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Inhibition of Human Dendritic Cell Activation by Hydroethanolic But Not Lipophilic Extracts of Turmeric (*Curcuma longa*)

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Abstract

Turmeric has been extensively utilized in Indian and Chinese medicine for its immune-modulatory properties. Dendritic cells (DCs) are antigen-presenting cells specialized to initiate and regulate immunity. The ability of DCs to initiate immunity is linked to their activation status. The effects of turmeric on human DCs have not been studied. Here we show that hydroethanolic (HEE) but not lipophilic “supercritical” extraction (SCE) of turmeric inhibits the activation of human DCs in response to inflammatory cytokines. Treatment of DCs with HEE also inhibits the ability of DCs to stimulate the mixed lymphocyte reaction (MLR). Importantly, the lipophilic fraction does not synergize with the hydroethanolic fraction for the ability of inhibiting DC maturation. Rather, culturing of DCs with the combination of HEE and SCE leads to partial abrogation of the effects of HEE on the MLR initiated by DCs. These data provide a mechanism for the anti-inflammatory properties of turmeric. However, they suggest that these extracts are not synergistic and may contain components with mutually antagonistic effects on human DCs. Harnessing the immune effects of turmeric may benefit from specifically targeting the active fractions.

Keywords

dendritic cells; turmeric; curcumin; inflammation; *Curcuma longa*; Zingiberaceae

Introduction

Turmeric is the dried rhizome powder of *Curcuma longa*, a perennial herb of the Zingiberaceae (ginger) family [1]. Turmeric is commonly used as a spice and food additive and has been utilized extensively in Indian and Chinese medicine for its medicinal properties, including antiinflammatory effects. The best-studied chemical constituents of turmeric are the curcuminoids, which impart the characteristic yellow color, and curcumin (diferuloylmethane) as the major constituent [2]. Due to its potential medicinal effects, turmeric extracts are extensively marketed in the U.S. as herbal supplements. Broadly, two methods for extraction have been utilized for turmeric preparations. A hydroethanolic extract contains 10–20% curcumin, whereas a “supercritical” lipophilic extraction process (www.new-chapter.com) yields predominantly alpha- and beta-tumerones. Commercially available supplements often contain a combination of these extracts, in order to putatively provide a “full spectrum” of the bioactive ingredients. However, the interactions between these constituents regarding bioactivity have been less studied.

Due to the putative anti-inflammatory properties of turmeric, several studies have examined the effects of turmeric on the immune system. The anti-inflammatory effects of curcumin include inhibition of TNF-induced activation of NF κ B [2], [3]. Several studies have shown that curcumin can modulate the function of human and murine lymphocytes, including T and B cells [4], [5]. Dendritic cells are professional antigen-presenting cells that play a critical role as immune sentinels in the initiation and regulation of immunity and inflammation [6]. Surprisingly, little is known about the effects of turmeric on human DCs.

Materials and Methods

Turmeric extracts

Three samples of turmeric extracts were analyzed. Details of extraction and characterization of the samples are described in the Supporting Information. Copies of certificates of analysis are also included in the Supporting Information. Two forms of turmeric samples extracted from *Curcuma longa* were supplied by New Chapter, Inc. The first is a supercritical extract (SCE), using carbon dioxide at specific pressure and temperature as solvent, that yielded an extract with 79% essential oil containing mainly alpha- and beta-tumerones, with a curcumin content of < 0.1 %. The second sample is a hydroethanolic extract (HEE), using water and ethanol as solvents, that yielded an extract with 15.3% curcumin. Another preparation of the hydroethanolic extract was obtained from the Institute of Chinese Medicine (ICM) with turmeric obtained from India and extracted with 60% ethanol. The final curcumin content was 11.8%. The raw botanical was identified according to the monograph as Rhizoma Curcumae Longae in the Pharmacopoeia of the People's Republic of China 2005. A voucher specimen (HK40400) is deposited at the Herbarium of the Agricultural and Fishery Conservation Department of Hong Kong. Turmeric from New Chapter was identified by Paul Spencer, and turmeric from ICM was identified by Mavis Lee.

All extracts were stored at 4 °C and dissolved in DMSO/water immediately prior to use in the experiments. The presence of endotoxin contamination was monitored by a limulus

lysate assay (Biowhittaker), and all preparations had endotoxin contamination of < 0.1 EU/mg. As a control, chemically pure curcumin (> 95 % purity) was obtained from Sigma.

Generation of human DCs

Dendritic cells were generated from blood monocytes as described [7]. Briefly, blood mononuclear cells were isolated from buffy coats purchased from the New York Blood Center by density gradient centrifugation using Ficoll Hypaque. CD14⁺ monocytes were isolated by immunomagnetic bead selection and cultured in the presence of GM-CSF and IL-4 to yield DCs.

Treatment of DCs with turmeric

DCs were pulsed with turmeric extracts (or media/solvent controls) for 2 h and then washed prior to activation. For inducing DC activation, DCs were cultured with a cocktail of inflammatory cytokines consisting of IL1 β , IL6, TNF α , and PGE2, as described [7].

Monitoring activation of DCs

The expression of costimulatory molecules (CD80, CD86) and CD83 (a marker of DC maturation) on DCs was monitored by flow cytometry, as described [7]. To evaluate the ability of activated DCs to stimulate mixed lymphocyte reaction, these DCs were cultured in the presence of allogeneic lymphocytes and proliferation was monitored based on tritium incorporation, as described [8].

Supporting information

The following documents are available as Supporting Information: turmeric study agent information, turmeric HE product specification, turmeric HE certificate of analysis (CoA), and turmeric SC certificates of analysis (CoA1 and CoA2).

Results

In order to evaluate the effects of turmeric on human DCs, we cultured human monocyte-derived DCs in the presence of different preparations of turmeric extracts. Functional properties of DCs are strongly linked to their maturation or activation status [6]. For example, culturing of DCs with a cocktail of inflammatory cytokines leads to strong upregulation of co-stimulatory molecules and to the ability of stimulating T cells [9]. We hypothesized that turmeric extracts may inhibit maturation of DCs in response to inflammatory cytokines. Activation of DCs was monitored by flow cytometry to evaluate the expression of co-stimulatory molecules CD80 and CD86 as well as expression of CD83, a marker associated with DC maturation. Culturing of DCs with hydroethanolic extracts of turmeric led to the inhibition of CD80, CD86, and CD83 compared with control DCs in the presence of vehicle control (Fig. 1A,B). No differences were observed between the effects of HEE from two different sources (data not shown). The effect of HEE on DC maturation was comparable to equimolar concentrations of curcumin, suggesting that this is likely to be the dominant active fraction in this extract (Fig. 1C).

A central issue in botanicals' research in general is that the presence of different chemical constituents in botanical extracts may be required for efficacy due to interactive effects. Investigation of additive, synergistic, or inhibitory effects offers a potential way to assess mechanism of action for individual components. Therefore, we hypothesized that the addition of another extract of turmeric, SCE, with differing biochemical properties (predominantly tumerones instead of curcuminoids) might alter the response of DC to HEE. As shown in Fig. 2A, culturing of DCs with the SC extract did not inhibit the maturation of DCs in response to inflammatory cytokines (CC). In the absence of CC, SCE also had no appreciable effect on the expression of DC markers. HEE partially inhibited expression of CD80 and had a less pronounced effect on CD86. Neither extract affected expression of CD83 in the absence of CC activation or influenced overall DC viability at the concentration tested. As shown, the addition of SC extracts did not lead to greater inhibition of DC maturation compared to HEE alone and in fact diminished the impact of HEE on CD80 expression (Fig. 2A). To further analyze the effects of these extracts on functional properties of DCs, we evaluated the ability of DCs to stimulate the mixed lymphocyte reaction (Fig. 2B). As expected, DCs cultured with HEE demonstrated a reduced ability to stimulate the MLR, while the DCs treated with SCE were comparable to control DCs. Interestingly, DCs treated with the combination of SCE and HEE were less inhibited compared with DCs treated with HEE alone.

Discussion

Together, these data demonstrate that HEE extracts of turmeric can inhibit the activation of human DCs in response to inflammatory cytokines. The ability of turmeric extracts to inhibit maturation may therefore provide another mechanism for its putative anti-inflammatory effects. These studies with human DCs extend prior observations made by Kim et al. [10] with murine DCs. They reported that curcumin suppressed CD80, CD86, and MHC class II expression, but not MHC class I expression, in murine DCs. The finding that HEE but not SCE mediated this effect is consistent with the curcuminoids being the active fraction responsible for the anti-inflammatory effects of turmeric. The mechanism of the effects of turmeric in these experiments is not known, but it is consistent with the known effects of curcumin on inhibition of the NF κ B pathway [11]. Indeed, Sandur et al. recently demonstrated that tumerones do not inhibit TNF-in-duced NF κ B activation [3].

The finding that different extracts of turmeric are not synergistic and may well be antagonistic for some effects deserves further evaluation, as this might have implications for optimal formulations of these botanicals in the clinic. This principle is potentially applicable to other botanicals as well. In other words, biologic effects of botanical preparations may depend not only on the mixture and proportion of active fractions but also on the other modulating components in the extract. As shown here, the study of DC activation may be especially useful in defining critical interactions when immune modulation is the objective. Optimizing the bioactivity of these agents may then depend on isolation and administration of pure bioactive compounds rather than as crude mixtures. Inflammation plays an important role in early carcinogenesis and has been postulated as an important target for cancer prevention [12]. These data support further evaluation of hydroethanolic extracts for anti-inflammatory and tumor preventive properties in the clinic.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Hydroethanolic extracts (HEE) of turmeric inhibit maturation of human monocyte-derived DCs in response to inflammatory cytokines. **(A)** Monocyte-derived DCs were cultured with $10 \mu\text{g/mL}$ of turmeric extracts or controls for 2 h, prior to the addition of the inflammatory cytokine cocktail. After overnight culturing, the expression of CD80, CD86, and CD83 was monitored by flow cytometry. Data are representative of 4 separate experiments. Numbers represent mean fluorescent intensity. **(B)** Summary of experiments. $*P < 0.05$. **(C)** DCs were treated with hydroethanolic extracts or with curcumin (CCM) at $10 \mu\text{M}$, and the expression of CD80, CD86, and CD83 was monitored after the addition of inflammatory cytokines, as in Fig. 1A. $*P < 0.05$ compared with vehicle controls.

Fig. 2.

Lack of synergy between HEE and SCE of turmeric. **(A)** DCs were cultured with 10 $\mu\text{g/mL}$ of each extract separately or together, prior to activation with a cocktail of inflammatory cytokines (CC) as described in the methods. After overnight culturing, the expression of markers associated with DC maturation was analyzed by flow cytometry. Data are representative of 3 similar experiments. **(B)** Cytokine-matured DCs obtained from the cultures as in Fig. 2A were used to stimulate allogeneic lymphocytes. The proliferation of responding cells was analyzed based on tritium incorporation (CPM: counts per minute). Data are representative of two similar experiments.