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Inflammatory genes in rat livers from cardiac- and brain death donors

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ABSTRACT

Background: Liver transplantation (LT) is the only life-saving treatment for patients with end-stage liver disease. The increase in patients has prompted the use of not only donation after brain death (DBD) donors but also living donors (LD) and donation after cardiac death (DCD) donors. Donor-type affects early graft function and graft survival as evidenced by an increased risk of developing ischemic type biliary lesions and higher risk of graft loss in DCD as compared with those in DBD grafts.

Methods: Using a rat model, we used quantitative reverse transcription-polymerase chain reaction to examine expression levels of proinflammatory, cytoprotective, and injury genes and determined apoptosis in DCD and DBD livers at different time points after retrieval.

Results: After retrieval, early mediators of inflammation MCP-1, HMGB1, and toll-like receptor (TLR 4) were increased in DCD livers, whereas the proinflammatory genes interleukin 6, interleukin 1β, tumor necrosis factor alpha, P-selectin, and E-selectin were massively upregulated in DBD compared with those in LD livers. HO-1 was increased in both postmortem groups. After cold ischemia, DCD livers showed increased levels of MCP-1, TLR4, and HMGB1, whereas expression of proinflammatory genes in DBD liver remained high. During 12 h of cold storage, expression levels remained stable except Hif-1α and HMGB1. DCD showed higher number of apoptotic cells compared with DBD livers.

Conclusions: Compared with LD, DCD livers showed only mild upregulation of inflammatory markers, but increased levels of MCP-1, HMGB1, and TLR4, and more apoptotic cells. In contrast, DBD livers showed a massive inflammatory response. These differences in tissue injury and inflammatory response might be relevant for the outcome after LT.

1. Introduction

Liver transplantation (LT) is the only curative treatment for patients with end-stage liver disease. Over the years, the outcome after LT has been improved, and this has resulted in more patients eligible for an LT. LT is limited because of the shortage of liver grafts and the increase in patients on the waiting list [1,2]. In an attempt to overcome this discrepancy,

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the donor pool is being enlarged by using not only donation after brain death (DBD) donors but also living donors (LD) and non–heart-beating donors also known as donation after cardiac death (DCD) donors [3,4].

LD livers are retrieved from healthy people and have experienced minimal warm and cold ischemia, which results in decreased inflammation in the transplanted liver [5]. Experience with LD transplantation procedure plays an important role in graft and recipient survival and influences the risk of surgical complications for the donor [5]. Organs from DBD donors suffer from physiological perturbations such as severe hemodynamic changes, hormonal changes, electrolyte imbalances, pulmonary changes, hypothermia, and inflammation, which can contribute to organ damage and inferior posttransplant outcome [6–10]. In DCD donors, death is based on irreversible loss of cardiopulmonary function. Thus, in contrast to organs from DBD donors, organs from DCD donors are exposed to a period of hypotension, hypoxia, and prolonged warm ischemia time, which affect early graft function as well as graft survival [11–13]. DCD liver grafts have an almost 11 times increased risk of developing ischemic type biliary lesions and 1.8 higher risk of graft loss as compared with those of DBD grafts [12,14], leading to a higher number of graft failures, retransplantations, and decreased patient survival [11,13].

The process of brain death is relatively well described in literature [6,7,9], and strategies to optimize the organ quality of brain death donors are emerging [15,16]. In contrast, experimental studies comparing perioperative gene expression profiles in liver grafts from LD, DBD, and DCD donors are sparse. Studies are limited to a small number of time points, and a relatively small time span [10], and are focused on results after transplantation. Usually "t = 0 biopsies" are obtained at the end of the cold storage period and are unable to take into account the major pathophysiological perturbations that occur during brain death, cardiac arrest, recovery of organs, and the cold storage period. Comparative studies in organs from LD, DBD, and DCD donors are currently lacking, and the molecular mechanisms that may account for the differences in transplant outcome are unclear [15,16].

The aim of this study therefore was to compare gene expression profiles of inflammatory, cytoprotective, and injury genes in livers from LD, DBD, and DCD donors at the time of retrieval, after clinically relevant cold ischemia times, and over a time-course during cold storage.

2. Materials and methods

2.1. Experimental design

The experimental protocol was approved by the Animal Experiments Committee under the Dutch National Experiments on Animals Act and complied with the 1986 directive 86/609/EC of the Council of Europe. Male Brown Norway rats of ages 12–14 wk, weighing 250–300 g, were purchased from Harlan-CPB (Austerlitz, the Netherlands). Rats were randomly assigned to an LD, DCD, or DBD group ($n = 7$ per group). The LD group served as control for both postmortem groups. After explantation, livers were stored in University of Wisconsin (UW) solution. At 0, 2, 4, 6, and 12 h, the liver tissue samples were collected and snap frozen in liquid nitrogen until further use. Messenger RNA (mRNA) expression levels in LD, DBD, and DCD livers were examined by quantitative reverse transcription-polymerase chain reaction and compared at, and between different preservation times. Time-point 0 was defined as the time directly after retrieval. Clinically relevant cold ischemia times were defined as 2 h in the LD group and 12 h in the DBD and DCD groups. During cold storage, mRNA expression levels were determined at 0, 2, 4, 6, and 12 h. In each group, mRNA expression levels were compared with the previous time point to determine possible changes during cold storage. For immunohistochemistry, DCD and DBD livers were used directly after retrieval and after 12 h of cold ischemia and stained for cleaved caspase-3.

2.2. Experimental models

Animals were anesthetized with isoflurane. After intubation, anesthesia was maintained using a mixture of N_2O/O_2/2% isoflurane. Animals were pressure control ventilated on a Siemens Servo 900C ventilator (Maquet Critical Care AB, Solna, Sweden) with 14-cm H_2O PIP, 4-cm H_2O PEEP with a frequency of 40 breaths per minute. In the DBD group, a frontolateral trepanation was made and a balloon catheter (Fogarty Arterial Embolectomy Catheter: 5F; Baxter Healthcare Co, Irvine, CA) was introduced in the extradural space and slowly inflated, causing a gradually increasing intracranial pressure [17]. Herniation of the brain stem and brain death was confirmed by dilated and fixed pupils, the absence of corneal reflexes, and an apnea test. DBD animals received ventilation but no further anesthesia. Intra-arterial pressure was continuously monitored via a PE50 catheter (Baxter Healthcare Co., Irvine, CA) placed in the carotid artery. Only rats with stable mean arterial pressure (>80 mm Hg) during the 6 h of brain death were included in the experiment. After 6 h, the liver was removed, flushed with phosphate-buffered saline, and stored in UW solution at 4°C. In the DCD group, cardiac arrest was induced by isoflurane overdose. The cardiac arrest period started when the blood pressure had dropped to 5–7 mm Hg, and lasted 20 min, the time commonly needed before starting with cold storage of the liver. The DCD donors are type III of the Maastricht criteria [18]. In the LD group, livers were collected after 1 h of mechanical ventilation using an N_2O/O_2/2% isoflurane mixture.

2.3. mRNA expression analysis

Liver sections were homogenized for 30 s using a Pro 200 homogenizer equipped (PRO Scientific Inc, Oxford, CT) in 1.0-mL Trizol (Invitrogen, Waltham, MA). Total RNA was isolated according to the manufacturer's guidelines of Trizol (Invitrogen), precipitated, and subsequently dissolved in diethyl pyrocarbonate-treated water. RNA concentration was determined using a NanoDrop ND-1000 UV-VIS spectrophotometer (NanoDrop Technologies, Wilmington, DE) and stored at −80°C until further use.

For complementary DNA (cDNA) synthesis, approximately 1 μg of the total RNA was used as template for first-strand cDNA synthesis using M-MLV Reverse Transcriptase (Invitrogen) according to the manufacturer's protocol with 250 ng