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A bis-malonic acid fullerene derivative significantly suppressed IL-33-induced IL-6 expression by inhibiting NF-κB activation

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ABSTRACT

IL-33 functions as a ligand for ST2L, which is mainly expressed in immune cells, including mast cells. IL-33 is a potent inducer of pro-inflammatory cytokines, such as IL-6, and has been implicated in the pathogenesis of allergic inflammatory diseases. Therefore, IL-33 has recently been attracting attention as a new target for the treatment of inflammatory diseases. In the present study, we demonstrated that a water-soluble bis-malonic acid fullerene derivative (C_{60}-dicyclopropane-1,1,1′,1′-tetracarboxylic acid) markedly diminished the IL-33-induced expression of IL-6 in bone marrow-derived mast cells (BMMC). The bis-malonic acid fullerene derivative suppressed the canonical signaling steps required for NF-κB activation such as the degradation of IκBα and nuclear translocation of NF-κB by directly inhibiting the IL-33-induced IKK activation. Although p38 and JNK also contributed to IL-33-induced expression of IL-6, the bis-malonic acid fullerene derivative did not affect their activation. Furthermore, the bis-malonic acid fullerene derivative had no effect on the NF-κB activation pathway induced by TNFα and IL-1. These results suggest that the bis-malonic fullerene derivative has potential as a specific drug for the treatment of IL-33-induced inflammatory diseases by specifically inhibiting the NF-κB activation pathway.

1. Introduction

ST2L is a member of the interleukin (IL)-1 receptor (IL-1R)/Toll-like receptor (TLR) superfamily and expressed on several immune cells, including Th2 lymphocytes, and mast cells [1,2]. Schmitz et al. identified IL-33 as a functional ligand of ST2L in 2005 [3], and the physiological function of IL-33 through ST2L has since been elucidated in detail. The finding of alterations in the mRNA and protein expression of IL-33 in the serum and mucosa of patients with inflammatory diseases was of importance. The expression of IL-33 was previously shown to be enhanced by asthma, and anaphylactic shock [4,5]. Furthermore, the secretion of chemical mediators from mast cells was augmented in patients with these conditions, and was attributed to the stimulating effects of IL-33, suggesting that IL-33 contributes to the pathogenesis of chronic autoimmune diseases and inflammatory diseases [6]. These findings clearly demonstrate the importance of IL-33 in the onset of inflammatory diseases, and have prompted research with a focus on IL-33 as a target for new therapeutic anti-inflammatory drugs.

Once stimulated with IL-33, ST2L forms a protein complex with the IL-1R accessory protein (IL-1RAcP) that activates downstream signaling molecules such as the mitogen-activated protein (MAP) kinase family and nuclear factor-kappa B (NF-κB), which is a critical transcription factor in inflammation [7,8]. The ST2L-IL-1RAcP complex has also been shown to recruit signaling molecules including myeloid differentiation factor 88 (MyD88), IL-1R-associated kinase-1 (IRAK-1), and tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6), similar to other members of the IL-1R/TLR superfamily [3,9]. The formation of this signaling complex is known to activate the canonical pathway of NF-κB. IκB kinase (IKK), which is downstream of TRAF6, phosphorylates two N-terminal serine residues of the inhibitor of NF-κB (IκBα). Phosphorylated IκBα is recognized by the ubiquitin ligase mechanism, which results in polyubiquitination and proteasomal degradation [10]. The released NF-κB complex has been shown to translocate to the nucleus, in which it binds to specific sequences in the promoter or enhancer regions of target genes involved in inflammatory reactions [11,12].

Fullerene (C_{60}) is a spherical molecule with a diameter of 0.7 nm and is a new kind of organic compound with a cage-like structure [13]. Chemical modifications with several water-soluble groups into the

Abbreviations: BMMC, bone marrow-derived mast cells; BSA, bovine serum albumin; CARMA 3, CARD-containing MAGUK protein 3; DMSO, dimethyl sulfoxide; FBS, fetal bovine serum; IκBα, inhibitor of NF-kappaB; IKK, IκB kinase; IL, interleukin; IL-1R, interleukin 1 receptor; IL-1RAcP, IL-1R accessory protein; IRAK-1, IL-1R-associated kinase-1; JNK, c-Jun. N-terminal kinase; LPA, lysophosphatidic acid; MAP kinase, mitogen-activated protein kinase; MEKK1, MEK kinase 1; MyD88, myeloid differentiation factor 88; NF-κB, nuclear factor-kappa B; NIK, NF-κB inducing kinase; PBS, phosphate-buffered saline; RT-PCR, reverse transcription-polymerase chain reaction; ROS, reactive oxygen species; TIR, Toll/IL-1 receptor; TLR, Toll-like receptor; TNF, tumor necrosis factor; TRAF6, TNF receptor-associated factor 6; WST-1, water-soluble tetrazolium-1.

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fullerene core have increased its solubility, and water-soluble fullerene derivatives were previously reported to possess various biological and pharmacological properties [14]. A pyrrolidinium fullerene derivative has been shown to exhibit anti-cell proliferative activities by inducing apoptosis, which was attributed to the generation of reactive oxygen species (ROS), indicating that fullerene derivatives have potential as anti-cancer drugs [15,16]. A previous study reported that proline-modified fullerene derivative exhibited inhibitory activities against human immunodeficiency virus (HIV)-reverse transcriptase and Hepatitis C virus RNA polymerase, which demonstrated that fullerene derivatives may be candidates for antiviral agents [17].

In the present study, we found that a bis-malonic acid fullerene derivative significantly inhibited the IL-33-induced expression of IL-6 by inhibiting the NF-κB activation pathway. These results clearly show the potential of the bis-malonic acid fullerene derivative as a novel anti-inflammatory drug.

2. Materials and methods

2.1. Antibodies and reagents

A bis-malonic acid fullerene derivative was synthesized as previously described [18,19]. Recombinant murine IL-33, IL-1β, and TNFα were purchased from PEPROTECH (Rocky Hill, NJ, USA). Antibodies against NF-κB (p65), Lamin B, IκBα, IKKγ, and β-actin were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Anti-GST antibody was purchased from Nacalai Tesque (Tokyo, Japan). Antibodies against JNK, phosphorylated JNK at Thr^{183}/Tyr^{185}, p38, phosphorylated p38 at Thr^{180}/Tyr^{182}, phosphorylated ATF-2 at $Thr^{69/71}$, phosphorylated IκBα at Ser^{32}, and phosphorylated NF-κB (p65) at Ser^{536} were purchased from Cell Signaling Technology (Danvers, MA, USA). Horseradish peroxidase-conjugated anti-rabbit, anti-goat, and anti-mouse polyclonal IgG antibodies were purchased from Dako-Japan (Tokyo, Japan).

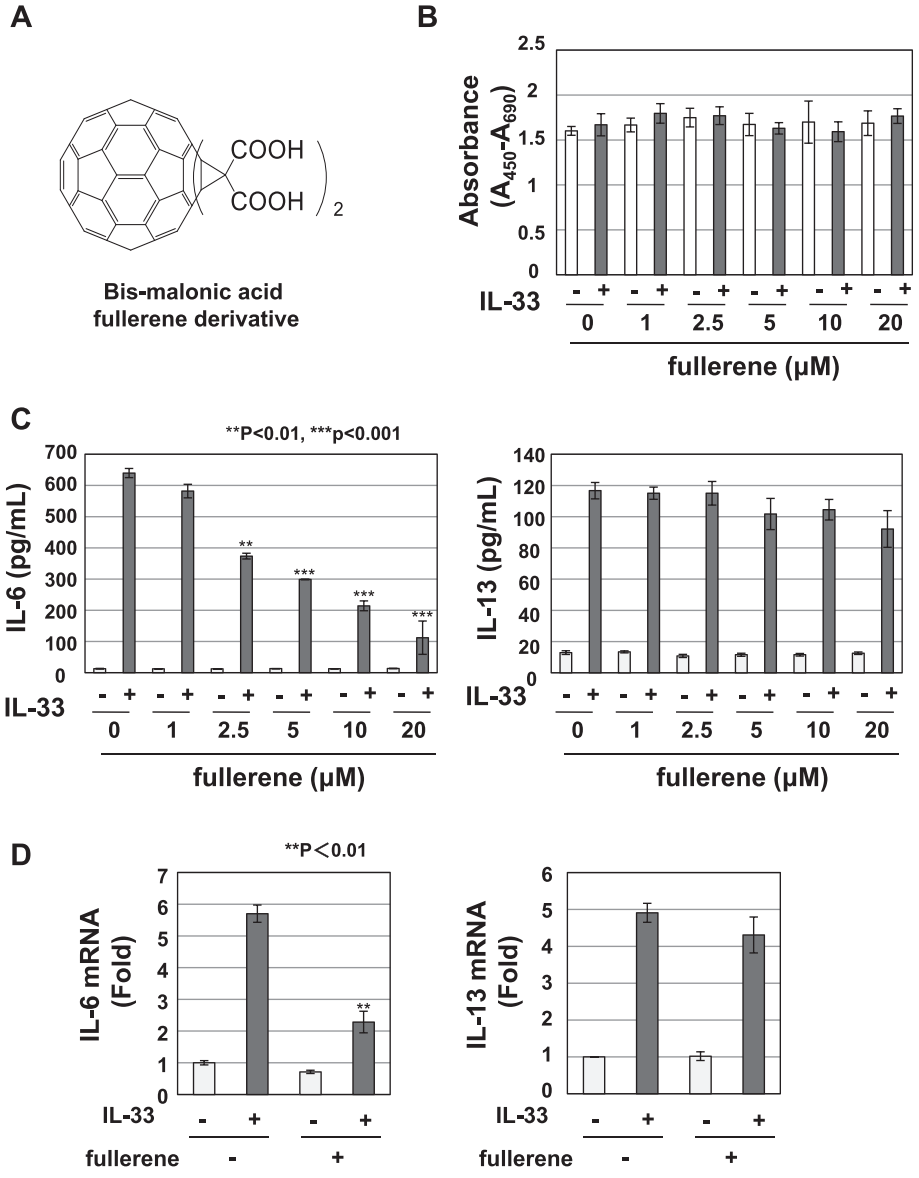

Fig. 1. The bis-malonic acid fullerene derivative significantly inhibited the IL-33-induced expression of IL-6. (A) Structure of the bis-malonic acid fullerene derivative. (B, C) BMMC were pretreated with DMSO (0.1%) or the bis-malonic acid fullerene derivative (1, 2.5, 5, 10, 20 μM) at 37 °C for 1 h prior to the stimulation with IL-33 (10 ng/mL) for 18 h. (B) WST-1 was added to these cells, which were then incubated for 2 h. Absorbance was measured using a microplate spectrophotometer. Values are the mean ± S.D. of three independent experiments. (C) IL-6 and IL-13 in the cultured supernatants was measured by ELISA. Data are shown as the mean ± S.D. of three independent experiments. ** and *** indicate $p < 0.01$ and $p < 0.001$, respectively. (D) BMMC were pretreated with DMSO (0.1%) or the bis-malonic acid fullerene derivative (10 μM) at 37 °C for 1 h prior to the stimulation with IL-33 (10 ng/mL) for 1.5 h. Total RNA was prepared and the expression of IL-6 mRNA and IL-13 mRNA was analyzed by quantitative real-time PCR. GAPDH mRNA was analyzed as an internal control. Data are the mean ± S.D. of the relative expression levels in three experiments. ** indicates $p < 0.01$.