

Establishment of Acute Liver Failure Model in Tibet Miniature Pig and Verified by a Non-bioartificial Liver

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Research Article

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Abstract

Background and aims Acute liver failure (ALF) is a severe liver disease with high morbidity and mortality. Animal model is very important to research ALF. This study aimed to establish a reproducible, D-galactosamine-induced, Tibet miniature pig model of ALF and verified by a non-bioartificial liver (NBAL).

Methods Thirteen Tibet miniature pigs were randomly divided into four groups (A, B, C, D) after central venous catheterization, then D-galactosamine (D-gal) at 0.45, 0.40 and 0.35g/kg body weight were injected through the central venous catheter in group A, B, C. While in group D, D-gal at 0.35g/kg body weight was injected and treated by a NBAL at 48 h after D-gal administration. Vital signs, blood index values were recorded at every 12 h after D-gal administration and every 2 h during NBAL treatment. Meanwhile, clinical manifestations, survival times and results of H&E staining, Tunel, Ki67, Masson assays and Picrosirius red staining were performed.

Results Tibet miniature pigs developed different degrees of debilitation, loss of appetite and jaundice after D-gal administration. Survival times of groups A, B, C and D were 39.7 ± 5.9 h, 53.0 ± 12.5 h, 61.3 ± 8.1 h and 61 ± 7 h, respectively. Blood levels of ALT, AST, TBIL, Cr, BUN, Amm, PT and inflammation factors (such as TNF- α , IL-1 β , IL-6) levels significantly increased compared with baseline in different groups ($P_s < 0.05$). Pathological results showed obvious liver cell necrosis positively correlated to the dose of D-gal. However, the NBAL did not increase the survival times of ALF, neither Amm and liver cell necrosis, it just decreased some biochemistry indexes (such as TBIL, ALT, AST).

Conclusions: We successfully established a reproducible, D-galactosamine-induced, Tibet miniature pig model of ALF, the NBAL did not improve the survival times of ALF and we think the dosage of 0.35 g/kg is optimal.

Introduction

Acute liver failure (ALF) is a rare and often presentation of severe liver dysfunction (such as jaundice, abnormal coagulation) in a patient with no pre-existing liver disease[1,2]. Which is often caused by viral hepatitis, drug poisoning, autoimmunity and other reasons, and has high morbidity and mortality, about one million people die of ALF every year in the world[3]. For patients with severe hepatitis and liver failure, the current treatment methods are mainly internal medicine therapy and liver transplantation, but the effect of internal medicine therapy is not ideal, the most effective treatment is liver transplantation. However, it is restricted by the source of donor liver and difficult to meet the needs of patients with ALF, which limits the wide application of liver transplantation[4]. As a new method to treat ALF, artificial liver system has made great progress in recent years[5]. In order to verify the safety and effectiveness of artificial liver system, it is of great significance to build a suitable animal model which is similar to clinical symptoms of ALF.

At present, the commonly used modeling methods are mainly surgical model[6] and drug model[7,8]. Compared with drug-induced model, surgical model needs high technical requirements, which is not

suitable for extensive promotion, and surgical trauma may affect the pathophysiology of liver, which is different from the physiological and biochemical manifestations of clinical liver failure. The main model animals are pigs, dogs, rabbits and rats[8-11]. However, the rat model is mainly used to study the molecular pathological mechanism of ALF[9]. Pigs and dogs are mainly used to study the safety and effectiveness of artificial liver system in treatment of ALF[8,12]. The anatomical structure and physiological metabolism of the liver of pigs are similar to that of human, which is helpful to guide the clinical application and the effective evaluation of artificial liver system. Therefore, the simulation of human ALF model with pig is of great significance for the study of the cause, treatment and prognosis of human ALF. Most of the existing studies mainly used Bama pig[8] and big white pig[12], and the dose and route of drug administration were diverse in different studies. At present, there is no previous study report the construction of ALF model with Tibet miniature pigs. Therefore, the dosage of drug needs to be further explored due to the different strains.

D-galactosamine (D-gal) is a kind of aminosaccharide selective hepatotoxic drug. It is metabolized through galactose pathway in the liver, which consumes the intermediate metabolite uridine diphosphate (UDP) of this pathway, thus inhibiting the metabolism of uridine, [impede](#) the synthesis of RNA nucleoprotein, leading to liver cell damage and hepatocyte necrosis[13]. Compared with other drugs, D-galactosamine has better repeatability, no obvious extrahepatic toxicity, and liver damage is similar to clinical ALF. Therefore, it is an ideal model drug for ALF.

Dual plasma molecular adsorption system (DPMAS) is a treatment mode of non-bioartificial liver which is developed recently[14], it primarily consists of a resin adsorber (HA330-II)[15] and a bilirubin adsorber (BS330)[16]. DPMAS system can not only adsorb bilirubin and bile acid, but also adsorb some inflammatory factors and others harmful small molecule substances (such as cytokines, endotoxin) [17,18]. But this system does not have biosynthesis function which has no significant effect on hepatic encephalopathy, a fatal complication of ALF.

In this study, we used different doses of D-gal through the internal jugular vein to construct the ALF model of Tibet miniature pig, and compared the clinical manifestations, [physiology and biochemistry](#), coagulation, survival time, histopathological and immunohistochemical characteristics of model animals, in order to build a stable, with suitable therapeutic time window, drug-induced large animal model of ALF, and verified by a non-bioartificial liver (NBAL).

Materials And Methods

Animals

Thirteen Tibet miniature pigs, 1-3 years old, all males, weighing 35-45kg (Tab.1), purchased from Dongguan Songshan Lake Pearl Laboratory Animal Science and Technology Co., Ltd., license No. SCXK (Guangdong) 2017-0030. All experimental animals were quarantined and kept in cage. Feeding with special food and drinking water freely. All animals were fed adaptively for one week before experimenting. All the animals were fasting for 12 hours before the experiment.

Study design

According to the results of the literature search and the results of the pilot test of the ALF model induced by D-gal in the early stage of our research group, 13 Tibet miniature pigs were randomly divided into four groups (A, B, C, D) after central venous catheterization, then D-gal at 0.45, 0.40 and 0.35g/kg body weight was injected through the central venous catheter in group A, B, C. While in group D, D-gal at 0.35g/kg body weight was injected and treated by a non-bioartificial liver (NBAL) at 48 h after D-gal administration (Fig 1), that is:

Group A (n=3): 0.45 g/kg D-gal;

Group B (n=3): 0.40 g/kg D-gal;

Group C (n=3): 0.35 g/kg D-gal;

Group D (n=4): 0.35 g/kg D-gal and treated by a NBAL at 48 h.

Animal anesthesia

Tibet miniature pigs fasted for 12 h before experiment. The basic anesthesia was performed by intramuscular injection of Sumianxin-II 1.5 ml, sodium pentobarbital (10 ml) and atropine (0.5 mg/kg). Weighing the Tibet miniature pigs after basic anesthesia and preparing D-gal solution according to their weights, then lay the Tibetan miniature pigs on the operating table with a thermal insulation blanket, carry out the neck skin preparation, disinfect the paving sheets, and perform rapid jugular vein catheterization under the condition of local anesthesia with lidocaine.

Venous catheterization

The catheter was inserted and remained in the internal jugular vein using the improved seldinger puncture technique. The detail catheterization process in Group A, B, C (Central venous catheter) were shown in Fig.S1. While in Group D (Double chamber hemodialysis catheter) was shown in Fig.S2.

Preparation of D-gal solution

According to the weight of Tibet miniature pig, the D-gal was weighed. Then the D-gal were dissolved in 5% glucose solution, and the drug concentration was 1 g/10 ml. The pH value of D-gal solution was adjusted to 6.8 with 1 mol/L NaOH solution. A 0.22 μ m filter was used to remove bacteria and impurities, then injected into the infusion bag for reserve and all of them were used up within 1 hour after preparation, all the operational process were in the super-clean workbench.

ALF model establishment

After the jugular vein catheterization were successfully implemented, blood samples were taken as baseline (0 h), then the D-gal solution was injected through the central venous catheter within 30 min, and

then 500 ml saline was added. The catheter was sealed by heparin and covered with heparin caps. The Tibetan miniature pig was put into the cage, and it was naturally revived and free to water after catheter was properly fixed.

Evaluation of Tibet miniature pig ALF model

General conditions

After the infusion of D-gal, the general conditions of Tibet miniature pigs, such as walking and standing ability, skin and eyelid color, vomiting, jaundice, and the time of occurrence were observed. Model animals were recorded every 6 h at wakefulness, and every 1 h after coma. At the same time, the survival time of the model animals were also recorded. If the model animal lived for more than 96 h, it was considered alive.

Detection of serum biochemical indexes

Blood was collected through central venous catheter before D-gal infusion (0 h), every 12 h until 96 h, before animals died, and the serum was separated immediately. Then alanine aminotransferase (ALT), aspartyl transferase (AST), albumin (ALB), urea nitrogen (BUN), creatinine (Cr), total bilirubin (TBIL) and blood glucose (GLU) were measured. At the same time, the serum samples were kept in the refrigerator at -80°C for reserve.

Detection of blood ammonia and coagulation indexes

The blood ammonia (Am_m) was detected by multi-channel biochemical analyzer using blood ammonia determination reagent tablets within 30 min after blood were collected. The prothrombin time (PT), international standardized ratio (INR) and activated partial thromboplastin time (APTT) were also examined.

Detection of inflammatory factors

All blood samples were collected and centrifuged at 4000 rpm for 10 min for plasma collection. Cytokines were assessed using the Luminex 200 system with the Porcine Cytokine 13-plex Panel Magnetic Bead Kit (Luminex, Austin, USA).

Pathological examination

The autopsy was immediately performed after model animal dead. The heart, liver, spleen, kidney, lung and intestine tissues were taken. All tissue specimens were fixed with polyformaldehyde solution, trimmed into 5 mm³, fixed with 10% formaldehyde, dehydrated with alcohol step by step, embedded in paraffin, dyed with HE staining and observed under light microscopy. Ki67 and Tunel staining were performed to detect the proliferation, apoptosis and necrosis of hepatocytes in each group. Masson assays and sirius red staining were used to observe the fibrosis of liver tissue in each group.

NBAL treatment

NBAL treatment was initiated at 48 h after D-gal administration. Propofol (0.10 mg/kg/min) was used to maintain anesthesia during the treatment. In order to prevent possible hypotension 500 ml saline were infusion before the treatment. Blood biochemistry and coagulation function were tested every 2 h during the treatment. The vital signs of ALF model animal, pre- and post-filter pressure and transmembrane pressure (TMP) were recorded every 1 h during the treatment. Heparin was infusion to maintain the APTT between 175 s and 250 s.

Statistical methods

Data were expressed by Mean \pm SD. Variance analysis of repeated measurements was used between groups. LSD-t test was used to compare the two groups. Kaplan-Meier survival analysis was used to analyze the survival time. Log-rank method was used to compare the comparison between groups. All data were analyzed by SPSS 21.0 statistical software and GraphPad Prism 5.0 software were used for plotting. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

General conditions and survival analysis

After D-gal administration, the food intake of Tibet miniature pig in group A decreased 12 hours, one animal vomited significantly, and all Tibet miniature pigs showed unstable standing, listless and yellow urine 24 hours. Then the disease continued to develop rapidly, with drowsiness and coma, all died within 48 hours, with an average survival time of 39.7 ± 5.9 h; one Tibet miniature pig in group B nausea and vomiting occurred 24 hours after D-gal administration, limb convulsion occurred in one Tibet miniature pig at 39 hours and died of liver coma in a short time. The survival time of group B was 53.0 ± 12.5 h; While one Tibet miniature pig in group C occurred nausea and vomiting, yellow urine and [lags in response](#) 36 hours after administration, the survival time of group C was 61.3 ± 8.1 h; However, the survival time of group D was 61 ± 7 h. Kaplan Meier survival analysis showed that there was significant difference in survival time of Tibet miniature pigs in each group ($\chi^2 = 10.97$, $P = 0.012$). The survival time of group A was significantly shorter than that of group C ($P = 0.025$) and Group D ($P = 0.010$). There was no significant difference in the survival time between group A and group B ($P = 0.197$), group B and group C ($P = 0.486$), group C and group D ($P = 0.583$), as shown in Fig.2.

Changes in serum biochemical, coagulation indexes and Amm

The serum ALT, AST, Amm, TBIL, BUN, Cr and PT at peak values in each group were significantly increased after D-gal administration in comparison with baseline ($P_s < 0.05$). While GLU at peak values in each group were significantly decreased after D-gal administration in comparison with baseline ($P_s < 0.05$). The serum ALT, AST, TBIL and ALB in group D were significantly decreased after 8 h NBAL treatment ($P_s < 0.05$). While PT in group D was significantly increased because the use of heparin.

However, the GLU, BUN and Cr in group D were no significant changes after 8 h NBAL treatment (As shown in Fig.3).

Changes in inflammatory markers

As shown in Fig.4, IL-1 α , IL-1RA, IL-1 β , IL-2, IL-4, IL-6, IL-8, IL-10, IL-18 and TNF- α at peak values in each group were significantly increased after D-gal administration when compared with baseline values ($P < 0.05$ for all). However, the IL-12 had no significantly changed. Although IL-1 α , IL-1RA, IL-1 β , IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18 and TNF- α had no significantly changed in group D after 8 h NBAL treatment, NBAL treatment may remove some inflammatory factors which prevent the rapid progression of ALF.

NBAL treatment

The NBAL was running steadily with no pipeline and filter coagulation, filter rupture of membranes and blood leak during treatment. All ALF model animal were completed 8 h NBAL treatment except one dead at 2 h. The values of ALT, AST, TBIL, PT and ALB were significantly decreased when compared with 0 h during NBAL treatment. The values of Amm, Cr and BUN were increased when compared with 0 h during NBAL treatment, but there had no statistic difference (Fig.5A-H). The vital signs of ALF model animal were steadily during treatment (Fig.5I-J). The pre- and post-filter pressure and TMP were also running steadily during treatment (Fig. 5K-L).

Gross specimens and Histopathology

Results of examination of gross specimens of main organs are shown in Fig.6. The results of HE staining of main organs are shown in Fig.7. The HE pathological examination of main organs in each group showed that renal tissue contour was visible, glomerular capillaries and renal interstitial blood vessels were slightly dilated and congested. Spleen: The splenic sinus is mildly to moderately dilated with a large number of red blood cells. There was no obvious cardiac abnormality. Lung: The bronchioles and alveoli of the lung tissue are complete, the pulmonary interstitial capillaries are diffusely dilated and congestive, and local bleeding is scattered in focal form.

In group A, the HE staining of liver cells presented with extensive necrosis, had visible nuclear fragments, and a large number of vacuolar structures after D-gal administration. The situation in groups B and C was similar, with areas of necrotic lesions with diffuse swelling of liver cells having cytoplasmic and vacuolar degeneration. In group D, liver cells had mainly degenerative edema and the liver sinus structure was visible (Fig.7). The Tunel assay demonstrated obvious positive cells in group A and group B, while in groups C and D positive cells were present in comparatively lower quantities. However, the Ki67 assay demonstrated obvious positive cells in group C and group D, while in groups A and B positive cells were present in comparatively lower quantities. Masson and picosirius red staining revealed mild fibrosis in groups A, B, C, and D (Fig.8).

Discussion

Animal model is an important verification platform for demonstrating the effectiveness of new therapeutic methods. It is also an important tool for studying pathological and molecular mechanisms of diseases. Terblanche and Hickman[19] considered that the ideal criteria of ALF animal model mainly include: reversibility; repeatability; death from liver failure; certain treatment window period; large animals; and minimal harm to environment and experimenter. Fournea et al.[20] and Newsome et al. [21] considered that ideal animal models should also include: (1) model animals should be conscious and easy to assess encephalopathy; (2) model animals should have similar physiological and metabolic functions of human beings; and (3) model animals should conform to ethics.

At present, there are two main methods to construct ALF animal models: drug model[7,22,23] and surgical model[6,24,25]. Compared with the drug model, surgical ALF animal model requires high surgical techniques, and surgical trauma affects the pathophysiology of liver, which is different from the physiological and biochemical manifestations of clinical ALF. For drug models, although there are great differences in drug tolerance and metabolism between different animals and different species, the most common cause of ALF in clinic is still drug factors, so it is still the focus of current research.

The most common used drugs for inducing ALF mainly include D-galactosamine (D-gal)[7,22], acetaminophen (APAP)[26-29], thioacetamide (TAA)[30], and carbon tetrachloride (CCl₄)[31]. In most drug models of ALF, there are nephrotoxicity, cardiotoxicity and other organ damage besides hepatotoxicity. However, D-gal has good reproducibility, less extrahepatic toxicity and dose easy to control compared with other drugs. Furthermore, D-gal induced ALF is similar to clinical symptoms. Therefore, D-gal is an ideal drug for inducing ALF animal model.

As we all know, drug dosage and administration method are two important factors in constructing ALF model. The same drug may have different reactions due to different species or individuals, and individual differences are great. Glorioso et al[12] successfully established a pig ALF model by injecting 0.75 g/kg D-gal into the external jugular vein, and successfully applied it to the study of artificial liver. Li LJ et al [4] constructed Chinese miniature pig (Bama pig) ALF model through the internal jugular vein incision and catheterization with D-gal dose at 1.3g/kg and 1.5g/kg, respectively, and successfully used it to verify the efficacy of artificial liver. Shi XL et al[32] also successfully establish the ALF model of Chinese miniature pig (Bama pig) by injected D-gal through the internal jugular vein. However, the dose was 0.40 g/kg. Drug-induced ALF model has been reported in many ways[8,12,32]. At present, the most common used methods are external jugular vein catheterization and intraperitoneal injection. Intraperitoneal administration is simple and convenient, but the drug absorption effect is quite different. However, the existing literature on external jugular vein administration requires incision and intubation, and the operation is relatively complex. Furthermore, operative trauma may affect the effect of drugs.

On the basis of preliminary experiments and literature review, we used the improved seldinger puncture technique(non-incision) to inserted and remained catheter in the internal jugular vein. This method is simple, less traumatic and short anesthesia time, thus minimizing the factors which may affecting the drug effect. An ALF model was successfully established by infusion different doses of D-gal (0.45, 0.40

and 0.35 g/kg) through central venous catheter. The clinical manifestations, survival time, liver and kidney function, coagulation function, histopathology and immunohistochemistry of Tibet miniature pigs were compared.

The results showed that all the Tibet miniature pigs showed different degrees of anorexia, mental malaise, skin yellow staining and abnormal coagulation function after D-gal administration, which were similar to those of clinical ALF. The survival time of Tibet miniature pigs with 0.45, 0.40 and 0.35 g/kg D-gal were 39.7 ± 5.9 h, 53 ± 12.5 h and 61.3 ± 8.1 h, respectively. There was a significant negative correlation between the survival time of Tibet miniature pigs and the dose of D-gal. The biochemical indexes (ALT, AST, TBIL, PT, etc.) of Tibet miniature pigs increased gradually After D-gal administration. The higher of the D-gal dosage, the earlier the biochemical indexes reached the peak value. The survival time in group D (NBAL treatment) has no significant difference when compared with Group C. Furthermore, the NBAL treatment just reduced some biochemical indexes, such as ALT, AST, TBIL, etc.

When ALF occurs, due to a mass of necrosis of hepatocytes, decreased metabolism of hepatocytes and abnormal ammonia metabolism, blood Amm increases, which leading to the occurrence of hepatic encephalopathy. In this study, the Amm of Tibet miniature pigs gradually increased after D-gal administration. The average level of Amm in group A (0.45 g/kg) was 207.7 $\mu\text{mol/L}$ at 36 hours after D-gal administration, which was about 5 times over the baseline value. The average level of Amm in group B (0.40 g/kg) was 370.5 $\mu\text{mol/L}$ at 60 hours after D-gal administration, which was about 8.7 times over the baseline value. The level of serum Amm in group C (0.35 g/kg) also increased progressively after D-gal administration, and reached 323.3 $\mu\text{mol/L}$ at 60 hours, which was about 10 times over the baseline value. The Amm in group A increased faster than that in group B and C, and that in group B was also faster than that in group C. Coma, convulsions and other symptoms occurred in all of the experimental animals before death. The significant increase of Amm proved that hepatic encephalopathy occurred in the model animals. However, the Amm in group D didn't significant decreased after NBAL treatment.

Furthermore, we examined the histopathological changes of the experimental animals after the animals died. The HE staining showed different damage degrees of hepatocyte cord and disordered arrangement of hepatocytes in different group; TUNEL showed a large number of apoptotic and necrotic hepatocytes; Ki67 showed a small amount of regeneration of hepatocytes in group C and group C; Masson and picosirius red staining could see different fibrosis degrees of liver tissue. These results further confirmed that ALF occurred in Tibet miniature pigs after infusion of D-gal. Unfortunately, the NBAL treatment didn't significantly improved the pathology of ALF model.

All in all, we successfully established an ALF model of Tibet miniature pigs with different doses of D-gal through the central venous catheter and verified by a NBAL. We think that 0.35 g/kg is the most ideal dosage. After 48 h, the biochemical indexes of ALF model animals all increase rapidly, which is the suitable treatment time of artificial liver. The NBAL did not increase the survival times of ALF, it just reduced some biochemical index. The successful establishment of ALF model in Tibet miniature pig laid a solid foundation for us to further evaluate the safety and effectiveness of bio-artificial liver system.

Declarations

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Author contributions This manuscript was written through the contributions of all authors. All authors have given approval to the final version of the manuscript.

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Conflict of interest All authors declare that they have no conflict of interest.

Ethics approval This protocol and procedure employed were ethically reviewed and approved by the Zhujiang Hospital Institutional Review Board.

Consent for publication Not applicable.

Availability of data and material All data generated or analyzed during this study are included in this published article.

References

1. Shah NJ, Royer A, John S: Acute liver failure. 2021
2. Wang F, Gong S, Wang T, Li L, Luo H, Wang J, et al. Soyasaponin ii protects against acute liver failure through diminishing yb-1 phosphorylation and nlrp3-inflammasome priming in mice. *THERANOSTICS* 2020;10:2714-2726.
3. Grek A, Arasi L: Acute liver failure. *AACN Adv Crit Care* 2016;27:420-429.
4. Zhang J, Zhao X, Liang L, Li J, Demirci U, Wang S. A decade of progress in liver regenerative medicine. *BIOMATERIALS* 2018; 157:161-176.
5. Tandon R, Froghi S. Artificial liver support systems. *J Gastroenterol Hepatol* 2021; 36:1164-1179.
6. Cai L, Weng J, Feng L, He G, Qin J, Zhang Z, et al. Establishment of a novel simplified surgical model of acute liver failure in the cynomolgus monkey. *BIOMED RES INT* 2016; 2016:3518989.
7. Feng L, Cai L, He GL, Weng J, Li Y, Pan MX, et al. Novel d-galactosamine-induced cynomolgus monkey model of acute liver failure. *World J Gastroenterol* 2017; 23:7572-7583.
8. Zhou N, Li J, Zhang Y, Lu J, Chen E, Du W, et al. Efficacy of coupled low-volume plasma exchange with plasma filtration adsorption in treating pigs with acute liver failure: a randomised study. *J HEPATOL* 2015; 63:378-387.

9. Conrad E, Resch TK, Gogesch P, Kalinke U, Bechmann I, Bogdan C, et al. Protection against ma-induced liver damage by myeloid cells requires type i interferon and il-1 receptor antagonist in mice. *HEPATOLOGY* 2014; 59:1555-1563.
10. Diaz-Buxo JA, Blumenthal S, Hayes D, Gores P, Gordon B. Galactosamine-induced fulminant hepatic necrosis in unanesthetized canines. *HEPATOLOGY* 1997; 25:950-957.
11. Fu T, Li H, Zhao Y, Cai E, Zhu H, Li P, et al. Hepatoprotective effect of alpha-mangostin against lipopolysaccharide/d-galactosamine-induced acute liver failure in mice. *BIOMED PHARMACOTHER* 2018; 106:896-901.
12. Glorioso JM, Mao SA, Rodysill B, Mounajjed T, Kremers WK, Elgilani F, et al. Pivotal preclinical trial of the spheroid reservoir bioartificial liver. *J HEPATOL* 2015; 63:388-398.
13. Morikawa A, Sugiyama T, Kato Y, Koide N, Jiang GZ, Takahashi K, et al. Apoptotic cell death in the response of d-galactosamine-sensitized mice to lipopolysaccharide as an experimental endotoxic shock model. *INFECT IMMUN* 1996; 64:734-738.
14. Chen G, Wu M, Wu B, Liu F, Liu J, Liu L. Effects of dual plasma molecular adsorption system on liver function, electrolytes, inflammation, and immunity in patients with chronic severe hepatitis. *J CLIN LAB ANAL* 2019; 33:e22926.
15. Pomarè MD, Ankawi G, Lorenzin A, Neri M, Caprara C, Ronco C. Biocompatibility and cytotoxic evaluation of new sorbent cartridges for blood hemoperfusion. *Blood Purif* 2018; 46:187-195.
16. Su R, Rao Y, Shen X, Zhu J, Ji A, Jin G, et al. Preparation and adsorption properties of novel porous microspheres with different concentrations of bilirubin. *Blood Purif* 2016; 42:104-110.
17. Wan YM, Li YH, Xu ZY, Yang J, Yang LH, Xu Y, et al. Therapeutic plasma exchange versus double plasma molecular absorption system in hepatitis b virus-infected acute-on-chronic liver failure treated by entercavir: a prospective study. *J Clin Apher* 2017; 32:453-461.
18. Zhong S, Wang N, Zhao J, Zhang L, Luo L, Zeng WQ, et al. [plasma exchange combined with double plasma absorption therapy improve the prognosis of acute-on-chronic liver failure]. *Zhonghua Gan Zang Bing Za Zhi* 2018; 26:744-749.
19. Terblanche J, Hickman R. Animal models of fulminant hepatic failure. *Dig Dis Sci* 1991; 36:770-774.
20. Fourneau I, Pirenne J, Roskams T, Yap SH. An improved model of acute liver failure based on transient ischemia of the liver. *Arch Surg* 2000; 135:1183-1189.
21. Newsome PN, Plevris JN, Nelson LJ, Hayes PC. Animal models of fulminant hepatic failure: a critical evaluation. *Liver Transpl* 2000; 6:21-31.

22. Zhang Z, Zhao YC, Cheng Y, Jian GD, Pan MX, Gao Y. Hybrid bioartificial liver support in cynomolgus monkeys with d-galactosamine-induced acute liver failure. *World J Gastroenterol* 2014; 20:17399-17406.
23. Li Y, Wu Q, Wang Y, Weng C, He Y, Gao M, et al. Novel spheroid reservoir bioartificial liver improves survival of nonhuman primates in a toxin-induced model of acute liver failure. *THERANOSTICS* 2018; 8:5562-5574.
24. Detry O, Gaspar Y, Cheramy-Bien JP, Drion P, Meurisse M, Defraigne JO. A modified surgical model of fulminant hepatic failure in the rat. *J SURG RES* 2013; 181:85-90.
25. Chen HS, Joo DJ, Shaheen M, Li Y, Wang Y, Yang J, et al. Randomized trial of spheroid reservoir bioartificial liver in porcine model of posthepatectomy liver failure. *HEPATOLOGY* 2019; 69:329-342.
26. Lee KC, Baker LA, Stanzani G, Alibhai H, Chang YM, Jimenez PC, et al. Extracorporeal liver assist device to exchange albumin and remove endotoxin in acute liver failure: results of a pivotal pre-clinical study. *J HEPATOL* 2015; 63:634-642.
27. Li L, Huang W, Wang S, Sun K, Zhang W, Ding Y, et al. Astragaloside iv attenuates acetaminophen-induced liver injuries in mice by activating the nrf2 signaling pathway. *MOLECULES* 2018;23
28. Schneider KM, Elfers C, Ghallab A, Schneider CV, Galvez E, Mohs A, et al. Intestinal dysbiosis amplifies acetaminophen-induced acute liver injury. *Cell Mol Gastroenterol Hepatol* 2021; 11:909-933.
29. Chauhan A, Sheriff L, Hussain MT, Webb GJ, Patten DA, Shepherd EL, et al. The platelet receptor clec-2 blocks neutrophil mediated hepatic recovery in acetaminophen induced acute liver failure. *NAT COMMUN* 2020; 11:1939.
30. Afifi NA, Ramadan A, Erian EY, Sedik AA, Amin MM, Hassan A, et al. Synergistic effect of aminoguanidine and l-carnosine against thioacetamide-induced hepatic encephalopathy in rats: behavioral, biochemical, and ultrastructural evidence. *Can J Physiol Pharmacol* 2021; 99:332-347.
31. Engelmann C, Sheikh M, Sharma S, Kondo T, Loeffler-Wirth H, Zheng YB, et al. Toll-like receptor 4 is a therapeutic target for prevention and treatment of liver failure. *J HEPATOL* 2020; 73:102-112.
32. Shi XL, Gao Y, Yan Y, Ma H, Sun L, Huang P, et al. Improved survival of porcine acute liver failure by a bioartificial liver device implanted with induced human functional hepatocytes. *CELL RES* 2016; 26:206-216.

Tables

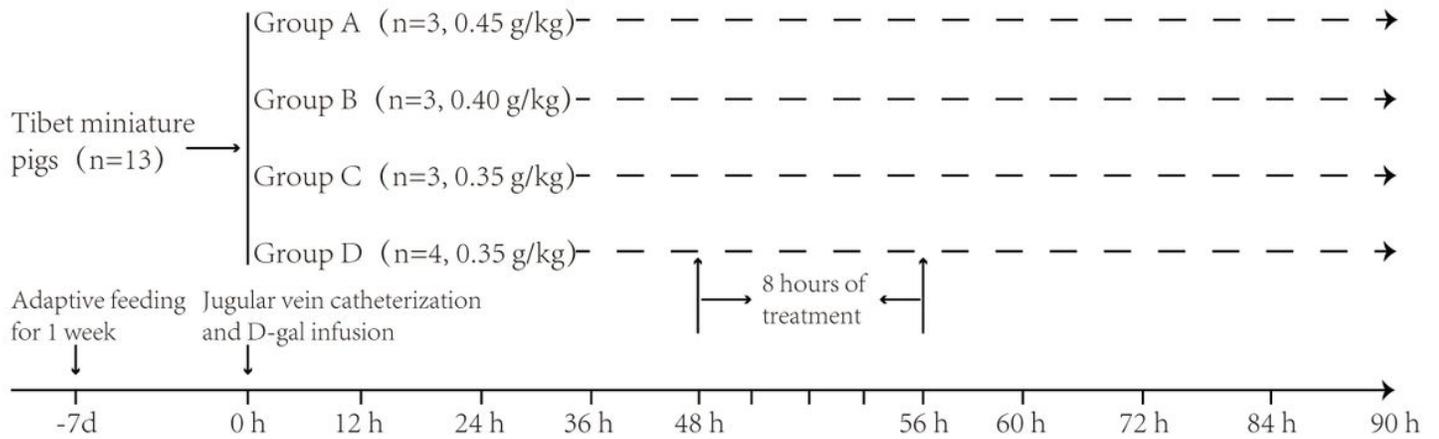
Tab.1 The general conditions of experimental animals before D-gal administration

Group	Age(y)	Weight(kg)	Sexual (F/M)	Dose (g/kg)	BP (mmHg)	Amm (μmol/L)	PT (s)
A	1.0	40	M	0.45	109/76	35	13.1
A	1.2	41	M	0.45	113/78	58	12.6
A	2.0	40	M	0.45	120/75	30	12.6
B	1.0	40	M	0.40	118/80	41	14.1
B	1.0	41	M	0.40	116/79	54	13.8
B	2.0	40	M	0.40	124/87	33	11.2
C	2.5	45	M	0.35	118/78	23	12.0
C	1.0	44	M	0.35	117/69	42	11.1
C	1.5	41	M	0.35	121/79	33	14.6
D	1.0	45	M	0.35	112/70	43	11.4
D	1.5	40	M	0.35	109/81	43	12.8
D	2.5	37	M	0.35	121/84	29	15.1
D	1.0	35	M	0.35	116/90	24	12.3
F: Female; M: Male; BP: Blood pressure; Amm: Ammonia; PT: Prothrombin time.							

Figures

A Preparation

Sample collection and animal behavior observation



B

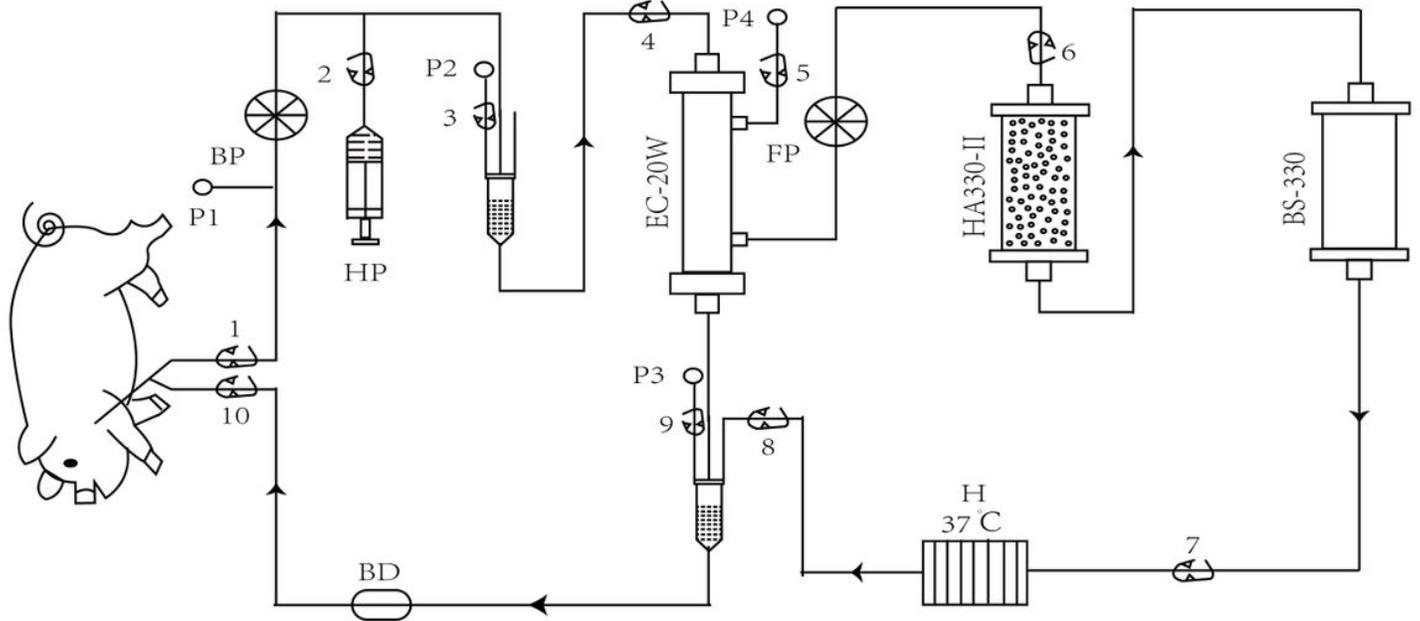


Figure 1

Study design (A) and NBAL schematic diagram (B). D-gal: D-galactosamine; BP: blood pump; FP: points slurry pump; BD: bubble detector; P1-4: pressure detector; EC-20W: plasma separator; HA330-II: resin adsorber; BS-330: bilirubin adsorber; H: heater; 1-10: pinch valve.

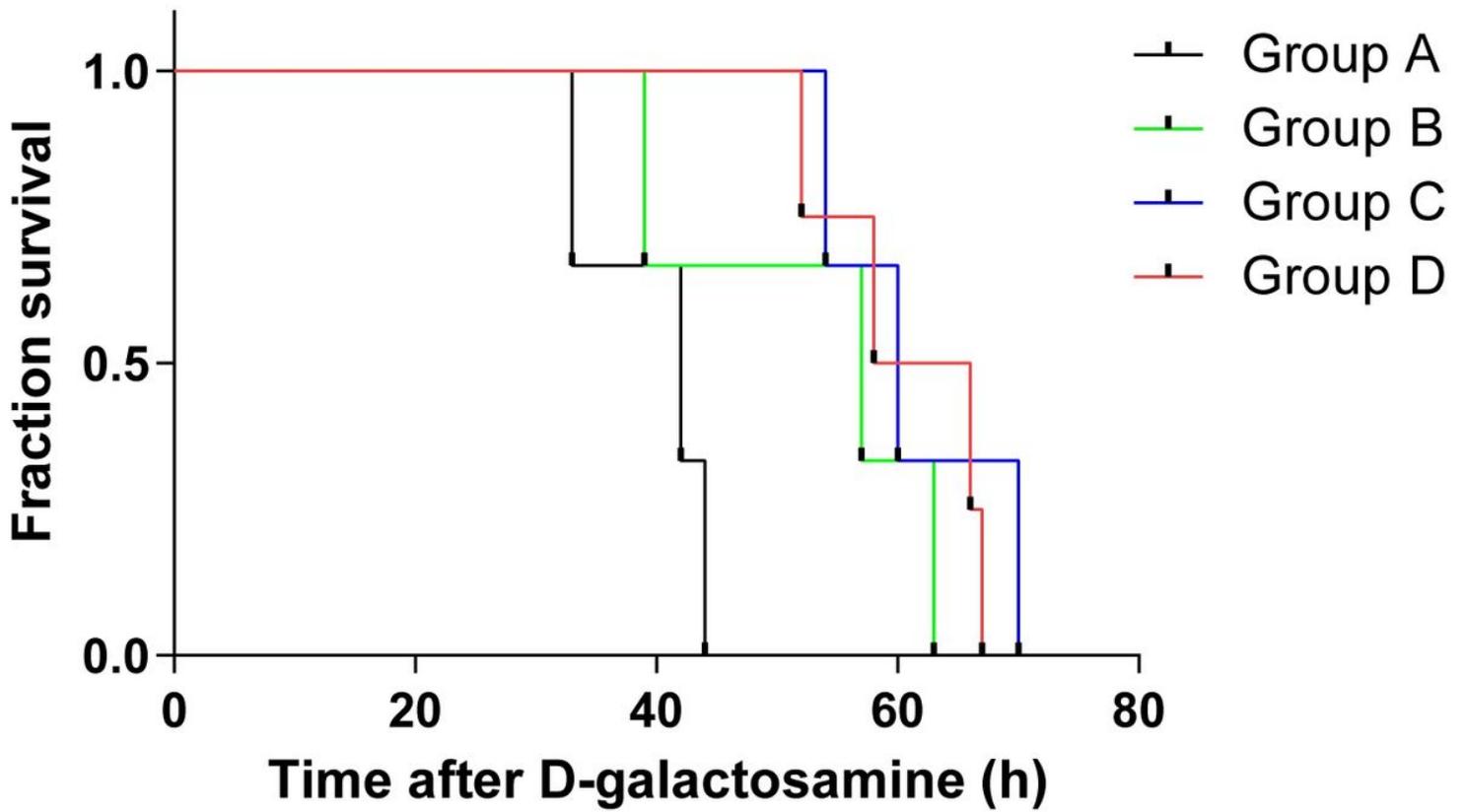


Figure 2

Survival times in different groups. Group A vs group B: $P = 0.197$; group A vs group C: $P = 0.025$; Group A vs group D: $P = 0.010$; Group B vs group C: $P = 0.486$. Group C vs group D: $P = 0.583$.

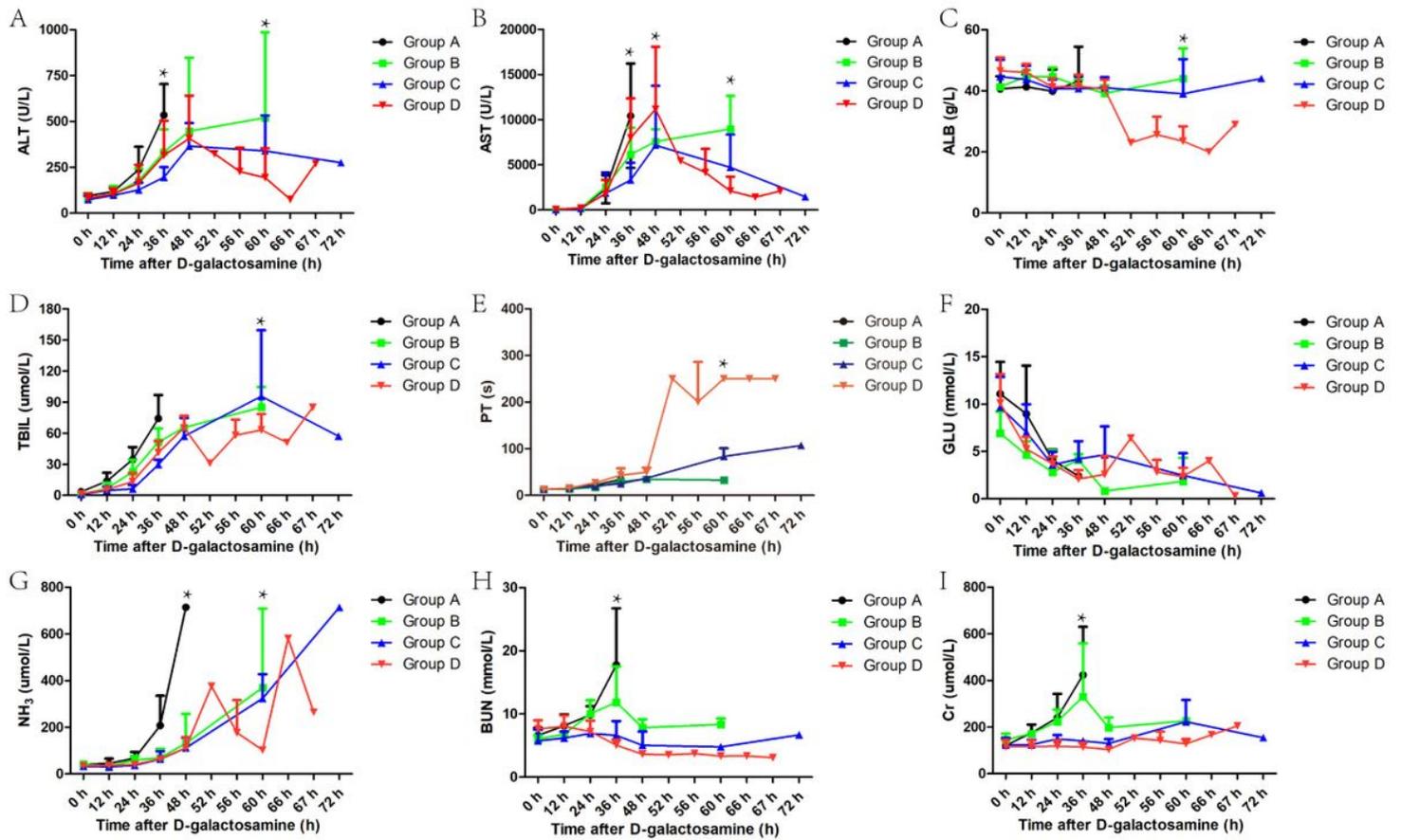


Figure 3

Changes of biochemical indices at different time points in each group. All data points are mean \pm SD. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALB: Albumin; TBIL: Total bilirubin; PT: Prothrombin time; GLU: glucose; Amm: ammonia; BUN: Blood urea nitrogen; Cr: Creatinine; *: P < 0.05.

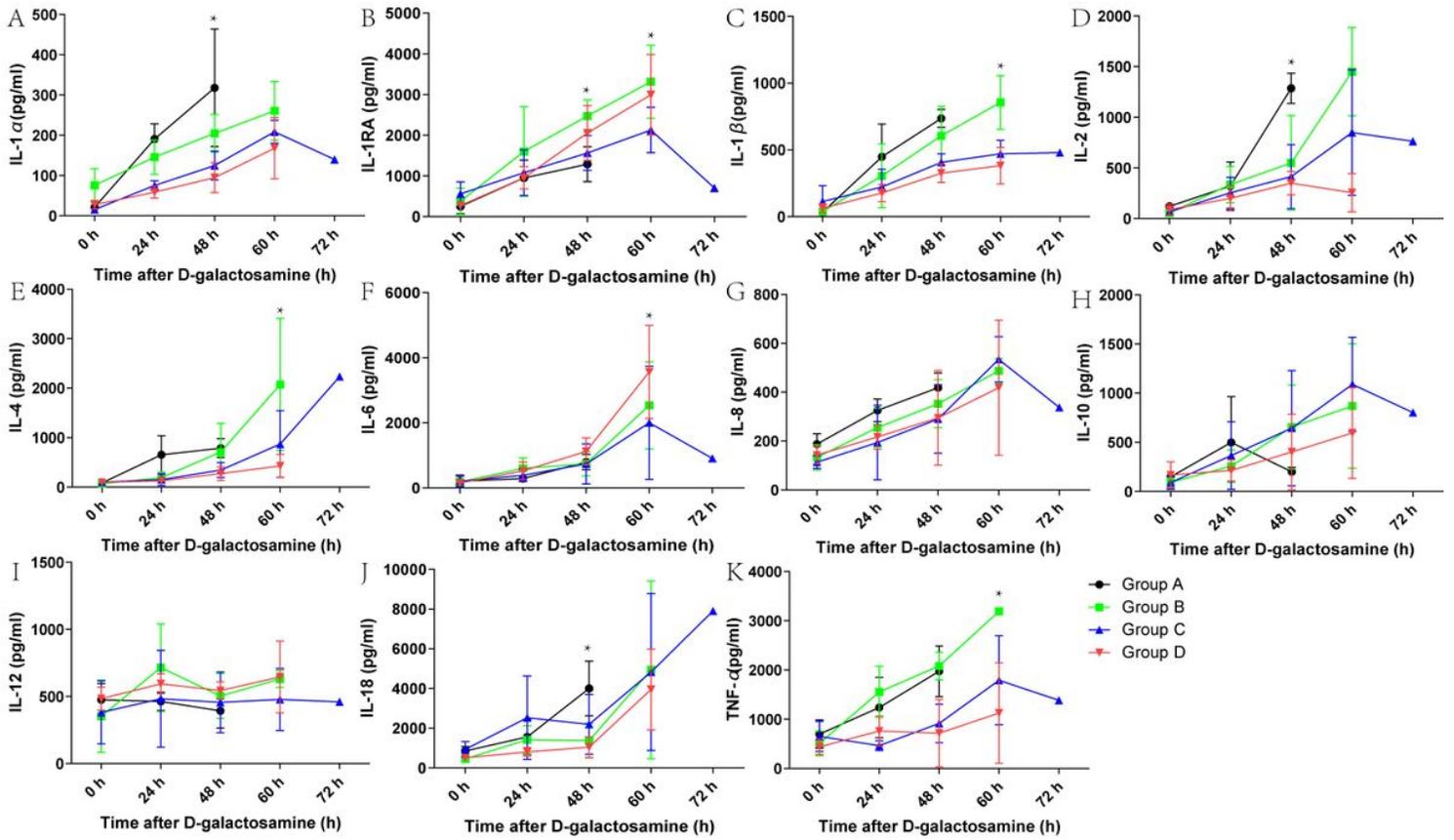


Figure 4

Changes of inflammation markers at different time points in each group. All data points are mean \pm SD, IL-1 α : Interleukin-1 α ; IL-1RA: Interleukin-1receptor antagonist; IL-1 β : Interleukin-1 β ; IL-2: Interleukin-2; IL-4: Interleukin-4; IL-6: Interleukin-6; IL-8: Interleukin-8; IL-10: Interleukin-10; IL-12: Interleukin-12; IL-18: Interleukin-18; TNF- α : Tumor necrosis factor- α ; * \square P < 0.05.

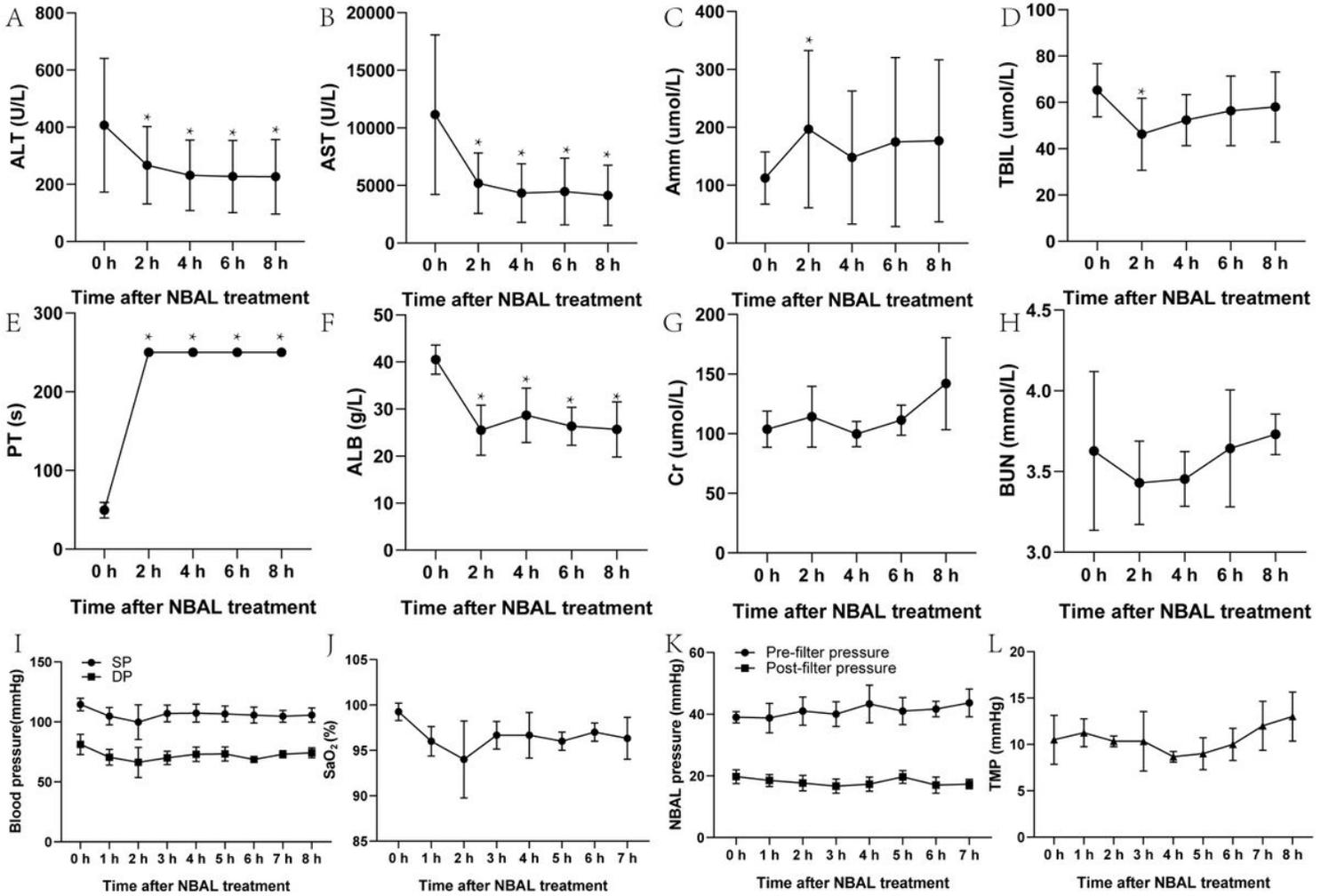


Figure 5

Changes of biochemical indexes, vital signs and machine pressure during NBAL treatment. All data points are mean ± SD. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; Amm: ammonia; TBIL: Total bilirubin; PT: Prothrombin time; ALB: Albumin; GLU: glucose; Cr: Creatinine; BUN: Blood urea nitrogen; SP: systolic pressure; DP: diastolic pressure; SaO₂: Blood oxygen saturation; TMP: transmembrane pressure; NBAL: Non-bioartificial liver. * compared with 0 h P < 0.05.

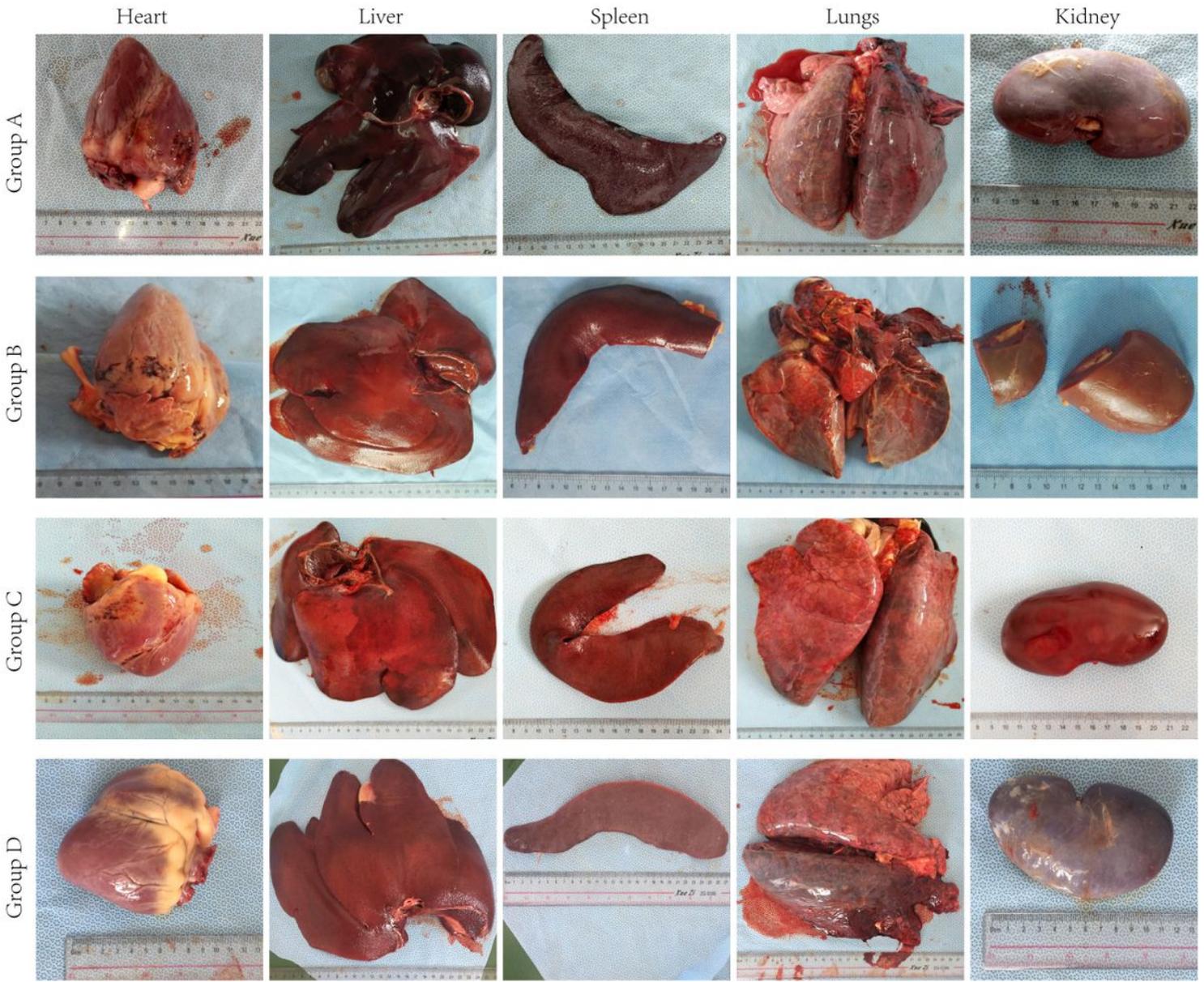


Figure 6

Gross specimens of main organs post-mortem in different groups.

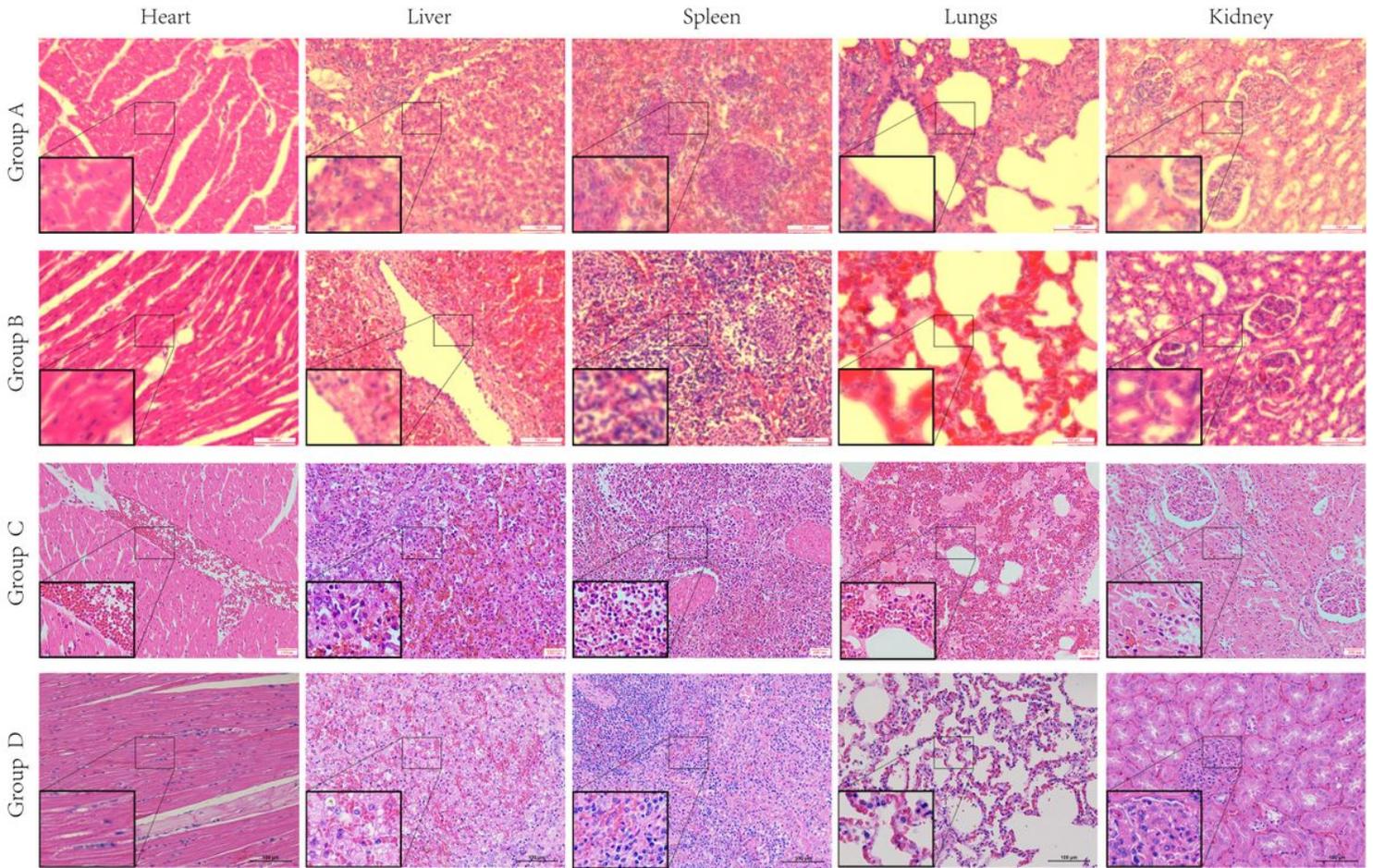


Figure 7

HE staining of main organs post-mortem in different groups. Lower left corner detail: enlarged scale (scale bars: 100 µm)

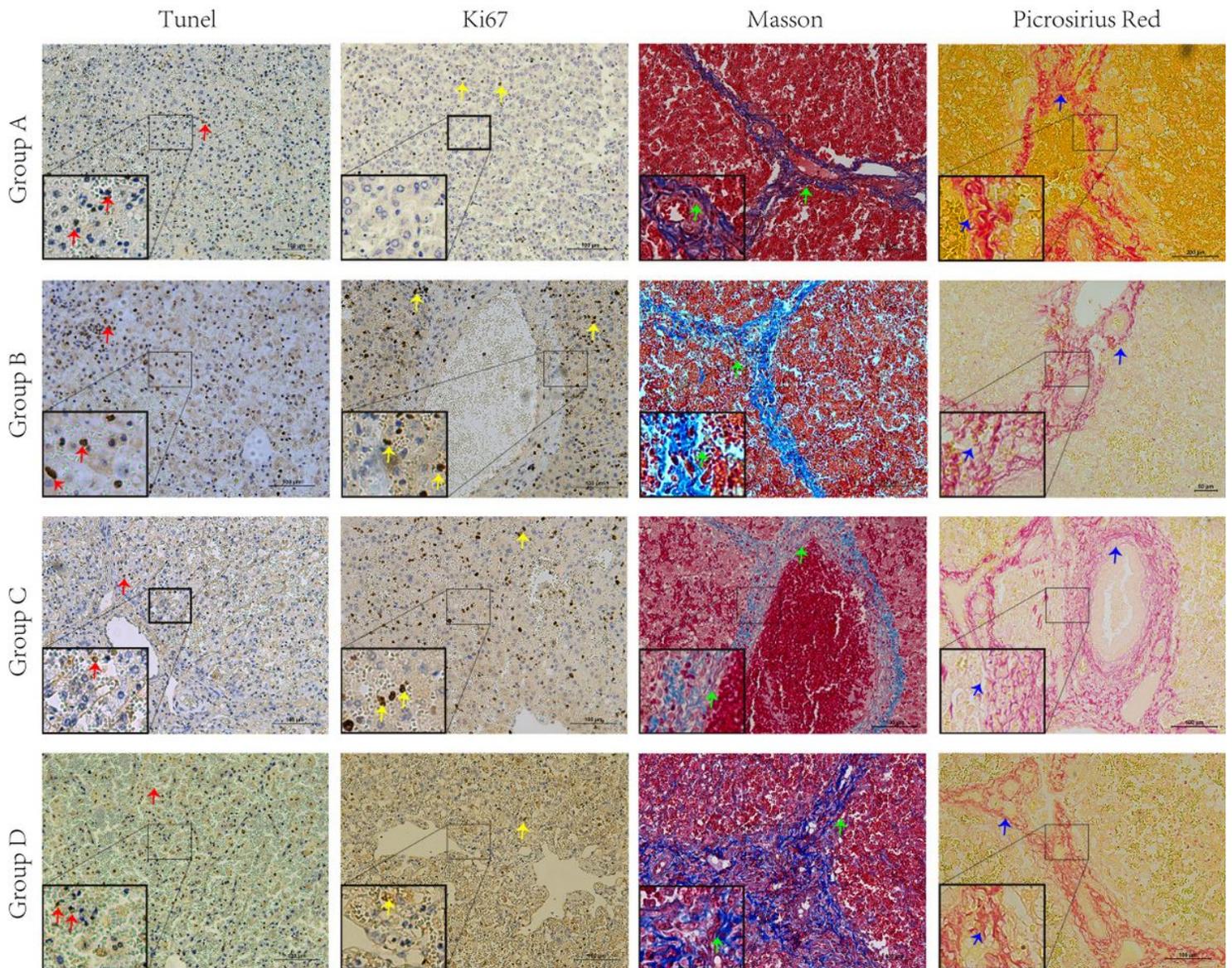


Figure 8

Tunel, Ki67, Masson assays and Picrosirius Red staining of post-mortem liver specimens from different groups. Tunel: Terminal-deoxynucleotidyl transferase mediated nick end labeling; Arrows: positive result. Lower left corner detail: enlarged scale (scale bars: 100 μ m).

Supplementary Files

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