Adding Anti-inflammatory Agents During Warm Ex-Vivo Liver Perfusion Improves the Preservation of Pig Liver Grafts before Transplantation

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Introduction

Normothermic and subnormothermic ex vivo liver perfusion has been developed as an alternative preservation method to cold storage (CS) for liver transplantation. So far most studies have focused on using warm perfused (WP) preservation as a technique to avoid CS. However, WP graft preservation extends beyond changing CS to WP. The active metabolism during the WP preservation time offers the opportunity to assess graft function, but also to provide mediators against preservation injury prior to reperfusion. Warm perfused graft preservation represents a new platform to administer anti-inflammatory compounds before graft reperfusion where pro-inflammatory signaling occurs. The optimal perfusate composition and temperature has not been defined yet and the impact of adding anti-inflammatory agents to the perfusion system is unclear. The addition of different anti-inflammatory agents during ex vivo perfusion at physiological or near-to-physiological conditions might have additional protective effects on the graft besides avoiding cold ischemic injury.

Hypothesis

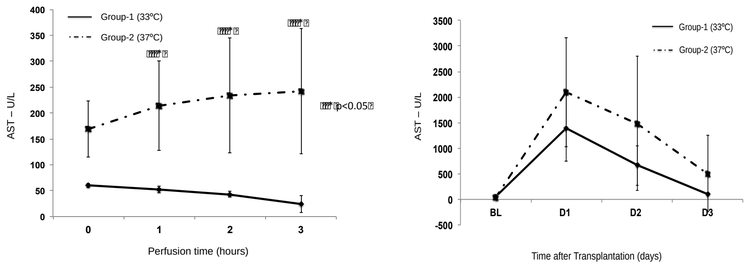
Addition of anti-inflammatory agents during ex vivo perfusion improves organ preservation before pig liver transplantation

Methods

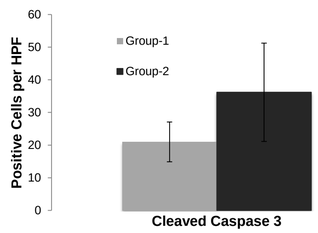
- Livers retrieved under heart beating conditions were perfused for 4 hours. Following the preservation period, pig LT was performed. In the study group (n=5) anti-inflammatory strategies (**alprostadil, n-acetylcysteine, CO and sevoflurane**) were applied during subnormothermic temperature (33˚ C). This was compared to a control group were livers (n=5) were perfused at 37ºC without anti-inflammatory agents, similar to the setup in current European clinical trials. During 3-day follow-up, markers of reperfusion injury, bile duct injury and liver function were examined.

Results

AST levels during ex vivo liver perfusion and after pig liver transplantation

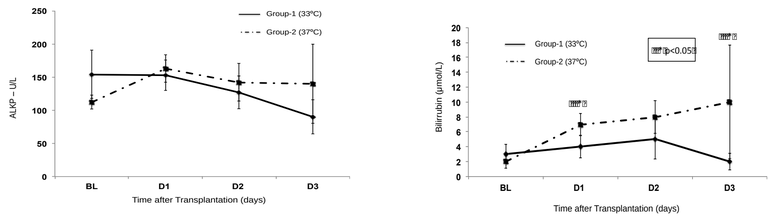


Cleaved Caspase-3 staining as marker of early apoptosis in biopsies 2 hr after pig liver transplantation (p=0.06)



Alkaline Phosphatase & Total Bilirubin

Post-transplant marker of bile duct integrity tended to have lower values in G1 vs G2.

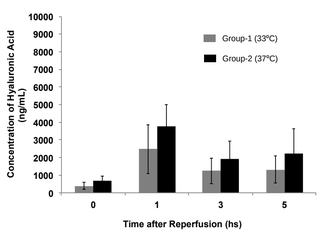


Endothelial cell (EC) preservation assessed by CD31 immunohistochemistry & Serum Hyaluronic Acid levels

EC viability was assessed in liver tissue obtained 2hrs after reperfusion and stained with CD31 immunohistochemistry. All slides were scored for integrity of the sinusoidal endothelial cell lining and evaluated by a blinded pathologist. All liver grafts in Group-1 had an intact sinusoidal EC lining and minimal EC injury. In contrast, Group-2 grafts showed disruption of the EC lining (p=0.01). In normal conditions, HA is removed by endothelial cells from the circulation. HA levels were lower at all three time points in Group-1 when compared to the control grafts correlating with a better EC function in the study group.

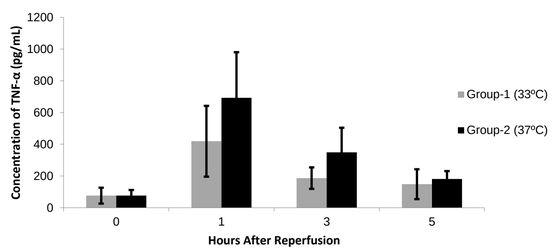
CD31-SCORE	Pattern
0	no staining
1	scattered staining without obvious architecture
2	reduced staining throughout lobule but intact architecture
3	reduced staining in zone 3 only
4	physiological cell lining

CD 31 - SCORE	0	1	2	3	4
Group-1	0%	0%	0%	50%	50%
Group-2	0%	0%	40%	40%	20%



TNF-α concentration after transplantation as marker of inflammatory response in Group-1 vs. Group-2

TNF-α levels were lower in Group-1 vs. Group-2



Conclusions

Our study demonstrates that warm ex vivo liver perfusion can be further improved by refining perfusate mediators and perfusion temperature. The protective effects of avoiding cold ischemic injury can be further improved if we capitalize on the new option to modify pro-inflammatory signaling and block multiple pathways prior to the initiation of the reperfusion injury cascade.