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EXPERIMENTAL STUDY

Ischemia-reperfusion injury in kidney transplantation from non--heart-beating donor – do antioxidants or antiinflammatory drugs play any role?

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Abstract: *Background:* Ischemia reperfusion injury (IRI) is a serious problem of transplanted kidneys from a non-heart-beating donor (NHBD). IRI is probably the main cause of primary disfunction or delayed graft function. The aim of this study was to demonstrate the reduction of IRI by intravenous application of antioxidants or immunosuppressives to the recipient before the kidney transplantation in an experimental model.
Method: Piglets weighing between 20–25 kg were used (n=45) for the experiment. Intravenous application of multivitamins (GI) and a combination of immunosuppressives (GII) was tested one hour before the kidney transplantation from the NHBD. In control group (GIII) simple NHBD modelling was used. Plasma levels of malondiadehyde (MDA) and reduced glutathione (GSH) were assessed at intervals of 0, 20, 60 and 120 minutes after the kidney transplantation. Concentrations of both MDA and GSH were also assessed in the transplanted kidney before and 120 minutes after transplantation.
Results: A permanent increase in MDA plasma concentrations occurred in GIII. In GI and GII, after a transient increase in MDA plasma levels within the first 20 minutes after reperfusion, it decreased permanently ($p<0.05$, $p<0.01$). MDA plasma levels were not significantly different between GI and GII groups, but both groups differed from GIII ($p<0.001$). GSH plasma levels and tissue concentrations of MDA and GSH were not statistically significant in any group in the course of the experiment.
Conclusion: Intravenous application of multivitamins or immunosuppressives before kidney transplantation could have a significant influence on the immediate function of transplanted kidneys from a NHBD *(Tab. 3, Fig. 1, Ref. 13).*
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Key words: ischemia reperfusion injury, non heart beating donor, multivitamins, immunosuppressives.

Ischemia reperfusion injury (IRI) is a very important factor that participates in the immediate and long-term function of transplanted kidneys from a non-heart-beating donor (NHBD). The more intensive the development of IRI after transplantation, the higher the probability of acute rejection of the graft, which is connected with the development of chronic rejection of the transplanted kidney. Kidneys procured from NHBD are burdened by long warm ischemia time and a significant development of IRI. This results in high incidence of primary non-function (PNF) or a delayed graft function (DGF) of the transplanted kidneys. It is generally known that kidneys with DGF have higher incidence of acute rejection and worse long-term results when compared to normally functioning kidneys immediately after transplantation. In our previous experimental studies (1, 2) we demonstrated a close connection between IRI development and the production of reactive oxygen species (ROS) and inflammatory cytokines in the kidneys retrieved from NHBD immediately after their transplantation.

Antioxidants and immunosuppressives can considerably influence the severity of IRI by reducing the inflammatory response immediately after reperfusion of the transplanted kidney from the NHBD, and can thus hypothetically improve its immediate and long-term function.

We hypothesized that the application of agents with antioxidant and anti-inflammatory effects would have an influence the on the severity of IRI. To test this hypothesis, we examined the effect of intravenous application of multivitamins and mycophenolate mofetil plus tacrolimus to the recipient one hour before kidney transplantation.

Material and method

Male Landrace pigs (n=45) weighing between 20 to 25 kg, were used for this experimental study. All procedures complied

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The animals were divided into three groups. Multivitamins - retinoli palmitas, thiamini hydrochloridum, riboflavini natrii phosphas dihydricus, nicotinamidum, dexpanthenolum, pyridoxini hydrochloridum, acidum ascorbicum, tocoferoli alfa acetas, tocoferolum alfa racemicum (Multibionta N, Merck KGaA, Darmstadt, Germany) were given intravenously (2 ml/min) in a dose of 10 ml in 100 ml of physiological solution per animal immediately after left kidney retrieval – group I (n=15). Immediately after the left kidney retrieval, the GII group (n=15) was given a continuous 60-minute intravenous infusion composed of mycophenolate mofetil (MMF, CellCept, Roche, Basel, Switzerland), in a dose of 20 mg/kg of the animal's weight together with tacrolimus (Prograf, Astellas Pharma, Prague, Czech Republic), 0.1 mg/kg, and hydrocortisone (Hydrocortisone Valeant, Valeant Czech Pharma, Prague, Czech Republic) in a dose of 3 mg/kg. GIII (n=15) served as a control group with NHBD simulation only. The immunosuppressive combination was chosen according to our clinical protocol of immunosuppression in patients after kidney transplantation from NHBD.

The quality of kidney procurement was evaluated by light and electron microscopy. The degree of kidney glomerular damage, tubular epithelium damage and interstitial inflammation was determined using the three-grade scale. The method of histological evaluation was described in our previous study (3). Kidneys in which one of the two scales overlapped the Stage 2 were eliminated from the study because of improper procurement, which could have influenced the graft perfusion after transplantation. At the end of our study, we thus evaluated 41 kidneys (GI – 14, GII – 14, GIII – 13).

The whole experiment was performed under general anaesthesia with regulated ventilation. The animals were premedicated intramuscularly with a combination of azaperone (5–6 mg/kg) and atropine (0.075 mg/animal). A peripheral venous catheter was inserted through the auricular vein and a central venous catheter through the jugular internal vein. During the introduction of total anaesthesia, before the tracheal intubation, thiopental in dose of 7.5–10 mg/kg was administered intravenously. After the tracheal intubation, the animals were ventilated by a combination of conventional regulated PCV ventilation with an average setting as follows: T=0.8 s, in P=16 cmH_2O, BR=28/min, PEEP=5 cmH_2O, FiO_2=0.3u. During the procedure, the animals were anaesthetized by an intravenous application of ketaminum (Calypsol) with an average dose of 0.2 mg/kg and pancurionium bromide at a dose of 0.1 mg/kg. A volume expander with a starch base was applied intravenously (10 % HES), as well as Ringer lactate 1/1 at a 1:1 ratio with a speed of 50=80 ml/hour. Inotropic support was given by an intravenous application of dopamine at an average dose of 2–3 ug/kg/min.

NHBD simulation was performed after midline laparotomy by clamping the hilum of the left kidney with a vascular clamp for 45 minutes. 4 minutes before applying the vascular clamp we administered intravenously 100 units of heparin/kg of animal's weight. After 45 minutes of left kidney hilum clamping, the left kidney was removed, washed by 200 ml of Histidine-Tryptophan-Ketoglutarate solution (Custodiol, HTK, Dr. Franz Kohler Chemie, GmbH,Germany). A wedge-shaped biopsy was taken from the upper pole of the kidney and subjected to evaluation of malondialdehyde (MDA) and reduced glutathione (GSH) cortex and medulla tissue levels. Tissue samples for biochemical analysis were frozen immediately and stored at a temperature of -80 °C. The left kidney was autotransplanted after 60-minute administration of intravenous medicaments (GI, GII). Heterotopic autotransplantation was performed via previous laparotomy. End-to-side aortic and inferior vena cava anastomoses were performed just above the iliac bifurcation. The ureter was inserted via the lower pole of laparotomy in a plastic bag. Venous blood was sampled directly from the renal vein at time intervals of 0, 20, 60 and 120 minutes for the assessment of MDA and GSH.

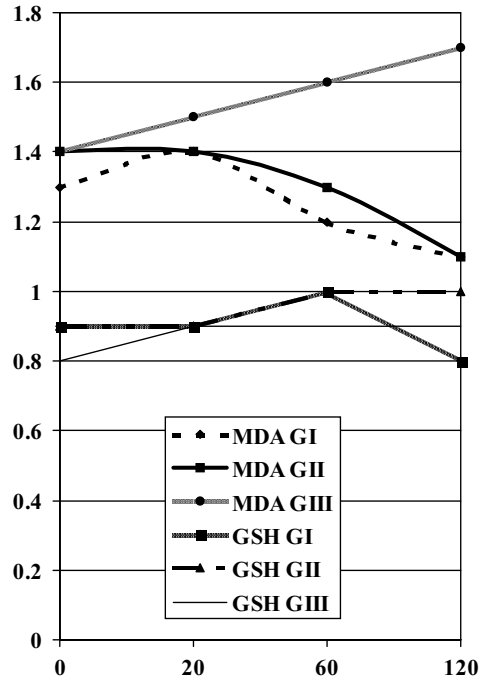

Fig. 1. Plasma levels of MDA (µmol/l) and GSH (nmol/l) in pigs during the experiment (mean ± SD; NS – non significant).

After 120 minutes of kidney reperfusion, a wedge-shaped biopsy was taken from the upper pole of the kidney and subjected to evaluation of MDA and GSH cortex and medulla tissue levels and the animals were killed by using an intravenous dose of 40 ml of 7.5 % potassium chloride.

GSH was determined spectrophotometrically: thiols react with 4-chlor-1-methyl-7-trifluormethylchinolinium methylsulphate forming thiothers; under strongly alkaline pH GSH-thiother was then converted to a colored thione. The result was expressed in mmol/l erythrocytes. Sodium heparinate was used as an anticoagulant, measurements were performed on an Olympus AU 400 Analyzer (Olympus, Japan) with reagent kits GSH 400 (OXIS, Portland, USA).

Tab. 1. Evolution of MDA (µmol/l) and GSH (mmol/l) plasma levels in GI (n=14), GII (n=14) and GIII (n=13) during 120 minutes of the experiment (mean ± SD; NS – not significant).

Time (min)	MDA GI (µmol/l)	MDA GII (µmol/l)	MDA GIII (µmol/l)	GSH GI (mmol/l)	GSH GII (mmol/l)	GSH GIII (mmol/l)
0	1.34±0.26	1.39±0.18	1.40±0.22	0.92±0.17	0.86±0.13	0.88±0.24
20	1.41±0.21	1.43±0.21	1.52±0.18	0.90±0.14	0.89±0.16	0.90±0.17
60	1.25±0.19	1.27±0.22	1.64±0.21	0.96±0.23	0.95±0.24	0.89±0.19
120	1.08±0.12	1.12±0.19	1.68±0.14	0.83±0.20	0.97±0.19	0.93±0.16
Kruskal-Wallis p<	0.05	0.01	0.05	NS	NS	NS

Tab. 2. Mean plasma levels of MDA and GSH in GI (n=14), GII (n=14) and GIII (n=13) calculated from measured levels of blood samples in 0, 20, 60 and 120 minutes of experiment.

	MDA (µmol/l)	GSH (mmol/l)
G I	1.27±0.19	0.90±0.18
G II	1.30±0.20	0.91±0.18
G III	1.56±0.18	0.90±0.19
ANOVA	p<0.01	NS
Wilcoxon	p<0.001	NS
Median test	p<0.001	NS

MDA was determined photometrically in heparin plasma after a reaction with thiobarbituric acid as thiobarbituric acid-reactive substances (TBARS), according to Jentzsch et al. Although this reaction was not specific for MDA, it was confirmed that most of TBARS are produced only during the process of lipid peroxidation.

MDA and GSH concentrations in the kidney cortex and medulla were measured after tissue homogenization in a cold physiological saline solution and expressed as nmol (MDA) or µmol (GSH) per gram of protein.

Statistical software STATISTICA 98 was used for statistical evaluation. The values of individual parameters were expressed as averages with standard deviations. For statistical processing of the data, which was intended to determine the over-time differences in values of individual parameters in the given groups, the non-parameter ANOVA test – Kruskal-Wallis test – was used. The differences in the given figures between groups at individual times were also evaluated by the Kruskal-Wallis test. $p<0.05$ was considered statistically significant.

Results

In the control group, there was a continuous increase in MDA levels during reperfusion, which served as a marker of IRI development with ROS production. In GI and GII groups, a temporary increase in MDA plasma levels in the first 20 minutes after reperfusion was followed by a decrease in their levels in the course of the experiment, which showed sufficient saturation of the recipient with substances preventing the development of ROS. In both groups the decrease in MDA on the time scale was statistically significant ($p<0.05$, $p<0.01$, respectively) (Tab. 1). The difference in MDA plasma levels between GI and GII groups did not reach any statistical significance while both groups differed considerably from the control group ($p<0.01$, $p<0.001$, respectively) (Tab. 2). GSH plasma levels were neither statistically significant during the experiment in any group nor did they reach any statistically significant differences when compared in all groups. MDA and GSH tissue levels in the medulla and cortex of the transplanted kidneys before, and two hours after transplantation did not reach any statistical significance (Tab. 3).

Discussion

The worldwide shortage of kidneys for transplantation and an increasing number of patients with end-stage renal disease (ESRD) prompt the search for new sources of kidneys suitable for transplantation. Kidneys retrieved from NHBD are a possible solution to the problem of increasing the volume of kidney transplantation. However, kidneys transplanted from NHBD are

Tab. 3. Comparison of MDA and GSH cortex (c) and medulla (m) concentrations before and two hours after kidney transplantation without any statistically significant (NS) difference.

Group (min)	MDAc (nmol/g)	MDAm (nmol/g)	GSHc (µmol/g)	GSHm (µmol/g)
I (0 min)	230.4±24.1	406.1±241.2	19.1±3.2	21.9±10.0
I (120 min)	215.8±57.4	341.3±109.2	20.4±8.5	24.6±9.1
II (0 min)	260.2±31.2	340.4±192.3	15.4±6.1	18.7±12.3
II (120 min)	223.5±47.2	271.9±153.4	16.2±9.2	20.5±9.1
III (0 min)	215.8±23.9	472.3±189.1	19.5±2.6	22.2±6.4
III (120 min)	346.1±236.8	546.8±390.4	24.1±10.2	24.1±8.3
ANOVA, Wilcoxon, Median test	NS	NS	NS	NS

still burdened with high DGF (60–70 %) and PNF (5–8 %) rate in comparison to kidneys transplanted from heart-beating donors (4, 5, 6). Probably the main problem of NHBD kidney dysfunction after transplantation is the extent of warm ischemia time, which determines the intensity of IRI.

IRI is one of the principal "antigen-independent" factors of post-transplant graft dysfunction. In addition, patients with ESRD, especially those on haemodialysis, suffer from oxidative stress owing to overproduction of ROS. During IRI after renal transplantation from NHBD, the patients are burdened with another load of ROS, which mediate the cell membrane damage through lipid peroxidation, direct oxidation of membrane proteins and the activation of endothelial cells. This results in functional and structural cell damage (7, 8, 9). IRI can have also a remote effect on other organs, e.g. liver, where it can lead to various pathophysiological changes (10). The clinical effect of this phenomenon will however require further studies.

Our recent study follows our previous experimental studies in the area of kidney transplantation from NHBD (11, 12). The problem of our previous studies was the length of WIT, which was only 30 minutes. This period however simulates the minimum of cardiopulmonal resuscitation (CPR) time in NHBD (13, 14), which is in fact longer for various reasons (length of cardiac arrest to CPR, length of CPR and "non-touch" period).

The present experimental study was therefore designed to imitate the true ischemic time of kidneys in a clinical situation during their procurement from NHBD. Therefore, we performed a 45-minute WIT of the left kidney by vascular clamp application on the left kidney hilus. We tested the simple saturation of a recipient by a complex of antioxidative (multivitamins) and antiinflammatory medicaments (immunosuppressives) which play an important role in protection from IRI. To exclude various antigenic factors during allotransplantation, we used our own experimental autotransplantation model, where one animal was simultaneously both donor and recipient.

Administration of antioxidants or immunosuppressives to the donor one hour before kidney transplantation led to a significant decrease in MDA plasma levels after 20 minutes of graft reperfusion. There were no differences in the levels of principal intracellular antioxidant – GSH and lipid peroxidation product of MDA in the graft tissue between the groups of animals. Tissue levels of GSH and MDA also did not differ between the groups of animals before and 120 minutes after the transplantation of kidneys. This phenomenon probably depends on the insufficiency in tissue saturation by ROS scavengers or immunosuppressives, owing to the relatively short interval of their intravenous application. This time interval, however, was effective in diminishing the effect of IRI in the experimental model of transplanted kidney from NHBD.

We can conclude that the intravenous application of drugs with antioxidative or antiinflammatory effects to the recipient one hour before kidney transplantation can have a significant influence on the immediate function of transplanted kidneys from NHBD.

References

1. Treska V, Kuntscher V, Molacek J, Kobr J, Racek J, Trefil L. Can the ischemia-reperfusion syndrome in transplanted kidneys procured from non-heart-beating donors be influenced by adding selenium into the reperfusion solution?An experimental study. Transplant Proc 2003; 35: 3125—3127.

2. Treska V, Molacek J, Kuntscher V, Liska V, Kobr J, Racek J, Kormunda S. Immunosuppresive agents have an influence on ischemia-reperfusion injury in kidneys procured from a non-heart-beating donor: experimental study. Transplant Proc 2004; 36: 2931—2934.

3. Treska V, Molacek J, Kobr J, Racek J, Trefil L, Hes O. Ichemic training and immunosuppressive agents reduce the intensity of ischemic reperfusion injury after kidney transplantation. Exp Clin Transplant 2006; 4:439—444.

4. White SA, Jain S, Absalom H, Murphy G, Williams ST, Knight A, Zickerman AM, Nicholson ML. Influence of Delayed Graft Function in Renal Transplants From Cadaveric or Non-Heart-Beating Donors. Transplant Proc 2000; 32: 189.

5. Brook NR, White SA, Waller JR, Veitch PS, Nicholson ML. Non-heart beating donor kidneys with delayed graft function have superior graft survival compared with conventional heart-beating donor kidneys that develop delayed graft function. Amer J Transplant 2003; 3: 614—618.

6. Rela M, Jassem W. Transplantation from non-heart-beating donors. Transplant Proc 2007; 39: 726—727.

7. Aquilar A, Alvarez-Vijande R, Capdevila S, Alcoberro J, Alcaraz A. Antioxidant patterns (superoxide dismutase, glutathione reductase, and glutathione peroxidase) in kidneys from non-heart-beating-donors: experimental study. Transplant Proc 2007; 39: 249—52.

8. Richer JP, Gibelin H, Planet M, Bardou A, Ben Amor I, Germoville T,Caritez JC, Carretier M, Eugene M, Hauet T. Ischemia-reperfusion injury is associated with inflammatory cell infiltration: evaluation in a pig kidney autotransplant model. Transplant Proc 2000; 32: 482—483.

9. Gulec B, Coskun K, Oner K, Aydin A, Yigitler C, Kozak O, Uzar A, Arslan I. Effects of perfusion solutions on kidney ischemia-reperfusion injury in pigs. Transplant Proc 2006; 38: 371—374.

10. Gulec B, Coskun K, Yigitler C, Yigit T, Aydin A, Oner K. Ischemia-reperfusion injury in the liver during renal transplantation: does perfusion solution play any role? Transplant Proc 2008; 40: 59—62.

11. Treska V, Racek J, Kuntscher V, Hasman D, Kobr J, Molacek J, Trefil L, Hes O, Reischig T. Plasma and tissue levels of free oxygen radicals in recipient sof kidneys from non-heart-beating donors: does recipient pretreatment with antioxidant drugs make sense? An experimental study in pigs.Transplant Proc 2002; 34: 3060—3064.

12. Hes O, Treska V, Hasman D, Boudova L, Kuntscher V. Determination of apoptotic cells in kidneys from non-heart-beating donors and heart-beating donors using the TUNEL assay: a limited immunohistochemical method? Transplant Proc 2002; 34: 1095—1097.

13. Navarro AP, Sohrabi S, Wilson C, Sanni A, Wyrley-Birch H, Vijayanand D, Reddy M, Rix D, Manas D, Talbot D. Renal transplants from category III non-heart-beating donors with evidence of pre-arrest acute renal failure. Transplant Proc 2006; 38: 2635—2636.

14. Sanni AO, Wilson CH, Wyrlex-Birch H, Vijayanand D, Navarfro A, Gok MA, Sohrabi S, Jaques B, Rix D, Soomro N, Manas D,Talbot D. Non-heart-beating kidney transplantation: 6-year outcomes. Transplant Proc 2006; 38: 3396—3397.

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