Medium- and Long-Chain Fatty Acids Differentially Modulate Interleukin-8 Secretion in Human Fetal Intestinal Epithelial Cells

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ABSTRACT The primary therapeutic effects of enteral nutrition in patients with Crohn’s disease have been reported previously. Although the quantity and type of fat in enteral nutrition are considered to be important, it is unclear how fat modulates mucosal inflammatory responses in the intestine. In the present study, we evaluated the effects of medium-chain and long-chain fatty acids (MCFA and LCFA) on interleukin (IL)-8 secretion in a fetal intestinal epithelial cell line, intestine-407 cells. IL-8 expression was evaluated at the protein and mRNA levels. The activation of nuclear factor-κB was assessed with an electrophoretic gel mobility shift assay. The addition of oleic acid (LCFA) micelles, but not octanoic acid (MCFA) micelles, weakly but significantly enhanced basal IL-8 secretion in the intestine-407 cells. The addition of MCFA (5 mmol/L) induced a 40% increase in IL-1β-induced IL-8 secretion and a 35% increase in tumor necrosis factor (TNF)-α-induced IL-8 secretion, respectively. The addition of LCFA (5 mmol/L) induced a 140% increase in IL-1β-induced IL-8 secretion and a 110% increase in TNF-α-induced IL-8 secretion, respectively. These responses were also observed at the mRNA levels. The electrophoretic gel mobility shift assay indicated that both MCFA and LCFA enhanced IL-1β- and TNF-α-induced nuclear factor-κB activation. We demonstrated the proinflammatory activities of MCFA and especially LCFA. It is likely that medium-chain triglycerides may be more suitable than long-chain triglycerides as an energy source in enteral diets in the treatment of patients with Crohn’s disease. J. Nutr. 130: 2636–2640, 2000.

KEY WORDS: • NF-κB • Crohn’s disease • inflammation

Inflammatory bowel disease (IBD) is a chronic inflammatory process, of which the cause and pathogenesis remain unclear. The contribution of genetic factors has been reported in family studies as well as genetic linkage analyses (Nakajima et al. 1995, Tysk et al. 1988), but it has also been suggested that environmental factors, including dietary components, might play an important role (Tragonone et al. 1995). Furthermore, nutritional support in the correction and maintenance of nutritional status in patients with IBD is widely accepted (Greerling et al. 1999, O’Sullivan et al. 1998).

Several studies have demonstrated the primary therapeutic effects of enteral nutrition in patients with Crohn’s disease (CD) (O’Morain et al. 1984, O’Sullivan et al. 1998, Saverymuss et al. 1985). In these studies, the remission rates among patients treated with enteral diets were comparable to those of patients administered corticosteroid therapy (Saverymuss et al. 1985, Seidman et al. 1986). However, the mode of action of enteral nutrition as the primary treatment of active CD patients remains conjectural. Theoretically, several mechanisms have been proposed: bowel rest, elimination of dietary antigen uptake, alterations in intestinal microbial flora, nutritional repletion and modification of intestinal permeability (O’Sullivan et al. 1998). Among these factors, the quantity and type of fat in enteral diets may have important therapeutic effects. In previous reports of enteral feeding trials in CD patients, it has been demonstrated that high-fat formulas resulted in poorer outcomes, whereas low-fat diets were associated with more favorable results (Fernandez-Banares et al. 1994). Furthermore, fatty acid chain length affects the response to enteral diets. Middleton et al. (1995) demonstrated that remission rates of active CD patients were negatively correlated with amounts of long-chain triglycerides (LCT), whereas Khoshoo et al. (1996) reported that there were no differences in therapeutic efficacy between low and high medium-chain triglyceride (MCT) diets in active CD patients. Recently, we observed that LCT feeding markedly enhanced mucosal damage compared with MCT feeding in trinitrobenzene sulfonic acid–induced experimental enteritis in rats (Tsujikawa et al. 1999). These findings suggest the possibility that fats, especially LCT, may enhance local inflammatory responses in the intestine. However, how fat absorption stimulates inflammatory responses in the intestinal mucosa has not been fully investigated.

The cytokine interleukin (IL)-8 is a potent chemoattractant for neutrophils, T cells and basophils. IL-8 induces the accumulation and activation of neutrophils and initiates and
promotes acute inflammatory responses. In the intestinal mucosa, epithelial cells have been regarded as a site of IL-8 secretion (Eckmann et al. 1993, McDermott et al. 1998, van Deventer 1997). It has also been reported that epithelial IL-8 secretion is potently enhanced by the proinflammatory cytokines tumor necrosis factor (TNF)-α and IL-1β, released by activated monocytes/macrophages. To evaluate the effects of MCT and LCT on the inflammatory response in the intestine, we tested the effects of medium- and long-chain fatty acids (MCFA and LCFA) on IL-8 secretion in the human fetal intestinal epithelial cell line intestine-407 (Henle and Denhardt, 1957), LCT and MCT are considered to be absorbed as LCFA and MCFAs, respectively. The present study provided data that indicate LCFA and MCFA differentially modulate IL-1β- and TNF-α–induced IL-8 secretion in intestinal epithelial cells.

MATERIALS AND METHODS

Reagents. Recombinant human IL-1β (specific activity 2 × 10^6 U/mg by mouse thymocyte proliferation assay) was kindly provided by Osuka Pharmaceutical (Tokushima, Japan). Recombinant human TNF-α (specific activity 2.5 × 10^6 U/mg by cytotoxic assay against LM cells) was kindly provided by Dainippon Pharmaceutical (Osaka, Japan). All other reagents used in this study were purchased from Sigma Chemical Co. (St. Louis, MO).

Cells. The intestine-407 cells were obtained from American Type Culture Collection (Rockville, MD). The cells were established from the small intestine of a human fetus (Henle and Denhardt 1957), retain a normal karyotype (data from American Type Culture Collection) and exhibit typical epithelial morphology and growth. The cells are used as a model of normal intestinal epithelial cells in vitro. For example, the expression of the TNF-α receptor has been identified in these cells (Kawanishi 2000). The cells were cultured as a monolayer and maintained in Dulbecco’s modified Eagle’s medium (GIBCO, Grand Island, NY) containing 10% fetal bovine serum (GIBCO), 5 × 10^-4 U/L penicillin and 50 mg/L streptomycin. The cells were seeded at a density of 2.5 × 10^5 cells/L, and the cell culture medium was changed every 3rd d. All experiments were performed after cells reached confluence.

Preparation of micellar solutions. Micellar solutions were prepared according to the method described by Johnston and Bergstrom (1964). We used oleic acid (18:1) as the LCFA and octanoic acid (8:0) as the MCFA. Oleic acid and mono-olein were dissolved in benzene, dried under nitrogen and dissolved in 40 mmol/L taurocholate. The solution was diluted with an equal volume of 0.125 mol/L NaCl plus 0.017 mol/L phosphate buffer (pH 7.4). The final concentration contained 20 mmol/mL sodium taurocholate, 19.2 mmol/mL oleic acid and 9.6 mmol/mL mono-olein. A solution of 19.2 mmol/mL octanoic acid in phosphate buffer with 20 mmol/mL taurocholate was similarly prepared. A solution of 20 mmol/mL taurocholate in phosphate buffer was also prepared as a control.

Quantification of human IL-8. The amounts of antigenic IL-8 in the samples were determined with enzyme-linked immunosorbent assay kits (Cytoscreen Human IL-8, catalogue no. KHC0082; Bio Source, Camarillo, CA). Intestine-407 cells were incubated for 12 h, and then IL-8 levels in supernatants were determined with enzyme-linked immunosorbent assay kits (Cytoscreen Human IL-8, catalogue no. KHC0082; Bio Source, Camarillo, CA). Intestine-407 cells were incubated for 12 h, and then IL-8 levels in supernatants were determined with enzyme-linked immunosorbent assay kits (Cytoscreen Human IL-8, catalogue no. KHC0082; Bio Source, Camarillo, CA). Intestine-407 cells were incubated for 12 h, and then IL-8 levels in supernatants were determined with enzyme-linked immunosorbent assay kits (Cytoscreen Human IL-8, catalogue no. KHC0082; Bio Source, Camarillo, CA). Intestine-407 cells were incubated for 12 h, and then IL-8 levels in supernatants were determined with enzyme-linked immunosorbent assay kits (Cytoscreen Human IL-8, catalogue no. KHC0082; Bio Source, Camarillo, CA). Intestine-407 cells were incubated for 12 h, and then IL-8 levels in supernatants were determined with enzyme-linked immunosorbent assay kits (Cytoscreen Human IL-8, catalogue no. KHC0082; Bio Source, Camarillo, CA).

Statistical analysis. The data are expressed as means ± SD. The variance was analyzed by the Bartlett test (Statview for Macintosh Version 4.5; Abacus Concepts, Berkeley, CA). Subsequently, statistical significance of differences was determined by the Fisher’s LSD (Protected Least Significance Difference) test (Statview for Macintosh Version 4.5). Differences resulting in P-values of <0.05 were considered statistically significant.

RESULTS

MCFA did not affect basal IL-8 secretion in intestine-407 cells. LCFA weakly but significantly enhanced basal IL-8 secretion (Control 15.1 ± 1.6 ng/10^6 cells, LCFA 26.5 ± 2.2 ng/10^6 cells, mean ± SD, n = 4, P < 0.05). MCFA dose-dependently enhanced IL-1β– and TNF-α–induced IL-8 secretion (Fig. 1). These effects were observed at concentrations as low as 1.0 mmol/L (P < 0.05). LCFA dose-dependently enhanced IL-1β– and TNF-α–induced IL-8 secretion (Fig. 2). These effects were also observed at concentrations as low as 1.0 mmol/L (P < 0.05). When the effects of MCFA (5 mmol/L) and LCFA (5 mmol/L) were compared, LCFA enhanced IL-1β–induced IL-8 secretion more strongly than MCFA (IL-1β plus MCFA 94.6 ± 3.8, IL-1β plus LCFA 185.6 ± 13.3, P < 0.01). Similarly, the effects of LCFA (5 mmol/L) on TNF-α (10 μg/L)–induced IL-8 secretion were significantly stronger than those of MCFA (5 mmol/L) (TNF-α plus MCFA 85.6 ± 3.8, TNF-α plus LCFA 138.8 ± 10.2, P < 0.01).

The addition of either MCFA or LCFA enhanced both IL-1β– and TNF-α–induced IL-8 mRNA expression (Fig. 3). The effects of LCFA on mRNA were stronger than those of MCFA (P < 0.05), compatible with findings at the protein level.

Stimulation with IL-1β and TNF-α for 2 h increased NF-kB–DNA binding activity (Fig. 4, lanes 2 and 5). The specificity of this reaction was confirmed by the addition of cold
oligo-DNA, in which the reactive band disappeared (lane 8). The addition of antibodies to a 50,000 molecular weight subunit (p50) of NF-κB and the 65,000 molecular weight subunit (p65) induced supershifts of the binding complexes (lanes 9 and 10), indicating that this binding complex was a heterodimer that consists of the p50 and p65 subunits. The addition of MCFA enhanced the IL-1β– and TNF-α–induced IL-8 secretion (lanes 3 and 6). Similarly, LCFA enhanced the IL-1β– and TNF-α–induced IL-8 secretion (lanes 4 and 7). The effects of LCFA were stronger than those of MCFA (P < 0.05), suggesting that the effects of both MCFA and LCFA were mediated by signal transduction through the NF-κB activation pathway.

**DISCUSSION**

IL-8 acts as a chemoattractant and an activator of neutrophils and plays an important role in the initiation and maintenance of local inflammatory responses (Baggiolini et al. 1994, Miller et al. 1992). Although many different cell types, including monocytes/macrophages, fibroblasts and endothelial cells, can secrete IL-8 (Baggiolini et al. 1994), epithelial cells have been established as one of the main biosynthetic sites in the intestine (Eckmann et al. 1993, McDermott et al. 1998, van Deventer 1997). IL-8 is a key cytokine for the recruitment and activation of neutrophils, which are abundant in the intestinal lesions of IBD (McDermott et al. 1998, van Deventer 1997). In the present study, we tested the effects of MCFA and LCFA on IL-8 secretion in human fetal intestinal epithelial cells. Our results indicated that MCFA and LCFA significantly enhanced IL-1β– and TNF-α–induced IL-8 secretion in these cells. LCFA alone also weakly but significantly stimulated basal IL-8 secretion. These findings suggest that the absorption of MCFA and LCFA may play an important role in the progression and maintenance of local inflammation in the intestine. It is likely that the enhancement of IL-8 secretion in intestinal epithelial cells may result in the continuous recruitment of neutrophils and the prolongation of local inflammation in the intestine, reducing the remission rate or response to therapy in IBD patients. LCFA enhanced IL-1β– and TNF-α–induced IL-8 secretion more than did MCFA. These results are consistent with the clinical reports that high LCT feeding, but not MCT feeding, reduces the remission rate of active CD patients (Khoshoo et al. 1996, Middleton et al. 1995). Thus, the proinflammatory nature of MCFA and LCFA was demonstrated, and there were differences between their proinflammatory effects.

The transcription factor NF-κB is important in the transcriptional activation of genes encoding the proteins that...
participate in inflammatory and immune responses (Lenardo and Baltimore 1989). NF-κB activation is regulated by its cytoplasmic association with IκB molecules (inhibitors of nuclear factor for immunoglobulin κ chain in B cells), which mask the nuclear localization signal of NF-κB. In most cells, IκBα is the predominant inhibitory molecule, and the activation and translocation of NF-κB into the nucleus are contingent on its release from IκBα. Numerous stimuli, including IL-1β and TNF-α, rapidly induce the proteolytic degradation of IκBα and the consequent activation of NF-κB. The promoter region of the human IL-8 gene has been cloned, sequenced and shown to contain putative consensus binding motifs for NF-κB (Kunsh et al. 1994, Yasumoto et al. 1992). In vivo mucosal NF-κB activation has been reported to correlate with the disease activity of IBD patients (Schreiber et al. 1998). Our results indicated that the enhancing effects of MCFA and LCFA on IL-8 secretion were correlated with the increase in NF-κB activation in intestinal epithelial cells. Furthermore, IL-1β- and TNF-α-induced NF-κB activation was more potently enhanced by the addition of LCFA than of MCFA. These findings suggest that various inflammatory responses, which are mediated by NF-κB activation, may be enhanced by MCFA and especially LCFA in the intestinal mucosa. In the therapeutic strategies for CD patients, these results suggest that MCT rather than LCT should be used as an energy source in enteral diets because of their lower proinflammatory activity.

The replacement of dietary LCT by MCT reduces both steatorrhea and diarrhea as well as fecal electrolyte excretion in patients with a reduced small intestinal mucosal area due to resection or disease (Greenberger and Skillmann 1969, Hot 1968, Jeppensen and Mortensen 1998). These effects are considered to be associated with the rapid absorption of MCT, which is not dependent on micelle formation, intraluminal hydrolysis and mucosal reesterification (Greenberger and Skillmann 1969, Hot 1968). The human colon is not usually considered to be a site of fat absorption, but several experiments have indicated that because of their water solubility, MCFA are effectively absorbed in the colon (Jeppensen and Mortensen 1998). These nutritional characteristics of MCT or MCFA also make them suitable energy sources for CD patients, because multiple ulceration and mucosal inflammation in the small intestine markedly decrease absorption. Combined results suggest that MCT are potentially beneficial in the nutritional treatment of CD patients for two reasons:}

**FIGURE 3** Northern blot analysis of interleukin (IL)-8 mRNA expression in intestine-407 cells. Cells were cultured in the absence or presence of IL-1β (10 μg/L) or tumor necrosis factor (TNF)-α (10 μg/L) in combination with medium- or long-chain fatty acids [MCFA (5 mmol/L) or LCFA (5 mmol/L)] for 3 h, and then total cellular RNA was extracted. Medium containing taurocholate (20 mmol/L) was used as control. A representative blot is shown (A). The radioactivity of each band was measured by the Instant Imager Electronic Autoradiography system (Packard). The radioactivity relative to that of medium alone was calculated (B). Values are expressed as means ± SD, n = 4; values not sharing a letter are significantly different (P < 0.05).

**FIGURE 4** Electrophoretic gel mobility shift assays for nuclear factor (NF)-κB DNA-binding activities of intestine-407 cells. Cells were incubated with medium alone, interleukin (IL)-1β (10 μg/L) or tumor necrosis factor (TNF)-α (10 μg/L) in combination with medium- or long-chain fatty acids [MCFA (5 mmol/L) or LCFA (5 mmol/L)] for 2 h, and then the nuclear extracts were prepared. Medium containing taurocholate (20 mmol/L) was used as control. Dotted arrow indicates nonspecific band. A representative gel is shown (A). The radioactivity of each band was measured by the Instant Imager Electronic Autoradiography system (Packard). The radioactivity relative to that of medium alone was calculated (B). Values are expressed as means ± SD, n = 4; values not sharing a letter are significantly different (P < 0.05).
more rapid absorption in the small and large bowel and 2) less proinflammatory activity.

In conclusion, we demonstrated differences between MCFA and LCFA in their proinflammatory activities. Both MCFA and LCFA enhanced IL-1β and TNF-α-induced inflammatory responses in intestinal epithelial cells, but the effects of LCFA were stronger than those of MCFA. To our knowledge, this is the first report demonstrating differences in proinflammatory activity between MCFA and LCFA. Our findings suggest that MCT may be more suitable than LCT as an enteral energy source in the treatment of CD patients.

LITERATURE CITED


