Preclinical development of the AMC-HepaRG-Bioartificial Liver

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Citation for published version (APA):
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GENERAL INTRODUCTION

This thesis revolves around the development of the AMC-bioartificial liver (BAL), an innovative device aimed at the treatment of patients suffering from acute liver failure (ALF) and Acute on chronic liver failure (ACLF).

Definition and prognosis of ALF and ACLF

Over 40 definitions of ALF can be found in literature [1]. The European Association for the Study of the Liver (EASL) defines ALF as severe acute liver injury, characterized by markers of liver damage (elevated serum transaminases) and impaired liver function (jaundice and International Normalized Ratio> 1.5) in combination with the clinical appearance of hepatic encephalopathy, in the absence of a pre-existing liver disease. Acute presentations of chronic auto-immune hepatitis, Wilson disease and Budd-Chiari syndrome are to be considered ALF when fulfilling the above criteria, despite the presence of pre-existing liver disease [2]. ALF is associated with high mortality rates, especially in those who are not eligible for liver transplantation. Due to technical advances in supportive care in specialized liver units and, most importantly, the availability of emergency liver transplantation in the last decades, survival of ALF patients increased from 16.7% in 1973–1978 to 62.2% in 2004-2008 [3].

As for ALF, the definitions of ACLF that is used in clinical practice and in the scientific literature is ununiformed. Working groups of the Asian, American and European scientific organizations, as well as the World Gastroenterology Organization all formulated their own definitions of ACLF. These definitions differ mainly in the criteria for organ failure and preexisting liver disease, as well as the requirement for the acute insult to be hepatic, all of them are associated with high mortalities [4]. The biggest problem that arises due to the non-uniformity of the term ACLF in the scientific literature is that results are not comparable and therefore very difficult to translate to the clinical context. Based on the results of the CANONIC study, ACLF was defined by the EASL chronic liver failure (CLIF) consortium as decompensated liver cirrhosis in the presence of at least one organ failure. In the case of single-organ failure, serum creatinine level must be ≥1.5 mg/dL, or hepatic encephalopathy present [5]. Based on the serial organ failure (SOFA) score, the CLIF-C ACLF score was developed and validated in an external cohort [6]. With this score, patients can be stratified from no ACLF to grade 3 ACLF with associated 180 days mortality rates ranging from 38% to 96%. This is currently the best defined scoring system available, with the restriction that is based on European data and may not be fully applicable to other geographic regions.
Etiology
ALF is triggered by acute liver injury, the cause of which varies geographically, but is most commonly an acute viral hepatitis (e.g. hepatitis A, B, D, E and, to a lesser extent, C), an intoxication (e.g. paracetamol or poisonous mushroom), or an idiosyncratic drug reaction. In a substantial proportion of cases, the cause remains unknown, although these may still be triggered by unrecognized infections, intoxications or idiosyncratic drug reactions. Less common causes of ALF include acute liver ischemia, Budd-Chiari syndrome, Wilson’s disease and acute fatty liver of pregnancy [7, 8].

Like ALF, the triggers for ACLF vary geographically: in Europe the most commonly identified triggers are bacterial infections (33%), active alcoholism (25%) and gastrointestinal hemorrhage (13%), while in China exacerbation of hepatitis B infection was reported as a trigger in 36% of cases [4].

Presentation
The time from liver insult to ALF varies from days to weeks. In general, the first symptoms are related to loss of liver synthetic and metabolic capacity, such as jaundice, coagulopathy, hypoglycemia, hyperlactatemia and accumulation of toxins, such as endogenous benzodiazepines and ammonia, leading to the development of hepatic encephalopathy. A more advanced disease feature is multi-organ failure, thought to be mediated by both the release of damage associated molecular patterns (DAMPs) from necrotic liver cells and pathogen associated molecular patterns (PAMPs) from secondary infections and bacterial translocation, leading to a self-reinforcing cytokine storm [9]. Another deadly complication of ALF is intracranial hypertension caused by cerebral edema, the precise pathogenesis of which is still not fully understood, but inflammation, hyperammonemia and increased cerebral glutamine levels appear to play key roles [10, 11].

ACLF, depending on the definition, generally develops within days, the current view is that preceding triggers, as described above, result in the release of DAMPs and/or PAMPs with a central role for bacterial translocation, leading to an escalation of the inflammatory response that cannot be tempered by the already compromised liver [4].

Treatment
Initial treatment of ALF and ACLF consists of supportive therapy of failing organs, with early endotracheal intubation and sedation when patients develop agitation, often invasive monitoring of intracranial pressure and measures to treat intracranial hypertension and special attention to the prevention of septic complications. Emergency liver transplantation is a treatment option for those cases that are considered to have a very poor prognosis without transplantation, and who have not yet suffered irreversible (brain) damage and are
fit enough to undergo surgery. The selection of these patients in an early stage remains challenging. The Kings College Criteria for this selection were first published in the 1980s and are still commonly used [12], with a reported sensitivity of 69% and specificity of 92% [13]. The 5-year survival after orthotopic liver transplantation for ALF in Europe is 72%, which is slightly lower compared to elective liver transplantation; a difference that is caused mainly by early septic and neurological complications [14]. In one randomized controlled trial, high volume plasma exchange in ALF has shown a survival benefit, suggesting this should also be considered as a treatment modality [15].

Artificial liver therapy
Artificial liver support systems, such as MARS and PROMETHEUS rely on albumin dialysis. These systems were tested in both ALF and ACLF patients and treatment resulted in a consistent improvement in clinical and biochemical parameters. However, despite multiple large randomized controlled trials, till date no study has shown a survival benefit in intention to treat analyses [16].

Bioartificial liver therapy and the AMC-BAL
BALs are envisioned to support patients suffering from ALF and ACLF in order to bridge them to either liver transplantation or recovery. BALs are extracorporeal devices, loaded with living cells (the biocomponent) and are generally connected to the patient via a plasmapheresis circuit.

The AMC-BAL was among the first generation of devices, conceived in the AMC in the 1990’s. It consists of a sheet of matrix to hold the biocomponent, that is wound around a core, and interleaved with gas-permeable capillaries for oxygenation [17]. The features that sets it apart from many other devices is that the biocomponent comes in direct contact with the patient’s plasma (Fig. 1), that the cells are in immediate contact with oxygen and are allowed to organize into three dimensions . Initially, the AMC-BAL was based on primary porcine hepatocytes. Efficacy was proven in a porcine model of ALF and a Phase I clinical trial was in progress [18, 19] when a change in regulation prohibited the further clinical use of xenogeneic cells in the European union [20]. The alternative biocomponent of first choice would have been primary human hepatocytes, however, these are not available in sufficient quantities. A quest for a human-derived proliferative cell source of sufficient quality eventually ended at the human progenitor cell line HepaRG [21]. In 2012, efficacy of the laboratory scale HepaRG-AMC-BAL was shown in a rat model of ALF [22].

The primary aim of this thesis was to further develop the HepaRG-AMC-BAL towards clinical application.
General introduction and thesis outlines

**PART I** of this thesis focusses on the selection of a biocomponent for the AMC-BAL:

In **Chapter 2**, we first describe the demands posed to biocomponents of BALs applied clinically, as well as those applied in drug safety studies and other *in vitro* applications. We then proceed to review the literature on available proliferative cell sources to suit these demands and propose a benchmark set of tests to assess candidate biocomponents. In **Chapter 3**, two such candidate biocomponents were compared head-to-head: the hepatic cell lines HepaRG and C3A. These were assessed in conventional monolayer cultures and

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**Figure 1. HepaRG-AMC-BAL.** Schematic representation of the HepaRG-AMC-BAL (A) with details (B-C) and hematoxylin/eosin-staining (D) showing medium/plasma inflow port (I), medium/plasma outflow port (II), gas inlet port (III) (gas outlet port at opposite position not visible), the non-woven matrix to which the cells attach (IV), gas capillaries (V) and inter-capillary space (VI) through which the culture medium/plasma flows). *From van Wenum et. al. Biofabrication. 2017 30;9(3):035001*
in laboratory-scale BALs. The results described led to the conclusion that both cell lines perform better in BALs than in monolayers, and that HepaRG is best suited for application as a BAL-biocomponent.

In PART II, HepaRG cells and their behavior as BAL-biocomponent under different culture conditions are further characterized:

Chapter 4 continues on the observation that culturing in the AMC-BAL has a beneficial effect on the functionality of HepaRG cells compared to conventional monolayer culturing. We investigated the importance of three dimensional configuration, medium perfusion, as well as oxygenation to this effect, and investigated its association with increased mitochondrial biogenesis through whole-transcriptome microarray analysis, mitochondrial abundance, as well as mitochondrial membrane potential.

In Chapter 5 we zoom in on the importance of pericellular oxygen concentration for the differentiation of HepaRG cells. We investigated the influence of oxygen concentration on their differentiation grade by functional and transcriptional assays, as well as immunostaining of immature and mature hepatocyte markers and of Hypoxia Inducible Factor 1α. We also applied hypoxia during the expansion phase of the cells in order to increase the proliferative capacity.

Chapter 6 focusses on the occurrence and reversibility of the toxic effect of blood plasma on HepaRG cells in monolayer and BAL cultures. Cultures were exposed to different fractions of healthy human plasma in order to identify the fraction containing the detrimental factor(s). A whole-genome transcriptomic study on cells exposed to this plasma for different durations was performed to identify the underlying mechanisms. Finally, we also assessed the detrimental effect of healthy versus ALF-plasma of pigs on mitochondrial functions.

In PART III the HepaRG-AMC-BAL was prepared for pre-clinical and clinical testing:

In Chapter 7, we explored cryopreservation of both HepaRG cells prior to BAL-loading, as well as fully differentiated BALs. We determined the conditions for transport of cell-loaded BALs to the clinic and developed a clinically applicable BAL-transport system. We assessed tumorigenicity of HepaRG cells in immunodeficient mice, and we successfully scaled up the HepaRG-AMC-BAL from the 9 mL laboratory-model to a 540ml clinical size model. Finally, in Chapter 8 the content of this thesis is summarized and future perspectives of the HepaRG-AMC-BAL are given.
REFERENCES


