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Tat-CBR1 inhibits inflammatory responses through the suppressions of NF-κB and MAPK activation in macrophages and TPA-induced ear edema in mice

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ABSTRACT

Human carbonyl reductase 1 (CBR1) plays a crucial role in cell survival and protects against oxidative stress response. However, its anti-inflammatory effects are not yet clearly understood. In this study, we examined whether CBR1 protects against inflammatory responses in macrophages and mice using a Tat-CBR1 protein which is able to penetrate into cells. The results revealed that purified Tat-CBR1 protein efficiently transduced into Raw 264.7 cells and inhibited lipopolysaccharide (LPS)-induced cyclooxygenase-2 (COX-2), nitric oxide (NO) and prostaglandin E_2 (PGE_2) expression levels. In addition, Tat-CBR1 protein leads to decreased pro-inflammatory cytokine expression through suppression of nuclear transcription factor-kappaB (NF-κB) and mitogen activated protein kinase (MAPK) activation. Furthermore, Tat-CBR1 protein inhibited inflammatory responses in 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced skin inflammation when applied topically. These findings indicate that Tat-CBR1 protein has anti-inflammatory properties in vitro and in vivo through inhibition of NF-κB and MAPK activation, suggesting that Tat-CBR1 protein may have potential as a therapeutic agent against inflammatory diseases.

Introduction

Carbonyl reductase 1 (CBR1) is an NADPH-dependent short chain dehydrogenase/reductase with 277 amino acid residues and is found in the liver, epidermis, stomach, smooth muscle fibers, neuronal cells, small intestine, and kidneys (Forrest and Gonzalez, 2000). Among the four known isoforms (CBR1–CBR4) of CBR, CBR1 has protective functions against protein and DNA damage by inactivating acrolein, 4-oxonon-2-enal (ONE), and 4-hydroxynon-2-enal (4-HNE) (Oppermann, 2007). Other studies have also demonstrated that overexpression of CBR1 protects against reactive oxygen species (ROS)-induced cell damage while CBR1 mutation causes neurodegeneration in *Drosophila melanogaster* by oxidative stress thus suggesting that CBR1 plays an important role in cell survival and in protecting against oxidative stress response (Kelner et al., 1997; Botella et al., 2004). In addition, many reports have shown that CBR1 exerts a beneficial role in a variety of diseases such as cancer, diabetes, and neuronal diseases (Forrest and Gonzalez, 2000; Ismail et al., 2000; Doorn et al., 2004; Rashid et al., 2010; Maser, 2006). However, to date the biological function and mechanism of CBR1 protein in inflammation related diseases remain poorly understood.

Inflammatory cells such as macrophages regulate the inflammatory response and play important roles in a number of disease processes by producing various pro-inflammatory cytokines including tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), interleukin 1-β (IL-1β) and IL-6 and inflammatory mediators such as nitric oxide (NO), prostaglandin E_2 (PGE_2), and cyclooxygenase-2 (COX-2) (Pierce, 1990; Marks-Konczalik et al., 1998; Yang et al., 2003). When the cells were stimulated by lipopolysaccharide (LPS), two distinct downstream signaling pathways were activated: nuclear factor kappa B (NF-κB) and mitogen-activated protein kinases (MAPKs). Both pathways induce the pro-inflammatory cytokines and inflammatory mediators therefore providing targets for the development of therapeutic approaches to treating inflammatory diseases (Surh et al., 2001; Li and Verma, 2002; Krakauer, 2004; Ivashkiv, 2011; Sica and Mantovani, 2012).

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Although CBR1 has great therapeutic potential in various diseases, the low permeability of CBR1 protein is an obstacle to its development for use. Protein transduction domains (PTDs) such as Tat or PEP-1 can efficiently transduce into cells and tissues including the blood brain barrier (BBB) with exogenous macromolecules (Schwarze et al., 1999; Morris et al., 2001; Wadia and Dowdy, 2002). A growing body of research has demonstrated that various recombinant proteins fused with a PTD are capable of transducing target proteins in vitro and in vivo and offer protection against cell death in various diseases (Kubo et al., 2008; Dietz, 2010; van den Berg and Dowdy, 2011; Kim et al., 2011, 2013a,b; Sohn et al., 2012; Ahn et al., 2013; Liu et al., 2014).

In the present study, we examined the protective effect of Tat-CBR1 protein on LPS- and 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation. We found that Tat-CBR1 protein demonstrated anti-inflammatory properties in vitro and in vivo through inhibition of NF-κB and MAPK activation.

Materials and methods

Materials and animals. LPS and TPA were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ni^{2+}-nitrilotriacetic acid Sepharose Superflow was purchased from Qiagen (Valencia, CA, USA). Dulbecco's modified Eagle's medium was purchased from Lonza (DMEM; Walkersville, MD, USA). The fetal bovine serum (FBS) and antibiotics were purchased from Gibco BRL. Synthetic Tat peptide was purchased from Peptron (Daejeon, Korea). The indicated primary antibodies and actin were obtained from Cell Signaling Technology (Beverly MA, USA) and Santa Cruz Biotechnology (Santa Cruz, CA, USA), respectively. All other chemicals and reagents were of the highest commercial grade available.

Male 6–8 week-old ICR mice were obtained from the experimental animal center at Hallym University. The mice were housed at a constant temperature (23 °C), relative humidity (60%) with fixed 12 h light:12 h dark cycle and free access to food and water. All experimental procedures involving animals and their care conformed to the Guide for the Care and Use of Laboratory Animals of the National Veterinary Research and Quarantine Service of Korea and were approved by the Hallym Medical Center Institutional Animal Care and Use Committee.

Construction, expression and purification of Tat-CBR1 proteins. A Tat expression vector was prepared in our laboratory as described previously (Kim et al., 2013c). To construct a Tat-CBR1 protein, CBR1 cDNA was amplified using the following primers; sense primer 5′-CCGCTCGAGT CGTCCGGCATC-3′ and antisense primer 5′-CGGGATCCTTATCACCACTG TTCAAC-3′, through polymerase chain reaction (PCR). After subcloning the PCR product into a TA cloning vector, it was ligated into the Tat expression vector. A CBR1 expression vector without the Tat PTD was also constructed to be used as a control.

The Tat-CBR1 and control CBR1 plasmids were expressed in *Escherichia coli* BL21 cells treated with 0.1 mM isopropyl-β-D-thiogalactoside (IPTG; Duchefa, Haarlem, Netherlands) at 18 °C for 8 h and purified using a Ni^{2+}-nitrilotriacetic acid Sepharose affinity column and PD-10 column chromatography (Amersham, Braunschweig, Germany) according to the manufacturer's instructions. The purified proteins were treated using Detoxi-Gel™ endotoxin removing gel (Pierce, Rockford, IL, USA) to remove endotoxins. Endotoxin levels in the proteins were below the detection limit (<0.1 EU/ml) as tested using a Limulus amoebocyte lysate assay (BioWhittaker, Walkersville, MD, USA). The purified Tat-CBR1 and control CBR1 proteins were estimated by Bradford assay (Bradford, 1976).

Transduction of Tat-CBR1 protein into Raw 264.7 cells. Raw 264.7, mouse macrophage cells, were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 20 mM Hepes/NaOH (pH 7.4), 5 mM $NaHCO_3$, 10% FBS, and antibiotics (100 μg/ml streptomycin, 100 U/ml penicillin) at 37 °C under humidified conditions of 95% air and 5% CO_2.

To assess the transduction ability of Tat-CBR1 protein into Raw 264.7 cells, cells were treated with 0.1–1 μM of proteins for 1 h or 1 μM of proteins for 15–60 min. Then the cells were treated with trypsin–EDTA and washed with phosphate-buffer saline (PBS). The cells were harvested for the preparation of cell extracts to perform Western blot analysis. To examine the intracellular stability of Tat-CBR1 protein, cells were treated with 1 μM of proteins for 1 h and harvested at various time periods (1–48 h). The intracellular stability levels of Tat-CBR1 protein were analyzed by Western blotting using a His antibody.

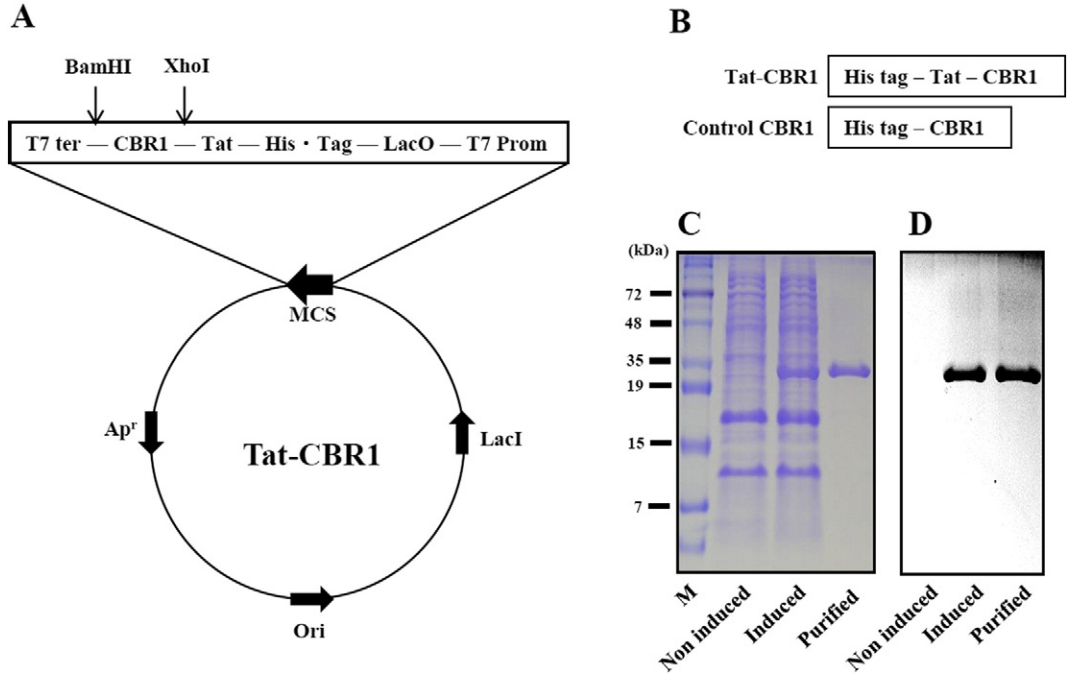

Fig. 1. Construction and purification of Tat-CBR1 protein. A schematic representation of Tat-CBR1 expression vector. A human CBR1 gene was cloned into the *Xho*I and *Bam*HI sites of a Tat expression vector (A). Diagram of the Tat-CBR1 and control CBR1 proteins (B). Each protein consists of six histidine residues at the N-terminal and is expressed by adding IPTG. Expressed and purified Tat-CBR1 proteins were analyzed by 15% SDS-PAGE (C) and confirmed by Western blot analysis using an anti-rabbit polyhistidine antibody (D).