Immediately started pulsatile machine perfusion of a kidney graft from a DCD (donor after cardiac death) as a way to improve its properties

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Introduction: The number of kidneys available for transplantation still does not match the number of patients on the waiting list, which gets longer every year. At the same time, kidney transplantation is the only chance for the long-term survival of patients with good quality of life. Therefore, organs from marginal donors, including donors after cardiac death (DCD), have recently been used. The most common cause of death of these donors is severe trauma, especially craniocerebral trauma, or sudden cardiac arrest, followed by unsuccessful resuscitation. Kidneys can be harvested once an individual is declared dead according to the exact criteria. This involves initial washing of the grafts in the donor's body using a perfusion solution, followed by their removal and storage using selected preservation method. Mechanical pulsatile perfusion using special instrumentation is the most frequently used technique in the area of DCD. Despite of good long-term outcomes of grafts from DCDs, this group is burdened by a greater number of delayed graft function (DGF) and primary nonfunction (PNF).

Objective: To summarize the essential knowledge about DCD and its subsequent use in experimental work. Graft quality from DCD donors is significantly determined by the period just after the donor’s circulatory arrest and subsequent graft perfusion. Using a perfusion system of our own design, we first explored the ROP (retrograde oxygen persufflation) technique. This experiment led us to the idea of mechanical perfusion of the kidneys with a preservation solution already in the donor's body. The aim is to reduce the incidence of delayed onset of graft function or its primary nonfunction after graft transplantation.

Methodology: The ROP methodology was tested in a population of 10 animals (pigs) divided into two groups. All animals were subjected to simulated warm ischemia for 20 minutes. Immediately after the explantation, the ROP method was used in the first group (n = 5) and mechanical machine perfusion in the second group (n = 5) to preserve the graft, for a period of 60 minutes in both groups. Subsequently, the organ was re-transplanted to the original animal. Venous blood samples were collected at given time intervals over a period of 2 hours to determine the urea level. Biopsy samples of kidney tissue taken during and at the end of the experiment were also evaluated. The subsequent experiment studied the immediately initiated pulsatile perfusion. The first part consisted of an experiment on a small animal (rabbit). Again, all the animals were subjected to simulated warm ischemia for 30 minutes. Subsequently, one kidney in the animal body was routinely perfused using hydrostatic pressure in the first group (n = 5) and using a mechanical perfusion pump in the second group (n = 5), in both groups for 30 minutes. The kidney flow parameters and the temperature inside the organ were recorded. Histological examination of the perfused kidneys was performed after ending the experiment. The second experiment, evaluating the immediately initiated pulsatile perfusion, was conducted on a large animal (pig). Seven (7) animals were subjected

to simulated warm ischemia of one kidney for 30 minutes. Subsequently, the kidneys in the animal body were routinely perfused under hydrostatic pressure in the first group (n = 3) and using a mechanical perfusion pump in the second group (n = 5) for 60 minutes. The flow parameters and the temperature decrease in the organ were measured. Subsequently, the original blood flow was restored and autotransplantation was performed. Over the next 6 hours, diuresis was monitored and biochemical values and markers of renal damage in the serum and urine were measured. After perfusion and at the end of the experiment, samples were taken for histological examination.

Results: In an experiment to examine ROP, we found no difference in serum urea levels in venous blood taken after organ autotransplantation ($p=0.843$). Similarly, no statistically significant difference was found in the quality of renal graft perfusion when examined by a pathologist at times I, II, and III ($p=1.00$). Both methods were therefore equivalent. The first experiment with the immediately initiated perfusion on a small animal showed a significantly higher maximum flow rate when using mechanical perfusion compared to the control group using only hydrostatic pressure ($p=0.004$). Similarly, we observed a statistically significant difference in graft temperature decrease in favor of mechanical perfusion ($p<0.001$). The qualitative histopathological evaluation of the graft perfusate was also statistically significant, again in favor of mechanical perfusion ($p=0.005$). In the second experiment on a large animal, we again achieved a statistically significant difference between the two groups when using mechanical perfusion, for both peak flow and flow compared to the maximum flow rate in hydrostatic perfusion ($p=0.007$ and 0.019, respectively). Concerning the decrease in the measured organ temperature, the level of statistical significance ($p=0.071$) was closely not reached. Similarly, diuresis after the transplantation was not significantly different, either ($p=0.602$). Comparison of the observed biochemical markers and markers of renal parenchymal damage in the serum and urine showed no significant difference at any of the time points of 0, 1, 3, and 6 hours.

Discussion: The results confirm the identical quality of grafts preserved by classical intraarterial perfusion and retrograde oxygen persufflation. We found no significant differences in either the histopathological evaluation of the grafts taken or the urea levels in the animals after transplantation of the respective grafts. The results are in accordance with the literature data. We did not confirm the opinion of some authors that the ROP may even be superior to conventional perfusion or to "cold storage". However, we undoubtedly confirmed that even simple ROP can protect the kidney parenchyma. In our opinion, the aim is not to replace the usual intra-arterial perfusion with retrograde persufflation, but there is a possibility of a certain combination of both methods. In the first experiment with immediately started mechanical perfusion, we observed statistically significant faster cooling of the kidney grafts with machine perfusion, as well as a statistically significantly higher perfusion solution flow rate per minute through the kidney. In this way, the organ can be perfused with a greater amount of perfusate during the same time. The method used so far uses cold perfusate, which, however, warms up from ambient room temperature when administered to the body of a deceased donor. In our perfusion system, we were able to achieve a very low perfusate temperature of about 4 degrees Centigrade by incorporating a special cooler of the medium just before the entrance to the donor's body. Therefore, subsequent cooling of the organ is

more effective. We demonstrated by histological examination that kidney perfusion using a mechanical perfusion system in the animal's body is significantly better than perfusion under hydrostatic pressure. Histological preparations did not contain blood elements in the glomeruli of a kidney perfused with the perfusion pump. Controlled mechanical in-situ perfusion appears to be able to remove even small microthrombi already formed in the kidney and preserve maximum of kidneys microcirculation. In the second experiment on a large animal, mechanical perfusion again achieved a statistically significant difference between the two groups, both for the peak flow in mechanical pulsatile perfusion (flow at peak systole of the pump) and for the mean flow (calculated identically as in clinical practice) compared to the maximum flow rate under hydrostatic perfusion ($p=0.007$ and 0.019, respectively). When comparing the markers of renal parenchymal damage, neither groups differed significantly. We conclude from the above that pulsatile graft perfusion immediately initiated in a donor’s body after irreversible circulatory arrest is better able to perfuse the graft, thus preserving a greater number of undamaged glomeruli and nephrons in the parenchyma. More efficient cooling can result in early arrest of ongoing ischemic changes and reduce the percentage occurrence of PNF and DNF after the transplantation. This method would seem to be optimal when combined with ROP prior to the transplantation to the recipient.

Conclusion: Kidney donors after irreversible circulatory arrest are an integral part of transplantation medicine today. Their use can significantly increase the number of organs that can be used for transplantation, thereby improving the quality of life of many patients in the terminal phase of renal failure. It is also the most challenging group of donors in terms of organization and performance of the organ removal, as it is associated with a number of logistical, social and ethically sensitive issues. In the vast majority of cases, machine perfusion is currently used to preserve organs from DCD donors, which guarantees the best functional results after subsequent transplantation to the recipient. Therefore, it is advisable to initiate machine perfusion as soon as possible, already in the body of the deceased donor. In our experiment, we examined the efficacy of retrograde oxygen persufflation in line with the literature data. Further, we demonstrated the possibility of better cooling and washing of the kidney graft in the donor's body in two experimental models. The optimal goal of our research would be to introduce a new methodology, a perfusion algorithm for kidneys removed from marginal donors. Its introduction into clinical practice certainly requires a significant amount of work to be done in the experimental field, yet the low financial and logistics requirements of this method are promising for the future.