International Immunopharmacology 54 (2018) 46–51

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Dexmedetomidine promotes liver regeneration in mice after 70% partial hepatectomy by suppressing NLRP3 inflammasome not TLR4/NFκB

Meihong Lv[a], Huizhi Zeng[b], Yi He[c], Jiashu Zhang[d], Guang Tan[e,*]

[a] Department of Anesthesiology, First Affiliated Hospital of Dalian Medical University, Dalian, Liaoning, China
[b] Department of General Surgery, Second Affiliated Hospital of Dalian Medical University, Dalian, Liaoning, China
[c] Department of Urology, Second Affiliated Hospital of Dalian Medical University, Dalian, Liaoning, China
[d] College of pharmacy, Dalian Medical University, Dalian, Liaoning, China
[e] Department of General Surgery, First Affiliated Hospital of Dalian Medical University, Dalian, Liaoning, China

ARTICLE INFO

Keywords:
Dexmedetomidine
Liver regeneration
TLR4/NFκB
NLRP3 inflammasome

ABSTRACT

Inflammasome activation is mediated by NOD-like receptors (NLRs) that play important role in cellular proliferation. NLRP3 senses the widest array of stimuli. But its role in the liver regeneration after partial hepatectomy (PHx) is still unknown. Dexmedetomidine (Dex) has been documented to protect the liver against ischemia-reperfusion injury via the suppression of the TLR4/NF-κB pathway, which is important for NLRP3 inflammasome activation and liver regeneration. We tested whether Dex contributes to liver regeneration, and investigated its consequent effect on inflammasome activation. In vitro, L02 human liver cells were treated with Dex at different concentrations. The 70% PHx was performed in C57 BL/6 mice as PHx group, and sham-operated animals as Sham group, Dex-treated animals were assigned into two groups: Dex + PHx, which received single intraperitoneal injections of Dex (25 μg/kg) before PHx 30 mins; Dex + PHx + Dex, which received additional Dex (25 μg/kg) after PHx for 3 days. Dex significantly inhibited the proliferation of Lo2 cells in vitro and decreased the expression of TLR4/NFκB. In vivo, Dex + PHx exhibited promoted effect on liver regeneration and liver function recovery via inhibiting NLRP3 inflammasome activation. Dex + PH + Dex inhibited the liver regeneration, which may be associated with suppressed expression levels of TLR4/NFκB pathway. Though Dex pretreatment contributed to liver regeneration and function recovery via inflammation suppression, excessive inflammation suppression accompanied with TLR4 suppression could be related to the diminished liver regeneration, suggesting that TLR4/NFκB played important role in liver regeneration and Dex + PHx might be a useful therapeutic strategy to promote liver regeneration in clinical.

1. Introduction

The liver is an organ that has a known regeneration capability. Developments in surgical techniques have led to rapid advances in liver surgery. Nowadays, the most important limiting factor in liver surgery is liver failure after major liver resections. The remaining liver tissue after liver resection is capable of regeneration. This process is a complex condition in which many different mechanisms play a role [1]. Many regulatory pathways are involved in liver regeneration after partial hepatectomy (PHx), to initiate growth, protect liver cells and sustain remnant liver functions [2]. There are many studies related to exposing liver regeneration steps but this topic still remains up to date. Identifying mechanisms that initiate liver regeneration will lead to the development of new therapeutic strategies to enhance recovery from liver disease.

As reported, inflammation played critical role in liver regeneration, particularly important for the early priming phase of regeneration [3,4], depending on many cytokines production, whose importance were underscored by findings that liver regeneration is impaired in their absence, like IL-6 [5], TNF-receptor-1 [6,7] and NF-kB signaling [8,9]. However, the regulatory mechanism which cytokines depend on is still unknown. TLR-4/NF-κB signaling is a classic pathway, which has been reported to regulate inflammation response through controlling pro-inflammatory cytokines releases. As reported [10,11], TLR-4/NF-κB is also involved in the regulation of regenerative and apoptotic genes and plays a key role in liver regeneration. In this study, to investigate the molecular mechanism by which the inflammatory response was dependent on, TLR-4/NF-κB signaling pathway was investigated.

Inflammasomes are a group of large caspase-1-activating protein complexes in response to the evoke of innate immunity and production

* Corresponding author at: 222, Zhongshan Road, Xigang District, Dalian 116011, China.
E-mail address: tanguang_gd@163.com (G. Tan).

http://dx.doi.org/10.1016/j.intimp.2017.10.030
Received 8 July 2017; Received in revised form 30 September 2017; Accepted 25 October 2017

of pro-inflammatory cytokines [12]. Damage-associated molecular patterns (DAMP) and pathogen-associated molecular patterns (PAMP) bind to pattern-recognition receptors inducing an intracellular signaling cascade [13]. Inflammasomes, particularly NLRP3 inflammasome, are shown to be activated in a variety of liver diseases, including drug-induced liver injury [14], ischemia–reperfusion injury [15], fibrosis [16], and non-alcoholic fatty liver disease [17] However, to date, little is known about the role of NLRP3 inflammasome in the liver regeneration.

Dexmedetomidine (Dex) is a highly selective agonist of α2-adrenergic receptors, which is widely used in clinical anesthesia [18]. Beneficial effects of Dex include effective sedation, analgesia, hemodynamic stabilization, as well as anti-inflammatory and diuretic effects without respiratory depression and drug-dependency issues [19]. In addition, Dex has been shown to ameliorate liver ischemia-reperfusion injury [20]. Several studies have demonstrated that dexmedetomidine may inhibit inflammatory mediator levels including IL-1, IL-6 and TNF-α [21–23]. However, few studies have been conducted on the mechanism of its anti-inflammatory effects. Therefore, this study aims to observe the hepatic protective effects of Dex after PH, to explore its relevant mechanisms.

In this study, we further investigate the role of inflammation response during the liver regeneration after partial hepatectomy, and determine whether Dex contributes to liver regeneration, and also investigated the consequent effect on inflammasome activation in liver cells.

2. Material and method

2.1. Cell culture

Human hepatocytes L02 cells were purchased from the Cell Bank of Chinese Academy of Sciences (Shanghai, China) and was cultured in Dulbecco's modified eagle medium (DMEM; Invitrogen, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; Wisent Biotechnology Co., Ltd., Nanjing, China), 100 U/ml penicillin and 100 mg/ml streptomycin, and grown in a 95% air and 5% CO_2 humidified atmosphere at 37 °C.

2.2. Proliferation assay

Cell viability was determined using an MTT assay. L02 cells were seeded in 96-well microplates, 4×10^3 cells/well for one day and then treated with 200 μl of DMEM medium containing with various concentrations of Dex (Meilun Biotechnology Co., Ltd. Dalian, China). After incubation for 24 h, 20 μl (5 mg/ml) of MTT solution was added per well and further incubated for 4 h. The medium was removed, and formazan was solubilized by adding 150 μl/well of DMSO. Relative cell viability was obtained on a microplate reader with a 570 nm filter.

2.3. Animals

Male C57 BL/6 mice (8 weeks, 20–25 g) were purchased from the Experimental Animal Center of Dalian Medical University (Dalian, China). Mice were treated in accordance with the Guide for the Care and Use of Laboratory Animals of the National Academy of Sciences (NIH publication No. 85–23, revised 1996). Animals were housed under standard conditions with 12 h light/12 h dark cycle, and were given free access to food and water, and were monitored every 12 h during the experimental procedure. All animal experiments are approved by the ethics committee of Dalian Medical University and performed in accordance with the institutional guidelines. There was no animals died prior to the experimental endpoint.

2.4. 70% partial hepatectomy (PHx)

70% partial hepatectomy (PHx) was performed [24] to study their immune responses. The animals were anesthetized with chloral hydrate injections. A midline incision was created under microscopic guidance, and the middle and left hepatic lobes of the liver were fully freed, vascular sutures were used to ligate the branches of the hepatic artery and portal vein of the median and left lateral lobes of the liver. Then, the bile duct was ligated with vascular sutures, and the gallbladder was removed. Finally, the median and left lateral lobes of the liver were resected after a silk suture ligation was secured around the base of each lobe.

2.5. Grouping and drug treatment

Sham group: mice subjected to abdomen dissection and isolation of the hepatic peripheral vessels;

Model group: mice underwent the PHx procedure as described above, and no drug was utilized;

Dex + PHx group: mice received 25 μg/kg Dex, via intraperitoneal injection 30 min before the operation;

Dex + PHx + Dex group: mice received 25 μg/kg Dex via intraperitoneal injection, at 30 min before and 24 h, 48 h, 72 h after operation;

In the current study, the dose selected for Dex was on the basis of the previous studies [25] and our preliminary experiments.

2.6. Liver weight/body weight ratio

The animals recovered for 72 h then were euthanized. And body weights of all mice in each group were measured, blood plasma was taken and the remaining liver tissue was removed, weighed, and frozen or fixed in 10% buffered formalin and embedded in paraffin. The liver weight/body weight ratio was then calculated to observe the liver regeneration conditions.

2.7. Examination of liver injury

To assess the damage to the hepatic parenchyma, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured using detection kits according to the manufacturer's instructions (Nanjing Jiancheng Institute of Biotechnology, Nanjing, China) The paraffin embedded liver tissue sections were then stained with hematoxylin and eosin (HE), and examined for morphological changes.

2.8. Western blot analysis

In vitro study, the L02cells (5×10^5) were seeded in a 6 cm dish, grown until 80% confluent, and then incubated with Dex for 24 h. Cells were collected and lysed with RIPA buffer. In vivo study, the mice were euthanized at 12 h and 72 h after hepatectomy, and liver samples were obtained for Western blot analyses. According to the manufacturer's instructions, proteins were extracted from mice livers with protein extraction kit (KeyGen Biotech, Nanjing, China).

Protein was measured according to the procedure of bicinchoninic acid (BCA) (Solarbio, Beijing, China), with bovine serum albumin as the standard. Proteins (20 μg) were resuspended in electrophoresis sample buffer containing β-mercaptoethanol and separated by electrophoresis on a pre-cast 10% SDS-polyacrylamide gel (Bio-Rad, Hercules, CA), followed by electrotransfer to a PVDF membrane (Millipore, Bedford, MA). Membranes were blocked using 5% non-fat milk in Tris-buffered saline with 0.1% Tween-20 (TBST) for 2 h at 37 °C. β-Actin served as loading control. Membranes were incubated overnight at 4 °C with a 1:1000 dilution of polyclonal antibody for TLR4(WL00196), NFκB (WL01980), NLRP3(WL02635), cleaved caspase-1 p20 (WL02996a) and