

Effect of perfusate composition during long-term machine perfusion of livers using a precision-cut liver slice model

J. Hofmann¹, A.T. Meszaros¹, V. Hackl¹, N. J. Ortner², C. Bogensperger¹, T. Hautz¹, S. Schneeberger¹, T. Resch¹

¹organLife Laboratory and Daniel Swarovski Research Laboratory, Department of Visceral, Transplant and Thoracic Surgery, Medical University of Innsbruck, Innsbruck, Austria

²Department of Pharmacology and Toxicology, Institute of Pharmacy, Center for Molecular Biosciences Innsbruck, University of Innsbruck, Innsbruck, Austria

INTRODUCTION

Long-term normothermic machine (NMP) perfusion may open the opportunity for *ex vivo* regeneration of marginal organs.^{1,2} Thus, perfusion protocols need to be adapted for extended perfusion durations. One important point to consider is that perfusate electrolyte levels may exceed physiological levels during isolated liver perfusion since filtration is lacking.

AIM

Development of a precision-cut liver slice (PCLS)³ model to evaluate the cellular effects of changing **perfusate composition** during long-term machine perfusion.

METHOD

- **8 mm punch biopsies** obtained of cold preserved livers before they underwent NMP.
- **PCLS (300 μM)** were generated using a Leica Vibratome.
- Cultivation for 7 days in DMEM supplemented with defined concentrations of **Na⁺, K⁺ and urea**.
- For viability and functionality assessment MTS assay was performed and albumin secretion was assessed.⁴
- Cell damage was evaluated by LDH assessment in the supernatant.⁵

RESULTS

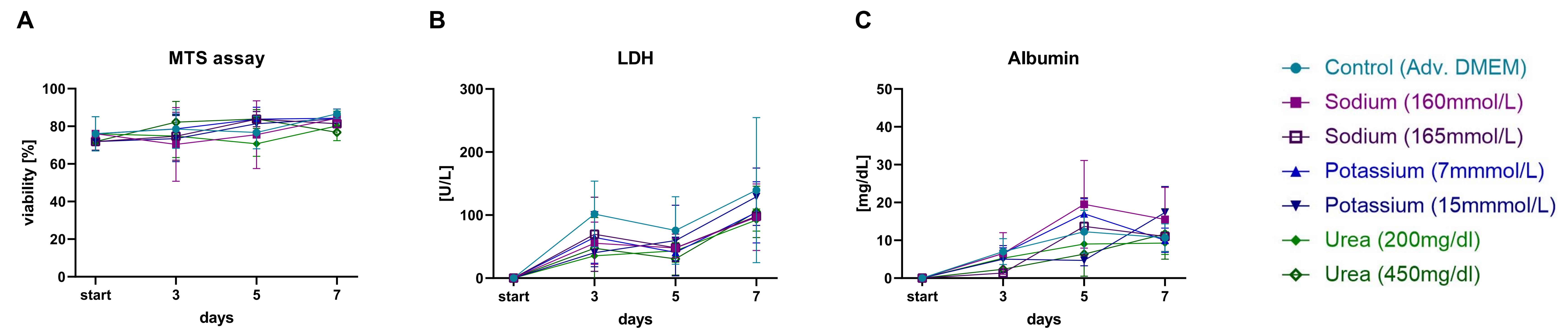


Figure 1: Analysis of viability by MTS assay (A), cell damage by LDH analysis (B) and functionality by albumin production (C).

PCLS of a 8 mm punch biopsy could be generated and slicing process did not cause any damage. Viability was maintained for all conditions with no significant compared to the control group and to the viability after slicing (Figure 1A).

LDH levels increased at day 3 due to the slicing process, but they remained stable in the further course of cultivation for all conditions (Figure 1B).

For all conditions we observed albumin production with no significant differences (Figure 1C).

CONCLUSIONS

Na⁺, K⁺ and urea levels above the physiological range do not cause damage to PCLS.

Elevated perfusate levels of those analytes may not lead to tissue injury during long-term machine perfusion.

REFERENCES

1. Lascaris B, Thorne AM, Lisman T, Nijsten MWN, Porte RJ, de Meijer VE. Long-term normothermic machine preservation of human livers: what is needed to succeed? *Am J Physiol Gastrointest Liver Physiol* 2022; **322**(2): G183-G200.
2. Eshmuminov D, Becker D, Bautista Borrego L, Hefti M, Schuler MJ, Hagedorn C, et al. An integrated perfusion machine preserves injured human livers for 1 week. *Nat Biotechnol* 2020; **38**(2): 189-98.
3. de Graaf IA, Olinga P, de Jager MH, Merema MT, de Kanter R, van de Kerkhof EG, et al. Preparation and incubation of precision-cut liver and intestinal slices for application in drug metabolism and toxicity studies. *Nat Protoc* 2010; **5**(9): 1540-51.
4. Wu X, Roberto JB, Knupp A, Kenerson HL, Truong CD, Yuen SY, et al. Precision-cut human liver slice cultures as an immunological platform. *J Immunol Methods* 2018; **455**: 71-9.
5. Palma E, Doornebal EJ, Chokshi S. Precision-cut liver slices: a versatile tool to advance liver research. *Hepatol Int* 2019; **13**(1): 51-7.

ACKNOWLEDGEMENT

We thank all members of the NMP and organLife teams at the Department of Visceral, Transplant and Thoracic Surgery, University Hospital Innsbruck.

CONTACT INFORMATION

Julia Hofmann, MSc
organLife – Organ Regeneration Center of Excellence Innsbruck
Dept. of Visceral, Transplant and Thoracic Surgery
Medical University of Innsbruck
julia.hofmann@i-med.ac.at