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Caprylic acid and medium-chain triglycerides inhibit IL-8 gene transcription in Caco-2 cells: comparison with the potent histone deacetylase inhibitor trichostatin A

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1 Medium-chain triglyceride (MCT) is often administered to patients with Crohn's disease (CD) or short-bowel syndrome. However, little is known about the effects of medium-chain fatty acids (MCFAs) and MCT on intestinal inflammation. In this study we examined whether caprylic acid, one of the MCFAs, and MCT suppress IL-8 secretion by differentiated Caco-2 cells.

2 We found for the first time that caprylic acid and MCT suppress IL-8 secretion by Caco-2 cells at the transcriptional level when precultured together for 24 h. We also tried to clarify the mechanism of IL-8 gene inhibition by examining the activation of NF-κB and other transcription factors by electrophoretic mobility shift assay (EMSA), and found that caprylic acid did not modulate their activation.

3 The result of dual-luciferase assay using Caco-2 cells transfected with IL-8 promoter/luciferase reporter plasmid revealed that caprylic acid inhibited the activation of IL-8 promoter.

4 Similar results were observed when cells were precultured with the well-known potent histone deacetylase inhibitor trichostatin A (TSA).

5 We examined the state of H4 acetylation in IL-8 promoter using the technique known as chromatin immunoprecipitation (Chr-IP). TSA rapidly induced H4 acetylation in IL-8 promoter chromatin, whereas caprylic acid did not. These results suggest that the inhibition of IL-8 gene transcription induced by caprylic acid and TSA does not necessarily require the marked suppression of transcription factors, and the mechanism of inhibition of IL-8 gene transcription may be different between caprylic acid and TSA.

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Abbreviations: CD, Crohn's disease; Chr-IP, chromatin immunoprecipitation; EMSA, electrophoretic gel shift assay; IBD, inflammatory bowel diseases; MCFA, medium-chain fatty acid; MCT, medium-chain triglyceride; NF-κB, nuclear factor-kappa B; TSA, trichostatin A

Introduction

The epithelium is a line of defense against an array of substances in the intestine, including resident and pathogenic micro-organisms (Sanderson & Walker, 1994). In inflammatory conditions where the mucus barrier is lost, such as Crohn's disease (CD) or necrotizing enterocolitis, intestinal epithelial cells secrete a wide array of cytokines after stimulation with pro-inflammatory cytokine or bacterial products (Eckmann *et al*., 1993). Recently, it was demonstrated that increasing concentrations of butyrate, one of the short-chain fatty acids (SCFAs), led to a dose-dependent reduction in IL-8 secretion by intestinal epithelial cells (Huang *et al*., 1997; Andoh *et al*., 1999a). Clinically, butyrate enema is effective for patients with active distal ulcerative colitis (Scheppach *et al*., 1992; Steinhart *et al*., 1994), and although the precise molecular mechanism that leads to such responses has not been well characterized, it is assumed to be partly based on the inhibitory action of butyrate on NF-κB activation, leading to inflammatory cytokine gene inhibition in enterocytes (Inan *et al*., 2000; Andoh *et al*., 1999b). However, little is known about the effects of medium-chain fatty acids (MCFAs) and medium-chain C8 triglyceride (MCT) on intestinal inflammation. MCT has been used therapeutically when assimilation of dietary long-chain triglyceride (LCT) is diminished (Winawer *et al*., 1966; Tandon *et al*., 1972), and in patients whose small bowel has been resected, replacement of dietary LCT by MCT has been reported to cause considerable improvement in fat absorption, resulting in a gain in total energy absorption (Jeppesen & Mortensen, 1998). It is also often administered to patients with CD. Although dietary fat intake has been thought to be a risk factor of relapse in inflammatory bowel diseases (IBD), whether MCFAs and MCT actually have an inflluence on intestinal inflammation has not been examined thoroughly either *in vivo* or *in vitro*.

We examined whether caprylic acid, a MCFAs and MCT suppress IL-8 secretion by Caco-2 cells, a colon cancer cell line that is an established model for the human intestinal

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epithelium (Pinto *et al*., 1983). IL-8 is an α-chemokine that possesses potent neutrophil chemo-attractant and activating properties (Oppenheim *et al*., 1991; Baggiolini *et al*., 1989) and is one of the most important chemokines in the pathogenesis of IBD. It is increased in the intestinal mucosa in patients with active ulcerative colitis (UC) and active CD, and is a good target for therapy (Mazzucchelli *et al*., 1994). To clarify the mechanism of IL-8 gene transcription, we examined the expression of IL-8 mRNA by Northern blotting and activation of its transcription factors by electrophoretic mobility shift assay (EMSA). We then performed a dual-luciferase assay, using Caco-2 cells transfected with IL-8 promoter/luciferase reporter plasmid, to evaluate the activation of the IL-8 promoter.

Cousens *et al*. (1978) demonstrated that all SCFAs containing up to six carbon atoms inhibited calf thymus deacetylases *in vitro*, leading to H4 acetylation in a rat hepatoma cell line (HTC cells). Reversible histone acetylation, which occurs in the ε-amino group of specific internal lysine residues located at the highly basic N-terminal domains of core histones, is often reported to affect gene transcription (Allfrey, 1980; Norton *et al*., 1989), and several lines of evidence have suggested that histone H4 acetylation may play a role in the inhibition of IL-8 gene in Caco-2 cells (Huang *et al*., 1997). In addition, it has become well established for several genes that targeting of histone acetylation to promoters is required for the regulation of transcription (Kuo *et al*., 2000; Vogelauer *et al*., 2000). In the present study, we used polymerase chain reaction (PCR) with chromatin immunoprecipitation (Chr-IP) to examine the state of H4 acetylation in IL-8 promoter gene chromatin. Using this technique, it is possible to assess the acetylation states of promoters of individual genes (Braunstein *et al*., 1993). Furthermore, we compared the action of caprylic acid on IL-8 gene transcription with that of trichostatin A (TSA), a well-known inhibitor of histone deacetylase (Yoshida *et al*., 1990). Although the molecular structure of TSA is quite different from those of SCFAs and MCFAs, TSA induces a marked accumulation of highly acetylated histones, and therefore, is considered to be useful for analysing the role of histone hyperacetylation in regard to the fatty acid-mediated intracellular regulation of gene transcription.

Methods

Reagents

Recombinant human IL-1β was obtained from Sigma Chemical Co. Ltd (St. Louis, MO, U.S.A.) and caprylic acid from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). MCT was a gift from Snow Brand Milk Products Co. Ltd (Tokyo, Japan). The MCT used in this study consisted mainly of tricaprylin and contained caprylic acid abundantly as a free fatty acid.

Cell culture

Caco-2 cells were obtained from the American Type Culture Collection (Rockville, MD, U.S.A.) and were grown in Dulbecco's modified Eagle's medium (Sigma) containing 15% foetal bovine serum (FBS; Sigma), 2 mM glutamine (Gibco BRL, Grand Island, NY, U.S.A.), 0.1 mM nonessential amino acid (Gibco), 10,000 U l^{-1} penicillin and streptomycin (Gibco), and 5 mg l^{-1} iron-saturated human transferrin (Gibco). On day 8, the media were changed to media without FBS. All experimental protocols, except the dual-luciferase reporter assay, were initiated on day 14, when cells were confluent and their state of differentiation stable (Oguchi *et al*., 1995). The dual-luciferase reporter assay was initiated as described below according to the manufacturer's instructions.

Cell viability

An assay using 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate (WST-1) was performed to measure cell viability as described previously (Ishiyama *et al*., 1995). Cells were incubated with various concentrations of fats for 24 h. Four hours after addition of WST-1 to the media, the amount of WST-1 product was colourimetrically assayed by measuring the optical density at 570 nm using a microplate reader (MPR-A4i, TOSOH, Yamaguchi, Japan). Accordingly, caprylic acid and MCT, water-soluble at pH 7.4, were added to media at the highest level not toxic to cells (caprylic acid, up to 1.3 mM; MCT, up to 0.6%).

Assay of IL-8

Cells were plated into 24-well plates at a density of 5×10^4 cells cm^{-2} and maintained as described above. On day 14, cells were precultured in the presence or absence of fat for 24 h, followed by stimulation with 1 ng ml^{-1} of IL-1β for 12 h. IL-8 levels in the culture supernatants were then measured by enzyme-linked immunosorbent assay (ELISA; Amersham Pharmacia Biotech, Buckinghamshire, U.K.).

RNA extraction and Northern blot analysis

Cells were precultured in the presence or absence of fat for 24 h, followed by 1 ng ml^{-1} of IL-1β for 2 h. Then the cells were scraped into tubes, and total RNA was extracted according to the manufacturer's instructions of the RNAzol kit (TEL-TEST, Inc. Friendswood, TX, U.S.A.). Total RNA (15 μg per lane) from each 10 cm dish was electrophoretically separated, capillary-transferred to a nylon membrane, and UV crosslinked. A cDNA probe for IL-8 was prepared by RT–PCR of total RNA from Caco-2 cells stimulated with IL-1β for 2 h. Random 9 mers (TaKaRa, Shiga, Japan) were used for reverse transcription of RNA. The product of RT reaction was amplified by PCR, and then labelled with digoxigenin (DIG) (Roche, Mannheim, Germany) by re-PCR. Hybridization of the Northern blots was performed using DIG Easy Hybri, and chemiluminescent detection was performed using anti-DIG AP and CSPD (Roche) according to the manufacturer's instructions (Trayhum *et al*., 1995).

Nuclear extracts and EMSA

Cells were precultured in the presence or absence of fat for 24 h, followed by 1 ng ml^{-1} of IL-1β for 1.5 h, and nuclear extracts were prepared by the method of Schutze *et al*. (1992). Briefly, cells grown on 6 cm dishes were washed with phosphate-buffered saline (PBS) and incubated on ice for 8 min in 750 μl of buffer A (0.5% Nonidet P-40, 2 mg ml^{-1}

leupeptin (in mM) HEPES–KOH 10, EDTA 0.1, dithiothreitol (DTT) 1, $MgCl_2$ 2, phenylmethylsulfonyl fluoride (PMSF) 0.5, sucrose 500). The cells were then scraped into tubes and centrifuged for 10 min ($12,000 \times g$). The nuclear pellets were resuspended with 45 μl of a high salt buffer (2 mg ml^{-1} leupeptin (in mM) HEPES–KOH 20, EDTA 0.1, DTT 1, $MgCl_2$ 1.5, PMSF 0.5), incubated on ice for 40 min and centrifuged for 10 min at 4°C ($12,000 \times g$). The resulting supernatant, nuclear extracts, was then mixed with 67.5 μl of a low salt buffer (20% glycerol, 2 mg ml^{-1} leupeptin (in mM): HEPES–KOH 20, EDTA 0.2, DTT 1, PMSF 0.5). Consensus oligonucleotides of NF-κB (TGAGGGGACTTTCCCAGGC), AP-1 (CGCTTGATGAGTCAGCCGGAA), and NF-IL-6 (TGCAGATTGCGCAATCTGCA) (Promega, Madison, WI, U.S.A.) were end-labelled with γ-^{32}p-adenosine triphosphate. Binding reactions were performed by mixing 10 μg of nuclear extracts with 1 μl of labelled oligonucleotide and gelshift binding buffer to a final volume of 10 μl. Reaction products were separated in a 4% acrylamide/60 : 1 acrylamide : bisacrylamide gel and analysed by autoradiography.

Transient transfection of Caco-2 cells with an IL-8 promoter/reporter gene construct and dual-luciferase assay

The IL-8 promoter region (−135 to +46 bp) was amplified by PCR using human blood genomic DNA with the primers as described previously (Huang *et al*., 1997). This fragment was ligated into the *Sst*I and *Xho*I sites of the luciferase reporter plasmid pGL3–Basic (Promega), yielding the IL-8 promoter/pGL3 reporter construct. At 24 h after plating, Caco-2 cells were co-transfected with IL-8 promoter/pGL3 reporter construct and pRL–TK vector (Promega) at a ratio of 40 : 1, respectively, using the liposome-mediated method (Leonard *et al*., 1994). On day 2, transfected cells were precultured with caprylic acid for 12 h, and stimulated with 1 ng ml^{-1} of IL-1β. After 6 h, the cells were lysed with 1 × lysis buffer (Promega), and the cytoplasmic extract was then assayed for luciferase activity using a luminometer (Lumat LB9501, Wallac Berthold, Wildbad, Germany) according to the manufacturer's instructions. The data obtained were evaluated as firefly luciferase activity (fluc)/*Renilla* luciferase activity (*R*luc) to correct the difference of transfection efficiency (Grentzmann *et al*., 1998).

Chromatin immunoprecipitation

The method of Braunstein *et al*. (1993) was adapted as follows. Cells from a 6 cm dish were cross-linked by addition of 1% HCHO to the medium for 10 min. The cells were then scraped and resuspended in 100 μl SDS lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris–HCl), sonicated under conditions that reduced DNA length to between 200 and 1000 base pairs, and debris removed by centrifugation. The chromatin solution was diluted 10-fold in IP buffer. The chromatin solution was then incubated with anti-acetylated H4 antibody (Upstate Biotechnology, Lake Placid, NY, U.S.A.) overnight at 4°C, and immune complexes collected with protein A heads pre-adsorded with sonicated single-stranded DNA. Following washes and elution, cross-links were reversed by heating 65°C for 4 h. DNA was recovered by phenol extraction and ethanol precipitation. Specific sequences (IL-8 promoter region) in the immunoprecipitates were detected by PCR under conditions in which product yield was dependent on input DNA dose, using the following primers. IL-8 (−135) 5′-GAAGTGTGATGACTCAGG-3′ IL-8 (+46) 5′-GAAGC-TTGTGTGTGCTCAGC-3′

Statistical analysis

The data obtained were expressed as mean ± s.e.mean. The intensity of each band was analysed by the NIH Image program. The means of the different groups were compared by analysis of variance (ANOVA) followed by Student's unpaired test. P values less than 0.05 were considered significant.

Results

Concentration of fats not toxic to Caco-2 cells

Previous reports have shown that IL-8 production and IL-8 mRNA expression increase at levels of butyrate that are toxic to Caco-2 cells and lead to significant cell death (Huang *et al*., 1997). In the present study, the concentrations of fats added to culture media were determined on the basis of the result of the WST-1 assay (data not shown). As a result, caprylic acid did not affect cell viability at concentrations up to 1.3 mM and MCT up to 0.6%.

Caprylic acid and MCT suppressed IL-8 secretion by Caco-2 cells at the mRNA transcription level when precultured together for 24 h

When cells were precultured with caprylic acid and MCT for 24 h before stimulation with 1 ng ml^{-1} of IL-1β, IL-8 secretion by stimulated Caco-2 cells decreased with increasing concentrations of caprylic acid and MCT (Figure 1). It was also confirmed that preculture for at least several hours with caprylic acid and MCT was necessary to suppress IL-8 secretion. When caprylic acid was added to the media at the time of stimulation with IL-1β, IL-8 production stayed almost constant (data not shown). Then, when caprylic acid was removed completely from the media just before stimulation with IL-1β, the suppression of IL-8 secretion remained intact (data not shown).

We performed Northern blot analysis to investigate whether IL-8 gene transcription was suppressed, and we found that pretreatment with caprylic acid and MCT for 24 h weakly inhibited IL-8 mRNA accumulation by stimulation with IL-1β (Figure 2).

Pre-incubation with caprylic acid and MCT failed to modulate the activation of nuclear transcription factors

To clarify the effect of caprylic acid and MCT on IL-8 gene transcription, we investigated the expression of NF-κB by EMSA, as well as the nuclear transcription factors AP-1 and NF-IL-6. Caprylic acid and MCT did not inhibit the NF-κB and overall level of its DNA binding activity in IL-1β-stimulated cells (Figure 3A). AP-1- and NF-IL-6-DNA binding activities could already be observed in nuclei of

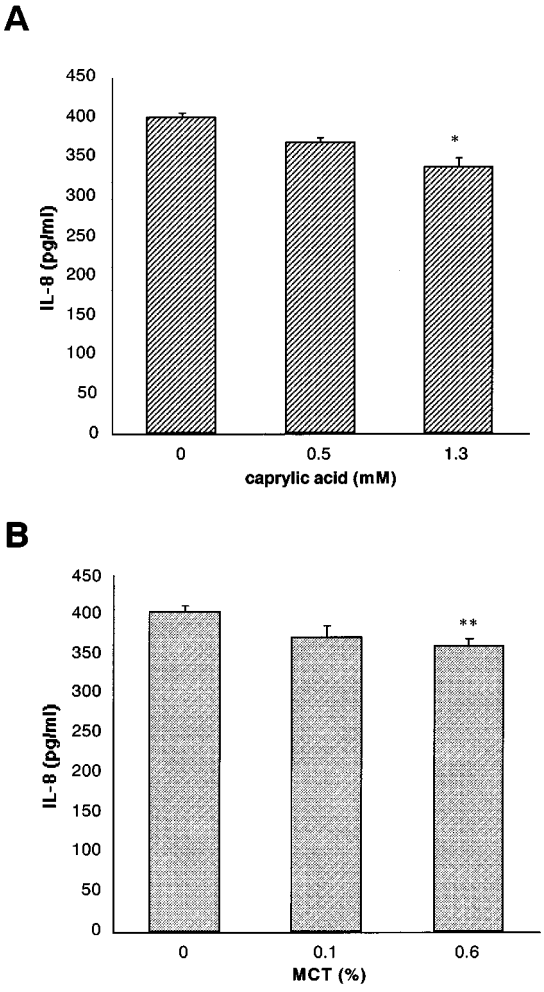

Figure 1 Effects of caprylic acid (A) and MCT (B) on IL-8 secretion by IL-1β-stimulated Caco-2 cells. Cells were incubated with medium alone, caprylic acid or MCT for 24 h and then stimulated with 1 ng ml^{-1} of IL-1β. The concentrations of caprylic acid and MCT were determined by the results of WST-1 assay, and IL-8 levels were measured by ELISA. Values are expressed as means$\pm$s.e.mean (*$P<0.05$, **$P<0.05$).

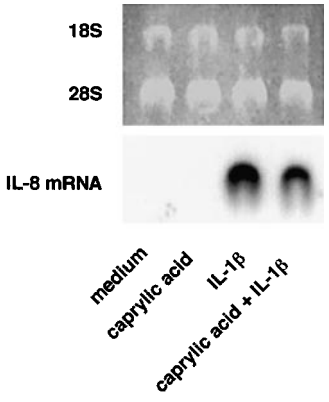

Figure 2 Effects of caprylic acid on IL-8 mRNA expression in Caco-2 cells. Cells were incubated with medium alone, caprylic acid (1.3 mM) or MCT (0.6%) for 24 h and then stimulated with 1 ng ml^{-1} of IL-1β for 2 h. IL-8 mRNA expression was evaluated by Northern blotting. Similar results were observed when cells were incubated with MCT.

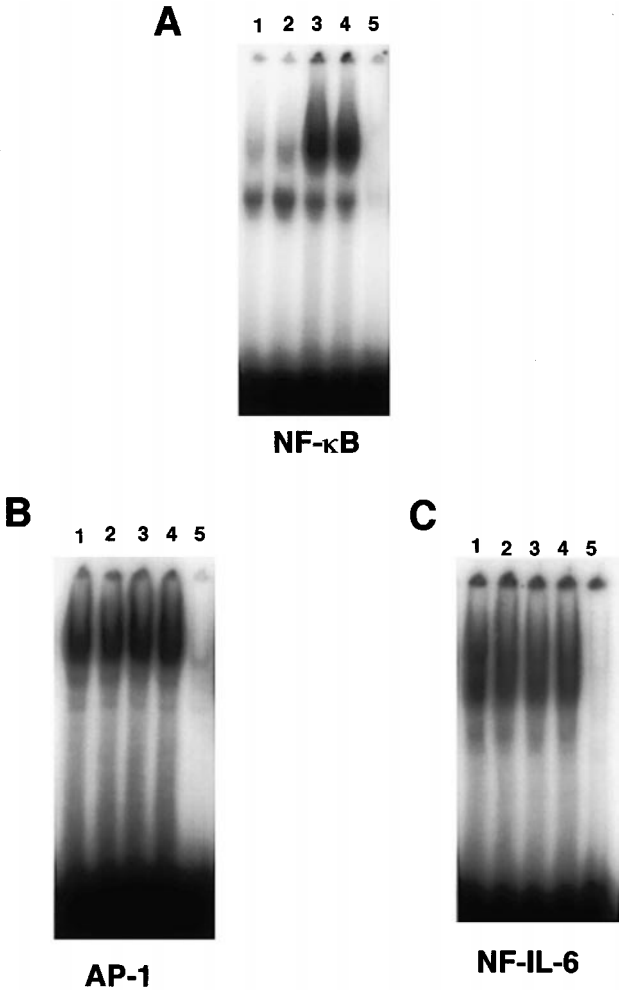

Figure 3 Effects of caprylic acid on the activation of NF-κB, AP-1 and NF-IL-6. Cells were incubated with medium alone, caprylic acid for 24 h. After stimulation with 1 ng ml^{-1} of IL-1β for 1.5 h. nuclear extracts were prepared. The activation of transcription factors described above was evaluated by electrophoretic mobility shift assay (EMSA) (A)–(C) lane 1, medium alone; lane 2, caprylic acid alone; lane 3, stimulation with 1 ng ml^{-1} of IL-1β; lane 4, preculture with caprylic acid for 24 h followed by 1 ng ml^{-1} of IL-1β; lane 5, 1 ng ml^{-1} of IL-1β plus cold probe; Similar results were observed when cells were incubated with MCT.

unstimulated cells, and were not enhanced or inhibited by the addition of fat or IL-1β. Similarly, pretreatment with caprylic acid or MCT did not modulate AP-1- and NF-IL-6-DNA binding activities in IL-1β-stimulated cells (Figure 3B,C).

Trichostatin A strongly inhibited IL-8 mRNA expression without modulating the activation of transcription factors

Trichostatin A, a specific inhibitor of histone deacetylase, also inhibited IL-8 mRNA expression when cells were jointly preincubated for 6 h before stimulation with IL-1β (Figure 4A). This effect was almost dose-dependent, but then disappeared immediately because of its short half-life (data not shown). At the same time, we examined the activation of the transcription factors described above, and found that preculture with trichostatin A had no effect on their activation (Figure 4B).

Caprylic acid inhibited activation of IL-8 promoter in Caco-2 cells transfected with IL-8 promoter/luciferase reporter plasmid

Because the activation of gene transcription is mainly regulated in its promoter region, we investigated the activation of IL-8 promoter in Caco-2 cells transiently transfected with IL-8 promoter/luciferase reporter plasmid. As shown in Figure 5, pretreatment of transfected cells with caprylic acid inhibited IL-1β-induced luciferase activity and thus also transcription factor-driven reporter gene activation. Similar results were observed when cells were precultured with TSA (data not shown).

Trichostatin A induced rapid histone H4 acetylation in IL-8 promoter region in differentiated Caco-2 cells, whereas caprylic acid did not

Finally, we investigated whether caprylic acid and trichostatin A actually increased the density of H4 actylation in genomic IL-8 promoter region using the techniques known as chromatin immunoprecipitation (Chr-IP). As shown in Figure 6, pretreatment with trichostatin A caused rapid H4 acetylation in IL-8 promoter chromatin in Caco-2 cells. However, in contract, caprylic acid did not induce promoter-specific H4 hyperacetylation. In this assay, immunoprecipitation was specific, since it was not observed with nonspecific IgG (data not shown). In addition, there was no contamination of DNA in our eluates, as no PCR band was observed when no antibody was used.

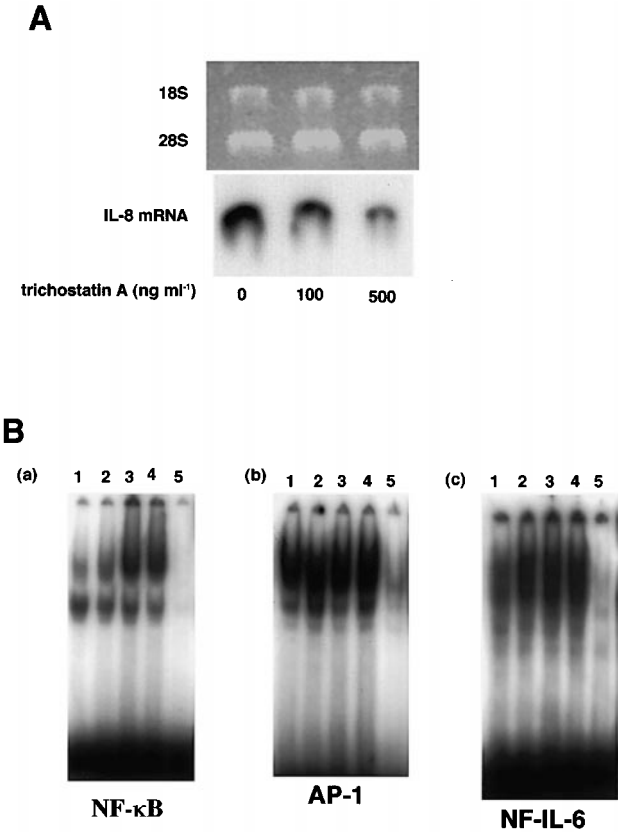

Figure 4 Effects of trichostatin A (TSA) on IL-8 mRNA expression and activation of its transcription factors. (A) Cells were incubated with various concentrations of TSA for 6 h, and then stimulated with 1 ng ml^{-1} of IL-1β for 2 h. IL-8 mRNA expression was evaluated by Northern blotting. (B) Cells were incubated with TSA (100 ng ml^{-1}) for 6 h. After stimulation with 1 ng ml^{-1} of IL-1β for 1.5 h, nuclear extracts were prepared. (a)–(c) lane 1, medium alone; lane 2, TSA alone; lane 3, stimulation with 1 ng ml^{-1} of IL-1β; lane 4, preculture with TSA followed by 1 ng ml^{-1} of IL-1β; lane 5, 1 ng ml^{-1} of IL-1β plus each cold probe.

Discussion

Most fat digestion begins in the duodenum, and pancreatic lipase is one of the most important enzymes involved. Fatty acids are liberated by the action of pancreatic lipase on dietary triglyceride and, in the presence of bile salts, form micelles, which diffuse through the unstirred layer to the

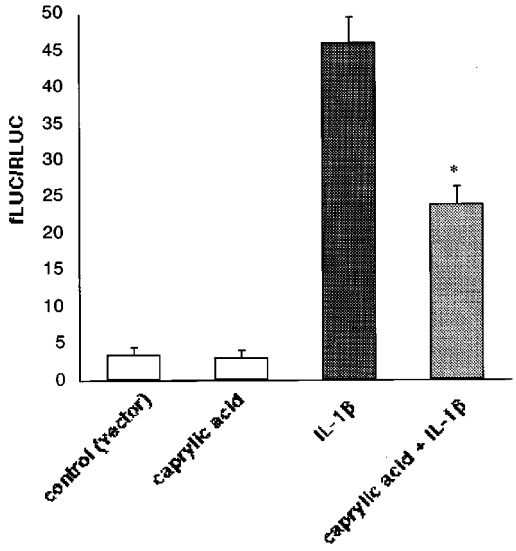

Figure 5 Effects of caprylic acid on activation of IL-8 promoter. Cells were transiently co-transfected with the IL-8 promoter/luciferase reporter plasmid and pRL-TK vector at a ratio of 40 : 1, and cultured with or without caprylic acid for 24 h. After stimulation with 1 ng ml^{-1} of IL-1β for 6 h, dual-luciferase assay was performed. The data obtained were evaluated as firefly luciferase activity/*Renilla* luciferase activity (fluc/Rluc). Similar results were observed when cells were incubated with trichostatin A. Values are expressed as means $\pm$ s.e.mean ($^*P<0.05$).

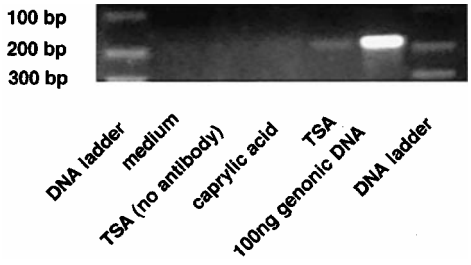

Figure 6 Effects of caprylic acid and trichostatin A (TSA) on IL-8 promoter-specific H4 acetylation in differentiated Caco-2 cells. Cells were cultured as indicated, cross-linked, and nuclear protein prepared. After precipitation with anti-acetylated H4 antibody, the specific sequence of the IL-8 promoter region was detected by PCR.

mucosal surface and enter enterocytes. Fatty acids containing less than 10–12 carbon atoms pass from the mucosal cells directly into the portal blood, where they are transported as free fatty acids. Finally, they are broken down in mitochondria by β-oxidation to acetyl-CoA, which enters the citric acid cycle. Because MCFAs and SCFAs can enter mitochondria without carrier protein, their β-oxidation can easily proceed (Ganong, 1997); in other words, they are a good source of energy. MCT is a fatty emulsion that contains abundant caprylic acid and is often administered to short-bowel patients. It is known that the luminal SCFA butyrate suppresses IL-8 production in a human colonic epithelial cell line (Huang *et al*., 1997; Andoh *et al*., 1999b), but the possibility that caprylic acid, one of the MCFAs, and MCT improve intestinal inflammation by suppressing IL-8 production in enterocytes has not been documented.

In this study we observed for the first time that caprylic acid and medium-chain C8 triglycerides suppressed IL-8 secretion by Caco-2 cells at the transcription level when precultured together for 24 h. The effect of MCFAs has been examined in a few recent studies. Tanaka *et al*. (2001) demonstrated that capric acid (C10) increased IL-8 production in IL-1β-stimulated Caco-2 cells. Andoh *et al*. (2000) demonstrated that octanoic acid (C8) dose-dependently enhanced IL-1β-induced IL-8 secretion in intestine-407 cells. We assume that there are several reasons for the apparent dichotomy between our results and those of others. First, the concentrations of MCFAs are considered significant. Previous reports have shown that IL-8 production and IL-8 mRNA expression increase at levels that are toxic to Caco-2 cells (Huang *et al*., 1997). Some investigators have applied much higher concentrations of fatty acids to culture media in their studies. Second, the length of MCFAs is important. We observed that caprylic acid (C8) suppressed IL-8 secretion by Caco-2 cells as described above, whereas capric acid micelle (C10) did not (data not shown). Third, different conditions of cell incubation with caprylic acid or IL-1β often produce different results. When caprylic acid is applied to the media at the same time of stimulation with IL-1β, IL-8 production is almost constant. Preincubation with caprylic acid for at least several hours is required to suppress IL-8 production as described above. Finally, the different status of Caco-2 cells has a pronounced effect on the experimental outcome. Mariadason *et al*. (2001) have shown that SCFA maximally induced a number of endpoint read-out analyses including IL-8 secretion in undifferentiated Caco-2 cells, but had a different effect in the normal colonic epithelium (differentiated Caco-2 cells). In the present study, differentiated Caco-2 cells (day 14), which may reflect the actual *in vivo* system, were used.

Because most of the pro-inflammatory cytokine genes including IL-8 contain κB-binding motifs in their promoter regions, their transcriptions are dependent on NF-κB activation (Baldwin, 1996; Barnes & Karin, 1997), which in IL-8 gene transcription is the most critical step. As AP-1 and NF-IL-6 co-operatively facilitate its transcription with NF-κB (Kunsh *et al*., 1994; Yamamoto *et al*., 1992), we examined the expressions of these three transcription factors by EMSA. Our results showed that caprylic acid and TSA did not decrease the amount of these transcription factors-DNA binding complex, suggesting that the inhibition of IL-8 gene transcription they induced does not necessarily require the marked suppression of transcription factors.

One attractive hypothesis for why caprylic acid and TSA inhibited IL-8 gene transcription in our study was that when they are used for pretreatment, they modulate the chromatin structure by acetylating histone as does butyrate. As described above, reversible histone acetylation is often reported to affect gene transcription, and Huang *et al*. (1997) reported that histones isolated from Caco-2 cells treated with various concentrations of sodium butyrate showed a direct correlation between the degree of H4 hyperacetylation and the degree to which the IL-8 gene is inhibited. Recently, in addition, not global patterns of acetylation but targeting of histone acetylation to promoters has been ascribed to a particular regulatory function (Smith *et al*., 2001). We used chromatin immunoprecipitation (Chr-IP) to investigate the state of H4 acetylation in IL-8 promoter in differentiated Caco-2 cells. Our results showed that a high dose of TSA induced H4 acetylation in IL-8 promoter chromatin in Caco-2 cells, whereas caprylic acid did not. Cousens *et al*. (1978) demonstrated that fatty acids containing up to six carbon atoms induced H4 acetylation in HTC cells. However, we could not observe the action of caprylic acid (C8) on targeted histone acetylation to IL-8 promoter in differentiated Caco-2 cells.

The results of the dual-luciferase assay in our study showed that caprylic acid and TSA inhibited the IL-1β-induced activation of IL-8 promoter in Caco-2 cells transfected with IL-8 promoter/pGL3 reporter construct. Reeves *et al*. (1985) demonstrated that mammalian cells were capable of rapidly assembling non-integrated circular plasmids into typical mini-chromosomes containing nucleosomes with a 190 bp repetitive spacing. Furthermore, they demonstrated that mini-chromosomes isolated from butyrate-treated cells contained highly acetylated forms of histone H4. Thus we may be able to compare the activation of our reporter construct with that of human genomic IL-8 promoter. TSA may inhibit the IL-1β-induced activation of IL-8 promoter via the chromatin modification induced by histone acetylation. However, the mechanism by which caprylic acid inhibits the IL-1β-induced activation of IL-8 promoter remains unclear.

In conclusion, this study demonstrates the inhibitory effect of caprylic acid (C8) and MCT on IL-8 gene transcription in differentiated Caco-2 cells. The mechanism by which they inhibit IL-8 gene transcription is not dependent on transcription factor inhibition or the induction of H4 hyperacetylation in its promoter. However, the inhibitory effect of the potent histone deacetylase inhibitor TSA on IL-8 gene transcription may be dependent on the state of H4 acetylation in its promoter. Further studies from various perspectives will be needed to reveal the effects of MCFAs and MCT on cytokine gene transcription in intestinal epithelial cells.

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References

ALLFREY, V.G. (1980). *A Comprehensive Treatise*. Vol.3, pp. 347–437. New York: Academic Press.

ANDOH, A., BAMBA, T. & SASAKI, M. (1999a). Physiological and anti-inflammatory roles of dietary fiber and butyrate in intestinal function. *J. Parent. Ent. Nutr.,* **23,** S70–S73.

ANDOH, A., FUJIYAMA, Y., HATA, K., ARAKI, Y., TAKAYA, H., SHIMADA, M. & BAMBA, T. (1999b). Counter-regulatory effect of sodium butyrate on tumor necrosis factor-alpha induced complement C3 and factor B biosynthesis in human intestinal epithelial cells. *Clin. Exp. Immunol.,* **118,** 23–29.

ANDOH, A., TAKAYA, H., ARAKI, Y., TSUJIKAWA, T., FUJIYAMA, Y. & BAMBA, T. (2000). Medium- and long-chain fatty acids differentially modulate interleukin-8 secretion in human fetal intestinal epithelial cells. *J. Nutr.,* **130,** 2636–2640.

BAGGIOLINI, M., WALZ, A. & KUNKEL, S.L. (1989). Neutrophil-activating peptide-1/interleukin-8, a novel cytokine that activates neutrophils. *J. Clin. Invest.,* **84,** 1045–1049.

BALDWIN, A.S. (1996). The NF-κB and IκB proteins, new discoveries and insights. *Annu. Rev. Immunol.,* **14,** 649–683.

BARNES, P.J. & KARIN, M. (1997). Nuclear factor-κB: a pivotal transcription factor in chronic inflammatory diseases. *N. Engl. J. Med.,* **336,** 1066–1091.

BRAUNSTEIN, M., ROSE, A.B., HOLMES, S.G., ALLIS, C.D. & BROACH, J.R. (1993). Transcriptional silencing in yeast is associated with reduced nucleosome acetylation. *Genes Dev.,* **7,** 592–604.

COUSENS, L.S., GALLWITZ, D. & ALBERTS, B.M. (1978). Different accessibilities in chromatin to histone acetylase. *J. Biol. Chem.,* **254,** 1716–1723.

ECKMANN, L., KAGNOFF, M.F. & FIERER, J. (1993). Epithelial cells secrete the chemokine interleukin-8 in response to bacterial entry. *Infect. Immun.,* **61,** 4569–4574.

GANONG, W.F. (1997). *Review of Medical Physiology,* pp. 442–444. Stamford: Simon & Schuster.

GRENTZMANN, G., INGRAM, J.A., KERRY, P.J., GESTELAND, R.F. & ATKINS, J.F. (1998). A dual-luciferase reporter system for studying recording signals. *RNA,* **4,** 479–486.

HUANG, N., KATZ, J.P., MARTIN, D.R. & WU, G.D. (1997). Inhibition of IL-8 gene expression in Caco-2 cells by compounds which induce histone hyperacetylation. *Cytokine,* **9,** 27–36.

INAN, M.S., RASOULPOUR, R.J., YIN, L., HUBBARD, A.K., ROSENBERG, D.W. & GIARDINA, C. (2000). The luminal short-chain fatty acid butyrate modulates NF-kappaB activity in a human colonic epithelial cell line. *Gastroenterology,* **118,** 724–734.

ISHIYAMA, M., TOMINAGA, H., SHIGA, M., SASAMOTO, K., OHKURA, Y., UENO, K. & WATANABE, M. (1995). Novel cell proliferation and cytotoxicity assays using a tetrazolium salt that produces a water-soluble formazan dye. *In vitro Toxicology,* **8,** 187–190.

JEPPESEN, P.B. & MORTENSEN, P.B. (1998). The influence of a preserved colon on the absorption of medium chain fat in patients with small bowel resection. *Gut,* **43,** 478–483.

KUNSH, C., LANG, R.K. & ROSEN, C.A. (1994). Synergistic transcriptional activation of the IL-8 gene by NF-kappa B p65 (RelA) and NF-IL-6. *J. Immunol.,* **153,** 153–164.

KUO, M.H., VOM BAUR, E., STRUHL, K. & ALLIS, C.D. (2000). Gcn4 activator targets Gcn5 histone acetyltransferase to specific promoters independently of transcription. *Mol. Cell,* **6,** 1309–1320.

LEONARD, D., MICHAEL, K. & JAMES, B. (1994). *Basic Method in Molecular Biology,* 2nd edn. pp. 633–635. Connecticut: Appleton & Lange.

MAZZUCCHELLI, L., HAUSER, C., ZGRAGGEN, K., WAGNER, H., HESS, M., LAISSUE, J.A. & MUELLER, C. (1994). Expression of interleukin-8 gene in inflammatory bowel disease is related to histological grade active inflammation. *Am. J. Pathol.,* **144,** 997–1007.

MARIADASON, J.M., VELCICH, A., WILSON, A.J., AUGENLICHT, L.H. & GIBSON, P.R. (2001). Resistance to butyrate-induced cell differentiation and apoptosis during spontaneous Caco-2 cell differentiation. *Gastroenterology,* **120,** 889–899.

NORTON, V.G., IMAI, B.S., YAU, P. & BRADBURY, E.M. (1989). Histone acetylation reduces nucleosome core particle linking number change. *Cell,* **57,** 449–457.

OGUCHI, S., WALKER, W.A. & SANDERSON, I.R. (1994). Differentiation and polarity alter the binding of IGF-1 to human intestinal epithelial (Caco-2) cells. *J. Pediatr. Gastroenterol Nutr.,* **20,** 148–155.

OPPENHEIM, J.J., ZACHARIAE, C.O.C., MUKAIDA, N. & MATSUSHIMA, K. (1991). Properties of the novel inflammatory supergene 'intercrine' cytokine family. *Annu. Rev. Immunol.,* **9,** 617–648.

PINTO, M., ROBINE-LEON, S., APPAY, M.-D., KEDINGER, M., TRIADOU, N., DUSSAULX, E., LACROIX, B., SIMON-ASSMAN, P., HAUFFEN, K., FOGH, J. & ZWEIBAUM, A. (1983). Enterocyte-like differentiation and polarization of the human colon carcinoma cell line Caco-2 in culture. *Biol. Cell,* **47,** 323–330.

REEVES, R., GORMAN, C.M. & HOWARD, B. (1985). Minichromosome assembly of non-integrated plasmid DNA transfected into mammalian cells. *Nucleic Acids Res.,* **13,** 3599–3615.

SANDERSON, I.R. & WALKER, W.A. (1994). *Handbook of Mucosal Immunology*. pp. 41–48. New York: Academic Press.

SCHEPPACH, W., SOMMER, H., KIRCHNER, T., PAGANIELLI, G., BARTRAM, P., CHRISTL, S., RICHTER, F., DUSEL, G. & KASPER, H. (1992). Effect of butyrate enemas on the colonic mucosa in distal ulcerative colitis. *Gastroenterology,* **103,** 51–56.

SCHUTZE, S., POTTHOFF, K., MACHLEIDT, T., BERKOVIC, D., WEIGMANN, K. & KRONKE, M. (1992). TNF activates NF-κB by phosphatidyl choline-specific phospholipase C-induced 'acidic' sphingomyelin breakdown. *Cell,* **71,** 765–776.

SMITH, E.R., ALLIS, C.D. & LUCCHESI, J.C. (2001). Linking global histone acetylation to the transcription enhancement of X-chromosomal genes in *Drosophila* males. *J. Biol. Chem.,* **276,** 314883–314886.

STEINHART, A.H., BRZEZINSKI, A. & BAKER, J.P. (1994). Treatment of refractory ulcerative proctosigmoiditis with butyrate enemas. *Am. J. Gastro.,* **89,** 179–183.

TANAKA, S., SAITOH, O., TABATA, K., MATSUSE, R., KOJIMA, K., SUGI, K., NAKAGAWA, K., KAYAZAWA, M., TERANISHI, T., UCHIDA, K. & HIRATA, I. (2001). Medium-chain fatty acids stimulate interleukin-8 production in Caco-2 cells with different mechanisms from long chain-fatty acids. *J. Gastroenterol. Hepatol.,* **16,** 748–754.

TANDON, R.K., RODGERS, JR. J.B. & BALINT, J.A. (1972). The effects of medium-chain triglycerides in the short bowel syndrome. Increased glucose and water transport. *Am. J. Dig. Dis.,* **17,** 233–238.

TRAYHUM, P., DUNCAN, J.S., NESTOR, A., THOMAS, M.E., EASTMOND, N.C. & RAYNER, D.V. (1995). Rapid chemiluminescent detection of mRNAs on Northern blots with digoxigenin end-labeled oligonucleotides. *Electrophoresis,* **16,** 341–344.

VOGELAUER, M., WU, J., SUKA, N. & GRUNSTEIN, M. (2000). Global histone acetylation and deacetylation in yeast. *Nature,* **408,** 495–498.

WINAWER, S.J., BROITMAN, S.A. & WOLOCHOW, D.A. (1966). Successful management of massive small-bowel resection based on assessment of absorption defects and nutritional needs. *N. Engl. J. Med.,* **274,** 72–78.

YAMAMOTO, K., OKAMOTO, S. & MUKAIDA, N. (1992). Tumor necrosis factor and interferon γ synergistically induce interleukin 8 production in a human gastric cancer cell line through acting concurrently on AP-1 and NF-κB-like binding sites of the interleukin 8 gene. *J. Biol. Chem.,* **267,** 22506–22511.

YOSHIDA, M., KIJIMA, M., AKITA, M. & BEPPU, T. (1990). Potent and specific inhibition of mammalian histone deacetylase both *in vivo* and *in vitro* by trichostatin A. *J. Biol. Chem.,* **265,** 17174–17179.

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