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Direktorin: Prof. Dr. med. Julia Mayerle

Benefits of albumin administration for patients with chronic liver diseases

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Hao Lin

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Erster Gutachter:	Prof. Dr. Christian J. Steib
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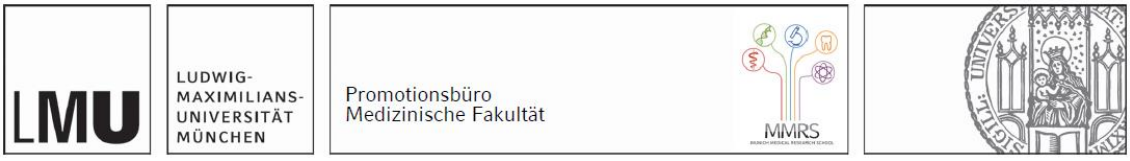

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Table of content

List of abbreviations

CLDs	chronic liver diseases;
SBP	spontaneous bacterial peritonitis;
KCs	Kupffer cells;
HSA	human serum albumin;
LPS	lipopolysaccharide;
TLR	Toll-like receptor;
ERK	extracellular signal-regulated kinase;
NF-κB	Nuclear Factor-κB;
MELD	Model for End-Stage Liver Disease;
HSCs	hepatic stellate cells;
SECs	sinusoidal endothelial cells;
PBS	phosphate-buffered saline;
PMA	phorbol myristate acetate;
LDH	lactate dehydrogenase;
CRP	C-reactive protein.

List of publications

Paper I

Lin H, Fan Y, Wieser A, Zhang J, Regel I, Nieß H, Mayerle, J, Gerbes, A.L, Steib, C.J. Albumin Might Attenuate Bacteria-Induced Damage on Kupffer Cells for Patients with Chronic Liver Disease. Cells. 2021;10(9):2298.

Paper II

Zhang J, Wieser A, **Lin H**, Fan Y, Li H, Schiergens TS, Mayerle, J, Gerbes, A.L, Steib, C.J. Pretreatment with zinc protects Kupffer cells following administration of microbial products. Biomedicine & pharmacotherapy. 2020;127:110208.

Paper III

Zhang J, Wieser A, **Lin H**, Li H, Hu M, Behrens IK, Schiergens, T.S, Gerbes, A.L, Steib, C.J. Kupffer cell activation by different microbial lysates: Toll-like receptor-2 plays pivotal role on thromboxane A(2) production in mice and humans. European journal of immunology. 2020;50(12):1988-97.

Your contribution to the publications

1.1 Contribution to paper I

The author contributed to conceiving and designing the study, performing the experiments, data collection and analysis, preparation of figures, and writing of the manuscript. Thus, the author is the sole first author for this paper.

1.2 Contribution to paper II

The author contributed to performing the experiments, data collection and analysis, revision of the manuscript. Thus, the author is the co-author for this paper.

1.3 Contribution to paper III

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2. Introduction

2.1 Background and aim

Chronic liver diseases (CLDs) are a long-term pathologic process that involves developing devastation and regeneration of livers, eventually leading to cirrhosis. The mechanism of CLD progression is difficult and unknown, however chronic inflammation and bacterial infections may play a key part in this process (1, 2). One of the most common complications of CLDs is spontaneous bacterial peritonitis (SBP) (3). Some certain bacterial strains are regarded as the most common reasons for SBP, such as *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Streptococcus pneumoniae* (*S. peneumoniae*), *Enterobacter cloacae* (*E. cloacae), Staphylococcus aureus* (*S. aureus*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Enterococcus faecium* (*E. faecium*) (4). However, few researchers have explored the impact of these prevalent bacteria species on CLD patients and human hepatic cells.

Kupffer cells (KCs) are the most numerous innate immune cells in livers, as well as resident macrophages (5). Through the interaction with pattern recognition receptors, KCs phagocyte and scavenge cell debris, senescent red blood cells, tiny particles, and complexes of proteins. KCs also remove harmful substances and pathogens from the intestine such as endotoxic lipopolysaccharide (LPS) (5-

7). KCs play a vital role in protecting the host by maintaining homeostasis as well as in triggering immunogenic and tolerogenic immune responses. However, because isolating KCs from human livers is challenging, there has been little research into their function, instead, most studies concentrated on other macrophages such as peripheral blood mononuclear cells and bone marrow-derived macrophages. Understanding the function of KCs in patients with CLDs may need thorough research of their role and mechanism.

CLD patients are typically treated with human serum albumin (HSA), an important plasma protein. The long-term benefits of albumin in individuals with cirrhosis remain controversial for years. The long-term administration of albumin to CLD patients decreased the cumulative incidence of sequelae of cirrhosis and systemic inflammation, such as SBP and non-SBP bacterial infections (8-10). The expander role of albumin, which is based on its recognized oncotic characteristics, is well-known among the reasons for albumin's advantages in cirrhosis patients. However, it is now undeniably evident that albumin's immunomodulatory activity becomes increasingly essential during liver cirrhosis treatments (11-13). HSA binds inflammatory mediators such as lipoteichoic acid, peptidoglycan, and LPS, which are Gram-negative and Gram-positive bacteria's surface components that trigger the innate immune system as well as inflammation via Toll-like receptor 2/4 (TLR 2/4) (12-15). The activation of Nuclear

Factor-B (NF-B) by albumin therapy reduced LPS-induced inflammation and elevated monocyte interleukin-6 (IL-6) gene production via NF-κB as well as the extracellular signal-regulated kinase (ERK) pathways (16, 17). Following albumin infusion, albumin can regulate innate immune responses to cirrhosis-associated prostaglandin E2-mediated immunosuppression (18). Therefore, a comprehensive investigation of albumin's role and mechanism in patients with CLDs and human livers might be beneficial in providing a better understanding of the dispute of albumin therapy in CLD patients.

In conclusion, our findings highlighted the protective role of albumin in CLD patients and KCs, implying that albumin therapies intend to benefit KCs by reducing microbial product damage apart from improving plasma osmolality, providing a compelling rationale for albumin use in CLD patients.

2.2 Albumin and infection

The therapeutic advantages of albumin in patients with CLDs have been discussed for years. Several studies have failed to establish that giving albumin to CLDs has any benefit other than postponing the development of renal failure (19, 20). Nonetheless, the vast variety of potential advantages of albumin treatment, including antioxidant function, immunomodulation, anti-inflammatory action, and the transportation of multiple endogenous and exogenous chemicals, has to be explored further (21). Some clinical trials have recently been

undertaken in order to provide more compelling evidence of the advantages of albumin supplementation in patients with CLDs. Long-term albumin supplementation to patients with CLDs and ascites increased survival time, reduced hospitalizations and complications, according to the findings (8, 10, 22). Our study comprised 138 outpatients or consecutive hospitalized patients with CLDs from July 2016 to March 2019, including chronic viral hepatitis B, primary sclerosing cholangitis, autoimmune hepatitis, chronic viral hepatitis C, liver cirrhosis, primary biliary cholangitis, steatosis hepatitis, nonalcoholic steatohepatitis, alcoholic liver disease, cryptogenic cirrhosis, cystic liver disease, Budd–Chiari Syndrome, hemochromatosis, liver adenoma, M. Wilson disease, sarcoidosis, and toxic liver disease. A variety of blood indicators (KCl, Zinc, NaCl bilirubin, GOT, Gamma-GT, INR, creatinine, and MELD Score) were examined. To evaluate whether albumin levels have influences on inflammation and infection of CLD patients, all 138 patients with CLDs were classified into two groups: normal albumin (≥3.5 g/dl) and low albumin (<3.5 g/dl) according to their albumin levels. The correlation between albumin and various clinical indicators was assessed by linear regression with the Pearson statistic. In our investigation, we discovered that blood albumin levels were shown to be negatively linked with CRP concentration in CLD individuals, indicating that CLD patients with high albumin levels had less infection. In addition, albumin levels were positively

correlated with KCl, Zinc, and NaCl levels in patients with CLDs, but bilirubin, GOT, Gamma-GT, INR, creatinine, and MELD Score were negatively correlated with albumin levels, suggesting that low albumin levels might lead to more severe liver damage in CLD patient. Albumin has the capacity to bind and deactivates considerable blood inflammatory mediators, including bioactive lipid metabolites, nitric oxide, reactive oxygen species, and pathogen-associated molecular patterns, which might explain why CLD patients with higher albumin have less inflammation.

2.3 Protective effects of albumin

2.3.1 THP-1 cells

Albumin's immunomodulatory role has lately received a lot of attention. In patients with cirrhosis, lower blood albumin binds fewer prostaglandin E2 (PGE2) molecules, resulting in raised PGE2 bioavailability and decreased the immune response of macrophages to LPS (18), suggesting that albumin may boost immunological activities in certain ways. KCs are a kind of immune cell that plays a key role in the immune response. Thus, KCs were employed in our study to investigate the relative mechanism of albumin in weakening inflammation. Despite the fact that there were three clinical studies to verify the efficacy of albumin on patients with CLDs alluded to above, one research found no favorable effects in patients with cirrhosis [36]. Intriguingly, in the three clinical

trials, a loading dosage of albumin administration was thought to be the cause of having positive effects in CLD patients [12], suggesting that the concentration of albumin administration is essential in demonstrating its benefits. Furthermore, 0.4 mg/ml albumin has been shown to promote PGE2-mediated immunosuppression [18]. Therefore, we utilized 0.4 mg/ml albumin in our *in vitro* experiments. Isolating KC from human livers is a difficult technique, and cell number was restricted. THP-1 is a human monocytic cell line obtained from an acute monocytic leukemia patient, and PMA-differentiated THP-1 cells are widely used as a model for human macrophage function and biology [36]. As a result, we employed THP-1 instead of KCs to assess the injury caused by various microbial isolates and multiple concentrations of bacterial products as well as the toxicity of albumin. The lactate dehydrogenase (LDH) in the supernatant was used to assess the cell damage. First, we applied varied amounts of bacterial products on THP-1 cells (1, 8, 16 g/ml) to assess the damage caused by microbial isolates. The cell damage produced by 8 bacterial strains linked to SBP, including Gram-negative and Gram-positive bacteria *E. coli, E. faecium, K. pneumoniae, E. cloacae, P. aeruginosa, S. peneumoniae, and S. aureus*, was then examined. Additionally, we investigated whether increasing albumin concentrations (0.01-0.4 mg/ml) had any toxicity on THP-1 macrophages. Our findings showed that bacterial product stimulation at 8 g/ml and 16 g/ml caused

substantial damage to THP-1 cells. THP-1 cells were harmed by bacterial products from all studied strains, regardless of Gram-negative or Gram-positive bacteria, but with a 0.01-0.4 mg/ml concentration range, albumin had no toxic effects on THP-1 cells as concentrations increased. Therefore, 8 g/ml bacterial products and 0.4 mg/ml albumin were selected in the subsequent experiments in term of albumin treatment. We stimulated THP-1 cells with 8 g/ml bacterial products of four tested bacteria, including *E. cloacae* and *E. coli TOP10* (Gram-negative bacteria strains) and *S. aureus* and *S. peneumoniae* (Gram-positive bacteria strains), in combination with 0.4 mg/ml albumin, to determine whether albumin protects macrophages from bacterial injury. THP-1 cells were either pre-treated with albumin (24 hours before microbial stimulation) or peri-treated with albumin (at the same time with microbial stimulation). After 24 hours of microbial isolate stimulation, the supernatant was collected for LDH measurement. Our findings revealed that both pretreatment and peritreatment of albumin protected THP-1 from microbial isolate damage.

2.3.2 Primary hepatic non-parenchymal cells

We isolated primary hepatic non-parenchymal cells (KCs, hepatic stellate cells (HSCs), and sinusoidal endothelial cells (SECs)) from normal liver tissues from patients with liver metastasis to determine whether albumin has the same effects on these cells. Primary KCs, HSCs, and SECs were treated with albumin and

stimulated with bacterial products as we did in THP-1 cells. In both pretreatment and peritreatment conditions, albumin remarkably decreased cell damage induced by bacterial isolates in KCs, showing identical results in THP-1 cells. We presumed that the protective effects of albumin in THP-1 and KCs might be attributed to albumin's capacity to bind inflammatory components, as described previously. Another possibility is that albumin strengthens KC phagocytosis and increases albumin levels inside cells, resulting in an additional synergistic promotion of KC protection. Unexpectedly, bacterial products greatly increased cell damage in HSCs, while albumin treatment did not show protective effects to counteract cell damage. The polymerized albumin receptor has been discovered on macrophages [37], and its involvement in albumin endocytosis has also been characterized [38]. Stellate cells can also actively absorb albumin from external sources [39]. Nonetheless, it is prominent that macrophages have greater phagocytosis than stellate cells, which might be a cause of this surprising outcome. Furthermore, neither bacterial product stimulation nor albumin therapy had any considerable effects on cell injury in SECs. In brief, we hypothesized that people with CLDs and normal albumin may inactivate inflammatory stimuli and bacterial products, which permits KCs to function correctly and aids the immune system's reaction to invading germs. Taken together with clinical data above, we conjectured that CLD patients with normal albumin might experience

elevated CRP and more severe inflammation for a short period (acute inflammatory reaction) due to a healthy immune system's response, however eventually get back to normal thanks to the effective immune responses maintained by albumin's protective effects on KCs, which helps to explain the link between our CRP results in CLD patients and the cell damage data in *in vitro* experiments. Consequently, we speculated that albumin's protective effects on KCs could offer some advantages in terms of reducing inflammation in CLD patients.

2.4 Relevant mechanism

TLRs, which are key molecules involved in bacteria-induced inflammation in macrophages, are activated by bacteria or bacterial metabolites. TLR also regulated the ERK and NF-κB signaling pathways, both of which are important for a healthy immunological response [29,30]. ERK and NF-κB pathways are the most important pathways participating in the TLR/MyD88/Interleukin 1 Receptor Associated Kinase 4 axis that reacts to Gram-positive and Gram-negative bacteria in livers (21-23). In *In vitro* experiments, albumin treatment activates ERK and NF-B pathways, causing a dose-dependent increase in inflammatory cytokines (acute inflammation responses) [40-43]. Throughout the MAPK-ERK and NF-B pathways, metabolic waste products of bovine serum albumin increased monocyte IL-6 gene expression (22). Besides, Zinc protects KCs

against cell damage produced by bacterial isolates through ERK and NF-κB pathways [28]. Thus, we reasoned that because Zinc and albumin have comparable protective properties, there could be an interactive mechanism between them. We postulated that ERK pathway and NF-κB pathway are involved in albumin's protective effects on liver macrophages. As a result, we performed immunofluorescence staining of p-ERK and p-NF-κB in KCs with *E. coli*, and *E. coli* plus albumin treatment to identify the activation of both pathways. Relative fluorescence intensity was utilized to evaluate the activation of both pathways. Our results showed that *E. coli* stimulation enhanced the activation of ERK and NF-κB pathways in KCs. Moreover, when compared to *E. coli* stimulation, albumin had an amplifying influence on the initiation of the ERK and NF-κB pathways triggered by bacterial products, revealing that potential pathways for albumin's protective benefits might be ERK and NF-κB pathways, and assisting in the comprehension of albumin's therapeutic advantages in patients with CLDs.

3. Summary

Chronic liver diseases (CLDs) are complicated illnesses that cause long-term inflammation and infection, hastening their progression. For years, there has been a debate on whether albumin should be applied in patients with CLDs. HSA (human serum albumin) plays a prominent part in immunomodulation throughout the CLD process. This study aimed to illustrate the immune-related advantages of albumin treatment in CLD patients.

To determine the effects of albumin levels in CLD patients, we included 138 outpatients or consecutive hospitalized patients with CLDs from July 2016 to March 2019 into our study cohort. Multiple blood indicators were evaluated, including KCl, Zinc, NaCl, bilirubin, GOT, Gamma-GT, INR, creatinine, and MELD Score. All 138 patients with CLDs were divided into two groups based on their albumin levels: normal albumin (≥3.5 g/dl) and low albumin (<3.5 g/dl). The association between albumin and various clinical indicators was assessed by linear regression using the Pearson statistic. In our research, we observed that blood albumin levels in CLD patients were negatively correlated with CRP levels, implying that CLD patients with higher albumin levels had less infection. Additionally, albumin levels were positively linked with KCl, Zinc, and NaCl levels in patients with CLDs, but were negatively correlated with bilirubin, GOT, Gamma-GT, INR, creatinine, and MELD Score, indicating that low albumin levels

may contribute to more severe liver damage in CLD patients. Then, we performed *in vitro* experiments to investigate further the effects of albumin in human livers. First, we utilized varied quantities of bacterial products (1, 8, 16 g/ml) in THP-1 cells to examine different levels of cell damage. The cell damage caused by eight bacterial strains associated with SBP, including *E. coli, E. faecium, K. pneumoniae, E. cloacae, P. aeruginosa, S. peneumoniae, and S. aureus*, was then examined. We also explored whether increasing albumin concentrations (0.01-0.4 mg/ml) had any toxicity on THP-1. The cell damage was measured using lactate dehydrogenase (LDH) in the supernatant. According to our findings, THP-1 cells were enormously damaged by bacterial product stimulation at 8 g/ml and 16 g/ml and by bacterial products from all strains examined, irrespective of Gram-negative or Gram-positive bacteria, while albumin had no harmful effects on THP-1 cells when its concentrations were in the range of 0.01-0.4 mg/ml. To determine whether albumin protects macrophages from bacterial damage, we stimulated THP-1 cells and primary hepatic non-parenchymal cells (Kupffer cells (KCs), hepatic stellate cells (HSCs), and sinusoidal endothelial cells (SECs)) with 8 g/ml bacterial products from four different bacteria, including *E. cloacae* and *E. coli TOP10* (Gram-negative bacteria species) and *S. aureus* and *S. peneumoniae* (Gram-positive bacteria species), in combination with 0.4 mg/ml albumin. Albumin was either pre-treated (24 hours before microbial stimulation) or peri-

treated (at the same time as microbial stimulation) in THP-1 cells. After 24 hours of stimulation of microbial products, the supernatant was harvested for LDH measurement. We found that THP-1 and KCs were protected from the injury caused by microbial isolate under both pretreatment and peritreatment with albumin. However, in HSCs, bacterial products notably increased cell damage, but albumin therapy did not present its protective benefits to neutralize cell damage. Furthermore, neither bacterial product stimulation nor albumin treatment to SECs caused any change of cell damage. To illuminate the potential mechanism of the protective effects of albumin, we applied immunofluorescence labeling of p-ERK and p-NF-κB in KCs with *E. coli* and *E. coli* plus albumin treatment. We observed that the ERK and NF-B pathways were activated when *E. coli* was stimulated in KCs. In addition, when compared to *E. coli* stimulation, albumin had an amplifying effect on the ERK and NF-κB signaling pathways triggered by bacterial products, indicating that the ERK and NF-κB signaling pathways may be underlying mechanisms for albumin's protective benefits.

In conclusion, our findings indicated that albumin administration may assist patients with CLDs in overcoming cell damage induced by bacterial infection, which might be attributed to the protective effects of albumin in KCs, indicating immunomodulatory advantages of albumin administration on patients with CLDs.

As a result, we speculated that albumin supplementation may help patients with CLDs reduce bacterial infection.

4. Zusammenfassung

Chronische Lebererkrankungen (CLD) sind mit Entzündungen und Infektionen assoziiert, die wiederum ein Fortschreiten der CLD beschleunigen. Seit Jahren wird darüber diskutiert, ob Albumin bei Patienten mit CLD prophylaktisch eingesetzt werden sollte. HSA (humanes Serumalbumin) scheint eine wichtige Rolle bei der Immunmodulation zu spielen. Ziel dieser Studie war es, die immunologischen Vorteile einer Albuminbehandlung bei CLD-Patienten zu untersuchen.

Um die Auswirkungen des Albuminspiegels bei CLD-Patienten zu bestimmen, haben wir 138 ambulante oder hospitalisierte Patienten mit CLD von Juli 2016 bis März 2019 in unsere Studienkohorte aufgenommen. Mehrere Blutwerte wurden ausgewertet, darunter Zink, Bilirubin, GOT, GPT, Gamma-GT, INR, Kreatinin und berechnet daraus auch der MELD-Score. Alle 138 CLD-Patienten wurden anhand ihres Albuminspiegels in zwei Gruppen eingeteilt: normales Albumin (≥3,5 g/dl) und niedriges Albumin (<3,5 g/dl). Albumin und weitere Blutwerte wurden durch lineare Regression unter Verwendung der Pearson-Statistik ausgewertet. Bei unseren Untersuchungen stellten wir fest, dass die Albuminwerte im Blut von CLD-Patienten negativ mit den CRP-Werten korrelierten, was bedeuten könnte, dass CLD-Patienten mit höheren Albuminwerten weniger Infektionen aufweisen. Darüber hinaus war der

Albuminspiegel bei CLD-Patienten positiv mit dem KCl-, Zink- und NaCl-Spiegel korreliert, aber negativ mit Bilirubin, GOT, Gamma-GT, INR, Kreatinin und MELD-Score, was darauf hindeutet, dass niedrige Albuminspiegel zu schwereren Leberschäden bei CLD-Patienten beitragen könnten. Anschließend führten wir *in-vitro*-Experimente durch, um die Albumin-Effekte in menschlichen Lebern weiter aufzuarbeiten. Zunächst verwendeten wir unterschiedliche Dosierungen für bakterielle Produkte (1, 8, 16 g/ml) bei der Inkubation der THP-1-Zellen. Hiermit wurden verschiedene Stufen der Zellschädigung untersucht. Anschließend wurde die Zellschädigung durch 8 verschiedene, mit einer SBP assoziierten, Bakterienstämme untersucht, darunter *E. coli*, E. faecium, K. pneumoniae, E. cloacae, P. aeruginosa, S. peneumoniae und S. aureus. Wir untersuchten auch, ob steigende Albumin-Konzentrationen (0,01-0,4 mg/ml) eine Toxizität für THP-1 bedeuten. Die Zellschädigung wurde anhand der Laktatdehydrogenase (LDH) im Überstand gemessen. Unseren Ergebnissen zufolge wurden THP-1-Zellen durch die Stimulierung mit bakteriellen Produkten bei 8 g/ml und 16 g/ml sowie durch bakterielle Produkte aller untersuchten Stämme stark geschädigt, unabhängig davon, ob es sich um Gram-negative oder Gram-positive Bakterien handelte, während Albumin bei Konzentrationen im Bereich von 0,01-0,4 mg/ml relativ wenig Auswirkungen auf THP-1-Zellen hatte. Um festzustellen, ob Albumin Makrophagen vor bakteriellen Schäden schützt,

stimulierten wir THP-1-Zellen und primäre hepatische nicht-parenchymale Zellen (KCs, HSCs und SECs) mit verscheidenen bakteriellen Produkten in einer Dosierung von 8 g/ml (E. cloacae und *E. coli* TOP10 (Gram-negative Bakterienarten) und S. aureus und S. peneumoniae (Gram-positive Bakterienarten)), in Kombination mit 0,4 mg/ml Albumin. Albumin wurde entweder im Sinne einer Vorbehandlung eingesetzt (24 Stunden vor Stimulation) oder gleichzeitig mit der mikrobiellen Stimulation der THP-1-Zellen. Nach 24 Stunden Stimulation mit mikrobiellen Produkten wurde Überstand für die LDH-Messung gewonnen. Es zeigte sich, dass THP-1- und KC-Zellen sowohl bei der Vorbehandlung als auch bei der Behandlung mit Albumin vor der durch das mikrobielle Isolat verursachten Schädigung geschützt waren. Bei den HSC hingegen erhöhten die bakteriellen Produkte die Zellschädigung deutlich, aber die Albumintherapie zeigte keine protektive Wirkung. Darüber hinaus bewirkte weder die Stimulierung durch bakterielle Produkte noch die Behandlung von SECs mit Albumin eine Veränderung der Zellschädigung. Um den potenziellen Mechanismus der schützenden Wirkung von Albumin zu beleuchten, haben wir die Immunfluoreszenzmarkierung von p-ERK und p-NF-κB in KCs mit *E. coli* und *E. coli* plus Albuminbehandlung durchgeführt. Wir stellten fest, dass die Stimulation mit *E. coli* die Aktivierung der ERK- und NF-κB-Signalwege in Kupfferzellen erhöhte. Darüber hinaus hatte Albumin im Vergleich zur *E. coli*-

Stimulation einen verstärkenden Effekt auf die Aktivierung der ERK- und NF-κB-Signalwege, die durch bakterielle Produkte ausgelöst werden, was darauf hindeutet, dass die ERK- und NF-κB-Signalwege möglicherweise die zugrundeliegenden Mechanismen für die schützenden Wirkungen von Albumin sind.

Unsere Ergebnisse deuten darauf hin, dass die Verabreichung von Albumin Patienten mit CLD bei der Überwindung von Zellschäden helfen kann, die durch eine bakterielle Infektion ausgelöst werden, was auf die schützende Wirkung von Albumin in Kupfferzellen zurückzuführen sein könnte. Infolgedessen spekulierten wir, dass eine Albumin-Supplementierung Patienten mit CLD helfen könnte, bakterielle Infektionen zu reduzieren.

References

1. Acharya C, Dharel N, Sterling RK. Chronic liver disease in the human immunodeficiency virus patient. Clinics in liver disease. 2015;19(1):1-22.

2. Fernández J, Acevedo J, Wiest R, Gustot T, Amoros A, Deulofeu C, et al. Bacterial and fungal infections in acute-on-chronic liver failure: prevalence, characteristics and impact on prognosis. Gut. 2018;67(10):1870-80.

3. Bernsmeier C, Triantafyllou E, Brenig R, Lebosse FJ, Singanayagam A, Patel VC, et al. CD14(+) CD15(-) HLA-DR(-) myeloid-derived suppressor cells impair antimicrobial responses in patients with acute-on-chronic liver failure. Gut. 2018;67(6):1155-67.

4. Gravito-Soares M, Gravito-Soares E, Lopes S, Ribeiro G, Figueiredo P. Spontaneous fungal peritonitis: a rare but severe complication of liver cirrhosis. European journal of gastroenterology & hepatology. 2017;29(9):1010-6.

5. Vollmar B, Menger MD. The hepatic microcirculation: mechanistic contributions and therapeutic targets in liver injury and repair. Physiological reviews. 2009;89(4):1269-339.

6. Bilzer M, Roggel F, Gerbes AL. Role of Kupffer cells in host defense and liver disease. Liver international : official journal of the International Association for the Study of the Liver. 2006;26(10):1175-86.

7. Kolios G, Valatas V, Kouroumalis E. Role of Kupffer cells in the pathogenesis of liver disease. World journal of gastroenterology. 2006;12(46):7413-20.

8. Caraceni P, Riggio O, Angeli P, Alessandria C, Neri S, Foschi FG, et al. Long-term albumin administration in decompensated cirrhosis (ANSWER): an

open-label randomised trial. Lancet (London, England). 2018;391(10138):2417-29.

9. Sort P, Navasa M, Arroyo V, Aldeguer X, Planas R, Ruiz-del-Arbol L, et al. Effect of intravenous albumin on renal impairment and mortality in patients with cirrhosis and spontaneous bacterial peritonitis. The New England journal of medicine. 1999;341(6):403-9.

10. Fernandez J, Claria J, Amoros A, Aguilar F, Castro M, Casulleras M, et al. Effects of Albumin Treatment on Systemic and Portal Hemodynamics and Systemic Inflammation in Patients With Decompensated Cirrhosis. Gastroenterology. 2019;157(1):149-62.

11. Spinella R, Sawhney R, Jalan R. Albumin in chronic liver disease: structure, functions and therapeutic implications. Hepatology international. 2016;10(1):124-32.

12. Bernardi M, Angeli P, Claria J, Moreau R, Gines P, Jalan R, et al. Albumin in decompensated cirrhosis: new concepts and perspectives. Gut. 2020;69(6):1127-38.

13. Arroyo V, García-Martinez R, Salvatella X. Human serum albumin, systemic inflammation, and cirrhosis. Journal of hepatology. 2014;61(2):396-407.

14. Jürgens G, Müller M, Garidel P, Koch MH, Nakakubo H, Blume A, et al. Investigation into the interaction of recombinant human serum albumin with Re-lipopolysaccharide and lipid A. Journal of endotoxin research. 2002;8(2):115-26.

15. Fukui H. Relation of endotoxin, endotoxin binding proteins and macrophages to severe alcoholic liver injury and multiple organ failure. Alcoholism, clinical and experimental research. 2005;29(11 Suppl):172s-9s.

16. Wheeler DS, Giuliano JS, Jr., Lahni PM, Denenberg A, Wong HR, Zingarelli B. The immunomodulatory effects of albumin in vitro and in vivo. Adv Pharmacol Sci. 2011;2011:691928.

17. Shen CY, Wu CH, Lu CH, Kuo YM, Li KJ, Hsieh SC, et al. Advanced Glycation End Products of Bovine Serum Albumin Suppressed Th1/Th2 Cytokine but Enhanced Monocyte IL-6 Gene Expression via MAPK-ERK and MyD88 Transduced NF-κB p50 Signaling Pathways. Molecules (Basel, Switzerland). 2019;24(13).

18. O'Brien AJ, Fullerton JN, Massey KA, Auld G, Sewell G, James S, et al. Immunosuppression in acutely decompensated cirrhosis is mediated by prostaglandin E2. Nature medicine. 2014;20(5):518-23.

19. Thévenot T, Bureau C, Oberti F, Anty R, Louvet A, Plessier A, et al. Effect of albumin in cirrhotic patients with infection other than spontaneous bacterial peritonitis. A randomized trial. Journal of hepatology. 2015;62(4):822-30.

20. Pulimood TB, Park GR. Debate: Albumin administration should be avoided in the critically ill. Critical care (London, England). 2000;4(3):151-5.

21. Merli M, Lucidi C, Lattanzi B, Riggio O. Albumin infusion in cirrhotic patients with infections other than spontaneous bacterial peritonitis: End of the story? Journal of hepatology. 2015;63(3):767-8.

22. Di Pascoli M, Fasolato S, Piano S, Bolognesi M, Angeli P. Long-term administration of human albumin improves survival in patients with cirrhosis and refractory ascites. Liver international : official journal of the International Association for the Study of the Liver. 2019;39(1):98-105.

23. Tsuchiya S, Yamabe M, Yamaguchi Y, Kobayashi Y, Konno T, Tada K. Establishment and characterization of a human acute monocytic leukemia cell line (THP-1). International journal of cancer. 1980;26(2):171-6.

24. Thung SN, Gerber MA. Presence of receptors for polymerized albumin in HBsAg-containing hepatocytes and hepatoma cell line. Hepatology (Baltimore, Md). 1981;1(2):132-6.

25. Yoshioka T, Yamamoto K, Kobashi H, Tomita M, Tsuji T. Receptor-mediated endocytosis of chemically modified albumins by sinusoidal endothelial cells and Kupffer cells in rat and human liver. Liver. 1994;14(3):129-37.

26. Beljaars L, Olinga P, Molema G, de Bleser P, Geerts A, Groothuis GM, et al. Characteristics of the hepatic stellate cell-selective carrier mannose 6-phosphate modified albumin (M6P(28)-HSA). Liver. 2001;21(5):320-8.

27. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nature immunology. 2010;11(5):373-84.

28. Arthur JS, Ley SC. Mitogen-activated protein kinases in innate immunity. Nature reviews Immunology. 2013;13(9):679-92.

29. Cohen P. The TLR and IL-1 signalling network at a glance. Journal of cell science. 2014;127(Pt 11):2383-90.

30. Drumm K, Gassner B, Silbernagl S, Gekle M. Albumin in the mg/l-range activates NF-kappaB in renal proximal tubule-derived cell lines via tyrosine kinases and protein kinase C. European journal of medical research. 2001;6(6):247-58.

31. Tang S, Leung JC, Abe K, Chan KW, Chan LY, Chan TM, et al. Albumin stimulates interleukin-8 expression in proximal tubular epithelial cells in vitro and in vivo. The Journal of clinical investigation. 2003;111(4):515-27.

32. Poteser M, Wakabayashi I. Serum albumin induces iNOS expression and NO production in RAW 267.4 macrophages. British journal of pharmacology. 2004;143(1):143-51.

33. Ralay Ranaivo H, Patel F, Wainwright MS. Albumin activates the canonical TGF receptor-smad signaling pathway but this is not required for activation of astrocytes. Experimental neurology. 2010;226(2):310-9.

34. Zhang J, Wieser A, Lin H, Fan Y, Li H, Schiergens TS, et al. Pretreatment with zinc protects Kupffer cells following administration of microbial products. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2020;127:110208.

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