

Novel anti-inflammatory effects of 3RS, 7R, 11R-phytanic acid

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Objective: 3RS, 7R, 11R-phytanic acid (3RS-PHY) is an oxidation product of phytol which exists as a constituent of chlorophyll in green plants. Some microorganisms inhabiting the rumen of ruminants are involved in release of phytol from chlorophyll, and also oxidation of phytol to 3RS-PHY. Therefore, large amounts of 3RS-PHY are formed in the rumen and the resultant 3RS-PHY is translocated to fat containing tissues and their derived foods such as ruminant meat. Because humans are not capable of producing phytol from chlorophyll, 3RS-PHY in the human body is exclusively derived from the above foods. 3RS-PHY has strong agonist activity at the peroxisome proliferator activated receptor (PPAR)¹. Because PPAR-regulated genes play important roles in glucose metabolism and immune regulation, 3RS-PHY intake may have possible health benefits through prevention of metabolic and auto-immune diseases². Recently, we demonstrated that 3RS-PHY inhibited T-cell production of interferon (IFN)- γ ³; however, the overall immunomodulatory effects were not evaluated. In this study, we investigated the effects of 3RS-PHY on the production of interleukin (IL)-2, IL-4, IL-10 and IL-17A in addition to IFN- γ . We also evaluated effects of 3RS-PHY on antibody production by B-cells and on nitric oxide production by macrophages, to reveal the overall immunomodulatory effects of 3RS-PHY and potential health benefits of meat intake through prevention of autoimmune disease.

Materials and Methods: The spleens of female C57BL/6 mice were aseptically removed and teased into single-cell suspensions and suspended in RPMI1640 medium containing 10% fetal calf serum, 100 units/ml penicillin, and 100 μ g/ml streptomycin. To investigate effects of 3RS-PHY on T-cell cytokine production, mouse splenocytes were incubated in medium containing 10 μ g/mL poke-weed mitogen (PWM) at 37 °C for 72 h, along with 30 μ M 3RS-PHY which was dissolved in dimethyl sulfoxide (DMSO) and added as a final DMSO concentration of 0.1%. Palmitic acid whose length of carbon chain is same as that of 3RS-PHY was also evaluated as a control fatty acid. After incubation, culture supernatants were collected and concentrations of IL-2, IL-4, IL-10, IL-17A and IFN- γ were detected using commercially available enzyme-linked immunosorbent assay (ELISA) kits. Using B-cells isolated magnetically from splenocytes, cytokine and antibody secretions were also evaluated to address potential effects of 3RS-PHY on B-cell functions. B-cells were stimulated with lipopolysaccharide (LPS) and incubated with 30 μ M 3RS-PHY, after which culture supernatants were subjected to ELISA for determination of IgM and IgG in addition to the above cytokines. To investigate effects of 3RS-PHY on macrophage functions, J774.1 cells were stimulated by LPS along with IFN- γ and incubated with 30 μ M 3RS-PHY. Nitric oxide concentrations in the culture supernatant were measured by a Griess reaction. The culture supernatant was also used for detection of tumor necrosis factor (TNF)- α and IL-6 by ELISA.

Results and Discussion: The present study demonstrated that 3RS-PHY significantly inhibited PWM-induced productions of IFN- γ , IL-10 and IL-17A. 3RS-PHY showed lower IL-2 concentration than palmitic acid, although there was no obvious difference between 3RS-PHY and the solvent DMSO control. PWM-induced IL-4 secretion was decreased in splenocytes incubated with 3RS-PHY, although more strong effects were elicited by palmitic acid. Our results also demonstrated that 3RS-PHY directly inhibited LPS-induced IgM and IgG productions by B-cells. 3RS-PHY was shown to inhibit IL-10 production by B-cells, albeit without significant change in IL-4. Furthermore, 3RS-PHY strongly suppressed nitric oxide production by J774.1 cells. 3RS-PHY also significantly inhibited secretions of TNF- α and IL-6, albeit similar or stronger effects were elicited by palmitic acid. Because T-cell production of cytokines such as IFN- γ and IL-17A, antibody secretion by B-cells, and nitric oxide production by macrophages are associated with human autoimmune diseases including rheumatoid arthritis and inflammatory bowel disease, the present findings imply that 3RS-PHY intake from ruminant meat has the potential to prevent or attenuate these diseases.

References:

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