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The effect of the use of a TNF-alpha inhibitor in hypothermic machine perfusion on kidney function after transplantation

Piotr Diuwe[a], Piotr Domagala[a,*], Magdalena Durlik[b], Janusz Trzebicki[c], Andrzej Chmura[a], Artur Kwiatkowski[a]

[a] Department of General and Transplantation Surgery, The Medical University of Warsaw, Warsaw, Poland
[b] Department of Transplant Medicine, Nephrology and Internal Medicine, The Medical University of Warsaw, Warsaw, Poland
[c] Department of Anaesthesiology and Intensive Care, The Medical University of Warsaw, Warsaw, Poland

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ABSTRACT

One of the most important problems in transplantation medicine is the ischemia/reperfusion injury of the organs to be transplanted. The aim of the present study was to assess the effect of tumor necrosis factor-alpha (TNF-alpha) inhibitor etanercept on the machine perfusion hypothermia of renal allograft kidney function and organ perfusion. No statistically significant differences were found in the impact of the applied intervention on kidney machine perfusion during which the average flow and vascular resistance were evaluated. There were no statistically significant differences in the occurrence of delayed graft function (DGF). Fewer events in patients who received a kidney from the etanercept treated Group A compared to the patients who received a kidney from the control Group B were observed when comparing the functional DGF and occurrence of acute rejection episodes, however, there was no statistically significant difference. In summary, no effect of treatment with etanercept an inhibitor of TNF-alpha in a hypothermic machine perfusion on renal allograft renal survival and its perfusion were detected in this study. However, treatment of the isolated organ may be important for the future of transplantation medicine.

1. Introduction

One of the most important problems in transplantation medicine is the ischemia/reperfusion injury of the organs, developing at different stages: in the body of the donor undergoing brain death, in organ harvesting and storage with the potential for ischemia, in reperfusion of the organ, and in the early post-transplantation stage. This damage may affect both early and late results of organ transplantation [1–4].

The factors that have an effect on kidney injury before the transplantation include, but are not limited to: free oxygen radicals [5], proteolytic enzymes, reactive oxygen species [6,7], and apoptosis [8,9]. Endothelial cells, leukocytes, platelets [10], and many cytokines and modulators of inflammatory reactions are also activated, including the proinflammatory tumor necrosis factor alpha (TNF-alpha) cytokine [11–13]. Its expression increases in relation to brain death and after organ reperfusion [14–17]. Increased levels of circulating proinflammatory mediators that accompany brain death and reperfusion may enhance the immunogenic potential of the transplanted organs and thus contribute to alloimmunization of the recipient and to increased probability of transplant rejection reaction [4,18,19].

Interventions to limit these changes may be directed at the time before organ harvesting, when the organ is in situ in the body of the donor, during organ storage, during transplantation, and early post-transplantation. The purpose of these interventions is to modify biochemical and physical processes related to ischemia and reperfusion, and to minimize the factors that may have a destructive effect on the transplanted organ.

The use of mechanical perfusion (hypothermic machine perfusion; HMP) during hypothermic kidney storage reduced the risk of delayed graft function (DGF) and improved graft survival [20–23]. Using HMP for kidney storage is the standard in many centers worldwide. It also enables the assessment of ischemic organ injury through analysis of perfusion parameters, such as vascular resistance and flow, and the relevant chemistry tests of the perfusion fluid. A potential for organ

Abbreviations: AR, acute rejection; CI, confidence interval; CIT, cold ischemia time; CS, cold storage; DGF, delayed graft function; ECD, expanded criteria donors; fDGF, functional delayed graft function; HD, hemodialysis; HMP, hypothermic machine perfusion; HR, hazard ratio; PD, peritoneal dialysis; PNF, primary non-function; PRA, panel reactive antibody; PRU, peripheral resistance unit; RA, rheumatoid arthritis; SCD, standard criteria donors; TIT, total ischemia time; TNF, tumor necrosis factor; Tx, treatment; UW, university of Wisconsin; WIT 2, second warm ischemia time

* Corresponding author at: Department of General and Transplantation Surgery, The Medical University of Warsaw, 59 Nowogrodzka Street, 02-006 Warsaw, Poland.
E-mail address: piotr.domagala@wum.edu.pl (P. Domagala).

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therapy has emerged, because specific drugs may be administered during perfusion. These treatments include attempts to inhibit secretion of proinflammatory molecules and their precursors by inhibiting their expression at the genetic level, by blocking their target sites, or by using monoclonal antibodies against specific antigens. Such interventions may contribute to expansion of therapeutic opportunities for contemporary transplantation medicine, to improve of transplantation outcomes and to increase of the pool of organs used for transplantation.

2. Patients and methods

The study included 100 kidneys harvested from 50 organ donors deceased between April 2011 and February 2014 and 94 organ recipients who were transplanted with these organs. After harvest in the facility of the donor, the kidneys were placed in a preservation fluid in simple hypothermia (simple cold storage; CS) and transported in thermostable containers filled with crushed ice (to maintain the temperature of about 4 °C) to the transplantation clinic. The kidneys were surgically prepared in operating theater conditions. After identification of anatomic structures, a vascular cannula was placed on the renal artery and kidneys (each one separately), and the kidneys were placed in the cassette hypothermic organ perfusion LifePort Kidney Transporter (*Organ Recovery System, IL*) cassette filled with the KPS-1 fluid.

Kidneys from each pair were randomly, alternately (left/right) assigned to one of two study groups: Group A (etanercept +), where a p75 Fc receptor protein (TNF-alpha inhibitor) known as etanercept was added to the perfusion fluid after the first hour of perfusion, or the control Group B (etanercept −) without any intervention. Six of 100 kidneys included in the study were disqualified from transplantation. Four kidneys were disqualified due to elevated values of vascular resistance during machine perfusion and two kidneys were disqualified due to the diagnosis of prostate cancer in the donor found upon autopsy. In all, 94 kidney transplantation procedures were performed and each group included 47 kidneys. The kidneys that were not transplanted were excluded from the study.

2.1. Organ donors

Kidneys were harvested from deceased people after the relevant commission had confirmed brain death. The organs were obtained from 47 donors, 70.2% of whom were males. The mean age of donors was 45.3 years. The most prevalent cause of death of the donors was the cerebrovascular disease (55.3%). The donors that died of craniocerebral injuries represented 34% of the study population. Donors' mean serum creatinine levels preceding death was 1.2 mg/dL and the mean urea level was 45.1 mg/dL. The kidneys were collected from standard and expanded criteria donors. United Network for Organ Sharing criteria were adopted for the expanded criteria donors (ECD). Of 47 donors, five (10.6%) met the ECD criteria (Table 1).

2.2. Organ recipients

No statistically significant differences were found when comparing characteristics of Group A and Group B kidney recipients (Table 2). No significant differences were found in the immunological match between the recipients and the donors or in the pre-transplantation recipient immunization, measured by the level of PRA (the maximum and the last measured values) (Table 3). The most common basic immunosuppression regimen was a three-drug regimen combining a corticosteroid, tacrolimus, and mycophenolic acid ester or sodium salt. No differences were shown between groups with respect to immunosuppression regimens used.

Table 1
Kidney donor characteristics (mean ± SD).

	Donors n = 47
SCD/ECD	42/5 (89.4%/10.6%)
Age (± SD) (years)	45.3 ± 12.2
Female/male	14/33 (29.8%/70.2%)
BMI (± SD) (kg/m^2)	25.8 ± 3.6
Cause of death	
CNS trauma	16 (34%)
Cerebrovascular accidents	26 (55.3%)
Other	5 (10.6%)
Hypertension (%)	17.0
Hypotension (%)	59.6
ICU stay (days) (IQR)	5 (4. 7)
Vasopressors (%)	87.2
Urine output (last day) (mL)	4392 ± 1643
Terminal creatinine level (mg/dL)	1.24 ± 0.6

Abbreviations: BMI, body mass index; CNS, central nervous system; ECD, expanded criteria donors; ICU, intensive care unit; SCD, standard criteria donors.

Table 2
Recipient characteristics of treated (etanercept +) and untreated (etanercept −) patient groups.

	Group A (etanercept +)	Group B (etanercept −)	*P* value
Age (years)	47.1 ± 14	51.9 ± 13.8	0.102
Female/male	14/33 (29.8%/70.2%)	15/32 (31.9%/68.1%)	0.823
BMI (kg/m^2)	24.1 ± 4.2	24.5 ± 3.6	1.0
HD treatment prior to transplant	39 (83%)	38 (80.9%)	
PD treatment prior to transplant	9 (19.1%)	12 (25.5%)	0.620
Duration of HD before transplantation (months) (IQR)	32 (18. 48)	32 (17. 60)	0.763
Duration of PD before transplantation (months) (IQR)	12 (8. 39)	28 (15. 41)	0.319
Preemptive transplant	0 (0%)	1 (2.1%)	1.0
Hypertension (%)	93.6	97.9	0.617
Diabetes (%)	21.3	8.5	0.082
Ischemic heart disease (%)	14.9	19.1	0.583
Urine output before transplantation (per day) (mL)	514 ± 593	824 ± 764	0.037

Abbreviations: BMI, body mass index; PD, peritoneal dialysis; HD, hemodialysis.

Table 3
Immunological status of treated (etanercept +) and untreated (etanercept −) patient groups.

HLA mismatch (average)	Group A (etanercept +)	Group B- (etanercept −)	*P* value
loc A	0.98	1.13	NS
loc B	1.07	1.2	NS
loc DR	0.56	0.74	NS
Totality (± SD)	2.56 ± 1.2	3.07 ± 1.4	0.071
Most recent PRA (%)	2.59	3.22	0.295
Highest PRA (%)	4.21	5.88	0.868

Abbreviations: PRA, panel reactive antibody.

2.3. Etanercept

Etanercept (Enbrel) is a receptor protein, p75 Fc (manufactured by use of genetic DNA recombination) that binds the human tumor necrosis factor (TNF). Etanercept is a dimer of a chimeric protein. It is a combination of the human TNF receptor 2 domain (TNFR2/p75),