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Transient hyperthermia during oxygenated rewarming of isolated rat livers

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SUMMARY

Pretransplant machine perfusion of donor grafts has gained clinical appreciation to improve graft function and survival after transplantation. This study was aimed as pilot investigation to evaluate the additive potential of a transient *ex vivo* heat shock treatment of the isolated organ during machine perfusion to further protect the graft from subsequent reperfusion injury. Rat livers were retrieved after 20 min of cardiac arrest and preserved for 18 h by cold storage in HTK solution. Prior to reperfusion, livers were subjected to 2 h of reconditioning machine perfusion with gradual increase in perfusion temperature up to 35 °C. In half of the livers ($n = 7$), a brief hyperthermic impulse (10 min perfusion at 42 °C) was implemented in the machine perfusion period. Functional recovery of the grafts was observed upon normothermic reperfusion *in vitro*. Induction of heat shock protein 70 was followed on the mRNA and protein level. Chaperone induction by transient hyperthermia was associated with a significant improvement of bile production upon reperfusion and significantly reduced enzyme loss of mitochondrial GLDH. Heat shock treatment further affected pro-inflammatory upregulation in the graft in significantly reducing gene expression as wells as protein release of TNF-alpha. It is concluded, that graft conditioning by controlled hyperthermia *ex vivo* may represent a feasible and useful tool to improve liver recovery after preservation.

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Key words
ex vivo perfusion, heat shock, hyperthermia, ischemia reperfusion injury, organ preservation and procurement

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Introduction

Liver transplantation still remains the therapy of choice in end-stage liver diseases. Increasing shortage of donor organs during last centuries has driven efforts to find strategies for enhanced accessibility of donor grafts [1]. The use of extended criteria donor (ECD) organs is a way to counter the rising demand on organs for transplantation. However, these "less quality" grafts are more prone to ischemia–reperfusion injury (IRI) and are associated with higher risks of delayed graft function and decreased survival [2]. In this regard, *ex vivo* organ preservation represents an important tool to condition and regenerate the graft and offers possibilities to improve organ quality prior to implantation [3].

Nowadays, machine perfusion (MP) of donor grafts has found its way in clinical application and successfully contributes to better graft function and survival outcome after transplantation of ECD organs [4].

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Hypothermic oxygenated machine perfusion (HMP) is an already established method, intensively evaluated in experimental and clinical settings [5,6]. A more modern approach is constituted by normothermic machine perfusion (NMP) providing perfusion under physiological conditions, thereby enabling therapeutic interventions [7]. However, abrupt temperature shifts, that appear when the cold-stored organ is transferred to normothermic perfusion, were found to negatively impact organ quality [8,9]. In order to combine favorable transport logistic of simple cold storage and the advantage of organ reconditioning by NMP prior to reperfusion, controlled oxygenated rewarming (COR) was introduced to circumvent dramatic shifts in temperature and to enable a mild adaption of metabolic activity in the vulnerable cold-stored tissue [3,10].

Notwithstanding that, MP is not only a strategy to supply the graft with nutrients and oxygen to minimize events of IRI, but also provides an accessible isolated organ and thus offers a platform for a broad range of treatment options. These include pharmacological applications [11], genetic and immunomodulatory modifications [12], but also physical interventions to improve organ recovery and to reduce vulnerability to postreperfusion injury and rejection.

Heat shock treatment is a method, known to trigger protective signal transduction in tissue cells and induces, for example, anti-inflammatory and anti-apoptotic mechanisms. Hyperthermic conditions stimulate the production of heat shock proteins (HSP), which are protective molecular chaperones, stabilizing protein structures during heat exposure [13].

The present pilot study was aimed to delineate the potentially beneficial impact of applying a controlled hyperthermic impulse as additive physical conditioning tool during liver preservation.

In an isolated rat liver preparation, it will be shown that integration of a brief hyperthermic period during oxygenated machine perfusion effectively induces an increased heat shock response in the tissue and actually improves functional recovery upon reperfusion.

Materials and methods

The experiments were performed according to the federal law regulating the protection of animals and follow the principles of laboratory animal care (NIH publication vol 25, No 28, revised 1996).

Male Wistar rats with a weight between 250 and 300 g were sacrificed by intracardiac injection of potassium chloride in deep isoflurane anesthesia. The abdomen was exposed by midline incision and 20 min after cardiac arrest, the portal vein was cannulated and the liver rinsed via the portal vein with 60 ml of histidine-tryptophan-ketoglutarate (HTK) solution (Köhler Chemie, Bensheim, Germany). After hepatectomy the livers were finally cold-stored overnight in HTK for 18 h at 4 °C and then randomly assigned to one of the following groups:

Controlled oxygenated rewarming ($n = 7$)

After 18 h of static preservation at 4 °C, some grafts were put on a home-made machine perfusion circuit and 200 ml of Aqix RS-I solution were recirculated through the portal vein in a constant pressure mode. The solution was passed through a thin wall silicone tubing curled in a box that was equilibrated with a 95% O_2–5% CO_2 gas mixture. The oxygenated perfusate ($pO_2 > 500$ mmHg) was subsequently passed through a heat exchanger and a bubble trap prior to entering the liver.

After initial perfusion in hypothermia, controlled rewarming of the perfusate was performed up to 35 °C as described earlier [14,15] using a programmable, external circulating cryo-thermostat connected to heat exchanger and reservoir. Overall perfusion time was 120 min; the maximal temperature was reached within the first 60 min. Portal pressure was measured by a pressure sensor that was placed in the inflow line immediately prior to the portal vein cannula. The sensor was connected to a servo-controller, ruling the roller-pump as to maintain an initial perfusion pressure of 3 mmHg, which was adjusted up to 5 mmHg during later perfusion at higher temperatures.

Transient hyperthermia during oxygenated rewarming ($n = 7$)

Livers were put on the machine perfusion circuit after 18 h of cold storage and perfused as described above. After 60 min of perfusion, reaching a temperature of 35 °C, the temperature of the perfusate was temporarily elevated to 42 °C for 10 min. Subsequently, extracorporeal perfusion was continued at 35 °C as usual for the rest of the 120 min (cf. Fig. 1).

Isolated liver perfusion

Prior to reperfusion all livers were rinsed with 20 ml of cold physiologic saline and exposed to 20 min of 2nd warm ischemia—simulating the ischemic period during surgical implantation *in vivo*—by placement in a double jacketed glass beaker that was connected to a circulating thermostat regulated to 22 °C.

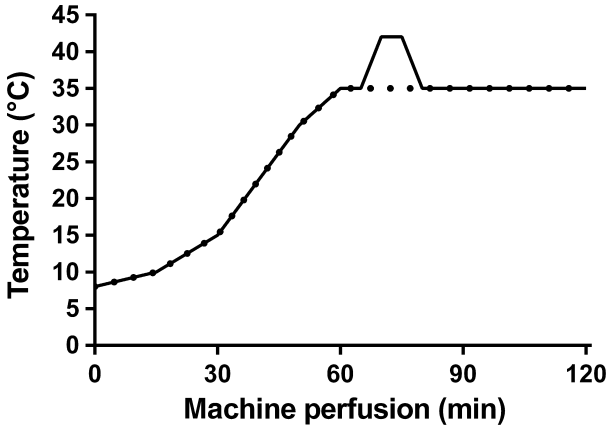

Figure 1 Temperature kinetics upon reconditioning perfusion after cold storage: (a) controlled oxygenated rewarming—dotted line. (b) Transient hyperthermia during oxygenated rewarming—solid line.

Reperfusion was then started with oxygenated (95% O_2–5% CO_2; $pO_2 > 500$ mmHg) Williams E solution (Sigma-Aldrich, Taufkirchen, Germany), supplemented with 3 g/100 ml of bovine serum albumin. The setup has been described in detail previously and was shown to allow for adequate approximation of tissue integrity and detection of structural changes in rat livers after hypothermic preservation [16].

Liver function upon reperfusion

The common bile duct of the livers was cannulated with a 27-gauge polyethylene tubing. Bile was collected during the whole reperfusion period and hepatic bile production was calculated as µl/g/h.

Recovery of energy metabolism was approximated by analysis of tissue levels of ATP at the end of reperfusion. Specimens were taken with precooled steel tongs, immersed in liquid nitrogen and stored at −80 °C for later analysis. Wet weight of the frozen tissue samples was measured before they were lyophilized in a vacuum freezer (−60 °C; <0.025 mbar) for at least 7 days to evaporate tissue water. Freeze-dried specimens were weighed again, and proteins were extracted with perchloric acid as described previously [17]. Aliquots of the neutralized supernatant were used for determination of adenosine triphosphate (ATP) by means of a commercial test kit (Abcam, Cambridge, UK) according to the manufacturer's instructions. The results were corrected for the respective dry weight to wet weight ratio of the tissue samples and expressed as µmol/g dry weight.

Western Blot

Quantification of target proteins was performed in whole-cell extracts by immunoblotting as described earlier [15]. Primary antibodies were used against induced Heat shock protein 70 (HSP70; Stressmarq, Victoria, BC, Canada) and ß-actin (Thermo Fischer Scientific, Waltham, MA, USA). Quantification of protein content was performed densitometrically with UN-SCAN-IT gel v 6.1 (Silk Scientific Corporation, Orem, UT, USA) as normalized against the expression of ß-actin.

TNF-alpha

TNF-alpha was analyzed as a readout for pro-inflammatory reactivity of the grafts. Quantification of mRNA upregulation was done using standard RT-PCR as detailed elsewhere [15]. Reagents and primers (TNF – PPR06411F, GAPDH – PPR06557B) were purchased from Qiagen GmbH (Hilden, Germany).

Perfusate levels of TNF-alpha were determined using standard ELISA test kit (Abcam), according to the instructions of the manufacturer.

Hepatic enzyme loss during perfusion

Enzyme activities of cytosolic alanine aminotransferase (ALT) and mitochondrial glutamate dehydrogenase (GLDH) in the perfusate were assessed in a routine fashion at the laboratory center of the University Hospital.

Histology

Liver tissue was collected at the conclusion of the experiments, cut into small blocks (3 mm thickness) and fixed by immersion in 4% buffered formalin. The blocks were embedded in paraffin, and 2–4 mm tissue slides were prepared using a microtome (SM 2000R; Leica Instruments, Nußloch, Germany). Hematoxylin Eosin (H & E) staining was conducted adherent to in-house standards and used to assess morphological integrity of the parenchyma. Sections were examined at 200-fold magnification, and the extent of necrotic injury was semi-quantitatively graded in a 4-stage system ranging from 0 (no necrosis) to 3 (severe necrosis with disintegration of hepatic cords) as described elsewhere [18] by two independent examiners.

Statistics

All values are expressed as means ± SEM. Differences between the groups were tested by nonparametric comparison using the Mann–Whitney test. Statistical significance was set at P less than 0.05.

Results

Reconditioning perfusion

During reconditioning perfusion, temporary hyperthermia resulted in slightly increased levels of lactic acid (4.4 ± 0.8 vs. 3.3 ± 0.5 μmol/l) as compared with controlled rewarming to 35 °C. Lower levels were found in the transient hyperthermia during oxygenated rewarming (THOR) group for enzyme leakage of ALT (16.4 ± 3.8 vs. 23.6 ± 7.7 U/l; THOR versus COR) and GLDH (8.0 ± 2.4 vs. 15.3 ± 7.7 U/l) but the differences did not reach statistical significance for any of these parameters.

Documentation of the heat shock response

Molecular confirmation of the heat shock response, induced by the transient hyperthermia during machine perfusion, was seen in an enhanced induction of HSP70 mRNA as well as the expression of the actual protein (cf. Fig. 2).

Western blot analysis disclosed an approximately threefold increase of actual protein reactivity of HSP70 upon reperfusion in THOR treated livers when compared to the control group.

Recovery upon reperfusion

Functional recovery of the livers was positively affected by the transient hyperthermia during reconditioning. Cumulative bile production upon reperfusion was found increased to nearly twice the values observed in the nonhyperthermia treated group (Fig. 3a).

Likewise, THOR treatment entailed a significant reduction in hepatic leakage of the intra-mitochondrial enzyme GLDH upon reperfusion while untreated livers exhibited higher starting levels as well as a steeper increase during ongoing perfusion (Fig. 3b).

No differences, however, were disclosed with respect to the leakage of ALT, maximal perfusate concentrations during reperfusion amounted to 244 ± 79 vs. 239 ± 39 U/l (THOR versus COR, n.s.).

The energetic recovery of the livers, as a supplementary readout for mitochondrial function, was judged by measurement of tissue ATP at the end of reperfusion period.

Tissue levels of ATP were notably, albeit not quite significantly affected by the reconditioning protocol in that THOR tendentially resulted in higher values in comparison to COR (2.89 ± 0.47 vs. 1.71 ± 0.34 μmol/g dw; $P = 0.053$).

Perfusate levels of TNF-alpha upon reperfusion were used to approximate pro-inflammatory reactivity of the graft with or without previous exposure to transient hyperthermia. It was found that THOR significantly reduced hepatic release of TNF-alpha (135.0 ± 67.7 vs. 220.1 ± 39.6 pg/nl; $P < 0.05$). This result was corroborated by gene expression analysis that revealed upregulation of TNF-alpha mRNA to be mitigated by the factor 2 after THOR as compared to COR (15.4 ± 2.9 vs. 32.9 ± 6.3 fold baseline; $P < 0.05$).

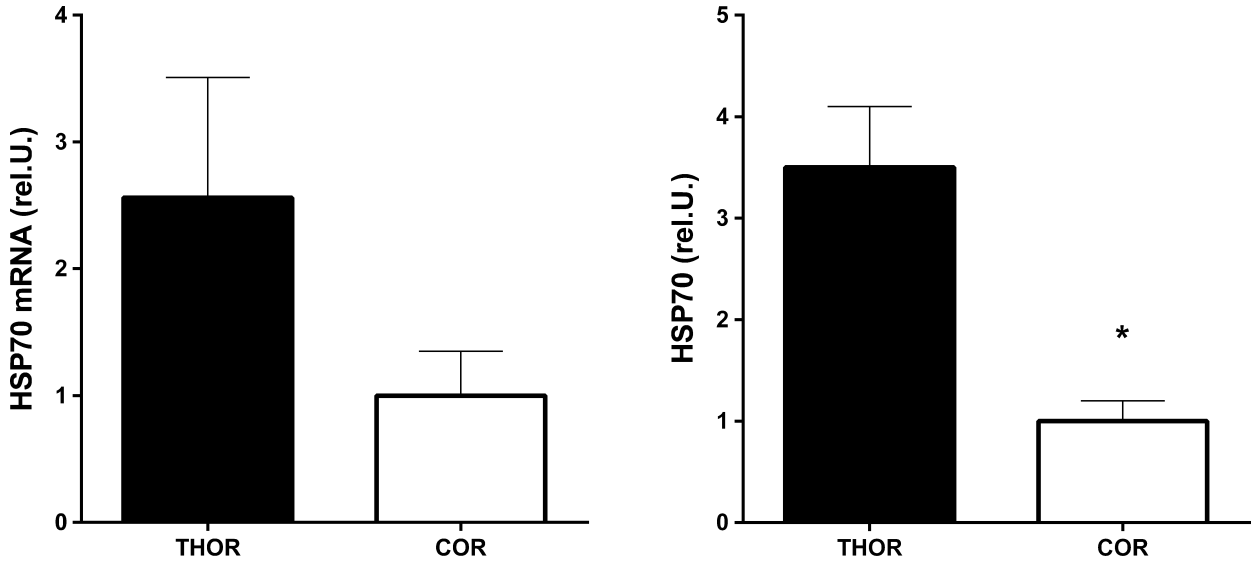

Figure 2 Gene expression (left) and protein expression (right) of HSP 70 after end-ischemic reconditioning by controlled oxygenated rewarming or by transient hyperthermia during oxygenated rewarming. Results expressed as relative amounts with COR set as 1 (*$P < 0.05$).

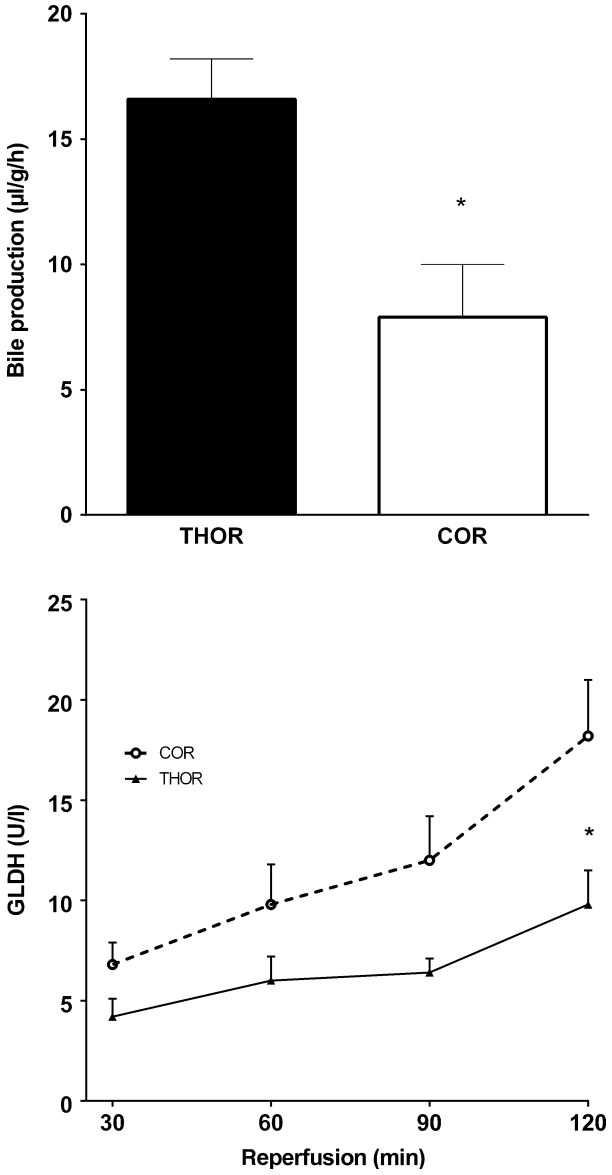

Figure 3 Upper panel: hepatic bile production during isolated reperfusion after end-ischemic reconditioning by controlled oxygenated rewarming (COR) or by transient hyperthermia during oxygenated rewarming (THOR); $*P < 0.05$. Lower panel: enzyme release of glutamate dehydrogenase upon isolated reperfusion after end-ischemic reconditioning by COR or by THOR, $*P < 0.05$.

Histological evaluation showed only mild alterations of hepatocellular morphology in either group. Mean injury scores were slightly, but not significantly lower after THOR than after COR: (0.7 ± 0.1 vs. 0.95 ± 0.1; n.s.).

Discussion

Heat shock treatment has been recognized as an effective method to protect the liver from subsequent ischemia reperfusion injury. The heat shock response is a powerful cellular mechanism that induces protective proteins which serve as potent chaperones that help tissues to resist stress situations. Synthesis of these HSP is induced at temperatures from 41 to 43 °C in rodents as well as in human beings [19].

Thus, whole-body exposure to hyperthermic conditions in rats, achieving core temperatures of 42–43 °C, has been shown to protect from subsequent warm or cold ischemic injury in various organs like liver [20] or heart [21].

However, the cumbersome requirement to heat up the whole body, along with the inconvenience this procedure may represent to the patient, has impeded a wider clinical use of the maneuver. In this regard, the pretransplant management opens new perspectives, because the isolated perfused organ graft, by contrast, can easily be subjected to firmly controlled thermal conditions and hence offers a privileged access to induced heat shock proteins without any/systemic effects to the patient.

Moreover, large parts of ischemia-related disorders that occur during hypothermic preservation do not constitute irreversible cell injury. Primarily the re-establishment of normal metabolism in the dyshomeostatic tissue at the onset of reperfusion triggers the occurrence of functional deficits eventually culminating in structural damage [9,22,23].

Thus, activation of intracellular signal pathways and chaperones that favor regeneration and cell survival may still salvage endangered tissue when initiated upon early reperfusion.

Similar effects have already been reported for the ischemic postconditioning in the heart [24] in showing a reduction of ischemia–reperfusion injury by tissue conditioning only immediately upon reperfusion. The protective effect afforded by mere postischemic manipulation of the early reperfusion period was equivalent to the benefit that could be obtained by classic ischemic preconditioning [25].

In contrast to the abundant knowledge about heat shock treatment in protecting against subsequent tissue ischemia [13,26], data on possible benefits of hyperthermia as a tool for delayed conditioning at the time of reperfusion are scarce [27]. In our model, we could initiate a significant increase in HSP 70 expression as a result of an isolated heat shock treatment during *ex vivo* graft perfusion after cold preservation.

It has already been shown in yeast that only short periods of hyperthermia may be sufficient to result in an upregulation of HSP's within 10–20 min [26].

One of the most prominent members of the heat shock protein family is the heat shock protein 70, which can be considered the prototype player in the heat shock response [28] and comprises a variety of protective functions during cellular stress situations.

Heat shock chaperones shelter mitochondria from oxygen free radicals (OFR) [27], stabilize lysosomal membranes [29], protect protein structure and support the repair and refolding of damaged proteins [30].

In our setting, the brief hyperthermic challenge was associated with an improved secretory function and less mitochondrial enzyme loss upon early reperfusion. However, no differences could be seen with regard to cytosolic loss of ALT.

As being confined to the mitochondrial compartment, GLDH has been seen a marker of mitochondrial damage and indicative of severe, mostly irreversible hepatocyte injury [31] whereas leakage of transaminases may occur already during less severe and potentially transient cell injury due to increased permeability of the outer cell membrane. GLDH has hence been described as an actually more effective biomarker of acute hepatic injury than ALT in the rat [32].

The discordant results in our study concerning the release of ALT and GLDH are surprising.

It may be conjectured that the protective potential of the heat shock protocol could have been effective enough to reduce major/irreversible injury to the cell as reflected by mitochondrial damage and release of GLDH, but not as potent as to also prevent acute permeability changes of the outer cell membrane that resulted in the release of transaminases. Nonetheless, THOR still exerted a beneficial functional triggering of cellular metabolism.

Chaperone mediated refolding or repairing of damaged proteins is far less energy consuming than disposal and re-synthesis and thus be particularly advantageous during reperfusion of the ischemically stressed and energy depleted graft.

The tendentially increased recovery of hepatic ATP concentrations in the heat-treated group is pointing in the same direction.

It is known from cell culture experiments that HSP70 contributes to the maintenance of mitochondrial membrane potential after glucose deprivation in astrocytes and preserves physiological respiratory function (of state 3 and state 4) [33].

In line with this, infarct size and apoptosis after neuronal ischemic injury were found to be reduced by overexpression of HspP70 in mice [34].

A limitation of our model must be seen in the relatively short follow-up period that was inherent to the *in vitro* approach. Maximal expression of heat shock proteins usually requires longer time after heat exposure than observed in our experiments. Possible further improvement of tissue regeneration during later reperfusion periods might thus be underestimated in the present study. Postischemic inflammatory upregulation like TNF-alpha production could, for instance, be documented in our model, but putative repercussions on ulterior graft integrity after transplantation, including the extent of protection provided by heat shock treatment, can only be speculated at.

Nonetheless, the results of the present study indicate that acute hyperthermic *ex vivo* graft conditioning after ischemic preservation is effective to improve early functional outcome upon reperfusion.

Based on the outstanding accessibility of isolated organ grafts in the pretransplant setting and the ease of the procedure, further studies will thus be warranted to confirm the value of the procedure with longer follow-up times *in vivo*. Likewise, variations in intensity and/or duration of the hyperthermic stimulus might further optimize the impact of *ex vivo* heat shock treatment.

Authorship

CvH: performed research, analyzed and interpreted data, wrote paper. TM: designed research, performed research, wrote paper.

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Conflicts of interest

The authors declare no conflicts of interest.

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REFERENCES

1. Abouna GM. Organ shortage crisis: problems and possible solutions. *Transplant Proc* 2008; **40**: 34.
2. Gastaca M. Extended criteria donors in liver transplantation: adapting donor quality and recipient. *Transplant Proc* 2009; **41**: 975.
3. Minor T, von Horn C, Paul A. Role of temperature in reconditioning and evaluation of cold preserved kidney and liver grafts. *Curr Opin Organ Transplant* 2017; **22**: 267.
4. Yuan X, Theruvath AJ, Ge X, *et al.* Machine perfusion or cold storage in organ transplantation: indication, mechanisms, and future perspectives. *Transpl Int* 2010; **23**: 561.
5. Guarrera JV, Henry SD, Samstein B, *et al.* Hypothermic machine preservation in human liver transplantation: the first clinical series. *Am J Transplant* 2010; **10**: 372.
6. Minor T, Paul A. Hypothermic reconditioning in organ transplantation. *Curr Opin Organ Transplant* 2013; **18**: 161.
7. Ravikumar R, Jassem W, Mergental H, *et al.* Liver transplantation after ex vivo normothermic machine preservation: a phase 1 (first-in-man) clinical trial. *Am J Transplant* 2016; **16**: 1779.
8. Kaths JM, Cen JY, Chun YM, *et al.* Continuous normothermic ex vivo kidney perfusion is superior to brief normothermic perfusion following static cold storage in donation after circulatory death pig kidney transplantation. *Am J Transplant* 2017; **17**: 957.
9. Minor T, von Horn C. Rewarming injury after cold preservation. *Int J Mol Sci* 2019; **20**: 2059.
10. Hoyer DP, Mathe Z, Gallinat A, *et al.* Controlled oxygenated rewarming of cold stored livers prior to transplantation: first clinical application of a new concept. *Transplantation* 2016; **100**: 147.
11. Yamanaka K, Houben P, Bruns H, Schultze D, Hatano E, Schemmer P. A systematic review of pharmacological treatment options used to reduce ischemia reperfusion injury in rat liver transplantation. *PLoS One* 2014; **10**: e0122214.
12. Figueiredo C, Carvalho Oliveira M, Chen-Wacker C, *et al.* Immuno-engineering of the vascular endothelium to silence MHC expression during normothermic ex vivo lung perfusion. *Hum Gene Ther* 2019; **30**: 485.
13. Kalmar B, Greensmith L. Induction of heat shock proteins for protection against oxidative stress. *Adv Drug Deliv Rev* 2009; **61**: 310.
14. Minor T, Efferz P, Fox M, Wohlschlaeger J, Luer B. Controlled oxygenated rewarming of cold stored liver grafts by thermally graduated machine perfusion prior to reperfusion. *Am J Transplant* 2013; **13**: 1450.
15. von Horn C, Minor T. Improved approach for normothermic machine perfusion of cold stored kidney grafts. *Am J Transl Res* 2018; **10**: 1921.
16. Minor T, Manekeller S. Assessment of hepatic integrity after ischemic preservation by isolated perfusion in vitro. The role of albumin. *Cryobiology* 2007; **54**: 188.
17. 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**: 331.
18. Camargo CA Jr, Madden JF, Gao W, Selvan RS, Clavien PA. Interleukin-6 protects liver against warm ischemia/reperfusion injury and promotes hepatocyte proliferation in the rodent. *Hepatology* 1997; **26**: 1513.
19. Polla BS. A role for heat shock proteins in inflammation? *Immunol Today* 1988; **9**: 134.
20. Saad S, Kanai M, Awane M, *et al.* Protective effect of heat shock pretreatment with heat shock protein induction before hepatic warm ischemic injury caused by Pringle's maneuver. *Surgery* 1995; **118**: 510.
21. Gowda A, Yang C, Asimakis GK, Rastegar S, Motamedi M. Heat shock improves recovery and provides protection against global ischemia after hypothermic storage. *Ann Thorac Surg* 1998; **66**: 1991.
22. Schlegel A, Kron P, Dutkowski P. Hypothermic oxygenated liver perfusion: basic mechanisms and clinical application. *Curr Transplant Rep* 2015; **2**: 52.
23. Minor T, Saad S, Koetting M, Nagelschmidt M, Paul A. Endischemic oxygen persufflation to improve viability of marginally preserved donor livers. *Transplant Int* 1998; **11**: S400.
24. Zhao ZQ, Corvera JS, Halkos ME, *et al.* Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. *Am J Physiol Heart Circ Physiol* 2003; **285**: H579.
25. Vinten-Johansen J, Zhao ZQ, Zatta AJ, Kin H, Halkos ME, Kerendi F. Postconditioning–a new link in nature's armor against myocardial ischemia-reperfusion injury. *Basic Res Cardiol* 2005; **100**: 295.
26. Richter K, Haslbeck M, Buchner J. The heat shock response: life on the verge of death. *Mol Cell* 2010; **40**: 253.
27. O'Neill S, Harrison EM, Ross JA, Wigmore SJ, Hughes J. Heat-shock proteins and acute ischaemic kidney injury. *Nephron Exp Nephrol* 2014; **126**: 167.
28. Aufricht C. Heat-shock protein 70: molecular supertool? *Pediatr Nephrol* 2005; **20**: 707.
29. Daugaard M, Rohde M, Jaattela M. The heat shock protein 70 family: highly homologous proteins with overlapping and distinct functions. *FEBS Lett* 2007; **581**: 3702.
30. Chebotareva N, Bobkova I, Shilov E. Heat shock proteins and kidney disease: perspectives of HSP therapy. *Cell Stress Chaperones* 2017; **22**: 319.
31. Frederiks WM, Marx F. Changes in cytoplasmatic and mitochondrial enzymes in rat liver after ischemia followed by reperfusion. *Exp Mol Pathl* 1987; **47**: 291.
32. O'Brien PJ, Slaughter MR, Polley SR, Kramer K. Advantages of glutamate dehydrogenase as a blood biomarker of acute hepatic injury in rats. *Lab Anim* 2002; **36**: 313.
33. Ouyang YB, Xu LJ, Sun YJ, Giffard RG. Overexpression of inducible heat shock protein 70 and its mutants in astrocytes is associated with maintenance of mitochondrial physiology during glucose deprivation stress. *Cell Stress Chaperones* 2006; **11**: 180.
34. Tsuchiya D, Hong S, Matsumori Y, *et al.* Overexpression of rat heat shock protein 70 reduces neuronal injury after transient focal ischemia, transient global ischemia, or kainic acid-induced seizures. *Neurosurgery* 2003; **53**: 1179.