Anti-arthritic Effect of Eugenol on Collagen-Induced Arthritis

Experimental Model

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This study was designed to test the efficacy of eugenol, a compound obtained from the essential oil of cloves (Syzygium aromaticum) in collagen-induced arthritis (CIA), a well characterized murine model of rheumatoid arthritis. Macroscopic clinical evidence of CIA manifests first as periarticular erythema and edema in the hind paws. Treatment with eugenol starting at the onset of arthritis (day 25) ameliorated these clinical signs of CIA. Furthermore, eugenol inhibited mononuclear cell infiltration into the knee joints of arthritic mice and also lowered the levels of cytokines (tumor necrosis factor (TNF)-α, interferon (IFN)-γ and tumor growth factor (TGF)-β) within the ankle joints. Eugenol treatment did not affect the in vitro cell viability as assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Therefore, eugenol ameliorates experimental arthritis and could be useful as a beneficial supplement in treating human arthritis.

Key words eugenol; collagen-induced arthritis; inflammation; cytokine; mononuclear cell

Eugenol (4-allyl-2-methoxyphenol) is the major component in the essential oil of many aromatic plants, including clove (Syzygium aromaticum) and it is known to possess antioxidant, analgesic and neuroprotective properties among others.1,2 In addition, eugenol and related compounds exhibit anti-inflammatory activities, e.g., inhibition of lipopolysaccharide (LPS)-stimulated nuclear factor-kappa B (NF-κB) activation, cytokine release and cyclooxygenase-2 (COX-2) expression by macrophages in vitro3 and inhibition of 5-lipoxygenase activity in polymorphonuclear cells.4 However, despite the anti-inflammatory effects of eugenol described previously, there is only one report of it exhibiting antirheumatic effects in a model of adjuvant arthritis.5 This new therapeutic strategy involving natural products has been devised because the anti-inflammatory and immunosuppressive drugs currently available cause many side effects and show limited efficacy in the treatment of rheumatoid arthritis.

Collagen-induced arthritis (CIA) is used as an experimental model that resembles human rheumatoid arthritis. Both T cell and B cell responses to the autoantigen as well as the production of cytokines by systemic and tissue-specific cell populations are critical for the development (and eventual diminution) of the autoimmune response to CIA and to the pathology in CIA.6 Due to the paucity of information on the effect of eugenol on experimental models of arthritis, this study was undertaken to investigate the efficacy of eugenol on CIA model.

MATERIALS AND METHODS

Animals This study was conducted on male DBA1/J mice, 2–3 months old, 20–25 g body weight, obtained from the University of São Paulo, Department of Pharmacology, Faculty of Medicine of Ribeirão Preto, SP, Brazil. All mice had access to water and standard rodent diet ad libitum. All experimental procedures were approved by the Ethics Committee of the State University of Maringá, Pr, Brazil.

Induction and Assessment of CIA Mice received 100 µg of bovine collagen type II (CII) (kind gift of David D Brand, Memphis, Tennessee, U.S.A.) dissolved in 25 µL of 0.05 mM acetic acid and emulsified with an equal volume of complete Freund’s adjuvant (CFA, 4 mg/mL) (Difco Laboratories, Detroit, Michigan, U.S.A.). Emulsion (50 µL) was injected intra-dermically (i.d.) into the base of the tail (day 0). Mice were monitored daily for signs of arthritis and graded using a scale of 0–4, where 0 = normal, 1 = mild swelling with erythema, 2 = significant joint swelling, 3 = severe swelling and digit deformity, and 4 = maximal swelling with ankylosis. Each joint was scored, with a maximum possible score of 4 per mouse that did have disease, since we sum the values of four paws and divide by four to put in the results.6

Treatment Protocol Mice received 100 µg of eugenol (Biodinâmica Laboratory, Ibiporã, Paraná, Brazil) or vehicle (saline containing 1% Tween 80, v/v) orally, daily, from the disease onset (day 25) until the end of the experiment. The normal mice were immunized with CII but without treatment or vehicle.

Cell Migration To evaluate leukocyte migration, the articular cavities of knee joints were washed twice with 5 µL phosphate buffered saline (PBS) containing 1 mM ethylene-diaminetetraacetic acid (EDTA) and then diluted to a final volume of 100 µL with PBS/EDTA. Total cell counts were performed in a Neubauer chamber under optical microscopy (Nikon Eclipse E-200), and differential cell counts (100 cells) were performed on cytocentrifuge slides (Cytospin 3; Shandon, Pittsburgh, PA, U.S.A.) stained with Rosenfeld’s stain. Differential cell counts were performed using a light microscope (Zeiss, Wetzlar, Germany), and results were expressed as the number of mononuclear cells per cavity.

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measurements of cytokine levels by enzyme-linked immunosorbent assay (ELISA) to measure cytokine concentrations in the inflammatory site, articular tissues were harvested and triturated in 500 µL of PBS containing EDTA by tissue-trimmer. Articular homogenates were centrifuged and supernatants collected and stored at −70°C for subsequent determination of interferon (IFN)-β, interleukin (IL)-10, tumor growth factor (TGF)-β, and tumor necrosis factor (TNF)-α concentrations by ELISA (R & D system), according to the manufacturer’s instructions.

Cell Viability Assay The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide] assay is based on the mitochondrial enzyme reduction of a tetrazolium dye to detect and determine cell viability. Briefly, the cells were plated at a density of 1×10⁵ cells/well into 96-wells plates. After 4 h exposure to eugenol (1, 3, 10, 30 or 90 µg/mL) of 0.05% dimethyl sulfoxide (DMSO), 20 µL of MTT (5 mg/mL, Sigma, St. Louis, U.S.A.) stock solution was added to each well. After 2 h incubation at 37°C, the medium was removed and 200 µL of DMSO were added to each well. Cells were incubated for a further 10 min and then the absorbance was read on a Bio-Tek (ELX 808IU) ELISA reader at the wavelength of 540 nm. Blank values were subtracted from each treated and control reading. Cell viabilities were expressed as percentage of viable cells determined as follows:

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\% \text{ viable cells} = \frac{(\text{the absorbance of the treated cells}) - (\text{the absorbance of the blank})}{(\text{the absorbance of the control})} \times 100 \div (\text{the absorbance of the blank})
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RESULTS AND DISCUSSION

Among the several constituents of plant essential oils, studies have shown that eugenol has antioxidant, anti-inflammatory, DNA-protective, analgesic and antimicrobial properties.1,2 Our previous findings indicate that Syzygium aromaticum, whose major compound is eugenol, has an immunomodulatory effect.7 In the present study, we tested if the presence of eugenol has any effect on an established arthritis in mice. Although only a few advances in understanding the etiology of human autoimmune diseases has been achieved with the use of experimental models, they do offer an opportunity to investigate new potential therapeutic applications to improve joint inflammation. As shown in Fig. 1A, eugenol-treated arthritic mice exhibited clinical scores lower than the control group which received vehicle only. Even though eugenol started to produce beneficial effects on the inflammation observed in CIA at the beginning of the treatment, a statistically significant reduction in the clinical score of CIA by eugenol became apparent after 35–40d after immunization. Besides, it was not observed any difference between vehicle-arthritic mice and normal mice (Fig. 1).

Given the fact that infiltration of leukocytes plays an essential role in experimental arthritis and could contribute to articular damage,8 we tested whether eugenol could modulate leukocyte recruitment to the knee joints and also cell viability. As shown in Fig. 1B, eugenol significantly reduced mononuclear cell migration to the knee joint when compared with the vehicle-treated arthritic mice. Cell viability was measured using an MTT test and expressed in terms of relative absorbance of eugenol-treated cells versus control cells. Our results show that cells treated with eugenol remain viable (>80%) even when exposed to the highest concentration (90 µg/mL) (Fig. 2).

Taking into account that cytokines are involved in direct cell-to-cell communication and in the tissue damage observed in rheumatoid arthritis,9 we investigated the effect of eugenol on the concentrations of TNF-α, IFN-γ, TGF-β and IL-10 in the affected ankle joints. Paw samples from arthritic mice treated with vehicle contained significantly higher concentrations of all of the above-mentioned cytokines when compared to mice treated with eugenol.
to those of naïve mice. On the other hand, mice treated with eugenol showed a significant reduction in TNF-α (Fig. 3A), IFN-γ and TGF-β (Fig. 3B) levels. It has been previously reported that IL-10 inhibit autoimmune inflammation in CIA. In our results, we found a slight tendency for eugenol to enhance levels of the anti-inflammatory cytokine IL-10 (Fig. 3A) when compared with vehicle-treated arthritic mice.

In conclusion, the results presented herein give additional insight into the previously described antiinflammatory beneficial effects of eugenol, suggesting that this compound may be an alternative and/or supplemental treatment to chronic inflammatory diseases such as rheumatoid arthritis.

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REFERENCES


