European Journal of Pharmacology 791 (2016) 157–167

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Immunopharmacology and inflammation

Associations between autophagy, the ubiquitin-proteasome system and endoplasmic reticulum stress in hypoxia-deoxygenation or ischemia-reperfusion

Tao Fan [a,1], Zhixin Huang [b,1], Lei Chen [a], Wei Wang [a], Boyou Zhang [a], Yao Xu [a], Shize Pan [a], Zhangfan Mao [a], Hao Hu [a], Qing Geng [a,*]

[a] *Department of Thoracic Surgery, Renmin Hospital of Wuhan University, Wuhan, China*
[b] *Department of Gynecology and Obstetrics, Renmin Hospital of Wuhan University, Wuhan, China*

A R T I C L E I N F O

Article history:
Received 25 June 2016
Received in revised form
25 August 2016
Accepted 25 August 2016
Available online 26 August 2016

Keywords:
Endoplasmic reticulum stress
Autophagy
Proteasome
Hypoxia-reoxygenation injury
Ischemia-reperfusion injury
Alveolar macrophage

Chemical compounds studied in this article:
MG-132 (PubChem CID: 462382)
Adriamycin (PubChem CID: 31703)
3-MA (PubChem CID: 1673)
Rapamycin (PubChem CID: 5284616)

A B S T R A C T

The activation of autophagy has been demonstrated to exert protective roles during hypoxia-reoxygenation (H/R)-induced brain injuries. This study aimed to investigate whether and how preconditioning with a proteasome inhibitor (MG-132), a proteasome promoter (Adriamycin, ADM), an autophagy inhibitor (3-methyladenine, 3-MA) and an autophagy promoter (Rapamycin, Rap) affected endoplasmic reticulum stress (ERS), the ubiquitin-proteasome system (UPS), autophagy, inflammation and apoptosis. Ubiquitin protein and 26S proteasome activity levels were decreased by MG-132 pretreatment but increased by ADM pretreatment at 2 h, 4 h and 6 h following H/R treatment. MG-132 pretreatment led to the increased expression of autophagy-related genes, ER stress-associated genes and IκB but decreased the expression levels of NF-κB and caspase-3. ADM pretreatment led to the decreased expression of autophagy-related genes, ERS-associated genes and IκB but increased the expression of NF-κB and caspase-3. Pretreatment with 3-MA reduced the expression of autophagy-related genes, autophagy and UPS co-related genes, as well as apoptosis-related although the latter was increased by Rap pretreatment at 2 h, 4 h and 6 h following H/R treatment. In vivo, pretreatment of rats with ADM, MG-132, 3-MA or Rap followed by ischemia-reperfusion (I/R) treatment resulted in similar changes. Proteasome inhibition preconditioning strengthened autophagy and ER stress but decreased apoptosis and inflammation. Autophagy promotion preconditioning exhibited similar changes. The combination of a proteasome inhibitor and an autophagy promoter might represent a new possible therapy to treat H/R or I/R injury-related diseases.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Protein metabolism is a process involving degradation and synthesis to maintain dynamic equilibrium. The ubiquitin-proteasome system (UPS) and the autophagy-lysosomal system are two major protein degradation pathways in eukaryotic cells. Proteasomes are widely distributed in all eukaryotes and are responsible for the proteolysis activity that eliminates excessive amounts of damaged proteins (Hershko and Ciechanover, 2000). The protease complex consists of a core subunit (20S) and two regulatory subunits (19S) (Peters et al., 1994). It has been well-defined that UPS is the primary mechanism for the proteolysis of misfolded or short-lived proteins and is involved in regulating the cell cycle and cell apoptosis (Nandi et al., 2006).

Autophagy, another intracellular protein degradation mechanism, is responsible for degrading and recycling cell constituents, such as defective organelles and long-lived proteins, by converting them into double-membraned autophagosomes and transferring them to lysosomes (Lin et al., 2013). It has been confirmed that there is a complementary association between UPS and autophagy in cellular stress and protection against neurodegenerative disease (Nedelsky et al., 2008). Ubiquitin-binding proteins and ubiquitin play important roles in selective autophagy (Kraft et al., 2010a). Autophagy is also involved in the degradation of ubiquitinated proteins (Lamark, 2010).

The unfolded protein response (UPR) is a protective mechanism in response to endoplasmic reticulum stress(ERS), which is activated when excess amounts of misfolded or unfolded proteins amass in the ER (Schroder, 2005; Schroder and Kaufman, 2005). The UPR clear these excessive proteins by promoting misfolded protein degradation and refolding and suppressing protein

* Corresponding author.
E-mail address: szgqing@126.com (Q. Geng).
[1] Tao Fan and Zhixin Huang contributed equally to this work.

http://dx.doi.org/10.1016/j.ejphar.2016.08.026
0014-2999/© 2016 Elsevier B.V. All rights reserved.

translation (Walter and Ron, 2011). ER stress is known to elicit autophagy through UPR pathways (Li et al., 2008). Furthermore, there is in vitro evidence that crosstalk exists between the UPS, autophagy and ER stress to regulate cell apoptosis (Kawaguchi, 2011). These observations suggest that it is of considerable significance to characterize the associations between UPS, autophagy and ER stress.

Reoxygenation is critical for end-stage lung disease, such as chronic obstructive pulmonary disease (COPD) and pulmonary artery hypertension, which need to be treated with lung transplant. Ischemia-reperfusion (I/R) is an important part during lung transplant. Reducing ischemia-reperfusion (I/R) injury is the key to increase success rate of surgery (Hatachi et al., 2014). Hypoxia-reoxygenation(H/R) or ischemia-reperfusion (I/R) injury has been reported to activate autophagy and induce ER stress (Jian et al., 2011; Wang et al., 2012). However, little is known about whether crosstalk exists between UPS, autophagy and ER stress and how crosstalk might affect inflammation and cell apoptosis following H/R or I/R injury. This study aimed to investigate the effects of proteasome and autophagy inhibition or promotion on UPS, ER stress, autophagy, inflammation and cell apoptosis in an alveolar macrophage cell line (NR8383) following H/R injury and in vivo following I/R injury. Because alveolar macrophage activation plays a crucial role in the development of lung ischemia reperfusion injury (Zhao et al., 2006), the study selected an alveolar macrophage cell model. Our results shed new light on the design of effective therapy strategies against H/R or I/R injury-associated diseases.

2. Materials and methods

2.1. Cell culture

The study used the rat alveolar macrophage cell line NR8383 (ATCC, CRL-2192) as an in vitro model. The cells were maintained in F-12K medium (Gibco, New York, USA #21127) at 37 °C in a humidified 5% CO_2 atmosphere. The medium as supplemented with 10% heat-inactivated fetal calf serum (FCS, Invitrogen, Beijing, China, #26400044). When the cells reached 80% confluence, they were digested with 0.25% trypsin and passaged.

2.2. Establishment of a stable PAsRFP-N1-LC3/NR8383 cell line

In accordance with the manufacturer's instructions, the PAsRFP-N1-LC3 (a sensitive autophagy reporter) plasmid was transfected into NR8383 cells using Lipofectamine 2000 reagent (Invitrogen, Beijing, China, #11668027). After culturing for 24 h, the cells were transferred and cultured in F-12K medium containing 600 μg/ml G418 (Invitrogen, Beijing, China, #10131027). Approximately 2 weeks of post-transfection and expansion, cell colonies exhibiting strong red fluorescence were selected under a fluorescence microscope (Olympus, Tokyo, Japan); these fluorescent cells were considered stable PAsRFP-N1-LC3/NR8383 cells and were cultured in medium containing 100 μg/ml G418 and 10% FBS for further experiments.

2.3. Cell viability assay (MTT assay)

Adriamycin (AD, Sigma, #25316-40-9) andMG132 (MERCK-Calbiochem, #474790) were employed as a proteinase inhibitor and a proteinase promotor, respectively. 3-MA (Sigma, #M9281) and Rap (Alexis Biochemicals, #380-004-M001) were used as an autophagy inhibitor and an autophagy promotor, respectively. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was carried to detect the effects of ADM, MG-132, 3-MA and Rap on the cell viability of PAsRFP-N1-LC3/NR8383 cells. The four drugs were diluted in DMSO. The procedure was conducted in accordance with the manufacturer's instructions. Briefly, cells incubated in a 96-well plate were treated with different concentrations of ADM (5 μM, 10 μM and 15 μM), MG-132 (2.5 μM,5 μM and 10 μM), 3-MA (5 mM, 10 mM and 15 mM) and Rap (150 nM, 200 nM and 250 nM) for 48 h. Untreated cells were defined as controls. Then, 50 μl of MTT (1 mg/ml; Sigma, USA, #M2128) were added to the cells in each well, and the cells were maintained for 4 h. Finally, formazan crystals were solubilized with DMSO (150 μl; Sigma, #D2650), and the absorbance was measured with a microplate reader (Multiskan MK3, THERMO, USA) at 570 nm. The cell inhibition rates (%) at each concentration were calculated by dividing by control values. Each experiment was performed in triplicate.

2.4. Cell treatment

PAsRFP-N1-LC3/NR8383 cells were randomly divided into ADM, MG-132, Rap, 3-MA and control groups in which the cells were individually incubated (the optimal concentrations were obtained from the MTT assay) for 4 h. The control group was pretreated with an equal volume of DMSO. Then, the cells underwent H/R treatment for 2 h, 4 h or 6 h based on the results of a previous study (Farivar et al., 2005). Briefly, the cells were placed in a humidified incubator with 0.5% oxygen for specific amounts of time (2 h, 4 h and 6 h) for hypoxia treatment. The cells were then moved to a normoxic incubator and cultured for similar periods of time (2 h, 4 h or 6 h) for reoxygenation treatment. Cells that were unstimulated under H/R conditions were designated as control cells. After appropriate treatment, a fluorescence microscope (Olympus, Tokyo, Japan) was utilized to observe and photograph the cells in the different groups. The relative RFP expression rate was calculated as the percentage of RFP^{+} cells in total cells.

2.5. Detection of 26S proteasome activity

The 20S core particle of the 26S proteasome is a multi-functional threonine protease that possesses three primary catalytic activities: chymotrypsin-like activity, trypsin-like activity, and glutamyl peptide-hydrolysing (PGPH)-like activity. The three activities were measured as described by a previous study. The cell lysates were incubated with Suc-LLVY-7-amido-4-methylcoumarin (proteasome-specific substrate, Sigma, USA) at 37 °C for 30 min after the appropriate treatments. The reaction was terminated by the addition of cold ethanol. The fluorescence absorbance was measured with a fluorescence photometer (BioTek, USA) at 380/440 nm. Each experiment was performed in triplicate.

2.6. Cell ultrastructure observation

The cells were fixed in 3% glutaraldehyde overnight and then fixed in 1% osmic acid to observe the ultrastructure in different groups with H/R treatment. The samples were subjected to dehydration with a graded ethanol series and embedded in epoxide resin. Then, an ultra-microtome (LKB-V, Bromma, Sweden) was used to cut the samples into ultrathin sections that were sequentially stained in uranyl acetate and lead citrate. Finally, using a transmission electron microscope (H-600, Hitachi, Tokyo, Japan), the sections were observed and photographed. The experiment was conducted in accordance with a previous study (Shimizu et al., 2002).

2.7. Western blot

Total proteins were extracted from cells undergoing H/R

Download English Version:

https://daneshyari.com/en/article/2530804

Download Persian Version:

https://daneshyari.com/article/2530804

Daneshyari.com