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Reversible mitochondrial uncoupling in the cold phase during liver preservation/reperfusion reduces oxidative injury in the rat model ☆

Alexander Y. Petrenko [a,*], Daria V. Cherkashina [a], Alexander Y. Somov [a,b], Elena N. Tkacheva [a], Olga A. Semenchenko [a], Alexander S. Lebedinsky [a], Barry J. Fuller [b]

[a] Institute for Problems of Cryobiology and Cryomedicine, Kharkov, Ukraine
[b] Division of Surgery & Interventional Sciences, Royal Free and University College Medical School, UCL, London, UK

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ABSTRACT

Reversible uncoupling of the mitochondrial electron-transport chain may be one strategy to prevent intracellular oxidative stress during liver cold preservation/warm reperfusion (CP/WR) injury. 2,4-Dinitrophenol (DNP) is a potent water-soluble uncoupling agent for supplementation of the hepatic CP solution. The aim of this work was to investigate the possible influence of DNP in the CP solution on the isolated rat liver state during CP/WR. Livers were subjected to CP at 4 °C in sucrose–phosphate based solution (SPS) for 18 h, followed by WR for 60 min *in vitro*. The final concentration of DNP was 100 μM. DNP presence during the CP stage led to partial ATP level decrease accompanied by a significant diminution in liver TBARS and a prevention of antioxidant enzyme activity decrease (catalase, GSH-PO, GSH-Red) when compared with livers stored without DNP. After DNP wash-out during WR, an improvement in the mitochondrial functional state (higher respiratory control indices and ATP levels, and a decrease in V_4 respiration rates) were observed. This was concurrent with lower TBARS levels, higher antioxidant enzyme activities and significant increase of bile production. The results suggest that reversible uncoupling may be one way to influence oxidative stress associated with hepatic cold preservation.

Introduction

Although our understanding of the organ and cellular changes during cold preservation has increased significantly in the past 20 years, cold storage injury remains a significant negative factor in clinical liver transplantation [7,13,21]. The involvement of mitochondria in hepatic cold preservation/warm reperfusion (CP/WR) injury liver has long been recognised [31], and the relationship between mitochondrial dysfunction and production of damaging reactive oxygen species (ROS) production has been identified [15]. However, the mitochondrial responses which dictate irreversible injury or potential cell survival are complex and not easy to predict. On the one hand, good coupling of oxidative metabolism with mitochondrial ATP generation is seen as essential for recovery after CP [23], but on the other hand, partial uncoupling and dissipation of the strong net negative mitochondrial transmembrane charge may reduce ROS production and injury in some models of liver hypoxia [27]. Intracellular oxidative stress development is one of the main injurious factors after long-term hepatic cold ischemia and following re-oxygenation [15]. ROS produced during electron transport processes result mainly from the mitochondria; although to a lesser extent microsomal and nuclear processes also contribute. Cold hypoxia also creates the prerequisites for disturbance in cellular energy transformation, with a switch to anaerobic glycolysis as the main energy source to support cell essential cell requirements [23].

Since oxidative phosphorylation is blocked during cold hypoxia, there are very few options to dissipate the strong negative charge accumulated on the mitochondrial enzymes complexes, and these contribute to ROS production when oxygen supply is initially restored during reperfusion. One possible way to avoid this would be to uncouple the mandatory link between accumulation of reduced metabolic intermediates during CP and passage of electrons along the electron-transport chain to the terminal mitochondrial complex, by using pharmacological 'uncoupling' agents [34]. However, this would need to be achieved using agents which could be rapidly reversed and non-toxic during reperfusion, because aerobic re-synthesis of ATP is essential to organ survival. It is already clear that strong, sustained mitochondrial uncoupling, as produced by increases of free fatty acids during hepatic CP, is very damaging to subsequent liver function [17].

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* Corresponding author. Address: Department of Biochemistry, Institute for Problems of Cryobiology and Cryomedicine, 23 Pereyaslavskaya Str., Kharkov 61015, Ukraine.
E-mail addresses: alexander_petrenko@cryo.org.ua, payua@yahoo.com (A.Y. Petrenko).

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