Effect of Remote Ischemic Conditioning on Local Graft Metabolism after Renal Transplantation from a Brain Dead Donor - An Experimental Study

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Abstract

Purpose: Small pediatric recipients receiving adult size kidneys from brain dead donors are at increased risk of vascular thrombosis and delayed graft function (DGF). We evaluated the local renal metabolic changes after graft reperfusion and the effect of remote ischemic conditioning (rIC) by use of microdialysis in a porcine DGF model.

Methods: Kidneys were recovered in brain dead pigs after 21h46min mean cold ischemia time. Recipient pigs were nephrectomized and randomized to rIC or control (15 kg, n=8 in each group). A control group of large recipient pigs was included as well (60 kg, n=8). The rIC protocol was four 5-minutes distal abdominal aorta clamping and unclamping cycles. Microdialysis catheters were placed cortically and extracapsularly. Follow-up was 10 hours from graft reperfusion, with hourly microdialysis samples.

Results: Cortical glucose developed significantly different (p=0.038) in the two small recipient groups, particularly in the middle of the observation period, with a higher glucose level after rIC. Glutamate decreased after reperfusion, with a systematic difference between groups (p=0.0075), due to lower levels in rIC pigs the first 4 h after reperfusion. Local lactate and glycerol showed high levels after reperfusion and decreased during follow up, with no differences between groups.

Conclusion: A gradual normalization of the local metabolism after reperfusion was demonstrated in all groups by use of microdialysis. rIC seemed to induce a faster metabolic recovery, this was significant for glutamate and glucose in the early hours after graft reperfusion and may be relevant for organ protection.

Keywords
- Delayed graft function
- Kidney transplantation
- Microdialysis
- Remote ischemic conditioning
- Swine

ABBREVIATIONS

DGF: Delayed Graft Function; rIC: Remote Ischemic Conditioning; GFR: Glomerular Filtration Rate; CIT: Cold Ischemia Time.

INTRODUCTION

Renal transplantation remains the treatment of choice for patients with end stage renal disease resulting in improved health and quality of life as well as decreased health expenses [1,2]. Small pediatric recipients receiving adult size kidneys from brain dead donors are at increased risk of vascular thrombosis and delayed graft function (DGF), most likely due to decreased renal blood flow in relation to the size of the kidney [3]. DGF is associated with impaired long-term graft function and more rejections [4]. Remote ischemic conditioning (rIC) is brief, repetitive, non-damaging ischemia and reperfusion periods in distant tissue before, during or after ischemia/reperfusion of the target organ, which may induce systemic organ protection [5-7].

Microdialysis is a minimally invasive technique for continuous monitoring of local metabolism or drug distribution. It is based on
simple diffusion over a semi-permeable membrane and the thin microdialysis catheter is placed directly in the tissue of interest. Its value has previously been established in renal metabolism monitoring and ischemia detection [8-10].

Little is known about the local metabolic changes early after reperfusion of DGF kidneys. We evaluated this and the effect of rIC by use of microdialysis in a porcine transplantation model with a high incidence of DGF.

MATERIALS AND METHODS

Experimental setup

Large healthy female Danish farm pigs were used as donors (n=16). Nephrectomy was initiated four hours after induction of brain death. The following day, the kidneys were transplanted into a total of sixteen paired small recipients, randomized to rIC or control (Figure 1) and further eight large recipients, all Danish farm pigs. Local renal metabolism was monitored by cortical and extra capsular microdialysis every 60 minutes for 10 hours after reperfusion. The study was approved by The Danish Ministry of Justice, The Animal Experimentation Inspectorate (No.2008-561-1584). Other data obtained from the same experimental setup has previously been published by Soendergaard et al [7].

Animals and anesthesia

Female Danish Landrace pigs weighing 63±2 kg (donors, n=16), 15 ± 1 kg (small recipients, n=16) or 63 ± 2 kg (large recipients, n=8) were used. Anesthesia was induced with intravenous midazolam 0.5 mg/kg and ketamine 5.0 mg/kg and in addition intramuscular atropine 0.02 mg/kg. Anesthesia was maintained with intravenous Mebumal 11.5 mg/kg/h and Fentanyl 11.5 µg/kg/h. A 750 mg Cefuroxim bolus was administered intravenously and repeated after 3 hours. Furthermore, the recipients received intravenously Ringer Acetate (small: 225 ml/h; size matched: 999 ml/h), and the small pigs additionally received 500 ml saline at experiment start and prior to graft reperfusion as well as continuous glucose monohydrate infusion (1.0 g/h). The large controls received 1000 ml saline at experiment start and 500 ml before graft reperfusion. The pigs were monitored 10 hours after reperfusion, and then put down with a pentobarbital overdose still under anesthesia. This procedure has previously been described thoroughly and published [7].

Surgical procedures

Induction of brain death in donors was achieved with a gradually inflated epidural 22 Fr Foley catheter balloon (60 mL/cc) during continuous intracranial pressure monitoring [11]. Both kidneys were removed through a midline incision in random order four hours after brain death confirmation. After flushing with heparinized saline, the kidneys were perfused with 4°C Custodiol and stored at 4°C. In the recipients the native kidneys were removed extraperitoneally through a midline incision. The grafts were Anastomosed to the distal caval vein and abdominal aorta. Grafts were placed in the space exposed after right-sided nephrectomy. Heparin was administered just before reperfusion, 4000 IE for the small and 10.000 IE for the large pigs. Microdialysis catheters were inserted superficially in the lateral renal cortex and fixed with a 5-0 suture laterally on the renal capsule [9]. A catheter was placed in ureter for urine collection and lymphatic vessels around the anastomotic site were ligated to prevent fluid loss.

Remote ischemic conditioning

Following randomization for the small recipient pigs, rIC was induced prior to graft reperfusion by abdominal aortic clamping. A corresponding aortic part was exposed in all control pigs. The rIC protocol was four cycles of 5-minutes ischemia or a similar sham period and 5-minutes unclamping followed by 15 minutes unclamping before graft reperfusion at time 0.

Microdialysis

CMA60 microdialysis catheters (CMA Microdialysis, Solna, Sweden) with a 30-mm membrane and 20,000 Dalton cutoff were perfused with ringer chloride at 0.5 µl/min by a CMA 400 syringe pump. The inlet tube was extended with a four meter custom made tube, as the pigs underwent MRI scans (data not shown here). The microdialysis catheter perfusion was initiated several hours before experiment start in order to remove air from the system. Samples were analyzed on CMA 600 microdialysis analyzer for concentrations of glutamate, glycerol and additionally lactate and glucose for cortical samples [9].

Statistics

Baseline characteristics are presented as means with standard deviations. Data for glycerol and extracapsular glutamate did not follow a normal distribution, hence a log-transformation was made. All data were analyzed using a two-way repeated measurement ANOVA with an additional random donor effect. The increasing within recipient variation with increasing levels of the parameter was taken into account by including a power of the mean variance function with a group dependent power. Serial correlation between residuals within recipients was modeled by
a Gaussian spatial autocorrelation function. Model validation was performed by inspecting within recipient standardized residuals. The data were analyzed in R 2.14.1 - R Development Core Team (2011). P<0.05 was considered as statistically significant.

RESULTS

The rIC grafts were removed from the donor 4h48min ± 44min after brain death, the small control grafts 4h47min ± 44min (p=0.96). Mean cold ischemia time (CIT) was 21h34min ± 86min for the rIC group and 21h43min ± 74min for small controls (p=0.83). For the large controls it was 21h03min ± 72min (p=0.29 vs. small controls). Two small control pigs died after 3 and 6 hours due to hypotension and arrythmia. One large control pig died after 6 hours due to circulatory collapse.

Cortical microdialysis

For the rIC group, 8 of 80 samples were missing due to technical errors; they were randomly distributed. In the small control pigs, microdialysis data were complete for all surviving animals but one, where the last 6 measurements were missing due to a defect microdialysis pump.

Local glucose (Figure 2) was higher after rIC with a significantly different development (p=0.038) in the two small recipient groups. Excluding the two recipients that died made little difference (p=0.014 for the interaction between treatment and time). A significant interaction between group and time (p=0.0043) was observed for the two control groups with significant higher concentration in the large compared to small controls from 4-6 hours after reperfusion.

Glutamate (Figure 3) decreased after reperfusion, with a systematic difference between small recipient groups (p=0.0075), due to lower levels in rIC pigs in the early hours. The difference between these two groups persisted until four hours after reperfusion. Excluding the two recipients that died made little difference (p=0.027 for the interaction between treatment and time). Comparing the large and small control groups, there was no significant interaction between group and time (p=0.31) and no significant difference between the groups (p=0.22) was observed.

Local lactate (Figure 4) showed high levels just after reperfusion and decreased during follow up with identical development for rIC and small controls (p=0.93) and no difference between these two groups (p=0.71). Comparing the large and small controls, there was a borderline significant interaction between group and time, which was mainly due to higher levels in the large group the first hour as the interaction was not significant if the analysis was confined to the time-points greater than 1 (p=0.95). Subsequently, there was no significant difference between the two groups (p=0.57).

Cortical glycerol concentration was high after reperfusion and it decreased rapidly (Figure 5). There was no significant difference in the development over time between the small recipient groups (p=0.26). Subsequently there was no significant difference in the concentration between the two small groups (p=0.63). Excluding the two recipients that died made little difference (p=0.46 for the interaction between treatment and time and p=0.85 for no difference between the two groups). Comparing large and small controls, there was the same development over time in both groups (p=0.55), with a significantly higher glycerol concentration for the large pigs (P=0.036).

Extracapsular microdialysis

For the rIC group, 18 out of 80 samples were missing,
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DISCUSSION

Microdialysis provides the opportunity for continuous in vivo investigation and monitoring of local metabolism by a minimal invasive procedure. By use of this technique, a gradual normalization of the local metabolites after graft reperfusion was demonstrated. Adult-size kidneys given to small recipients had metabolic changes suggesting slight ischemia compared to what was found in the size-matched recipients. Small recipients treated with rIC had a faster metabolic recovery than small recipients without rIC, statistically significant for glutamate and glucose in the early hours after graft reperfusion.

A highly clinically relevant model was used, with large brain dead pigs donating kidneys to small recipients. Following several pilot studies, an extraperitoneal approach was chosen in the small pigs, as pigs are extremely prone to intraabdominal fluid loss from the lymphatic system when operated intraperitoneally. This is thought to be particularly crucial in small pigs, however including all samples from one animal. For the large controls it was 32 out of 80, including all samples from two animals. For the small controls it was 22 out of 80, including missing samples due to the defect pump and the two deceased pigs.

For glutamate, the interaction between group and time was just significant (p=0.042). Considering small control recipients and rIC recipients only there was still a borderline significant interaction between group and time (p=0.041), and this was due to a difference at the first hour with lower levels after rIC (Figure 6). Regarding the groups of small and large controls, there was no significant interaction between group and time (p=0.44), and subsequently no difference between the two groups (p=0.29) was observed.

For all groups there was a clearly significant decrease of the glycerol concentration over time after 2 hours (p<0.0001). The interaction between group and time was significant (p=0.0043) (Figure 7). After 2 hours there was no significant difference between the three groups (p=0.14 for the interaction between group and time, and subsequently p=0.42 for no group effect).

Figure 4 Lactate (mmol/l) measured by microdialysis in renal cortex of small recipients. Black circles indicate the group subjected to remote ischemic conditioning. The 95% confidence intervals are shown. There were found no lactate differences between small recipients groups (p=0.71).

Figure 5 Glycerol (µmol/l) measured by microdialysis in renal cortex of small recipients. Black circles indicate the group subjected to remote ischemic conditioning. The 95% confidence intervals are shown. There were no significant differences between the small recipient groups (p=0.26).

Figure 6 Glutamate (µmol/l) measured by microdialysis on the renal capsule of small recipients. Black circles indicate the group subjected to remote ischemic conditioning. The 95% confidence intervals are shown. There were no statistical differences between the two small recipient groups (p=0.29).
many studies with large porcine transplantation models fail to
describe the exact transplantation procedure with regard to
peritoneum [12,13]. To our knowledge this problem has not
been reported within human renal transplantations. One of
the challenges managing small pediatric recipients in the clinic
involves aggressive fluid therapy [3], as graft hypoperfusion is
thought to play a key role in the higher incidence of DGF and
thrombosis in small children. Initial relative hypoperfusion may
also deteriorate the capacity for later increase of Glomerular
Filtration Rate (GFR) in a growing pediatric recipient [14].
In spite of intensive fluid therapy and meticulous anesthetic
monitoring, one of our small control pigs died due to irreversible
hypotension. Another small recipient died of arrhythmia. It
has previously been described that rIC decreases the rate
of malignant arrhythmias in pigs with coronary ischemia [15].
This study does not have sufficient power to conclude whether
rIC reduces malignant arrhythmias. As a control, a size matched
control group was included, and only slight differences were
observed in the local metabolism between the two groups. We
found significantly higher glycerol in the size-matched controls,
but it was decreasing in both groups and differences in the relative
recovery may have contributed to this difference. Glucose on the
other hand showed different development with a decline in the
middle of the observation period for the small pigs that may be
explained by graft hypoperfusion in the small recipient.

Remote ischemic conditioning (rIC) is a further development
of local ischemic preconditioning first described more than
25 years ago [16]. Instead of applying the protective ischemic
event to the target organ, ischemia is induced in a remote
tissue, typically by an inflatable cuff on an arm or leg, thereby
potentially inducing a protective response against ischemia/
reperfusion (injuries) [17]. rIC has been investigated in various
clinical and experimental studies, predominantly focusing on
the heart, where it increased myocardial salvage [6,18,19].
Both in porcine and rodent animal models there are conflicting
results regarding the potential effect of rIC on salvaging renal
function after ischemia/reperfusion [5,20-22]. In clinical studies
the renal protective effect of rIC has been demonstrated after
major cardiovascular surgery known to cause renal injury [23].
However, as more rIC studies are emerging, conflicting results
have been described [24-26]. So far rIC has not definitely been
proved to prevent acute kidney injury, although our porcine
study suggested better preservation of GFR and renal plasma
perfusion after transplantation from a brain dead donor [7].

rIC induced a lower glutamate release and glucose levels
were increased compared to the untreated small recipients -
similar to the levels observed in the large control recipients. A
decrease in reperfusion glutamate levels in response to ischemic
preconditioning has previously been described in the heart [27]
and in brain tissue [28]. Post ischemic glutamate administration
reduced myocardial infarct size to the same extent as ischemic
preconditioning, without additive effect, suggesting involvement
of glutamate metabolism in cardioprotection [27,29]. In the
kidneys, several studies have demonstrated a glutamate increase
in response to warm ischemia [8-10]. However, a recent porcine
study with prolonged cold ischemia before transplantation and
high susceptibility to thrombosis found no glutamate increase
in spite of changes in other ischemia markers [13]. The authors
speculate that the extended CIT (24h) impaired the cellular K+-
glutamate exchange due to higher K+ levels, and thus constant low
levels of glutamate persisted. This is in contradiction to a recent
study with DGF pigs subjected to renal ischemia [30] where a
glutamate increase was seen. In our present study we found
the lower glutamate in response to rIC in the early hours after
reperfusion. This may suggest an organ protective effect of rIC,
but further studies are warranted on the exact role of glutamate
in different compartments. The increased extracellular glucose
concentration in the presence of similar lactate levels after rIC is
consistent with reduced glucose uptake due to reduced glucose
oxidation. This finding supports the induction of a gradual wake
up of oxidative metabolism during reperfusion following rIC as
observed in the heart [29].

Placement of microdialysis catheters next to the tissue of
interest has previously been described for the kidney with the
extra capsular approach [9,10] and for other tissues, for example
the Achilles tendon, where a significant correlation has been found
between peri-tendinous and intra-tendinous concentrations of
lactate and glucose [31]. For detection of ischemia or anastomotic
leakage after gastro surgery, peritoneal microdialysis close to the
anastomosis has been suggested [32,33], as placement in the
intestinal wall, may be too traumatic with a severe inflammatory
response as a consequence [34]. Also ischemia in the myocardium
can be detected by microdialysis catheters placed on the surface
[35]. In our set-up we experienced an unacceptable high rate of
missing samples from the extracapsular catheters. The catheters
were perfused by the same pumps as the cortical catheters where
we did not see these problems. A plausible explanation could
be that the suture fixing the microdialysis catheter to the renal
capsule may have damaged the fragile microdialysis membrane
when the pigs were moved during the study. This is an important

Figure 7 Glycerol (µmol/l) measured by microdialysis on the renal
capsule of small recipients. Black circles indicate the group subjected
to remote ischemic conditioning. The 95% confidence intervals are
shown. No statistical significant differences were observed between
the small recipient groups (p=0.42).
observation, as the same is very likely to occur in a patient who is no longer confined to the bed after the operation and hence we do not recommend the extracapsular approach.

CONCLUSION

A gradual normalization of the local renal metabolism after graft reperfusion was demonstrated in all groups by use of microdialysis. Remote ischemic conditioning seemed to induce a faster metabolic recovery after graft reperfusion, and this was significant for glutamate and glucose in the early hours. Cortical placement of the microdialysis catheters was not associated with any complications.

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