Experimental treatment modalities for liver failure

Sosef, M.N.

Citation for published version (APA):
Sosef, M. N. (2003). Experimental treatment modalities for liver failure
Chapter 9

Summary and discussion
Acute liver failure is a complex and devastating consequence of acute liver injury. Acute orthotopic liver transplantation is currently the only effective treatment for those patients who are unlikely to spontaneously recover. Donor shortages however remain a serious problem and many patients die on the waiting list. This has generated interest in designing therapies that would support or replace normal liver function until a donor liver became available for transplant, or the patient's own liver recovered. Chapter 1 gives an overview of hepatic failure, liver transplantation and possible alternative treatment modalities including bioartificial liver (BAL) support and hepatocyte transplantation. In addition, complexities in storing hepatocytes are discussed.

Thorough preclinical evaluation of a bioartificial liver is a necessity before clinical studies are commenced. Unfortunately, one single animal model that perfectly mimics the pathophysiology of acute liver failure in humans is not available. Instead, the safety, feasibility and efficacy of a BAL device should be assessed in different animal models. In order to test the AMC-BAL in the anhepatic model in the pig, the surgical procedure of total hepatectomy had to be adapted to BAL testing. Chapter 2 describes a revised method for vascular reconstruction after total hepatectomy, using a newly designed rigid 3-way vascular prosthesis. A survival study showed a mean survival time of 46 ± 6 hours. The procedure proved a straightforward, well-reproducible technique with relatively minor, concomitant surgical trauma and is regarded well suited for testing of liver assist devices.

Using the aforementioned technique, the AMC-BAL was evaluated in the anhepatic pig model of acute liver failure. After 24 hours of anhepatic state, three treatment modalities were compared: 1. standard medical care alone, 2. the same, coupled with extracorporeal plasma perfusion of an AMC-BAL without hepatocytes (device control group), and 3. the same, but with perfusion of the BAL loaded with autologous hepatocytes. Chapter 3 describes a survival benefit in the AMC-BAL treated group of approximately 20 hours over both control groups. In addition, mean blood ammonia levels during BAL-treatment were significantly lower in the BAL-treated group in comparison to both control groups. An unexpected finding in this study was increasing direct bilirubin levels in all 3 groups, indicative of extrahepatic bilirubin conjugation.

The AMC-BAL has showed its efficacy in several animal studies, prolonging survival time as well as improving biochemical parameters. In order to assess the synthetic function of the hepatocytes inside the bioreactor in vivo, the contribution of BAL treatment on blood coagulation parameters was measured as is reported in chapter 4. Treatment of anhepatic pigs with the AMC-BAL appeared not to restore prothrombin time or clotting factor levels. However, increased levels of thrombin-antithrombin complexes and prothrombin fragments F1+2 during treatment of anhepatic pigs indicated synthesis and direct activation of coagulation factors, leading to thrombin generation. This proved in vivo the presence of synthetic capacity by hepatocytes inside the AMC-BAL.

Another approach to replacement of liver function is the transplantation of isolated hepatocytes. Next to correcting inherited metabolic diseases of the liver, hepatocyte transplantation also has the potential to support liver function in acute liver failure. Most studies however have been performed in rodents using intraportal or intrasplenic transfusion. Chapter 5 describes a study to explore ectopic sites for
hepatocyte transplantation in the pig, serving as a preclinical stage between rodents and human application. The spleen, pancreas, mesentery and the subserosa of stomach, jejunum and ileum were used to implant isolated autologous hepatocytes. Radionuclear and histopathological evaluation after 1-3 months demonstrated that in pigs, the mesentery and small bowel wall provide more favorable sites for transplantation of hepatocytes, compared to the pancreas or the spleen.

Hepatocytes are demanding cells to handle both for BAL purposes as for hepatocyte transplantation. The specialized processes of cell isolation and cell culture will prohibit hepatocyte-based therapies to be widely employed in non-specialized centers, unless the cells can be shipped and handled on the consumer end with only basic skills needed for further cell use. Cryopreservation could provide in this need. Numerous attempts however have been unsuccessful in cryopreserving isolated primary hepatocytes while maintaining high viability and cellular function. Chapter 6 describes the development of a new protocol to cryopreserve primary hepatocytes, utilizing HypoThermosol (a cold-storage solution), as the freezing medium. Results detailing overall survival and function over 14 days of culture in double collagen gel configuration indicated high viability combined with high long-term hepatospecific function and an intact response to cytokine challenge. Only albumin synthesis capacity was 70% instead of 100% when compared with non-frozen cells. This hepatocyte cryopreservation protocol is regarded the first to meet the rigorous demands for successful hepatocyte cryopreservation.

In chapter 7, cryopreserved rat hepatocytes are studied while cultured in the hepatocyte/ fibroblast coculture system. This is a well-characterized long-term culture system suited for application as a flat-plate bioreactor. Long-term survival and hepatospecific function were comparable with the former study, including the reduced level of protein synthesis. Cellular morphology and polarity, which were determined by the localization of actin filaments and connexin-32, were successfully maintained in hepatocytes following cryopreservation. Additional experiments confirmed the maintenance of viability and function during storage times in liquid nitrogen for three months.

Events associated with cryopreservation place extreme physical and physiological demands on cells during the process, especially on highly metabolically active systems such as hepatocytes. In chapter 8 microarray analysis is used to study the possible effects of cryopreservation on gene expression in rat hepatocytes following system recovery. No significant changes were found in transcription levels of genes belonging to the gene ontology groups encompassing protein biosynthesis and secretion, nitrogen metabolism, Cytochrome P450, ATP biosynthesis and apoptosis 24 hours following thawing and system recovery. The origin of reduced protein synthesis is believed to be at the post-transcriptional level.
In the past years we have witnessed the fascinating growth of hepatocyte-based therapies for acute liver failure from drawing table to the first clinical trials. An enormous amount of work brought valuable insight in the process of acute liver failure and the opportunities, but also the limitations of bioartificial liver support.

Nevertheless, the end of this enormous challenge in bioengineering is not in sight, we just left base. A second generation of bioartificial livers will profit from the development of immortalized cell lines or stem cells, and from further understanding how to maintain a large mass of hepatocytes viable and highly functional inside a bioreactor. With less emphasis on the race to show clinical efficacy, hopefully even more research will be aimed at the thorough characterization and testing of BAL systems in vitro, as well as in vivo.

With anticipation, studies are awaited on the cryopreservation of porcine and human hepatocytes in suspension. Successful cryopreservation of these cells will greatly assist implementation of BAL support and hepatocyte transplantation in clinical practice.