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ORIGINAL ARTICLE

Effects of polyphenols on human Th1 and Th2 cytokine production

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KEYWORDS
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Summary
Background: Numerous phenolic compounds are consumed in the diet in a range of foods. There are very few studies of the effects of these compounds on the production of lymphocyte-derived cytokines.
Aim of the study: To investigate the effects of five phenolic compounds on cytokine production by cultured human lymphocytes.
Methods: Human whole blood cultures were stimulated with the T cell stimulant concanavalin A for 48 h in the presence of phenolic compounds (vanillic acid, syringic acid, kaempferol, oleuropein and tyrosol) at concentrations up to 10^{-4} M. Interleukin (IL)-2, IL-4 and interferon-γ (IFN-γ) concentrations were measured in the culture supernatants by ELISA.
Results: IFN-γ concentration was significantly lower in cultures containing 10^{-4} M kaempferol than in cultures with kaempferol at 10^{-7}, $10^{-6}$and 10^{-5} M or without kaempferol. The other phenolic compounds did not affect IFN-γ concentration and none of the phenolics tested affected IL-2 or IL-4 concentrations.
Conclusions: Some, but not all, phenolic compounds can decrease IFN-γ production by stimulated human whole blood cultures.

Introduction

Phenolic compounds are present in all plants and thus are commonly consumed in the diet.[1] They have been described to have a range of biological activities which may be of benefit to human health.[2–4] Recently, interest has been focussed on the anti-inflammatory effects of some of these phenolic compounds. Phenolic compounds from tea have been shown to decrease interleukin (IL)-1β and enhance IL-10 production by human leucocytes in culture.[5] Phenolic compounds have also been shown to inhibit IL-1β production at the level of mRNA and protein in macrophage cell lines.[6] We have shown

Abbreviations: Con A, concanavalin A; IFN, interferon; IL, interleukin; Th, T helper

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that the olive oil-derived phenolics oleuropein and caffeic acid were able to decrease IL-1β production by human whole blood cultures.[7] In addition prostaglandin E_2 (PGE_2) production was decreased in these cultures in the presence of kaempferol.[7] However, very little is known about the effect of phenolic compounds on production of cytokines by T cells. Of particular interest are the T helper (Th) cells which are very important in orchestrating immune responses. These Th cells can be classified into subtypes according to their profiles of cytokine production. Th1 cells produce cytokines such as IL-2 and interferon (IFN)-γ which activate monocyte/macrophages, natural killer cells and cytotoxic T cells and are associated with host defence against bacteria, viruses and fungi.[8] In contrast, Th2 cells produce cytokines such as IL-4, IL-5 and IL-13 and are associated with allergic responses.[9] The balance between the production of Th1- and Th2-type cytokines is believed to be important in regulating cell-mediated immune versus allergic reactions.[8,9] The Mediterranean diet is associated with human health benefits (including protection against the development of cancer and coronary heart disease),[10,11] some of which may be ascribed to its phenolic constituents.[4,12,13] There are very few reports of the effects of phenolic compounds on the production of cytokines by human lymphocytes. Therefore, in this study we investigate the effect of five phenolic compounds on the production of Th1-type cytokines (IL-2, IFN-γ) and Th2-type cytokines (IL-4) by human whole blood cell cultures stimulated with the T cell stimulant concanavalin A (Con A). The phenolics tested were vanillic acid, syringic acid, kaempferol, oleuropein and tyrosol.

Materials and methods

Materials

RPMI 1640 culture medium (without glutamine) was purchased from Autogen Bioclear, Calne, UK. L-glutamine, benzyl penicillin, streptomycin sulphate, Con A, vanillic acid, syringic acid, and kaempferol were purchased from Sigma Chemical Co., Poole, UK. Oleuropein and tyrosol were purchased from Extrasynthase, Genay, France. Cytokine ELISA kits were purchased from Biosource Europe, Nivelles, Belgium.

Whole blood culture

The collection of blood from healthy human volunteers was approved by the local ethical committee. Blood was collected from fasted healthy male volunteers (aged 18–25 years) into heparin ($n = 6$). The whole blood was diluted 1/5 with RPMI 1640 medium supplemented with 0.75 mM L-glutamine and antibiotics (0.05 μg/ml each of benzyl penicillin and streptomycin sulphate). The diluted blood (1.7 ml/well) was placed in the wells of a 24-well culture plate. Polyphenols were added to give final concentrations of 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M. Polyphenols were added in 80 μl of 2.5% ethanol to give a final concentration of 0.1% ethanol in the culture. Control cultures contained 0.1% ethanol but no polyphenol. Con A (0.2 ml; 75 μg/ml final concentration) was added to stimulate cytokine production; cultures without Con A stimulation were not performed. The addition of 20 μl RPMI 1640 medium gave a final culture volume of 2 ml. Cultures were incubated at 37 °C in a 5% CO_2 atmosphere for 48 h. At the end of the culture period, the plates were centrifuged at 1000 rpm for 5 min. The supernatant medium was then carefully removed to avoid disturbance and aliquots were frozen at until analysis.

Measurement of cytokine concentrations

Cytokine (IL-2, IL-4, and IFN-γ) concentrations in cell culture supernatants were measured by ELISA in accordance with the manufacturer's instructions (Biosource Europe, Nivelles, Belgium). Limits of detection were 8 pg/ml for IL-2; 2 pg/ml for IL-4; 2 pg/ml IFN-γ.

Statistics

Data are presented as medians and 25th and 75th quartiles. Friedman's test was used to determine differences among concentrations of each polyphenol. Where a significant difference was seen among concentrations of each polyphenol, a Wilcoxon Signed Rank test was used to test differences between pairs. Differences with a P value of $\leqslant 0.05$ were deemed significant. All statistical analyses were performed using SPSS version 11.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Interleukin 2

IL-2 concentrations in the supernatants of Con A-stimulated control cultures were 708 (375, 907) pg/ml. These were not significantly altered by the addition of any of the phenolic compounds tested