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Original Article

Anti-inflammatory effect of cinnamaldehyde and linalool from the leaf essential oil of *Cinnamomum osmophloeum* Kanehira in endotoxin-induced mice

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ABSTRACT

Cinnamomum osmophloeum Kanehira is a Taiwan native plant that belongs to genus *Cinnamomum* and is also known as pseudocinnamomum or indigenous cinnamon. Its leaf is traditionally used by local people in cooking and as folk therapy. We previously demonstrated the chemical composition and anti-inflammatory effect of leaf essential oil of *Cinnamomum osmophloeum* Kanehira of linalool chemotype in streptozotocin-induced diabetic rats and on endotoxin-injected mice. The aim of the present study is to evaluate whether cinnamaldehyde and linalool the active anti-inflammatory compounds in leaf essential oil of *Cinnamomum osmophloeum* Kanehira. Before the injection of endotoxin, C57BL/6 mice of the experimental groups were administered cinnamaldehyde (0.45 or 0.9 mg/kg body weight) or linalool (2.6 or 5.2 mg/kg body weight), mice of the positive control group were administered the leaf essential oil (13 mg/kg body weight), and mice of the negative group were administered vehicle (corn oil, 4 mL/kg body weight) by gavage every other day for two weeks. All mice received endotoxin (i.p. 10 mg/mL/kg body weight) the next day after the final administration and were killed 12 h after the injection. Normal control mice were pretreated with vehicle followed by the injection with saline. None of the treatment found to affect body weight or food or water intake of mice before the injection of endotoxin. Cinnamaldehyde and linalool were found significantly reversed endotoxin-induced body weight loss and lymphoid organ enlargement compared with vehicle ($P < 0.05$). Both compounds also significantly lowered endotoxin-induced levels of peripheral nitrate/nitrite, interleukin (IL)-1β, IL-18, tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and High-mobility group box 1 protein (HMGB-1), and levels of nitrate/nitrite, IL-1β, TNF-α, and IFN-γ in spleen and mesenteric lymph nodes (MLNs) ($P < 0.05$). Endotoxin-induced expression of toll-like receptor 4 (TLR4), Myeloid differentiation primary response gene 88 (MyD88), myeloid differentiation protein 2 (MD2), Nod-like receptor family, pyrin domain containing 3 (NLRP3), apoptosis-associated speck-like protein

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containing a caspase-recruitment domain (ASC), and caspase-1 in spleen and mesenteric lymph nodes (MLNs) were inhibited by all tested doses of cinnamaldehyde and linalool ($P < 0.05$). Subsequently, the activation of nuclear factor (NF)-κB and the activity of caspase-1 in spleen and MLNs were also suppressed by these two compounds ($P < 0.05$). In addition, cinnamaldehyde and linalool at the dose equivalent to their corresponding content in the tested dose of the leaf essential oil, which was 0.9 mg/kg and 5.2 mg/kg, respectively, showed similar or slightly less inhibitory activity for most of these inflammatory parameters compared with that of the leaf essential oil. Our data confirmed the potential use of leaf essential oil of *Cinnamomum osmophloeum* Kanehira as an anti-inflammatory natural product and provide evidence for cinnamaldehyde and linalool as two potent agents for prophylactic use in health problems associated with inflammations that being attributed to over-activated TLR4 and/or NLRP3 signaling pathways.

1. Introduction

Pattern recognition receptors (PRRs) are proteins expressed mainly by cells of the innate immune system. Upon the recognition of pathogen-associated molecular patterns that associate with microbial pathogens (PAMPs), and damage-associated molecular patterns (DAMPs) that associate with cell components of damaged cell, PRRs initiate cascades of cellular signal transduction that eventually result in the generation of various inflammatory regulators, mainly a series of cytokines which help to conduct a complicated process for sterilizing purpose [1]. However, at time when such inflammatory response is out of control, it leads to tissue/organ damage. Currently, inflammation has been established as a major underlying mechanism of a great variety of acute and chronic clinical conditions associated with infectious and non-infectious problems. Consequently, various approaches with specific strategy to target inflammatory response have been developed for prophylactic and/or therapeutic use, among which the modulation of the activation of PRRs and downstream signaling pathways has raised great interest [2,3].

The membrane bound Toll-like receptors (TLRs) and the cytoplasmic Nod-like receptors (NLRs) are two of the mostly studied PRR families among which TLR4 and NLRP3 have been demonstrated to interact closely and contribute to the progressing of a great variety of inflammatory clinical conditions including several chronic and systemic inflammatory diseases [4,5]. Endotoxin-initiated inflammation is commonly used as a prototypical example to study the priming of such interaction due to the fact that this molecule from Gram-negative bacteria is the most well known TLR4 specific ligand and is recently established to be able to activate NLRP3 inflammasome [5]. When induced by endotoxin, TLR4 activates intracellular signaling pathway through the interaction with the co-receptor, MD2, which subsequently recruits the adaptor protein MyD88 thus activate the downstream signaling molecules [6]. In mice, the induction of either TLR4 mutation or MyD88 dysfunction caused lowered inflammatory response to endotoxin stimulation *in vivo* [7,8]. Although NLRP3 is not the corresponding receptor for endotoxin; however, the critical role of NLRP3 signaling pathway in endotoxin-induced inflammation has been revealed [5]. The activation of NLRP3 involves in two stages that require NF-κB to increase the expression of NLRP3 and downstream signal(s) of TLR4 to help deubiquitinate NLRP3 and to phosphorylate the adaptor protein, ASC, to allow the assembling of NLRP3 and ASC which then conjugate with procaspase-1 to transfer this enzyme to its active form, caspase-1 [4]. The activity of caspase-1 is essential for endotoxin-induced generation of IL-1β and IL-18 through the cleavage of pro-IL-1β and pro-IL-18 [5]. In either ASC- or caspase-1-deficient mice, it showed resistance to lethal dose of endotoxin-induced shock [9].

In general, the inflammatory status is indicated by level of molecules that elevated as a consequence of the activation of PPRs and commonly include IL-1β, TNF-α, and interferon IFN-γ. IL-18, the IFN-γ-inducing factor, is relatively rare to be used as a parameter of inflammation status. This may be due to the fact that this cytokine induces the production of IFN-γ by T lymphocytes [10], thus the presence of IFN-γ could well reflect the production of IL-18. Indeed, in murine the induction of endotoxinemia elevated levels of IL-18 and IFN-γ in lung while the treatment with anti-IL-18 antibody suppressed leukocyte infiltration in lung with decreased IFN-γ level [11]. High-mobility group protein 1 (HMG-1), also known as HMGB1, is a DAMP that secreted by endotoxin-, IFN-γ-, TNF-α-, or IL-1β-activated monocytes and macrophages that plays the role as a ligand of TLR4 and has recently been suggested a target for anti-inflammation therapy [12].

A variety of indigenous lauraceous plants in Taiwan has been found to possess anti-inflammatory activity that exerts by both volatile and nonvolatile components from these plants [13]. *Cinnamomum osmophloeum* Kanehira, a species of genus *Cinnamomum* that belongs to the plant family Lauraceae, is native in Taiwan which is also known as pseudo-cinnamomum or indigenous cinnamon and has been cultivated widely on the island and is traditionally used in cooking and as folk therapy to relief fever, arthritis, cold, gout, and general nerve pains. Despite these folk uses of *C. osmophloeum*, scientific evidences for prophylactic and/or

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