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ORIGINAL ARTICLE

CLA and n-3 PUFA differentially modulate clinical activity and colonic PPAR-responsive gene expression in a pig model of experimental IBD

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Summary
Background & aims: Conjugated linoleic acid (CLA) and n-3 polyunsaturated fatty acids (PUFA) have been proposed as important pharmaconutrients for modulating mucosal immunity and therapeutic responses in patients with inflammatory bowel disease (IBD). We evaluated the ability of CLA and n-3 PUFA alone or in combination to modulate IBD in a pig model of dextran sodium sulfate (DSS)-induced colitis.
Methods: Sixty-four, 15-day-old pigs were used to evaluate the effect of CLA, n-3 PUFA and a 50:50 mixture of CLA and n-3 PUFA on growth, clinical activity and colonic PPAR-responsive gene expression. Diets were formulated to contain: 1.33% soybean oil (control); 1.33% CLA; 1.33% fish oil; or 1.33% of a 50:50 mixture of CLA and fish oil. Intestinal inflammation was induced by an intragastric challenge with DSS on day 42 of dietary supplementation. The colonic expression of peroxisome proliferator-activated receptor-γ (PPAR-γ), PPAR γ- and δ-responsive genes, keratinocyte growth factor (KGF) and tumor necrosis factor (TNF-α) were assayed by quantitative RT-PCR.
Results: The onset of IBD was delayed, colitis less severe and growth suppression attenuated in pigs fed CLA, which correlated with induction of colonic PPAR γ and its responsive gene PPAR γ-coactivator-1α (PGC1-α) and downregulation of TNF-α. However, dietary supplementation with n-3 PUFA alone or in combination with CLA resulted in an early onset of disease (i.e., day 2) and faster recovery on days 6 and 7, which correlated with a marked induction of the PPAR δ-responsive gene uncoupling protein 3 (UCP3). CLA and n-3 PUFA acted synergistically to upregulate colonic KGF

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expression in DSS-challenged pigs but n-3 PUFA blocked CLA-induced PPAR γ activation.
Conclusion: Dietary CLA-supplementation upregulated colonic PPAR γ expression and contributed to delaying the onset of experimental IBD, whereas n-3 PUFA failed to protect from IBD, although it accelerated colonic regeneration and clinical remission by activating PPAR δ.

Introduction

Inflammatory bowel disease (IBD) is a widespread and debilitating illness afflicting over 3.5 million people worldwide[1] characterized by an unrelenting destruction of the gut mucosa. In addition to some identified genetic factors such as NOD2/CARD15,[2] which function by activating nuclear factor-κB (NF-κB), the prevailing notion is that IBD is initiated and maintained by a defective downregulation of mucosal immunity.[3] Indirect evidence demonstrates the potential impact of nutrition in general and lipid nutrition in particular in modulating the course of IBD.[4–6] For instance, we previously investigated the ability of conjugated linoleic acid (CLA) to ameliorate IBD in a pig model of bacterial-induced colitis.[7] We found that CLA ameliorated intestinal lesion development, prevented growth suppression and maintained or induced PPAR γ expression while repressing IFN-γ expression in pigs experimentally inoculated with *Brachyspira hyodysenteriae*.[8,9] More recently we generated more definitive molecular evidence in vivo of the effects of CLA in IBD. Specifically, we have found that the beneficial effects of CLA in dextran sodium sulfate (DSS)-induced colitis were abrogated in tissue-specific PPAR γ null mice lacking PPAR γ in hematopoietic and epithelial cells.[10] In addition, dietary CLA modulated the expression of PPAR γ- and δ-responsive genes and suppressed NF-κB activation in colons of mice with DSS-induced colitis.[10] Thus, CLA prevents or ameliorates experimental IBD through a PPAR γ-dependent mechanism.

n-3 Polyunsaturated fatty acids (PUFA) [i.e., docosahexaenoic (DHA) and eicosapentaenoic (EPA)] elicit potent anti-inflammatory and immunoregulatory properties directly[11,12] or following transcellular processing that results in the generation of hydroxy-containing n-3 PUFA metabolites.[13] In similar action to dietary CLA, n-3 PUFA have been reported to ameliorate intestinal inflammation in animal models of IBD.[6] However, previous IBD preclinical studies examined the efficacy of doses over 4 g of n-3 PUFA per 100 g diet, which would be unattainable in a human clinical setting. Conversely, the effective dose of CLA for regulating inflammatory responses in a pre-clinical setting ranged from 0.5 to 1.33 g/100 g. Even though no CLA clinical studies in humans with IBD have been performed, the effective CLA dose for modulating systemic immune responses in humans ranges from 3 to 6 g/day.[14] We found that in contrast to CLA, dietary n-3 PUFA-supplementation at 1.33 g/100 g did not prevent DSS colitis in mice.[10] Even though n-3 PUFA may not be effective in preventing IBD, it remains unknown whether they would accelerate clinical remission and colonic regeneration following an episode of IBD.

Herein we will also investigate a possible novel mechanism by which dietary PUFA supplementation can favorably modulate tissue regeneration: by activating PPAR δ and inducing the expression of epithelial growth factors. PPAR δ is important for the rapid epithelialization of a skin wound by regulating survival, migration and differentiation of skin epithelial cells.[15] Thus, it is likely that activation of PPAR δ in the gut will also contribute to the maintenance of the epithelial barrier. Our previous studies did not investigate whether dietary supplementation with n-3 PUFA and/or CLA would accelerate recovery or whether CLA and n-3 PUFA would act synergistically or antagonically in the gut. The present study aims to investigate the ability of CLA and fish oil fed alone or in combination to modulate clinical activity and PPAR-responsive gene expression in healthy pigs and in pigs with DSS colitis.

Materials and methods

Dietary treatments

Sixty-four, 15-day-old, early weaned pigs averaging 6.9 ± 0.7 kg were randomly distributed from outcome groups based on litter, gender and body weight into 16 blocks of 4 contiguous individual pens. Four dietary treatments were randomly