Research Article

Capsaicin, a TRPV1 Ligand, Suppresses Bone Resorption by Inhibiting the Prostaglandin E Production of Osteoblasts, and Attenuates the Inflammatory Bone Loss Induced by Lipopolysaccharide

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Capsaicin, a transient receptor potential vanilloid type 1 (TRPV1) ligand, regulates nerve-related pain-sensitive signals, inflammation, and cancer growth. Capsaicin suppresses interleukin-1-induced osteoclast differentiation, but its roles in bone tissues and bone diseases are not known. This study examined the effects of capsaicin on inflammatory bone resorption and prostaglandin E (PGE) production induced by lipopolysaccharide (LPS) in vitro and on bone mass in LPS-treated mice in vivo. Capsaicin suppressed osteoclast formation, bone resorption, and PGE production induced by LPS in vitro. Capsaicin suppressed the expression of cyclooxygenase-2 (COX-2) and membrane-bound PGE synthase-1 (mPGES-1) mRNAs and PGE production induced by LPS in osteoblasts. Capsaicin may suppress PGE production by inhibiting the expression of COX-2 and mPGES-1 in osteoblasts and LPS-induced bone resorption by TRPV1 signals because osteoblasts express TRPV1. LPS treatment markedly induced bone loss in the femur in mice, and capsaicin significantly restored the inflammatory bone loss induced by LPS in mice. TRPV1 ligands like capsaicin may therefore be potentially useful as clinical drugs targeting bone diseases associated with inflammatory bone resorption.

1. Introduction

Bone remodeling is regulated by osteoclastic bone resorption and new bone formation by osteoblasts. Prostaglandin E₂ (PGE₂) is mainly produced by osteoblasts during bone resorption associated with inflammation and acts as a potent stimulator of bone resorption [1]. Inflammatory cytokines, such as interleukin (IL)-1 and tumor necrosis factor α (TNFα) which induces PGE₂ production by osteoblasts, and PGE₂ are able to induce the expression of receptor activator of NF-κB ligand (RANKL) on the surface of osteoblasts. Two types of cyclooxygenase (COX), COX-1 and COX-2, are expressed in osteoblasts, and the expression of COX-2 is markedly induced by inflammatory stimulants. The blockage of PGE₂ synthesis by nonsteroidal anti-inflammatory drugs (NSAIDs) could suppress RANKL-dependent osteoclastic bone resorption associated with inflammation [2, 3]. Membrane-bound PGE synthase-1 (mPGES-1) is also essential for PGE₂ synthesis in osteoblasts and PGE-mediated osteoclast differentiation [2, 3]. An mPGES-1-null mouse demonstrated that PGE₂ production by osteoblasts is essential for inflammatory bone loss induced by lipopolysaccharide (LPS) in vivo [4].

The transient receptor potential (TRP) subfamily of ion channels contains six members (TRPV1–TRPV6), most of which were thermal-sensitive cation channels. TRPV1 has
been cloned from a cDNA library of the dorsal root ganglion in sensory neurons, and the TRPV1 signal is suggested to be a potential regulator of pain associated with thermal stimulation [5, 6]. Capsaicin is a natural ligand for TRPV1 that regulates nerve-related pain-sensitive signals, inflammation, and cancer growth [7]. Mouse osteoblasts express TRPV1, and capsaicin suppresses IL-1-induced osteoclast differentiation in the cocultures of mouse bone marrow cells and osteoblasts [8]. Rossi et al. [9] reported that human preosteoclasts express TRPV1 and its signal modulates RANKL-mediated osteoclast differentiation. Recent studies suggesting some of the possible roles of TRPV1 in bone metabolism are controversial, and the physiological and pharmacological effects of TRPV1 signals are not known in bone tissues. The present study examined the effects of capsaicin, a natural TRPV1 ligand, on inflammatory bone resorption in vitro and in vivo. These results showed that the TRPV1 signal suppressed osteoclastic bone resorption associated with PGE2 production in vitro and attenuated inflammatory bone loss induced by LPS in vivo.

2. Materials and Methods

2.1. Materials and Animals. Capsaicin (Figure 1(a)) was obtained from Wako Pure Chemical Co. Ltd. (Tokyo, Japan). LPS (Escherichia coli O55:B5) was purchased from Becton Dickinson Co. Ltd. (Franklin lakes, NJ). PGE2 was obtained from Sigma-Aldrich Co. LLC (St Louis, MO). Newborn (2-day-old and 5-day-old) and adult (6-week-old) ddy mice were obtained from Japan SLC Inc. (Shizuoka, Japan). A PGE2 enzyme immunoassay (EIA) kit was purchased from Amersham Co. Ltd. (Aylesbury, UK). All other chemicals were of analytical grade.

2.2. Primary Mouse Osteoblastic Cells. Primary osteoblastic cells were isolated from newborn mice after five routine sequential digestions with 0.1% collagenase (Wako Pure Chemical) and 0.2% dispase (Godo Shusei Co. Ltd, Tomakomai, Japan) as previously described [3]. Osteoblastic cells collected from fractions 2–4 were combined and cultured in α-modified MEM (αMEM) supplemented with 10% fetal calf serum (FCS) at 37°C under 5% CO2 in air.

2.3. Osteoclast Formation. Mouse bone marrow cells were cocultured with the primary osteoblastic cells for 7 days. The cells adhering to the well surface were stained for tartrate-resistant acid phosphatase (TRAP), and TRAP-positive multinucleated cells containing three or more nuclei per cell were counted as osteoclasts as previously described [10]. The concentrations of PGE2 in the cultured medium were determined using an enzyme immunoassay, as previously described [10].

2.4. Bone-Resorbing Activity in Organ Culture of Mouse Calvariae. Mouse calvariae were collected from 5-day-old mice and then were cultured for 24 h in BG5b medium containing 1 mg/mL bovine serum albumin (BSA). The calvaria was transferred into new medium, with or without LPS after 24 h, and then was cultured for 5 days at 37°C under 5% CO2 in air. The bone-resorbing activity was determined by measuring the concentration of calcium in the conditioned medium using a calcium kit (calcium C test; Wako Pure Chemical) as reported previously [2]. The bone-resorbing activity was expressed as an increase in medium calcium, which is consistent with the osteoclastic bone resorption as shown in the previous studies [2].

2.5. RT-PCR Analysis. Primary mouse osteoblastic cells were cultured for 24 h in αMEM containing 1% FCS then treated with LPS for 3 h. The total RNA was extracted from mouse osteoblasts using the acid guanidium-phenol-chloroform method. cDNA was synthesized from 10 μg of total RNA by reverse transcriptase (Superscript II Preamplification System; Invitrogen Life Technologies Co., Carlsbad, CA) and amplified using PCR. Primers for the mouse COX-1, COX-2, mPGES-1, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes were used in PCR as reported previously [4]. The PCR product was separated on a 1% agarose gel and stained with ethidium bromide. The signals were quantified using an image analyzer.

2.6. Inflammatory Bone Loss in Mice. Six-week-old mice were i.p. injected with LPS (10 mg/kg body weight) on days 0 and 4. The LPS was dissolved in PBS for injection. The mice in the control group were injected with PBS. Some mice were i.p. injected with capsaicin (1 mg/kg body weight). The femurs were collected 8 days after the first injection of LPS or phosphate-buffered saline (PBS). For each group, 6–9 mice were used. The bone mineral density (BMD) of femurs was measured by dual X-ray absorptiometry (model DCS-600R; Aloka, Co. Ltd, Tokyo, Japan) as reported previously [4]. The bone mineral content of the femurs was closely correlated with the ash weight [4]. The BMD was calculated by dividing the bone mineral content of the measured area by the area.

2.7. Statistical Analysis. The data are expressed as the mean ± SEM. The significant differences were analyzed using Student’s t-test.

3. Results

LPS markedly induced osteoclast differentiation in cocultures of mouse bone marrow cells and osteoblasts on day 7, while 30 μM of capsaicin completely suppressed the osteoclast formation induced by LPS (Figure 1(b)). The conditioned coculture medium was examined to ELISA to determine the effects of capsaicin on PGE2 production induced by LPS. The level of PGE2 in the conditioned medium treated with LPS was higher than that of the control and was clearly suppressed by adding 30 μM capsaicin in the coculture (Figure 1(c)). Bone-resorbing activity was measured by the increase in calcium in the conditioned medium. LPS markedly induced bone-resorbing activity in calvarial cultures. The addition of 30 μM capsaicin significantly suppressed the bone-resorbing activity induced by LPS (Figure 1(d)). These results indicate that capsaicin clearly
suppressed the osteoclastic bone resorption associated with inflammation in vitro.

PGE₂ is mainly produced by osteoblasts in bone tissues. The expression of COX-2 and mPGES-1 is essential for PGE₂ production by osteoblasts treated with bone-resorbing cytokines such as IL-1 and LPS, and the PGE₂ production is essential for inflammatory bone resorption [4]. An RT-PCR analysis showed the expression of COX-2 and mPGES-1
4. Discussion

The present study showed that capsaicin, a TRPV1 ligand, suppresses LPS-induced osteoclastic bone resorption associated with inflammation by inhibiting PGE₂ production by osteoblasts. PGE₂ is a typical inducer of bone resorption associated with inflammation. We have reported that PGE₂ binds to the EP4 receptor, one of the PGE receptor subtypes EP1-EP4, and induces the RANKL expression to stimulate bone resorption, using agonist and antagonist of EPs and respective EP-knockout mice [2, 11]. PGE₂ produced by osteoblasts binds to EP4 in osteoblasts and induces RANKL expression via EP4-mediated signals. LPS markedly induces the expression of COX-2 and mPGES-1 mRNAs in osteoblasts, and the PGE₂ production is essential for RANKL-dependent osteoclast formation [4]. The transcriptional regulation is important to understand the biological significance in target tissues because mPGES-1 is an inducible terminal enzyme associated with PGE2 biosynthesis. The mRNA expression of mPGES-1 is coupled with COX-2, and the induction of COX-2 mRNA proceeded mPGES-1 after adding LPS. The mouse COX-2 gene promoter possesses functional regulatory elements for NF-κB, NFIL-6, AP-1, and c/EBPα [12]. In contrast, the mouse mPGES-1 gene promoter possesses AP-1 and c/EBP, but not NF-κB [13]. Capsaicin clearly suppressed the LPS-induced expression of COX-2 and mPGES-1 mRNAs in osteoblasts in the present study. Further studies are needed to define the transcriptional regulation of COX-2 and mPGES-1 by capsaicin in osteoblasts.

Capsaicin, a typical ligand for TRPV1, is derived from chili peppers, which elicit a burning sensation through TRPV1 [5, 6]. Mouse primary osteoblasts and osteoblast cell line MC3T3-E1 express TRPV1, but expression of TRPV1 mRNA is not detected in bone marrow macrophages, and that capsaicin suppresses IL-1-induced osteoclast differentiation in the cocultures of mouse bone marrow cells and osteoblasts [8]. In addition, both resiniferatoxin, a natural TRPV1 agonist, and olvanil, a synthetic TRPV1 agonist, suppress osteoclast formation induced by IL-1 in the cocultures (Miyaura and Inada; unpublished data). The present study
TRPV1 antagonist, acts on preosteoclasts and suppresses the LPS in mice (Figure 3). Therefore, TRPV1 ligands possess potential as clinical drugs targeting bone diseases associated with inflammation. The current study showed that capsaicin significantly restored inflammatory bone loss in the femur induced by LPS in mice (Figure 3). Therefore, TRPV1 ligands possess potential as clinical drugs targeting bone diseases associated with inflammatory bone resorption. Sancho et al. [16] reported that capsaicin has the potential to protect against inflammatory bowel diseases associated with severe inflammation, thus indicating the anti-inflammatory effects of TRPV1 signal in intestinal diseases. Idris et al. [15] reported that the TRPV1 antagonist capsazepine inhibits bone loss due to estrogen deficiency in ovariectomized (OVX) mice. However, the mechanisms of bone loss in OVX animals differ from that in LPS-treated animals accompanied with severe inflammation. In addition, Clark et al. [17] reported that TRPV4, a calcium-permeable ion channel, possesses chondroprotective role and deletion of TRPV4 gene resulted in severe osteoarthritis using TRPV4 knockout mice. Further studies using several animal models for bone and cartilage diseases are needed to define the possible roles of TRPVs signals in bone.

References


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