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Detrimental effects of controlled reperfusion on renal function after porcine autotransplantation are fully compensated by the use of Carolina rinse solution

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Abstract Despite extensive efforts in the fields of donor selection and management, standardisation of organ retrieval procedures, storage solutions, and novel immunosuppressive protocols, the rates of delayed graft function (DGF) after renal transplantation have been stagnating between 30% and 50%. As DGF exerts negative influences on acute rejection episodes and long-term organ function, the early phase of transplantation immediately following reperfusion deserves special interest. Several studies on machine-controlled reperfusion showed promising results in various organs, in experimental and clinical settings. Moreover, the flushing of organs with Carolina rinse solution (CR) immediately prior to reperfusion has been proven beneficial and is being clinically applied in human liver transplantation in recognised departments. In our study, we set up an autogenic porcine kidney transplantation model and assessed the normal values (control group) for creatinine clearance (ClCr) and urine output per hour (U/h) after "standard" reperfusion similar to clinical transplantation. Subsequently, kidneys of the experimental group 1 were reperfused at a blood pressure (RR) under the systemic level by means of a roller pump. Group 2 kidneys were rinsed with CR before controlled reperfusion, analogous to group 1. Both groups were compared with each other and with the assessed normal values. Our findings for Group 1 are that pressure-reduced reperfusion negatively affected immediate graft function. ClCr was reduced from 9.9 (control group) to 3.4 ml/min, U/h from 233 to 132 ml ($P < 0.05$). Group 2 showed that rinsing the kidneys with CR before reperfusion improved functional parameters highly significantly, compared with group 1 (ClCr: 13.5 vs 3.4 ml/min, U/h: 384 vs 132 ml; $P < 0.05$) and even showed a positive trend compared with the control group (ClCr: 13.5 vs 9.9 ml/min, U/h: 384 vs 233 ml; $P = 0.0546$). We can conclude that in a model of porcine renal autotransplantation, pressure-reduced reperfusion via a roller pump is detrimental to early kidney graft function. The flushing of organs with CR prior to controlled reperfusion significantly improves ClCr as well as urine output.

Keywords Porcine renal transplantation · Experimental · Immediate graft function · Autotransplantation

Introduction

Delayed graft function (DGF), usually defined as the need for more than one haemodialysis after kidney transplantation (NTX), is an ongoing problem. Acute rejection is more likely to occur as long as the newly transplanted organ lacks stable graft function. Most authors agree, furthermore, that long-term organ function rates are negatively influenced [23, 26, 31]. Additionally, hospital stays are shorter, and the overall costs of transplantation are lower without DGF [21].

Several approaches have been chosen to improve early function of transplanted kidneys [11, 12, 15, 16, 17, 20, 28]. Despite positive effects in liver transplantation and some initially optimistic studies [27], introduction of new solutions for cold storage did not alter DGF rates in NTX, still ranging up to more than 40% [21]. Storage of kidneys under pulsatile machine perfusion seems to yield better results [19], but bears enormous logistic problems and makes organ exchange almost impossible.

Negative effects caused by ischaemia and reperfusion have been a major target of scientific research for years, and innumerable papers have been published on these topics [12, 15, 16, 17, 20, 28]. Most authors of experimental and clinical studies assess early and delayed graft function by daily measurement of serum creatinine only, starting on the day after transplantation [15, 16, 17, 28]. Usually, serum creatinine values of all animals rise initially for several days, until, approximately 5 or 6 days after transplantation, the course of the survivors starts to decline again, fading towards uraemia [12, 17, 20]. As this scenario does not at all resemble human NTX (with periods of haemodialysis for up to several weeks), the authors have tried to find an experimental setting to study kidney function immediately after reperfusion; only a few experimental papers on the isolated perfused kidney model [10] specifically focus on these first postoperative hours.

The concept of controlled reperfusion as a means of overcoming various detrimental effects of the reperfusion phase was first described in cardiac surgery [1], and thereafter clinically applied to peripheral vascular surgery [4, 5] and lung transplantation [18, 30]. Besides reduction of initial reperfusion pressure and oxygen content, various theoretical concepts have been developed [4]: reduced pressure and haematocrit, free radical scavengers, osmotic gradients, and energy substrates, to mention only a few. Haab and co-workers [9] reported on a study of warm ischaemia in the isolated perfused pig-kidney model: compared with a group that underwent "standard reperfusion" at normal RR, renal function was better if kidneys were gently brought back to circulation at only 60 mmHg.

A less complicated idea than reperfusion through one or more roller pumps originated in human liver transplantation, with the need to rinse out storage solutions rich in potassium before bringing the organ to circulation again. Carolina rinse solution (CR) which has been proven beneficial experimentally [2, 8] and has been applied in clinical hepatic transplantation for more than 10 years [3, 17] has also been successfully tested in canine renal transplantation [29].

Goals of this study were to develop an experimental setting for investigation of kidney function immediately after reperfusion, to examine the influence of pressure- and haematocrit-reduced, pump-driven reperfusion, and to study the influence of CR on immediate graft function.

Material and methods

For practical reasons, white landrace pigs were chosen. In order to exclude immunological influences as far as possible, we decided to work in the autogenic setting. As the main intention was to investigate renal function immediately after reperfusion, all animals were killed after 6 h. This decision was influenced by the wish to spare the animals a second postoperative phase with problems such as pain, herniation, and bowel obstruction.

Consent of the Austrian board for animal experiments was obtained. All experiments were performed in accordance with the principles of the Austrian Law for Animal Experiments as well as *Principles of Laboratory Animal Care* (NIH publication No. 86-23, revised 1985). Animals were maintained under the guidelines of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg 1986).

The basic technique of renal autotransplantation was studied at the transplantation department of the university of Hartford/Connecticut (see Acknowledgements).

The study comprised three stages:

1. The setting up of the operating technique and assessment of normal values (control group).
2. Controlled reperfusion (group 1); see Fig. 1.
3. CR (group 2).

The setting up of the operating technique and assessment of normal values (control group)

Animals underwent autotransplantation after a cold ischaemia time (CIT) of 24 h; blood samples were drawn before reperfusion and immediately prior to cessation of the experiment. The total amount of urine was recorded, ClCr was calculated. This group consisted of 11 animals, as all experiments, from the "set up" phase onward, were included.

Controlled reperfusion (group 1)

For the group 1 controlled reperfusion (Fig. 1) arterial blood was drawn from the left-sided common iliac artery into a silicone tube which was guided through a roller pump, similarly to a second tube with a sectional area of 17% of the first one and containing Ringer's solution. Both pumps' speed being coupled, a constant 1:6 ratio of blood:Ringer's was warranted. Tubes were connected through a Y-piece forming a single-lumen reperfusion line, which, after passing through a heat exchanger and pressure transducer, ended in a cannula.

We completed both vascular anastomoses without tying the running suture at the venovenostomy, the vascular clamps

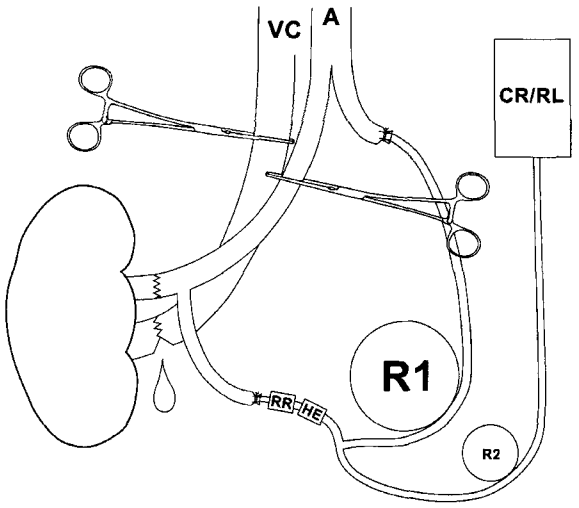

Fig. 1 Sketch of operation setting for groups 1 and 2. Fluid drop at venous anastomosis symbolises effluent rinse solution before anastomosis is tied and systemic circulation commenced by removal of vascular clamps. *A* aorta, *VC* vena cava, *CR/RL* bag containing CR or Ringer's, respectively, *R1*, *R2* roller pumps, *HE* heat exchanger, *RR* pressure transducer

remaining on the common iliac vessels. Subsequently, the kidney was flushed with 100 ml of Ringer's through the reperfusion cannula tied into the orifice of the transsected internal iliac artery. By the starting of only the smaller (R2) roller pump, effluent was drained through the open venous anastomosis. After the suture thread had been tied and the venous vascular clamp removed, reperfusion through the common reperfusion line was commenced at a pressure of 60 mmHg. For 30 min, bypass pressure was slowly increased in steps of 5 min up to systemic RR until, finally, the arterial vascular clamp was released, thus bringing the kidney into systemic perfusion. The perfusion cannula was removed, the stump of the internal iliac artery ligated, and the observation period started.

Carolina rinse solution (group 2)

The operations for group 2 were performed exactly as for group 1, the only difference being that CR was used instead of Ringer's (see Table 3 for composition of CR).

The technically demanding setting of controlled reperfusion with two roller pumps for 15 pigs undergoing transplantation caused three initial technical failures. Furthermore, although groups 1 and 2 were scheduled to consist of six animals each, an additional pig of group 2 had to be excluded from analysis due to technical difficulties, leaving six animals in group 1 and five in group 2.

For a comparison of Ringers and CR see Table 1.

Operating technique

Organ retrieval

Female pigs between 25 and 35 kg bodyweight were intubated orotracheally, and ventilation was started. Via a subcostal incision of approximately 12–15 cm, the left kidney was approached extraperitoneally. After careful dissection, systemic heparinisation, and drawing of blood samples, the ureter was ligated and cut, and both vascular pedicles were ligated close to the aorta and vena cava. We transsected the renal artery and vein and inserted a small vascular perfusion catheter into the artery, taking care not to injure the intimal layer. Kidneys were flushed with Custodiol solution (Dr. F. Koehler Chemie, Alsbach, Germany) under a hydrostatic pressure of 100 cm, and perfusion was continued for 10 min. Warm ischaemia time from ligation of the artery to start of perfusion ranged between 1 and 2 min. After perfusion, the kidney was packed into plastic bags containing Custodiol and stored on ice. Wound closure was performed in three layers.

Transplantation

Skin incision at the left side of the neck was followed by dissection of the vessels. Cannulae were inserted into the carotid artery for pressure monitoring (RR) and drawing of blood samples, as well as into the jugular vein for fluid therapy.

After laparotomy via a midline incision, blood and urine samples were drawn, and the bladder was evacuated. The right kidney was dissected and resected after ligation of vessels and ureter. The iliac vessels of the right side were ligated and transsected at the level of the inguinal ligament after systemic heparinisation. The renal vein and artery were anastomosed to the corresponding iliac vessels in an end-to-end fashion with Prolene 6.0 and 7.0 respectively; arterial anastomoses were performed to the stump of the external iliac artery; the orifice of the internal artery was spared for the reperfusion cannula. A soft-tip catheter was inserted and tied into the ureter to ensure complete urine drainage into a connected bag. After reperfusion the abdomen was closed with clamps.

Animals that could not maintain a mean systemic blood pressure of at least 100 mmHg were excluded from analysis. Six hours after reperfusion, blood and urine samples were drawn, the total amount of urine was assessed, and the animals killed by means of intravenous infusion of 2 g thiopentone followed by 80 mmol of potassium.

Creatinine clearance (ClCr) and urine output per hour (U/h) were chosen as endpoints; additionally, urine was tested for urea, creatinine, sodium, potassium and glucose. We performed the statistical analyses using the Mann-Whitney U-test, and $P = 0.05$ was regarded as a significant difference. Data are expressed as median (range). Calculations were carried out with "Statview 5.0" (SAS Institute) on an Apple Powermac G4 computer.

Table 1 Composition of Ringer's and CR

Ingredient	Ringer's	CR	Unit
Allopurinol	–	1	mmol/l
Deferoxamine (mesylate)	–	1	mmol/l
Glutathione	–	3	mmol/l
Adenosine	–	1	mmol/l
Nicardipine	–	0.002	mmol/l
Fructose	–	10	mmol/l
Glucose	–	10	mmol/l
Insulin	–	100	U/l
Hydroxyethyl starch	–	50	g/l
NaCl	154	115	mmol/l
KCl	4.02	5	mmol/l
$CaCl_2$	2.74	1.3	mmol/l
KH_2PO_4	–	1	mmol/l
$MgSO_4$	–	1.2	mmol/l
(*N*-morpholino) propane sulphonic acid	–	20	mmol/l
pH	6	6.5	

Results

Control group

Values for ClCr and U/h after a CIT of 24 h were 9.9 (1.9–18.6) ml/min and 233 (45–769) ml respectively. For results, see Tables 2 and 3.

Controlled reperfusion (group 1)

After controlled reperfusion ClCr (3.4 vs 9.9 ml/min; $P=0.0367$) as well as U/h (132 vs 233 ml; $P=0.0441$) were reduced significantly.

Carolina rinse solution (group 2)

When compared with group 1, the group 2 ClCr (13.5 vs 3.4 ml/min; $P=0.0062$) and U/h (384 vs 132 ml; $P=0.0106$) improved highly significantly. In comparison with the control group there seemed to be a trend ($P=0.0682$ and $P=0.0546$) in favour of CR, without statistical significance being reached. For values of urine analyses see Table 3.

Table 2 Results: values are expressed as median (range) (*NS* not significant)

Parameter	Value	*P* vs group 1	*P* vs group 2
ClCr (ml/min)			
Normal	9.9 (1.9–18.6)	0.0367*	0.0682 NS
Group 1	3.4 (1.8–7.1)	–	0.0062*
Group 2	13.5 (6.6–17.7)	0.0062*	–
U/h (ml)			
Normal	233 (45–769)	0.0441*	0.0546 NS
Group 1	132 (58–229)	–	0.0106*
Group 2	384 (199–564)	0.0106*	–

*Statistically significant; Mann-Whitney U-test

Table 3 Urine median values and comparison between groups. Values for urea, creatinine and glucose are in mg/100 ml, those for sodium and potassium are in mmol/l (*NS* not significant, Mann-Whitney U-test)

Group	Urea	Creatinine	Sodium	Potassium	Glucose
1	38	5.1	139	8.3	65
2	52	3.3	147	11.3	61
3	92	4.1	130	8	58
1 vs 2, *P*=	NS	0.0402	NS	NS	NS
1 vs 3, *P*=	0.0089	NS	NS	NS	NS
2 vs 3, *P*=	0.0281	NS	NS	0.0176	NS

Discussion

Most authors of studies referring to larger patient cohorts agree that DGF exerts negative influences on long-term outcome after renal transplantation [22, 23, 26, 31, 32], although this is still a matter of some debate. Some risk factors have already been detected: rejection episodes [31] prolonged CIT [22, 26], high age of organ donor [22, 26] the cause of the donor's death [26] anastomosis time [26], number of previous transplantations [14], and sensitisation [22].

Most studies on renal function after porcine transplantation start assessing serum creatinine values 24 h after transplantation (TX) [12, 15, 17, 20]. Animals of the treated and the control groups were followed between 8 [17] and 14 [20] days, respectively. Two or 3 days post TX, the pigs were anaesthetised again, and ClCr assessed [12, 15]. Renal function immediately following reperfusion has been examined only in "isolated perfused kidney" models [9]. To our knowledge, Bugge et al. [7] have published the only study on the very early phase of organ function. Eight recipients of living-donor TXs were followed and their renal function parameters compared with those of the remaining kidney of the donor. Starting at 70% of the native kidney's glomerular filtration rate, transplanted kidneys had improved to equal values within 3 h. Although interesting, the living-donation setting with perfect pre-operative status of the donor and a CIT of fewer than 3 h obviates comparison of these data with cadaveric donation. Nevertheless, crucial investigation of the reperfusion phase itself and of the first hours of the transplanted organ with circulation could be the key to our understanding and avoidance of DGF [23]. For practical reasons, a clinical study cannot focus on renal function immediately after reperfusion; this task demands a specifically designed experimental model.

Renal TX in the pig has been established for years [12, 13, 14, 15, 20, 24, 25] and, as opposed to "isolated kidney" or "warm ischaemia" models, seems the ideal way to study early organ function as closely as possible to clinical TX. Many publications on ischaemia/reperfusion use allogenic models [12, 13, 15]. Nonetheless, to work in the autogenic setting [11, 17, 20] and thus exclude immunological influences seems preferable.

Having set up the experimental design and found normal values (control group), we first tried to reproduce the findings of Haab et al. [9] regarding controlled reperfusion. In an isolated kidney model in Yucatan mini-pigs, after 60 min of warm ischaemia "low-pressure machine-reperfused" organs showed better functional and circulatory parameters than those reperfused in a "standard manner". In our model of 24 h of CIT, we could not validate these results – far from it: controlled reperfusion significantly reduced ClCr as well as U/h. Whether the addition of 1/7th of Ringer's solution in our experiment was detrimental, or the shorter study period in Haab's work (2 h as opposed to 6) obviated the development of acute tubule necrosis is not yet clear and demands further investigation.

Our following of the concept of Allen et al. [1] and Beyersdorf and colleagues [4, 5] and replacing Ringer's with Carolina rinse, a flush solution developed for hepatic TX [2, 8] that has been in clinical use for years [3, 17], resulted in a highly significant improvement in both ClCr and U/h. Even compared with the control group of kidneys reperfused similarly to those in clinical TX, CR-reperfused kidneys showed a trend towards better function, although our findings failed to reach statistical significance.

Our results show that reperfusion with less than systemic blood pressure and reduced oxygen content (achieved by addition of 1/7th part of Ringer's) is detrimental to early renal function. Controlled reperfusion with addition of CR, a specially designed rinsing solution, is able to improve renal function considerably. No single component of CR is capable of counterbalancing the multifactorial damage taking place during reperfusion of an ischaemic organ, but allopurinol and glutathione as antioxidants, adenosine as a vasodilator, hydroxyethyl starch to prevent interstitial oedema, fructose, glucose, and insulin to improve the energy status, and nicardipine as a calcium channel blocker, have all been proven effective experimentally [6, 8, 12, 13, 15, 17, 29].

Whether Carolina rinse or similar solutions will render any benefit in "standard renal transplantation" without controlled reperfusion, demands further investigation.

In conclusion, during the first 6 h after porcine autotransplantation with a CIT of 24 h, normal values for ClCr and U/h are 9.9 ml/min and 233 ml, respectively. Controlled reperfusion with less than systemic blood pressure and addition of 1/7th of Ringer's solution significantly worsens renal function. Replacing Ringers with CR improves ClCr and U/h significantly, possibly even to a "better-than-normal" level.

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