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Short communications

Novel immunomodulatory effects of phytanic acid and its related substances in mice

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ABSTRACT

Phytanic acid is one of the constituents of animal products with possible health benefits through improving lipid and glucose metabolism; however, its immunomodulatory effects remain undetermined. The *in vitro* effects of phytanic acid and its related substances on T-cell functions were investigated. Mouse splenocytes were stimulated by T-cell mitogens and incubated with phytanic acid, phytol, and pristanic acid followed by evaluation of cell proliferation and cytokine production. All phytanic acid-related substances significantly reduced IFN-γ, IL-4, and IL-10 production, with varying potencies, and had no significant effects on IL-2 production. Moreover, IL-17A production was inhibited by phytol and phytanic acid. Phytanic acid elicited immunomodulatory effects without inhibition of T-cell proliferation, and its effective concentration was less than 10 μM, which corresponds to the serum concentration of healthy humans. The present study suggests that phytanic acid has a potentially beneficial effect for amelioration of T-cell mediated autoimmune diseases.

1. Introduction

Increasing evidence indicates that dietary fatty acids are strongly related to human health outcomes and disease. Omega-3 fatty acids are one of the most thoroughly researched functional fatty acids, and have been shown to prevent coronary artery disease, hypertension, diabetes and inflammatory disorders (Kaushik, Dowling, Barrow, & Adhikari, 2015). Conjugated linoleic acid, which refers to a group of positional and geometric isomers of linoleic acid, has also received much attention due to its health effects including reduction of obesity, carcinogenesis and inflammation (Yang et al., 2015). The demand for foods enriched in the above fatty acids and

Chemical compounds: Phytanic acid (PubChem CID: 26840); Phytol (PubChem CID: 5280435); Pristanic acid (PubChem CID: 123929); Palmitic acid (PubChem CID: 985).

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Abbreviations: PPAR, peroxisome proliferator activated receptor; DMSO, dimethyl sulphoxide; PHA, phytohaemagglutinin; PWM, pokeweed mitogen; IL, interleukin; IFN, interferon

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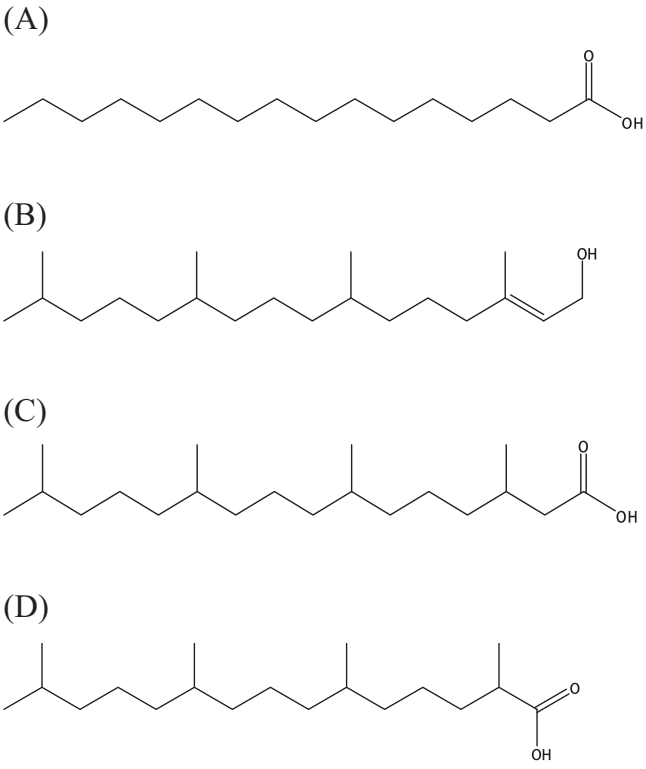

Fig. 1 – Chemical structures of palmitic acid (A), phytol (B), phytanic acid (C) and pristanic acid (D).

these supplements is increasing in the functional food market. Although lipids in foods contain a wide variety of fatty acids, only a few have proven beneficial to human health until now. Because individual fatty acids differ widely in their biological effects, finding health-promoting effects of specific fatty acids is considered to facilitate the development of novel functional foods.

Phytanic acid is a naturally occurring branched-chain fatty acid and originates from the phytol side chain of chlorophyll (Fig. 1). Some microorganisms inhabiting the rumen of ruminants produce phytol from chlorophyll, after which phytanic acid is formed via oxidation of phytol to phytenic acid. Therefore, dairy products and ruminant meat as well as some marine lipids are rich sources of phytanic acid. A previous study has reported that whole milk and lean organic beef contained phytanic acid with concentrations of 9.7 and 4.3 mg/100 g, respectively (Brown et al., 1993). Because humans are not capable of producing phytol from chlorophyll, phytanic acid in the human body is derived exclusively from the above foods.

Previous studies have shown that phytanic acid and its metabolite, pristanic acid (Fig. 1), are natural ligands for several subtypes of peroxisome proliferator activated receptor (PPAR) (Heim et al., 2002; Zomer et al., 2000), which form heterodimers with the retinoid X receptor and regulate gene expression related to fatty acid oxidation and glucose metabolism (Grimaldi, 2007). As PPARs are attractive molecular targets of functional foods for metabolic diseases (Chen, Kao, Tseng, Chang, & Hsu, 2014) and type 2 diabetes (Hsu, Liao, Lee, Hsu, & Pan, 2013), recent studies on health-improving properties of phytanic acid-related substances have pointed towards these diseases (Hellgren, 2010). Indeed, it has been demonstrated in rat hepatocytes that phytanic acid modulates gene expression involved in glucose metabolism via PPAR isoforms, suggesting a potential role for phytanic acid in the management of insulin resistance (Heim et al., 2002). Studies with primary porcine myotubes have shown that phytanic acid plays a role in stimulating glucose uptake (Che, Oksbjerg, Hellgren, Nielsen, & Young, 2013). Based on these findings, phytanic acid is now recognized as a constituent in animal products with possible health benefits (Young et al., 2013).

Recent studies have revealed important roles of PPARs on immunity besides lipid and glucose metabolism. In addition to the regulation of inflammatory responses of macrophage and endothelial cells (Clark, 2002), PPARs have been shown to regulate T-cell survival, activation, and CD4 positive T helper cell differentiation into Th1, Th2 and Th17 lineages (Choi & Bothwell, 2012). Several studies have demonstrated that PPARα agonists ameliorate experimental autoimmune encephalomyelitis in mouse models of T-cell mediated disorders (Yang, Gocke, Lovett-Racke, Drew, & Racke, 2008). Furthermore, PPARγ agonists have also been shown to exhibit potent anti-inflammatory effects in mouse models of encephalomyelitis (Niino et al., 2001) as well as inflammatory bowel disease (Lewis et al., 2011). These data strongly indicate that PPARs are potential therapeutic targets for the treatment of autoimmune diseases. However, questions of whether phytanic acid can modulate immune function and show beneficial properties for the treatment of autoimmune diseases have not been answered.

Here, we investigated the immunomodulatory effects of phytanic acid and its related substances, focusing on the cytokine production profile of T-cells, to address the potential for prevention or treatment of autoimmune diseases.

2. Materials and methods

2.1. *Animals*

Adult female C57BL/6 mice were purchased from Japan SLC, Inc. (Shizuoka, Japan). Animals were used in accordance with the *guidelines for the care and use of laboratory animals* at the University of Miyazaki and Law No. 105 of the Japanese government. The experimental design for the present study was approved by the University of Miyazaki (approval number: 2014-002).

2.2. *Cellular toxicity and T-cell proliferation*

The spleens of mice aged eight weeks were aseptically removed and teased into single-cell suspensions and incubated in RPMI1640 medium containing 10% foetal calf serum, 100 units/mL penicillin, and 100 μg/mL streptomycin. Assays were performed in flat-bottomed microtitre plates, with each well containing 1.5×10^5 splenocytes. For the assessment of toxicity on resting immune cells, splenocytes were incubated in the presence of various concentrations of phytanic acid and its related substances, which were dissolved in dimethyl sulphoxide (DMSO) and added as a final DMSO concentration of 0.1%. Palmitic acid, whose carbon chain is the same length as that of phytanic acid, was also evaluated as a control fatty