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Assessing warm ischemic injury of pig livers at hypothermic machine perfusion

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ABSTRACT

Background: Livers originating from donation after circulatory death (DCD) donors are exposed to warm ischemia (WI) before liver transplantation (LTx). Currently, there are no objective tests to evaluate the damage sustained before LTx. This study aims to identify surrogate markers for liver injury that can be assessed during hypothermic machine perfusion (HMP) preservation. In addition, we want to use mathematical equation modeling combining these markers to improve our assessment of DCD livers for transplantation.
Materials and methods: Porcine livers were exposed to incremental periods of WI (0–120 min) and subsequently HMP preserved for 4 h. Biochemical and hemodynamic parameters were repeatedly measured in the perfusate during HMP. Subsequently, to mimic LTx, normothermic isolated-liver perfusion was applied for 2 h and the injury assessed using a morphological score.
Results: With increasing WI periods, the perfusate became more acidotic, and levels of aspartate aminotransferase (AST), liver fatty acid binding protein, redox-active iron, and arterial vascular resistance increased. A damage index, combining AST and pH (damage index $= 2 - 37 \times \beta_{AST} - 257 \times \beta_{pH}$) based on multifactorial analysis of the changing pattern of these markers, had increased sensitivity and specificity to reflect WI and reperfusion injury.
Conclusions: This proof of concept study demonstrated the potential role for objective evaluation of DCD porcine livers during HMP and the advantage to use multifactorial analysis on the markers' changing pattern.

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1. Introduction

Liver grafts from donation after circulatory death (DCD) donors are more and more used to increase access to liver transplantation (LTx) and reduce waiting-list mortality [1,2]. Before preservation livers originating from DCD donors sustain an unavoidable period of warm ischemia (WI) which is variable in time and results in an enhanced

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ischemia–reperfusion injury (IRI) after LTx. Clinically, this translates into inferior graft survival because of an increased risk of graft dysfunction and ischemic-type biliary strictures which are the main reasons why the initial enthusiasm among transplant surgeons for DCD liver grafts has blunted. Although increasing length of WI is associated with poorer outcome, there is still no exact definition on the duration of WI; consequently, the exact injury is not known before LTx from DCD donors.

Today, the surgeon's evaluation remains the sole basis to accept or refuse a liver graft while taking into account the donor's medical history, biochemistry, and macroscopic aspect of the liver [3]. Additionally, the histologic aspect of the liver reflecting preexisting damage such as anoxic vacuoles can be taken into consideration [4,5].

No objective parameters, albeit identified in experimental settings to objectively assess liver injury and viability, are nowadays routinely used clinically [6]. Such biomarkers are potentially present in the effluent collected after static cold storage (SCS) [7,8]; alternatively phosphorus-31 magnetic resonance spectroscopy can be used to study the metabolic parameters of the organ during SCS [9,10].

Hypothermic machine perfusion (HMP) preservation is an alternative method to SCS for the preservation of donor organs [11–14]. For all types of kidney grafts, HMP offers superior preservation compared with SCS [15,16]. In addition, HMP provides a window between procurement and transplantation during which grafts can be assessed. During kidney HMP, some biochemical parameters of the released perfusate and hydrodynamic parameters are found to independently correlate with the outcome, which can help clinicians in their decision making [17].

In contrast to the kidney, data on HMP of the liver are very scarce. Today, one pilot trial has proven the feasibility of human liver HMP to preserve predominantly standard criteria donor livers suggesting attenuation of IRI [18]. In this study, a correlation between the aspartate aminotransferase (AST) release in the perfusate during HMP and the peak of AST after LTx was observed. However, such markers have not yet been studied during HMP of livers from DCD donors.

The selection of biomarkers that we investigated for this study was guided by current viability testing used during HMP preservation of kidneys and livers. These biomarkers include lactate dehydrogenase, alanine aminopeptidase, and fatty acid binding protein (FABP). All these biomarkers are intracellular enzymes and proteins released from the WI-damaged cells into the preservation solutions [19,20]. However, they were not independent to each other for the viability of DCD kidneys [20–22]. Another marker in the perfusate was redox-active iron reported by de Vries *et al*. It was shown to be an independent marker of the aforementioned enzymes and proteins for the viability of DCD kidneys [21]. Besides these biomarkers, the vascular resistance (VR) during HMP has been used as a hydrodynamic marker for DCD kidney viability clinically [19,23]. Experimentally, in small animal studies, hepatocellular enzymes in the effluent such as lactate dehydrogenase, alanine transaminase, and AST were found to be useful indicators of graft viability [24]. The energy metabolism of the cells was also monitored (glucose and adenosine triphosphate [ATP]).

An additional major consideration for us was that the markers we want to use for damage assessment should be widely available as a clinical test and the time between sample collection (perfusate) and obtaining the result should fit the decision process of the transplant surgeons on the transplantability of the liver grafts.

The aim of this study was firstly to identify in a preclinical model of DCD-LTx during HMP objective and reliable parameters reflecting the WI injury. Second, we aimed to evaluate the value of curve fitting of markers' changing pattern and multifactorial analysis to evaluate the WI injury.

2. Materials and methods

2.1. *Liver procurement, exposure to WI, and HMP*

We used our previously described preclinical large animal model of DCD-LTx, but the transplantation was replaced by normothermic isolated-liver perfusion [25]. Briefly, 36 inbred female Landrace pigs weighing 25–40 kg were treated in accordance to national and international guidelines on animal welfare [26,27]. Pigs were fasted 12 h before surgery with free access to water. Under general anesthesia, the livers were dissected free from the peritoneal attachments, and the gallbladder was removed. Hepatic WI injury was provoked by clamping the portal vein (PV), thoracic aorta, and *vena cava* after administration of intravenous heparin. Livers were exposed to incremental WI time (0–120 min; six livers in each group) as used previously [4,25,28] and then flushed with 5 L of 4°C–6°C histidine tryptophan ketoglutarate solution by gravity through PV and suprarenal aorta.

After flush out and removal, all livers were connected to a liver HMP device derived from the kidney Lifeport (Organ Recovery Systems, Chicago, IL). Livers were perfused via a pressure-controlled continuous perfusion (20 mm Hg for the hepatic artery (HA) and 3 mm Hg for the PV). To avoid excessive shear stress, portal flow was limited to $\leq$0.5 mL/g of liver/min. All livers were machine-perfused during 4 h with 2 L of oxygenated University of Wisconsin machine perfusion solution (4°C–6°C). The temperature was kept constant around 4°C–6°C, and in accordance with our previous HMP experiments, oxygenation of the perfusate at infusion was set for partial oxygen pressure (pO_2) of 330 $\pm$ 90 mm Hg [29].

2.2. *Evolution of biochemical parameters and VR during HMP*

A total of eight biochemical parameters related to liver injury were determined in the perfusate at different time points as shown in Figure 1. Levels of pH, potassium, lactate, and glucose were determined immediately after sampling using a Radiometer ABL blood gas and electrolyte analyzer (Radiometer, Copenhagen, Denmark). Samples of the perfusate were stored at −20°C until use. AST was measured using standard spectrophotometric technique; liver FABP (L-FABP) [30] using a commercially available ELISA kit (Hycult Biotechnology, Uden, the Netherlands); ATP release [31] by the commercially available ATPlite 1-step kit (Perkin Elmer, Zaventem, Belgium); and redox-active iron was measured by a bleomycin assay as previously described [21].

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