BOS12- ISCHEMIA REPERFUSION INJURY IN EXPERIMENTAL LIVER TRANSPLANTATION

BO111 N-ACYL-DOPAMINE DERIVATIVES INDUCE THE UNFOLDED PROTEIN RESPONSE IN ENDOTHELIAL CELLS: IS THIS IMPORTANT FOR A PROTECTIVE PHENOTYPE?

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The Unfolded Protein Response (UPR) is an intracellular signaling pathway that is activated by the accumulation of unfolded or misfolded proteins in the endoplasmic reticulum. In hibernating mammals it has been suggested that induction of the UPR is part of the cell protective program entering torpor. Moreover, organs obtained for animals during torpor are more resistant to cold preservation damage compared to those obtained from animals that are not hibernating. We have previously shown that N-acyl-dopamine derivatives (NADD) are able to protect endothelial - and epithelial cells against cold-induced injury. Current evidence indicates that this is likely due to direct structural features of NADDs, i.e. hydrophobicity and redox activity. Since it is not known if NADD may induce the UPR, and if this in turn may also contribute to resistance against cold preservation injury, we tested if synthetic NADDs are able to induce the UPR. To this end, we tested if N-octanoyl-dopamine (NOD), N-pivanoyl-dopamine (NPD) and NOD in which both hydroxy groups are acetylated (A-NOD) are able to induce UPR target genes, activate ESRE or ATF6 driven luciferase reporters, phosphorylate eIF2á and cause XBPI slicing in Human Umbilical Vein Endothelial Cells (HUVEC). All of the tested synthetic NADDs induced the UPR in between 2 and 24 h. NPD was less effective in this regard. Activation was abolished after 48 h. No selective activation of the three branches was observed for either of the compounds. UPR activation was transient and did not result in apoptosis. Cyclohexamide treatment did not abrogate the protective effect of NADDs against cold preservation injury, suggesting that induction of the UPR does not underlie resistance to cold preservation injury when cells are pre-treated with NADD. Nonetheless it remains to be assessed if prolonged NADD treatment brings the the cells in a state of hypometabolism and/or renders cells more resistant to cold preservation.

BO112 ENZYME-TRIGGERED CO-RELEASING MOLECULES (ET-CORMS): EVALUATION OF BIOLOGICAL ACTIVITY IN RELATION TO THEIR STRUCTURE

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Background and Purpose: Acyloxydiene-Fe(CO)3 complexes act as enzyme triggered CO releasing molecules (ET-CORMs) and can deliver CO intracellular via esterase mediated hydrolysis. The protective properties of structurally different ET-CORMs on hypothermic preservation damage and their ability to inhibit VCAM-1 expression were tested on cultured human umbilical vein endothelial cells (HUVEC) and renal proximal tubular epithelial cells (PTEC) using a structure-activity approach.
Experimental Approach: Cytotoxicity of ET-CORMs, protection against hypothermic preservation damage, and inhibition of VCAM-1 expression were assessed.
Key Results: Cytotoxicity of 2-cyclohexenone and 1,3-cyclohexanedione-derived ET-CORMs was more pronounced in HUVEC compared to PTEC and was dependent on the position and type of the ester (acyloxy) substituent(s) (acetate > pivalate > palmitate). Protection against hypothermic preservation injury was only observed for 2-cyclohexenone derived ET-CORMs and was not mediated by the ET-CORM decomposition product 2-cyclohexenone itself. Structural requirements for protection by these ET-CORMs were different for HUVEC and PTEC. Protection was affected by the nature of the ester functionality in both cell lines. VCAM-1 expression was inhibited by both 2-cyclohexenone- and 1,3-cyclohexanedione-derived ET-CORMs. 2-cyclohexenone but not 1,3-cyclohexanedione, also inhibited VCAM-1 expression.
Conclusions and Implications: We demonstrate that structural alterations of ET-CORMs significantly affect their biological activity. Our data also indicate that different ET-CORMs behave differently in various cell types (epithelial versus endothelial). These findings warrant further studies not only to elucidate the structure-activity relation of ET-CORMs in mechanistic terms but also to assess if structural optimization will yield ET-CORMs with restricted cell specificity.

BO114 ASSOCIATION BETWEEN SEVERE ISCHEMIA REPERFUSION INJURY AND PATIENT SURVIVAL AFTER LIVER TRANSPLANTATION

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Background: Peak Aspartate aminotransferase (AST) reflects Ischemia Reperfusion Injury (IRI) severity after Liver Transplantation (LTx). Because of concerns between IRI severity and outcome post-LTx, we aimed to (i) identify risk factors for severe IRI (peak AST>2000 IU/l) and (ii) study its impact on patient survival.
Methods: Between 01/2000 to 12/2010, we performed 552 LTx. Donor/recipient demographics, procurement/preservation/LTx data and patient survival were analyzed retrospectively.
Results: In 83 recipients, peak AST was >2000 IU/l (15%). Univariate and multivariate analyses revealed imported livers (versus locally procured) ($p = 0.01$) and intra-operative warm ischemic time ($p < 0.01$) as independent risk factors to develop a peak AST>2000 IU/l. Recipient ICU/hospital stays and death were significantly associated with peak AST>2000 IU/l ($p < 0.01$). The 1 & 5-years patient survival was lower in recipients with a peak AST>2000 IU/l (80.1, 61.6%) vs. recipients with a peak AST<2000 IU/l (92.7, 80.8%) ($p < 0.01$ respectively).
Conclusion: Imported (contrary to locally procured) livers and intra-operative warm ischemia are risk factors for severe IRI (peak AST>2000 IU/l) post-LTx in our series. Severe IRI is associated with a lower patient survival. This study supports the need to develop appropriate strategies aimed at decreasing IRI.

BO115 IGL-1 PRESERVATION SOLUTION PROTECTS RAT LIVER AGAINST APOPTOSIS IN ORTHOTOPIC LIVER TRANSPLANTATION

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Background: Injury due to cold ischemia reperfusion (I/R) is a major cause of graft non-function following liver transplantation and the use of an appropriate preservation solution is crucial for the viability of liver grafts. Glycogen synthase kinase-3b (GSK3b) phosphorylates voltage-dependent anion channel (VDAC), the most abundant protein in the outer membrane of mitochondria, and this in turn increases cell death during the ischemic insult. Thus, the inhibition of GSK3b activity is crucial for mitochondrial protection and the subsequent suppression of apoptosis. In this communication, we investigated the involvement of GSK3b in livers subjected to orthotopic liver transplantation (OLT) when IGL-1 preservation solution was used.
Methods: Male Sprague Dawley (200–250 g.b.w.) rats were classified as follows: Group1 = Sham; Group2 (UW) = Livers were flushed and preserved in UW for 8 h at 4°C and subjected to OLT. Rats were sacrificed at 24 h after transplantation according to Kamada 's cuff technique; Group 3 (IGL-1) = Livers were flushed and preserved in IGL-1 for 8 h at 4°C and subjected to OLT. Rats were sacrificed at 24 h after transplantation and then blood and liver samples were collected.ALT/AST, AMPK, PI3K/AKT and their direct substrate, GSK3-b and pVDAC were determined by Western blot. Mitochondrial injury (GLDH) and oxidative stress (MDA) were measured. Measure of apoptosis parameters was also performed.
Results: (i) IGL-1 significantly reduced liver injury (AST/ ALT) and mitochondrial damage when compared to UW. This graft protection was accompanied by increased AKT phosphorylation and subsequent GSK3-b inhibition, (ii) A significant reduction in pVDAC and release of Cytochrome C and caspases levels were found.
Conclusion: IGL-1 preservation solution increases the tolerance of the liver graft against IRI through inhibition of GSK3b, preventing thus liver apoptosis.

BO116 RELEVANCE OF SIRTUIN 1 IN LIVER PRESERVATION

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Background: Prolonged cold storage remains a risk factor for liver graft outcome, especially when steatosis is present. Steatotic livers exhibit relevant alterations in AMP-activated protein kinase (AMPK) activity and endoplasmic reticulum stress (ERS) in response to cold ischemia reperfusion injury (IRI). Sirtuin 1 (SIRT1) was shown to regulate cellular metabolism and to be associated with ER alterations. In this communication, we report by the first time the relationship between SIRT1, AMPK, ERS and liver autophagy when steatotic and non steatotic rat livers are preserved in IGL-1 solution.
Methods/Materials: Livers were preserved for 24 h (4°C) in IGL-1 solution and then washed with Ringer lactate solution. We assessed hepatic injury (transaminases, histology), SIRT1, AMPK, eNOS protein level and ERS (GRP78, p-elf2, ATF4, IRE1α and Caspase 12), as well as Beclin-1 (a protein associated with autophagy).