Title

Exploring the Protective Effect of Nrf2 Activator On Ischemia Reperfusion Injury in Rat Liver.

Authors

Masuda, Y
Takasu, C
Pham, C
et al.

Publication Date

2014-07-01

DOI

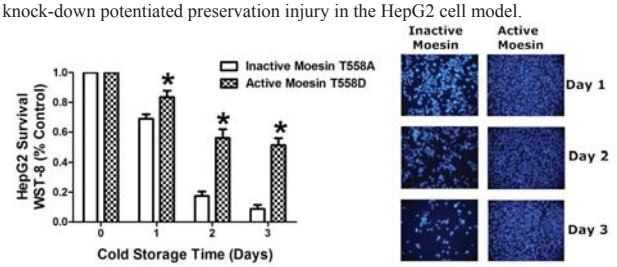
10.1097/00007890-201407151-01216

Peer reviewed

Abstract# C2062

Moesin Functionality and Rho Signaling in Hypothermic Liver Preservation Injury. A. Limkemann, T. Tian, S. Lindell, C. Kowalski, M. Mangino. *Department of Surgery, Virginia Commonwealth University, Richmond, VA.*

Purpose: Liver preservation injury is largely caused by cold ischemia-induced loss of cell adhesion and survival signaling. We describe a novel molecular mechanism of this effect involving the membrane bound cytoskeletal protein moesin by evaluating the role and mechanism of moesin activation in hypothermic liver preservation injury.
Methods: Mouse livers were cold stored and reperfused on an isolated perfused liver to study moesin function during preservation injury. Human hepatocytes (HepG2) and human or murine sinusoidal endothelial cell were cold stored and rewarmed to induce hypothermic preservation injury. The cells were transfected with: wild type moesin, a moesin specific siRNA duplex, and the moesin mutants T558D (active) and T558A (inactive). Tissue and cell moesin expression and actin binding were measured by western blot. Cell viability was used to test the effect of a specific Rho-A inhibitor and Rho-kinase inhibitor in HepG2 cells in a hypothermic preservation injury model.
Results: Cell viability progressively declined with increasing preservation time that correlated with moesin inactivation. Transfection of a moesin containing plasmid and a moesin specific siRNA duplex into HepG2 cells resulted in increased and decreased expression, respectively. Overexpression of moesin protected and moesin knock-down potentiated preservation injury in the HepG2 cell model.



Hepatocytes expressing inactive and active moesin binding mutants showed significantly more and less preservation injury, respectively (Figure-Left). Cold storage time dependently caused hepatocyte detachment from the matrix and cell death, which was prevented by the active moesin mutation (Figure-Right, DAPI fluorescence). Rho-A and Rho-kinase inhibition significantly potentiated hypothermic preservation injury in human hepatocytes.
Conclusion: Moesin is causally involved in hypothermic liver cell preservation injury through control of its active binding molecular functionality. Likely, moesin is protective because of the linker role that is essential for cell adhesion to the matrix, and moesin inactivation during cold preservation may be Rho-dependent.

Abstract# C2063

Exploring the Protective Effect of Nrf2 Activator On Ischemia Reperfusion Injury in Rat Liver. Y. Masuda,[1] C. Takasu,[1] C. Pham,[1] A. Le,[1] S. Li,[1] L. Robles,[1] N. Vaziri,[2] M. Stamos,[1] H. Ichii.[1] *[1]Surgery, University of California, Irvine, Orange, CA; [2]Medicine, University of California, Irvine, Irvine, CA.*

Background: Liver ischemia/reperfusion injury (I/RI) is a common pathologic process caused by various clinical settings, such as liver resection, liver transplantation, hypovolemic shock, and trauma. The Nrf2 pathway regulates host cell defense responses against oxidative stress and maintains the cellular redox balance. The aim of this study is to investigate the effect of Nrf2 activator, bardoxolone methyl analog (dh404) on warm ischemia reperfusion injury in rat liver.
Methods: SD rats were divided into 2 groups: CTL (n=5) non-treatment; DH (n=5) dh404 treatment. dh404 (1.5mg/kg/rat) was orally administered the night before and 5 hours before the procedure. All rats were subjected to 60 minutes of 70% ischemia followed by 3 hours reperfusion. After reperfusion, blood and liver tissues were collected to measure alanine aminotransferase (ALT), and malondialdehyde (MDA) levels in the serum, to evaluate histological change, and to determine the expression of Nrf2 target antioxidant enzymes.
Results: There was no significant difference in the scores of necrosis in the liver between groups (2.0±0.5 in CTL and 1.6±0.5 in DH). The levels of serum ALT in the DH treated group (542±234 IU/ml) was significantly lower when compared with the CTL group (1794±680 IU/ml). Moreover, the levels of MDA in the DH treated group were significantly lower when compared to the CTL group (23.9±3.8μM, vs. 31.5±4.1, respectively, $P<0.05$). Although the relative expression of Nrf2 in whole tissue was not different between the groups by western blot analysis, the expressions of GCLC and GCLM in the DH treated liver were significantly higher when compared to the CTL group.
Conclusions: Administration of dh404 significantly improved liver function, attenuated oxidative stress and raised anti-oxidant status in rats with hepatic I/RI, however not histological scores. These findings suggest that fortification of the antioxidant defense system by pharmacological activation of Nrf2 pathway in liver may be a strategy to improve clinical outcome in patients with liver I/RI.

Abstract# C2064

Provisional Data On the Effect of Anterograde Persufflation On Energy Charge and Hepatocyte Function in Donation After Cardiac Death Livers. S. Khorsandi, S. Jitraruch, W. Jassem, H. Vilca-Melendez, A. Prachalias, A. Dhawan, N. Heaton, P. Srinivasan. *Institute of Liver Studies, King's College Hospital, London, United Kingdom.*

Donation after cardiac death (DCD) livers, are considered to be a marginal organ for solid organ and cell transplantation. Low energy charge and purine quantity within liver parenchyma has been associated with poor outcome after liver transplantation. The aim of this work was to assess the effect of anterograde persufflation (A-PSF) using an electrochemical concentrator on DCD liver energy status and hepatocyte function. Organs utilized for research were DCD livers considered not suitable for transplant. The liver was formally split and the control non PSF section stored in University of Wisconsin Solution (UW) at 4°C. The A-PSF liver was immersed in UW on ice and A-PSF undertaken via the portal vein with 40% oxygen. Tissue samples were taken 2 hours after A-PSF from the A-PSF and control non PSF liver for snap freezing. Purine analysis was performed with photo-diode array detection. Hepatocytes were isolated from A-PSF and control non-PSF liver using a standard collagenase perfusion technique. Hepatocyte function was assessed using mitochondrial dehydrogenase activity (MTT) and the sulforhodamine B (SRB) assay for cell attachment.In <30% steatotic DCD (n=6) A-PSF increased the energy charge from 0.197 ± 0.025 to 0.23 ± 0.035 ($p=0.04$). In >30% steatotic DCD (n=4) A-PSF had no beneficial effect. After isolation (n=4, <30% steatosis) A-PSF was found to improve MTT from 0.92 ± 0.045 to 1.19 ± 0.55 ($p<0.001$) and SRB from 2.53 ± 0.12 to 3.2 ± 0.95 ($p<0.001$). In conclusion, A-PSF can improve the energy charge and function of isolated hepatocytes from <30% steatotic DCD livers.

Abstract# C2065

Indicators of Liver Viability in the *Ex Vivo* Perfused Human Liver. B. Bruinsma,[1] G. Sridharan,[1] P. Weeder,[1] M. Izamis,[1] P. Martins,[2] H. Yeh,[3] K. Uygun.[1] *[1]Center for Engineering in Medicine, Massachusetts General Hospital, Boston, MA; [2]Transplant Unit, Massachusetts General Hospital, Boston, MA; [3]Transplant Surgery, UMass Memorial, Worcester, MA.*

Machine perfusion of the liver is rapidly gaining support for the recovery and preservation of grafts injured by long warm ischemia (WI). A major advantage of machine perfusion at warm temperatures is the ability to assess the viability of the liver pre-transplantation. However, the best indicators need to be identified to accurately assess the liver during perfusion. To this end, we perfused 20 human livers discarded for transplantation with varying durations of warm ischemia (range 14-54 minutes) in our subnormothermic perfusion system. We monitored the liver by assessment of blood gas analysis, bile flow, biochemical markers of function (albumin, urea) and injury (alanine aminotransferase, alkaline phosphatase), tissue analysis (ATP), clearance of indocyanine green (ICG), and (electron-)microscopy. These parameters were correlated to characteristics of the donor and WI of the liver. We show here that metabolic and liver function parameters are sustained or improved during perfusion, with an increase in oxygen consumption, albumin and urea output and overall sustained bile production. Minimal injury was observed biochemically or microscopically. ATP content of the liver tissue improved significantly (61.1 to 204.3 $p=0.012$). ATP content showed a good correlation with WIT ($r^2=.83$, $p=.0045$). As ATP measurement requires tissue biopsy non-invasive and real-time parameter are preferred. Not the total production of bile, but whether bile production increased during perfusion correlated best with the low WIT ($p<0.006$)(Figure). A positive correlation with WIT was also found between the release of alanine aminotransferase (ALT) ($r^2=.58$, $p=.016$) and clearance of ICG ($r^2=.95$, $p=.002$). In conclusion, ATP content, sustained bile production and ALT and clearance of ICG are best correlated to WIT and may be useful as indicators of liver quality. Ongoing work aims to formulate predictive scores using these and other parameters of viability that can predict transplant outcome.