Human liver cell lines for the AMC-bioartificial liver
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CHAPTER 1

General introduction and outline of the thesis
Chapter 1
GENERAL INTRODUCTION

Severe liver failure (SLF), caused by loss of more than 60% functional liver mass, is a devastating syndrome with a high morbidity and mortality. SLF comprises two different syndromes: acute liver failure (ALF) and acute-on-chronic liver failure (ACLF). For ALF more than 40 different definitions can be found in literature. A common feature of all definitions is the development of hepatic encephalopathy (HE) within a number of weeks (2 to 26) after the onset of liver disease in a person with a previously normal liver. In contrast, ACLF is defined as an acute deterioration of liver function, including the development of HE, in a patient with pre-existing liver disease.

The etiology of ALF is diverse and incidences differ per geographic region, but is most commonly related to acute viral hepatitis (most frequently hepatitis A, B, and E), drug overdose (most frequently acetaminophen), and idiosyncratic drug reactions. In contrast, in the case of ACLF previously well-compensated liver disease derails by an acute insult such as variceal bleeding, a bacterial infection, or dehydration.

Pathologically, ALF and ACLF are both characterized by massive hepatocellular necrosis and/or apoptosis, in the case of ACLF often superimposed on cirrhosis. Clinically, both ALF and ACLF give rise to a number of severe and life-threatening complications, such as coagulopathy, hepatic encephalopathy, systemic vasodilatation, metabolic acidosis, uncontrolled sepsis, and finally multi-organ failure.

Although the pathophysiological events that occur and give rise to these complications in both syndromes are immensely complex and still not fully understood, a number of sequelae has been described. In the case of ALF, these pathophysiological processes can grossly be divided into three major sequelae. The first is the attenuation of hepatic protein synthesis, which leads to coagulopathy and alterations in plasma and brain amino acid composition, e.g., an increase in aromatic amino acids and a decrease in branched chain amino acids. The second is the reduced clearance of endogenous and exogenous toxins, such as ammonia, lactate, mercaptans, indoles, and benzodiazepine-like substances, which plays an important role in the development of HE, renal failure, and metabolic acidosis. Of note, astrocyte swelling in the brain is a key event in the development of HE, and hyperammonemia plays an important role in astrocyte swelling. Ammonia cannot efficiently be removed from the circulation by the synthesis of urea in the liver, but ammonia can still be incorporated into glutamine in muscle and brain, as an alternative route for ammonia detoxification. Interestingly however, there is accumulating evidence that the temporary fixation into glutamine is a crucial step in the pathogenesis of astrocyte swelling (Trojan Horse hypothesis). Glutamine is taken up by the astrocyte’s mitochondrion, and subsequent hydrolysis of through the activity of the enzyme glutaminase yields high levels of ammonia again. Here, ammonia induces oxidative stress that results in mitochondrial swelling and dysfunction, which leads to alterations in energy metabolism, signalling mechanisms,
astrocytic glutamate uptake, and cell swelling. The third major pathophysiological sequela in ALF is the development of immune dysfunction in which elevated systemic endotoxin levels play an important causative role. There is accumulating evidence that this is initiated by increased intestinal leakage, reduced Kupffer cell mass, and portal-hypertension induced portosystemic shunting. In addition, release of intracellular debris (DNA, RNA, and cytoskeletal fragments) from the necrotic liver into the circulation elicits an inflammatory reaction. The endotoxins and cellular fragments induce the release of various pro-inflammatory cytokines that fuel systemic inflammation and induce a release in endothelial-derived nitric oxide, the latter resulting in vasodilatation and a hyperdynamic circulation. In ACLF, the interaction between immune dysfunction, bacterial translocation from the gut, and circulatory dysfunction are the most important pathophysiological events.

Despite the progress made in supportive care, only 20% of ALF and ACLF patients survive without liver transplantation, which increases the survival rates to over 80% However, liver transplantation is limited by the scarcity of donor organs. In the US, about 20% of the patients with severe liver failure who are on the waiting list for liver transplantation die while waiting for a donor liver. Moreover, improving the patient’s condition before liver transplantation substantially increases post-transplant survival. Lastly, temporary liver support could potentially create time for the diseased liver to regenerate, thereby rendering liver transplantation superfluous. Taken together, there is an urgent need for liver support therapy.

Liver support systems can be divided into non-biological (artificial) and biological (bioartificial) systems. Non-biological systems typically use adsorbents and/or dialyzing membranes to detoxify the patients’ plasma, but have not yet shown a clear effect on survival of ALF and ACLF patients. Probably the potential of these systems is limited by their lack of metabolizing and synthesizing capacity, as well as the fact that detoxification in these systems is non-specific, which might lead to the removal of beneficial proteins, such as growth factors and clotting factors.

In contrast to non-biological systems, bioartificial livers (BALs) typically comprise an extracorporeal bioreactor that is loaded with a biocomponent, e.g. hepatocytes, that can be perfused with the patient’s blood or plasma. These systems can thereby potentially replace the majority of the liver’s many functions. To date, BAL devices with various configurations, biocomponents and culture strategies have been developed.

The AMC-BAL that was developed in the AMC is based on a bioreactor with an internal oxygenator and a spirally wound, non-woven polyester matrix, yielding a three-dimensional culture environment that provides direct cell-plasma contact and equal and optimal oxygenation of the biocomponent (Fig. 1 and 2).

Primary human hepatocytes are of course the ideal cell source for a BAL, but their availability is limited. Alternatively, BALs based on primary porcine hepatocytes have proven efficacious in animal models of ALF. However, the use of animal-derived cells is compromised by
xenotransplantation-related risks. Therefore, there is an urgent need for a human biocomponent with sufficient hepatic functionality that is also available in large quantities. In addition, the safety of this biocomponent should be warranted, which implies the absence of pathogens and preferably also of tumorigenicity.

The main objective of this thesis was to develop a BAL based on a human biocomponent with characteristics as indicated above, and to test its efficacy in an animal model of ALF.

Figure 1. Photograph of the AMC-BAL. For color figure, see page 211.

Figure 2. Schematic cross section of the AMC-BAL showing the polycarbonate housing (A), the spirally wound non-woven polyester matrix (B), the hollow semi-permeable oxygenation capillaries (C), and the extra-capillary space (D) through which the culture medium or patients plasma is perfused.
Chapter 1

OUTLINE OF THE THESIS

Firstly, in Chapter 2, we reviewed all proliferative human cell sources that have been applied in BAL systems so far. We analyzed their potential for clinical BAL application by comparing their *in vitro* hepatic functionality with primary hepatocytes and discussed their efficacy in animal models of acute liver failure and/or their clinical efficacy as assessed in patient studies.

In Chapter 3, we described the development and *in vitro* evaluation of the novel cell line cBAL119, that was generated by overexpressing the *pregnane X receptor* gene, a master regulator of detoxification, in the human hepatoma cell line HepG2. We assessed its potential for BAL application by studying its hepatic functionality cultured on monolayer as well as in a laboratory model of the AMC-BAL.

Chapter 4 describes the potential of another human hepatoma cell line for BAL application: the HepaRG cell line. This bipotent liver progenitor cell line differentiates *in vitro* into two distinct types of cells upon reaching confluence in monolayer cultures: hepatocyte-like cells and biliary-like cells. Subsequent culturing in the presence of 2% dimethyl sulfoxide (DMSO) further increases its detoxification function, but other hepatic functions are largely unknown. In this chapter, the HepaRG cells have been cultured on monolayer using different cell culture protocols – with and without DMSO. We studied transcript levels of various hepatic genes, cell damage parameters, and several liver-specific functions.

In Chapter 5 we cultured the HepaRG cells in a laboratory scale AMC-BAL, also with and without DMSO, and studied the morphology of the HepaRG cells, transcript levels of various hepatic genes, and a number of hepatic functional parameters. Subsequently, we tested if treatment with this HepaRG-AMC-BAL could increase the survival time of rats with ALF.

As opposed to a static monolayer culture that relies on passive diffusion for oxygenation, cell culture in the AMC-BAL is three-dimensional, actively oxygenated, and continuously perfused. In Chapter 6, we studied whether, for HepaRG cells, these differences in culture environment translate in differences in morphology, gene expression, and hepatic functionality. In addition, the effect of BAL culture time on these parameters was studied.

During treatment of ALF patients, the biocomponent of a BAL is exposed to ALF plasma that contains various toxic metabolites. Prolonged exposure to ALF plasma may therefore negatively affect the functionality of a BAL. Firstly, in Chapter 7, we tested this hypothesis on monolayer cultures of HepaRG cells by exposing them to rat derived ALF plasma and by studying the effects histologically, on cell damage parameters, on transcript levels of hepatic genes, and on several hepatic functions. In Chapter 8, this hypothesis was tested again, but this time on the HepaRG-AMC-BAL. Essentially the same parameters were studied, but in this study we additionally studied the effects of exposure to plasma of rats with both mild and severe ALF, *i.e.* BALs were tested prior to connection to the rats (control group), after 5 hours of exposure to rats developing mild ALF (mild ALF group), and after 10 hours of exposure to rats developing from mild ALF to death (severe ALF group).
In Chapter 9, we assessed the optimal medium perfusion flow rate for the HepaRG-AMC-BAL. To this end we perfused laboratory scale HepaRG-AMC-BALs with culture medium at four different perfusion flow rates and studied the effects on hepatic functionality and cell damage. In addition, we calculated mass balances of ammonia and lactate over the HepaRG-AMC-BAL with the purpose of evaluating their potential in monitoring BAL condition during the treatment of ALF patients.

The HepaRG cell line has been recognized as a promising source for in vitro testing of the metabolism and toxicity of drugs and other compounds. However, the hepatic differentiation of these cells has so far relied on exposure to DMSO, which has a damaging effect and represses all-round hepatic functionality. In Chapter 10 therefore, the potential of the HepaRG-AMC-BAL cultured without DMSO was evaluated for testing the metabolism and toxicity of drugs and compounds. To this end, these HepaRG-AMC-BALs were tested for phase 1 and phase 2 drug metabolism, as well as bile acid metabolism.

Lastly, Chapter 11 summarizes the studies of this thesis and discusses future studies.
REFERENCES


