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Production and application of HMGB1 derived recombinant RAGE-antagonist peptide for anti-inflammatory therapy in acute lung injury

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ABSTRACT

Acute lung injury (ALI) is an inflammatory lung disease caused by sepsis, infection, or ischemia-reperfusion. The receptor for advanced glycation end-products (RAGE) signaling pathway plays an important role in ALI. In this study, a novel RAGE-antagonist peptide (RAP) was produced as an inhibitor of the RAGE signaling pathway based on the RAGE-binding domain of high mobility group box-1 (HMGB1). Recombinant RAP was over-expressed and purified using nickel-affinity chromatography. In lipopolysaccharide- or HMGB1-activated RAW264.7 macrophage cells, RAP reduced the levels of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). RAP decreased the levels of cell surface RAGE and inhibited the nuclear translocation of nuclear factor-κB (NF-κB). These results imply that RAP decreases RAGE-mediated NF-κB activation and subsequent inflammatory reaction. For in vivo evaluation, RAP was delivered to the lungs of ALI-model animals via intratracheal administration. As a result, RAGE was down-regulated in the lung tissues by pulmonary delivery of RAP. Consequently, TNF-α, IL-6, and IL-1β were also reduced in broncoalveolar lavage fluid and the lung tissues of RAP-treated animals. Hematoxylin and eosin staining indicated that inflammation was decreased in RAP-treated animals. Collectively, these results suggest that RAP may be a useful treatment for ALI.

1. Introduction

Acute lung injury (ALI) is an inflammatory disease of the lungs caused by a variety of etiologies such as infection, sepsis, and ischemia-reperfusion (Matute-Bello et al., 2008). ALI is characterized by increased permeability of the lung tissue to protein-rich fluid resulting in lung edema and subsequent respiratory failure (Aman et al., 2011; Minamino and Komuro, 2006). Although many efforts have been made to develop effective treatments for ALI, the available therapeutic options are limited to mechanical ventilation and a few anti-inflammatory drugs (Mei et al., 2007). Mechanical ventilation with high oxygen pressure is currently the main therapy for ALI. However, it is not a fundamental treatment for the disease, but rather a respiration aid. In addition, hyperoxia due to excessive oxygen supply may damage lung tissues (Murray et al., 2008). Anti-inflammatory medications, such as dexamethasone, have been studied as alternative therapeutic options (von Bismarck et al., 2009). However, their effects remain controversial and the potential side effects have limited their application in ALI (von Bismarck et al., 2009). Therefore, new therapeutic agents need to be developed to effectively treat ALI.

A prior pathophysiological study indicated that the receptor for advanced glycation end-products (RAGE) has an important function in ALI (Uchida et al., 2006). Endogenous damage-associated molecular patterns (DAMPs) bind to RAGE and induce RAGE-mediated signal transduction (Fritz, 2011). DAMPs include high mobility group box-1 (HMGB1), S100 family proteins, and the β-amyloid peptide (Fritz, 2011; Izushi et al., 2016). In addition, pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharides (LPSs), are recognized by RAGE (Yamamoto et al., 2011). RAGE-ligand binding induces the nuclear translocation and activation of nuclear factor-κB (NF-κB) (He et al., 2013). NF-κB promotes the expression of pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6), in ALI and other inflammatory diseases (Barnes and Adcock, 1997; Yang et al., 2012). Therefore, inhibition of the interaction between RAGE and its ligands may be a possible treatment strategy for ALI (Hiscott et al., 2001). For this purpose, anti-RAGE antibody and soluble RAGE (sRAGE) were investigated as therapeutic agents for acute lung injury (Blondonnet et al., 2017; Izushi et al., 2016). As a result, anti-RAGE antibody or sRAGE blocked the RAGE-mediated signal transduction and reduced inflammation in the ALI mouse model. These results suggest that RAGE plays an important role in the progress of ALI and may be a potential therapeutic target.

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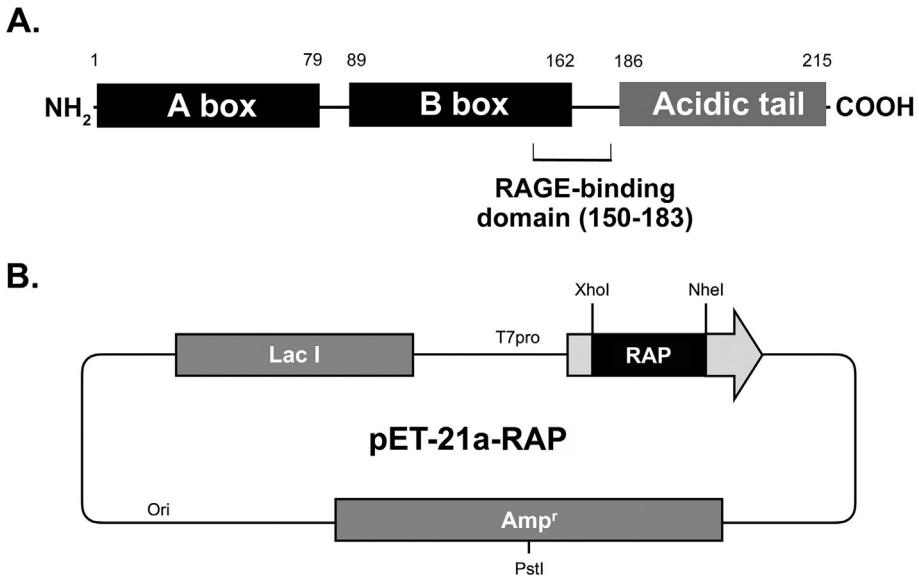

Fig. 1. Structure of HMGB1 (A) and map of the RAP expression plasmid, pET-21a-RAP (B).

One of the most important DAMPs in ALI is HMGB1. It was previously reported that antagonistic inhibition of HMGB1 reduced severe inflammation in ALI (Oh and Lee, 2014; Song et al., 2016; Wang et al., 2013). HMGB1 is composed of three domains: box A, box B, and an acidic tail (Fig. 1) (Thomas, 2001). HMGB1 Box A (HMGB1A) is an antagonist of wild-type HMGB1 (wtHMGB1) (Kokkola et al., 2003; Oh and Lee, 2014) and has been widely investigated as a therapeutic agent for various inflammatory diseases (Gong et al., 2009; Huang et al., 2007; Kokkola et al., 2003). In an animal stroke model, HMGB1A injection into the brain (Jin et al., 2011) resulted in a reduced inflammatory reaction and infarct volume in the ischemic brain tissue. HMGB1A has also been tested as a therapeutic agent for ALI (Gong et al., 2009; Oh and Lee, 2014; Song et al., 2016). The mechanism of HMGB1A's inhibition of wtHMGB1 remains unclear, although it had been suggested that HMGB1A might compete with wtHMGB1 for receptor binding (Kokkola et al., 2003). However, we recently proved that the binding of HMGB1A to wtHMGB1 in the cytoplasm reduced the secretion of wtHMGB1 from cells resulting in a decreased inflammatory reaction (Hwang et al., 2016).

Although wtHMGB1 is one of the ligands for RAGE and HMGB1A has an antagonist effect against wtHMGB1 in ALI, there are several other ligands for RAGE aside from wtHMGB1. Therefore, general RAGE-inhibiting molecules may be more effective than HMGB1A in the down-regulation of the RAGE-mediated signaling pathway, since they can inhibit various RAGE ligands including wtHMGB1. Previously, a RAGE-binding site was identified in HMGB1 box B (HMGB1B) consisting of amino acids 150–183 of HMGB1 (Andersson et al., 2002; Li et al., 2003). We hypothesized that the RAGE-binding fragment of HMGB1B, when produced as an independent peptide using recombinant DNA technology, might compete with various RAGE ligands for RAGE binding and reduce the inflammatory reaction in ALI. In this study, a novel RAGE-antagonist peptide (RAP) based on the sequence of the HMGB1B RAGE-binding domain was synthesized via recombinant DNA technology. RAP was over-expressed in bacteria and purified using nickel-affinity chromatography. The RAP was physically and biochemically characterized, and its anti-inflammatory effects were evaluated in ALI models using intratracheal administration. The results of this study suggest that the RAP may be a useful anti-inflammatory peptide for the treatment of ALI.

2. Materials and methods

2.1. Materials

*Nhe*I and *Xho*I were purchased from New England Biolabs (Ipswich, MA). The pET-21a plasmid was purchased from Novagen (Billerica, MA). Isopropyl-β-D-thiogalactopyranoside (IPTG) was acquired from Sigma-Aldrich (St. Louis, MO). Phenylmethyl sulfonyl fluoride (PMSF) was obtained from AMRESCO (Solon, Ohio). Ni-NTA Agarose was purchased from Qiagen (Valencia, CA). Raw264.7 cell lines were obtained from the Korean Cell Line Bank (Seoul, Korea). Cells with passages < 20 were used in these experiments. The Spectra/Por dialysis membrane (molecular weight cut-off 2000) was acquired from Millipore (Billerica, CA). The bicinchoninic acid (BCA) assay kit and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide (MTT) were purchased from Thermo Fisher Scientific (Waltham, MA). TNF-α, IL-6, and IL-1β ELISA kits were obtained from eBioscience (San Diego, CA). Alexa Fluor 488, NF-κB, the nuclear extraction kit, the p65 transcription factor assay kit, and 6× histidine antibody were acquired from Abcam (Cambridge, MA). Immunohistochemistry Accessory kits were purchased from Bethyl Laboratories (Montgomery, TX). NF-κB antibodies were obtained from Santa Cruz Biotechnology (Minneapolis, MN).

2.2. Construction of the RAP plasmid

HMGB1 cDNA was amplified via RT-PCR previously (Kim et al., 2008). The cDNA fragment encoding RAP (amino acids 150–186 of human HMGB1) was amplified using PCR with HMGB1 cDNA as a template. The primer sequences were as follows (*Nhe*I and *Xho*I sites are underlined): forward primer, 5′-GTATGCTAGCAAGCTGAAGGAAAAA TACGAAAAGG-3′ and reverse primer, 5′-ACCGCTCGAGACATTCCTTC TTTTTCTTGCTTTTTTC-3′. The amplified RAP cDNA was digested with *Nhe*I and *Xho*I and inserted into pET-21a at the *Nhe*I and *Xho*I sites, resulting in the pET-21a-RAP plasmid. The subsequent RAP amino acid sequence was KLKEKYEKDIAAYRAKGKPDAAKKGVVKAEKSKKKKEC.

2.3. RAP expression

An *Escherichia coli* BL21 strain was transformed with pET-21a-RAP. Transformed bacteria were isolated and cultured in 5 ml of Luria broth (LB) medium containing 50 μg/ml ampicillin in a shaking incubator at 37 °C for 18 h. The bacterial culture was transferred to 1 l of LB