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Bromelain Treatment Decreases Secretion of Pro-Inflammatory Cytokines and Chemokines by Colon Biopsies *In Vitro*

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Abstract

Oral bromelain has been anecdotally reported to decrease inflammation in ulcerative colitis (UC). Proteolytically active bromelain is known to decrease expression of mRNAs encoding pro-inflammatory cytokines by human leukocytes *in vitro.* To assess the effect of bromelain on mucosal secretion of cytokines in inflammatory bowel disease (IBD), endoscopic colon biopsies from patients with UC, Crohn's disease (CD), and non-IBD controls were treated *in vitro* with bromelain or media, then cultured. Secretion of pro-inflammatory cytokines and chemokines was measured. Significant increases in granulocyte colony stimulating factor (G-CSF), interferon (IFN)-γ, interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF) were detected in the media from actively inflamed areas in UC and CD as compared with non-inflamed IBD tissue and non-IBD controls. *In vitro* bromelain treatment decreased secretion of G-CSF, granulocyte-macrophage colony stimulating factor (GM-CSF), IFN-γ, CCL4/macrophage inhibitory protein (MIP)-1β, and TNF by inflamed tissue in IBD. Bromelain may be a novel therapy for IBD.

Keywords

proteinase therapy; inflammatory bowel disease; cytokines

Introduction

Inflammatory bowel diseases (IBDs) such as Crohn's disease (CD) and ulcerative colitis (UC) are currently hypothesized to result from an aberrant immune response to components of enteric bacteria that occurs in a genetically predisposed host (1,2). The ensuing immunologic reaction results in unbalanced production of Th1 (e.g. IFN-γ, IL-2, and lymphotoxin) vs. Th2 cytokines (e.g. IL-4, IL-5, IL-10, and IL-13). Tumor necrosis factor (TNF) is also over-expressed in both CD and UC. Pro-inflammatory cytokines affect colonic mucosal integrity by altering expression of growth factors and metalloproteinases (3). In addition, they affect leukocyte recruitment by altering production of chemokines (4). Accumulation and activation of inflammatory cells within the colonic mucosa leads to the tissue damage that is characteristic

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of IBD and results, directly and indirectly, in the clinical symptoms of bloody diarrhea and abdominal pain.

An anecdotal report by Kane et al. (5) described 2 patients with UC refractory to conventional treatments who rapidly entered and remained in clinical and endoscopic remission after self-treatment with oral bromelain obtained from a health food store. Bromelain is a mixture of proteolytic enzymes derived from pineapple stem that has demonstrated beneficial effects in various models of inflammation. These include carrageenan-induced pleurisy in the rat (6–8), immunologically mediated arteriosclerosis in rat aortic allografts (9), the experimental allergic encephalomyelitis (EAE) model for the human autoimmune disease multiple sclerosis (10), and several human rheumatologic diseases (11–13). Although the exact composition of bromelain preparations can vary according to source and method of purification, the major protein components are stem bromelain (~80%), fruit bromelain (~10%), and ananain (<5%). These proteinases have different substrate specificities toward both model substrates and cell surface molecules. The activity of individual proteinases within pharmaceutical bromelain preparations can be monitored using model substrate assays or by flow cytometric assessment of their effects on specific bromelain-sensitive cell surface molecules (14).

We and others have previously shown that *in vitro* treatment with bromelain removes certain cell surface molecules that affect leukocyte migration and activation (15–18). Treatment with proteolytically active bromelain affects T cell expression of mRNAs encoding IL-2, IL-4, and interferon (IFN)-γ and inhibits ERK-2-mediated signal transduction in leukocyte and colon epithelial cell lines *in vitro* (19,20). We recently showed that oral bromelain can retain sufficient proteolytic activity within the gastrointestinal tract of mice to remove bromelain-sensitive molecules from intestinal epithelial cells and macrophages (21). Furthermore, treatment with oral bromelain decreases the incidence of spontaneous colitis and the severity of both spontaneous and established colitis in C57BL/6 IL-$10^{-/-}$ mice (22). The anti-inflammatory effect of bromelain requires proteolytic activity, since treatment with inactivated bromelain has no effect on the incidence and severity of colitis in these models (22). However, the specific cell types affected when bromelain is given orally and the cell surface molecules that must be removed from these cells to achieve beneficial anti-inflammatory effects are still under intense investigation.

The purpose of this study was to determine whether *in vitro* bromelain treatment could alter the secretion of pro-inflammatory cytokines and chemokines by colon biopsy tissue obtained from patients with IBD and from normal, non-IBD controls.

Materials and Methods

Study population

All patients ≥ 18 years of age with a histopathologically confirmed diagnosis of Crohn's disease (involving the colon) or ulcerative colitis who presented to the Duke Endoscopy Unit for colonoscopy on selected days between January 1, 2003 and September 30, 2007 were invited to participate in the study. During the same time periods, patients who presented to the Endoscopy Unit for routine, average risk colorectal cancer screening (i.e. no history of symptoms and no family history of colorectal malignancy) or for routine follow up of prior colon polyps were invited to participate as the normal, non-IBD control group. Patients with a history of prior colon malignancy, those in whom colonoscopy was being performed to evaluate symptoms, or those with findings suspicious for malignancy or inflammation at the time of colonoscopy were excluded from the control group.

Clinical information on each patient was obtained from the medical record, from a brief interview prior to colonoscopy, and from the colonoscopy report. Data collected included: age,

gender, diagnosis (CD, UC, normal control), and endoscopic rating of disease activity. The clinical characteristics of the population studied are shown in Table 1. This study was approved by the Institutional Review Board, and all patients gave written informed consent prior to participation.

Mucosal biopsies

Paired endoscopic biopsy samples of colonic mucosa were obtained from all participants. At the time of colonoscopy, the endoscopic appearance of the colitis in the study population was classified as “active” or “inactive”. The term “inactive” was applied to areas that were either grossly normal in appearance or showed evidence of scarring from previous inflammation. Areas of the colon that were inflamed as evidenced by mucosal erythema, edema, friability, or frank ulceration were termed “active”. If inflammation was present, biopsies were obtained from the most severely inflamed region. For patients whose colons contained areas of active as well as inactive disease, two pairs of biopsies were obtained, one pair each from the active and inactive areas. These paired biopsies from a single patient were accordingly labeled “inflamed” and “non-inflamed”. Patients without evidence of active disease at the time of colonoscopy had a single pair of biopsies labeled “non-inflamed”. Patients whose colitis was active throughout had a single pair of biopsies labeled “inflamed”. Control patients had a single pair of biopsy specimens labeled “control”.

Culture of Colon Biopsies and Cytokine Assays

Biopsies were washed three times in RPMI1640 media (Gibco BRL, Grand Island, NY) containing 500 U/ml penicillin and 500 μg/ml streptomycin (5X pen/strep) then weighed. One biopsy of each set was treated with 1 mg/ml bromelain (catalog #B-4882; Sigma-Aldrich Chemical Co., St. Louis, MO) in RPMI + 1X pen/strep (media) for 1 hour at 37°C while the other was treated with media alone. These treatment conditions have previously been shown to be sufficient to remove bromelain-sensitive molecules from a variety of different cell types (15,16,21). All biopsies were treated using aliquots derived from the same lot of bromelain. Bromelain proteolytic activity was monitored periodically using model substrate assays as described (14). The biopsies were washed with media + 10% FBS to remove and/or inactivate any residual bromelain, then cultured in 0.5 ml media. Culture media was collected after 24 hrs, filtered through a 0.2 μm filter, and stored at −80°C until analysis. For some samples, bromelain that had been inactivated chemically or with heat (14) was compared with active bromelain to determine the role of proteolytic activity in any effects observed.

The cytokines present in spent media were quantitated using a Luminex bead-based fluorescent multiplex immunoassay (BioRad, Hercules, CA). The cytokines tested were granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12 (p70), IL-13, IL-17, MCP-1, MIP-1β, and TNF. Cytokine levels were expressed as pg cytokine/ml/mg biopsy tissue using standard curves generated at the time of biopsy analysis. The lower limit of detection was defined as the concentration that yielded a signal >2 SD above background; this varied from 1 – 10 pg/ml for the analytes tested. According to the manufacturer’s literature, the reagents used had negligible cross-reactivity with other analytes.

Statistical Analysis

Since the data was not normally distributed and there were some extreme values, all analyses were performed using non-parametric methods. Thus, medians and ranges are reported rather than medians and standard deviations. To determine whether mucosal levels of pro-inflammatory cytokines differed between normal (non-IBD) controls and IBD patients, or between patients with CD and those with UC, distributions were compared using the Wilcoxon

rank sum test. Comparisons between pairs of biopsies obtained from the same subjects (non-inflamed vs. inflamed tissue samples, media vs. bromelain-treated) were made using the Wilcoxon signed-rank test. All tests were two-sided and significant was defined at an α level of 0.05. SAS, Version 8.2 (Cary, NC) was used for all analyses.

Results

Cytokine secretion

Human IBD is characterized by accumulation and activation of immune cells within the intestinal mucosa. We first determined whether increased secretion of pro-inflammatory cytokines and chemokines could be detected in media when colon biopsies from IBD patients were cultured *in vitro*. Low levels of IL-2, IL-4, IL-5, IL-7, IL-12 (p70), IL-13, and IL-17 were detected in many biopsy cultures, but the levels of these cytokines did not differ significantly between normal and IBD biopsies (p = NS). Levels of IL-10 were quite variable and also did not differ significantly between tissues derived from patients with and without IBD.

Secretion of G-CSF, GM-CSF, IFN-γ, IL-1β, IL-6, MCP-1, MIP-1β, and TNF by cultured colon tissues obtained from non-IBD control patients (n = 8) was similar to that observed in non-inflamed tissues from patients with UC (n = 20) and CD (n = 23). However, increased levels of G-CSF, IFN-γ, IL-1β, IL-6, and TNF were secreted by biopsies obtained from inflamed portions of the colon in patients with either UC (n = 12) or CD (n = 18) compared with normal biopsies (Table 2, Figure 1). These increases were generally more dramatic in the UC patients. Analysis of the 26 matched pairs of inflamed vs. non-inflamed tissues within individual UC and CD patients also found significantly higher levels of G-CSF, IFN-γ, IL-1β, IL-6, and TNF in the inflamed tissues (all $p \leq 0.0001$).

Bromelain effect on secretion of pro-inflammatory cytokines and chemokines

We next examined whether *in vitro* bromelain treatment affected production of the 8 pro-inflammatory cytokines and chemokines that were produced in at least moderate levels by human colon tissue. Bromelain treatment significantly reduced levels of G-CSF, IFN-γ, and MIP-1β present in culture media from inflamed and non-inflamed UC tissue (except for IFN-γ in non-inflamed UC tissue where p = 0.06), inflamed and non-inflamed CD tissue, and normal tissues. Bromelain also significantly reduced secretion of TNF from inflamed UC and CD tissues and GM-CSF from inflamed UC tissues (Table 2, Figure 1). Decreases in secretion of MCP-1 were observed both in normal tissue (p = 0.023) and non-inflamed CD tissue (p < 0.001) exposed to bromelain and also approached significance for inflamed tissues from IBD patients (p=0.058). However, bromelain treatment did not appear to have an effect on the levels of IL-1β or IL-6 detected in the culture media of normal tissues or inflamed or non-inflamed IBD tissues. Continued secretion of these cytokines following bromelain treatment provides evidence of the viability of the colon biopsies and demonstrates that their ability to produce cytokines is not adversely affected by bromelain treatment. Comparison of matched biopsies (either both active or both inactive) from the same patient showed that bromelain treatment did not influence IL-10 production, which remained low (median 6 pg/ml for bromelain-treated normal biopsies; median 4 pg/ml for bromelain–treated inflamed UC biopsies).

To exclude the possibility that residual bromelain might cause decreased detection of cytokines by either inactivation of the antibodies used to detect the cytokines or direct proteolytic degradation of cytokines, known quantities of the 16 cytokines analyzed were incubated with 10 μg/ml bromelain at 37°C for 1 hr then analyzed by Luminex multiplex fluorescent immunoassay. The 10 μg/ml bromelain concentration models a 1% carryover based on the 1 mg/ml used for bromelain treatment of the biopsies. Less than 10% change in cytokine concentrations were observed after incubation with bromelain (n = 3 tests per cytokine).

To determine the role of bromelain proteolytic activity on cytokine secretion, 8 additional sets of biopsies from IBD patients were obtained in triplicate and incubated with either media alone, proteolytically active bromelain, or inactive bromelain and their cytokine production determined. Effects on secretion of G-CSF was used as the primary endpoint for this study, since this cytokine is reliably secreted in at least moderate amounts by both non-inflamed and inflamed biopsies and is markedly decreased by exposure to active bromelain. The median G-CSF secreted by these predominately non-inflamed biopsies treated with proteolytically inactive bromelain was 128 pg/ml/mg tissue (range 25 – 237), a value that did not differ from that seen using media alone (179 pg/ml/mg tissue, range 47 – 1150) ($p = 0.156$, Wilcoxon signed rank test). In contrast, proteolytically active bromelain significantly deceased G-CSF secretion to a median of 11 pg/ml/mg tissue (range 1 – 102; $p = 0.008$ compared with inactive bromelain). Thus, the effects of bromelain treatment on cytokine secretion by colon biopsies appears to be dependent upon the proteolytic activity of bromelain.

Discussion

Cultured biopsies from clinically inflamed portions of the colon of IBD patients produced increased levels of the pro-inflammatory cytokines G-CSF, IFN-γ, IL-1β, IL-6, and TNF compared with non-inflamed colon tissues from the same patients. These cytokines have previously been shown to be increased in media derived from organ cultures of colon tissues from patients with active IBD (23–25). This provides an additional validation of the use of organ culture methods used here to study the effects of experimental bromelain treatment on cytokine secretion. Multiplex fluorescent immunoassays allowed us to quantitate multiple cytokines simultaneously in the small quantity of media derived from culture of each individual colon biopsy.

Although Th2 cytokines have been implicated in IBD, particularly in UC, the levels of Th2 cytokines (IL-4, IL-5, IL-10, IL-13) that we observed to be secreted into the media were low and were not increased in the IBD biopsies cultured. One possible explanation for this is that the environment during organ culture may not provide immune stimulation to mimic that present in the colon *in vivo*. Alternatively, this may reflect the effects of therapy that the IBD patients received *in vivo* prior to biopsy excision.

Treatment of colon biopsies with bromelain before culture decreased secretion of G-CSF, IFN-γ, and MIP-1β by biopsies from all patient groups, with decreased secretion of GM-CSF also observed in inflamed biopsies from UC patients and decreased TNF secretion in inflamed biopsies from patients with either CD or UC (Figure 1). Decreased MCP-1 secretion was additionally observed in normal and non-inflamed IBD tissues. The effect of bromelain treatment on secretion of cytokines and chemokines by colon biopsy cultures was specific, since some of the cytokines analyzed (e.g. IL-1β, IL-6) were clearly not affected by bromelain treatment. These effects required proteolytic activity and were not seen when biopsies were treated with proteolytically inactive bromelain.

The effects of bromelain on cytokine secretion have previously been studied only in leukocytes. Mynott *et al* showed that bromelain treatment prevented increases in mRNAs encoding IL-2, IL-4, and IFN-γ in GA15 Th0 cells following stimulation with phorbol ester plus calcium ionophore, but not with CD3 mAbs (19). The results reported here both confirm and extend these previous observations. Bromelain treatment decreased IFN-γ production by cultured biopsies, similar to what was previously reported for leukocyte cell lines (19). However, the production of IL-2 and IL-4 by the cultured colon biopsies was too low to reliably detect differences following bromelain treatment. The studies reported here also show that, in addition to decreased IFN-γ secretion, bromelain treatment decreased G-CSF and MIP-1β secretion by all groups of colon biopsies. Other effects of bromelain treatment included decreased secretion

of GM-CSF from inflamed UC tissues, decreased TNF secretion from inflamed CD and UC colon tissues, and decreased MCP-1 secretion from normal colon tissue. These changes in cytokine and chemokine secretion were observed without the addition of exogenous stimulants. It is likely that residual bacteria and bacterial lipopolysaccharides (LPS) present in the cultures stimulated cytokine production by cells present within the biopsies. Although washing colon biopsies removes residual fecal material and allows short-term cultures in the presence of antibiotics, it does not eliminate bacterial contamination. Furthermore, Swidsinski *et al* showed that IBD patients, particularly those with CD, have increased numbers of mucosally-associated bacteria that are not removed by washing (26). Thus, cytokine production during culture of colon biopsies may reflect stimulation that occurred *in vivo* or may be due to mucosally-adherent bacteria and their products that remain present at low levels within the cultures themselves. Any differences in mucosally-associated bacteria between biopsies obtained from normal and IBD patients would reflect differences that are also present *in vivo* (26).

IFN-γ is a pro-inflammatory cytokine produced predominately by activated T and NK cells. Increased tissue levels of IFN-γ are a hallmark of CD (27). IFN-γ increases mucosal permeability through effects on epithelial tight junctions and by inducing cellular adhesion molecules. This increased permeability can potentially increase immune system exposure to antigens derived from the intestine. Induction of adhesion molecules on endothelial and immune cells increases the ability of neutrophils, monocytes, and lymphocytes to adhere to blood vessels and extravasate into inflamed tissues (28). However, despite the important role of IFN-γ in the inflammatory process, a humanized IgG_1 monoclonal antibody to IFN-γ (HuZAF) failed to show any benefit over placebo in a small trial in moderate to severe CD (29).

TNF has been targeted as a key cytokine in the inflammatory cascade, with particular importance in CD. The central role identified for TNF led to the approval of infliximab (Remicade®), a monoclonal antibody to TNF, as the first biologic therapy for the treatment of patients with active CD. Two large, phase III, randomized placebo-controlled trials have also demonstrated efficacy of infliximab in UC (30). The data in the present study demonstrate similar mucosal TNF production in biopsies from patients with active UC and CD, providing indirect evidence supporting the use of anti-TNF therapies for both these forms of IBD.

The chemokine MIP-1β, also known as CCL4, is known to be produced by T cells and macrophages and has been shown to be chemotactic for monocytes, activated T cells, NK cells, and immature dendritic cells that express its receptor, CCR5. Although it has been suggested that MIP-1β is increased in UC (31), our studies did not confirm elevated secretion of MIP-1β by inflamed tissues from IBD patients compared with non-inflamed or normal tissues. However, bromelain treatment markedly decreased secretion of this chemokine by normal tissue and by both non-inflamed and inflamed tissue from IBD patients.

The chemokine MCP-1 (also known as CCL2 or MCAF/JE) has previously been shown to increase when colon cells are exposed to inflammatory stimuli (32) and to be elevated in UC (30,32). Previous work has shown that MCP-1 is produced by fibroblasts and smooth muscle, endothelial, epithelial, and activated peripheral blood mononuclear cells (27,34). MCP-1 binds to the CCR2 chemokine receptor and stimulates migration of monocytes and macrophages, eosinophils, and lymphocytes into the inflamed tissue (27). In our study, MCP-1 secretion did not vary significantly between normal controls and non-inflamed or inflamed IBD colon tissues. However, secretion of MCP-1 by normal colon tissue was decreased following bromelain treatment.

G-CSF stimulates the production and survival of neutrophils. G-CSF has previously been noted to be increased in organ culture supernatants of colon tissues from patients with IBD (24). G-

CSF was demonstrated to delay apoptosis of neutrophils, an activity that could lead to accumulation of neutrophils in mucosal tissues of IBD patients (25). However, CD has also been associated with neutropenia and with impaired neutrophil migration. A clinical trial involving 20 patients showed that systemic administration of pharmacologic doses of recombinant human G-CSF (filgrastim) led to decreased CD activity scores in 55% and sustained remission in 25% (35). G-CSF therapy restores neutrophil migration in CD patients (36), which has been proposed to account for the therapeutic effect of G-CSF. Bromelain treatment markedly decreased secretion of G-CSF by colon tissues. Whether this has systemic effects and will prove to be beneficial or detrimental *in vivo* will require direct testing. However, it is likely that the overall effect of bromelain on colon inflammation will be related to the sum of its effects on the total cytokine milieu, rather than effects on any individual cytokine.

In summary, bromelain treatment reduced secretion of several pro-inflammatory cytokines and chemokines that have been shown to be elevated in IBD and to play a role in the pathogenesis of IBD. Bromelain treatment could thus potentially result in decreased leukocyte migration to the colon and decreased overall inflammatory activity if similar changes also occur when colon tissues are exposed to bromelain *in vivo*. However, these studies did not directly address potential mechanisms by which bromelain may decrease cytokine and chemokine production by colon tissues. Bromelain removes cell surface molecules that govern leukocyte activation thresholds (e.g. the CD14 receptor for bacterial LPS on monocytes and the CD21 activation regulator on B cells; 16). Bromelain also removes cell surface receptors that can serve as receptors for intestinal bacteria (16,37-41), which may decrease exposure of immune cells to bacterial antigens that stimulate detrimental immune responses in IBD. Proteolysis by bromelain may also affect production of pro-inflammatory cytokines and chemokines by altering concentrations of regulatory cytokines such as IL-10 or TGF-β that was not analyzed in the current study.

In contrast to the current study, several *in vitro* studies have shown that bromelain treatment can actually increase production of pro-inflammatory cytokines such as IL-6, TNF, IFN-γ, GM-CSF by macrophages, NK cells, and mixed lymphocyte cultures (42–44). Additional studies are needed to address the mechanisms by which bromelain affects colon tissues. Further studies will also be required to determine the duration of bromelain effects in this system. Our previous work demonstrated that the time required for re-expression of cell surface molecules removed by bromelain proteolysis depended on the specific cell type and cell surface molecule affected. Replacement times ranged from 15 minutes for CD62L on peripheral blood leukocytes (L.P. Hale, unpublished) to >48 hrs for CD44 on T cells (15). Thus, it is of critical importance to determine the specific cell type in the intact intestinal biopsies that is mediating the down-regulatory effects of bromelain in this system.

Based on the work of Mynott *et al*, bromelain treatment most likely results in inhibition of cytokine/chemokine expression at the transcriptional level (19). Under the conditions used for our studies in which bromelain was removed and/or inactivated prior to biopsy culture, direct degradation of the cytokines and chemokines studied by bromelain was unlikely. However, disruption of cytokine or chemokine function post-translationally by direct proteolysis or through other mechanisms (e.g. interfering with binding to receptors) would also be predicted to be beneficial in providing anti-inflammatory activity in IBD. Clearly, further investigation as to how bromelain treatment affects pro-inflammatory cytokine and chemokine secretion by cells present in inflamed colon is needed. Such studies may lead to new insights into the pathogenesis of IBD and stimulate development of novel therapies for treatment of IBD.

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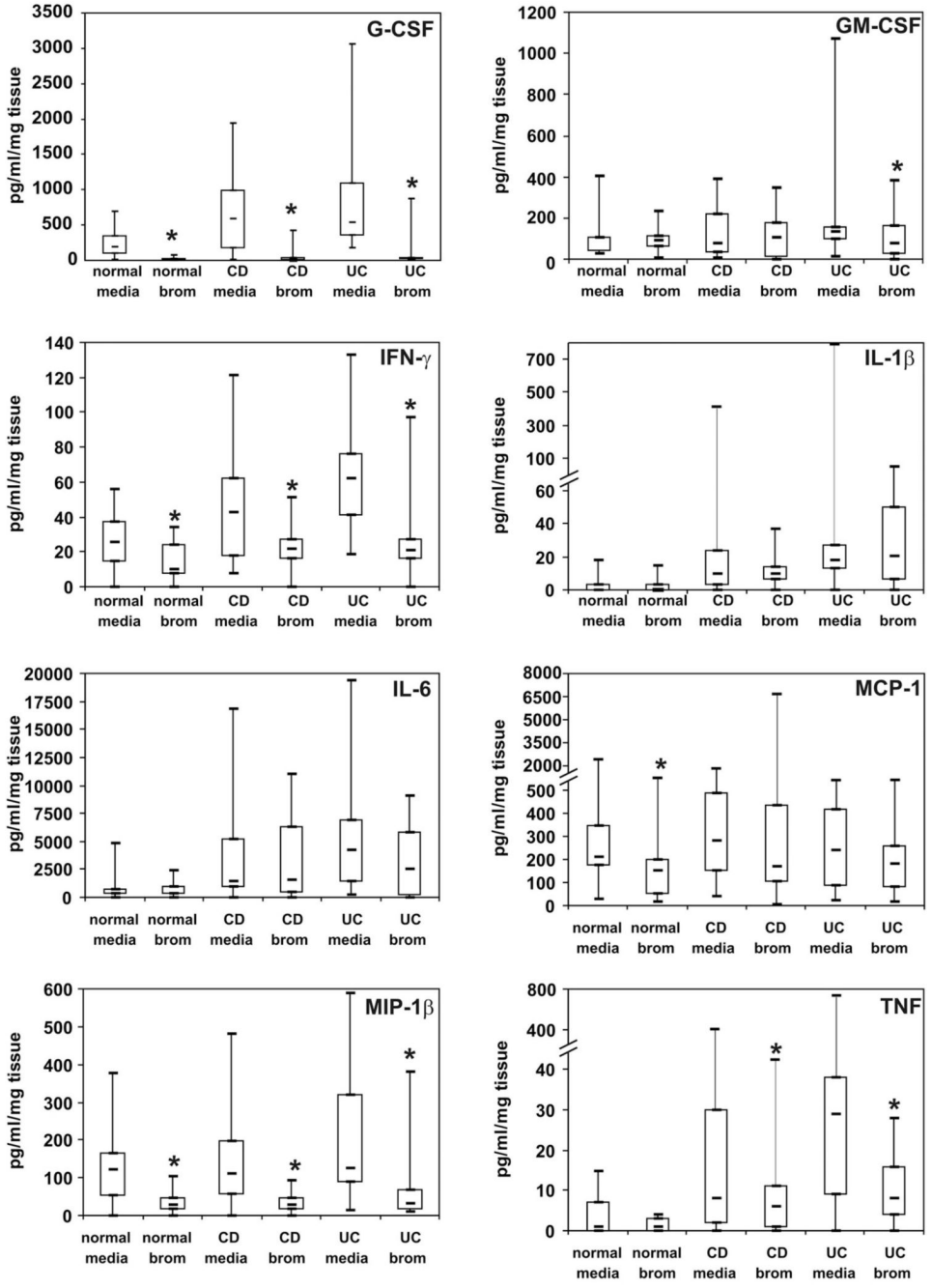

Figure 1. Effect of bromelain on cytokine and chemokine secretion by human colon tissues

Paired colon biopsies derived from normal patients without IBD (n = 8) and inflamed colon from patients with CD (n = 18) and UC (n = 12) were treated with media alone or 1 mg/ml bromelain in media at 37° for 1 hr, then washed to remove and inactivate bromelain prior to culture. Cytokine levels detected in the media following organ culture for 24 hrs are reported as pg/ml/mg tissue. The box represents the lower through upper quartiles of values obtained. Median values are indicated by horizontal lines within the boxes. The minimum and maximum values obtained are indicated by the endpoints of the vertical lines. Vertical lines that are longer than the 1.5 times the height of the box indicate the observance of outliers. Note that the axes are broken in panels reporting results for IL-1β, MCP-1, and TNF to reflect that wide ranges

of values observed for those cytokines. * indicates $p \leq 0.03$ when cytokine levels produced by media-treated and bromelain-treated tissues are compared using the Wilcoxon signed- rank test. Cytokine secretion from non-inflamed biopsies from patients with CD (n = 22) and UC (n = 20) did not differ from that seen with normal tissues, either at baseline (Table 2) or after bromelain treatment. Therefore, these data are not shown in this figure.

Table 1

Clinical Characteristics of Colon Biopsy Donors

	Normal	UC	CD
Gender	4 male/4 female	11 male/11 female	5 male/19 female
Mean Age (Range)	62 (50 – 78)	47 (23 – 77)	46 (25 – 70)
N active/N Inactive *	not applicable	12/10	17/7

* Disease activity was assessed endoscopically as described in Methods.

Table 2

Baseline Secretion of Pro-Inflammatory Cytokines and Chemokines by Cultured Human Colon Tissues

		Median and ranges of cytokine secretion per 24 hrs (pg/ml/mg tissue)*							
	N	**G-CSF**	**GM-CSF**	**IFN-α**	**IL-1β**	**IL-6**	**MCP-1**	**MIP-1β**	**TNF**
Normal(N)	8	199(16–690)	89 (30–402)	26(0–56)	2 (0–18)	468 (0–4856)	209 (30–2123)	122 (0–377)	1 (0–15)
Non-inflamed CD(nCD)	23	347 (9–3231)	45 (0–508)	25 (0–84)	1 (0–8)	433 (0–4084)	301 (31–2751)	84 (1–440)	0 (0 –112)
P, nCD vs. N		0.608	0.840	0.623	0.659	0.9259	0.639	0.771	0.942
Non-inflamed UC(nUC)	20	356 (24–2227)	40 (0–605)	22 (0–84)	1 (0–94)	638 (109–3677)	225 (24–3083)	78 (14–219)	3 (0–88)
P, nUC vs. N		0.381	0.188	0.960	0.879	0.706	0.980	0.355	0.457
Inflamed CD(iCD)	18	590 (10–1940)	76 (7–391)	43 (8–121)	10 (0–452)	1427 (0–16811)	281 (43–1940)	111 (0–481)	8 (0–375)
P, iCD vs. N		0.114	0.805	0.169	**0.047**	**0.022**	0.805	0.912	0.134
Inflamed UC(iUC)	12	542 (179–3066)	135 (16–1071)	62 (19–133)	18 (0–776)	4234 (252–19440)	242 (21–1347)	127 (14–591)	29 (0–760)
P, iUC vs. N		**0.030**	0.247	**0.018**	**0.019**	**0.035**	0.790	0.386	**0.017**
p,(iCD +iUC) v N		**0.038**	0.709	**0.040**	**0.015**	**0.012**	0.986	0.619	**0.033**

* p values were obtained using the Wilcoxon rank sum test.