The importance of adequate oxygenation during hypothermic machine perfusion

To the Editor:

We read with interest the recent article by Alix *et al.* describing the effect of M101 (a marine worm hemoglobin) added to the static cold storage (SCS) solution for liver preservation.[1] In this study, the authors compared SCS+M101 to SCS alone and to hypothermic oxygenated machine perfusion (HOPE). The authors claim that adding the oxygen carrier M101 to SCS could be an alternative to HOPE for liver graft preservation. Although the authors should be commended for investigating an innovative approach to prevent ATP depletion during graft preservation, there are several important methodological issues to address.

First, we disagree with the author's definition of HOPE and believe that this control group is flawed. The authors state that, during HOPE, a continuous oxygen flow was delivered to the surface of the preservation solution. However, this approach is substantially different from the previously described (and currently clinically used) method of HOPE, where a membrane oxygenator in the machine perfusion circuit allows for active oxygenation of the preservation solution to achieve partial oxygen pressures (pO_2) of (at least) 70 kPa (500 mmHg).[2,3] Delivering oxygen only to the surface of the preservation solution is unlikely to achieve an adequate pO_2. Unfortunately, there is no information on the oxygen content in the perfusate.

Secondly, the different oxygenation strategy may account for the decreasing ATP levels during machine perfusion, which is a very unlikely phenomenon during HOPE if the preservation solution is adequately oxygenated. In fact, several groups have previously shown increased ATP levels at the end of both single and dual HOPE, in experimental and clinical studies (Table 1).[2,4–10] The observed decrease in ATP levels during machine perfusion supports our hypothesis that the HOPE methodology applied by the authors does not achieve adequate perfusate pO_2 levels. Adequate oxygenation under hypothermic conditions remains the key strategy to decrease reperfusion injury, as mitochondria are reprogrammed by cold oxygenation, leading to metabolism of accumulated succinate and NADH with significant increases in ATP levels within just 1–2 hours of HOPE.[11]

Third, in the results presented by the authors, mitochondrial activity and post-transplantation injury markers are similar between livers preserved with SCS supplemented with M101 *vs.* HOPE. However, we postulate that by using HOPE with an adequate pO_2 of the preservation solution, mitochondrial function would have been substantially improved. Notably, high (100%) oxygen delivery best preserves functional and structural liver integrity compared to low (20%) oxygen delivery.[12] In addition, machine perfusion in the absence of oxygen is not able to prevent reperfusion injury and triggers hepatocyte death.[12,13]

Fourth, the effect of M101 supplementation in SCS solutions should be further tested in experimental models, such as animal models or preclinical research models using severely injured grafts, *e.g.* in livers exposed to extended donor warm ischemia, or in severely steatotic livers.

Fifth, we believe there are several other benefits of HOPE, such as the ability to assess liver viability or extend preservation time, which are currently unlikely to be achieved by SCS.

Taken together, we believe that it is currently overstated that M101 supplemented to the SCS solution could be an alternative to HOPE for liver graft preservation. Based on the results presented, supplementation of M101 to the SCS solution seems better than SCS alone, but this requires more research. Additionally, this method still does not achieve the results that can be obtained with HOPE.

Table 1. Studies using HOPE for donor liver preservation with measurement of ATP.

Reference	Protocol	Fold ATP increase
Brüggenwirth *et al.* (2020)[5]	20-hour dual HOPE of discarded human livers	5
Van Rijn *et al.* (2017)[2]	2-hour dual HOPE of human livers	11
Westerkamp *et al.* (2016)[6]	2-hour dual HOPE of discarded human livers	15
Monbaliu *et al.* (2012)[7]	24-hour dual HOPE of discarded human livers	2
Stegeman *et al.* (2009)[8]	90-minute HOPE of rat livers	6
Vekemans *et al.* (2007)[9]	24-hour HOPE of porcine livers	1
Dutkowski *et al.* (2006)[10]	1-hour HOPE of rat livers	1

ATP, adenosine triphosphate; HOPE, hypothermic oxygenated machine perfusion.

Keywords: Liver preservation; Liver transplantation; ATP; Hypothermic oxygenated machine perfusion.
Received 17 August 2020; received in revised form 29 September 2020; accepted 5 October 2020; Available online 9 October 2020

Financial support

The authors received no financial support to produce this manuscript.

Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

IB, RP, VM wrote the manuscript in consultation with OL, MM, PD, DM and PM. All authors critically revised the final manuscript and approved the submitted version.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2020.100194.

References

Author names in bold designate shared co-first authorship

[1] **Alix P**, **Val-Laillet D**, Turlin B, Ben Mosbah I, Burel A, Bobillier E, et al. Adding the oxygen carrier M101 to a cold-storage solution could be an alternative to HOPE for liver graft preservation. JHEP Rep 2020;2(4):100119.

[2] van Rijn R, Karimian N, Matton APM, Burlage LC, Westerkamp AC, van den Berg AP, et al. Dual hypothermic oxygenated machine perfusion in liver transplants donated after circulatory death. Br J Surg 2017;104(7):907–917.

[3] 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):764–770. discussion 770–1.

[4] **Kron P**, **Schlegel A**, Mancina L, Clavien P-A, Dutkowski P. Hypothermic oxygenated perfusion (HOPE) for fatty liver grafts in rats and humans. J Hepatol 2018;68(1):82–91.

[5] Brüggenwirth IMA, van Leeuwen OB, de Vries Y, Bodewes SB, Adelmeijer J, Wiersema-Buist J, et al. Extended hypothermic oxygenated machine perfusion enables ex situ preservation of porcine livers for up to 24 hours. JHEP Rep 2020;2(2):100092.

[6] **Westerkamp AC**, **Karimian N**, Matton APM, Mahboub P, van Rijn R, Wiersema-Buist J, et al. Oxygenated hypothermic machine perfusion after static cold storage improves hepatobiliary function of extended criteria donor livers. Transplantation 2016;100(4):825–835.

[7] Monbaliu D, Liu Q, Libbrecht L, De Vos R, Vekemans K, Debbaut C, et al. Preserving the morphology and evaluating the quality of liver grafts by hypothermic machine perfusion: a proof-of-concept study using discarded human livers. Liver Transpl 2012;18(12):1495–1507.

[8] Stegemann J, Minor T. Energy charge restoration, mitochondrial protection and reversal of preservation induced liver injury by hypothermic oxygenation prior to reperfusion. Cryobiology 2009;58(3):331–336.

[9] Vekemans K, Liu Q, Brassil J, Komuta M, Pirenne J, Monbaliu D. Influence of flow and addition of oxygen during porcine liver hypothermic machine perfusion. Transpl Proc 2007;39(8):2647–2651.

[10] Dutkowski P, Furrer K, Tian Y, Graf R, Clavien P-A. Novel short-term hypothermic oxygenated perfusion (HOPE) system prevents injury in rat liver graft from non-heart beating donor. Ann Surg 2006;244(6):968–977.

[11] **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(7527):431–435.

[12] Lüer B, Koetting M, Efferz P, Minor T. Role of oxygen during hypothermic machine perfusion preservation of the liver. Transpl Int 2010;23(9):944–950.

[13] **Schlegel A**, **de Rougemont O**, Graf R, Clavien P-A, Dutkowski P. Protective mechanisms of end-ischemic cold machine perfusion in DCD liver grafts. J Hepatol 2013;58(2):278–286.

Isabel M.A. Brüggenwirth[1]
Otto B. van Leeuwen[1]
Matteo Müller[2]
Philipp Dutkowski[2]
Diethard Monbaliu[3]
Paulo N. Martins[4]
Robert J. Porte[1]
Vincent E. de Meijer[1,*]

[1]*Department of Surgery, Section of Hepatobiliary Surgery and Liver Transplantation, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands;*
[2]*Department of Surgery and Transplantation, University Hospital Zurich, Zurich, Switzerland;*
[3]*Department of Abdominal Transplant Surgery, University Hospitals Leuven, Catholic University Leuven, Leuven, Belgium;*
[4]*Department of Surgery, Division of Organ Transplantation, UMass Memorial Medical Center, University of Massachusetts, Worcester, Massachusetts, United States*

**Corresponding author. Address: Department of Surgery, Section of HPB Surgery and Liver Transplantation, University Medical Center Groningen, P.O. Box 30.001, 9700 RB Groningen, The Netherlands.*

E-mail address: v.e.de.meijer@umcg.nl (V.E. de Meijer).