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Capsicum ethanol extracts and capsaicin enhance interleukin-2 and interferon-gamma production in cultured murine Peyer's patch cells ex vivo

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Abstract

We investigated the effects of red pepper (*Capsicum annuum* Lin.) extracts (capsicum extract) and its main pungent capsaicin on T helper 1 (Th1) and 2 (Th2) cytokine production in cultured murine Peyer's patch (PP) cells in vitro and ex vivo. Direct administration of capsicum extract (1 and 10 μg/ml) and capsaicin (3 and 30 μM) resulted in suppression of interleukin (IL)-2, interferon (IFN)-γ, IL-4 and IL-5 production. In an ex vivo experiment using PP cells removed from the mice after oral administration of capsicum extract (10 mg/kg/day for 4 consecutive days), IL-2, IFN-γ and IL-5 increased in response to concanavalin A (Con A). Oral administration of 3 mg/kg/day capsaicin, one active constituent of the extract, also enhanced IL-2, INF-γ and IL-4 production in response to Con A stimulation but did not influence the production of IL-5. Orally administered capsazepine (3 mg/kg/day), a selective transient receptor potential vanilloid 1 (TRPV1) antagonist, slightly enhanced IL-2 production also irrespective of Con A stimulation. The capsaicin-induced enhancement of both IL-2 and IFN-γ production was not reduced by oral administration of capsazepine (3 mg/kg/day), suggesting a TRPV1 receptor-independent mechanism. Flow cytometric analysis revealed that the population of $CD3^+$ cells in the PP cells was significantly reduced while $CD19^+$ cells increased after oral administration of capsicum extract (1 and 10 mg/kg/day) and capsaicin (0.3 and 3 mg/kg/day). Capsazepine (3 mg/kg/day) weakly but significantly reversed these effects. Orally administered capsicum extract and capsaicin did not change the T cell subset ($CD4^+$ and $CD8^+$), Th1 ($IFN\text{-}\gamma^+$) and T2 ($IL\text{-}4^+$) ratio. These findings indicate that capsicum extract and capsaicin modulate T cell-immune responses, and their immunomodulatory effects on murine PP cells are partly due to both TRPV1-dependent and -independent pathway.

Keywords: Capsicum; Capsaicin; Peyer's patch; Th1/Th2; Cytokines; TRPV1

Introduction

The inner surface of the intestinal tract possesses a large area of mucosal membranes, which are continuously exposed to various substances in the intestinal lumen (Mowat, 2003). Gut-associated lymphoid tissues exist on the intestinal mucosal site and play an important role in the immune system. Peyer's patches (PP) are considered to be lymphoid tissues where mucosal immune responses such as local IgA production and systemic immunological responses are induced (Mowat, 2003). Antigen presentation in PP is important in determining systemic immune responses including T and B cell-dependent immunity (Mowat, 2003; Yoshida et al., 2002). Orally administrated hot water extract of cultured mycelia of *Cordyceps sinensis* (Berk.) Sacc was previously shown to increase interleukin (IL)-6 and granulocyte-colony stimulating factor (GM-CSF) production by PP cells in mice (Koh et al., 2002). Juzen-taiho-to, a Kampo prescription, was also shown to enhance production of these cytokines in PP cells from C3H/HeJ mice (Hong et al., 1998). Moreover, we recently reported that culture filtrate of the medicinal entomogenous fungi *Paecilomyces tenuipes* (Peck) Samson (= *Isaria japonica* Yasuda or *Isaria tenuipes*) selectively

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enhanced T helper 1 cytokine production in cultured murine PP cells from C57BL/6J mice (Takano et al., 2005), and suggested that oral administration of the culture filtrate might increase immune responses (Takano et al., 1996), in part due to enhancement of these cytokines. Therefore, to elucidate the mechanisms of oral immune responses, examination of the cytokine modulating activities in cultured PP cells will be beneficial.

Capsicum annuum (Solanaceae) is used worldwide not only as a food, due to its pungency, but also in traditional medicine against gastric ulcers, rheumatism, alopecia and toothache (Szallasi and Blumberg, 1999). Capsaicin is the most well-known and pungent active ingredient of Capsicum. Large numbers of studies have established that capsaicin shows various pharmacological effects and is endowed with a pleiotropic pattern of biological activities, some of which are mediated by the activation of cellular targets different from vanilloid receptor 1 (Szallasi and Blumberg, 1999). Capsaicin has also been shown to have immunomodulatory effects, as indicated by its ability to modulate lymphocyte proliferation and immunoglobulin A, E and G production (Nilsson et al., 1991; Eglezos et al., 1990), regulate the expression of substance P and its receptor in monocytes (Ho et al., 1997), and inhibit cytosolic Ca^{2+} mobilization induced by platelet activating factor in the monocyte cell lines HL-60 (Choi et al., 2000). However, the systemic T-cell-dependent immune response after oral administration of capsaicin remains to be clarified.

In this study, we therefore examine the effects of ethanolic extracts of *C. annum*, capsaicin and a vanilloid receptor-specific antagonist, capsazepine, on the production of T-helper cytokines in cultured PP cells in vitro and ex vivo.

Materials and methods

Animals

Male C57BL/6J mice, 6 to 10 weeks of age, were purchased from Japan SLC (Shizuoka, Japan). Mice were housed in groups of five in plastic cages with a 12 h light: 12 h dark cycle and free access to water and food ad libitum. Adaptation to these conditions for at least 1 week was allowed before commencing the experiment. The experimental procedures complied with the guidelines of the Council for Experimental Animals, the Faculty of Pharmaceutical Sciences, Kanazawa University, Japan. Mice were sacrificed by anesthetization with an overdose of ether.

Materials

RPMI-1640 medium, phosphate-buffered saline (PBS), fetal bovine serum (FBS), penicillin and streptomycin were obtained from Invitrogen Corp. (Carlsbad, CA USA). Concanavalin A (Con A) (type IV), type I collagenase, capsaicin and capsazepine were obtained from Sigma Chem. Co. (St. Louis, MO). All other reagents were purchased from Wako Pure Chemical Co. (Tokyo, Japan). For analysis of the T, B and T cell subset ($CD4^+$ and $CD8^+$) in PP cells, the following monoclonal antibodies (mAb, Beckman Coulter Inc., Hialeah, FL) were used: anti-CD45RA-fluorescein isothiocyanate (FITC) antibodies (RA3-6B2, IgG2a), anti-CD3-FITC antibodies (KT3, IgG2a), anti-CD4-FITC antibodies (YTS191.1, IgG2b), anti-CD8-phycoerythin (PE) antibodies (KT15, IgG2a), anti-IFN-γ-FITC antibodies (XMG1.2, IgG1) and anti-IL-4-FITC antibodies (BVD-24G2, IgG1). The isotype-matched controls used in this experiment were IgG1 conjugated to FITC, IgG2a conjugated to PE, IgG2a conjugated to FITC and IgG2b conjugated to FITC.

Extraction and isolation

Capsicum annum L. for medicinal use was purchased from Uchida Wakanyaku Co. Ltd. (Tokyo, Japan). A voucher specimen of this plant (C05205) was deposited in our laboratory at the Faculty of Pharmaceutical Sciences, Kanazawa University, Japan.

The dried fruits (1.0 kg) of *C. annum* were macerated in 95% ethanol (EtOH) (1 L, 3 times) at room temperature for 24 h. After filtration, the ethanol solution was evaporated under reduced pressure to give ethanol extract (capsicum extract) (26 g). Capsicum extract (10 g) was then subjected to column chromatography on silica gel (500 g). Elution with a mixture of hexane–ethylacetate (AcOEt) and methanol (MeOH) gave 14 fractions: Fr-1 (5% AcOEt, 1200 ml, 698 mg), Fr-2, (25% AcOEt, 300 ml, 675 mg), Fr-3 (25% AcOEt, 300 ml, 120 mg), Fr-4 (25% AcOEt, 300 ml, 46 mg), Fr-5 (25% AcOEt, 300 ml, 54 mg), Fr-6 (35% AcOEt, 1200 ml, 458 mg), Fr-7 (50% AcOEt, 600 ml, 78 mg), Fr-8 (50% AcOEt, 600 ml, 210 mg), Fr-9 (65% AcOEt, 1200 ml, 1104 mg), Fr-10 (75% AcOEt, 600 ml, 119 mg), Fr-11 (75% AcOEt, 600 ml, 80 mg), Fr-12 (90% AcOEt, 600 ml, 141 mg), Fr-13 (100% AcOEt, 1200 ml, 2844 mg), and Fr-14 (100% MeOH, 1200 ml, 4319 mg). Fr-2 (10 mg) eluted with 25% AcOEt was determined as being

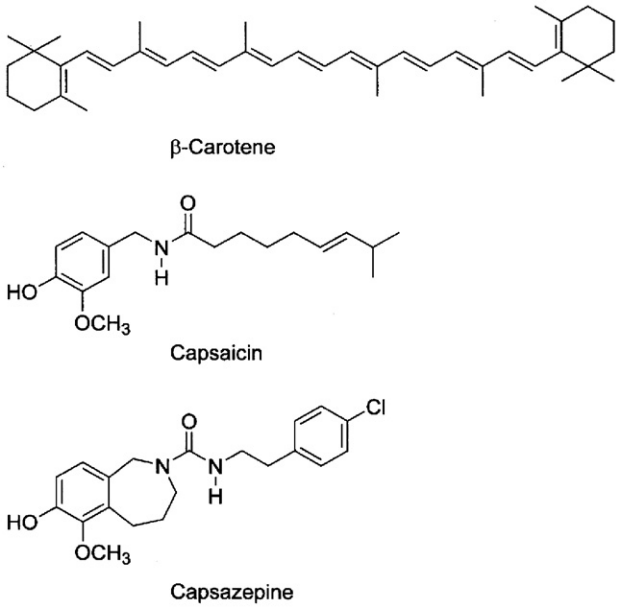

Fig. 1. Chemical structures of β-carotene, capsaicin and capsazepine.