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Original Contribution

Soluble receptor for advanced glycation end-products protects against ischemia/reperfusion-induced myocardial apoptosis via regulating the ubiquitin proteasome system

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ABSTRACT

Aim: Apoptosis participated in the pathological process of myocardial ischemia/reperfusion (I/R) injury. Previous studies have reported that endogenous substance sRAGE protect against I/R injury through inhibiting myocardial apoptosis. But the mechanisms are currently unknown. Prior work has demonstrated that ubiquitin proteasome system (UPS) dysfunction is closely related to apoptosis. We explored the potential role of UPS in the effect of sRAGE inhibition on I/R-induced myocardial apoptosis.

Methods and results: Adult male C57BL mice treated with sRAGE (100 μg/day, i.p.) or saline were performed to ligate left anterior descending coronary artery (LAD) as an *in vivo* model. As an *in vitro* model, primary murine cardiomyocytes pretreated with sRAGE or sRAGE-containing adenovirus were simulated I/R by "ischemia buffer". The TUNEL and caspase-3 activity were assessed. Also the activity and expression of proteasome were detected. sRAGE decreased the number of TUNEL-positive cardiomyocytes and caspase-3 activity, however, the inhibition of sRAGE on I/R-induced apoptosis was abolished by proteasome inhibitor Bortezimb (BTZ). sRAGE inhibited the decreased proteasome activity, also the reduction in protein and gene levels of β1i and β5i following I/R. Suppression of STAT3 blocked the inhibition of sRAGE on apoptosis induced by I/R. The chromatin immunoprecipitation (CHIP) results confirmed that sRAGE promoted activating STAT3 binding to β1i and β5i promoter.

Conclusions: Our data suggest that the inhibition of sRAGE on I/R-induced apoptosis is associated with activation and expression of proteasome, including improved proteasome activity and elevated β1i and β5i expression mediated by STAT3 activation. We predict that sRAGE is a novel intervention to target UPS activation for preventing and treating myocardial apoptosis.

1. Introduction

Myocardial ischemia/reperfusion (I/R) injury restricted the prognosis of ischemic heart disease. More and more interventions are explored to mitigate myocardial I/R injury, such as increase in endogenous substances released. One of the most important endogenous substances is soluble receptor for advanced glycation end-products (sRAGE). sRAGE is the soluble isomer of receptor for advanced glycation end-products (RAGE), which is a transmembrane receptor of the immunoglobulin superfamily. Ligand-RAGE axis is involved in many diseases, such as myocardial I/R injury [1,2], diabetes complications [3], and inflammatory diseases [4,5]. The effect of sRAGE inhibition on RAGE-mediated signaling pathway activation by competitive binding to RAGE ligands [6,7]. Bucciarelli LG et al. have demonstrated that the binding of RAGE to advanced-glycation end products (AGEs) affects myocardial energy metabolism and function during I/R [1]. To further clarify the effect of RAGE in myocardial I/R injury, sRAGE was used as an antagonist. The previous papers have showed that administration of sRAGE protects the myocardial injury from I/R, including heart function,

Abbreviations: sRAGE, soluble receptor for advanced glycation end-products; I/R, Ischemia/Reperfusion; UPS, Ubiquitin proteasome system; LAD, Left anterior descending coronary artery; STAT, Signal transducer and activator of transcription; RAGE, Receptor for advanced glycation end-products; AGEs, Advanced glycation end-products; MAPK, Mitogen activated protein kinase; JNK, c-Jun N-terminal kinase; IFN-γ, Interferon-γ; BTZ, Bortezimb; MOIs, Multiplicities of infection; PFI, Proteasome function insufficiency

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apoptosis [8,9], and myocardial fibrosis [10].

The ubiquitin proteasome system (UPS) is involved in degradating the majority of the cellular proteins and keeping protein quality control in eukaryotic cells. The UPS activity is attributed to three β-subunits, β1, β2 and β5, respectively. However, the constitutively β-subunits are replaced by inducible β-subunits (β1i, β2i, β5i) in response to inflammatory cytokines, and then the UPS activity is attributed to both constitutively and inducible β-subunits [11]. The UPS dysfunction participated in the progression of many kinds of heart disease. The UPS function insufficiency, including decrease in proteasome activity and the ability to degradate proteins, has been demonstrated in myocardial I/R in mice [12,13]. Otherwise, apoptosis plays a virtual role in I/R injury. Most important, apoptosis is triggered by damaged proteins. The occurrence of apoptosis can result due to UPS dysfunction [14].

The mechanism of sRAGE inhibits I/R-induced apoptosis apart from blocking the interaction of RAGE with ligands. However, the downstream of UPS involves in regulating key apoptosis-related signaling pathways, such as MAPK JNK, calcineurin, FOXO, p53, et al. [14,15]. Moreover, Liliensiek et al. have reported that sRAGE decrease the delayed-type hypersensitivity response in both $RAGE^{-/-}$ and $RAGE^{+/+}$ mice in a similar manner, indicating sRAGE not only purely blocking cell surface RAGE activation but also has other regulation pathways [16].

The aim of the present study is to define whether the inhibition of sRAGE on myocardial and cardiomyocytes apoptosis induced by I/R via regulating ubiquitin proteasome system mediated by STAT3.

2. Materials and methods

2.1. Animals

Adult Male C57BL/6 mice of 6–8 weeks were provided from Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). All mice were housed in a pathogen-free environment, kept under a 12/12-h light–dark cycle at a temperature of 23–25 °C and offered standard laboratory animal chow and water in the Laboratory of Animal Experiments at Capital Medical University. The animal procedures were performed conform the NIH Guidelines on the Use of Laboratory Animals (NIH Publication no. 85-23, revised 1996). The animal care and experiments were approved by the Animal Subjects Committee of Capital Medical University. Mice have 2 weeks adaptation time before any experimental procedures. So animals were used for experiments at 8–10 weeks of age (weight 23–25 g).

2.2. Experimental groups

For *in vivo* regional I/R protocol, mice ($n=10$–12 per group) were randomly subject to one of the following treatments: (1) sham-operated animals underwent the same procedure except the ligature placed under LAD was not fasten, and received equal volumes of vehicle (100 μg/day) twice; (2) sham-operated animals were administered sRAGE (100 μg/day) by intraperitoneal injection twice; (3) I/R group animals received equal volumes of vehicle (100 μg/day), the administer route and time of vehicle were same to the sham-operated group; (4) I/R group animals received sRAGE (100 μg/day), the administrate route and time of sRAGE were same to the sham-operated group [2].

For *in vitro* I/R protocol, we used the following groups: (1) Sham/Sham+Ad-GFP, or (2) Sham+sRAGE (900 ng/mL)/Sham+Ad-sRAGE [9], (3) I/R/I/R+Ad-GFP, (4) I/R+sRAGE (900 ng/mL)/I/R+Ad-sRAGE, (5) I/R+Ad-sRAGE+Bortezimb (BTZ) (7 nmol/L) [17], I/R+Ad-sRAGE+AG 490(5 μmol/L) [18], I/R+Ad-sRAGE+S3I-201(100 μmol/L) [19]. Cardiomyocytes were pretreated with sRAGE for 10 min, AG490, S3I-201 and BTZ for 30 min before simulating I/R.

2.3. Myocardial ischemia/reperfusion model protocol in vivo

The method of I/R surgery or Sham operation were performed in C57BL/6 mice has been described previously in details [20]. The animals were anesthetized with 2% isoflurane inhalation with an isoflurane delivery system but not ventilated and were fixed on a heating pad, then the left-sided thoracotomy of mice was performed by making a small skin cut and dissecting the pectoral major and minor muscle to make a hole at the fourth intercostal space, after that the heart was exposed and the origin of the LAD was occluded by a 7-0 silk ligature, sham-operated underwent the similar procedure without ligation. After 30 min of ischemia, the slipknot was released by pulling the long end of slipknot suture and the myocardium began to be reperfused. The myocardium undergo 30 min of ischemia and 24 h of reperfusion. sRAGE (100 μg/day) was administered into mice by intraperitoneal injection (i.p.) twice, which were performed at 12 h prior to LAD ligation and 12 h of reperfusion, respectively. As control, the equal volume of vehicle was administered into Sham and I/R mice. At the end of the reperfusion, all mice were euthanized by thoracotomy with 1–4% inhalant isoflurane for evaluating the myocardial apoptosis, peptidase activities and expression of proteasome.

2.4. Cell culture and ischemia/reperfusion protocol in vitro

To isolate and culture primary neonatal cardiomyocytes, an enzymatic technique described previously was used [21]. Simulated ischemia/reperfusion was performed as described previously [22]. Briefly, cardiomyocytes were stimulated ischemia by “ischemia buffer” (pH 6.3), including the following (mmol/l) substances: 118 NaCl, 24 $NaHCO_3$, 1.0 NaH_2PO_4, 2.5 $CaCl_2$-$2H_2O$, 1.2 $MgCl_2$, 20 sodium lactate, 16 KCl and 10 deoxyglucose, and cardiomyocytes were incubated in an composed of 5% CO_2 and 95% N_2 at 37 °C for 2 h, and then the “ischemia buffer” were replaced by normal medium for a further 24 h of reperfusion. For control groups, cardiomyocytes were cultured with normal medium in an atmosphere of 5% CO_2 at 37 °C for 24 h.

2.5. Transfection of adenovirus

sRAGE expression adenovirus was designed based on sequence of human RAGE (reference sequence: NM_001136) from residue 23–340 (i.e.the ectodomain of RAGE) followed with a stop codon [23] and purchased from Shanghai hanbio Co., Ltd. Gene transfer with adenovirus encoding GFP was used as an internal control. Cardiomyocytes were infected with 100 multiplicities of infection (MOIs) of adenovirus and cultured in serum-free DMEM medium for 6–8 h at 37 °C in 5% CO_2 incubator, and then the serum was added into medium. The cardiomyocytes infected by adenoviral were incubated at an composed of 5% CO_2 and 95% N_2 at 37 °C for 24 h before simulating ischemia experiments [24].

2.6. Evaluation of myocardial apoptosis

After the end of 24 h reperfusion, the apoptosis of heart tissue and cardiomyocytes was evaluated by detection of TUNEL assay and caspase-3 activity. The experimental protocols as described previously in details [25,26].

2.7. Proteasome activity assays

Caspase-like, trypsin-like and chymotrypsin-like proteolytic