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Chamomile, a novel and selective COX-2 inhibitor with anti-inflammatory activity

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ABSTRACT

Aims: Inducible cyclooxygenase (COX-2) has been implicated in the process of inflammation and carcinogenesis. Chamomile has long been used in traditional medicine for the treatment of inflammatory diseases. In this study we aimed to investigate whether chamomile interferes with the COX-2 pathway.
Main methods: We used lipopolysaccharide (LPS)-activated RAW 264.7 macrophages as an *in vitro* model for our studies.
Key findings: Chamomile treatment inhibited the release of LPS-induced prostaglandin $E_{(2)}$ in RAW 264.7 macrophages. This effect was found to be due to inhibition of COX-2 enzyme activity by chamomile. In addition, chamomile caused reduction in LPS-induced COX-2 mRNA and protein expression, without affecting COX-1 expression. The non-steroidal anti-inflammatory drug, sulindac and a specific COX-2 inhibitor, NS398, were shown to act similarly in LPS-activated RAW 264.7 cells. Our data suggest that chamomile works by a mechanism of action similar to that attributed to non-steroidal anti-inflammatory drugs.
Significance: These findings add a novel aspect to the biological profile of chamomile which might be important for understanding the usefulness of aqueous chamomile extract in the form of tea in preventing inflammation and cancer.

Introduction

There is increasing evidence that longstanding inflammation plays a critical role in the initiation and development of various human illnesses, including cancer (Federico et al. 2007; MacLennan et al. 2006; Khansari et al. 2009). Inflammation and disease are linked through the production of inflammatory mediators by macrophages and neutrophils (Federico et al. 2007; O'Shea and Murray 2008). Inflammation results in induced expression and enzyme activity of cyclooxygenase-2 (COX-2), which produces inflammatory mediators such as prostaglandin E_2 (PGE_2) (Hussain et al. 2003). COX-2, unlike COX-1 which is constitutively expressed in most mammalian tissue, is not detectable in normal tissues, but is rapidly induced by growth factors, tumor promoters, oncogenes and carcinogens (Hussain et al. 2003; Simmons et al. 2004). Aberrant or increased expression of COX-2 has been implicated in the pathogenesis of various inflammatory disorders including lupus, multiple sclerosis, arthritis, Alzheimer's disease and cancer (Kapoor et al. 2005; Wang et al. 2007). It was observed in early clinical studies that levels of prostaglandins and COX-2 were higher in tumor tissue than in normal tissue, suggesting a role of COX-2 in tumorigenesis (Taketo 1998; Buskens et al. 2002). Experimental studies demonstrate that deletion of COX-2 suppresses the development of intestinal polyps in Apc delta716 knockout mice, whereas overexpression of COX-2 is sufficient to induce mammary gland tumors in multiparous (nonvirgin) females, suggesting a pivotal role of COX-2 in tumorigenesis (Oshima et al. 1996; Liu et al. 2001). In light of the above findings, COX-2 has become the focal point for the development of anti-inflammatory and anticancer drugs. Non-steroidal anti-inflammatory drugs (NSAIDs), nonselective non-aspirin NSAIDs and COX-2 selective inhibitors are being widely used for various inflammatory disorders and cancer prevention (Thun and Blackard 2009). Selective inhibitors of COX-2, however, are associated with a small but definite risk of myocardial infarction and stroke. In view of the gastric side-effects of conventional NSAIDs and the recent withdrawal of selective COX-2 inhibitors from the market due to their adverse cardiovascular side-effects, there is considerable impetus to develop alternative anti-inflammatory agents with reduced gastric and cardiovascular side-effects (Ortiz 2004; Coruzzi et al. 2007). Plant-derived natural agents may potentially be useful in this regard.

Chamomile has been used for centuries as a medicinal plant for its anti-inflammatory and analgesic properties (McKay and Blumberg 2006; Srivastava and Gupta 2009). It is consumed in the form of tea at a frequency of more than a million cups per day (Speisky et al. 2006).

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Chamomile has been approved by the German Commission E for oral consumption in the management of various inflammatory diseases of the gastrointestinal tract, and for topical application in the treatment of various skin disorders and inflammatory disorders of certain mucosal surfaces, such as the oral cavity and ano-genital areas (Ross 2008). Recent studies have demonstrated its antioxidant, hypocholesteroemic, anti-parasitic, anti-aging, and anticancer properties, supporting its longstanding traditional use for treating various human ailments (Babenko and Shakhova 2006; Lee and Shibamoto 2002; Srivastava and Gupta 2009). Several constituents of chamomile, including apigenin 7-*O*-glucoside, luteolin, terpene compounds, chamazulene, and (-)-alpha-bisabolol, patuletin, quercetin, myricetin, and rutin have been studied with respect to their anti-inflammatory activities. Of these, chamazulene, alpha-bisabolol, and apigenin have been shown to possess the highest anti-inflammatory activity against pro-inflammatory agents (McKay and Blumberg 2006). The anti-inflammatory effects of azulenes may be related to an influence on the pituitary and adrenal glands, through increased cortisone release and reduced histamine production (Rekka et al. 1996). In cell culture studies, both bisabolol and bisabolol oxide have been shown to inhibit 5-lipoxygenase activity (Braga et al. 2009). Apigenin 7-*O*-glucoside application has been shown to inhibit skin inflammation caused by application of xanthine-oxidase and cumene hydroperoxide in rats (Fuchs and Milbradt 1993). In light of accumulated investigative evidence, we speculated that chamomile may contain constituents that interfere with the actions of COX-2. To investigate this hypothesis, we used LPS-activated murine RAW 264.7 macrophages as a cell model, since they express high levels of COX-2 and are the most relevant model for our studies.

Materials and methods

Materials

Dry chamomile flower of Egyptian origin was purchased from Bec's Tea Nirvana, Cleveland, Ohio. Cell culture medium, DMEM, fetal bovine serum, penicillin–streptomycin cocktail and phosphate buffer saline were purchased from Cellgro Mediatech, Inc. (Herndon, VA). Lipopolysaccharide (LPS, *E coli*), acetylsalicylic acid, sulindac, arachidonic acid and apigenin 7-*O*-glucoside (>95% pure) were purchased from Sigma (St. Louis, MO). NS398 was purchased from Calbiochem. All reagents used in the experiments were of analytical reagent grade or HPLC grade where applicable.

Preparation of extracts

Dry chamomile flowers were weighed and crushed to powder with a marble pestle and mortar and a 5% w/v suspension was prepared in a flask by adding hot boiled water. The flask was then placed on a shaker (200 rpm) for 4 h and the temperature was maintained at 37 °C. After shaking, the flask was brought to room temperature and the suspension was filtered through a series of Whatman filters and finally passed through 0.22 micron filter (Millipore, Billerica, MA). The filtered aqueous extract was freeze-dried and stored at −20 °C until use. For cell culture studies, the dried material from aqueous extract was weighed and dissolved in culture medium to achieve desired concentration.

Cell culture

Murine RAW 264.7 macrophages were obtained from the American Type Culture Collection (ATCC) and cultured in Dulbecco's modified essential medium in appropriate culture conditions. Cell stimulation was performed with 1 μg/mL of LPS.

PGE_2 release

To determine PGE_2 accumulation from endogenous arachidonic acid, cells were seeded in 96-well plates (5×10^4/200 μL/well), cultured for two days and, after supernatants were replaced by fresh medium, incubated with or without LPS in the absence or presence of the test agents for 24 h. PGE_2 was measured in cell culture supernatants and cell lysate of RAW 264.7 macrophages by using PGE_2 enzyme immunometric EIA kit (Cayman Chemical, Ann Arbor, MI). Experiments were performed at least three times in triplicate.

COX-2 enzyme activity

RAW 264.7 cells (1×10^5 cells in a 96 well plate) were pretreated with acetylsalicylic acid (250 μM) for 30 min to irreversibly inactivate COX-1. Thereafter, cells were washed with PBS and fed with fresh medium. Induction of COX-2 was achieved by adding LPS for 24 h. Then, medium was aspirated and cells washed with PBS again and supplied with fresh medium (fetal bovine serum-free). Test compounds were pre-incubated for 30 min before exogenous arachidonic acid was added. After 15 min, supernatants were removed and analyzed by PGE_2 enzyme immunometric EIA assay. Experiments were performed at least three times in triplicate.

Cell viability assay

Cell respiration, an indicator of cell viability, was determined by the mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) to formazan. After the supernatants were removed for PGE_2 determination, cells were incubated at 37° with MTT (0.5 mg/mL) for 45 min. The medium was aspirated and cells were solubilized in dimethyl sulfoxide (250 μL) for at least 2 h in the dark. The extent of reduction of MTT was quantified by optical density measurement at 550 nm.

Western blot analysis

Macrophages, grown in 6-well plates to confluence, were incubated with or without LPS in the absence or presence of the test agents. Cells were washed with ice-cold PBS and stored at −70 °C until further analysis. Frozen plates were put on ice and cells were lysed in 1% Triton X-100, 0.15 M NaCl, and 10 mM Tris–HCl pH 7.4 for 30 min. Lysates were homogenized through a 22 G needle and centrifuged at 10,000*g* for 10 min at 4 °C. The supernatants were collected and protein was measured by the method according to Bradford 1976. Cell lysates, containing equal amounts of protein, were boiled in SDS sample buffer for 5 min before running on a 10% SDS–polyacrylamide gel. Proteins were transferred to polyvinylidene fluoride membranes (Invitrogen, Carlsbad, CA). Membranes were blocked with 5% fat-free dry milk in TBS-T pH 8.0 (Tris-buffered saline [50 mM Tris, pH 8.0, and 150 mM NaCl] with 0.1% Tween 20) and then incubated with a mouse immunoglobulin G_1 against COX-1 (SC-7950) and COX-2 (SC-7951) or monoclonal anti-β-actin (SC-47778) antibody obtained from SantaCruz, SantaCruz, CA, diluted to 1:250 and incubated overnight at 4°. After washing 3 times with TBS-T, COX-1 and COX-2 was visualized by an anti-mouse IgG:horseradish peroxidase conjugate and the enhanced chemiluminescence system (ECL™, Amersham Pharmacia Biotech). Signal intensities were evaluated by densitometric analysis (Kodak Digital Science™ Image Station 2000R Life Science Products).

Reverse transcriptase (RT)-PCR analysis

RAW 264.7 cells (5×10^6 cells–10 cm dish) were incubated for 24 h with or without various concentrations of chamomile and LPS (1 μg/ml). After washing with PBS twice, total RNA was isolated from the cell