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Anti-Inflammatory Activities of Digested Green Curry Paste in Peripheral Blood Mononuclear Cells from Rheumatoid Arthritis Patients

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ABSTRACT

Introduction: Several individual spices have been shown to have anti-inflammatory activity. However, the anti-inflammatory activities of mixed spices consumed as components of habitual diets are limited. We aimed to assess the anti-inflammatory activities of '*Green curry paste* '(GCP) consisting of nine different spices in cell culture models. **Methods:** Non-toxic diluted bioaccessible fraction generated by simulated digestion of GCP was added to the apical compartment of Caco-2 cells grown on bicameral inserts for 4 h. Basolateral media (bioavailable fraction) was added to cultures of peripheral blood mononuclear cells (PBMC) from 12 patients with rheumatoid arthritis in the absence or presence of lipopolysaccharide (LPS). Cytokines in culture media were quantified by ELISA. Caco-2 cells also were activated with IL-1β following exposure to the bioaccessible fraction of GCP to determine its effect on chemokine expression. **Results**: Spontaneous and LPS-induced secretions of IL-6 were significantly decreased when PBMC were exposed to the basolateral medium from Caco-2 cell cultures incubated with the bioaccessible fraction of GCP compared to control cultures. In a similar procedure, secretions of TNF-α and IL-8 were also decreased, but the changes were not significantly different from the controls. Pre-treatment of Caco-2 cells with the bioaccessible fraction of GCP also significantly suppressed IL-1β -induced expression of IL-8 (62% protein and 63% mRNA) and MCP-1 (47% protein and 51% mRNA) (p<0.05). **Conclusion:** These results suggest the use of GCP as a food component towards attenuating the severity of inflammatory conditions.

Key words: Anti-inflammatory activity, Caco-2 human intestinal cells, green curry paste, peripheral blood mononuclear cells

INTRODUCTION

Dietary spices have long been used in the Eastern world for culinary and alimentary purposes. Their potential ability to ameliorate the adverse effects of inflammation associated metabolic syndrome has been extensively reported (Jungbauer & Medjakovic, 2012). Although

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individual phytochemicals in various spices and herbs have been demonstrated to suppress inflammatory mediator gene expression (Aggarwal *et al.*, 2009), such activities have been rarely reported for mixtures of spices that often comprise standard components in habitual diets (Tuntipopipat *et al.*, 2011). Increasing evidence suggests that the health benefits of dietary plants result from the additive and synergistic interactions of the phytochemicals present in whole diets that modulate multiple signal transduction pathways (Liu, 2013). Curry paste, a basic constituent of Asians cuisine, is composed of various herbs and spices including chili, garlic, galangal, lemongrass, kaffir lime, pepper seed, cumin, coriander and turmeric. Crude extracts or pure compounds isolated from the spices in curry pastes have been shown to suppress inflammatory response (Aggarwal *et al.*, 2009). Due to their anti-inflammatory potency, dietary spices may serve as a complementary medicine for the management of inflammatory diseases such as rheumatoid arthritis (RA) and inflammatory bowel diseases (IBD).

The gastrointestinal epithelium is the initial site in the body exposed to consumed nutrients, non-nutrients, xenobiotics and noxious agents. Nutrients and many of the other compounds and their metabolites generally are absorbed and delivered to target tissues. Continuous exposure to noxious agents may be chemical insults to the intestinal immune system causing over-production of cytokines, growth factors, adhesion molecules and neuropeptides that in turn lead to chronic inflammation of the gut. Inflammatory bowel disease (IBD) is characterised by chronic inflammatory conditions of the gastrointestinal tract (Yang, Taboada & Liao, 2009). IBD affects 0.5% of the population in developed countries, and the incidence in developing countries continues to increase (Loftus, 2004). Increased concentrations of pro-inflammatory factors such as TNF-α, IL-8 and MCP-1 are present in sera of IBD patients and correlated with the severity of the condition (Khan *et al.*, 2006; Yang *et al.*, 2009). Treatments for IBD include corticosteroids, aminosalicylates, immunomodulators and the Food and Drug Administration (FDA)-approved anti-TNF-α humanised antibodies (Camilleri, 2003). Although these therapeutic approaches ameliorate IBD by inducing and maintaining clinical remission, the long-term management of the disease often results in adverse side effects such as immunosuppression, enhanced susceptibility to malignancies and suppressed resistance against infectious diseases (Bassaganya-Riera *et al.*, 2004). Thus, attention is being given to the possible use of cost effective natural products possessing anti-inflammatory activities that induce fewer side effects (Viladomiu *et al.*, 2013).

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disorder characterised by leukocyte recruitment and activation, cell proliferation, angiogenesis, and pannus formation leading to joint destruction (Scott, Wolfe &Huizinga, 2010). The global prevalence of RA is approximately 1% (de Grauw, van de Lest & van Weeren, 2009). Abnormal proliferation of synovium and excessive secretion of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), IL-6, IL-8, monocyte chemo-attractant protein-1 (MCP-1) and proteases secreted by immune cells contribute to pathological complications in the RA joint (Chen, 2010). Uncontrolled RA results in progressive joint destruction and functional decline. Due to its chronic immune-mediated pathology, long-term treatment with immunomodulatory drugs is generally required. Inhibitors of pro-inflammatory cytokines TNF-α, IL-1 and IL-6, as well as T- and a B-lymphocyte targeting agents, are currently approved to treat patients who fail to respond to conventional disease-modifying anti-rheumatic drugs (DMARDs) (Vivar & Van Vollenhoven, 2014). However, such

treatments are costly (Moller & Villiger, 2006) and increase susceptibility to infection (Keisuke *et al.*, 2011). Due to the severe side effects and high cost of these treatments, new and safe natural alternatives or co-therapies are needed. Because many spices are consumed with meals daily, these natural products are presumably considered to be safe and inexpensive relative to the conventional pharmacological agents.

The primary objective of the present study was to assess the anti-inflammatory activity of green curry paste (GCP) that contains nine spices. GCP was first digested *in vitro* for exposure of Caco-2 cell cultures to compounds in the bioaccessible fraction of digesta. Basolateral media was collected for selection of those compounds and or their metabolites from GCP likely absorbed and thus available for modulating immune cell (peripheral blood mononuclear cells) activities. The second objective was to test the anti-inflammatory activities of the bioaccessible fraction from digested GCP on the response of Caco-2 cells to pro-inflammatory insult.

METHODS

Dulbecco's modified Eagle's medium (DMEM), RPMI-1640 medium, lipopolysaccahride (LPS; *E. coli* O11:B4), digestive enzymes and reagents were purchased from Sigma (St. Louis, MO, U.S.A.). Fetal bovine serum (FBS) was obtained from PAA Laboratories (Haidmannweg, Pasching, Austria). L-glutamine, non-essential amino acids (NEAA), penicillin and streptomycin, fungizone were purchased from Invitrogen (Grand Island, NY, USA). IL-1β was purchased from Peprotech (Rocky Hill, NJ). All chemicals were of analytical grade.

Preparation of green curry paste (GCP)

All raw materials were purchased from the local market. The constituents of the green curry paste (GCP) per g fresh weight were as follows: 34.6 % fresh green chili pepper fruits (*Capsicum annuum*); 23.5% fresh lemon grass stem (*Cymbopogon citratus*); 20.7% fresh garlic bulb (*Allium sativum*); 9.5% fresh galangal root (*Alpinia galangal*); 1.1% dry kaffir lime peel (*Citrus hystrix*); 1.1% dry coriander seed (*Coriandrum sativum*); 0.5% dry pepper seed (*Piper nigrum*); 0.5% dry cumin (*Cuminum cyminum*); 0.5% fresh tumeric root (*Curcuma longa*); and 4.5% shrimp paste with 3.4% reduced sodium salt. These materials were collectively pounded to a homogeneous consistency. The GCP product was freeze dried by lyophilisation and homogenised by an electric kitchen blender. The freeze dried sample was stored in vacuum packed aluminum foil bags and stored at -20 °C until use.

In vitro digestion

The *in vitro* digestion procedure was slightly modified from Garrett, Failla & Sarama (1999). The freeze dried sample (0.7 g) with 5% soybean oil (v/v) was homogenised with 120 mM NaCl containing 150 µM butylated hydroxytoluene. The pH was adjusted to 2.0 ± 0.1 with HCl prior to addition with 2 ml of acid pepsin (40 mg/ml in HCl) and incubated for 1 h at 37 °C in a shaking water bath. The pH was adjusted to 6.0 ± 0.1 with $NaHCO_3$. Porcine bile extract, pancreatin and lipase were added to achieve final concentrations of 2.4 mg/ml, 0.2 mg/ml and 0.1 mg/ml, respectively, in the reaction mix. The pH was increased to 6.5 ± 0.1 with total reaction volume of 50 ml and incubated at 37 °C for 2 h in a shaking water bath. The digested sample was centrifuged at 10,000 g (Becton Dickinson Primary Care Diagnostics, Sparks, MD, U.S.A.) for 1 h to isolate the aqueous fraction and the supernatant was then filtered (0.22 μm pores) to obtain the bioaccessible fraction from digested GCP. A control sample was identically prepared by performing the *in vitro* digestion procedure in the absence of GCP.

Cytotoxicity of the bioaccessible fraction

Caco-2 cells (HTB37, American Type Culture Collection; passages 26–34) were maintained as previously described (Garrett *et al.,* 1999). Sulforhodamine B assay was used to assess the possible cytotoxicity of the bioaccessible fraction of GCP. Monolayers (10-11 days post-confluency) were washed once with DMEM before incubation with the diluted bioaccessible fraction (1:3-1:4 with basal DMEM) with/ without GCP for 4 h at 37°C in humidified atmosphere of 95% air/5% CO_2. Test media were removed after 4 h and monolayers were washed once with sterile warm PBS before initiating the SRB assay (Vichai & Kirtikara, 2006). Absorbance at 500 and 690 nm for cultures is proportionate to cell number. Corrected absorbance after exposure of cells to diluted (1: 4) bioaccessible fraction was not significantly different from untreated cultures.

Preparation of bioavailable fraction

Caco-2 cells were seeded at 2.5×10^4 cells/ml on 0.4 μm Polyethylene Terephthalate membrane 6-transwell cell culture inserts (Griener-bio-one, Miami, FL, USA). Cells were cultured and medium changed until 21–25 days post-confluence to obtain a fully differentiated, polarised intestinal like-epithelium. Lipoprotein synthesis and secretion by Caco-2 cells are maximal 3-4 weeks after the monolayer becomes confluent (Mehran *et al.*, 1997). The experimental design for obtaining the bioavailable fraction from the bioaccessible fraction of digested GCP is shown in Figure 1. Briefly, monolayers were incubated for 4 h with 2 ml of the diluted bioaccessible fraction and 2 ml basal RPMI 1640 in the apical and basolateral compartment, respectively. Basolateral medium (referred to as the bioavailable fraction) was collected to assess anti-inflammatory activity with peripheral blood mononuclear cells (PBMC) (Figure 1). The bioavailable fraction obtained after apical exposure to diluted bioaccessible fraction without GCP served as the control.

Anti-inflammatory activity of aqueous fraction from GCP against Caco-2 cells

Anti-inflammatory activity of the diluted bioaccessible fraction generated by digestion of GCP also was determined. After incubation of monolayers of Caco-2 cells with the diluted bioaccessible fraction for 4 h, apical medium were aspirated and the basolateral fraction (bioavailable fraction) was collected. Caco-2 cells were washed twice with phosphate buffer solution (PBS) containing 2g/l of albumin and induced inflammation by addition of 10 ng/ml of IL-1β to the apical compartment for another 20 h (Figure 1). The apical medium and cellular RNA were collected to measure IL-8 and MCP-1 proteins and mRNA, respectively.

Evaluation of anti-inflammatory activity of bioavailable fraction of GCP

Twelve active RA patients aged between 33-81 years were recruited from Ramathibodi Hospital, Mahidol University, Thailand. All patients in this study fulfilled the American College of Rheumatology Classification Criteria for diagnosis of RA. Stable-active RA was defined by the following criteria: (1) Erythrocyte Sedimentation Rate (ESR)>30 mm/h; (2) swollen joint count or tender joint count > 3 joints; (3) Disease Activity Score (DAS28) > 3.2. The exclusion criteria were diagnosis of other arthritic diseases, inflammation in other tissues, autoimmune disease such as systemic lupus erythematosus (SLE), consumption of dietary supplements including omega-3 fatty acids, vitamin C, vitamin E, *Moringa oleifera*, curcumin and fish oil, and infection or fever during the week prior to blood withdrawal. All experiments and study protocol were approved by the Ethical Committees of Ramathibodi Hospitaland Mahidol University. Patients were informed of the purpose of the study and the experimental design and

provided fully informed written consent. Whole blood in acid citrate dextrose (ACD) (Griener-bio-one, Kremsmunster, Austria) was collected and 9 ml of whole blood was layered on top of density gradient solution (Lymphoprep, Oslo, Norway) and subsequently centrifuged as described by manufacturer to obtain peripheral blood mononuclear cells (PBMC). PBMC from each patient were cultured in complete RPMI medium (basal RPMI-1640 supplemented with 10% (v/v) heat-inactivated FBS, 2 mM L-glutamine and 100 unit/ml penicillin and 100 μg/ml streptomycin) and incubated with/without 2 ng/ml of LPS (*E. coli* O11: B4) for 1 h prior to incubation with the bioavailable fraction for 18h from Caco-2 cell cultures treated with the bioaccessible fraction after simulated digestion without or with GCP (Figure 1). Media were collected to quantify TNF-α, IL-6 and IL-8 by ELISA.

Enzyme-Linked Immunosorbent Assay (ELISA) for cytokine production

TNF-α, IL-6, MCP-1 and IL-8 in culture medium were measured using paired antibody sandwich ELISA (Peprotech, Rocky Hill, NJ) (Tuntipopipat*et al.*, 2011). Concentration of TNF-α, IL-6, IL-8 and MCP-1 was calculated by comparing the absorbance with their respective standard curves.

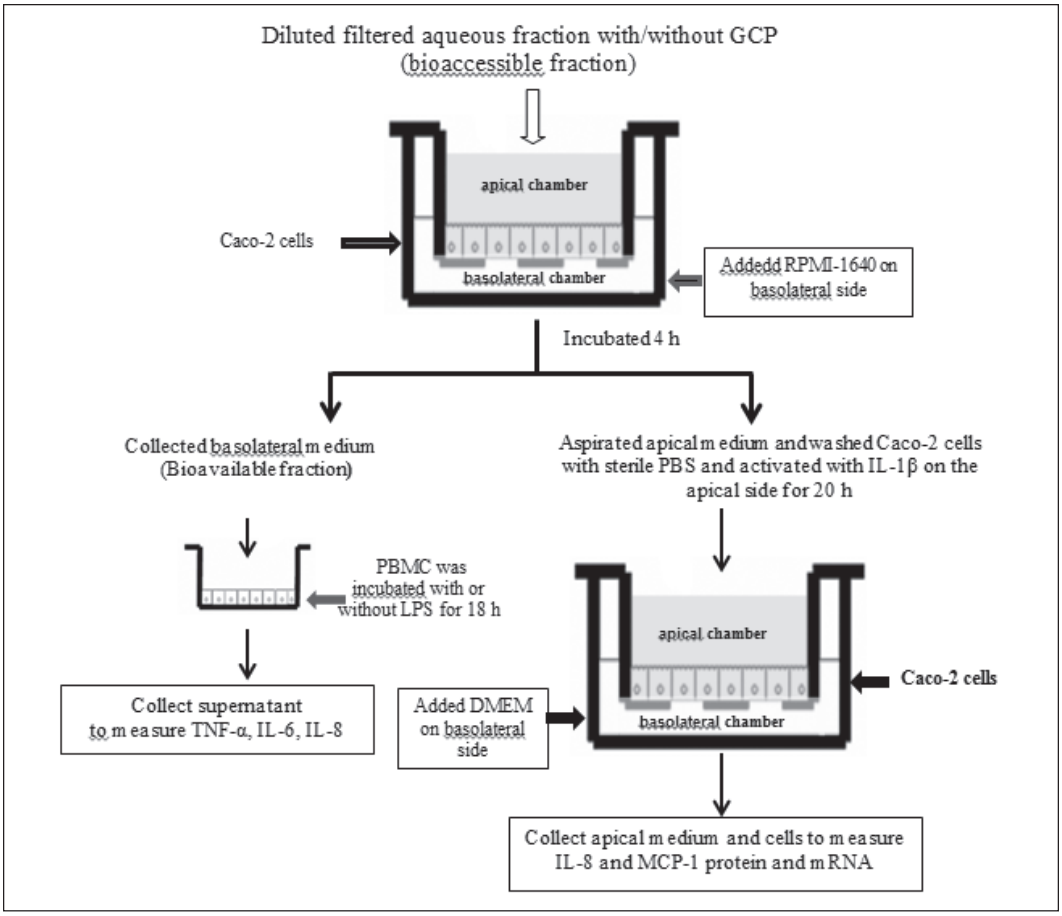

Figure 1. Schematic diagram of the experimental design

RNA extraction and RT-PCR

After Caco-2 cells were treated as described in the former session, RNA from treated Caco-2 cells was isolated by TRIZOL reagent (Invitrogen, Carlsbad CA, USA) according to the manufacturer's instructions. Complementary DNA (cDNA) was transcribed by reverse transcriptase with oligo-dT18 primer (Bio Basic Co., Ltd., Markham, ON, Canada) (final concentration 5 μM), 2 μl of 10 mM dNTP mix (Sib enzyme, Novosibirsk, Russia). The amplification of each target DNA were optimised to obtain a clear PCR product and amplified by Taq DNA polymerase in a MyCycler thermal cycler (BioRad, Hercules, CA, USA). The oligonucleotide primers used were as follows: IL-8 (forward) 5'-ATGACTTCCA-AGCTGGCCGTGGCT-3'(reverse) 5'-TCTCAGCCCTCTTCAAAAACTTCTC-3'; MCP-1 (forward) 5'-CTCGCTCAGCC AGATGCAATCAAT-3' (reverse) 5'-CCCAGGGGTAGAACTGTGGTTCAA-3'; and âactin (forward) 5'-GGCACCA CACCTTCTACAATG-3'(reverse); 5'-GGTCTCAAACATGATCTGGGTC-3'. The PCR products were separated on 2% agarose gel electrophoresis with 1x Tris-borate buffer containing SYBR® Safe DNA gel stain (Invitrogen). Analysis of band intensity was done by Image J program. The results were reported as relative ratio of band intensity between targeted mRNA/b-actin mRNA.

Statistical analysis

All statistical analyses were performed using SPSS (version 19.0, SPSS Inc., Chicago, Illinois). Representative values were expressed as mean ± SD or means ± SE. All data in the study were normal distribution. Paired student *t*-test was used to compare levels of TNF-α, IL-6 and IL-8 between those secreted from PBMC incubated with the bioavailable fraction with/without GCP. Statistical comparisons of more than two groups were determined by analysis of variance (ANOVA), followed by Duncan's multiple range test. The values were considered statistically significant when $p < 0.05$.

RESULTS

Bioavailable fraction of GCP suppresses spontaneous TNF-α, IL-6 and IL-8 secretion by PBMC from active RA patients

Average content of spontaneous TNF-α (37 vs. 52 pg/ml), IL-6 (69 vs. 93 pg/ml) and IL-8 (152 vs. 193 pg/ml) decreased when PBMC was exposed to bioavailable fraction with digested GCP compared to control bioavailable fraction lacking digested GCP as shown in Table 1. However, only the content of IL-6 decreased significantly ($p = 0.004$), while treatment effects on medium TNF-α ($p = 0.52$) and IL-8 ($p = 0.056$) failed to achieve statistical significance.

Bioavailable fraction of GCP inhibits LPS-activated TNF-α, IL-6 and IL-8 secretion by PBMC from active RA patients

Cultures of PBMC from RA patients were also treated with LPS to assess the effect of the bioavailable fractions from Caco-2 cells exposed to the diluted bioaccessible fraction with GCP. To be expected, cyto-/chemokine secretion by LPS-treated PBMC was much greater than in cultures not treated with LPS (Table 1). IL-6 secretion in LPS-activated cultures of PBMC from RA patients was significantly decreased ($p = 0.004$) by incubation in medium containing the bioavailable fraction from Caco-2 cells exposed to the bioaccessible fraction of digested GCP (Table1). The decreases in medium TNF-α and IL-8 in LPS-activated cultures of PBMC incubated with the bioavailable fraction generated by exposing Caco-2 cells to aqueous medium generated during digestion of GCP were not statistically significant (Table 1).

Table 1. Spontaneous and LPS-induced secretion of TNF-α , IL-6 and IL-8 by PBMC incubated with bioavailable fraction obtained after incubating Caco-2 cells with diluted bioaccessible fraction without (control) or with GCP (Figure 1).

Patient number	*TNF-α(pg/ml)*				*IL-6 (pg/ml)*				*IL-8 (pg/ml)*			
	Spontaneous		*LPS-stimulated*		*Spontaneous*		*LPS-stimulated*		*Spontaneous*		*LPS-stimulated*	
	Control[1]	*GCP*[2]	*Control*	*GCP*	*Control*	*GCP*	*Control*	*GCP*	*Control*	*GCP*	*Control*	*GCP*
1	28	25	789	837	49	34	5,287	4,585	156	98	600	558
2	39	26	962	854	22	21	4,422	4,435	329	181	927	789
3	38	14	1,268	1,368	131	60	6,187	5,845	143	118	676	576
4	18	16	659	558	71	43	8,366	8,007	269	211	688	563
5	27	17	561	434	47	33	8,079	7,029	145	135	428	421
6	16	27	600	571	24	37	5,225	5,035	291	298	280	230
7	78	70	461	319	94	81	4,015	3,421	132	77	284	249
8	29	21	1,341	1,138	33	20	6,001	5,315	258	100	668	559
9	12	7	1,116	977	148	98	5,466	4,789	156	130	405	385
10	80	62	1,062	1,110	145	122	4,210	3,924	126	170	298	407
11	62	52	998	1,049	143	111	4,276	3,626	161	84	396	191
12	198	116	1,371	1,657	211	177	4,969	5,420	155	221	290	367
mean	52.0	37.7	932	906	93.2	69.7	5,542	5,119	193	152	495	441
SE	14.8	9.1	91	113	17.7	14.1	412	389	20	19	61	50
P value[3]	0.054		0.52		0.004		0.004		0.056		0.06	

[1] Concentrations of pro-inflammatory factors in cultures of PBMC after incubation in basolateral medium from cultures of Caco-2 cells apically exposed to diluted bioaccessible fraction without GCP.

[2] Concentrations in cultures of PBMC after incubation in basolateral medium from cultures of Caco-2 cells apically exposed to dilute bioaccessible fraction containing GCP.

[3] Compares mean between of pro-inflammatory factors in cultures of PBMC after incubation in basolateral medium from cultures of Caco-2 cells apically exposed to diluted bioaccessible fraction with and without GCP.

Bioaccessible fraction of GCP suppresses IL-1β activated expression of IL-8 and MCP-1 expression on Caco-2 cells

Caco-2 cells secreted basal level of IL-8 and MCP-1 at 32 and 9 pg/ml, respectively (Figures 2a and 2c). Activation of Caco-2 cells with IL-1β significantly (p<0.05) increased IL-8 secretion to 167 pg/ml and MCP-1 to 65 pg/ml. These increases were associated with increased expression of IL-8 and MCP-1 mRNA (Figures 2b and 2d). Pre-incubation of Caco-2 cells with the diluted bioaccessible fraction of GCP significantly (p<0.05) decreased IL-1β -induced elevation of IL-8 protein and mRNA by 62% (Figure 2a) and 63% (Figure 2b), respectively, and MCP-1 protein and mRNA by 47% (Figure 2c) and 51% (Figure 2d), respectively, compared to cultures only treated with IL-1β.

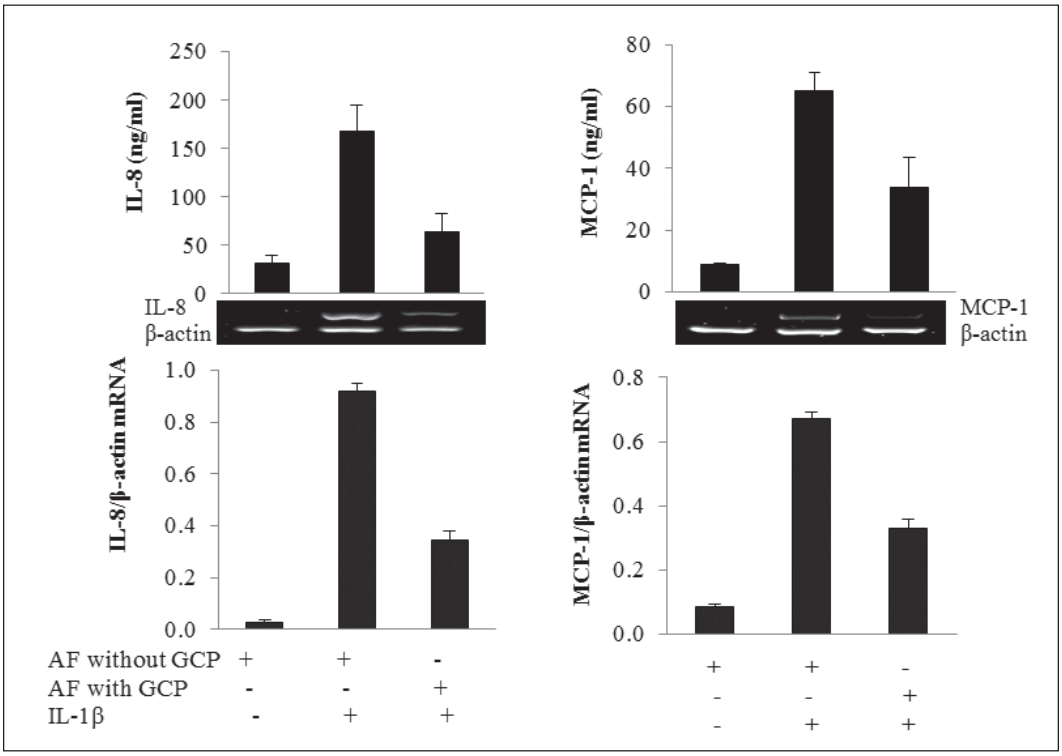

Figure 2. Diluted bioaccessible fraction (AF) of GCP suppressed expression of IL-8 and MCP-1 in IL-1β activated

Notes: Caco-2 cells. Differentiated Caco-2 cells grown on bi-cameral inserts were incubated with diluted bioaccessible fraction generated by digestion with or without GCP on apical compartment and RPMI-1640 was added on the basolateral compartment. Monolayers were washed after 4 h incubation and fresh medium containing 10 ng/ml IL-1β was added to the apical compartment and incubated for an additional 20 h. Culture medium and cells were collected to measure IL-8 (A) and MCP-1(C) proteins by ELISA and mRNA (B and D) by RT-PCR.

Results are presented as mean ± SD for three independent experiments.
Presence of asterisk above error bar exhibits significant differences in mean for cultures pre-treated with diluted bioaccessible fraction of GCP vs cultures pre-treated with diluted bioaccessible fraction without GCP (p<0.05).

DISCUSSION

This study showed that bioaccessible compounds from the digested GCP that transported across monolayers of Caco-2 cells decreased spontaneous secretion of TNF-α , IL-6 and IL-8 produced by PBMC from some RA patients (Table 1). Also, IL-6 secretion by LPS-activated PBMC from RA patients was attenuated by exposure of cells to the bioavailable fraction of Caco-2 cells incubated with the diluted bioaccessible fraction from digested GCP. It is possible that some of the bioactive compounds present in the basolateral medium containing bioavailable compounds transported across the monolayer of Caco-2 cells may have been metabolites of compounds in apical medium, i.e., Caco-2 cells transformed some of the compounds in GCP into bioactive metabolites. One or more of the compounds in the diluted bioaccessible fraction of digested GCP also markedly attenuated IL-8 and MCP-1 expression in cultures of IL-1β activated Caco-2 cells (Figure 2).

Elevated concentrations of pro-inflammatory cytokines have been reported in sera and synovial fluid of RA patients with its content associated with the severity of the pathology (Chen, 2010). RA patients treated with anti-TNF-α(infliximab) and the soluble TNF receptor (etarnecept) had reduced serum IL-1, IL-6, IL-8, and MCP-1, reduction of disease scores such as morning stiffness, assessment of pain, patient's and physician's global assessment scores, DAS28 and American College of Rheumatology (ACR) response, and markers of bone resorption (Klimiuk *et al.*, 2011). IL-6 is a cytokine with a central role in acute and chronic inflammation. It promotes bone resorption which has a pivotal role in synovitis, bone erosions and in the systemic features of inflammation. Humanised anti IL-6R monoclonal antibody (tocilizumab) effectively controls local and systemic inflammatory manifestations and blocks cartilage and bone destruction (Fonseca *et al.*, 2009). The suppression of IL-6 secretion in PBMC from RA patients by one or more bioavailable compounds or metabolites in digested GCP compounds suggests that GCP may be a complementary food for the management of rheumatoid arthritis.

This study also demonstrates the anti-inflammatory effect of bioaccessible fraction from GCP in an *in vitro* model of intestinal inflammation consisting of IL-1β-activated Caco-2 cell monolayers. IL-1β induces synthesis and secretion of IL-8 and MCP-1 (Ligumsky *et al.*, 1990) that leads to the recruitment and activation of neutrophils and monocytes within the inflamed intestinal mucosa. IBD, the most common and serious chronic inflammatory conditions of human bowel (Khan *et al.*, 2006), is the result of impaired integrity of the intestinal mucosa barrier and dysfunction of gut immunity (Corfield, Wallace & Probert, 2011). Expression of IL-8 and MCP-1 is up-regulated in intestinal mucosa of IBD patients (Khan *et al.*, 2006; Atreya & Neurath, 2010). One or more compounds in the bioaccessible fraction of digested GCP markedly attenuated IL-8 and MCP-1 expression in cultures of IL-1β activated Caco-2 cells (Figure 2). Data from the present study showed that the anti-inflammatory activity of GCP is retained after digestion. The possible use of GCP for the prevention and as an adjuvant for inflammatory bowel disorders merits examination.

The GCP contains 9 spices including fresh green chili pepper fruits, fresh lemon grass stem, fresh garlic bulb, fresh galangal root, dried kaffir lime peel, coriander seed, dried pepper seed, cumin and tumeric root. Insights regarding which of the hundreds of compounds in GCP and similar mixtures of spices have anti-inflammatory activity are provided in the literature. For example, we previously reported that the ethanol extract from red curry paste (RCP) significantly suppressed LPS-activated pro-inflammatory mediator gene expression including

inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), TNF-α, and IL-6 in LPS-induced murine macrophage cell line (Tuntipopipat *et al.*, 2011). The RCP contains seven spices, i.e., dry red chili pepper, dry garlic bulb, fresh lemon grass stem, dry shallot bulb (*Allium cepa*), fresh galangal root, dry kaffir lime peel, and dry pepper seed. Six of these spices are common ingredients in green and red curry pastes. Chili pepper is the main ingredient of both curry pastes. Anti-inflammatory activity has been reported for whole fruits, crude extracts and capsaicin. Injection of mice with chili extract alleviated carrageenan-induced pleurisy (Zimmer *et al.*, 2012). Red pepper juice showed anti-inflammatory activity against carragenan- and antigen-induced inflammation in rats by decreasing level of TNF-α and IL-1β (Spiller *et al.*, 2008). Treatment of mice with aqueous extract of lemongrass inhibited IL-1β secretion by LPS-induced peritoneal macrophages (Sforcin *et al.*, 2009). The sulfur compounds from garlic extract inhibited LPS-NO and PGE2 production and the expression of the TNF-α, IL-1β, IL-6, iNOS and COX-2 in activated RAW264.7 cells (Lee *et al.*, 2012). The suppressive effect, exerted through NF-kB inactivation, also markedly inhibited the LPS-induced phosphorylations of p38 mitogen-activated protein kinases and extracellular signal-regulated kinases (ERK) (Lee *et al.*, 2012). Diarylheptanoid isolated from galangal root significantly suppressed LPS-induced production of iNOS, IL-1β TNF-α and COX-2 gene expression in murine macrophage cells (Yadav, Liu & Rafi, 2003). Although the dry kaffir lime peel (*Citrus hystrix*) contributes only 1.1% in GCP's recipe, a recent study demonstrated the inhibitory effects of a product made from the peel of six citrus fruits in Japan on azoxymethane-induced colitis (Lai *et al.*, 2013). Feeding this product decreased the number of aberrant crypt foci (ACF), suppressed iNOS, COX-2, ornithine decarboxylase, vascular endothelial growth factor, and matrix metallopeptidase concentrations in colonic tissues. The GCP recipe also contains 0.5% turmeric whose active compound 'curcumin' has been shown to have numerous health promoting benefits effects including anti-collagen-induced arthritis activity (Huang *et al.*, 2013). The combined effects of the mixture of spices in curries with their numerous compounds possess anti-inflammatory effect on chronic inflammatory conditions which merits further investigation in animal models and humans.

CONCLUSION

This study provides preliminary data to support anti-inflammatory activity of dietary green curry paste. *In vivo* pre-clinical and clinical studies now are needed to investigate effects of the GCP to modulate the pathological complications associated with chronic inflammatory conditions such as RA and IBD patients. However, the primary limitations of the present study were the relatively small number of RA donors for the collection of PBMC and the cross-sectional nature of the study with a single clinical assessment and single collection of blood for isolation of PBMC from each patient. Also, the amount of green chili paste used in this *in vitro* experiment cannot be directly translated to the *in vivo* situation because simulated digestion as performed did not include other foods to which GCP is generally added and our cellular studies were limited to two types of cells (Caco-2 cells and PBMC) that were incubated separately. Future studies should be conducted on the use of traditional curry as a food component in attenuating the severity of inflammatory conditions.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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