Capsaicin attenuates LPS-induced inflammatory cytokine production by upregulation of LXRα

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Here, we investigated the role of LXRα in capsaicin mediated anti-inflammatory effects. Results revealed that capsaicin inhibits LPS-induced IL-1β, IL-6 and TNF-α production in a time- and dose-dependent manner. Moreover, capsaicin increases LXRα expression through PPARγ pathway. Inhibition of LXRα activation by siRNA diminished the inhibitory action of capsaicin on LPS-induced IL-1β, IL-6 and TNF-α production. Additionally, LXRα siRNA abrogated the inhibitory action of capsaicin on p65 NF-κB protein expression. Thus, we propose that the anti-inflammatory effects of capsaicin are LXRα dependent, and LXRα may potentially link the capsaicin mediated PPARγ activation and NF-κB inhibition in LPS-induced inflammatory response.

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1. Introduction

Capsaicin is an active component accounting for the pungency of chili peppers. It has been shown to have complex pharmacological actions [1]. Currently, it is used to relieve the pain of peripheral neuropathy such as post-herpetic neuralgia caused by shingles. It may also be used as a cream for the temporary relief of minor aches and pains of muscles and joints associated with arthritis, simple backache, strains and sprains [2–4]. In addition to the analgesic effect, capsaicin has anti-inflammatory properties, because it reduces expression of several pro-inflammatory cytokines, chemokines, cell adhesion molecules and the function of immune cells [5–8]. Transient receptor potential vanilloid 1 (TRPV1) is a specific receptor for capsaicin [9]. However, studies have shown that the inhibitory action of capsaicin on the release of pro-inflammatory molecules was not mediated by a TRPV1, indicating the involvement of an alternative mechanism [10,11].

Liver X receptor α (LXRα) and LXRβ are members of the nuclear hormone receptor superfamily. Originally, both of them have been described as regulators of cholesterol metabolism, but recent data suggest that these receptors may be directly implicated in inflammation and immune regulation [12,13]. In macrophages, LXR activation inhibits the gene transcription of various inflammatory genes, including TNF-α, COX-2, IL-1β, MMP-9 and iNOS [14]. In addition, LXR agonists demonstrate anti-inflammatory effects in various animal models of inflammation [15–17]. Our previous studies have also shown that activation of LXRs with T0901317 has a protective role in LPS-induced lung injury [18]. The ability of LXRs to integrate metabolic and inflammatory signaling makes them attractive targets for pharmacologic intervention in metabolic and inflammatory diseases [19–21].

LXRα has been identified as a target gene of peroxisome proliferator-activated receptor gamma (PPARγ). PPARγ activation induces the expression of LXRα [20,22]. Kim CS et al. have indicated that capsaicin induces apoptosis by activation of the PPARγ in HT-29 human colon cancer cells. Interestingly [23], Park JY et al. have shown that capsaicin directly acts as a PPARγ agonist and then inhibits the production of TNF-α in LPS-stimulated murine macrophages [11]. A recent study has suggested that capsaicin protects against oxLDL-induced lipid accumulation with the LXRα pathway in macrophages [24]. Thus, we hypothesized that the anti-inflammatory activity of capsaicin might be mediated by promotion of LXRα expression. In the present study, we demonstrated that capsaicin inhibits IL-1β, IL-6 and TNF-α production in LPS-stimulated THP-1 macrophage cells. This effect is, at least partially, mediated by up-regulation of LXRα expression.

2. Materials and methods

2.1. Materials

LPS (L4524, from E. coli 055:B5) and capsaicin were obtained from Sigma (St. Louis, MO). T0070907 was from Cayman Chemical Co. (Ann Arbor, MI). Rabbit polyclonal anti-LXRα, anti-p65 NF-κB, and anti-β-actin antibody were obtained from Abcam (Cambridge, UK).
2.2. Cell culture

Human THP-1 cells were cultured in RPMI-1640 supplemented with 0.1% nonessential amino acids, penicillin (100 U/mL), streptomycin (100 μg/mL) and 20% fetal bovine serum (FBS) at 37 °C in 5% CO₂ at a cell density of 0.2 to 1.0 × 10⁶/mL. After 3 to 4 days, cells were treated with PMA (160 nmol/L) for 24 h to become fully differentiated macrophages before their use in experiments.

2.3. Transfection of siRNA

The siRNA against LXRα and an irrelevant 21-nucleotide siRNA duplex which was used as a control were purchased from the Biology Engineering Corporation in Shanghai, China. THP-1 macrophage cells (2 × 10⁶ cells/well) were transfected with the siRNA of LXRα, or control, in the absence or presence of appropriate plasmids using Lipofectamine 2000 (Invitrogen). After 4 h incubation, the medium was changed to medium A, and real-time RT-PCR was performed. In comparison to the control siRNA, the siRNA of LXRα and STAT3 suppressed the expression of these proteins by 85%, according to immunohistochemistry analysis.

2.4. ELISA

After stimulation with LPS at the indicated time point, the cell supernatants were collected and analyzed using a Quantikine ELISA Kit for TNF-α, IL-1β, and IL-6 (R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions.

2.5. RNA isolation and RT-PCR

Total RNA from cells was extracted by TRizol reagent or the mirVana miRNA Isolation Kit (Ambion) in accordance with the manufacturer’s instructions. Real-time quantitative PCR, using SYBR Green detection chemistry, was performed on Roche light Cycler Run 5.32 Real-Time PCR System. The primer sequences used were the following: LXRα: forward primer: 5'-ACGAGCTATGCAGTGATATGTGGG-3', reverse primer: 5'-CTCTTCTTGATGCTTCAGTTTCCC-3' and GAPDH: forward primer: 5'-TCACATCTTCCAGGAGCGAG-3', reverse primer: 5'-TGTCGCTGTTGAAGTCAGA-3'. Melt curve analyses of all real-time PCR products were performed and shown to produce a single DNA duplex. Quantitative measurements were determined by using the ΔΔCt method and expression of β-actin was used as the internal control.

2.6. Western blot analyses

Cellular and whole-tissue proteins were extracted. Equal amount of total proteins (40 μg) was loaded onto 10% SDS-PAGE, fractionated by electrophoresis, and transferred to polyvinylidene fluoride (PVDF) membranes. The primary antibodies were used against p65 NF-κB and β-actin. Protein bands were visualized with ECL plus chemiluminescent substrate.

2.7. Immunohistochemistry

Cells were fixed in 4% paraformaldehyde in PBS for 30 min. The cells were washed with PBS and incubated for 20 min at 4 °C with
permeabilization buffer containing 0.1% Triton X-100 in PBS. Samples were blocked with 2% bovine serum albumin (BSA) in PBS for 30 min and incubated with rabbit anti-LRXα for 12 h at 4°C. After washing three times with PBS, the secondary antibody was added. Then DAB was used. Hematoxylin staining, 10 min. Fluorescent-labeled cells were observed using an inverted microscope.

2.8. Statistical analysis

Data are expressed as means ± S.D. Results were analyzed by one-way ANOVA and Student’s t test, using SPSS 13.0 software. Statistical significance was obtained when P values were less than 0.05.

3. Results

3.1. Capsaicin inhibits LPS-induced pro-inflammatory cytokine production

To confirm the role of capsaicin on LPS-induced inflammatory responses, the effects of capsaicin on IL-1β, IL-6 and TNF-α production in LPS-stimulated THP-1 macrophage cells were detected by ELISA. As shown in Fig. 1A–C, LPS increased the secretion of IL-1β, IL-6 and TNF-α in a time-dependent manner. However, simultaneous application of capsaicin with LPS significantly decreased IL-1β, IL-6 and TNF-α levels at 6 h and 12 h. To further evaluate the anti-inflammatory effects of capsaicin, different concentrations of capsaicin were used. As shown in Fig. 1D–F, LPS increased the secretion of IL-1β, IL-6 and TNF-α. However, the increased IL-1β, IL-6 and TNF-α levels were significantly diminished by simultaneous application of capsaicin in a dose-dependent manner. These data suggested that capsaicin inhibits LPS-induced pro-inflammatory cytokine production in a time- and dose-dependent manner.

3.2. Capsaicin upregulates LXRα expression through PPARγ pathway

Anti-inflammatory mechanism of capsaicin was associated with PPARγ activation [11]. Moreover, LXRα is one of the target genes of PPARγ [22]. To examine whether LXRα can be induced by capsaicin, we determined its expression level in THP-1 macrophage cells after capsaicin treatment by qRT-PCR, western blot analysis and immunohistochemistry analysis. As shown in Fig. 2A–D, capsaicin significantly increased the LXRα mRNA and protein expression. However, such action of capsaicin was abrogated by the specific PPARγ antagonist T0070907. These data suggested that capsaicin upregulates LXRα expression through PPARγ pathway.

Fig. 2. Effects of capsaicin on LXRα expression. A–C. THP-1 macrophage cells were treated with capsaicin (10 µM) or simultaneous with T0070907 (10 µM) for 6 h. The levels of LXRα mRNA and protein expression were analyzed by qRT-PCR and immunohistochemistry analysis. C. The IOD of LXRα positive signals in each group was calculated. Data are mean ± S.D. n = 3. *P < 0.05 vs control group, #P < 0.05 vs capsaicin. (magnification ×400).
3.3. LXRα participates in the inhibitory effects of capsaicin on LPS-induced pro-inflammatory cytokine production

To further confirm that LXRα was responsible for capsaicin inhibiting cytokine release, a lost-of-function approach was applied. As shown in Fig. 3A–C, treatment with siRNA for down-regulated LXRα protein expression. LXRα siRNA abrogated the inhibitory action of capsaicin on LPS-induced IL-1β, IL-6 and TNF-α production in THP-1 macrophage cells (Fig. 3D–F). These results implied that the inhibitory action of capsaicin against pro-inflammatory cytokine production in LPS-stimulated THP-1 macrophage cells is mediated by LXRα.

3.4. LXRα participates in the inhibitory effects of capsaicin on NF-κB activation

In non-neuronal cells, anti-inflammatory mechanism of capsaicin was also shown to associate with inactivation of NF-κB pathway [10, 25]. To test the role of LXRα in capsaicin mediated NF-κB inhibition, p65 NF-κB protein expression was evaluated by western blot analysis. As shown in Fig. 4, LPS induced the p65 NF-κB protein expression. Capsaicin markedly decreased the p65 NF-κB protein expression. However, LXRα siRNA abrogated the inhibitory action of capsaicin on p65 NF-κB protein expression. These data suggested that NF-κB pathway

Fig. 3. Effects of LXRα on capsaicin mediated pro-inflammatory cytokine production. A. THP-1 macrophage cells were transfected with or without LXRα siRNA (20 μM) for 24, and then LXRα protein expression was measured by immunohistochemistry analysis. B. The IOD of LXRα positive signals in each group was calculated. C–E. THP-1 macrophage cells were transfected with or without LXRα siRNA (20 μM) for 24 h, and then treated with capsaicin (10 μM) or LPS (100 ng/mL) for 6 h. The levels of IL-1β, IL-6 and TNF-α in the culture medium were measured by ELISA. Data are mean ± S.D. for three independent experiments performed in triplicate. #P < 0.05 vs LPS group, *P < 0.05 vs capsaicin + LPS group.

Fig. 4. Effect of LXRα on the inhibitory action of capsaicin on LPS-induced NF-κB activation. THP-1 macrophage cells were transfected with or without LXRα siRNA (20 μM) for 24 h, and then treated with capsaicin (10 μM) or LPS (100 ng/mL) for 6 h. Western blot analysis of p65 NF-κB protein of a representative experiment out of three performed was shown.

Fig. 5. Schematic showing the intracellular signaling pathways for the anti-inflammatory action of capsaicin in macrophages. CAP, capsaicin; PPARγ, Peroxisome proliferator activated receptors γ; PPRE, Peroxisome proliferator response element; LPS, Lipo-poly saccharides; TLR4, Toll like receptor 4; NF-κB, nuclear factor kappa B; IκB, I kappa B; LXRα, Liver X receptor α.
may act as the downstream signaling of LXRα on capsaicin-mediated anti-inflammatory effects.

4. Discussion

In the present study, we demonstrated that capsaicin reduces pro-inflammatory cytokines IL-1β, IL-6 and TNF-α production in LPS-stimulated THP-1 macrophage cells in a time- and dose-dependent manner. Moreover, capsaicin increases LXRα expression through PPARγ pathway. Pre-treatment with LXRα siRNA resulted in stronger inhibitory action of capsaicin on LPS-induced pro-inflammatory cytokine production in THP-1 macrophage cells. Additionally, LXRα siRNA abrogated the inhibitory action of capsaicin on p65 NF-κB protein expression. As illustrated in Fig. 5, our data suggested that the inhibitory action of capsaicin against LPS-induced pro-inflammatory cytokine production in THP-1 macrophage cells is mediated by LXRxα activation, and LXRα may potentially link the capsaicin mediated PPARγ pathway activation and NF-κB pathway inhibition in LPS-induced inflammatory response.

Excessive or prolonged inflammatory responses to infection are detrimental to health and may even be lethal. An important mechanism in inflammation is the recruitment of macrophages and the release of pro-inflammatory cytokines [26,27]. IL-1β, IL-6 and TNF-α are well-known proinflammatory key mediators in pathogenesis of acute inflammatory diseases. Myeloid cells such as monocytes and neutrophils produce IL-1β, IL-6, and TNF-α, the three inflammatory hallmark cytokines constituting the cytokine storm during septic shock [28,29]. Here, we demonstrated that capsaicin inhibits IL-1β, IL-6 and TNF-α production in LPS-stimulated THP-1 macrophage cells. Similar to our study, it has been shown that capsaicin acts directly on macrophages and inhibits production of inflammatory cytokines, including IL-8, MIP-1, GM-CSF, IFN-γ and IL-2 [30,31]. It has also been shown that capsaicin can also inhibit inflammatory cytokines release in SGC-7901 and adipocytes [10,32]. Combining these and our findings in the present study, the anti-inflammatory activity of capsaicin may be due to its inhibitory effect on various cytokines.

PPARγ belongs to the ligand-activated nuclear receptor superfamily. Strategy of activating PPARγ has been shown to attenuate the production of certain inflammatory mediators and thus protect cell from inflammation-induced injuries [33,34]. Previous studies have shown that some biological functions of capsaicin were directly mediated by PPARγ pathway [11,23]. LXRxα has been identified as a target gene of PPARγ. PPARγ activation induces the expression of LXRxα [20,22]. The above mentioned prompt us to test whether the inhibitory effect of capsaicin is mediated by LXRxα. In the present study, we showed that capsaicin inhibiting LPS-induced inflammatory responses is LXRxα dependent. This notion is further supported by findings that capsaicin induced upregulation of LXRxα in THP-1 macrophage cells. Inhibition of LXRxα activation by siRNA diminished the inhibitory action of capsaicin on LPS-induced pro-inflammatory cytokine production. When we further explored the role of PPARγ in capsaicin mediated LXRxα expression. We found that capsaicin mediated LXRxα upregulation was abrogated by specific PPARγ antagonist T0070907. Thus, these data suggested that capsaicin-mediated anti-inflammatory effects are LXRxα dependent, and PPARγ may act as the upstream signal protein of LXRxα.

NF-κB, a family of inducible transcription factors, regulates the expression of specific genes involved in inflammation [35]. It is widely understood that activation of LXRxα blocks NF-κB-mediated inflammatory gene expression [18,36,37]. As an anti-inflammatory agent, capsaicin also inhibits NF-κB, leading to the reduction of pro-inflammatory mediators [10,25,32]. When we examined the role of LXRxα in capsaicin mediated NF-κB inhibition, we found that the inhibitory action of capsaicin on NF-κB expression was blocked by LXRxα inactivation with siRNA. These results clearly reveal that the inhibition of NF-κB activation by capsaicin may be associated with LXRxα activation.

In conclusion, our study demonstrated that capsaicin inhibited pro-inflammatory cytokine production in LPS-induced THP-1 macrophage cells. This effect is, at least partially, mediated by promotion of capsaicin-stimulated LXRxα expression. These observations define a novel mechanism of capsaicin in the control of inflammatory cytokine expression and support the potential utility of capsaicin to prevent and treat inflammatory disease.

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