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Bovine Glycomacropeptide Is Anti-Inflammatory in Rats With Hapten-Induced Colitis1

ABSTRACT

Milk κ-casein-derived glycomacropeptide has immunomodulatory and bacterial toxin binding effects. The intestinal anti- inflammatory activity of glycomacropeptide was assessed in trinitrobenzenesulfonic acid-induced colitis in rats. Rats were administered glycomacropeptide daily starting either 2 d before (pretreatment) or 3 h after (post-treatment) colitis induction. Pretreatment with glycomacropeptide had a dose-dependent anti- inflammatory effect, characterized by lower body weight loss, decreased anorexia (57%), colonic damage (65%), and weight to length ratio (32%), as well as a reduction in colonic alkaline phosphatase activity (42%) and interleukin 1, trefoil factor 3, and inducible nitric oxide synthase mRNA levels ($P < 0.05$). The mechanism of action of glycomacropeptide is unknown but is consistent with an inhibition of the activation of immune cells. The magnitude of the anti-inflammatory effect was generally comparable to that of sulfasalazine, an established drug used in the treatment of inflammatory bowel disease. Bovine glycomacropeptide may play a role in the management of patients with inflammatory bowel disease. J. Nutr. 135: 1164-1170, 2005.

KEY WORDS: * glycomacropeptide * casein macropeptide * trinitrobenzenesulfonic acid * inflammatory bowel disease

Glycomacropeptide (GMP),3 also referred to as casein macropeptide, is a 64-amino acid peptide that contains varying amounts (0 to 5 U) of N-acetylneuraminic (sialic) acid. This peptide results from the enzymatic hydrolysis of milk κ-casein in the stomach of neonates due to the action of chymosin (1). In addition, GMP is present at 10-15% in milk whey as a result of the action of the same enzyme during the cheesemaking process. GMP is included among the biologically active components of milk that have the ability to control the growth of host friendly colonic microflora and to modulate immune functions, thus helping to control infections. GMP may therefore act as an anti-infectious factor, promoting the growth of bifidobacteria while inhibiting the proliferation of pathogens (2,3). In addition, GMP may combat infection by binding to lectins, viruses, and mycoplasma (1). The effects of GMP on immune function are complex, however. GMP has been reported to increase the proliferation and phagocytic activity of the macrophage-like cell line U937 (4), whereas other authors showed that GMP inhibits the proliferation of several immune cells such as spleen cells and Peyer's patch cells (5). An increase in IgG production by mouse B-lymphocytes (6) and the ability to interfere with interleukin-1β (IL-1β) receptor binding (7) have also been described.

In addition to its abilities to modulate immunity, GMP is quite unique in that its amino acid profile is high in BCAA and lacking in the aromatic amino acids, including phenylalanine, tryptophan, and tyrosine (8). Therefore, it is one of the few naturally occurring proteins safe for individuals with phenylketonuria. Because of its high BCAA content, several authors have indicated that this peptide could be useful in the management of some liver diseases (9).

The expression "inflammatory bowel disease" (IBD) refers to 2 distinct but closely related conditions, ulcerative colitis and Crohn's disease. Both are chronic relapsing diseases of the intestine. They cause a significant deterioration of the quality of life of patients and are highly (and increasingly) prevalent (10,11). Despite intense investigative efforts, IBD etiology remains unknown, but it is believed to involve an interplay of genetic, environmental, microbial, and immunological factors. A current view of IBD is that it may represent an uncontrolled and exacerbated response to luminal antigens that are innocuous for the normal population. Thus, intestinal inflammation would be the culmination of a cascade of events and processes initiated by antigens due to inadequate handling by the host's immune system. The net result is a self-perpetuating process in which disruption of the epithelial layer and absorption of luminal antigens play a central role. Although IBD can often be successfully managed pharmacologically, the drugs used, such as corticoids, aminosalicylates, or azathioprine, have a plethora of serious adverse effects that limit their application. Hence, the search for new treatments with a low profile of adverse effects is clearly warranted (11).

GMP has an excellent safety record as an integral part of the normal human diet. In

addition, its reported immunomodulatory properties may be beneficial in IBD. Thus, we set out to verify this hypothesis by testing the anti-inflammatory effect of GMP in a widespread model of IBD, namely, trinitrobenzenesulfonic acid (TNBS)- induced colitis in rats (12). We present evidence that GMP, preferably administered as a pretreatment, attenuates experimental hapten-induced colonic inflammation. Furthermore, the anti-inflammatory effect of GMP was comparable to that of an established drug used to treat IBD, namely, sulfasalazine.

MATERIALS AND METHODS

Except where indicated, all reagents and primers were obtained from Sigma. Taq polymerase was purchased from Amersham Biosciences. Antibodies were purchased from Santa Cruz Biotechnology and Sigma. GMP (BioPURE-GMP(TM)) was the kind gift of Davisco Foods International. According to the manufacturer, the GMP content was 93%; fat and lactose contents comprised 0.2 and <1%, respectively.

Animals. Female Wistar rats (175-225 g) obtained from the Laboratory Animal Service of the University of Granada were used; they were housed in macrolon cages (6/cage) and maintained in our laboratory in air-conditioned animal quarters with a 12-h light:dark cycle. The rats had free access to tap water and food (Panlab A04, Panlab). This study was carried out in accordance with the Directive for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes of the European Union (86/609/EEC).

Induction of colitis. Colitis was induced following the method of Morris et al. (13) with minor modifications. Briefly, rats were deprived of food overnight and anesthetized with halothane. Under these conditions, the rats were administered 10 mg of TNBS dissolved in 0.25 mL of 50% ethanol (v:v) by means of a Teflon cannula inserted 8 cm through the anus. Rats were kept in a head-down position for an additional 30 s and returned to their cage.

Experimental design. Rats were randomly assigned to 8 different groups (n = 6). Two groups, C and CG500, were control groups and did not receive the TNBS challenge but 0.25 mL of PBS intrarectally. In addition, group CG500 was administered GMP orally [500 mg/ (kg . d) in 1% methylcellulose] from the beginning of the study and for 7 d, whereas group C was administered vehicle only. Colitis was induced in all remaining groups with TNBS as described above. The T group was administered only TNBS to induce colitis and 1% methylcellulose orally, serving as disease control. Groups G500, G50 and G5 were administered different doses of GMP orally [500, 50 or 5 mg/(kg . d) in 1% methylcellulose, respectively] starting 2 d before the TNBS challenge. Group PG500 was administered 500 mg/(kg . d) of GMP orally in 1% methylcellulose, starting 3 h after the TNBS challenge. Two sulfasalazine groups were administered 500 mg/ (kg . d) of that drug starting 2 d before colitis (group SZ) or 3 h after colitis induction (group PSZ). Treatments continued for 5 d after the TNBS challenge. An esophageal catheter was used to deliver all GMP and sulfasalazine treatments. A second experiment with groups C, T, and G500 (n = 6) was conducted to obtain additional samples required for the study (because of the limits imposed by the use of longitudinally cut samples). All data were pooled and presented as such. In all cases, body weights as well as group water and food intake were recorded daily.

Assessment of colonic damage. Animals were killed by cervical dislocation and the entire colon was removed, gently flushed with saline, placed on an ice-cold plate, cleaned of fat and mesentery, and blotted on filter paper. Each specimen was weighed and its length measured under a constant load (2 g). The large intestine was opened longitudinally and scored for visible damage by an observer unaware of treatment on a 0-25 scale according to the criteria detailed in Table 1. The colon was subsequently divided longitudinally in several pieces for biochemical determinations. The fragments were immediately frozen in liquid nitrogen except for the sample used to determine total glutathione content, which was immediately weighed and frozen in 1 mL of 5% (wt/v) trichloroacetic acid (see below). All of the samples were kept at -80C until analysis.

Alkaline phosphatase (AP) activity was measured spectrophotometrically as described (14) and expressed as U/g protein. Total glutathione content was quantitated with a recycling assay (15). Results are expressed as nmol/g wet tissue.

Western blot. The colonic levels of inducible oxide nitric synthase (iNOS) were determined by immunoblotting. Colonic samples were homogenized in lysis buffer (0.1% SDS, 0.1% sodium deoxycholate, 1% Triton X-100 in PBS) with protease inhi\bitors (1 mmol/L 1,10-phenanthroline, 1 mmol/L phenylmethylsulfonyl fluoride, 18 mg/L aprotinin). The supernatants obtained after centrifugation (7000 g, 10 min at 4C) were boiled for 4 min in Laemmli buffer, separated by SDS-PAGE (10%), electroblotted to nitrocellulose membranes, and probed with the corresponding antibodies (1: 1000 overnight at 4C). The bands were detected by enhanced chemiluminescence (PerkinElmer) and quantitated with NIH software (Scion Image). After the transference of the samples to nitrocellulose membranes, equal loading was checked routinely by Coomassie blue staining. The composition of the Laemmli buffer (5X) was: 312 mmol/L SDS, 50% v:v glycerol, 1% v:v 2-mercaptoethanol, 22.5 mmol/L EDTA trisodium salt, 220 mmol/L Tris, and traces of bromphenol blue (pH = 6.8).

RT-PCR. The expressions of IL-1β, interleukin 1 receptor antagonist (IL-1ra),

transforming growth factor β (TGF- β), mucins (MUCs) 2, 3 and 4 as well as trefoil factor 3 (TFF3) were examined by RT-PCR. Total RNA was extracted with Trizol (Life Technologies). RNA (5 g/sample) was subjected to reverse transcription using the First-strand cDNA synthesis kit (Amersham Biosciences). PCR amplification was performed using 2 L of cDNA for a final PCR reaction volume of 25 L. The expression of the ribosomal 18S unit was routinely examined as a loading standard. The primers and size of the amplified fragments were: MUC2 (sense 5'-GCT CAA TCT CAG AAG GCG ACA G-3'; antisense 5'-CCA GAT AAC AAT GAT GCC AGA GC- 3' 875 bp); MUC3 (sense 5'-CAC AAA GGC AAG AGT CCA GA-3'; antisense 5'-ACT GTC CTT GGT GCT GAA TG-3' 515 bp); MUC4 (sense 5'-CGT ACT AGA GAA CTT GGA CAT G-3'; antisense 5'-GGT AGO AGA ACT TGT TCA TGG-3' 638 bp); TFF3 (sense 5'-ATG GAG ACC AGA GCC TTC TG-3'; antisense 5'- ACA GCC TTG TGC TGA CTG TA-3' 403 bp); IL-1β (sense 5'-AAT GAC CTG TTC TTT GAG GCT G-3'; antisense 5'-CGA GAT GCT GCT GTG AGA TTT GAA G-3' 115 bp; IL-1ra (sense 5'-GAG TCA GCT GGC CAC CCT G-3'; antisense 5'-CAG ACT TGA CAC AAG ACA GGC A-3' 230 bp); TGF-β (sense 5'-GCT AAT GGT GGA CCG CAA CAA C-3'; antisense 5'-CAC TGC TTC CCG AAT GTC TGA C-3' 200 bp); ribosomal 18S unit (sense 5'-CCA TTG GAG GGC AAG TCT GGT G-3'; antisense 5'-CGC CGG TCC AAG AAT TTC ACC- 3' 389 bp). To set up the PCR conditions, different amounts of colonic RNA from a pool of samples and different number of cycles were assayed (data not shown). The cycle numbers and hybridization temperatures for each PCR reaction were as follows: 23 cycles and 56C (for MUC2, MUC3 and TFF3); 27 cycles and 57C (for MUC4, TGFβ, IL-1β, and IL-1ra); 17 cycles and 60C (for the ribosomal 18S unit). After the PCR amplification, 5 L of each reaction was resolved in 2.5% (wt:v) agarose gels using ethidium bromide. Bands were quantitated with NIH software (Scion Image).

TABLE 1

Scoring criteria applied to the visible lesions in the rat colon1

TABLE 2

Food intake and body weight gain in the different experimental groups of rats treated with GMP or sulfasalazine1

Statistical analysis. Results are expressed as means SEM. Differences among means were tested for statistical significance by 1-way ANOVA and a posteriori Fisher least significance tests on preselected pairs (treatments vs. C and T groups). All analyses were carried out with the SigmaStat program (Jandel). Differences were considered significant at $P < 0.05$.

RESULTS

TNBS model. The administration of 500 mg GMP/ (kg . d) to control rats (group CG500) did not produce any observable changes in the rat colon nor did it affect food intake or weight gain (Tables 2 and 3). As expected, administration of TNBS induced a severe inflammatory response in the large intestine characterized by mucosal erosions, epithelial necrosis, submucosal fibrosis, and edema, resulting in a marked increase in the colonic damage score (Table 3). TNBS colitis was also characterized by anorexia and weight loss, colonic shortening, and an increase in the colonic weight: length ratio (Tables 2 and 3).

Effect of GMP. Pretreatment of colitic rats with GMP resulted in significant protection with the highest dose assayed, i.e., 500 mg/ (kg . d) (group G500). Thus, the G500 group had a lower colonic weight:length ratio, extension of necrosis, and damage score than the control (T group, Table 3, $P < 0.05$). Interestingly, colonic length did not differ from that of the normal rats (group C). In addition, GMP treatment increased food intake and reduced weight loss (d 2-5 postchallenge, Table 2). The benefits of GMP pretreatment tended to lessen when the dose was lowered, indicating that its effect is dose dependent. However, even the lowest dose assayed [5 mg/(kg . d)] was still associated with a lower colonic damage score ($P < 0.05$). On the other hand, the therapeutic effects of GMP were largely lost when it was administered as a post-treatment, as is usually the case. However, the PG500 group had a lower colonic damage score and less weight loss (Tables 2 and 3, $P < 0.05$).

Effect of sulfasalazine. Our data show a clear anti-inflammatory effect of this established drug administered as a pretreatment; this was characterized by a decrease in the colonic damage score, necrotic extension, and colonic weight:length ratio (Table 3, $P < 0.05$). However, sulfasalazine did not reduce anorexia or body weight loss, and colonic length was also unchanged (Tables 2 and 3). Of note, the aforementioned significant effects of sulfasalazine were generally comparable to those of GMP at the same dose, i.e., 500 mg/ (kg . d). On the other hand, when administered as a post-treatment, differences in colonic length ($P = 0.091$) and weight to length ratio ($P = 0.075$) with

sulfasalazine were not significant.

Biochemical characterisation of the effects of GMP and sulfasalazine. TNBS colitis was associated with a significant increase in AP activity, a parameter recently proposed as marker of inflammation (16) (Fig. 1A). GMP, but not sulfasalazine pretreatment, reduced AP colonic activity (57%, $P < 0.05$). However, when administered as a post-treatment, only sulfasalazine had a significant effect (62%, $P < 0.05$).

On the other hand, total glutathione levels were decreased, reflecting oxidative stress (Fig. 1B). Only sulfasalazine pretreatment prevented the reduction in glutathione levels associated with TNBS-induced colitis, whereas GMP had no effect. This is consistent with the known antioxidative properties of sulfasalazine (17). This effect disappeared when sulfasalazine was given after TNBS.

TABLE 3

Evolution of parameters of macroscopic damage in TNBS colitic rats treated with GMP or sulfasalazine1

FIGURE 1 Colonic AP activity (panel A) and total glutathione content (panel B) in normal and colitic rats. Values are means SEM, n = 6-12. + Different from C group, $P < 0.05$; * different from TNBS group, $P < 0.05$.

Further examination of the status of the rats after 5 d of colitis revealed that the production of iNOS and proliferating cell nuclear antigen, as well as the mRNA levels of IL-1β and IL- 1ra, were upregulated by the TNBS challenge (Figs. 2 and 3 and data not shown). Administration of 500 mg GMP/ (kg . d) to rats before the administration of TNBS resulted in a decrease in the expression of iNOS. This was not a uniform effect, i.e., some rats exhibited very low levels, whereas others had iNOS levels comparable to rats from the T group. When GMP was given after TNBS, the response was similar (Fig. 2). Sulfasalazine had a more uniform effect, resulting in a virtual normalization of iNOS as a pretreatment. When administered as a post-treatment, the effect was clearly lower but still significant ($P < 0.05$).

GMP pretreatment reduced the IL-1β and IL-1ra mRNA levels ($P < 0.05$, Fig. 3A and B). Interestingly, this effect disappeared when given as a post-treatment for IL-1β but largely persisted for IL-1ra. Sulfasalazine had similar effects.

We also measured the levels of TGF-β, a protecting peptide that induces migration, differentiation, and apoptosis and inhibits proliferation. Five days after the induction of colitis, TGF-β mRNA abundance did not differ among the groups studied (Fig. 3C).

We characterized the mucin expression profile at the mRNA level. Our results indicate that only MUC4 mRNA production was increased 5 d after the induction of colitis, whereas MUC2 or MUC3 mRNA production did not differ (Fig. 4). MUC4 mRNA levels in groups G500 and PG500 were lower than those of the T group ($P < 0.05$), indicating that the administration of the highest dose of GMP both before and after the TNBS challenge normalized the levels of this mucin. Unfortunately, we could not measure MUC4 mRNA in the sulfasalazine groups. Interestingly, both GMP-treated groups also showed diminished production of MUC3, although this change was not significant in the case of the PG500 group ($P = 0.114$). On the other hand, TFF3, an important peptide produced by goblet cells and involved in the maintenance and repair of the intestinal mucosal barrier, was also markedly induced in the T group ($P < 0.05$, Fig. 4A). Interestingly, this effect was completely prevented by GMP administered either as a pretreatment or as a post-treatment. Sulfasalazine, on the other hand, produced a nonsignificant decrease when given before TNBS ($P = 0.086$), whereas it had no effect when administered after colitis induction.

FIGURE 2 Colonic protein expression of iNOS (panel A) and densitometric analysis (panel B) in rats treated with GMP or sulfasalazine. Expression was assessed by Western blot. Values are means SEM, n = 6-12. + Different from C group, $P < 0.05$; * different from T group, $P < 0.05$.

FIGURE 3 Colonic mRNA expression of IL-1β (panel A), IL-1ra (panel B), and TGF-β (panel C) in rats treated with GMP or sulfasalazine. Expression was assessed by semiquantitative RT-PCR and densitometry was performed with the Scion Image software. Representative blots and dens\itometric analysis (normalized to the 18 S signal, panel D) are shown. Values are means SEM, n = 6. + Different from C group, $P < 0.05$; * different from T group, $P < 0.05$.

DISCUSSION

The search for new therapies for inflammatory bowel disease has been a primary focus of interest for many investigators in the last few years. Although efficacious drug treatments for IBD are currently available, the risk of adverse effects is high, especially considering the chronic and relapsing nature of this condition. The emerging concepts arising from a deeper knowledge of IBD pathophysiology has prompted the introduction of novel pharmacologic treatments such as the cytokine antagonists (18). At the same time, a parallel strategy has been developed in the field of nutritional management of

IBD (19). Thus, dietary fiber has been demonstrated to have an important protective role in intestinal inflammation by increased production of butyrate and modulation of the intestinal flora (i.e., acting as a prebiotic) (20,21). Similarly, PUFA tend to reduce inflammation by affecting eicosanoid generation and peroxisome proliferator-activated receptors (22,23). Probiotics have been used also in an attempt to modulate directly the intestinal flora (24). The incorporation (or enrichment) of these items into nutritional products has given rise to the concept of "functional food products," i.e., dietary products with an added health value. GMP is an inexpensive peptide derived from cow's milk during cheese making. It is derived from whey, which has immunomodulatory properties, including release of IL-1ra in monocytes and inhibition of IL-2 expression in CD4+ T cells (7,25). GMP effects are complex, however. Thus, immune cell proliferation can be either stimulated or inhibited, depending on the concentration of the peptide (26,27). On the other hand, GMP reportedly binds and inactivates bacterial toxins and has a modest capacity to promote the growth of bifidus species (1). Therefore GMP could plausibly have a beneficial effect on IBD. We selected TNBS-induced colitis because it is one of the most widely used models both to test new therapeutic treatments and to study the pathophysiology of IBD (28-31). TNBS acts as a hapten, modifying mucosal proteins; these in turn elicit or enhance the inflammatory response, which lasts for several weeks (13,31). At 5- 7 d, TNBS-induced colitis is characterized by a CD4+-driven, Th-1 type of inflammation with transmural involvement, fibrosis, infiltration of monocytes and lymphocytes, and profound disturbances in motility and ionic transport. Thus, the model most closely resembles Crohn's disease. The time point selected is possibly the best at which to evaluate the anti-inflammatory activity of experimental treatments because the effect of even established drugs tends to be negligible at later stages.

FIGURE 4 Colonic mRNA expression of TFF3 (panel A), MUC2 (panel B), MUC3 (panel C), and MUC4 (panel D) in rats treated with GMP or sulfasalazine. Expression was assessed by semiquantitative RT-PCR and densitometry was performed with the Scion Image software. Representative blots and densitometric analysis (normalized to the 18 S signal, panel E) are shown. Values are means SEM, n = 6. + Different from C group, $P < 0.05$; * different from T group, $P < 0.05$. MUC4 could not be measured in samples from sulfasalazine- treated rats.

Our results demonstrate that GMP gavage results in significant protection from inflammatory damage in TNBS-induced colitic rats. Thus GMP reduced the colonie damage score, weight:length ratio, and extent of necrosis, and increased AP colonie activity and iNOS expression. Furthermore, these effects did not differ from those obtained with sulfasalazine, an established drug treatment for IBD (11), suggesting that the therapeutic effect observed with GMP is quantitatively relevant. The effect was clearly dose dependent because the G50 and G5 treatment had a much smaller effect on intestinal inflammation. Consequently, only the G500 group was considered for further analysis. GMP exerted a clearly superior therapeutic effect when given before colitis induction than as a post-treatment; this is a common observation because most therapies offer superior effects as a pretreatment in experimental colitis, including sulfasalazine in the present study. It is interesting to note, however, that even after colitis induction, some of the beneficial effects of GMP were retained, suggesting that patients may benefit from GMP even when taken during inflammatory bouts of their condition.

Unlike sulfasalazine, GMP had a significant effect on anorexia and weight loss. In TNBS-induced colitis as well as in human IBD, anorexia occurs as a consequence of actions on the hypothalamus of systemically elevated IL-1β and other factors (32,33). Thus a greater food intake as detected in several GMP-treated groups may be interpreted as reflecting a lowering of the levels of these mediators secondary to prevention of colitic damage. Consistent with this hypothesis, peripheral blood mononuclear cells from GMP- treated rats produced lower levels of tumor necrosis factor than those obtained from untreated colitic rats (data not shown).

Interestingly, the expression of IL-1β, as judged by its mRNA level, was significantly diminished as a consequence of GMP treatment. This is in agreement with the previous observation by Monnai and Otani (7) that GMP stimulates IL-1ra release from monocytes. IL-1ra is a natural cytokine inhibitor that prevents IL- 1β signaling through its receptor by competitive binding with the latter, thus modulating the inflammatory cascade. This mechanism would be expected to reduce the activation of monocytes, themselves a major source of IL-1β. Hence we measured IL-1ra mRNA levels, which were reduced by GMP pre- and post-treatment. Because both IL- 1β and IL-1ra are subject to post-transcriptional in addition to transcriptional regulation (34,35), our data cannot establish whether the IL-1β:IL-1ra ratio is modified by GMP treatment. However, it is interesting to note that the IL-1ra mRNA levels were reduced only by GMP post-treatment, whereas those of IL-1β were unchanged. This suggests that the IL-1β:IL-1ra ratio may be reduced with GMP pretreatment but not with GMP post-treatment, a circumstance that would be consistent with the reduced anti- inflammatory activity in the latter case. Of note, very similar results were obtained with sulfasalazine, indicating that the same mechanism might account for its diminished effect when given after colitis induction.

We also measured other inflammatory markers in an attempt to define the mechanism of action of GMP. TFF3 is a bioactive peptide involved in the maintenance of gastrointestinal tissue as well as in tissue repair, particularly at the epithelial level (36).

Although the mechanisms that mediate TFF3 actions are ill defined, TFF3 appears to be upregulated in experimental colitis and is thought to exert a beneficial role in this context (37-39). Our data are in agreement with these findings because TFF3 expression [which appears to be transcriptionally regulated (40)] was significantly increased in the T group 5 d after colitis induction. However, the GMP- treated groups, G500 and PG500, had TFF3 mRNA levels comparable to those of the control group. This probably reflects the protection of these rats from colonic inflammation brought about by GMP rather than a mechanism of action, although this cannot be ruled out because the timing of TFF3 induction and effects in TNBS colitis are not characterized. On the other hand, it can be assumed that the mechanism of action of GMP is not related to antioxidative activity (because glutathione levels were unchanged) or to actions on TGF- β, an anti-inflammatory peptide that opposes Th-1 responses but that can produce excessive scarring of tissue (also unaffected by GMP treatment) (41).

iNOS upregulation was greatly reduced by GMP pretreatment and only modestly by GMP post-treatment ($P < 0.05$). Thus, iNOS inhibition may be a relevant mechanism of action of GMP. Of note, the effect of sulfasalazine on iNOS was very similar, although of a greater magnitude. Sulfasalazine is expected to decrease iNOS induction via its inhibitory effects on nuclear factor-κB (42).

The effects of GMP also include a normalization of MUC4 expression. MUC4 is a heterodimeric glycoprotein complex that consists of a peripheral O-glycosylated mucin subunit, ascites sialoglycoprotein-1 (ASGP-1), tightly but noncovalently linked to a N-glycosylated transmembrane subunit, ASGP-2 (43). The complex is expressed in a number of normal, vulnerable epithelial tissues, including the mammary gland, uterus, colon, cornea, and trachea. MUC4 was shown to bind ErbB2, a receptor tyrosine kinase, triggering a specific phosphorylation in the absence of other ErbB ligands and potentiating phosphorylation and signaling by the latter. Hence MUC4 may be involved in epithelial homeostasis in addition to its classically assigned protective function. The fact that neutrophil elastase has the potential to increase MUC4 expression (44) is consistent with its upregulation in TNBS-induced colitis because this model is characterized by intense neutrophil infiltration (13). Thus, the normalization of MUC4 expression observed in the G500 and PG500 groups likely reflects the amelioration of the inflammatory response. These results should be interpreted cautiously because of the existence of post-transcriptional regulation of the MUC4 gene. Furthermore, we do not know whether MUC4 is involved in sulfasalazine action because we were unable to amplify its mRNA. It is obvious, however, that the effects of GMP on TFF3, MUC3, and MUC4 are not as well correlated with the anti-inflammatory activity as the effects on AP and IL-1β and IL-1ra expression because these are the only variables affec\ted significantly in the pretreatment group (G500) but not in the post-treatment group (PG500). Because the period of colitis was relatively short, it is possible that the effects on mucins and TFF3 may play a more important role in the long term. However, as explained above, the TNBS model is not well suited for this type of mechanism.

In conclusion, GMP exerts a dose-dependent anti-inflammatory effect in TNBS-induced colitis, with a potency and efficacy similar to those of sulfasalazine. Its mechanism of action is unknown but may be related to actions on IL-1β and iNOS. GMP may be useful for the dietary management of IBD.

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3 Abbreviations used: AP, alkaline phosphatase; ASGP, ascites sialoglycoprotein; C, control (noncolitic) group; CG500, group of noncolitic rats treated with 500 mg/kg of GMP; GMP, glycomacropeptide; GMP500, GMP50, GMP5, groups of colitis rats pretreated with 500, 50, or 5 mg/kg of GMP; IBD, inflammatory bowel disease; IL-1β, interleukin 1 β; IL-1ra, interleukin 1 receptor antagonist; iNOS, inducible nitric oxide synthase; MUC, mucin; PG500, group of colitic rats post-treated with 500 mg/kg of GMP; SZ and PSZ, groups of colitic rats pre- or post-treated with 500 mg/kg sulfasalazine, respectively; T, TNBS control group; TFF, trefoil factor; TGF-β, transforming growth factor β; TNBS, trinitrobenzene-sulfonic acid.

LITERATURE CITED

1. Brody, E. P. (2000) Biological activities of bovine glycomacropeptide. Br. J. Nutr. 84: 539-546.

2. Bruck, W. M., Kelleher, S. L., Gibson, G. R., Nielsen, K. E., Chatterton, D.E.W. & [illegible] (2003) rRNA probes used to quantify the effects of glycomacropeptide and

alpha-lactalbumin supplementation on the predominant groups of intestinal bacteria of infant rhesus monkeys challenged with enteropathogenic Escherichia coli. J. Pediatr. Gastroenterol. Nutr. 37: 273-280.

3. Bruck, W. M., Graverholt, G. & Gibson, G. R. (2003) A two- stage continuous culture system to study the effect of supplemental alpha-lactalbumin and glycomacropeptide on mixed cultures of human gut bacteria challenged with enteropathogenic Escherichia coli and Salmonella serotype typhimurium. J. Appl. Microbiol. 95: 44-53.

4. Li, E.W.Y. & Mine, Y. (2004) Immunoenhancing effects of bovine glycomacropeptide and its derivatives on the proliferative response and phagocytic activities of human macrophagelike cells, U937. J. Agric. Food Chem. 52: 2704-2708.

5. Otani, H., Monnai, M., Kawasaki, Y., Kawakami, H. & Tanimoto, M. (1995) Inhibition of mitogen-induced proliferative responses of lymphocytes by bovine kappa-caseinoglycopeptides having different carbohydrate chains. J. Dairy Res. 62: 349-357.

6. Monnai, M., Horimoto, Y. & Otani, H. (1998) Immunomodificatory effect of dietary bovine κ-caseinoglycopeptide on serum antibody levels and proliferative responses of lymphocytes in mice. Milchwissenschaft 53: 129-132.

7. Monnai, M. & Otani, H. (1997) Effect of bovine κ- caseinoglycopeptide on secretion of interleukin-1 family cytokines by P388D1 cells, a line derived from mouse monocyte/macrophage. Milchwissenschaft 52: 192-196.

8. Nakano, T., Silva-Hernandez, E. R., Ikawa, N. & Ozimek, L. (2002) Purification of kappa-casein glycomacropeptide from sweet whey with undetectable level of phenylalanine. Biotechnol. Prog. 18: 409-412.

9. Nakay, S. & Modler, H. W. (1999) Food Proteins: Processing Applications. Wiley-VCH, New York, NY.

10. Ekbom, A. (2004) The epidemiology of IBD: a lot of data but little knowledge. How shall we proceed? Inflamm. Bowel Dis. 10 (suppl. 1): S32-S34.

11. Sands, B. E. (2000) Therapy of inflammatory bowel disease. Gastroenterology 118: S68-S82.

12. Neurath, M., Fuss, I. & Strober, W. (2000) TNBS-colitis. Int. Rev. Immunol. 19: 51-62.

13. Morris, G. P., Beck, P. L., Herridge, M. S., Depew, W. T., Szewczuk, M. R. & Wallace, J. L. (1989) Hapten-induced model of chronic inflammation and ulceration in the rat colon. Gastroenterology 96: 795-803.

14. Bessey, O. A., Lowry, O. H. & Brook, M. J. (1946) Rapid colorimetric method for the determination of alkaline phosphatase in five cubic milliliters of serum. J. Biol. Chem. 164: 321-329.

15. Akerboom, T. P. & Sies, H. (1981) Assay of glutathione, glutathione disulfide, and glutathione mixed disulfides in biological samples. Methods Enzymol. 77: 373-382.

16. Snchez de Medina, F., Martnez-Augustin, O., Gonzlez, R., Ballester, I., Glvez, J. & Zarzuelo, A. (2004) Induction of alkaline phosphatase in the inflamed intestine: a novel pharmacological target for inflammatory bowel disease. Biochem. Pharmacol. 68: 2317-2326.

17. Verspaget, H. W., Aparicio-Pages, M. N., Verver, S., Edelbroek, P. M., Hafkenscheid, J. C., Crama-Bohbouth, G. E., Pena, A. S., Weterman, I. T. & Lamers, C. B. (1991) Influence of sulphasalazine and mesalazine on cellular and biochemical oxygen metabolite production. Effect of in vivo administration and an in vitro analysis. Scand. J. Gastroenterol. 26: 779-786.

18. Hibi, T., Inoue, N., Ogata, H. & Naganuma, M. (2003) Introduction and overview: recent advances in the immunotherapy of inflammatory bowel disease. J. Gastroenterol. 38 (suppl. 15): 36- 42.

19. O'Sullivan, M. & O'Morain, C. (2001) Nutritional treatments in inflammatory bowel disease. Curr. Treat. Options Gastroenterol. 4: 207-213.

20. Rodriguez-Cabezas, M. E., Galvez, J., Camuesco, D., Lorente, M. D., Concha, A., Martinez-Augustin, O., Redondo, L. & Zarzuelo, A. (2003) Intestinal anti-inflammatory activity of dietary fiber (Plantago ovata seeds) in HLA-B27 transgenic rats. Clin. Nutr. 22: 463-471.

21. Kanauchi, O., Mitsuyama, K., Araki, Y. & Andoh, A. (2003) Modification of intestinal flora in the treatment of inflammatory bowel disease. Curr. Pharm. Design 9: 333-346.

22. Bassaganya-Riera, J., Reynolds, K., Martino-Catt, S., Cui, Y., Hennighausen, L.,

Gonzalez, F., Rohrer, J., Benninghoff, A. U. & Hontecillas, R. (2004) Activation of PPAR gamma and delta by conjugated linoleic acid mediates protection from experimental inflammatory bowel disease. Gastroenterology 127: 777-791.

23. Gil, A. (2002) Polyunsaturated fatty acids and inflammatory diseases. Biomed. Pharmacother. 56: 388-396.

24. Marteau, P., Lepage, P., Mangin, I., Suau, A., Dore, J., Pochart, P. & Seksik, P. (2004) Review article: gut flora and inflammatory bowel disease. Aliment. Pharmacol. Ther. 20 (suppl. 4): 18-23.

25. Otani, H., Horimoto, Y. & Monnai, M. (1996) Suppression of interleukin-2 receptor expression on mouse CD4+ T cells by bovine kappa-caseinoglycopeptide. Biosci. Biotechnol. Biochem. 60: 1017- 1019.

26. Otani, H., Monnai, M. & Hosono, A. (1992) Bovine κ- casein as inhibitor of the proliferation of mouse splenocytes induced by lipopolysaccharide stimulation. Milchwissenschaft 47: 512- 515.

27. Otani, H. & Hata, I. (1995) Inhibition of proliferative responses of mouse spleen lymphocytes and rabbit Peyer's patch cells by bovine milk caseins and their digests. J. Dairy Res. 62: 339- 348.

28. Whittle, B. J., Cavicchi, M. & Lamarque, D. (2003) Assessment of anticolitic drugs in the trinitrobenzene sulfonic acid (TNBS) rat model of inflammatory bowel disease. Methods Mol. Biol. 225: 209- 222.

29. Jurjus, A. R., Khoury, N. N. & Reimund, J. M. (2004) Animal models of inflammatory bowel disease. J. Pharmacol. Toxicol. Methods 50: 81-92.

30. Sanchez de Medina, F., Perez, R., Martinez-Augustin, O., Gonzalez, R., Lorente, M. D., Galvez, J. & Zarzuelo, A. (2002) Disturbances of colonic ion secretion in inflammation: role of the enteric nervous system and cAMP. Pflugers Arch. 444: 378-388.

31. Sanchez de Medina, F., Galvez, J., Romero, J. A. & Zarzuelo, A. (1996) Effect of quercitrin on acute and chronic experimental colitis in the rat. J. Pharmacol. Exp. Ther. 278: 771-779.

32. El-Haj, T., Poole, S., Farthing, M. J. & Ballinger, A. B. (2002) Anorexia in a rat model of colitis: interaction of interleukin-1 and hypothalamic serotonin. Brain Res. 927: 1-7.

33. McHugh, K. J., Collins, S. M. & Weingarten, H. P. (1994) Central interleukin-1 receptors contribute to suppression of feeding after acute colitis in the rat. Am. J. Physiol. 266: R1659-R1663.

34. Ohguchi, M., Yamato, K., Ishihara, Y., Koide, M., Ueda, N., Okahashi, N., Noguchi, T., Kizaki, M., Ikeda, Y., Sugino, H. & Nisihara, T. (1998) Activin A regulates the production of mature interleukin-1 beta and interleukin-1 receptor antagonist in human monocytic cells. J. Interferon Cytokine Res. 18: 491-498.

35. Harrison, L. M., van Haaften, W. C. & Tesh, V. L. (2004) Regulation of proinflammatory cytokine expression by Shiga toxin 1 and/or lipopolysaccharides in the human monocytic cell line THP-1. Infect. Immun. 72: 2618-2627.

36. Hollenbach, E., Neumann, M., Vieth, M., Roessner, A., Malfertheiner, P. & Naumann, M. (2004) Inhibition of p38 MAP kinase- and RICK/NF-κB-signaling suppresses inflammatory bowel disease. FASEB J. 18: 1550-1552.

37. Renes, I. B., Verburg, M., Van Nispen, D. J., Taminiau, J. A., Buller, H. A., Dekker, J. & Einerhand, A. W. (2002) Epithelial proliferation, cell death, and gene expression in experimental colitis: alterations in carbonic anhydrase I, mucin MUC2, and trefoil factor 3 expression. Int. J. Colorectal Dis. 17: 317-326.

38. Vestergaard, E. M., Brynskov, J., Ejskjaer, K., Clausen, J. T., Thim, L., Nexo, E. & Poulsen, S. S. (2004) Immunoassays of human trefoil factors 1 and 2: measured on serum fr\om patients with inflammatory bowel disease. Scand. J. Clin. Lab. Investig. 64: 146- 156.

39. Tran, C. P., Cook, G. A., Yeomans, N. D.,Thim, L. & Giraud, A. S. (1999) Trefoil peptide TFF2 (spasmolytic polypeptide) potently accelerates healing and reduces inflammation in a rat model of colitis. Gut 44: 636-642.

40. Blanchard, C., Durual, S., Estienne, M., Bouzakri, K., Heim, M. H., Blin, N. & Cuber, J. C. (2004) IL-4 and IL-13 up-regulate intestinal trefoil factor expression: requirement for STAT6 and de novo protein synthesis. J. Immunol. 172: 3775-3783.

41. Beck, P. L. & Podolsky, D. K. (1999) Growth factors in inflammatory bowel disease. Inflamm. Bowel Dis. 5: 44-60.

42. Hasko, G., Szabo, C., Nemeth, Z. H. & Deitch, E. A. (2001) Sulphasalazine inhibits macrophage activation: inhibitory effects on inducible nitric oxide synthase expression, interleukin-12 production and major histocompatibility complex II expression. Immunology 103: 473-478.

43. Price-Schiavi, S. A., Jepson, S., Li, P., Arango, M., Rudland, P. S., Yee, L. & Carraway, K. L. (2002) Rat Muc4 (sialomucin complex) reduces binding of anti-ErbB2 antibodies to tumor cell surfaces, a potential mechanism for herceptin resistance. Int. J. Cancer 99: 783-791.

44. Pizarro, T. T., Arseneau, K. O., Bamias, G. & Cominelli, F. (2003) Mouse models for the study of Crohn's disease. Trends Mol. Med. 9: 218-222.

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