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Research paper

Role of heme oxygenase-1 in potentiation of phagocytic activity of macrophages by taurine chloramine: Implications for the resolution of zymosan A-induced murine peritonitis

Wonki Kim[a], Seung Hyeon Kim[a], Jeong-Hoon Jang[a], Chaekyun Kim[b], Kyeojin Kim[a], Young-Ger Suh[c], Yeonsoo Joe[d], Hun Taeg Chung[d], Young-Nam Cha[b], Young-Joon Surh[a,e,f,*]

[a] Tumor Microenvironment Global Core Research Center and Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, Seoul 08826, Republic of Korea
[b] Department of Pharmacology and Toxicology, College of Medicine, Inha University, Incheon 22332, Republic of Korea
[c] College of Pharmacy, Institute of Pharmaceutical Sciences, Cha University, Pocheon 11160, Republic of Korea
[d] Department of Biological Sciences, University of Ulsan, Ulsan 44610, Republic of Korea
[e] Department of Molecular Medicine and Biopharmaceutical Sciences, Graduate School of Convergence Science, Seoul National University, Seoul 08826, Republic of Korea
[f] Cancer Research Institute, Seoul National University, Seoul 03087, Republic of Korea

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ABSTRACT

Phagocytosis of pathogens by macrophages is crucial for the successful resolution of inflammation induced by microbial infection. Taurine chloramine (TauCl), an endogenous anti-inflammatory and antioxidative substance, is produced by reaction between taurine and hypochlorous acid by myeloperoxidase activity in neutrophils under inflammatory conditions. In the present study, we investigated the effect of TauCl on resolution of acute inflammation caused by fungal infection using a zymosan A-induced murine peritonitis model. TauCl administration reduced the number of the total peritoneal leukocytes, while it increased the number of peritoneal monocytes. Furthermore, TauCl promoted clearance of pathogens remaining in the inflammatory environment by macrophages. When the macrophages isolated from thioglycollate-treated mice were treated with TauCl, their phagocytic capability was enhanced. In the murine macrophage-like RAW264.7 cells treated with TauCl, the proportion of macrophages clearing the zymosan A particles was also increased. TauCl administration resulted in elevated expression of heme oxygenase-1 (HO-1) in the peritoneal macrophages. Pharmacologic inhibition of HO-1 activity or knockdown of *HO-1* in the murine macrophage RAW264.7 cells abolished the TauCl-induced phagocytosis, whereas the overexpression of *HO-1* augmented the phagocytic ability of macrophages. Moreover, peritoneal macrophages isolated from *HO-1* null mice failed to mediate TauCl-induced phagocytosis. Our results suggest that TauCl potentiates phagocytic activity of macrophages through upregulation of HO-1 expression.

1. Introduction

Chronic inflammation occurs when an intracellular microbial infection is not properly resolved. If microbial pathogens or their toxic components are not adequately eliminated. The infectious process result in considerable damage to the host and cause infectious disease such as respiratory allergy, skin diseases and inflammatory bowel diseases [1,2]. Phagocytes, such as neutrophils, monocytes and macrophages, play pivotal roles in the effective elimination of pathogens, thereby maintaining the tissue homeostasis. Upon encountering inflammatory pathogens, polymorphonuclear neutrophils (PMNs) recruited to the inflamed area undergo oxidative burst, comprising the first line of host defense against pathogen infection. This leads to overproduction of reactive oxygen species with which the neutrophils kill and eliminate pathogens. The activated neutrophils acquire significantly enhanced ability to phagocytose pathogens [3]. Subsequently, macrophages, which are derived from monocytes circulating in the blood, are recruited, and then target uncleared inflammatory pathogens remaining in the inflammatory environment [4,5]. The phagocytic removal of the pathogens by macrophages, a process called 'phagocytosis', is essential for resolution of inflammation caused by microbial infections.

In addition to their functions in the removal of uncleared pathogens by phagocytosis, macrophages release pro-inflammatory cytokines, and

* Corresponding author at: College of Pharmacy, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 08826, South Korea.
E-mail address: surh@snu.ac.kr (Y.-J. Surh).

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this alarms cellular defense mechanisms of the host to fight the microbial infection. Moreover, they also produce anti-inflammatory substances, some of which are involved in the resolution of inflammation [6]. Heme oxygenase-1 (HO-1) is one of the major anti-inflammatory enzymes that plays a critical role in defending the body against microbial infection [7]. HO-1 induction is an important part of the host immune response to microbial infections including sepsis [8] and tuberculosis [9]. Although it has been reported that HO-1 has anti-inflammatory effects against invading pathogens, there is less certainty about the pro-resolving effects of HO-1 expressed in macrophages during microbial infection.

Taurine, a decarboxylation product of cysteine, is one of the most abundant free amino acids in inflammatory cells including neutrophils and plays important roles in several essential physiological processes, such as osmoregulation, membrane stabilization, calcium mobilization and immunity [10]. The stored taurine reacts stoichiometrically with hypochlorous acid (HOCl), a strong antibacterial oxidant produced from hydrogen peroxide (H_2O_2) by the myeloperoxidase activity of the activated neutrophils in the presence of chloride ion. This results in the generation of taurine chloramine (TauCl), which is then released from apoptotic neutrophils to the surrounding inflammatory tissue. In particular, TauCl has been reported to have microbicidal activity due to its antioxidant and anti-inflammatory properties [11–13]. However, molecular mechanisms underlying phagocytosis of pathogens stimulated by TauCl have yet to be established. Here, we report that TauCl upregulates HO-1 expression in macrophages and thereby facilitates the engulfment of a fungal component to allow the resolution of inflammation.

2. Materials and methods

2.1. Animals

C57BL/6 mice (8 weeks of age) were purchased from Central Lab Animal Inc. (Seoul, South Korea). *HO-1* knockout mice (BALB/c) were provided by Dr. M.A. Perrela (Harvard Medical School). All mice were maintained according to the Institutional Animal Care Guidelines. Animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Seoul National University.

2.2. Reagents and kits

Taurine was purchased from Sigma Aldrich (St Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), and fetal bovine serum (FBS) were obtained from GIBCO RBL (Grand Island, NY, USA). Fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-labeled zymosan A particles were purchased from invitrogen (Carlsbad, CA, USA). Small interfering RNA (siRNA) against the *HO-1* gene, and zinc protoporphyrin IX (ZnPP) were supplied by Santa Cruz Biotechnology (Santa Cruz, CA, USA). Primary antibody against actin was supplied by Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-HO-1 was the product of Stressgen (Ann, Arbor, MI, USA). PE-conjugated goat anti-rabbit IgG (H + L) secondary antibody and anti-mouse horseradish peroxidase-conjugated secondary antibody were provided by Thermo Fisher Scientific Inc. (Waltham, MA, USA). Polyvinylidene difluoride (PVDF) membranes were supplied from Gelman Laboratory (Ann, Arbor, MI, USA). The Enhanced Chemiluminescent (ECL) detection kit was obtained from Amersham Pharmacia Biotech (Buckinghamshire, UK).

2.3. Synthesis of N-chlorotaurine sodium

N-chlorotaurine sodium salt was synthesized by the reaction of taurine with an ethanolic solution of chloramine-T as a source of electrophilic chlorine and sodium salt. To a well stirred suspension of finely powdered taurine (5 g) in absolute ethanol (100 ml) was added chloramine-T (12 g). The reaction mixture was stirred for about 5 h at ambient temperature. The resulting sodium salt of *N*-chlorotaurine was filtered off and washed with 40 ml of ethanol. Its purity and identity were verified by UV and IR spectral data and further evidence has been gained by ^{13}C NMR spectroscopy and mass spectrometry.

2.4. Zymosan A-FITC-induced peritonitis

Zymosan A-FITC (30 mg/kg) was administered intraperitoneally at 12 h before giving TauCl (20 mg/kg, suspended in autoclaved water) or vehicle, and mice were sacrificed 6 h later. Peritoneal leukocytes were collected by washing with 3 ml of phosphate-buffered saline (PBS) containing 3 mM EDTA.

2.5. Total and differential leukocyte counts

Cells from peritoneal exudates were incubated for 1 h by Turk's solution (0.01% crystal violet in 3% acetic acid) to eliminate red blood cells, and then the number of the total leukocytes was counted by the hematocytometer. For the differential leukocyte counts, a cytospin centrifuge was used to concentrate cells from peritoneal exudates onto microscope slides in a circle with 6 mm diameter. The cells were then subjected to Wright-Giemsa staining.

2.6. Phagocytosis assay

To assess the percentage of phagocytosis of macrophages *in vivo*, peritoneal exudate cells from mice were labeled with allophycocyanin (APC)-conjugated F4/80 antibody (eBioscience, San Diego, CA, USA), and permeabilized with 0.1% Triton X-100. The proportion of macrophages engulfing zymosan A-FITC particles ($F4/80^+$/zymosan A-$FITC^+$) was determined by flow cytometry or immunocytochemistry. A sterile irritant, thioglycollate has been used to enhance the yield of peritoneal macrophages. For measuring phagocytosis *ex vivo*, mice were administered intraperitoneal injection of thioglycollate medium (3%). After three days, peritoneal macrophages were isolated from thioglycollate-treated mice, and incubated in six-well flat-bottomed microtiter plates for 24 h. Adherent monolayer cells were co-incubated for 1 h with zymosan A-FITC or zymosan A-PE particles. Zymosan A particles engulfed by macrophages were then visualized under a Nikon Eclipse Ti microscope (Nikon, Tokyo, Japan). To determine the phagocytic activity of macrophages *in vitro*. FITC-labeled zymosan A were then co-incubated with RAW264.7 cells, and the proportion of RAW264.7 cells containing zymosan A particles (FITC-positive cells) was assessed by FACSCalibur™ Flow Cytometer (BD, Franklin Lakes, NJ, USA).

2.7. Flow cytometry for measurement of HO-1-expressing peritoneal macrophages

To measure the HO-1 expressing macrophages in vivo, peritoneal exudate cells from mice were fixed with 10% neutral-buffered formation solution for 30 min. The cells were labeled with APC-conjugated F4/80 antibody cells were fixed with 10% neutral-buffered formation solution for 30 min at room temperature, permeabilized with 0.2% Triton X-100 for 5 min, and blocked with 2% BSA in PBS for 30 min. Anti-HO-1 antibody, diluted at 1:100 with 2% BSA in PBS, was applied overnight at 4 °C. After washing with PBS, cells were incubated with PE-conjugated anti-rabbit IgG secondary antibody diluted at 1:1000 for 1 h. Cells were analyzed using FACSCalibur™ Flow Cytometer (BD, Franklin Lakes, NJ, USA).

2.8. Immunocytochemical analysis of HO-1

Peritoneal exudates were spun in a cytocentrifuge onto a slide. The cells were then subjected to immunocytochemical analysis of HO-1. After fixation with 10% neutral-buffered formalin solution for 30 min at room temperature, cells were permeabilized with 0.2% Triton X-100,