

# **Report of the 2007 Joint International Congress of the ILTS, ELITA and LICAGE for the Novartis Transplantation Advisory Board Grant**

## **Concurrent Oral Abstract Session: Basic Science**

Thursday June 21, 2007

Room Location: Vidigal A&B, 2:30 – 4:00 pm

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### Introduction

During the ILTS 2007 meeting in Rio de Janeiro, Brasil, I presented my abstract “Complete Dearterialization of the Liver causes Intrahepatic Cholestasis due to Reduced Hepatobiliary Transporter Expression” in this session. Perhaps because of the multidisciplinary character of the subjects presented here, it turned out to be a very lively and interactive session. Besides ischemic cell damage of the bile ducts, historically complicating loss of arterial blood supply to the liver after transplantation, main point of discussion was that loss of bile secretory function and subsequent intrahepatic cholestasis seem to play an important role as well.

### Ischemic vs. cholestatic hepatobiliary injury

Hepatic artery thrombosis after human orthotopic liver transplantation is a common complication, however a devastating complication, associated with high morbidity and mortality. Outside the setting of transplantation, hepatic artery thrombosis appears to have limited consequences for the liver. For example in the treatment of multiple hepatic metastases, occlusion of the hepatic artery is well tolerated. This can largely be explained by the presence and rapid development of arterial collaterals, replacing the occluded artery. In this scenario, perfusion is maintained by portal venous and hepatic arterial input. The transplanted liver, however, differs from the native livers in that arterial blood supply from accessory arteries and collateralization is completely interrupted during hepatectomy. As a consequence of hepatic artery thrombosis, the transplanted liver is perfused by the portal vein alone.

Arterial blood supply is closely associated with biliary structures before entering the sinusoids. Arterial blood is supplied to the bile ducts through a network of arterioles and capillaries, called the peribiliary plexus, coming from the hepatic arteries. Due to this distribution and its greater oxygen content, complete loss of arterial blood supply to the liver

is generally followed by severe morbidity, mainly from biliary damage. This condition is often referred to as ischemic cholangiopathy. Serum alkaline phosphatase and gamma-glutamyltransferase levels are elevated, and cholestasis is the main presenting feature.

Of interest, hepatic artery blood flow seems to be critical for the recovery of bile secretory function after liver transplantation. *Ex vivo* completely dearterialized porcine livers perfused by the portal vein alone, have diminished choleresis carried on phospholipids. In human, phospholipids are secreted into bile via the concerted action of the multidrug resistance 3 MDR3 P-glycoprotein (gene symbol ABCB4), located in the canalicular membrane of hepatocytes. Recently, we have demonstrated (Hoekstra et al. Hepatology 2006) in mice heterozygous for the disruption of the Mdr2 gene (a homologue of human MDR3), that endogenous bile salts act cytotoxic after liver transplantation, due to intrahepatic cholestasis and intracellular bile salt retention. Intrahepatic cholestasis originates from altered bile transporter expression/function and altered secretory responses induced by proinflammatory cytokines.

Therefore, we have introduced the possibility that the observed hepatobiliary injury, when the graft loses arterial blood supply, can be in part attributed to the inability of the liver to maintain bile secretory function.

#### Cholestatic hepatobiliary injury

Formal evidence for such primary cholestatic injury after loss of arterial blood supply was still lacking. To confirm the critical involvement of bile secretory function in the pathogenesis of hepatobiliary injury, we compared arterial perfusion by the hepatic artery or peribiliary plexus alone and complete dearterialization of the liver in a mouse model. Hepatobiliary function was assessed by analysis of bile transporter expression, serum bile acids and bilirubin, and hepatic ATP-content. In addition, cellular injury was assessed by light microscopy and biochemical markers. Ligation of either the hepatic artery or the peribiliary plexus alone did not induce marked histological, biochemical or molecular changes, suggesting that both the hepatic artery and the peribiliary plexus have the capacity to complement for each other. Complete loss of arterial blood supply to the liver, however, resulted in decreased bile transporter expression as early as 24 hours, with intrahepatic cholestasis progressing the following weeks. Hepatobiliary damage and bile duct proliferation developed relatively late.

Our study provides evidence that endogenous bile salts may actively contribute to hepatocellular injury after loss of arterial blood supply of the liver graft after OLT. In our hypothesis, the principle insult occurred to the bile ducts, inducing ischemic cell damage of the bile ducts, portal inflammation, ductular proliferation, bile infarcts, and finally fibro-obliteration of the bile ducts. However, the primary event relates to the inability of

hepatocytes to maintain bile secretory function. As a consequence of early limited metabolic capacity of hepatocytes (ATP depletion) and late increased proinflammatory cytokines (TNF- $\alpha$  and IL1- $\beta$ ), bile transporter expression and function are reduced. Subsequently, bile salts and other bile compounds accumulate and aggravate hepatocellular injury. So, even when the principle insult occurs to the bile ducts, hepatocellular injury is an invariable feature of cholestasis, associated with accumulation of bile salts in the liver and blood.

Therapeutic strategies should therefore focus on maintaining choleresis and awaiting collateralization. Ursodeoxycholic acid is known to improve cholestatic disorders. It has been shown to stimulate canalicular transport and bile secretion. However, its choleric action also increases biliary pressure, which has been shown to aggravate bile infarcts and hepatocyte necrosis in the homozygous Mdr2<sup>-/-</sup> mice. Administration of fibrates or statins might provide an alternative therapeutic approach to modify hepatocellular injury after hepatic artery thrombosis, however, their use is not without side-effects.

### Conclusion

We have provided novel evidence about the pivotal role of endogenous bile salts in the pathogenesis of hepatocellular injury after loss of arterial blood supply. Our data indicates that arterial blood flow either by the hepatic artery or peribiliary plexus is mandatory to preserve normal liver architecture and function. In the absence of arterial blood flow, severe injury of the bile ducts and liver parenchyma occurs. As a consequence of loss of arterial blood supply, primary hepatocyte bile secretory function is decreased, resulting in intrahepatic cholestasis and subsequently aggravated hepatocellular injury. Although the principle insult is to the bile ducts, the key early event relates to whether the liver is able to maintain bile secretory function in the absence of arterial blood supply. Therapeutic strategies should therefore focus on maintaining choleresis and awaiting collateralization.

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