Generation of rat models of acute decompensation of cirrhosis developing ACLF

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Background/Purpose

Acute-on-chronic liver failure (ACLF) is a defined clinical disease with high morbidity and mortality and characterized by acute severe hepatic failure (acute decompensation) with one, two or more extrahepatic dysfunctions (organ failure) on a background of liver cirrhosis. Therefore, it is important to better understand the development of ACLF.

Effective treatments for ACLF are lacking and the mechanisms of ACLF development remain poorly understood. Therefore, it is necessary to develop an animal model with strict reference to the human outcome.

This study aims at generating animal models of acute decompensation (AD) of cirrhosis developing ACLF, to identify those best reflecting the patient populations appearing as potential responders to a combinatorial therapy.

Specifically, we create rat models of cholestasis or toxic liver injury. On top of CCl_4 injections we generate models of alcoholic (ASH) and non-alcoholic (NASH) steatohepatits.

To reflect human ACLF new rat models of cirrhosis with different precipitating events are urgently needed. Thus, we mimicked excessive alcohol consumption by binge drinking, pneumonia by transnasal inoculation and sterile inflammation by LPS injections.

Methods

Liver cirrhosis was induced using bile duct ligation (BDL) or carbon tetrachloride (CCl_4) injections in Sprague Dawley rats. Rats of the alcoholic steatohepatitis (ASH) group received ethanol (EtOH) in the drinking water *ad llibitum*. Starting with 4% EtOH (week 1), followed by 8% EtOH for one week (week 2). The final concentration for the rest of the study was 16% EtOH. Rats of the non alcoholic steatohepatitis (NASH) group were fed with Western diet (containing 20.9% crude fat and 1.25% cholesterol) *ad libitum*.

When ascites was present (21 days of BDL, 12-14 weeks of CCl_4, 5-6 weeks of CCl_4+WD, 8-9 weeks of CCl_4+EtOH), as a clear sign of portal hypertension, ACLF was induced. To induce ACLF by sterile inflammation, rats were twice injected with LPS (120h and 72h, 6.25µg/kg body weight) before the final experiment.

Acute alcoholic hepatitis was induced by binge drinking of EtOH (6g/kg body weight) 72h before the final experiment. As a model of pneumonia, we performed transnasal inoculation (TNI) 72h before the final experiment. For this, 0.1g of freshly collected stool was diluted in 2 mL sterile 0.9 % NaCl-solution and inoculated.

For organ dysfunction characterization, invasive pressure measurements, blood chemistry analyses and quantitative polymerase chain reactions (qPCR) (KIM-1) were performed. For liver fibrosis assessment picosirius red staining was performed in paraffin embedded liver sections. The neurological behavior test was performed according to Yarnell *et al.* (curr. Protoc. Neurosci., 2016). Brain water content was determined using the dry weight technique (Shah *et Al.*, Liver Transplant., 2013)

SpO_2 was measured using Pulsoxymeter (Pulox)and INR was measured using CoaguChek Pro (Roche).

Liver

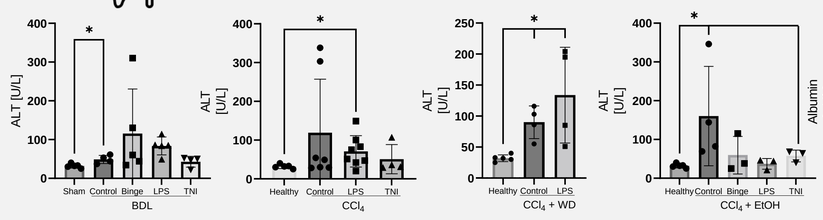


Fig 1a: Effect of ACLF induction on Alanine transaminase (ALT) and Aspartate transaminase (AST) levels in different rat models

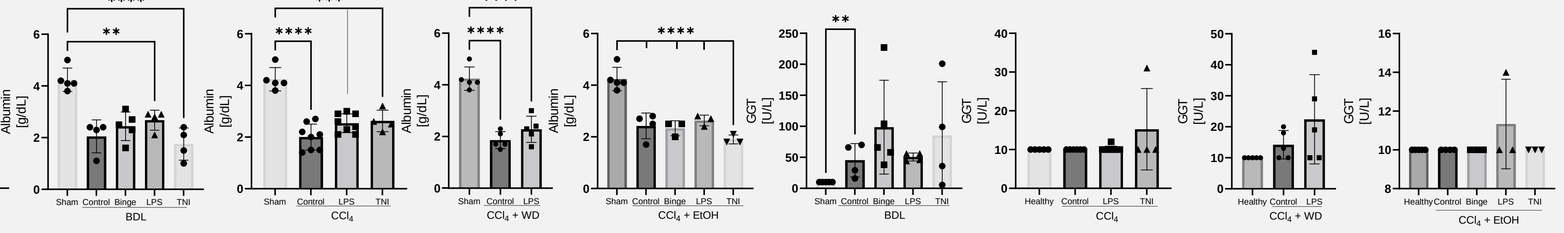
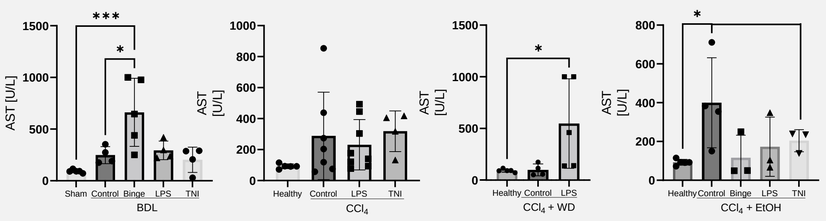


Fig 1b: Effect of ACLF induction on the Albumin (Alb) concentration in the different rat models



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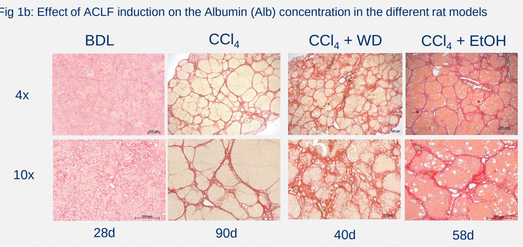


Fig 1c: Hepatic picosirius red stainings of cirrhosis development at different time points.

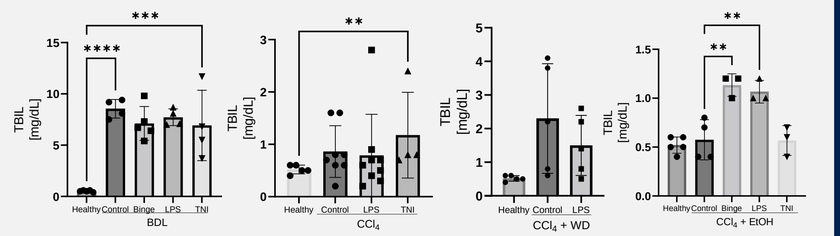


Fig 1d: Effect of ACLF Induction on Gamma-Glutamyltransferase (GGT) and Total Bilirubin (TBIL) levels in the different rat models

Brain

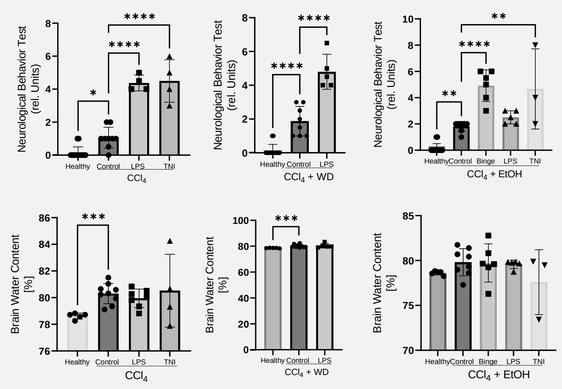


Fig 2: Effect of ACLF induction on Neurological Behavior Test and brain water content in different models.

Lung

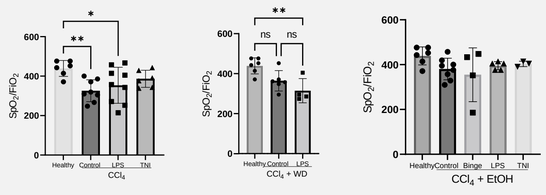
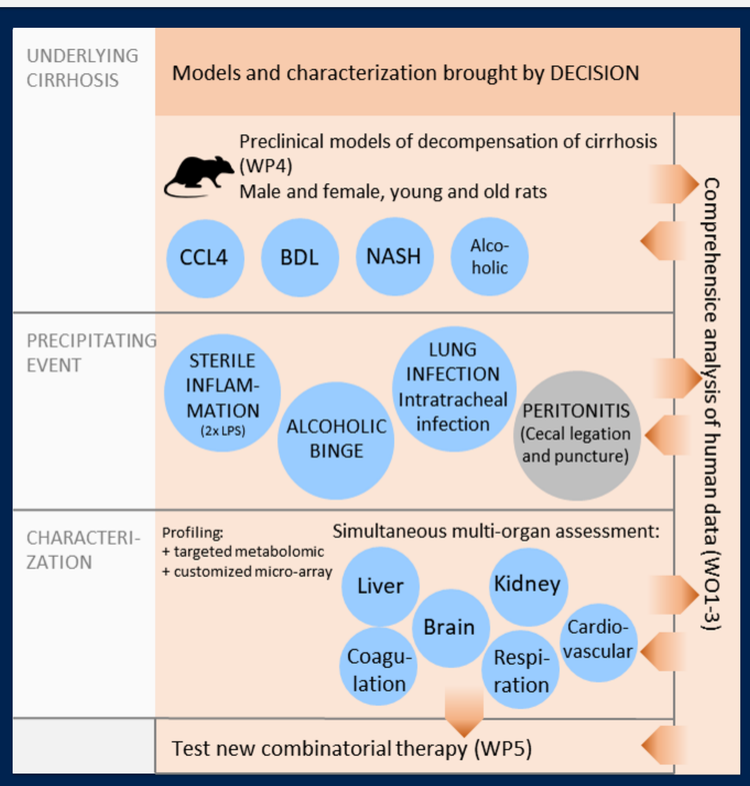


Fig 3: Effect of ACLF induction on SpO2/FiO2 in different models



Kidney

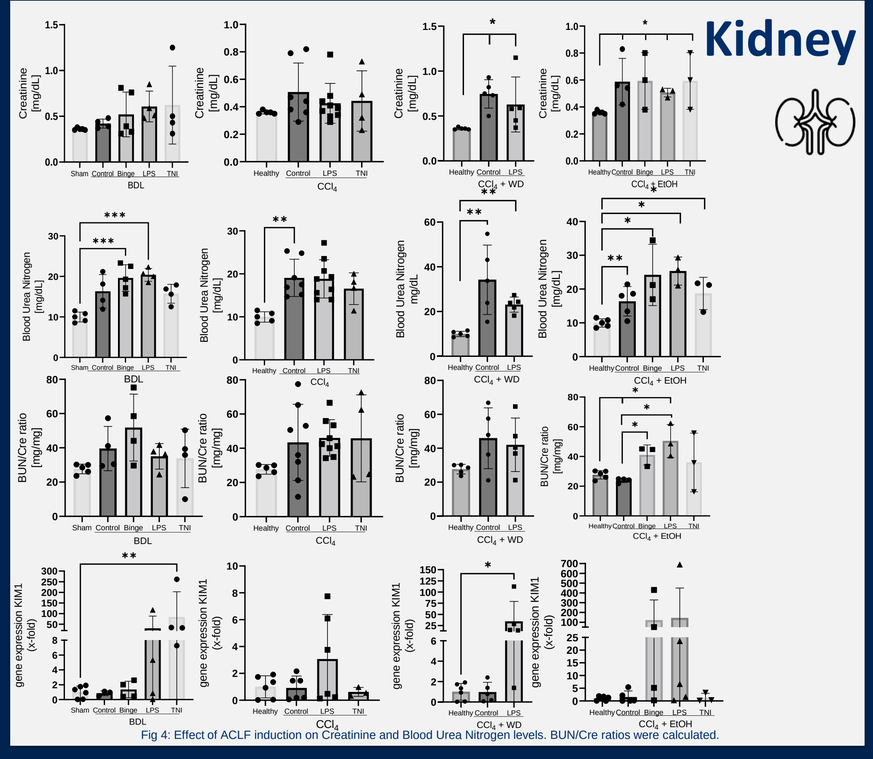


Fig 4: Effect of ACLF induction on Creatinine and Blood Urea Nitrogen levels. BUN/Cre ratios were calculated.

Coagulation

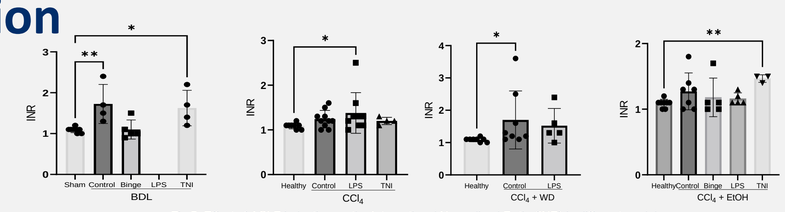


Fig 5: Effect of ACLF induction on the International Normalized Ratio (INR) in different models

Circulation

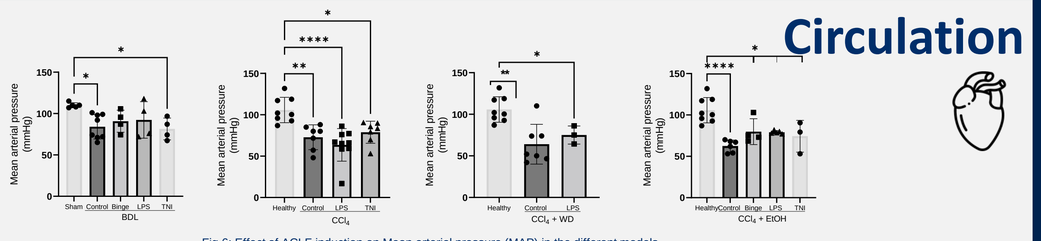


Fig 6: Effect of ACLF induction on Mean arterial pressure (MAP) in the different models.

Results

Rats developed a severe cirrhosis within 3 weeks after BDL characterized by liver, brain and circulation dysfunction. Only binge drinking as an ACLF precipitating event, led to further deterioration of liver function shown by increased levels of ALT, AST and GGT (Fig. 1a, 1d).

In toxic liver disease induced by CCl_4, rats developed a severe cirrhosis within 12-14 weeks characterized by liver, brain, circulation and respiratory dysfunction (Fig. 1-6). The injection of LPS and the TNI as precipitating events led to further deterioration in brain function, as shown in the neurological behaviour tests and the increased brain water content (Fig. 2). Blood coagulation was also affected by LPS injections, as shown by the increased INR in the ASH group (Fig. 5).

In the NASH model, rats developed a severe cirrhosis within 4-6 weeks characterized by liver, kidney, brain, coagulation and circulation dysfunction (Fig. 1-6).

Additionally, LPS injections as a precipitating event in the NASH model, led to a deterioration in liver, brain and respiratory function (Fig. 1-6).

In the ASH model, rats developed a severe cirrhosis within 8-9 weeks characterized by liver, brain and circulation dysfunction (Fig. 1-6). The three precipitating events of Binge drinking, LPS injections and TNI worsened the kidney function in all liver cirrhosis models (Fig. 4). Binge drinking, TNI and LPS injections impaired the brain function in all CCl_4 groups, as assessed by the neurological behaviour test and the brain water content (Fig. 2).

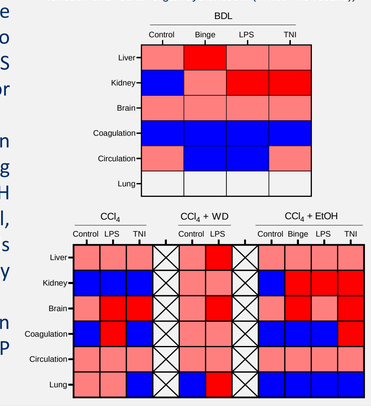
Discussion/Outlook

ACLF is a frequent syndrome in patients with liver cirrhosis and therapeutic options are urgently needed. Therefore animal models which mimick this severe disease needs to be established. In the BDL model the precipitating events of binge drinking, LPS injections and TNI induced hepatic and renal failure, but no brain, coagulation or circulation dysfunction.

In toxic liver disease LPS injections and TNI induced mental as well as cogaulation failure. In the NASH model, LPS injections induced severe liver, brain and lung dysfunction and represents a suitable model for ACLF studies. However, in the ASH model all three precipitating events, alcohol binge, LPS and TNI induced severe renal, mental and coagulation dysfunction (Tabl. 1). Taken together, the ASH model stands out as the most efficient cirrhosis modell for ACLF induction. However the respiratory function was not affected by any precipitating event.

The bacterial translocation (Peritonitis) as a precipitating event of ACLF is still in progress in BDL rats to accomplish the task. The animal models generated in this WP will contribute to a better understanding of the mechanism behind ACLF.

Table 1: Illustration of changes in Organe function in the cholestatic and toxic liver cirrhosis model. (Blue represents no change in organ function, Pink a mild change in organ function and red an organ dysfunction. (White= no results))



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