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Long-Term Perfusion of the Liver Outside the Body: Warming Up for *Ex Vivo* Therapies?

The term "game-changing technology" is every so often used as a hyperbole to underline the importance of research to the outside world. The experimental work of the ETH Zurich and University Hospital Zurich, recently published in Nature Biotechnology by Eshmuminov et al.,[(1)] is, however, no less than a real game changer in the field of liver transplantation. The authors achieved an increase in stable liver function *ex vivo* from a few hours to 7 days using a normothermic machine perfusion (NMP) device. This leap forward in perfusion time, while maintaining physiological balance, is incredibly important as it opens a window of opportunity to explore *ex vivo* organ repair therapies.

Driven by organ shortage and waiting list mortality, transplant physicians are pushed to use donor organs of marginal quality. NMP provides opportunities to test and even improve the quality of these grafts. However, with current commercially available NMP devices, safe perfusion is only warranted for up to 24 hours. In most clinically used perfusion protocols, the portal vein is perfused with highly oxygenated blood, resulting in a high demand for vasodilator medication. Furthermore, the perfusion systems lack sufficient physiological support, which is detrimental for long-term perfusion of donor livers. Obviously, *in vivo*, the liver is not an autonomous organ. Liver homeostasis depends on hormonal systems, such as pancreatic glucose regulation and kidney filtration to remove waste products, to regulate electrolyte levels, extracellular volume, and pH balance. Furthermore, motion created by the contraction of the diaphragm aids in perfusing the liver and provides biomechanical support for the functioning of the liver.

To achieve sustained *ex vivo* perfusion, *in vivo* physiological conditions were mimicked. Glucose levels were monitored in real time, and glucose homeostasis was automatically regulated by administering glucose, insulin, or glucagon. Furthermore, an integrated dialysis system continuously removed waste products and maintained electrolyte levels. Diaphragm movement

was simulated by inflation of a balloon under the liver, functioning like an antidecubitus bed and preventing nonperfused areas. Finally, oxygen saturation in the portal vein was set to the physiological venous saturation, resulting in a decreased need for vasodilators. This article is not the first to implement these individual components as, for example, hemodialysis and management of glucose homeostasis have been implemented by others.[2,3] However, this article is the first to implement all of these different elements together in one automated circuit.

With the new perfusion system, 10 discarded human livers were perfused. In six of these livers, physiological levels of glucose, electrolytes, and oxygen saturation were maintained. These livers were found viable at 7 days on the basis of adequate response to hormones and vasoactive agents and maintained histological integrity without endothelial activation and assessment of liver function/damage. During perfusion, a gradual decrease in nonviable cells was detected, and dividing cells were seen, indicating regeneration. Furthermore, glucose metabolism was preserved as determined by positron emission tomography/computed tomography imaging. This also revealed that these livers were fully perfused, without poorly perfused areas. Additionally, the metabolic and excretory liver functions were active throughout the perfusion as demonstrated by production of adenosine triphosphate, coagulation factor V, urea, and bile. Metabolic products such as lactate and ammonia were efficiently cleared. The other four livers were found to be nonviable as they did not meet the viability score and showed insufficient metabolic or excretory liver function.

The lack of knowledge on how the viability scores should be valued as predictive values for successful and safe liver transplantation is a limitation. Porcine livers were functional after transplantation, but follow-up was only 3 hours. The ultimate proof would be clinical transplantation of a human liver. Transplantation of a perfused human liver in an immune-incompetent porcine model could serve as an intermediate step for showing the safety of the procedure.

The novelty of this exciting publication does not lie within the possibility of testing liver function as an aid to deciding whether marginal or even discarded futile grafts can be used safely as this is also feasible during short-term (4-8 hours) NMP.[4] However, more time is required to sufficiently repair grafts on the pump. Clinical data from small-for-size living donor liver transplantation and the associated liver partition and portal vein ligation for staged hepatectomy procedure in extreme liver resections show that 5-7 days are needed to allow for sufficient cell proliferation and resumption of normal metabolic homeostasis by the liver.[5] Long-term stable perfusion can provide clinicians with enough time to improve those grafts which do not pass the required criteria for short-term NMP. Furthermore, this repair process can be aided by applying exogenous repair therapies.

These exogenous repair therapies or regenerative medicine strategies could include (stem) cell therapy or pharmacological interventions. An example of cell therapy is the infusion of mesenchymal stromal cells (MSCs) into the liver during perfusion, permitting the cells to engraft and exert their paracrine effects.[6] MSCs are shown to produce growth factors and interleukins, which benefit regeneration and reduce inflammation and hepatic injury.

Moreover, long-term NMP could be used to treat steatotic grafts with a "defatting cocktail," to reduce steatosis and associated inflammation before transplantation.[7] Severe steatosis is the main indication for declining donor livers as these livers are highly susceptible to reperfusion injury and primary nonfunction. Therefore, the increasing obesity of the donor population is a growing threat to liver transplantation. Being able to remove the fat from these grafts could enlarge the pool of suitable donor organs. Other potential applications could include a small liver graft incubator for a living donor or *ex vivo* complex liver resections or treatment of diseased liver (e.g., primary liver cancer) for improving treatment strategies.

The challenge ahead will be to translate from this excellent experimental work to clinical practice and to select patients who will benefit, while the technique is still in an experimental phase. If a donor liver is primarily acceptable for transplantation, subjecting this liver to the long-term NMP platform might jeopardize an otherwise successful transplantation. If a liver graft fails to pass short-term NMP testing and needs long-term repair, is it justifiable to subject a patient to the risk of receiving this "refurbished" liver, while there is still time to wait for another primarily acceptable offer? Obviously, these decisions will be dictated by waiting list mortality

and dropout rates per center/country. Theoretically, patients who potentially benefit the most are those with very high Model for End-Stage Liver Disease scores or acute liver failure. However, these patients may not have the time to wait for the repair process and are the least able to cope with perioperative or posttransplant complications, such as reperfusion syndrome, acute kidney injury, or delayed graft function. Given the sometimes troublesome graft reperfusion after short-term NMP (A. Schlegel to J. de Jonge, personal communication), not including these patients might initially be better. A logical choice would be to include patients with hepatocellular carcinoma threatening to cross transplantability criteria with otherwise stable liver disease or patients being denied access to liver transplantation (due to age, medical history of malignancy, etc.). In those cases, it should be clear if emergency retransplantation will be allowed in case of primary nonfunction.

Summarizing, this novel platform for *ex vivo* long-term NMP of human livers provides an exciting therapeutic playfield and is one of the most significant breakthroughs in hepatobiliary and transplant surgery of recent decades, enabling organ repair and regenerative therapy on the pump.

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