Pump-Priming Grant

Report – Syed Hussain Abbas

Title: The impact of normothermic machine perfusion and defatting on Hypoxia-Inducible Factor (HIF) expression in human steatotic livers

Hypoxia-inducible factors (HIFs) are cellular oxygen sensitive transcription factors which have been implicated as the 'master regulators' in response to hypoxia through activation of a number of hypoxia responsive genes. The HIF-1α isoform has demonstrated a protective effect through reduction in hepatic lipid synthesis, *de novo* lipogenesis and lipid peroxidation as well as promotion of fatty acid β-oxidation. However, the HIF-2α isoform is reported to activate genes involved in hepatic fatty acid synthesis and suppression of genes that regulate fatty acid β-oxidation resulting in progression of lipid accumulation and fibrosis.

Hypothesis: NMP with pharmacological stabilisation of HIF-1α and defatting interventions will improve graft tolerance to ischaemia-reperfusion injury (IRI).

Objective: The overarching aim of this study was to develop a platform of hypoxia-inducible factor (HIF) modulation during oxygenated normothermic machine perfusion of the liver and test this with the adjunct of defatting therapies. The specific objectives were as follows:

1. To optimise dosing schedules for pharmacological HIF modulation during human liver NMP
2. To optimise methods for liver HIF quantification using western blots and correlate this with a downstream HIF dependant target (erythropoietin, EPO)
3. To optimise discarded steatotic human livers through selective pharmacological induction of HIF-1α (achieved through HIF prolyl-hydroxylase inhibition combined with inhibition of HIF-2α dimerisation) during NMP with the adjunct of defatting therapies.

Methods

As part of this pre-clinical study, 5 livers were preserved using the OrganOx *metra* normothermic machine perfusion (NMP) device for a maximum of 24 hours. NMP involves maintaining the liver in an ex-situ functioning state with delivery of oxygen and nutrition at a normal body temperature. However, due to the presence of oxygen in the normothermic circuit, pharmacological HIF modulation was required. The pharmacological agents included deferoxamine (DFO) and the HIF-2α dimerisation inhibitor, PT2385. DFO is a potent activator of both the HIF-1α and HIF-2α isoforms and therefore to investigate the beneficial effects of HIF-1α alone, the use of a HIF-2α dimerisation inhibitor (PT2385) was required.

The livers received the following perfusion protocols: (i) Liver 1 (control): NMP alone protocol; (ii) Liver 2: NMP with defatting agents (L-carnitine and forskolin); (iii) Liver 3: NMP with DFO; (iv) Liver 4: NMP with DFO and PT2385; (v) Liver 5: NMP with a combination of defatting agents, DFO and PT2385.

HIF-1α and HIF-2α expression was quantified using western blots of snap frozen biopsies. Downstream HIF dependant target activation was determined through serial perfusate EPO measurements using Immulite 2000 XPi.

Results

Perfusate EPO measurements

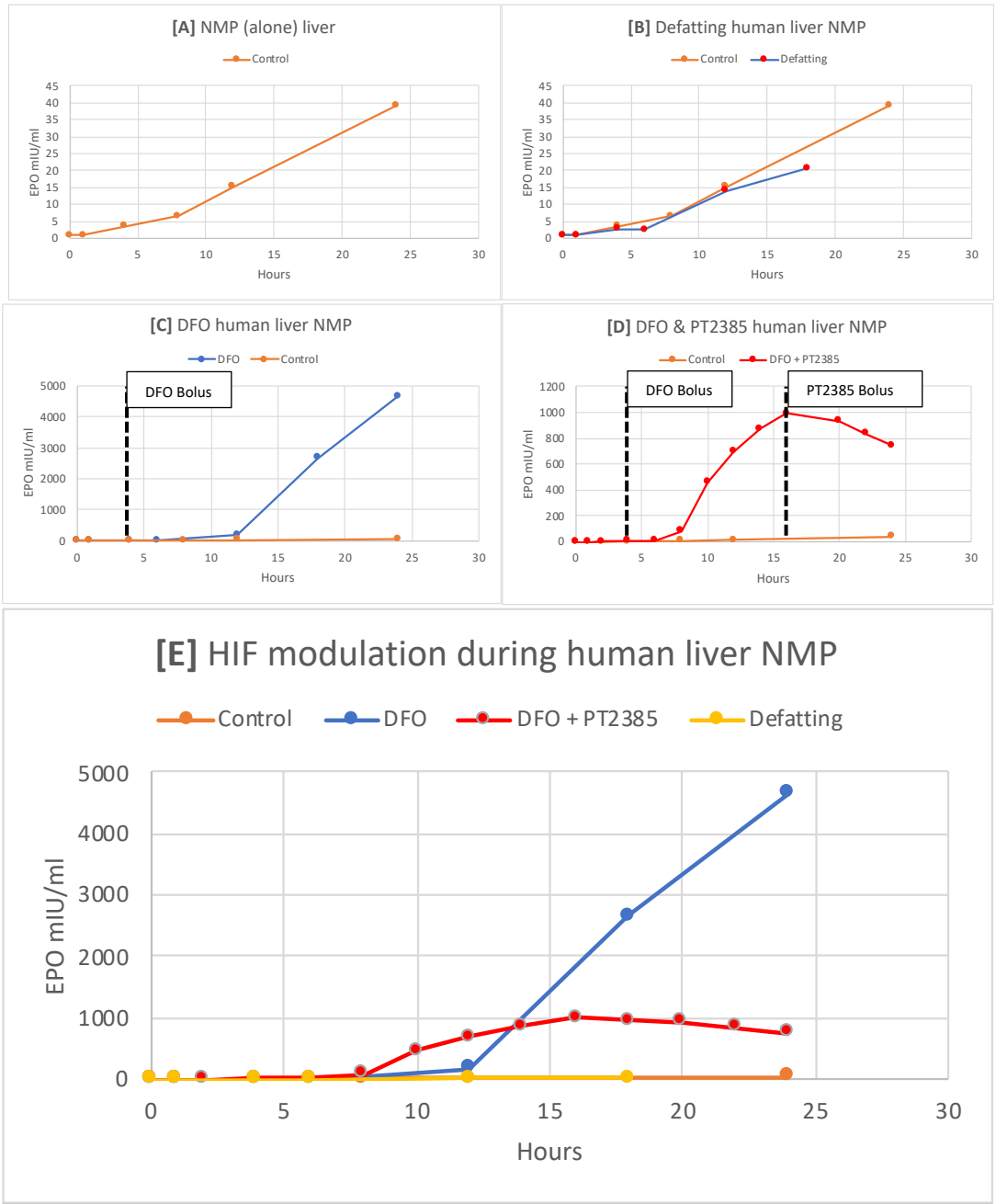

Figure 1: **[A]** Liver 1 (control): Low EPO levels during NMP alone; **[B]** Liver 2 (NMP with defatting agents, L-carnitine and forskolin) vs. control: Comparable levels of EPO compared to control; **[C]** Liver 3 (NMP with DFO): Bolus of DFO delivered at 4h of perfusion demonstrating an increase in perfusate EPO levels >4000 mU/ml towards the end of perfusion compared to the control; **[D]** Liver 4 (NMP with DFO and PT2385): Bolus of DFO delivered at 4h of perfusion demonstrating an increase of perfusate EPO, followed by reduction of perfusate EPO at 16h of perfusion post delivery of PT2385 compared to the control; **[E]** Combined perfusions (B, C & D) vs control (A). *Perfusate EPO data for Liver 5 (NMP with a combination of defatting agents, DFO and PT2385) is pending.

Western Blots: Tissue HIF-1α and HIF-2α expression

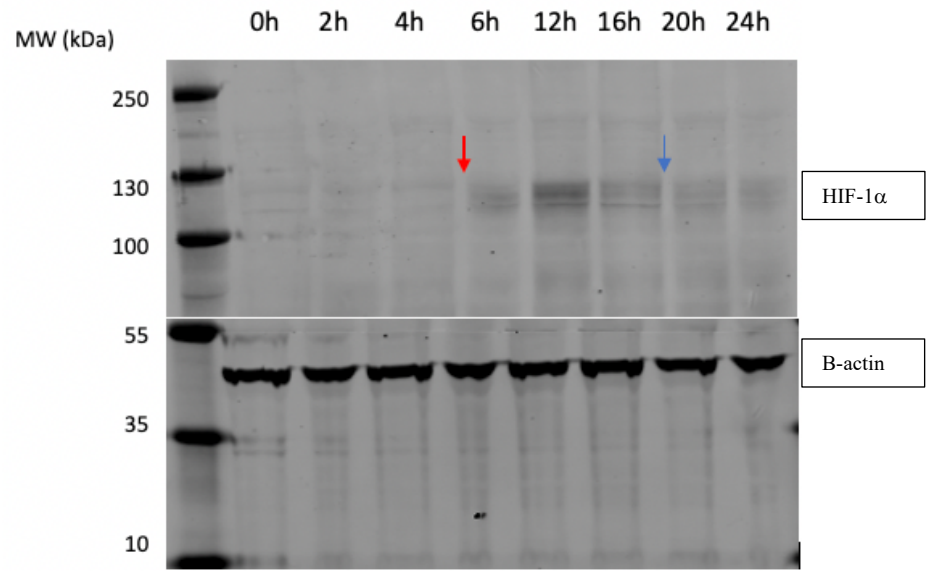

Figure 2:[A] Normothermic liver perfusion (Liver 4) with delivery of DFO & PT2385 over 24h. 0h signifies end of static cold storage (before liver is placed on machine). Between 0h-4h the liver is perfused with pRBC in normothermic oxygenated conditions. After 4h biopsy (red arrow), DFO is administered. **After administration of DFO, HIF-1α signal (100-130kDa) becomes apparent for remaining duration of perfusion (up to 24h) despite PT2385 being administered after 16h biopsy (blue arrow). [B]** B-actin control.

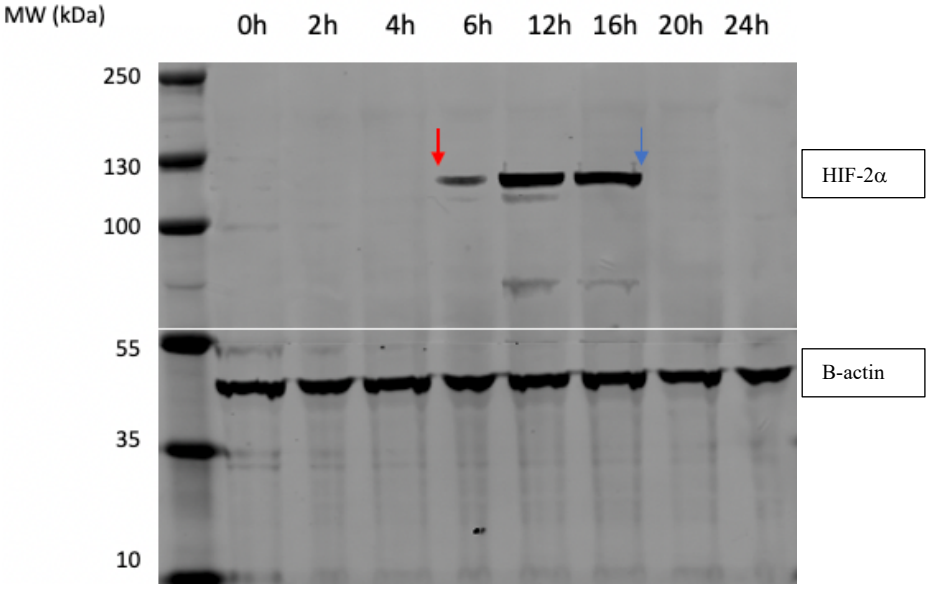

Figure 3:[A] Normothermic liver perfusion (Liver 4) with delivery of DFO & PT2385 over 24h. 0h signifies end of static cold storage (before liver is placed on machine). Between 0h-4h the liver is perfused with pRBC in normothermic oxygenated conditions. After 4h biopsy (red arrow), DFO is administered. **After administration of DFO, HIF-2α signal (100-130kDa) becomes apparent. However, following delivery of PT2385 after the 16h biopsy (blue arrow), the HIF-2α signal disappears for the remaining duration of perfusion. This is also correlates with the perfusate EPO data in Figure 1D. [B]** B-actin control.

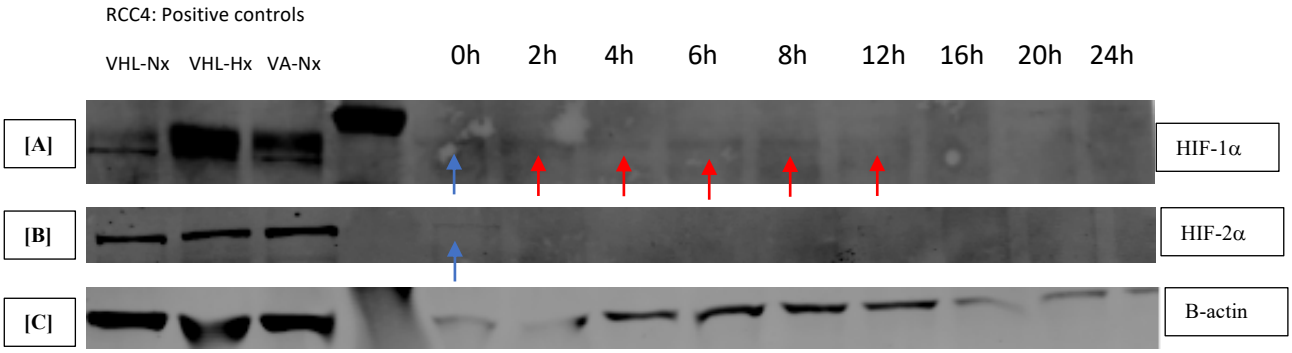

Figure 4: Normothermic liver perfusion (Liver 5) with delivery of defatting agents (L-carnitine & Forskolin), DFO & PT2385 over 24h. 0h signifies end of static cold storage (before liver is placed on machine). Following priming of the NMP circuit with pRBC (normothermic oxygenated conditions) the combination of defatting agents (L-carnitine & forskolin), DFO and PT2385 were administered and the liver was subsequently connected/perfused for 24h.

[A] HIF-1α signal (100-130kDa) is apparent at the end of cold storage (blue arrow) and persists for 12h during perfusion (red arrows). **[B]** HIF-2α signal (100-130kDa) is only apparent at the end of cold storage and does not persist for the duration of perfusion. **[C]** B-actin control.

Outputs (publications/presentations)

- Abstract due for submission to British Transplant Society Annual Congress
- Abstract due for submission to American Society of Transplant Surgeons Conference

Next Steps (what is it leading to)

These results demonstrate pharmacological modulation of the hypoxia inducible factor pathway during normothermic oxygenated machine perfusion. The results also demonstrate activation and stabilisation of the HIF-1α isoform with inhibition of the HIF-2α isoform using the combination of DFO and PT2385.

In order to optimise high-risk steatotic grafts and improve tolerance to ischaemia reperfusion injury, the next series of experiments will involve a model of ex-situ whole blood reperfusion following initial preservation with NMP where livers will be perfused using the defatting protocol or defatting with the adjunct of HIF modulation (DFO & PT2385). The results may support development of new perfusion protocols with application in the setting of a clinical trial.