Comparison of hypothermic and normothermic pulsatile machine perfusion in an ex-vivo porcine kidney model.

N.Vallant^{1,2}, F.W.K. Tam¹, B. Sandhu¹, N. Wolfhagen³, C.Pusey¹, V. Papalois¹

¹ Renal and Transplant Department, Imperial College London

² Department of Visceral-, Transplant- and Thoracic Surgery, Medical University Innsbruck

³ Erasmus University, Rotterdam

Background: Normothermic machine perfusion (NMP) offers great promise to preserve, preassess and even resuscitate donor organs ex-vivo, thereby offering the possibility of increasing the organ pool¹. However, as more experimental studies are needed to establish the optimal conditions for this to occur in the clinical routine, hypothermic machine perfusion (HMP) should not be overlooked as simple, though effective way of preserving and preconditioning organs ex-vivo prior to transplantation. In clinical trials in kidney transplantation, HMP has already been demonstrated to be superior to static cold storage (SCS) in terms of functional outcomes, most notably for organs from deceased donors². As comparative data is scarce, our aim was to directly compare NMP and HMP in an ex-vivo porcine kidney model.

Methods: 14 porcine kidneys from 7 donor pigs were retrieved and stored on ice for 24 hours. Thereafter, they either underwent HMP (n=5) or NMP (n=7) for 4 hours using an RM3 pulsatile perfusion machine (Fig. 1 a+b), or they remained on SCS for the same time (n=2). Two kidneys from one same donor pig were then reperused with whole blood for 2 hours on one machine at 37°C. During that time, the function of the kidneys was evaluated by measuring physiological parameters, including intrarenal resistance indices, perfusate flow rates and urinary outputs. Oxygen consumption rates were also calculated and compared between the groups. The effect at a cellular level was assessed by measuring mRNA expressions of inflammatory markers, including TNF α , IL-1 β , NGAL and EDN-1 using RT-PCR. The concentrations of NGAL in perfusate samples were analysed by ELISA.

Fig.1.: preconditioning phase: 4 hours of hypothermic machine perfusion (a) at 4°C with Belzer Machine Perfusion Solution, or normothermic machine perfusion using a packed red cell based perfusate (b) at 40mmHg systolic pressure and 36-37°C, respectively both on an RM3 pulsatile perfusion device.

Fig.2.: reperfusion phase: 2 hours on one machine using whole blood at 37°C, systolic perfusion pressure: 80-100mmHg.

Results: Hypothermically perfused kidneys showed a significantly higher urinary output at 60 minutes of reperfusion (189,5ml $\pm$ 73,5ml vs. 59,5ml $\pm$ 45,2ml, $p=0.0286$) during reperfusion than normothermically perfused kidneys. The oxygen consumption, resistance index and flow rates were better in hypothermically perfused kidneys, but this did not reach significance. Interestingly, on mRNA level, expressions of proinflammatory markers were higher upregulated after HMP than after NMP when compared to the control (tissue at time of retrieval). This was statistically significant for the expression of endothelin-1 EDN-1 (12,37 $\pm$ 7,44 vs 3,03 $\pm$ 1,96, $p=0,02$).

Fig.3. renal blood flow (a), oxygen consumption (b) and urinary output (c) during reperfusion were higher in kidneys after 4h of HMP than in kidneys after 4h of NMP or 28h of SCS. This only reached statistical significance for the urinary output at 60min of reperfusion. Creatinine clearance in 2 compared pairs of NMP and HMP was higher for kidneys after HMP than after NMP.

Fig.4. gene expression profiles after different treatments, followed by reperfusion: NGAL was not expressed in kidneys after SCS and stronger downregulated after HMP than after NMP compared to the control (T0-directly after retrieval) (a). Inflammatory cytokines were higher expressed in kidneys after HMP than after NMP and CSS, respectively (b-d).

Conclusion: We found that hypothermic machine perfusion of porcine kidneys improved physiologic parameters and led to an increased urinary output despite showing a higher upregulation of inflammatory markers on mRNA level when compared to normothermically perfused kidneys. Further experiments will be necessary to find underlying mechanisms responsible for this observation. Hypothermic machine perfusion is a well established method for preservation and preconditioning of organs coming from extended criteria donors and therefore, must not be overlooked in favor of normothermic machine perfusion until enough data exists.

References: 1 The use of ex-vivo normothermic perfusion for the resuscitation and assessment of human kidneys discarded because of inadequate in situ perfusion. Hosgood S Barlow A Dormer J Nicholson M. Journal of translational medicine; 2015 vol: 13 pp: 329. 2 Hypothermic machine perfusion reduces delayed graft function and improves one-year graft survival of kidneys from expanded criteria donors: a meta-analysis. Jiao B, Liu S, Liu H, Cheng D, Cheng Y, Liu Y. PLoS One. 2013 Dec 10;8(12):e81826. doi: 10.1371/journal.pone.0081826. eCollection 2013. Review.