluence, and they were incubated for various periods.

**Determination of TM Antigen**

Human umbilical vein endothelial cells treated with the PGI₂ analog were washed three times with serum-free medium, scraped off with a rubber policeman, and then collected by centrifugation at 1800 rpm for 10 min. The supernatant was discarded and the cell pellet was suspended in 1 ml of Tris-buffered saline (TBS; 50 mmol/l Tris–HCl, 100 mmol/l NaCl, pH 7.5) containing 0.5% Triton X-100 (Nacalai Tesque, Kyoto, Japan). The cells were incubated for 1 h at room temperature and centrifuged at 15 000 rpm for 10 min, and the supernatant was collected. Thrombomodulin antigen was then determined by a sandwich-type enzyme-linked immunosorbent assay using two monoclonal antibodies for human TM, as reported previously.

**In Vivo Study**

**Animal Models of an Extensive Hepatectomy**

Twenty adult mongrel dogs of both sexes weighing 10–15 kg raised in the Animal Experimental Laboratory, Mie University Graduate School of Medicine, were used for the following experiments. All dogs were cared for according to the National Research Council Guidelines for animal housing and care. All dogs were fasted for 24 h and thereafter underwent endotracheal intubation, then general anesthesia was induced with an intravenous injection of pentobarbital (Nembutal; 5 mg/kg). A mid-abdominal skin incision was made after dividing the animals into the following four groups: sham-operated control (simple laparotomy) (n = 5), 70% hepatectomy (n = 5), 84% hepatectomy (n = 5), and 84% hepatectomy with the administration of PGI₂ analog (OP-2507) (n = 5).