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Isolated kidney perfusion: the influence of pulsatile flow

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ABSTRACT
Within the scope of transplantation research, ex vivo kidney perfusion has been proven an attractive model to study ischemia-reperfusion and preservation injury. Renal perfusion techniques also occupy scientists with the aim to optimize organ reconditioning and preparation prior to transplantation. This study investigated the influence of a pulsatile perfusion pattern that brings flow conditions closer to physiological situations, on renal perfusion characteristic and kidney function in the isolated perfused kidney. Kidneys were perfused via a roller pump at constant pressure set to 90 mmHg, or with addition of pulsatile pressure peaks (90/70 mmHg; 60/min) using an adjustable positive displacement pump. It was found that pulsatile pressure significantly enhanced renal flow rates as compared to non-pulsatile perfusion mode, especially after preceding preservation of the kidney by static cold storage. The improved vascular conductivity went along with a notable improvement of clearance of creatinine, sodium reabsorption and reduced tubular cell injury (Loss of fatty acid binding protein). The better vascular conductance upon pulsatile perfusion could be attributed to improved endothelial release of nitric oxide and reduced secretion of endothelin-1 into the perfusate. It is concluded, that pulsatile perfusion mode should be preferred in isolated kidney perfusion as resulting in better preservation/recovery of renal perfusion and function.

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Introduction
The ex vivo perfusion of the isolated kidney has been used since decades as a useful tool for the study of renal physiology and as valuable model in the evaluation of various pathophysiologic conditions [1–3].

In the field of transplantation research, the isolated perfused kidney serves as a surrogate model for assessment of ischemia reperfusion or preservation injury and supplants actual in vivo transplantation in experimental animals [2]. The widespread use of isolated kidney perfusion in experimental screening studies thus contributes to reduced strain inflicted to recipient animals suffering from dys- or non-functioning kidneys in transplantation studies.

Moreover, the isolated kidney preparation is technically less demanding and therefore more reproducible than actual transplantation in vivo. Pathophysiological studies take benefit from the isolated approach, allowing for variations in the experimental setting that might be difficult or impossible in the living animal.

Recently, translational as well as clinical research has focussed on renal machine perfusion as alternative preservation method in competition with static cold storage.

This interest has fuelled a variety of refinements concerning the optimal physical perfusion conditions. Novel perfusates have been developed for better preservation of the kidney during hypothermic perfusion [1,4–6]. Systematic investigations have specified adequate perfusion pressures under hypothermic conditions [7] as well as at normothermia [8].

Less data are available concerning the mode of renal perfusion, e.g. if arterial pressure should be kept at a continuous level or if a pulsatile pattern, mimicking the physiological situation, should be preferred.

Recent upsurge in research on mechanistic background of perfusion preservation suggests pulsatile shear stress at the vascular endothelium to be a major effector for improved preservation of kidneys by hypothermic machine perfusion (HMP) [9,10] and several reports on HMP suggest pulsatile perfusion to be superior to constant pressure modes [9,11,12].

However, no systematic investigation has been carried out so far focussing on the use of the isolated kidney as reperfusion model or in physiological experiments.

Although pulsatile perfusion pattern is sometimes advocated [13], the use of a roller pump instead of non-pulsatile pumping systems is often thought to suffice for pulsatile stimulation without control of the actual pressure curve at the renal artery.

This study thus was designed in order to investigate the respective role of vascular pulsatility for isolated kidney...
perfusion in healthy (freshly excised) kidneys as well as in preparations that had suffered a longer period of static preservation as usually encountered in transplantation practice and organ preservation research.

Materials and methods

All experiments were performed in accordance with the federal law regarding the protection of animals. The principles of laboratory animal care (NIH publication no. 85-23, revised 1985) were followed.

Kidneys were removed from anesthetized, non-surviving female German Landrace pigs weighing between 25 and 30 kg by trans-peritoneal access through midline abdominal incision. The renal artery was cannulated on the back-table and the kidneys flushed by 100 cm gravity with 100 ml of Histidine-Tryptophan-Ketoglutarate (HTK) solution (Dr. F. Köhler Chemie, Bensheim, Germany) at 4°C.

The isolated kidneys were put on an ex vivo perfusion circuit for normothermic perfusion as detailed below either immediately or after 20 hours of static cold storage immersed in the preservation solution at 4°C for 20 h.

Isolated kidney perfusion

Prior to isolated perfusion, the ureter was cannulated with PE-tubing for collection of urine during the experiment.

Isolated normothermic organ perfusion was done in an established set up [12] with some modifications.

The system contained a thermostatically controlled (37°C) moist chamber and perfusate reservoir, a precision roller pump, a hollow fibre oxygenator (Hilite LT 1000, Medos, Stolberg, Germany) with heat exchanger also connected to the thermostat, a transit time flow meter with flow through probe interposed in the perfusion line (Hugo Sachs electronic, March-Hugstetten, FRG) and a pressure transducer connected to the arterial inflow line immediately at the renal artery.

The perfusate consisted of 1000 ml Krebs–Henseleit buffer supplemented with 2.2% bovine serum albumin and 20 ml of concentrated amino acid solution (RPMI 1640-50x). Creatinine (0.01 g/l) and urea (1 g/l) were also added to allow for calculation of renal clearances.

The perfusate was oxygenated with 95% oxygen and 5% carbon dioxide and flow through the kidneys in the moist chamber was adjusted to achieve a mean perfusion pressure of 90 mmHg by manual control of the roller pump speed.

In half of the cases, arterial inflow was subjected to an additional pulsatile pattern, generated by means of an adjustable positive displacement pump, which alternately (60 times/min) added or withdrew an adjusted pressure to/from a fluid column connected to the vent of the oxygenator. The actual driving pressure at the renal artery thus varied between approx. 70 mmHg and 90 mmHg during one cycle.

Urine was collected outside of the perfusion chamber and urinary fluid loss was replaced every 30 min by adding equal amounts of balanced salt solution to the perfusion medium.

Renal function

Perfusate and urine samples were analysed for concentrations of urea and creatinine in a routine fashion at the Laboratory centre of the University Hospital.

From these data and the volume of the produced urine, the respective clearances were calculated as urinary creatinine/urea × urine flow/perfusate creatinine/urea in ml/min.

Tubular reabsorption of sodium was approximated by calculating the fractional excretion of sodium (FENa) according to:

\[ \text{FE}_{\text{Na}} = 100 \times \frac{\text{urinary sodium}}{\text{perfusion sodium}} \times \frac{\text{urinary creatinine}}{\text{perfusion creatinine}}. \]

Oxygen consumption was calculated from the pO2 differences between arterial and venous sites, measured in a pH-blood gas analyser (ABL 815flex acid-base laboratory, Radiometer, Copenhagen) and expressed as ml min⁻¹ g⁻¹ according to trans-renal flow and kidney mass.

Tubular cell injury

Structural injury to renal tubular cells was analyzed using the release of the intracellular carrier protein L-type fatty acid binding protein (LFABP), as a compound predominantly expressed in liver tissue as well as in proximal tubular cells of kidney [14].

Measurements were done with a commercial ELISA kit (USCN life science, Wuhan, China) according to the instructions of the manufacturer on a fluorescence micro plate reader (Tecan, Grailsheim, Germany).

Vascular mediators

Concentrations of major mediators, involved in the control of vascular tone were determined in the perfusate using analytic kits from the following companies according to the instructions of the manufacturers to analyse perfusate levels of endothelin 1 (USCN life science, Wuhan, China) and total nitric oxide (R&D Systems, Wiesbaden, Germany).

Statistics

All values were expressed as means ± SEM. After proving the assumption of normality, differences between the pulsatile and non-pulsatile perfusion were tested by parametric comparison of the means using Student’s t-test or one-way analysis of variance and post hoc comparison with Tukey Kramer multiple comparison test, where appropriate (Instat 3.01, Graph Pad software Inc, San Diego, CA). Statistical significance was set at \( p < .05 \).

Results

Pulsatile perfusion pattern

The kinetics of the arterial perfusion pressure with or without proactive induction of pulsatile pressure peaks is depicted in Figure 1. It is seen that little fluctuations in perfusion pressure are induced by the rotations of the roller
pump, but that these do actually induce a pulse wave pattern during isolated kidney perfusion. By contrast, implementation of alternating pressure peaks in the arterial line resulted in a net fluctuation of perfusion pressure and a real pulsatile perfusion profile.

Vascular integrity during perfusion

In sham preparations that were perfused immediately after organ retrieval, renal perfusate flow was only moderately affected by pulsed vs non-pulsatile perfusion mode, although pulsed perfusion resulted in tendentially higher flow rates (cf. Figure 2).

After cold storage, however, these differences became much more evident and significantly higher flow rates were observed upon pulsed perfusion. Of note that in the latter case near normal values of renal perfusate flow could be obtained while vascular conductivity was found notably impaired upon non-pulsatile perfusion after ischemic preservation.

Molecular triggers of vascular conductivity

Vascular tone is dominantly influenced by the balanced interplay of the two major mediators of vascular relaxation (NO) and vasoconstriction (endothelin) at the luminal endothelium.

In our model, we could substantiate a significant increase in endothelin-1 activity after ischemic preservation in the perfusate of non-pulsatile perfused kidneys, which was not disclosed after pulsed perfusion (cf. Figure 3).

Similarly, a reduction of the endogenous vasodilator NO was apparent upon non-pulsatile perfusion as compared to pulsatile perfusion, which was most pronounced after preceding ischemic preservation, although these differences did not reach statistical significance.
The difference between pulsatile and non-pulsatile perfusion conditions became more evident when plotting the individual ratios of ET and NO as integral denominator of vascular tone. Pulsed perfusion consistently resulted in a significant reduction of this ratio and hence promoted a more vasoconductive phenotype.

Renal function

Glomerular function of the preserved kidneys was estimated by the calculation of renal clearances for creatinine (Figure 4). This was found to be notably impaired in both groups after ischemic preservation as compared to non-ischemic sham conditions. However, pulsatile perfusion conditions led to a more than three-fold and significant improvement of post-preservation function of the kidneys in comparison to non-pulsatile reperfusion.

Likewise, fractional excretion of sodium as a readout of renal tubular function was massively deteriorated after ischemic preservation and non-pulsatile perfusion, while only moderate alterations could be evidenced upon pulsed perfusion even after renal ischemia (FE Na, Figure 4).

Discussion

The main finding of the present study points out that significant impairments of vascular function and conductivity are observed upon renal perfusion in vitro after static preservation and this vascular dysfunction is in large parts mitigated by the implementation of a pulsatile perfusion pattern in the experimental set-up. The dependency of vascular conductivity on pulsatility of perfusion pressure was less pronounced but also noticeable in fresh preparations.

Of note, the effects of pulsatile pressure were not limited to mere vascular phenomenae but also have significant implications on renal function during isolated perfusion, e.g. clearance of creatinine and tubular cell integrity.

Renal tubular cells are strongly dependent on adequate oxygenation, as transmembranous transport systems are major energy demanding processes [15,16]. Therefore, maintenance of sufficient vascular perfusion of tubular epithelial cells in the kidney is considered to be pivotal to safeguard physiological cell function.

Likewise, impaired vascular perfusion also favours a reduction of glomerular filtration by simple reduction of transcapillary hydraulic pressure difference [17].

Under physiological conditions in vivo, endothelial cells are constantly exposed to mechanical stimuli exerted by pulsatile blood flow and pressure. This mechanical stimulation translates into a strong induction of endothelial nitric oxide synthase (eNOS) as well as inhibition of endothelin (ET-1) [18] as to guarantee for optimized vascular conductivity and tissue perfusion.

Experimental studies have further disclosed a subcellular network, orchestrated by the flow dependent activation of the transcription factor Krüppel-like factor 2 (KLF-2) and triggering the transcription of a variety of anti-inflammatory and anti-thrombogenic genes while depressing the upregulation of cellular adhesion molecules on endothelial cells [10,19,20].

To this regard, pulsatile flow has been shown to be much more effective than steady shear stress [12,20] or oscillatory flow [21].

The effects of endothelial stimulation are lasting for several minutes up to hours after cessation of vascular perfusion. Thus, they are already declining during early nonpulsatile perfusion after retrieval, but are still operative. The lack of mechano-stimulation to the endothelium during (prolonged) static preservation in contrast notably aggravates the endothelial dysfunction upon reperfusion [19].

Vascular shear stress directly stimulates the phosphorylation of eNOS in an endothelial protein kinase A (PKA) dependent manner resulting in the production of the endogenous vasodilator nitric oxide (NO) [22].

NO is known to maintain medullary blood flow by promoting vasodilation of descending vasa recta and juxtaglomerular arterioles [23], while oxygen free radicals, putatively arising after ischemic preservation, may reduce local bioavailability of NO.

Pulsatile endothelial stimulation has been shown to result in elevated activation of eNOS already within 20 min in a
cell culture model [24]. This indicates a swift effect of pulsatile perfusion even before the transcriptional machinery will be reactivated after static storage condition in favor of a vaso-protective endothelial phenotype.

In line with previous results, the present study disclosed a notable dysbalance of endogenous mediators that regulate vascular tone (NO and ET-1) upon nonpulsatile perfusion to the effect of a more vasoconstrictive reaction, which was attenuated by using the pulsatile perfusion mode.

Being closer to physiological conditions upon post-transplant reperfusion in vivo, we would thus recommend pulsatile perfusion also for use in ischemia reperfusion models aiming to supplant actual transplantation. As pulsatile perfusion actually also affected renal filtration and tubular cell function it is conjectured that in vitro experiments will better mimick renal physiology if performed in a pulsatile perfusion mode.

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Disclosure statement

The authors report no conflicts of interest.

References


