Transplant Immunology 31 (2014) 98–104

Contents lists available at ScienceDirect

Transplant Immunology

journal homepage: www.elsevier.com/locate/trim

No effect of remote ischaemic conditioning on inflammation in a porcine kidney transplantation model

Niels Secher [a,*], Peter Soendergaard [b], Kristian Ravlo [b], Christoffer Soelling [a], Asger Granfeldt [a], Lise Wogensen [c], Anna K. Keller [d,e], Ulla Moeldrup [e], Ernst O. Ostraat [e], Troels M. Joergensen [e], Bente Jespersen [b], Else Toennesen [a]

[a] Department of Anaesthesiology and Intensive Care Medicine, Aarhus University Hospital, Noerrebrogade 44, Aarhus C 8000, Denmark
[b] Department of Renal Medicine, Aarhus University Hospital, Brendstrupgaardsvej 100, Aarhus N 8200, Denmark
[c] The Research Laboratory for Biochemical Pathology, Aarhus University Hospital, Noerrebrogade 44, Aarhus C 8000, Denmark
[d] Institute of Clinical Medicine, Aarhus University Hospital, Brendstrupgaardsvej 100, Aarhus N 8200, Denmark
[e] Department of Urology, Aarhus University Hospital, Brendstrupgaardsvej 100, Aarhus N 8200, Denmark

ARTICLE INFO

Article history:
Received 11 April 2014
Received in revised form 26 May 2014
Accepted 27 May 2014
Available online 4 June 2014

Keywords:
Apoptosis
Cytokine
Inflammation
Kidney transplantation
Remote ischaemic conditioning

ABSTRACT

Main problem: Delayed graft function after kidney transplantation is associated with decreased graft survival and increased patient mortality but the pathogenesis is poorly understood. Remote ischaemic conditioning (rIC) may prevent delayed graft function by an anti-inflammatory effect. In a porcine model of transplantation from adults to children, we investigated the inflammatory response in the transplanted kidney and the effect of rIC.
Methods: Kidneys were recovered from brain dead donor pigs(63 kg) and transplanted into two groups of recipient pigs(15 kg) after 22 h of cold ischaemia. Recipients were randomised to either: rIC ($n = 8$) performed before the 10-h reperfusion period or no-rIC ($n = 8$). Non-transplanted kidneys from eight brain dead pigs served as controls.
Results: Compared to controls, transplantation increased the number of apoptotic cells, macrophages and neutrophils in the kidney. After transplantation, IL-10 levels increased and IL-6 levels decreased in the kidney, whereas levels of TNF-α and IL-8 were not affected. A significant rise in plasma IL-1β and IL-6 was observed in the recipients after transplantation. Plasma IL-10 was not affected by transplantation and TNF-α and IL-8 were below detection limit. No effect of rIC was found with regards to cell infiltration or cytokine production.
Conclusion: Renal transplantation elicits an inflammatory response in the kidney manifested as apoptotic cell death, macrophage and neutrophil infiltration, and an anti-inflammatory cytokine response 10 h after transplantation. This response was not modified by rIC.

1. Introduction

Delayed graft function (DGF) has implication on graft survival, rejection rate and patient mortality [1,2]. The risk of DGF is increased when organs are recovered from brain-dead donors or transplanted from large donors to small paediatric recipients [3,4].

Most of the current knowledge on ischaemic renal injury is based on rodent ischaemia-reperfusion (IR) models and the pathogenic pathways leading to DGF are not well understood. In man, pro-inflammatory cytokines may play a role in the deleterious effects of IR leading to DGF [5,6].

The presence of pro-inflammatory cytokines in the kidney leads to infiltration of macrophages and neutrophils—cells that may contribute to renal damage [7]. The release of pro-inflammatory cytokines may also damage the kidney by the induction of apoptosis [8].

There is no efficient prophylactic treatment to avoid DGF. Several clinical studies have confirmed that remote ischaemic conditioning (rIC) can prevent ischaemic myocardial injuries [9,10]. rIC is brief, repetitive, non-damaging periods of ischaemia, inducing a systemic protection against ischaemic reperfusion injuries in remote organs [11]. The mechanism of rIC has been extensively studied, and one proposed signalling pathway is a reduction of the inflammatory response [12].

Clinical studies in patients undergoing percutaneous coronary intervention support a beneficial effect of rIC on kidney IR injury [13,14]. In this porcine model, we have previously shown improved GFR and increased renal plasma perfusion in transplanted kidneys when the recipients were exposed to rIC [15]. Experimental studies have furthermore shown an attenuation of inflammation and cellular injury in transplanted kidneys [16].

Abbreviations: DGF, Delayed graft function; IR, Ischaemia-reperfusion; rIC, Remote ischaemic conditioning; MAP, Mean arterial pressure.

* Corresponding author at: Department of Anaesthesiology and Intensive Care Medicine, Aarhus University Hospital, Nørrebrogade 44, DK-8000. Tel.: +45 78 46 28 52; fax: +45 78 46 15 18.
E-mail address: niels.secher@me.com (N. Secher).

http://dx.doi.org/10.1016/j.trim.2014.05.005
0966-3274/

The present study is part of the experimental porcine study published by Soendergaard et al. in which adult kidneys are transplanted into piglets mimicking the clinical situation when adult kidneys are transplanted into children [15]. This donor–recipient combination was chosen to expose the transplanted kidney to a high risk of DGF [17].

2. Objective

The aim of the study is to describe the inflammatory and apoptotic response following brain death and renal transplantation and to investigate the effect of rIC on the transplanted kidney.

We hypothesise that transplantation causes apoptotic cell death, leukocyte infiltration and a pro-inflammatory cytokine response in kidneys from brain dead donors. Furthermore, we hypothesise that rIC attenuates the inflammatory response in the transplanted kidney.

3. Materials and methods

3.1. Experimental protocol

In a paired randomised study, eight kidney pairs were surgically removed from eight brain-dead donor pigs (60–64 kg) and transplanted to sixteen bilateral nephrectomised recipient pigs (14–16 kg) randomly allocated to "rIC" or "no-rIC." Remote ischaemic conditioning was carried out before reperfusion of the kidney, and animals were observed for 10 h after transplantation. Eight kidneys from unilateral nephrectomised brain-dead pigs (59–66 kg) served as controls (see Fig. 1).

The study was approved by the National Committee on Animal Research Ethics no. 2008-561-1584 (Animal Experiments Inspectorate, Copenhagen, Denmark) and conducted in accordance with the "Principles of Laboratory Animal Care" (NIH publication Vol. 25, No. 28 revised 1996).

In the same animals, renal function measured by GFR, renal plasma perfusion and renal biomarkers was evaluated. These data have been published by Soendergaard et al. [15].

3.2. Animal preparation and surgical procedures

Female Danish Landrace pigs were anaesthetised and mechanically ventilated. Ringer acetate was infused to maintain fluid balance and keep mean arterial pressure (MAP) above 60 mmHg. If fluid was insufficient to preserve MAP adrenalin and dopamine was administered. Brain death in the donor was induced by increasing intracranial pressure, as previously described [18]. Kidneys were removed, perfused and stored in 5 °C cold storage solution. Two recipients were transplanted simultaneously after having their native kidneys removed.

Experienced transplant surgeons carried out organ retrieval and transplantation.

rIC was carried out in four cycles by clamping the abdominal aorta. Each cycle included 5 min of ischaemia and 5 min of reperfusion. It was not possible to blind the surgeons or investigators to the treatment during the experiments; however, all subsequent analyses were carried out blinded. The method has been described in detail by Soendergaard et al. [15].

3.3. Samples

Arterial gasses, lactate, glucose and haemoglobin were analysed every hour (ABL 700, Radiometer, Copenhagen, Denmark).

Blood samples for cytokine analysis (TNF-α, IL-1β, IL-6, IL-8 and IL-10) were drawn at baseline, 15 min after rIC/no-rIC, and at 30, 60, 120, 240 and 360 min after reperfusion in recipients.

Renal biopsies from kidneys in the control group and the recipient group were taken before euthanisation and snap frozen in liquid nitrogen for cytokine analysis (TNF-α, IL-6, IL-8 and IL-10) or fixed in formaldehyde and paraffin embedded for immunohistochemistry.

3.4. Cytokine analysis

Plasma cytokines were analysed using a multiplex assay (Procarta® Porcine Cytokine Assay Kit, Panomics, CA, USA) following the manufactures recommendations. The assay was analyzed on a Luminex®$_{100}$ (Bio–Rad, CA, USA) using the BioPlex Software Manager 5.0. (Bio-Rad). Detection limits were 4.10 pg/mL(TNF-α), 3.93 pg/mL(IL-1β), 4.65 pg/mL(IL-6), 4.75 pg/mL(IL-8) and 3.69 pg/mL(IL-10).

Tissue cytokines were measured in triplicate (IL-8 in duplicate) by an in-house time-resolved immunofluorometric assay (TRIFMA) based on porcine-specific matched pairs of anti-cytokine antibodies in combination with recombinant cytokine standards (R&D Systems, UK) [19]. Detection limits were 391 pg/mL(TNF-α), 24 pg/mL(IL-6), 98 pg/mL(IL-8), and 391 pg/mL(IL-10).

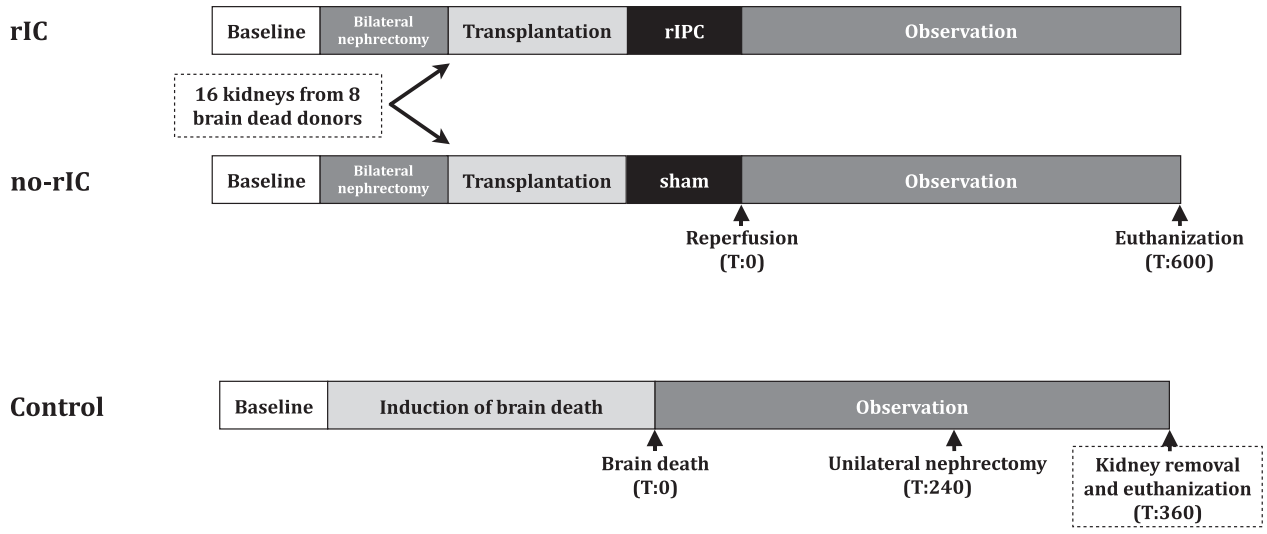

Fig. 1. Experimental protocol. Two animals were transplanted simultaneously and randomised to remote ischaemic conditioning (rIC, $n = 8$) or sham operation (no-rIC, $n = 8$) by the closed envelope principle. Kidneys from brain-dead donors where used as controls ($n = 8$). See text for details.