Biochemical and Biophysical Research Communications xxx (2018) 1–8

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Dynamic changes of proteasome and protective effect of bortezomib, a proteasome inhibitor, in mice with acute pancreatitis

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ARTICLE INFO

Article history:
Received 31 August 2018
Accepted 11 September 2018
Available online xxx

Keywords:
Acute pancreatitis
Proteasome
NF-κB
Bortezomib

ABSTRACTS

The proteasome is involved in the activation of NF-κB and can regulate the progression of inflammatory diseases. However, the role of proteasome in acute pancreatitis (AP) has not been demonstrated. In this study, we first observed that the protein level and activity of proteasome 20S were increased significantly in pancreatic injury tissues after caerulein-induced mild acute pancreatitis (MAP) induction, which was in consistent with the expression of the NF-κB nucleoprotein and positively correlated with the severity of AP. Then, bortezomib, a classical proteasome inhibitor, was used to intervene the progression of MAP in mice. The results showed that bortezomib administration reduced the serum amylase and lipase levels and mitigated histopathological manifestation of pancreatic injury in mice. Meanwhile, bortezomib decreased the expression of NF-κB p65 nucleoprotein as well as total proteasome 20S protein, and inhibited the activity of 20S in pancreatic tissues. In addition, we found that bortezomib could protect pancreatic acinar cell against necrosis and mitigate the severity of AP in a severe acute pancreatitis model induced by sodium taurocholate hydrate. Taken together, our study for the first time confirmed that the proteasome participated in the pathogenesis of AP and its inhibitor bortezomib could protect against AP in mice.

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1. Introduction

Acute pancreatitis (AP) is one of the most common gastrointestinal tract diseases in the world [1]. Now it has been the second most common cause of hospitalization and the fifth leading cause of death in hospital in the USA [2]. As an acute non-infectious inflammatory disease, AP involves the characteristics of acute onset, high mortality, recurrent attacks and so on. Most cases of AP are mild disease course with a recovery within 5–7 days by conservative treatment. However, there are about 15%–20% of patients developing severe AP (SAP) with approximately mortality up to 30% [3–5].

Nuclear factor-kB (NF-κB) is a protein complex that plays a vital role in DNA transcription, cytokine production and cell survival. In most animal quiescent cells, NF-κB is sequestered in an inactive form in the cytoplasm through a combination with the inhibitory protein IkBα [6–8]. The degradation of IkBα allows NF-κB p65 to translocate into the nucleus to regulate the host inflammatory, immune, and anti-apoptotic responses [9,10]. Although the pathogenesis of AP has not been completely understood, it is widely accepted that the activation of NF-κB may modulate the pathogenesis of AP. Many studies reported that NF-κB regulated the development of AP and played a pivotal role in the inflammatory response cascades at the early stage of AP [11–13].

The ubiquitin-proteasome pathway is the principle pathway for the degradation of IkBα. When stimulated by bacteria or virus,

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https://doi.org/10.1016/j.bbrc.2018.09.066
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Please cite this article in press as: Q. Zhu, et al., Dynamic changes of proteasome and protective effect of bortezomib, a proteasome inhibitor, in mice with acute pancreatitis, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.09.066

stress and cytokines, etc. IkBα is phosphorylated, ubiquitylated, and subsequently degraded by the proteasome 26S [14,15]. As we known, the proteasome is generally called proteasome 26S because of its sedimentation coefficient, which is composed of 11s and19s regulatory particles and 20S core particles [16]. The ubiquitin-proteasome pathway abnormalities may mediate several diseases such as genetic diseases, inflammatory diseases, autoimmune diseases, tumors and so on [17–19]. Bortezomib, a borate dipeptide, which is highly selective and reversible on proteasome 26S and exerts its effect by binding to the 20S core particles. The effect has been applied for clinical trials for the treatment of multiple myeloma [20,21]. However, whether the proteasome involved in the pathophysiology of AP is unknown yet. In this current article, we observed the dynamic changes of proteasome 20S in pancreatic tissues of AP and verified whether the proteasome inhibitor bortezomib could protect against AP in mice.

2. Materials and methods

2.1. Animals

ICR male mice weighing between 30 and 35 g were obtained from Yangzhou University Model Animal Center (Yangzhou, China) and housed in specific pathogen-free facilities. Before experiment, all mice were kept in an environment of controlled temperature (23 °C ± 2 °C), humidity and lighting (12 h light/12 h dark). This study was performed with the permission of the Science and Technology Commission of the Affiliated Hospital of Yangzhou University municipality, and all methods were carried out in accordance with the Principles of Laboratory Animal Care (NIH publication number 85Y23, revised 1996).

2.2. Regents

Bortezomib was purchased from Selleck (Selleckchem,Texas,USA); proteasome 20S activity assay kit, caerulein, sodium taurocholate hydrate (TLC) were purchased from Sigma (Sigma Aldrich, St. Louis, MO, USA); anti-NF-κB p65 antibody was purchased from Cell Signaling Technologies (Beverly,MA,USA); anti-LaminB1 antibody was purchased from Proteintech (Proteintech Group Inc, Chicago, USA); anti-β-actin antibody was purchased from Santa Cruz Biotechnology(Santa Cruz, CA, USA). Anti-20S proteasome, goat anti-rabbit and rabbit anti-mouse secondary antibodies were purchased from Abcam (Abcam, Cambridge, UK); Amylase kits were purchased from BioSino (BioSino Bio-Technology & Science Inc., Beijing, China) and lipase kits were purchased from Nanjing Jiancheng (Nanjing Jiancheng Corp., Nanjing, China).

2.3. MAP model preparation and bortezomib treatment

The mild AP (MAP) mice model was induced by intraperitoneal (i.p.) injection of caerulein (50 μg/kg, interval of 1 h, 10 times). Mice was sacrificed at 0, 12, 24 and 48 h after the first injection of caerulein to determine the dynamic changes of proteasome 20S and NF-κB [22,23]. To observe the effect of bortezomib, mice were randomized into five groups: control group, MAP group, low-dose (0.1 mg/kg), medium dose (0.5 mg/kg), and high dose (1 mg/kg) bortezomib group. All group except for the control group were developed a MAP model by caerulein and the control group was injected with equal amount of PBS. Bortezomib, dissolved in 200 μl PBS, was intraperitoneal injected at 0 h after the first caerulein, and the same amount of PBS was injected in the control group and MAP group. Mice were sacrificed at 12 h after the first caerulein administration [24].

2.4. SAP model preparation and bortezomib treatment

The SAP mice model was induced by retrograde infusion of TLC (1% 100 μl + methylene blue reagent 20 μl) dissolved in saline into the distal common bile duct and pancreatic duct. Briefly, after intraperitoneal injection of sodium pentobarbital (50 mg/kg), 2–3 cm longitudinal incisions were made in the abdomen of the mice to expose the abdominal cavity and the duodenum was turned over to expose its distal side. The retrograde duodenum was inserted into the biliary pancreatic duct using an insulin needle to slowly inject TLC solution. After injection, the abdominal cavity of mice was layered and closed. All the mice were placed on a 37 °C constant temperature heating table for 90 min [25].

SAP experimental mice were randomly divided into three groups: sham group, SAP group, and bortezomib group. In the sham group, only abdominal operation was performed and the SAP model was not induced. In bortezomib group, bortezomib (1 mg/kg, dissolved in 200 μl PBS) was intraperitoneal injected to mice before operation. The same dose of PBS (200 μl) was administrated in the control group and SAP group. Three groups of mice were all sacrificed at 12 h after TLC administration.

2.5. Sample collection and preparation

Mice were sacrificed under anesthesia by intraperitoneal injection of sodium pentobarbital (50 mg/kg). A portion of the pancreatic and pulmonary tissues were fixed in 4% paraformaldehyde in phosphate-buffered saline for histological analysis and the rest were stored at −80 °C for further investigation. Blood samples were collected at different time points for amylase and lipase detection. The analysis was performed according to the kit instructions.

2.6. Histological examination

Pancreatic tissue samples, fixed in the 4% phosphate buffered formaldehyde, were embedded in paraffin blocks, stained with hematoxylin and eosin and examined with a light microscope finally. The histopathological scoring analysis of pancreatic and pulmonary tissues was performed blindly according to our previously described methods [26,27].

2.7. Immunofluorescence detection

Fresh tissues of pancreas were embedded in optimal cutting temperature (OCT) compound, and samples were cut into 7 μm sections. Then, tissues were rinsed twice with 1X PBS, permeabilized using 0.1% triton X-100/PBS for 15 min and blocked in blocking solution (5% BSA in 1X TBST). The pancreatic tissues were incubated with primary antibody 20S proteasome (1:200) overnight at 4 °C and then washed 3 times in PBS (pH = 7.4) solution for 5 min each time and then incubated with secondary antibody for 1 h at room temperature. Tissues were incubated by DAPI solution at room temperature for 10 min and washed again. Finally, the slides were observed under the fluorescence microscope.

2.8. Western blot (WB) detection

Pancreatic tissue lysates were prepared in saline-containing protease inhibitor and/or complete phosphatase inhibitor cocktail. The protein lysates were separated by electrophoresis in 10% SDS-PAGE gels and transferred to polyvinylidene difluoride (PVDF) membranes and blocked with 5% skim milk at room temperature for 1 h (except 20S proteasome protein PVDF was blocked with 5% skim milk at room temperature for 0.5 h), and then incubated

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