Preclinical evaluation of a new organ preservation solution
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Chapter 9

Summary, conclusions, and discussion
Summary

In this thesis, the preclinical evaluation of Polysol, a new organ preservation solution is described. In chapter 1, the general introduction, an overview is presented of the history, principles, methods and current status of solid organ preservation. The current clinically employed kidney preservation methods cold static storage and machine perfusion preservation are described. Also, a detailed description is provided of the constituents present in Polysol.

Part I. Preclinical cold storage preservation studies

The quality of organ preservation is generally considered to be a major determinant of graft function and survival. After retrieval from the donor, the temperature of the graft must be reduced as rapidly as possible to reduce graft metabolism and to attenuate preservation injury. The graft is also washed out to remove blood remnants in order to prevent occlusion of the vascular bed before cold storage. Incomplete graft washout not only prevents the tissue from being protected by the preservation solution, but also the presence of blood remnants and cellular debris may contribute to impaired blood flow upon reperfusion. In chapter 2, the effect of different preservation solutions for washout of kidney grafts was evaluated regarding cooling rate, kidney weight alterations, remaining blood remnants and structural integrity after ex vivo washout using cold preservation solution followed by cold storage. Kidneys retrieved from pigs were washed out using the new, low viscosity, colloid containing solution Polysol, the University of Wisconsin (UW) solution, or histidine-tryptophan-ketoglutarate (HTK) followed by cold storage. Also, kidneys were retrieved after clamping of the renal vessels for thirty minutes, mimicking warm ischaemia damage, followed by washout using HTK or Polysol. After washout, only the weight of kidneys washed out with HTK had increased, indicating swelling of the tissue. Overall, kidneys washed-out with Polysol showed better preservation of structural integrity after cold storage compared with either UW or HTK. This study also demonstrated that washout of kidney grafts using the colloid-based preservation solutions Polysol and UW more rapidly decreased the core temperature compared with HTK. Also, fewer blood remnants remained in the vascular bed of warm ischaemia-damaged kidneys after washout using Polysol compared with HTK. The results of this study indicate that the presence of a colloid facilitates an effective washout and rapid cooling of the kidney grafts. The most widely used preservation method for heart-beating kidney grafts is cold static storage using UW solution. A drawback of UW, however, is its high viscosity, mainly due to the colloid hydroxyethyl starch. This
A high-molecular-weight molecule is known to cause obstructions of the microvasculature by accelerated aggregation of erythrocytes, resulting in incomplete washout of the donor graft. To date, new preservation solutions have not been able to significantly improve preservation quality of grafts. The aim of the study in chapter 3 was to compare Polysol and the UW solution for cold storage of porcine kidney grafts. In a porcine autotransplantation model, real-time parameters of the renal microcirculation were assessed using a combined laser Doppler and flowmetry system. Thereafter, kidneys were retrieved and washed out with Polysol or UW followed by twenty hours cold storage. After the preservation period, the contralateral kidneys were removed and the preserved kidneys autotransplanted. Polysol was able to better preserve the microcirculation compared to UW as expressed by higher values of capillary blood flow, blood flow velocity and tissue oxygen saturation values. In addition, cold storage using Polysol resulted in improved functional recovery as demonstrated by lower posttransplant serum creatinine and blood urea values in comparison to the UW group. Also, structural integrity was better preserved in the Polysol group. Therefore, this study demonstrated the feasibility of Polysol as a preservation solution for hypothermic cold storage preservation of heart-beating kidney grafts.

In an era of continuous organ shortage, alternative sources such as organs from non-heart-beating donors are increasingly being employed for transplantation. These organs have sustained warm ischaemia damage and are associated with higher primary non-function and delayed graft function rates than organs from heart-beating donors. Maintenance of organ viability during preservation is considered a prerequisite for successful outcome after transplantation. In chapter 4, Polysol was compared with HTK for the preservation of warm ischaemia-damaged kidney grafts. Pigs underwent a left nephrectomy after clamping of the renal vessels for thirty minutes. Kidney grafts washed out with Polysol or HTK were cold stored for twenty hours. After the preservation period, the contralateral kidneys were removed and the preserved kidneys implanted heterotopically. Renal function was assessed daily for seven days. All Polysol preserved grafts and three out of six grafts in the HTK group showed immediate function, as demonstrated by urine production within one day after reperfusion. Also, peak serum creatinine and blood urea values were lower in the Polysol group. Histologic evaluation showed less glomerular shrinking, tubular damage, edema, inflammatory infiltrates and necrosis in grafts preserved with Polysol compared with HTK. We therefore concluded that application of Polysol solution for washout and cold storage preservation of warm ischaemia-damaged kidney grafts resulted in improved renal function and structural integrity when compared with HTK.
Part II. Preclinical perfusion preservation studies

Following the publication of a landmark paper in 1967 by Belzer et al., kidney preservation by hypothermic machine perfusion has become an established clinical activity. To date, however, preservation of the liver by machine perfusion has not followed although in 1968, the first successful clinical application of liver machine perfusion was described. After the introduction of simple cold static storage, the clinical focus averted from machine perfusion since cold storage was easier to use, was cheaper and did not involve the logistical drawbacks associated with machine perfusion. Also, cold storage of liver grafts using the UW solution allowed for significantly longer preservation periods compared to previously used preservation solutions. The review in chapter 5 describes experimental and clinical liver perfusion preservation studies as well as current developments. Liver machine perfusion is increasingly reconsidered since experimental studies have shown that oxygenated perfusion can provide a complete wash-out and is able to restore parenchymal energy status, a phenomenon of particular importance in preservation of livers from compromised donors. The benefits of machine perfusion for both heart-beating and non-heart-beating liver grafts seem promising in view of expanding the donor pool. The current development of easier to use machine perfusion systems for liver preservation and new perfusion solutions exemplify the regained interest in liver preservation by machine perfusion. After an intermission of approximately forty years, clinical application of this promising technique now appears to be in sight also for the preservation of livers.

The objective of the study in chapter 6 was to investigate the effect of different perfusion pressures during machine perfusion on the preservation quality of kidney grafts. After assessment of the microcirculation, a left nephrectomy was performed. Immediately thereafter, the explanted kidneys were washed out with Polysol, followed by machine perfusion at a mean perfusion pressure of 30 mmHg or 25 mmHg. After the preservation period, the contralateral kidneys were removed and the preserved kidneys were heterotopically autotransplanted and subsequently, the microcirculation was reassessed. Machine perfusion using a mean pressure of 25 mmHg resulted in better preservation of microcirculation compared with 30mm Hg as demonstrated by higher capillary blood flow. Overall, an improvement in recovery of renal function and better preservation of structural integrity was seen when perfusion at a mean pressure of 25 mmHg was applied.

Application of machine perfusion for the preservation of kidney grafts is associated with a reduction of delayed graft function. Therefore, the use of machine perfusion is correlated with lower costs for hospitalization of transplant recipients as expressed by reducing both the need for postoperative dialysis and length of hospital stay. Recently, a new machine perfusion system for hypothermic oxygenated pulsatile perfusion was
developed. The aim of the study, described in chapter 7 was to assess the biological safety of this system in combination with Polysol in comparison with cold storage using Polysol or UW in a porcine autotransplantation model. Preservation using machine perfusion or cold storage with Polysol resulted in improved recovery of renal function and higher microcirculatory flow compared with cold storage using UW. Also, structural integrity was better preserved in the machine perfused grafts. This study demonstrated the biological safety of the novel machine perfusion system in a porcine autotransplantation model.

In chapter 8, the value of hypothermic oxygenated pulsatile perfusion in combination with Polysol was assessed for the preservation of warm ischaemia-damaged kidney grafts. After clamping of the renal vessels for thirty minutes, the kidneys were removed and preserved for twenty hours by machine preservation with Polysol or cold storage with either HTK or Polysol. Subsequently, the contralateral kidneys were removed and the preserved kidneys transplanted. Control pigs underwent a unilateral nephrectomy. Renal function was assessed daily for one week and kidney biopsies were analysed for morphology. In the machine perfused grafts, renal function was comparable to that of non-ischaemic controls. Both Polysol groups showed improved renal function compared with the HTK group, with more favorable results for the machine perfused grafts. This study demonstrated that the function of warm ischaemia-damaged kidney grafts after pulsatile perfusion preservation was comparable to that of non-ischaemic controls.

Conclusions

The severe shortage of donor organs has led to waiting lists for organ transplantation worldwide. In the quest to enlarge the donor pool, improvement of the quality of organs for transplantation is generally considered of vital importance. Therefore, development of new preservation methods and in particular of new preservation solutions has been the focus of research for the past decades. In this thesis, Polysol was evaluated as a preservation solution for cold storage and machine perfusion of kidney grafts.

From the preclinical cold storage preservation studies, described in Part I of this thesis, it can be concluded that application of Polysol enhanced the quality of preservation of kidney grafts. An improvement of functional recovery after cold storage was observed using Polysol compared with UW and HTK, the clinical standards for preservation of optimal and warm ischaemia-damaged grafts, respectively. Furthermore, it can be concluded that the presence of a colloid in the preservation solution facilitates an effective washout and rapid cooling of kidney grafts prior to preservation. In the preclinical perfusion preservation studies presented in Part II of this thesis, the feasibility of Polysol as a perfusion solution for kidney
grafts was demonstrated. Application of Polysol in combination with hypothermic oxygenated pulsatile perfusion proved to be advantageous over cold static storage for the preservation of optimal as well as warm ischaemia-damaged kidney grafts. By using perfusion preservation, the function of warm ischaemia-damaged kidney grafts after transplantation was comparable to that of non-ischaemic controls. Furthermore, a mean perfusion pressure of 25 mmHg during machine perfusion leads to an improvement in recovery of renal function and better preservation of structural integrity compared with 30 mmHg.

**Discussion**

To effectively assess the influence of the preservation solution on the quality of preservation, the porcine autotransplantation model was employed. This useful model however, has the disadvantage of absence of alloantigen-dependent immune mechanisms, which emerge after transplantation. Therefore, Polysol should be evaluated in a model of allogeneic transplantation to assess its potential effects on immune responses to the graft. The use of allotransplantation models would be able to demonstrate the clinical applicability of Polysol.