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# Retrograde Oxygen Persufflation of Kidney – Experiment on an Animal

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**Abstract.** *Aim: There is still a lack of organs for transplantation purposes. In the field of kidney and liver transplantation, one available solution is the use of organs from so-called marginal donors. These donors can be e.g. non-heart-beating donors. In these cases, perfusion and preservation of organs intended for transplantation is generally more difficult. Retrograde oxygen persufflation (ROP) may be a possible solution to this issue. This method is based on retrograde perfusion by oxygen through the renal vein thus reconditioning the organ. Materials and Methods: We operated on 10 animals (porcine models). Ischemic injury of the right kidney was simulated in all animals . In group A (N=5), kidneys were perfused with retrograde oxygen persufflation after explantation. In group B (N=5), kidneys were perfused intrarterially as in usual clinical practice. After perfusion all kidneys were transplanted to the original donor animal. Quality of graft restitution was evaluated by the urea level obtained from the renal vein and by histopathological analysis after explantation. Results: We found no statistically significant differences between groups A and B in urea levels after transplantation, nor did we find any significant differences in quality of kidney parenchyma restoration between these groups. Conclusion: Retrograde oxygen persufflation is able to protect and restore kidney parenchyma.*

Today, we still see a lack of organs for transplantation purposes. In the field of kidney and liver transplantation, one solution to this problem is the use of organs from so-called marginal donors. These donors can be *e.g.* non-heart-beating donors (NHBD). In these cases, perfusion and preservation of organs intended for transplantation is generally more difficult. In current clinical practice, intra-arterial renal perfusion is initially applied *in situ*. After explantation, the graft is perfused using a perfusion pump. An alternative is customary storage in an ice-cold solution ('cold storage') applied after *in situ* perfusion. Kidneys taken from NHBD are always injured by warm ischemia. A method which would improve the quality of organs is still being sought. Retrograde oxygen persufflation (ROP) may be a possible solution to this issue. This method is based on retrograde perfusion by oxygen through the renal vein. Our objective was to simulate warm ischemia in a porcine kidney and then to preserve the graft using only venous retrograde oxygen persufflation. After kidney retransplantation, we evaluated the graft function and its degree of injury, and compared such modalities with grafts subjected to standard intra-arterial perfusion by a preservation solution. The goal was to find an answer to the question as to whether the ROP is beneficial for the preservation and recondition of organs injured by warm ischemia.

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*Key Words:* Retrograde oxygen persifflation, kidney transplantation, non-heart-beating donor.

## Materials and Methods

Male pigs (domestic pig – *Sus scrofa domesticus*, supplied by the Agricultural Department, Pilsen, Czech Republic) with a weight of approx. 30 kg were used as models in this study. The operations were performed at the Experimental Medicine Centre of the Faculty of Medicine in Pilsen, Czech Republic. The animals were provided with care pursuant to legislation of the Czech Republic applicable to work with laboratory animals and of course this experiment followed EU regulations for research on laboratory animals. The project was approved by the Ethical Committee of Faculty l of Medicine in Pilsen, Charles University in Prague, LFP 38456/2014.

*Design of the experiment*. In group A (N=5 animals), the experimental animal was put under general anesthesia using thiopental, calypsol, and fentanyl. We reached the retroperitoneum through medial laparotomy, and exposed the renal vessels in the right kidney. A kidney pedicle clamp was applied for 20 minutes (simulation of warm ischemia). Explantation of the kidney was then

performed and at the same time the first renal biopsy sample (biopsy IA) was taken. The bioptic sample was taken in the form of a wedge excision of renal parenchyma including cortex as well as medulla. After explantation, the renal graft was connected to an insufflator (Faculty of Biomedical Engineering in Kladno, Czech Technical University in Prague, Czech Republic) by which the graft was perfused in a retrograde manner (ROP) with moisturized gaseous oxygen under a pressure of 18 mm Hg. The perfusion cannula was applied into the renal vein (Figure 1). During the procedure, the graft was placed in a cold perfusion solution (Custodiol; Dr. Franz Köhler Chemie GmbH, Bensheim, Germany) at 4˚C (for a detailed description, see below). It was necessary to needle-puncture several perforations into the renal parenchyma to allow gaseous oxygen release. The perfusion lasted 120 min. At the end of this interval, a second renal biopsy sample was taken (biopsy IIA). Then the renal graft was transplanted to original donor animal and nephrectomy of the other side (left-sided) was performed. During transplantation, the renal artery was anastomosed to the aorta (end-to-side anastomosis) and the renal vein to the inferior vena cava (end-to-side anastomosis) (Figure 2). After restoration of blood circulation through the graft, a sample of venous blood was taken from the renal vein of the transplanted kidney every 30 minutes to determine markers of renal function (in this phase of the experiment, only urea). This marker was determined directly in serum with common procedures) After 120 min, the graft was explanted, a third bioptic sample of the kidney (biopsy IIIA) was taken and the experimental animal was sacrificed using a cardioplegic solution.

In group B (N=5 animals), renal ischemia was also simulated for 20 minutes by kidney pedicle clamping. After nephrectomy, the renal graft was routinely perfused using Custodiol perfusion solution under constant hydrostatic pressure at 4˚C. The cannula was applied customarily into the renal artery (Figure 3). Perfusion was applied for the same period as in group A, 120 min. Then the kidney was transplanted in the same manner as was the other-sided nephrectomy. Blood samples were taken again from the renal vein. The bioptic samples were taken at identical intervals (biopsy IB-IIIB). After 120 min, the animals were sacrificed identically as in group A.

Histopathological examination of the graft was performed by a qualified pathologist (blinded) who generally assessed the degree of injury to the nephron. The items assessed were the quality of the glomerulara perfusion, the presence of microthrombi and, where applicable, the presence of acute tubular necrosis. Injury was assessed based on the following scale as: Level 1: complete perfusion, no erythrocytes in capillary vessels; level 2: incomplete perfusion, occasional groups of erythrocytes in capillary vessels (in particular in the glomerulus); level 3: the kidney remained non-perfused (physiological condition); level 4: non-perfused kidney, presence of acute tubular necrosis.

In this phase of the experiment, the level of urea in serum was evaluated from the venous blood samples.

*Description of the ROP procedure.*

*Experimental unit for monitoring and control of the mechanical perfusion parameters:* For the needs of the monitoring of parameters during the experiments, a multi-functional platform based on an experimental unit using the field-programmable gate array technology was designed. Specifically, an NI-PCI-7831R card (National Instruments, Austin, TX, USA) was installed in a personal computer. The individual sensors measuring pressure, flow rate, and temperature, were connected to this card together with the electronic system for the control of the pumps to provide forced mechanical perfusion. Pressure was measured both by MPXV5010 and MPXV4002 sensors (Freescale Semi., Austin,TX, USA) and pressure liquid chambers TruWave (Edwards Lifesciences, Irvine, CA, USA). Temperature measurement was performed by standard temperature sensor Pt-100 placed into glass cases (Theta90, Prague, Czech Republic) and sensors for surface temperature NTC 30k at 25˚C (LHL s.r.o., Ústí n. Labem, Czech Republic). Flow rate was measured usingSonoflow IL.52 (Sonotec, Halle, Germany) inline flow rate sensor . The sensors were completed with the necessary electronic system to achieve the required accuracy and stability. The forced perfusion was induced using gear pumps (Diener, Embrach, Switzerland) with an electronic performance control system based on pulse width modulation. Due to this control, it was possible to adjust the flow rate parameters infinitely, including the simulation of the pulsatile wave. For the needs of control of the experimental unit, parameter monitoring and log creation, a special-purpose software application was developed.

*ROP:* During ROP, the pressure of oxygen was set at 18 mm Hg (2.4 kPa) with a deviation of ±0.2 mm Hg. Pressure was measured in the tee-branch between the catheter inserted in the supply tube using the MPXV5010 pressure sensor. The temperature of the dialysate, in which the kidney was tempered, was 4˚C , maintained by the laboratory temperature controller equipped with a cooling system.

*Intra-arterial perfusion.* In the case of grafts subjected to intra-arterial perfusion by defined hydrostatic pressure, the flow rate through the kidney and the pressure, which was measured in the second lumen of the supply catheter, were also recorded. In this manner, the error of measurement caused by self-fluidic resistance was minimized. The liquid chamber was used as a sensor. The dialysate was cooled to 4˚C using the laboratory temperature controller equipped with a cooling system but due to its recirculation and the initial heating by the organ, the temperature during the experiment was actually maintained within the range of 3.9-6.4˚C. The temperature of the organ dropped to approx. 5.2˚C measured on the surface.

## Results

No technical problems with the graft perfusion or transplantation as such were found in any of the presented experimental animals. In two cases (one case in each group), aggravated segmental perfusion was discovered upon the pole artery interruption.

*Evaluation of urea level in serum.* Table I provides the values of urea in blood taken from the renal vein after the kidney transplantation at individual intervals (0, 30, 60, 120 min). We found slightly increasing levels of urea with increasing time in both groups but we did not find significant changes between those groups.

Repeated ANOVA tests for differences between groups A and B gave a value of $p$=0.843, *i.e.* no statistically significant difference.

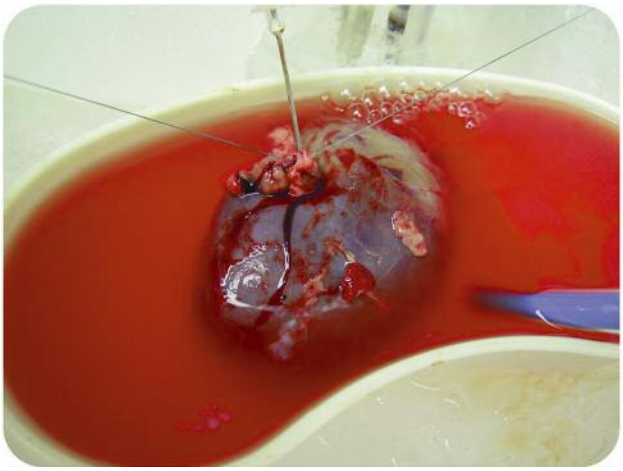

Figure 1. *Kidney retrograde oxygen persufflation.*

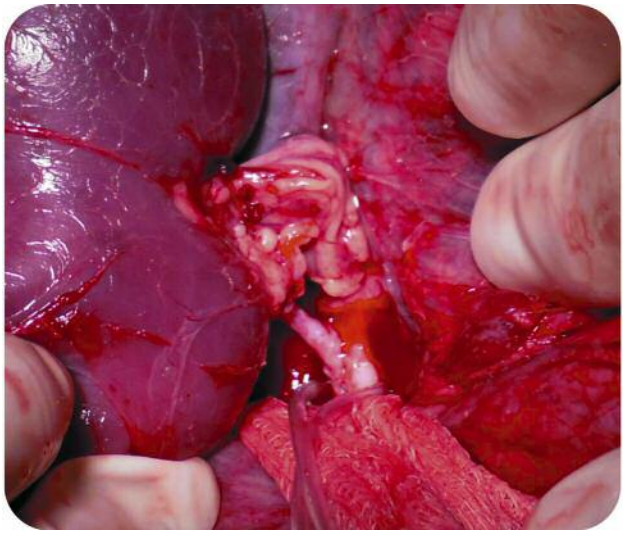

Figure 2. *Condition of the kidney after the graft transplantation.*

*Histopathological evaluation of the bioptic samples*. Table II provides the level of the quality of nephron perfusion plus the overall improvement of parenchymal quality (the presence of microthrombi, acute tubular necrosis). We also did not find any significant changes between groups in overall parenchymal quality (Figure 4 and 5).

Fisher's exact test for differences between groups at each time point was $p=1.00$, therefore the level of kidney perfusion did not differ statistically between groups A and B at at any time point.

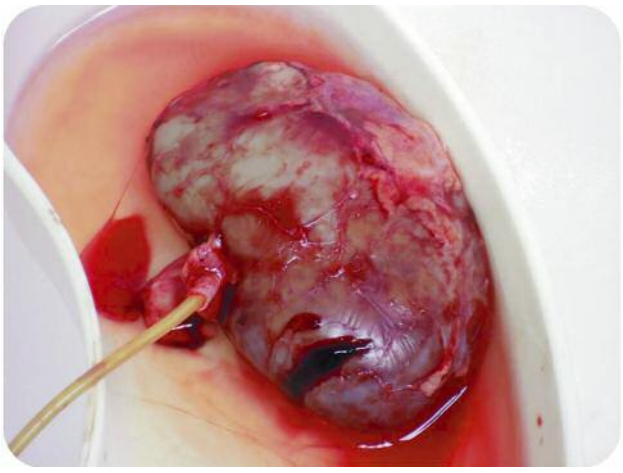

Figure 3. *Intra-arterial perfusion.*

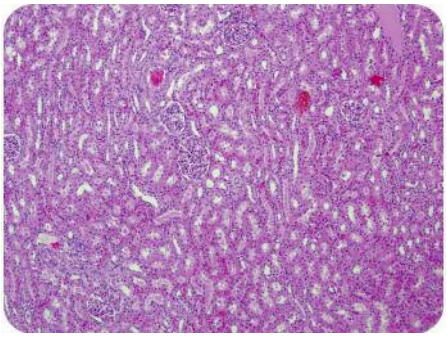

Figure 4. *Bioptic sample IIIA showing kidney after transplantation in the group with retrograde oxygen persufflation. Hematoxylin and eosin staining, magnification ×100.*

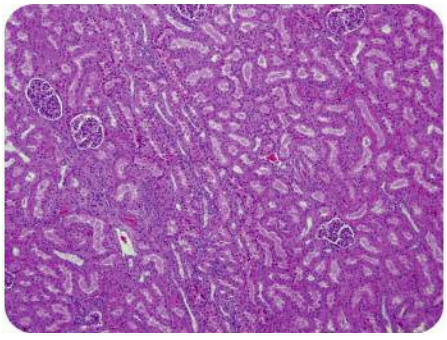

Figure 5. *Bioptic sample IIIB showing kidney condition after transplantation in the group with intra-arterial perfusion. Hematoxylin and eosin staining, magnification ×100.*

Table I. *Levels of venous urea in individual animals at the times ranging from 0 to 120 min after transplantation (time 0=declamping).*

| | Urea (mmol/l) at time | | | |
|---|---|---|---|---|
| Animal number (group) | 0 min | 30 min | 60 min | 120 min |
| 1 (A) | 4 | 4 | 6 | 7 |
| 2 (A) | 5 | 6 | 6 | 7 |
| 3 (A) | 8 | 8 | 8 | 10 |
| 4 (A) | 8 | 7 | 9 | 9 |
| 5 (A) | 6 | 7 | 8 | 8 |
| 6 (B) | 5 | 5 | 7 | 7 |
| 7 (B) | 7 | 7 | 8 | 9 |
| 8 (B) | 4 | 6 | 12 | 8 |
| 9 (B) | 7 | 6 | 8 | 8 |
| 10 (B) | 5 | 5 | 7 | 7 |

Repeated ANOVA tests for differences between the groups A and B gave a value of $p$=0.843, *i.e.* no statistically significant difference.

Table II. *Quality of the renal graft perfusion rated on the following scale as: Level 1: complete perfusion, no erythrocytes in capillary vessels; level 2: incomplete perfusion, occasional groups of erythrocytes in capillary vessels (in particular in the glomerulus); level 3: the kidney remained non-perfused (physiological condition); level 4: non-perfused kidney, presence of acute tubular necrosis.*

| | Biopsy sample | | |
|---|---|---|---|
| Animal (group) | I | II | III |
| 1 (A) | 2 | 2 | 3 |
| 2 (A) | 3 | 2 | 2 |
| 3 (A) | 1 | 2 | 1 |
| 4 (A) | 2 | 3 | 2 |
| 5 (A) | 2 | 2 | 3 |
| 6 (B) | 2 | 1 | 3 |
| 7 (B) | 3 | 2 | 2 |
| 8 (B) | 3 | 3 | 2 |
| 9 (B) | 1 | 1 | 2 |
| 10 (B) | 1 | 4 | 3 |

## Discussion

A permanent lack of organs for transplantation purposes results in a higher tolerance of the acceptance of marginal donors. This topic is closely related to the implementation of new methods for the preservation of grafts and a so-called recondition of organs after explantation from the donor This approach is justified by very good results in particular of renal grafts from NHBDs achieved in clinical practice (1-3). The primary factor injuring parenchyma is warm ischemia. Warm ischemia is especially found in NHBD. The procedures for renal graft preservation and recondition applied at individual clinics often differ (4-8).

ROP, which was discovered accidentally, was first described in the 1970s (9). A number of experimental studies have been performed in the past (10, 11). They used retrograde oxygen persufflation of organs used for transplantation (mainly kidney and liver). Results of these studies are still controversial and not very clear. For the first time probably in clinical practice, the ROP was applied to kidneys by Rolles in 1989 (12). Studies comparing the quality of grafts preserved by ROP with those routinely subjected to cold storage or intra-arterial perfusion have been performed on a repetitive basis (12, 13). It has been shown that ROP, from many aspects, can be even more beneficial than the customary preservation both for kidneys and for liver. The majority of experiments concerned small-sized animals (rats, and dogs). Other experiments with ROP dealt in particular with the bio-energy condition of grafts and their function after transplantation (13). More recent similar experiments were performed on porcine animals (14, 15). A recent article by Suszynski *et al.* describes all preservation methods for different organs very well (16).

In our experimental study, we wished to verify the suitability of ROP for a large experimental animal. From this point of view, a porcine animal is an absolutely ideal bio-model due to both its anatomy and the pathophysiology of the injury caused by warm ischemia.

The persufflator used here was designed specifically for this experimental project. Specific and fully controlled values of perfusion pressure and flow rate were provided. Technically, this is very easy and not at all costly. In this phase of the experiment, we opted to perform the evaluation of graft quality by histopathological analysis of the graft (both after preservation itself, and after the subsequent transplantation). Our scale of graft quality has been applied at our clinic on a long-term basis (in addition to the standard Remuzzi score) for the evaluation of grafts from NHBDs. The other evaluated factor was the function of the transplanted kidney, which was objectivised by regular sampling of venous blood from the graft. The marker of graft function at this point was the level of urea (contralateral nephrectomy). We are aware of the fact that the our view is simplifying to a certain extent. However, our goal was to determine whether ROP is a feasible alternative for the recondition of a renal graft.

Our results demonstrated the practically identical quality of grafts preserved both by standard intra-arterial perfusion, and ROP. We found no significant differences in the histopathological evaluation of the extracted grafts (tested using the Fisher's exact test in a contingency table for each of the times I, II and III independently), and in the values of urea in animals after the transplantation of the respective grafts. Our results are in accordance with references (10-12,

14, 15). We did not find evidence to support the opinion of some authors that ROP may even be superior compared to standard perfusion or cold storage (16). On the other hand, we have proven beyond reasonable doubt that mere ROP is able to protect and restore parenchyma.

In the next phase of the study, we would like to extend both the period of warm ischemia of the graft, and the period of restitution using ROP. In addition, we would like to extend the survival of the experimental animal at least in the order of days. It is also necessary to use new laboratory markers of renal graft function. A combination of ROP with the pharmacological application of free oxygen radical sweepers is also a possibility.

In our opinion, the aim is not to replace standard intra-arterial perfusion by retrograde persufflation. Instead, some combination of both methods seems to be promising. However, prior to clinical applications in other experimental research is necessary.

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