Ginger Extract Components Suppress Induction of Chemokine Expression in Human Synoviocytes

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ABSTRACT

Introduction: Ginger has a long history of medicinal use, particularly as an anti-inflammatory agent for a wide variety of diseases such as arthritis. Suppression of inflammation in arthritis is attributed to suppression of proinflammatory cytokines and chemokines produced by synoviocytes, chondrocytes, and leukocytes.

Objective: This study aimed to elucidate the effect of a combination ginger extract and its individual components on chemokine expression in human synoviocytes.

Methods: Human synoviocytes were incubated with 100 μg/mL combination ginger extract (GE) of Alpinia galanga (AG) and Zingiber officinale (ZO); AG extract alone; ZO extract alone; or control media, for 1 hour at 37°C, 5% CO₂. Cells were next activated with 1 ng/mL of tumor necrosis factor alpha (TNF-α) for 1 hour to determine macrophage chemotactic factor (MCP-1) and interferon-γ activated protein (IP-10) mRNA levels using reverse transcriptase polymerase chain reaction (RT-PCR). Secreted MCP-1 and IP-10 were quantified by enzyme-linked immunosorbent assay (ELISA) following a 24 hour incubation period.

Results: The GE combination was consistently more effective in decreasing chemokine mRNA and chemokine secreted protein levels than its individual components ZO or AG. In comparison, ZO was more effective than AG in suppressing chemokine expression.

Conclusion: The present study demonstrates that GE inhibits chemokine expression, and that the combination of ZO and AG components acts synergistically. This ginger formulation may be useful for suppressing inflammation due to arthritis.

INTRODUCTION

Arthritis affects as many as 36 million people in the United States and causes severe joint swelling, pain, and inflammation. The different forms of arthritis have distinctive symptoms, prognoses, and treatments (Altman, 1995; Mankin et al., 1994; Martel-Pelletier, 1999). Osteoarthritis (OA) is the most common form of arthritis, affecting approximately 70–80% of the population over 50 years of age. The second most prevalent form is rheumatoid arthritis (RA), a debilitating autoimmune inflammatory syndrome.

The inflamed joint is characterized by thickening and increased cellularity of the synovial tissue as well as cartilage degeneration. The pathogenesis of arthritis has been attributed to activation and release of proinflammatory mediators, leading to cartilage degradation. The inflammatory mediators include cytokines, degradative enzymes such as matrix metalloproteinases (MMPs), arachidonic acid metabolites, and reactive radicals (Dingle, 1991; Ehrich et al., 1999; Felisaz et al., 1999; Guh et al., 1999; Mathys et al., 2000; Moreland, 1999; Yoshida et al., 1999; Ziegler-Heitbroch et al., 1993). The increased cellularity in the inflamed synovium has been attributed to

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the recruitment of leukocytes, particularly monocytes from blood vessels, into the synovial tissue. Neutrophil influx into the inflamed joints of rheumatoid patients has also been noted in response to chemotactic molecules (Patterson et al., 2002).

Chemotactic molecules were originally identified as proinflammatory cytokines until they were designated as chemokines in the 1990s. There are at least 50 different chemokines, expressed and secreted by a variety of cells, that play a role in inflammation (Bagglioni, 2001). In the joint, chondrocytes and synoviocytes express and produce an array of chemokines. These chemotactic molecules not only induce the migration of leukocytes from the blood into the inflamed tissue but also mediate degradation of cartilage by decreasing proteoglycan synthesis and activating MMPs that break down cartilage (Katrib et al., 2001; Yuan et al., 2001). Non-steroidal anti-inflammatory drugs (NSAIDs) can suppress the activation of proinflammatory mediators, but long-term use of these drugs has known side effects. They induce gastrointestinal ulceration and disrupt proteoglycan metabolism (Day et al., 1987; Fries et al., 1989; Fries et al., 1991; Pincus and Griffin, 1991).

Herbal extracts have been used as anti-inflammatory agents for the treatment of rheumatic diseases and inflammatory disorders (Afzal et al., 2001; Bechwith, 1999; Bierhaus et al., 1997; Chopra et al., 2000; Jobin et al., 1999; Kumar et al., 1998; Singh and Aggarwal, 1995; Srivastava and Mustafa, 1989; Srivastava and Mustafa, 1992). In particular, extracts of ginger have well-documented anti-inflammatory effects, relieve pain, and decrease joint swelling in arthritic patients (Srivastava and Mustafa, 1989; Srivastava and Mustafa, 1992). In a randomized clinical trial of 57 cases, ginger extracts were noted in an exploratory test to be effective compared to placebo during the first period of treatment prior to crossover with NSAIDS (Bliddal et al., 2000). More recently, Altman and Marcussen (2001) demonstrated that a ginger extract combination from the rhizomes of Zingiber officinale (ZO) and Alpinia galanga (AG) to create a concentrate of aromatic compounds and terpenoids (255 mg combined GE extract = 3000 mg Z. officinale dry rhizome and 500 mg A. galanga dry rhizome). The extraction procedure was performed by standardized and controllable steps. The resulting extract has a high content of hydroxy-methoxy-phenyl compounds including 6-gingerol, 8-gingerol, 10-gingerol, gingerdione, and gingerdil. Other aromatic compounds in the extract include 1'-acetoxychavicol acetate, 1'-acetoxyeugenol acetate, trans-p-coumaryl diacetate, coniferyl diacetate, 1'-hydroxychavicol acetate, 1'-hydroxychavicol, P-hydroxy-trans-cinnamaldehyde, P-methoxy-trans-cinnamyl alcohol, and 3,4-dimethoxy-trans-cinnamyl alcohol. ZO and AG were prepared using the same method of extraction and the final extracts contained the same amounts of active ingredients as GE. The extracts were supplied by Ferrosan A/S.

Isolation and propagation of synoviocytes

Synovial tissue was obtained from patients with OA undergoing routine primary knee replacement surgery. The cells were isolated by tissue digestion using 0.1% collagenase (Boehringer, Mannheim, Germany) and incubated in monolayer culture at 37°C, 5% CO₂, for 2 to 8 weeks. Cells were retrieved using 0.25% trypsin, washed with Hank’s balanced salt solution (HBSS), and then resuspended in Dulbecco’s modified Eagle medium (DMEM) containing 10% heat-inactivated fetal calf serum. Synoviocytes displayed ovoid to spindle shape morphology and exhibited clearly demarcated nuclei and cytoplasm. The cytoplasm intensely stained for collagen type I while the nuclei vividly stained with toluidine blue. Semiquantitative reverse transcriptase polymerase chain reaction (RT-PCR) indicated that synoviocytes expressed high levels of collagen type I mRNA. The expression of type I collagen, continued proliferation, and survival in tissue culture over several weeks, indicated that the synoviocytes consisted of primarily fibroblastic cells. The viability of cells used in subsequent experiments was 100%.

Treatment procedure

To determine the effect of the extract preparations, synoviocytes were seeded onto 6-well plates at 5 × 10⁵ cells/well. Synoviocytes were next incubated with control media alone (C), or 100 µg/mL of GE, ZO, or AG, for 1 hour before activation with 1 ng/mL of TNF-α. The concentration of TNF-α (1 ng/mL) used in the present study has been well documented to activate inflammatory cells, including synoviocytes. Cells were reincubated for 1 or 24
hours at 5% CO₂ and 37°C. The sets of cells incubated for 1 hour were frozen at −70°C for subsequent RNA extraction. RT-PCR analysis indicated that the 1 hour incubation following addition of activator cytokine yielded the strongest signals and consistent results.

**RNA extraction and analysis by RT-PCR**

Cells were lysed and total RNA was extracted with Tri-zol reagent (Life Technologies, Gaithersburg, MD). Equal amounts (1 μg) of total RNA were subjected to reverse transcription into cDNA at 42°C for 1 hour with oligo(dT) 18 primers. The transcripts were then amplified by RT-PCR. Primers specific for MCP-1 and IP-10 were used. Two μL of cDNA template was used in each PCR reaction, with initial treatment at 94°C for 3 minutes. PCR reactions were then conducted in a Perkin Elmer thermal cycler for 30 cycles (MCP-1) or 35 cycles (IP-10) of denaturation (94°C, 30 seconds), annealing (55°C, 30 seconds), and extension (72°C, 2 minutes), with 72°C for 7 minutes at the end. RT-PCR products were analyzed on 1.5% agarose gel. The gels were scanned using the HP Precision Scan Pro and were normalized to the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) housekeeping gene (Phan et al., 2003).

**ELISA assays for chemokines**

The levels of MCP-1 and IP-10 were quantified by multiplexed sandwich enzyme-linked immunosorbent assay (ELISA). The SearchLight Proteome Arrays (Pierce Biotechnology, Woburn, MA) use a special spotting technique in which the target-specific antibodies are bound to each well of a microplate. A luminescent signal is imaged using a cooled charge-coupled device (CCD) camera following the ELISA procedure. The image is then analyzed using ArrayVision software (Imaging Research, St. Catharines, Ontario, Canada).

**RESULTS**

**Effect of ginger extract components on MCP-1 and IP-10 mRNA levels in human synoviocytes**

Nonactivated synoviocytes from two cell lines (A and B) had no detectable levels of MCP-1 and IP-10 mRNA. Stimulation with TNF-α upregulated the transcript levels of MCP-1 and IP-10. When synoviocytes exposed to 100 μg/mL of GE were activated with TNF-α, the MCP-1 mRNA levels were suppressed by 60% and the IP-10 mRNA levels were barely detectable in cell line A (Fig. 1A). In comparison, preincubation of cell line B with GE prior to activation showed a similar suppression of MCP-1. The levels of MCP-1 mRNA were reduced by 55%, and IP-10 mRNA levels by 40% (Fig. 1B). Preincubation of synoviocytes from cell line A with individual ginger components ZO or AG did not block subsequent activation of MCP-1, but inhibited IP-10 activation by 50%. In contrast, cell line

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**FIG. 1.** Synoviocytes (cell lines A and B) were incubated with control medium alone (C), or 100 μg/mL of combined ginger extract (GE), Alpinia galanga (AG), or Zingiber officinale (ZO), for 1 hour before activating with 1 ng/mL of TNF-α. The cells were reincubated for 1 hour and were subsequently analyzed by reverse transcriptase polymerase chain reaction (RT-PCR). RT-PCR profiles were visualized on agarose gels. Right and center panel, GAPDH housekeeping gene. Left panel, cell lines A and B. Transcript levels were normalized to GAPDH from cell lines A and B.
B showed no detectable suppression in both MCP-1 and IP-10 mRNA levels. There was no change in GAPDH mRNA levels following treatment with TNF-α or with the ginger extract preparations.

**Effect of ginger extract components on MCP-1 and IP-10 secreted protein levels in human synoviocytes**

Inhibition of chemokine activation by ginger extract preparations was verified by ELISA determination of secreted protein products. The combination ginger extract (GE) reduced the secreted MCP-1 levels in cell line A (5128 pg/mL) and in cell line B (1943.2 pg/mL) when compared to control (18587 and 32850 pg/mL, respectively) as shown in Figure 2A. In contrast, ZO had less pronounced inhibition of MCP-1 secreted levels in cell line A and B (11187 pg/mL and 34375 pg/mL, respectively). The AG preparation had no inhibitory effect on MCP-1 production in cell line A or B (17271 pg/mL and 30978 pg/mL, respectively) as shown in Figures 2A and 2B, left panel.

Production of IP-10 was remarkably reduced by GE in cell line A (48 pg/mL) and cell line B (10 pg/mL) compared to controls (153 pg/mL and 400 pg/mL, respectively). The ginger preparation ZO showed less inhibitory effect on IP-10 production in cell line A (71 pg/mL) and no inhibitory effect in cell line B. Compared to the other two ginger preparations, AG did not suppress IP-10 production in cell lines A and B: levels of secreted IP-10 were observed similar to those of untreated controls.

The extent of chemokine suppression by the ginger preparations is also dependent on the synoviocyte cell line studied. Nevertheless, exposure of both synoviocyte cell lines to GE consistently yielded the most profound suppression of chemokine expression. The detectable protein concentrations of MCP-1 and IP-10 did not show direct correlation with the mRNA levels in certain cell preparations. This may be attributed to the qualitative nature of the RT-PCR method used to assess transcript levels as well as other factors that regulate translation of these transcripts to the protein product.

**FIG. 2.** Synoviocytes (cell lines A and B) were incubated with control medium alone (C), or 100 μg/mL of combined ginger extract (GE), *Alpinia galanga* (AG), or *Zingiber officinale* (ZO), for 1 hour before activating with 1 ng/mL of TNF-α. The cells were reincubated for 24 hours and the supernatant was subsequently analyzed for MCP-1 and IP-10.
The present study demonstrates for the first time that ginger extract suppresses the expression of chemokines MCP-1 and IP-10, which induce migration of leukocytes and monocytes from the blood into inflammatory areas (Baggiolini, 2001). Both MCP-1 and IP-10 chemokines have been shown to be involved in the pathogenesis of RA and OA (Hanaoka et al., 2003; Katrib et al., 2001; Yuan et al., 2001). MCP-1 is a potent chemotactic agent for monocytes and macrophages, which provides ongoing recruitment of inflammatory cells to further fuel the inflammatory cascade (Baggiolini et al., 1997).

In addition to its chemotactic function, MCP-1 has also been implicated in inducing MMPs (Hayashida et al., 2001; Villiger et al., 1992). The induction of MMPs eventually leads to cartilage degradation in the joint. In inflamed joints, MCP-1 may be induced by cytokines such as TNF-α and other degraded insoluble tissue fragments. It has been reported that hyaluronan fragments produced from damaged osteoarthritic cartilage and synovial tissue enhance MCP-1 production (Katrib et al., 2001; Yuan et al., 2001). The persistent production of MCP-1, resulting in continued recruitment of monocytes and macrophages, may be a key factor in chronic joint inflammation.

In contrast, IP-10 is a chemoattractant for activated T cells and selectively targets type 1 helper (TH1) cells, which results in the upregulation of IFN-γ instead of peripheral blood T cells that produce IL-4 (Farber, 1993; Gangur et al., 1998). Thus IP-10 provides another pathway of inflammatory cell recruitment of lymphoid cells. In addition, IP-10 was reported to act as a chemoattractant for human monocytes and natural killer (NK) cells (Jose et al., 1994; Taub et al., 1995). The restricted selectivity of IP-10 for a single receptor on T cells suggests that this chemokine plays a critical role in the regulation of recruitment and formation of lymphoid infiltration in inflammatory lesions. The inhibition of MCP-1 and IP-10 expression in synoviocytes indicates an important mechanism by which ginger extract exerts its anti-inflammatory effect in arthritis.

CONCLUSIONS

In the present study, we compared the efficacy of the GE combination to its individual components, ZO and AG, on chemokine suppression in human synoviocytes. We discovered that the GE is more effective in suppression of both MCP-1 and IP-10 than its individual components, suggesting a synergistic effect of the two individual ginger extracts. This observation may indicate that the components exert their effect via parallel and partially overlapping mechanisms. Our discovery may explain the reported efficacy of the combined formulation on relief of pain and inflammation in patients with OA (Altman and Marcussen, 2001).

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