Advances in hypothermic perfusion

Clavien, Pierre-Alain ; Dutkowski, Philipp

DOI: https://doi.org/10.1002/lt.24844

Syllabus

Advances in Hypothermic Perfusion

Pierre-Alain Clavien and Philipp Dutkowski

Department of Surgery & Transplantation, University Hospital Zurich, Zurich, Switzerland

Word count: 1188
Figures: 1

Correspondence:

Pierre-Alain Clavien, MD, PhD

Department of Surgery & Transplantation

Ramistrasse 100

University Hospital Zurich

CH-8091 Zurich

Phone: +41 44 255 33 00

Mail: clavien@access.uzh.ch

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1002/lt.24844

Key words: Machine perfusion, extended criteria grafts, HOPE, viability testing

Abbreviations:

ROS: oxygen free radicals; DAMP: Danger associated molecular pattern; TLR-4: Toll-like-receptor-4; SEC: sinusoidal endothelial cells; DCD: donation after circulatory death; DBD: donation after brain death; HMP: Hypothermic machine perfusion, HOPE: Hypothermic oxygenated perfusion; ATP: adenosine triphosphate; RET: reverse electron flow;

Introduction

Liver transplantation is an overall success story and has led worldwide to much more candidates on waiting lists than donor organs available[1-2]. This discrepancy between liver supply and demand has forced professionals to consider grafts from so-called extended criteria donors (ECD). Such livers induce, however, for more post-transplant complications, including primary-non-function (PNF), early allograft dysfunction (EAD), biliary complications and graft loss[3-5]. Key factors for liver graft dysfunction include donor warm ischemia (donation after circulatory arrest, DCD), graft steatosis, and prolonged conventional cold storage of more than 10 hrs[1,2,6]. Graft optimization or repair strategies before implantation are therefore of high importance to improve outcome. This presentation focus on underlying mechanisms of injury throughout procurement and implantation with special emphasis on hypothermic machine perfusion.

1. Injury during procurement and implantation

Liver injury already starts before organ procurement, e.g. during brain death (DBD), or due to donor warm ischemia in donation after cardiac death (DCD). After such initial injury already in donors, liver grafts undergo the process of retrieval surgery, including cold flush and cold storage. While cooling is a well-established procedure to slow down energy demand, a number of negative effects have been reported, e.g. depletion of adenine nucleotide pool[7], lactate acidosis[8], increase of chelatable iron[9], intracellular calcium accumulation[10], calpain activation[11,12] and sinusoidal endothelial damage[13]. All these effects are triggered by anaerobic metabolism, and limit therefore the maximum time period for cold storage due to deleterious effects on plasma membrane lipids, cytoskeleton, microtubules, and mitochondria, disabling the ion-exchange pumps and consequently membrane swelling and cell lysis[14].

During implantation, liver grafts are further exposed to ischemic rewarming, once more aggravating anaerobic metabolism. Subsequently, a re-exposure with normothermic blood and oxygen during implantation triggers an immediate burst of reactive oxygen species within the first minutes of reperfusion[15]. The mechanism behind has been shown to be dependent on mitochondrial derived injury[16], caused by mitochondrial electron leaksmainly due to reverse electron flow from mitochondrial complex II to complex I (RET)[17]. Thiscorrelates with massive oxidative injury of mitochondrial membranes and also DNA[18] and results in downstream release of danger associated molecular patterns (DAMPs)[19], activating of toll-like-receptors (TLR) located on non-parenchymal liver cells, and in release of numerous inflammatory mediators (Figure 1)[20]. Any efforts to restore blood flow in hypoxic tissue, therefore, paradoxically induce potentially more destructive than beneficial effects, depending on the amount of accumulated injury during the ischemic period[21].

2. Hypothermic perfusion concept

2.1. Idea and Mechanism

The current data point to the fact that oxygenation of the mitochondrial electron chain under hypothermic conditions, e.g. hypothermic oxygenated perfusion (HOPE), is the key element for the protection of hypothermic machine perfusion against reperfusion injury[22]. Three effects emerge due to this. First, function of mitochondrial enzyme complexes is improved during cold oxygenated perfusion, probably leading to forward instead of reverse electron flow. Secondly, adenine nucleotides are significantly uploaded to high levels during cold oxygenation, consecutively to a “repair” of mitochondrial function[23]. Third, the cellular redox state changes from reduced to oxidized[23]. Treatment by HOPE results therefore in less oxidative injury, less release of DAMPs, less activation of toll like receptors and improved liver function upon implantation. This effect is similar for DCD and steatotic liver grafts[24,25].

Furthermore, HOPE is not only effective against reperfusion injury but prevents also down-stream activation of immune response pathways[26].

One of the major advantages of hypothermic machine perfusion is its easy applicability, e.g. an endischemic approach after initial cold storage is effective. Thus, machine perfusion under cold conditions does not necessarily need to start at the place of organ donation[25]. Furthermore, HOPE perfusion was equally protective in DCD kidneys in a rodent model of kidney transplantation[27].

2.2. Clinical Data

Hypothermic dual (portal vein and hepatic artery) perfusion of the first twenty standard DBD human livers has been reported 2010 by Guarerra et al[28]. Machine perfusion was applied without additional active oxygenation after previous cold storage and transport of organs to the perfusion center. Of note, despite the lack of oxygenators in the circuit, the perfusate pO_2 was sufficient in enabling aerobic conditions during perfusion[28]. The results showed, that perfusion resulted in significantly less peak enzyme release and shorter hospital stay, as well as less early graft dysfunction compared to a non-randomized control group[28]. In a further report, the same investigators recently showed less biliary complication after application of hypothermic perfusion to marginal DBD organs[29]. Consistent to these results, hypothermic perfusion including active oxygenation (HOPE) has been shown by our group to be protective in human extended DCD liver grafts, despite very long donor warm ischemia times[30], with no occurrence of intrahepatic biliary complications in contrast to matched un-perfused DCD livers[31]. Importantly, HOPE treatment appeared sufficient by single portal vein perfusion, as the entire intra- and extrahepatic biliary system is positively effected through multiple collaterals between portal vein and hepatic artery[32,33]. Randomized trials are

therefore initiated to further evaluate the effect of HOPE on DBD and DCD liver grafts (hope-liver.com - Zurich, Groningen Institute for Organ transplantation - GIOT)[34].

Of note, a hypothermic oxygenated perfusion approach was tested recently in Maastricht Type II DCD livers following normothermic regional perfusion (NRP), or after extended cold storage[35]. In addition, the first long-term outcome analysis of HOPE treated DCD livers has been completed, showing excellent 5-year graft survival comparable to DBD liver transplantations[36].

2.3. Viability Assessment

Similarly, to liver enzyme measurements during normothermic perfusion, detection of several parameters during HMP and HOPE is currently under evaluation, e.g. microRNA[37], DAMP release[38], citric acid metabolites[39], parameters of mitochondrial function[40], and nucleotid pool assessment[41]. Several of these compounds may correlate to liver function after transplantation, but remain to be further investigated.

2.4. Future Aspects

Hypothermic perfusion is currently applied end-ischemically, after initial cold flush and storage. Whether liver grafts can be again cold stored after short term HOPE treatment is currently under investigation. Graft optimization in perfusion centers, with afterwards cold storage transport to implantation centers, could help to provide more livers for more transplant centers that do not have access to machine perfusion yet.

3. Conclusion

Repair of injured liver grafts and prediction of organ function before implantation are the two major concerns to allow the safe use of organs that were previously regarded as unsuitable. Much effort should therefore be directed to further developments of dynamic preservation methods, which will likewise replace static cold storage in high-risk grafts. In this context, reliable thresholds need to be defined for the difficult decision, which graft will benefit from machine perfusion treatment to provide a lifesaving, but also affordable optimization. More research and international concepts are necessary to further improve *ex vivo* medical treatment before transplantation, for example by new perfusion devices, e.g. Transmedics® machine for normothermic perfusion, or Airdrive® for hypothermic perfusion[42], which will be compared to existing techniques. Highly attractive are, however, relative short perfusion and centre bound treatments of liver grafts before implantation, which can provide significantly uploads in cellular energy stores. In all perfusion technologies, modern analytical technologies (e.g. proteomics, metabolomics) will be tested on liver tissue and perfusate, and may help to search for new biomarkers assessing graft quality before implantation.

Table 1: Current clinical liver perfusion systems for hypothermic perfusion

	Company	Pump	Oxgenation	Temperature	Perfusion route	cost	Transportation
Liver assist	Organ Assist	Centrifugal pump	Yes	10-37 °C	single or dual	3500.-€ per disposable	in the OR
Airdrive	QRS international	Membrane pump	Yes	8-12°C	single or dual	single use whole system	car or plane carriage
Life port liver transporter	Organ recovery	Roller pump	Yes	4-10 °C	dual	not specified	car or plane carriage

4. References

1. Graham JA, Guarrera J V. "resuscitation" of marginal liver allografts for transplantation with machine perfusion technology. J Hepatol. 2014;61(2):418–31.
2. Jiménez-Romero C, Caso Maestro O, Cambra Molero F, Justo Alonso I, Alegre Torrado C, Manrique Municio A, et al. Using old liver grafts for liver transplantation: Where are the limits? Vol. 20, World Journal of Gastroenterology. 2014. p. 10691–702.
3. Dutkowski P, Oberkofler CE, Slankamenac K, Puhan MA, Schadde E, Mullhaupt B, et al. Are there better guidelines for allocation in liver transplantation? A novel score targeting justice and utility in the model for end-stage liver disease era. Ann Surg. 2011;254(5):745–53; discussion 753.
4. Olthoff KM, Kulik L, Samstein B, Kaminski M, Abecassis M, Emond J, et al. Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. Liver Transplant. 2010;16(8):943–9.
5. Jay C, Ladner D, Wang E, Lyuksemburg V, Kang R, Chang Y, et al. A comprehensive risk assessment of mortality following donation after cardiac death liver transplant - An analysis of the national registry. J Hepatol. 2011;55(4):808–13.
6. Merion RM, Goodrich NP, Feng S. How can we define expanded criteria for liver donors? J Hepatol 2006,45:484-488.
7. van Golen RF, Reiniers MJ, van Gulik TM, Heger M. Organ cooling in liver transplantation and resection: How low should we go? Vol. 61, Hepatology. 2015. p. 395–9.
8. Dutkowski P, Krug A, Krysiak M, Dünschede F, Seifert JK, Junginger T. Detection of mitochondrial electron chain carrier redox status by transhepatic light intensity during rat liver reperfusion. Cryobiology. 2003;47(2):125–42.
9. Petrat F, de Groot H, Sustmann R, Rauen U. The chelatable iron pool in living cells: A methodically defined quantity. Vol. 383, Biological Chemistry. 2002. p. 489–502.
10. Chang WJ, Chehab M, Kink S, Toledo-Pereyra LH. Intracellular calcium signaling pathways during liver ischemia and reperfusion. J Invest Surg. 2010;23(4):228–38.
11. Kohli V, Gao W, Camargo, JR. C a., Clavien PA. Calpain is a mediator of preservation-reperfusion injury in rat liver transplantation. Proc Natl Acad Sci USA. 1997;94(3247):9354–9.
12. Chen M, Won DJ, Krajewski S, Gottlieb RA. Calpain and mitochondria in ischemia/reperfusion injury. J Biol Chem. 2002;277(32):29181–6.
13. Malhi H, Gores GJ. Cellular and Molecular Mechanisms of Liver Injury. Gastroenterology. 2008;134(6):1641–54.
14. Takakuwa Y, Nishino H, Ishibe Y, Ishibashi T. Properties and kinetics of membrane-bound enzymes when both the enzyme and substrate are components of the same microsomal membrane. Studies on lathosterol 5-desaturase. J Biol Chem. 1994;269(45):27889–93.
15. Van Golen RF, Van Gulik TM, Heger M. Mechanistic overview of reactive species-induced degradation of the endothelial glycocalyx during hepatic ischemia/reperfusion injury. Vol. 52, Free Radical Biology and Medicine. 2012. p. 1382–402.

16. Pryde KR, Hirst J. Superoxide is produced by the reduced flavin in mitochondrial complex I. J Biol Chem 2011, 18056-18065.

17. Chouchani ET, Pell VR, Gaude E, Aksentijević D, Sundier SY, Robb EL, et al. Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. Nature. 2014;515(V):431–5..

18. Jaeschke H. Reactive oxygen and mechanism of inflammatory liver injury: Present concepts. J Gatroenterol Hepatol 2011: 173-179.

19. Schlegel A, Graf R, Clavien PA, Dutkowski P. Hypothermic oxygenated perfusion (HOPE) protects from biliary injury in a rodent model of DCD liver transplantation. J Hepatol. 2013 Nov;59(5):984–91.

20. Schlegel A, Kron P, Dutkowski P. Hypothermic machine perfusion in liver transplantation. Curr Opin Organ Transplant. 2016 Jun;21(3):308–14.

21. Blackstone C, Chang C-R. Mitochondria unite to survive. Nat Cell Biol. 2011;13(5):521–2.

22. Schlegel A, Rougemont O De, Graf R, Clavien PA, Dutkowski P. Protective mechanisms of end-ischemic cold machine perfusion in DCD liver grafts. J Hepatol. 2013 Feb;58(2):278–86.

23. Dutkowski P, Graf R, Clavien PA. Rescue of the cold preserved rat liver by hypothermic oxygenated machine perfusion. Am J Transpl 2006: 903-912.

24. Schlegel A, Kron P, Graf R, Dutkowski P, Clavien PA. Warm vs. cold perfusion techniques to rescue rodent liver grafts. J Hepatol. 2014;61(6):1267–75.

25. Schlegel A, Dutkowski P. Role of hypothermic machine perfusion in liver transplantation. Vol. 28, Transplant International. Blackwell Publishing Ltd; 2015. p. 677–89.

26. Schlegel A, Kron P, Graf R, Clavien P-A, Dutkowski P. Hypothermic Oxygenated Perfusion (HOPE) downregulates the immune response in a rat model of liver transplantation. Ann Surg. 2014;260(5):931-7-8.

27. Kron P, Schlegel A, de Rougemont O, Oberkofler CE, Clavien P-A, Dutkowski P. Short, Cool, and Well Oxygenated - HOPE for Kidney Transplantation in a Rodent Model. Ann Surg. 2016: 815-822.

28. Guarrera J V., Henry SD, Samstein B, Odeh-Ramadan R, Kinkhabwala M, Goldstein MJ, et al. Hypothermic machine preservation in human liver transplantation: The first clinical series. Am J Transplant. 2010;10(2):372–81.

29. Guarrera J V., Henry SD, Samstein B, Reznik E, Musat C, Lukose TI, et al. Hypothermic machine preservation facilitates successful transplantation of “orphan” extended criteria donor livers. Am J Transplant. 2015;15(1):161–9

30. Dutkowski P, Schlegel A, De Oliveira M, Müllhaupt B, Neff F, Clavien PA. HOPE for human liver grafts obtained from donors after cardiac death. J Hepatol. 2014;60(4):765–72..

31. Dutkowski P, Polak WG, Muiesan P, Schlegel A, Verhoeven CJ, Scalera I, et al. First Comparison of Hypothermic Oxygenated PErfusion Versus Static Cold Storage of Human Donation After Cardiac Death Liver Transplants: An International-matched Case Analysis. Ann Surg. 2015;262(5):764–71.

32. Schlegel A, Kron P, De Oliveira ML, Clavien PA, Dutkowski P. Reply to “Is single portal vein perfusion the best approach for machine preservation of liver grafts?” Vol. 64, Journal of Hepatology. 2016. p. 1195–6.

33. Schlegel A, Kron P, De Oliveira ML, Clavien PA, Dutkowski P. Is single portal vein approach sufficient for hypothermic machine perfusion of DCD liver grafts? Vol. 64, Journal of

Hepatology. 2016. p. 239–41.

34. Jochmans I, Akhtar MZ, Nasralla D, Kocabayoglu P, Boffa C, Kaisar M, et al. Past, Present, and Future of Dynamic Kidney and Liver Preservation and Resuscitation. Am J Transplant. 2016;16(9):2545–55.

35. De Carlis L, De Carlis R, Lauterio A, Di Sandro S, Ferla F, Zanierato M. Sequential use of normothermic Regional Perfusion and Hypothermic Machine Perfusion in Donation After Cardiac Death Liver Transplantation with Extended Warm Ischemia Time. Transplantation. 2016; October; 10, p e 101-e102.

36. Schlegel A, Muller X, Perera T, Clavien P.-A, Isaac J, Muiesan P, Dutkowski P, 5-year experience in human extended DCD liver transplantation treated by hypothermic oxygenated perfusion (HOPE) before implantation, Abstract Congress International Liver Transplantation Society Prague, 2017.

37. Verhoeven CJ, Farid WRR, De Ruiter PE, Hansen BE, Roest HP, De Jonge J, et al. MicroRNA profiles in graft preservation solution are predictive of ischemic-type biliary lesions after liver transplantation. J Hepatol. 2013;59(6):1231–8.

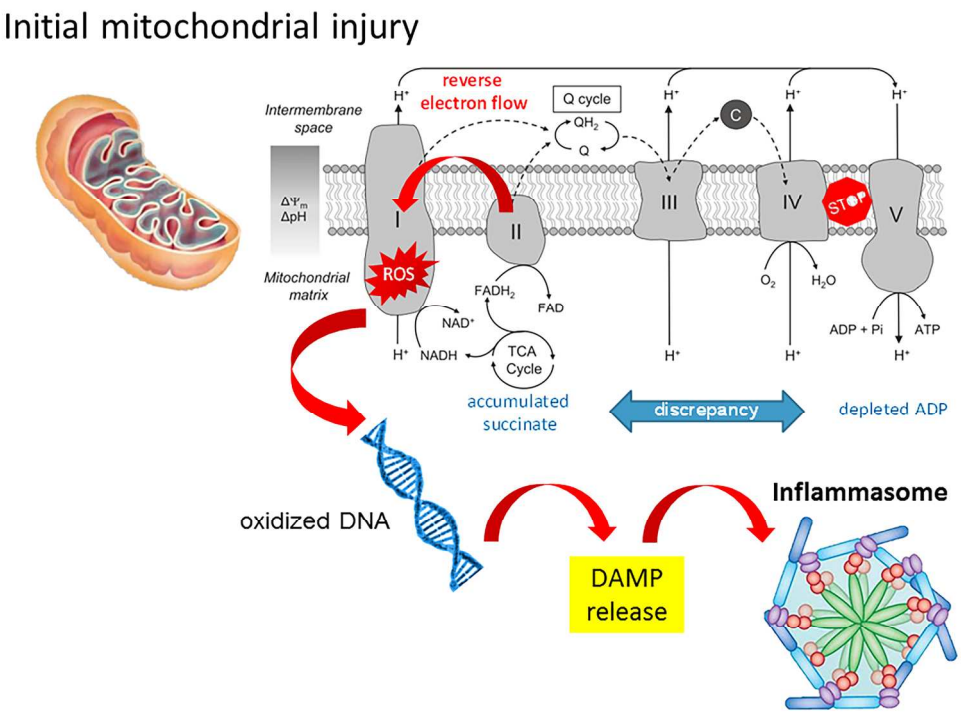
38. Sutton ME, Op Den Dries S, Karimian N, Weeder PD, De Boer MT, Wiersema-Buist J, et al. Criteria for Viability Assessment of Discarded Human Donor Livers during Ex Vivo Normothermic Machine Perfusion. PLoS One. 2014;9(11).

39. Yarmush G, Santos L, Yarmush J, Koundinyan S, Saleem M, Nativ NI, et al. Metabolic Flux Distribution during Defatting of Steatotic Human Hepatoma (HepG2) Cells. Metabolites. 2016;6(1):1.

40. Farid WRR, Verhoeven CJ, De Jonge J, Metselaar HJ, Kazemier G, Van Der Laan LJW. The ins and outs of microRNAs as biomarkers in liver disease and transplantation. Vol. 27, Transplant International. 2014. p. 1222–32.

41. Roedder S, Vitalone M, Khatri P, Sarwal MM. Biomarkers in solid organ transplantation: establishing personalized transplantation medicine. Genome Med. 2011;3(6):37

42. Compagnon P, Levesque E, Hentati H, Disabato M, Calderaro J, Feray C, Corlu A, Cohen JL, Ben Mosbah I, Azoulay D. An Oxygenated and Transportable Machine Perfusion System Fully Rescues Liver Grafts Exposed to Lethal Ischemic Damage in a Pig Model of DCD Liver Transplantation. Transplantation 2017: e205-e213.



Mechanism of ischemia reperfusion injury

254x190mm (300 x 300 DPI)