Impact of adhesion molecules of the selectin family on liver microcirculation at reperfusion following cold ischemia

Abstract We investigated the role of adhesion molecules in the early phase of reperfusion following cold ischemia. Livers of male Lewis rats were preserved for 0 h (group A) or 24 h in University of Wisconsin (UW) solution without additives (group B) or in UW solution with anti-ICAM-1 antibody (group C) or anti-E-selectin-1, SLe\(^x\) and SLe\(^a\) antibodies (group D). The livers were then reperfused with diluted rat whole blood (DWB; groups A and B), DWB containing anti-ICAM-1 and LFA-1 antibodies (group C) or DWB containing anti-L-selectin, SLe\(^x\) and SLe\(^a\) antibodies (group D). The reperfusion was performed at 37 °C for 1 h at 5 cm H\(_2\)O of perfusion pressure. During reperfusion, hepatic microcirculation was assessed by monitoring portal and peripheral tissue blood flow. Bile production was significantly reduced in group B livers compared with those in group A. Anti-ICAM-1 and LFA-1 antibodies failed to improve hepatic microcirculation, whereas anti-LECAM-1, SLe\(^x\) and SLe\(^a\) antibodies significantly improved the microcirculation. Bile production in group C and D livers was comparable to that in group B livers. Preservation for 24 h significantly increased the release of TNF-\(\alpha\) from 0.207 to 43.7 pg/g per hour during reperfusion. Monoclonal antibodies to the adhesion molecules did not suppress the release of TNF-\(\alpha\) in groups C and D. Histological examination demonstrated a lack of leukocyte infiltration or thrombus in hepatic microvessels. The extent of hepatocyte necrosis did not differ among groups B, C, and D. We conclude that the microcirculatory disturbance in the early phase of reperfusion occurs as a result of the tethering of leukocytes through the interaction of the selectin family and their ligands, and that the ICAM-1-LFA-1 pathway is not involved in this step. The lack of improvement in bile production with antibodies to the selectin family and their ligands strongly suggests that other mechanisms participate in the deterioration of hepatic function.

Key words Adhesion molecules, rat, liver transplantation. Liver transplantation, adhesion molecules, rat. Cytokines, liver transplantation, rat

Introduction

Because of the increased incidence of primary nonfunction with prolonged preservation time, the duration of organ preservation is limited to less than 24 h in clinical transplantation [8]. Several studies have examined the mechanisms of tissue injury, termed “cold ischemia-reperfusion injury”, which is responsible for primary nonfunction [6, 10, 37]. In the last decade, intensive studies have revealed several important mechanisms of cold is-
chemia-reperfusion injury. Briefly, during cold preservation of the liver with University of Wisconsin (UW) solution [39], sinusoidal lining cells rather than hepatocytes are damaged [7, 13, 24]. Upon normothermic reperfusion following cold preservation, the tissue injury is further exacerbated, even though reperfusion alone is not harmful. The deleterious effects of reperfusion following prolonged cold preservation is characterized by sinusoidal lining cell injury [7, 13, 24], leukocyte adhesion [17], platelet adhesion [19], and increased coagulation [30]. We previously demonstrated that depletion of leukocytes markedly improved hepatic microcirculation after reperfusion following 24-h cold ischemia [12]. It is known that mediators that can alter the endothelial cell surface are released after reperfusion. Among these, cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6), are thought to be important for the induction of cold preservation-reperfusion injury [27]. In addition, cell surface receptors, known as adhesion molecules and categorized as belonging to the immunoglobulin superfamily, integrin family, or selectin family, play an important role in the adhesion of leukocytes. Intracellular adhesion molecule-1 (ICAM-1) [1] and vascular cell adhesion molecule-1 (VCAM-1) [25] are the major components of the immunoglobulin superfamily, while the lymphocyte function-associated molecule-1 (LFA-1) [32] and Mac-1 [33] belong to the integrin family. The selectin family includes endothelial leukocyte adhesion molecule-1 (ELAM-1, E-selectin) [23], leukocyte-endothelial cell adhesion molecule (LECAM-1, L-selectin) [9], and granule membrane protein of 140 kD (GMP-140, p-selectin) [14]. Members of the selectin family have high affinity to the polysaccharide chain such as sialyl Lewis x (SLe^x) and sialyl Lewis a (SLe^a) as their ligands [29]. However, it is not clear whether these adhesion molecules are essential for the induction of cold ischemia-reperfusion injury, particularly in the early phase. Nor is the relationship between cytokines and adhesion molecules in this process fully understood.

In the present study, we examined the role of the immunoglobulin superfamily and of the integrin and selectin families on microcirculatory disturbances in the early phase of reperfusion following cold preservation using the isolated liver perfusion model. We also studied the effect of their monoclonal antibodies on TNF-α.

Materials and methods

Liver procurement

Male Lewis rats weighing 200–350 g were purchased from Charles River Japan (Yokohama, Japan) and used for this study. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation at our institution. The rats were anesthetized by inhalation of 1 % halothane (Takeda Pharmaceutical, Osaka, Japan) and 99 % oxygen at a flow rate of 0.2 l/min. The abdominal cavity was opened and an Intramedic PE-50 polyethylene catheter (Clay Adams, Parsippany, NJ, USA) was inserted into the common bile duct. Heparin (1000 U/kg body weight) was injected into the penile vein. A 14-G angiocatheter was inserted into the portal vein and fixed with a 4-0 silk suture. The liver was then perfused with 50 ml of chilled preservation solution at 15 cm H2O perfusion pressure. During perfusion, the connective tissues and vessels around the liver were dissected, and the liver was removed and soaked in chilled preservation solution. The liver was reperfused within a short period or 24 h after procurement.

Reperfusion

Pressure-dependent perfusion was performed according to the method described by Hamamoto et al. [11] with a slight modification, as shown in Fig. 1. Briefly, the circuit was placed in a thermoinsulated chamber (Shimizu Rikagaku Kiki, Tokyo, Japan). The liver was perfused by recirculating the perfusate through the portal vein to a Hamilton lung oxygenated with 95 % O2 + 5 % CO2. The volume of the perfusate was 20 ml. The portal pressure was continuously measured with a disposable pressure monitoring kit (Viggo-Spectramed, Singapore). The analog signal of the amplified pressure signal was digitized using an analog/digital (A/D) converter (AD112-SC98, Contec, Osaka, Japan) and stored on a personal computer (Model PC9801VX; NEC, Tokyo, Japan). The portal pressure was used as a feedback signal to control the revolution of a peristaltic rotary pump (Model Miniplus 3, Gilson Electronics, Villiers le Bel, France) and to maintain a constant perfusion pressure of 5 cm H2O. The peripheral tissue blood flow of the liver was continuously measured with a laser-Doppler tissue flow meter (Advance, Tokyo, Japan). The output signal was A/D converted and stored on the hard disk for later analysis. The temperature of the liver was monitored continuously with an electric thermometer using a zener diode (model LM335, National Semiconductor, USA) constructed in our laboratory. The temperature signal was also acquired and stored on the computer through the A/D converter board. The computer-controlled heating pad maintained the perfusion temperature at 37°C. The portal flow was calculated from the rotation of the rotary pump.

Preparation of the perfusate

For reperfusion of the liver, approximately 10 ml of whole blood was withdrawn from the abdominal aorta of an anesthetized and heparinized Lewis rat using a surgical procedure similar to that described above for the donor rats. The blood was diluted to 6 g/dl of hemoglobin with Krebs-Henseleit solution and used as diluted whole blood (DWB). For the perfusate used in Groups c and d, respective monoclonal antibodies were dissolved and left for 30 min at 4°C before use.

Monoclonal antibodies

Monoclonal antibodies to rat ICAM-1 and α Kogyo and β chains (CD18) of LFA-1 were purchased from Seikagaku (Tokyo, Japan). Monoclonal antibodies to rat L-selectin, human E-selectin, and SLe^x and SLe^a were also purchased from Seikagaku.