428n Development of a Physiologically Relevant Experimental Model for Organ-Scale Metabolic Analysis and Engineering: Meeting the Oxygen Requirements of an Isolated Perfused Rat Liver

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Purpose. The stable perfusion of an organ ex vivo under physiological conditions is a powerful tool for organ-scale metabolic analysis and engineering. The organ's response to systemic insults or drugs can be monitored in real time, whilst simultaneously allowing for the manipulation of its environment with the intent to treat, revive, and better store potentially transplantable organs. While liver perfusion systems have been in use since the early 1950s, due to limiting technologies and a perpetual disagreement regarding the extent of oxygenation required for stable perfusion, no gold standard perfusion protocol is currently available. Accurate measurements and maintenance of stable organ functionality are therefore compromised over long perfusion times. The requirements of a stable oxygen carrier, which determines organ viability, are that it is practical, functional and quantifiable. The goal of this study therefore, is to use such a carrier in approaching a physiological oxygen supply to the isolated perfused liver. In so doing, prove that any significant deviation from the norm results in a chronic debilitation of the liver structure and function. Method. First, we characterize the ideal system by careful measurement of in vivo flow properties into and out of the rat liver with particular emphasis on the parameters governing normal oxygen uptake rate. We then compare this data to reproductions of current literature-recommended perfusate oxygenation conditions, and attempt to systematically improve upon them. Hereby, livers are isolated using Mortimore's Technique for minimal organ disruption, and perfused with an isotonic buffered solution of Minimum Essential Medium fortified with metabolites. The perfusate forms the basis of one of four conditions investigated: A) No erythrocytes B) Rat erythrocytes C) Porcine erythrocytes D) Goat erythrocytes. Results. Using an ultrasonic flow probe, the blood flow rate (ml/min/g liver) into the liver via the portal vein was found to be: 1.4 (+/- 0.2, n=6) and via the hepatic artery: 0.4 (+/- 0.2, n=8). Using a blood gas analyzer, the rat blood hematocrit was found to be 40% (13.5g/dL) with a pO2 of 70mmHg (+/- 8, n=13) in the portal vein and 137mmHg (+/-18, n=11) in the hepatic artery. At 37°C, with a portal pressure of ~14mmHg, and a pH of 7.2, this implied a total oxygen flow rate into the liver of 0.07mlO2/min/g liver via the hepatic artery and 0.26mlO2/min/g liver via the portal vein. The amount of O2 exiting the liver via the suprahepatic vena cava was 0.05mlO2/min/g liver. Consequently, the average total oxygen uptake rate (OUR) is ~0.28mlO2/min/g liver. In the absence of erythrocytes, the liver was immediately oxygen deprived, allowing for an OUR of 0.054mlO2/min/g liver. The addition of porcine erythrocytes (10g/dL) improved the OUR to 0.16mlO2/min/g liver but showed obvious signs of congestion. Goat erythrocytes (12g/dL) caused no congestion yet elicited the formation of thrombi requiring further investigation into their usefulness. Conclusion. While further analysis will be appended to this study, it is apparent that the absence of an oxygen carrier is detrimental, while the use of xenogeneic erythrocytes can improve the oxygenation to the liver. The interaction of each species of erythrocyte with the rat liver must be appreciated individually however, and the oxygen supply to the liver optimized appropriately.