Cyclooxygenase-2 (COX-2) promotes intestinal wound healing but elicits also proinflammatory effects and has been implicated in colorectal carcinogenesis. Thus, a balanced expression of COX-2 is essential for intestinal homeostasis. This study was designed to evaluate the regulation of COX-2 by probiotic organisms and to characterize ligands and receptors involved. Colo320 and SW480 intestinal epithelial cells (IEC) were stimulated with gastrin or TNF-α and pre- or coincubated with commensales, bacterial supernatants, or distinct toll-like receptor (TLR) ligands. COX-2 promoter activity was determined by luciferase assays, protein expression by Western blotting, and secretion of prostaglandin E2 (PGE2) by ELISA.

Commensales differentially regulated COX-2 expression in IEC. E. coli Nissle 1917, the probiotic mixture VSL#3, and media conditioned by these organisms ameliorated induced COX-2 expression and PGE2 secretion. Heat inactivation and DNase treatment significantly decreased these regulatory capacities. Lactobacillus acidophilus, however, significantly increased COX-2 expression and PGE2 secretion. TLR agonists differentially ameliorated basal or induced COX-2 expression. Distinct probiotics specifically and significantly decrease induced COX-2 expression in IEC, most likely mediated by released factors and in part by bacterial DNA. A significant involvement of TLRs in these regulatory processes remains to be established.

INTRODUCTION

Colorectal cancer (CRC) is the second leading cause of death in the United States. This malignancy is also one of the most serious complications of inflammatory bowel disease (IBD), in particular ulcerative colitis (UC) but also Crohn’s disease (1,2). However, predictive and protective factors have not been fully elucidated yet, and the subgroup of patients at high risk can not clearly be identified. Further characterization of risk as well as protective factors is needed. In this context, the precise mechanisms of the IBD-related carcinogenesis process are largely unclear, although it is generally assumed that IBD-related carcinogenesis occurs as a result of chronic inflammation (3). A growing body of evidence suggests that COX-2 activity and prostaglandin synthesis may be involved in the multistep process of intestinal carcinogenesis. More specifically, aberrant Cyclooxygenase-2 (COX-2) expression has been detected in premalignant adenomatous polyps and colorectal carcinomas (4,5) as well as in other epithelial-derived malignancies (6,7). Furthermore, COX-2 expression is associated with increased epithelial cell proliferation, decreased apoptosis, and increased cell invasiveness (8), whereas chronic inhibition of COX activity by nonsteroidal anti-inflammatory drugs has been associated with chemopreventative effects on colon cancer (9). Epidemiological studies have shown that prolonged use of aspirin is associated with a reduced risk of CRC (10). Consistent with these data, several non-steroidal anti-inflammatory drugs (NSAIDs), including COX-2 inhibitors, suppressed the development of chemically induced colon carcinomas in rats (11) and intestinal polyps in Min mice with a nonsense mutation of the Apc gene (12). Consequently, the identification of the pathways and regulatory elements that control COX-2 expression in general and in intestinal cells in particular is a subject of major interest. However, the precise mechanisms regulating COX-2 expression in intestinal epithelial cells has not been fully clarified yet (13), although several inducers such as cytokines, mitogens, and stresses have
been described (14,15). AP-1 (16) and the MAPK p38 (17) are the only regulatory elements identified in IEC so far and the role of endogenous cell-surface receptors remains to be determined. Since the continuous use of NSAIDs has been shown to have detrimental effects on the mucosal integrity, it seems essential to further characterize the physiological regulation of COX-2 expression, that is, by epithelial microbial interaction with its known importance in intestinal homeostasis. Probiotics are viable microbial food ingredients supposed to be beneficial through their effect in the intestinal tract, and anticarcinogenic-antimutagenic effects of these organisms have been reported in vivo (18). In fact, Bifidobacterium longum supplementation have reduced colon and liver carcinogenesis by 2-amino-3-methylimidazo[4,5-f]quinoline as well as azoxymethane (AOM)-induced colon cancer in rats (19). In addition, dietary supplements of Lactobacilli have increased the latency of onset of experimental colon cancer in rats (20). The current study was therefore designed to evaluate the regulation of COX-2 expression by the endogenous luminal flora. We report that specific probiotic strains used as model organisms significantly decreased cytokine and growth factor induced expression of COX-2. Furthermore, probiotic culture supernatants but not defined microbial patterns that have been recognized as specific TLR ligands had the same regulatory properties.

MATERIALS AND METHODS

Reagents

TNF-α was obtained from ImmunoK (Abingdon, UK) and used in concentrations ranging from 1 to 100 ng/ml. Gastrin was obtained from Bachem (Weil am Rhein, Germany) and used in concentrations of 10−4–10−8 mol/l. LPS and LTA were purchased from Sigma-Aldrich (Munich, Germany) and CpGs were generated by TIB MOLBIOL (Berlin, Germany).

Cell Lines and Bacterial Strains

The human colon cancer cell lines Colo320 and SW480 were purchased from the American Type Culture Collection (LGC-ATCC; Wesel, Germany) and cultured in RPMI supplemented with 10% (vol/vol) heat-inactivated fetal bovine serum (GIBCO, Karlsruhe, Germany) and 2 mM l-glutamine (Invitrogen, Karlruhe, Germany) and 2% sodium dodecyl sulfate (SDS). Cultured cells were washed and the medium replaced by antibiotic-free cell culture medium. Bacteria grown overnight in LB broth were pelleted by centrifugation, resuspended in sterile buffered saline (PBS), and added to the cells in a concentration of 1 × 106 CFU/ml.

Expression Vectors

COX-2 promoter-reporter plasmid pTIS-10L contains luciferase cDNA under the control of the human COX-2 promoter (~963 to +70). Cells were furthermore transiently transfected with full-length plasmids coding for TLRs 2 or 4 and the adaptor protein MD-2.

Transfection Procedures

Cells were transfected with the COX-2 promoter and 1 μg of pRL-null (Promega) to correct for transfection efficiency. Firefly luciferase activity was normalized to Renilla. Transient transfections were performed using Effectene (Qiagen). After incubation at 22°C for 10 min, the DNA/Effectene mixture was added to cells covered with 10 ml complete medium. Twenty-four hours after transfection, cells were starved for 24 h and then stimulated as indicated. Following the stimulation periods, cell lysates were prepared for assessment of luciferase activity in which the dual luciferase assay reporter system (DLR) was performed on a manual luminometer (Berthold Technologies GmbH & Co. KG, Bad Wildbad, Germany).

Protein Isolation and Western Blot Analysis

Cells were lysed and homogenized in ice-cold buffer containing protease inhibitors. Protein contents were determined using the Bradford method. Equal amounts of total protein were resolved by 10% sodium-dodecyl sulfate polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes (Bio-Rad, Munich, Germany). Membranes were incubated overnight at 4°C with 5% dry milk in PBS containing 0.1% Tween 20 (TBS-T) followed by incubation for 2 h at room temperature with a polyclonal goat anti-COX-2 antibody (1:1000; Santa Cruz Biotechnologies, Santa Cruz, CA). Following multiple washing steps in TBS-T and incubation with a secondary antibody (1:1500; Santa Cruz) specific bands were detected by use of the enhanced chemiluminescence Western blotting system (GE Healthcare, Munich, Germany).

Equal loading was confirmed by stripping the blots in 62.5 mM Tris-HCl, pH 6.8, and 2% sodium dodecyl sulfate.
FIG. 1A. Strain-specific effects of live probiotics on COX-2 promoter activity, protein expression, and PGE2 secretion in IEC. Colo320 (white bars) and SW480 cells (black bars) were incubated with $1 \times 10^6$ CFU of the indicated probiotic strain for 6 h. COX-2 promoter activity was determined by luciferase assay (part I). Protein expression of COX-2 was analyzed by Western blotting. Part II shows an original blot (lower panel; lane 1: unstimulated control; lane 2: E. coli Nissle; lane 3: VSL#3; lane 4: Lactobacillus acidophilus) and a densitometric evaluation of repeated experiments (upper panel). PGE2 secretion was determined by ELISA following exposure of IEC to microorganisms as indicated in part III. AU, arbitrary unit; kDa, kilo Dalton. **$P < 0.01$; *$P < 0.05$ (vs. unstimulated control).

containing 100 mM 2-ME ($\beta$-mercaptoethanol) at 50°C for 30 min and subsequent reprobing with anti-GAPDH (1:1000; Santa Cruz). For quantification (Bio-Rad, München, Germany), blots were scanned (Epson Perfection 1640SU-Photo scanner; Epson, Meerbusch, Germany).

Enzyme-Linked Immunoabsorbent Assay (ELISA)

For a PGE2 ELISA, $1 \times 10^6$ cells were plated and grown to subconfluency. Cells were then stimulated with gastrin or TNF-$\alpha$, TLR ligands, live bacteria, or filtered supernatants from bacterial cultures as indicated. Subsequently, IEC supernatants were harvested and centrifuged for 10 min at 1,500 rpm to pellet residual cells. The PGE2 ELISA (Assay Design, Hines Drive, MI) was performed following the manufacturer’s instructions. Samples were read at 450 nm using a 7520 microplate reader (Tecan, Crailsheim, Germany). To exclude deviations by differences in cell viability, trypan blue exclusion assays were performed.

Statistical Analysis

All experiments were performed at least as triplicates or quadruplicates. The results of replicates were averaged and expressed as mean ± SEM. Comparison between experimental groups were performed using the Student’s $t$-test or analysis of variance (ANOVA) as appropriate. Results were considered statistically significant for $P$ values less than 0.05.

RESULTS

Strain-Specific Effects of Living Probiotic Bacteria and Conditioned Media on COX-2 Expression and PGE2 Secretion

Incubation of Colo320 and SW480 cells with probiotic organisms had a strain-specific effect on COX-2 promoter activity and PGE2 secretion. Whereas incubation for 6 h with $1 \times 10^6$ CFU of E. coli Nissle 1917 or the probiotic mixture VSL#3 did not significantly alter the COX-2 promoter activity (Fig. 1A-I).
or COX-2 protein expression (Fig. 1A-II) nor had any effect on basal PGE2 secretion (Fig. 1A-III). Incubation with $1 \times 10^6$ CFU of *Lactobacillus acidophilus* significantly increased the activity of the COX-2 promoter in both cell lines, with a subsequent significant increase in COX-2 protein levels and PGE2 secretion (Fig. 1AI-III). These effects were dose and time dependent. Maximal effects were observed when $1 \times 10^6$ CFU/ml were applied to IEC for 6 h. Whereas lower concentration and shorter incubation periods resulted in a less pronounced effect, probiotic organisms in higher concentrations ($10^8$ CFU/ml or more) as well as longer incubation periods (8–24 h) were associated with a decrease in the observed effects (data not shown). Figure 1B shows the effect of the probiotic strains on COX-2 promoter activity (part I), protein expression (part II), and PGE2 secretion (part III) following 12 h of incubation. Comparably, the supernatants of *Lactobacillus acidophilus* appeared to down-regulate COX-2 promoter activity and protein expression, whereas supernatants of *Lactobacillus acidophilus* significantly increased the activity and expression of this enzyme.

### Effects of Probiotics on Cytokine and Gastrin-Induced Expression of COX-2

Following a 6 h incubation period, gastrin ($10^{-4}$ to $10^{-8}$ mol) increased the activity of the COX-2 promoter in IEC up to 8-fold (Fig. 2A). Furthermore, the proinflammatory cytokine TNF-α (10 ng/ml) increased the COX-2 promoter activity up to 3-fold (Fig. 2A). Alterations in the COX-2 promoter activity were followed by an increased expression of COX-2 protein (data not shown). Subsequently, as detected by use of a specific ELISA, PGE2 levels were increased accordingly up to 6-fold in culture supernatants (Fig. 2A).
FIG. 2A. Effects of probiotics on cytokine and gastrin induced expression of COX-2. Colo320 (white bars) and SW480 (black bars) cells were stimulated with gastrin (10^{-6} mol) or TNF-\(\alpha\) (10 ng/ml) for 6 h or the times indicated. COX-2 promoter activity was analyzed by measuring luciferase activity and prostaglandin E\(_2\) (PGE\(_2\) secretion by ELISA (A). AU, arbitrary units.

Following preincubation with \(1 \times 10^6\) CFU of the probiotic strains \textit{E. coli Nissle 1917} or VSL#3 for 6 h, the effects of gastrin and TNF-\(\alpha\) on COX-2 promoter activity and COX-2 protein expression were significantly reduced up to 50\% (Fig. 2B). Consequently, PGE\(_2\) secretion was also significantly decreased to levels detected in unstimulated cells (Fig. 2C). On the contrary, preincubation with \(1 \times 10^6\) CFU \textit{Lactobacillus acidophilus} did not reduce growth factor or cytokine-induced COX-2 promoter activity in Colo320 or SW480 cells but had an additive effect on COX-2 promoter activity and PGE\(_2\) secretion (Figs. 2B, 2C). Preincubation of IEC with supernatants from probiotic cultures had effects comparable to those observed following exposure to the living organisms. Whereas \textit{E. coli Nissle} and VSL#3 supernatants significantly decreased gastrin or TNF-\(\alpha\) induced COX-2 promoter activity, COX-2 protein expression as shown in Fig. 2D and PGE\(_2\) secretion, preincubation with supernatants of \textit{Lactobacillus acidophilus} cultures had no such effect.

Effects of Heat Inactivation and DNase Treatment on the Regulation of COX-2 Promoter Activity and PGE\(_2\) Secretion by Probiotic Culture Supernatants

Following incubation with living organisms and sterile filtered supernatants, the effects of heat killed probiotics and culture supernatants on cytokine and growth factor induced COX-2 expression were analyzed. As shown in Fig. 3A, heat inactivation of living organisms abolished previously observed effects on COX-2 promoter activity as well as on COX-2 protein expression (data not shown) and PGE\(_2\) secretion (data not shown). Comparable effects were observed when cultures were treated with heat inactivated conditioned media (data not shown). Furthermore, also DNase treatment of culture supernatants had a similar effect as heat inactivation, although the decrease in induced COX-2 promoter activity, COX-2 protein expression, or PGE\(_2\) secretion was not as pronounced (Fig. 3B). Of note, heat inactivation abolished the COX-2 stimulatory effects of supernatants of \textit{Lactobacillus acidophilus} cultures, whereas effects were conserved following DNase treatment of these supernatants.

Role of TLR Ligands and TLRs in the Regulation of COX-2 Expression

To further characterize putative factors involved in the regulation of COX-2 expression in IEC, we tested the effect of specific TLR ligands (LTA: 5–20 \(\mu\)g/ml; LPS: 1–10 \(\mu\)g/ml; CpG: 1–5 \(\mu\)mol) on basal and induced (gastrin and TNF-\(\alpha\)) COX-2 promoter activity and PGE\(_2\) secretion in Colo320 and SW480 cells. Initially, cells were tested for the expression of TLRs and adaptor proteins. Subsequently, cells were transfected with plasmids spanning the entire coding region of TLR2, TLR4, or MD-2 to rule out ineffectiveness of the stimulation due to low or absent expression of individual TLR receptors. In agreement with previously published results, LPS significantly increased COX-2 promoter activity in IEC, whereas stimulation with LTA and CpG did not result in a significant alteration of COX-2 expression.
FIG. 2B. Colo320 cells were preincubated with $1 \times 10^6$ CFU of *E. coli Nissle 1917*, the probiotic mixture VSL#3, or *Lactobacillus acidophilus* for 6 h and then stimulated with gastrin ($10^{-6}$ mol) or TNF-α (10 ng/ml) for an additional 6 h. Again, promoter activity was analyzed by measuring luciferase activity (part I). Furthermore, effects on COX-2 protein expression were determined by Western blotting (part II). The lower panel shows a representative blot and the upper panel the densitometric evaluation of repeated analysis. Lane 1: unstimulated control; lane 2: gastrin stimulated cells; lane 3: TNF-α stimulated cells; lane 4: preincubation with *Lactobacillus acidophilus*; lane 5: preincubation with *E. coli Nissle*; lane 6: preincubation with VSL#3. AU, arbitrary units; kDa, kilo Dalton.

promoter activity (Fig. 4A) or PGE2 secretion (data not shown) in Colo320 or SW480. Furthermore, a combination of various TLR agonists had no additive effect on COX-2 promoter activity nor on PGE2 secretion (data not shown). Furthermore, preincubation for 12 h or coincubation for 6 h (data not shown) with a distinct TLR ligand did not decrease gastrin or TNF-α induced COX-2 promoter activity (Fig. 4B) or PGE2 secretion (data not shown). CpG treatment revealed a trend toward decreased activity of the promoter or secretion of PGE2 without reaching the level of significance.

FIG. 2C. Effects of preincubation with probiotic organisms on gastrin induced PGE2 secretion as determined by ELISA. ** $P < 0.01$ (vs. unstimulated control).
DISCUSSION

Of the two COX isozymes (21), COX-1 is expressed constitutively in many cell types including endothelial cells, platelets, and intestinal epithelial cells (15) and produces prostaglandins, which serve to maintain cellular homeostasis. Although COX-2 expression has been attributed to some extent with mucosal healing, COX-2 has also proinflammatory capacities, and abnormally high levels of this enzyme as detected in IBD patients (22) may be pathogenic during intestinal inflammation (23). It is well known that chronic inflammation is associated with cancerogenesis, and it has been demonstrated that COX-2 is induced during tumorigenesis by various stimuli including cytokines and growth factors (24) such as gastrin. Gastrin has been shown to induce COX-2 in human gastric and colorectal cancer cell lines partly by release of the chemokine IL-8 (25–28). Furthermore, we and others have previously shown that so called probiotics might induce IL-8 (29,30). In agreement with these results, we detected a significantly upregulated COX-2 promoter activity and PGE2 secretion following stimulation of colonic epithelial cells with gastrin or the proinflammatory cytokine TNF-α. The tight control of COX-2 expression might therefore be an important therapeutic goal not only in the prevention of inflammatory lesion but also in neoplastic transformation. Interestingly, COX-2 selective inhibitors reverse the trophic properties of gastrin in colorectal cancer (31–33). However, pharmacological regulation of COX-2 with, for example, NSAR is potentially associated with deleterious side effects in the intestine; and COX-selective inhibitors have been associated with cardio- and cerebrovascular disease (34). Therefore, we sought to determine whether the use of so called probiotic organisms might represent an alternative approach in the reversion of dysregulated COX-2 activity in the

FIG. 3A. Effects of heat inactivation and DNase treatment of probiotics culture supernatants on COX-2 promoter activation. A: Probiotics culture supernatants were heat inactivated or B: treated with DNase as indicated in the Material and Methods section. Inactivated conditioned media were subsequently applied to Colo320 and SW480 cultures for 12 h. IEC remained either untreated or were stimulated with gastrin (10^{-6} mol) or TNF-α (10 ng/ml) for another 6 h. Thereafter, COX-2 promoter activity was determined as described. Controls remained unstimulated. Shown are results of \( n = 4 \) independent experiments obtained with Colo320 cells. \( * P < 0.05 \) vs. gastrin or TNF-α stimulated cultures. ns, nonsignificant; AU, arbitrary units. (Continued on next page)
Application of probiotics aims at the preservation and reestablishment of intestinal homeostasis with known balanced activity of the COX enzymes.

Analysis were performed with microbial *E. coli* and *Lactobacillus* and organisms of the probiotic mixture VSL#3 that had previously been reported to have health supporting properties (35–38). However, we detected strain-specific effects on the COX-2 expression. Whereas incubation with the Gram-negative organism *E. coli Nissle* and with organisms of the probiotic mixture VSL#3 had no effect on basal COX-2 expression and decreased stimulated COX-2 activity, protein expression and PGE2 secretion, *Lactobacillus acidophilus*, significantly increased expression and activity of this enzyme. No results have so far been reported on the potential effect of *E. coli Nissle* and the probiotic mixture VLS#3 on the expression of COX-2. The probiotic mixture VSL#3 contains 4 strains of *Lactobacilli*. No data are available on the putative effect of *Lactobacillus bulgaricus* and *Lactobacillus plantarum* on COX-2 expression or PGE2 secretion. *Lactobacillus acidophilus*, however, has previously been shown to slightly upregulate COX-2 expression in another IEC culture system (39), whereas this strain has been reported to downregulate *Helicobacter pylori* induced COX-2

FIG. 3B. (Continued)

FIG. 4A. Role of TLR ligands and TLRs in the regulation of COX-2 expression. Colo320 (white bars) and SW480 (black bars) cells were transiently transfected with plasmids coding for TLR2, TLR4, and MD-2 and stimulated with specific ligands for TLR2 (LTA: 10 µg/ml), TLR4 (LPS: 2 µg/ml), or TLR9 (CpG: 1 mmol) as indicated (A). The effect on basal and induced COX-2 promoter activity was determined by luciferase assays. Results are means SEM of 6 independent experiments. ** P < 0.01. AU, arbitrary units.
expression in the gastric epithelium of Mongolian gerbils (40). However, in this in vivo model, a combination of two Lactobacilli was used with no further differentiation of the specific effects of the individual Lactobacilli. Furthermore, Lactobacillus casei has been shown to downregulate COX-2 expression in a rodent trinitrobenzenesulphonic acid (TNBS) colitis model (41). Observations made by use of a probiotic strain in a defined model system are always limited in respect of possible conclusions. Results obtained in this study with the probiotic mixture VSL#3 are furthermore limited because the contribution of individual strains could not be assessed.

We report that in particular preincubation of IEC with E. coli Nissle, VSL#3, or incubation with cultured supernatants conditioned by these probiotics significantly decreased COX-2 expression induced by gastrin or the proinflammatory cytokine TNF-α. Interestingly, heat-killed microorganisms or heat inactivated culture supernatants had not such an effect, and also DNase treatment significantly decreased the regulatory potential. Beneficial effects of probiotics have been attributed to factors released into culture supernatants in previous studies (30,37). Most of these studies have identified a heat sensitivity of these factors but failed to identify a distinct factor; and just recently, two proteins that promote intestinal homeostasis have been purified from Lactobacillus rhamnosus GG (37). Furthermore, metabolic factors from probiotic organisms have been suggested to mediate at least in part the observed health promoting effects; for example, butyrate and propionate have been shown to downregulate COX-2 expression in a Caco-2 cell culture model (39). In further search of microbial factors that might be associated with the regulation of COX-2 expression, we analyzed the effects of TLR ligands on basal and induced COX-2 expression. Specific bacterial CpG motifs had previously been shown to alleviate intestinal inflammation via activation of the Toll-like receptor (TLR) 9 (42,43). Furthermore, activation of TLR2 by its synthetic ligand Pam(3)Cys-SK4 has been demonstrated to significantly suppress mucosal inflammation and apoptosis by efficiently restoring the tight junction associated integrity of the intestinal epithelium, thereby stabilizing the intestinal barrier function (44). In agreement with a previous report, the TLR4 ligand LPS induced COX-2 promoter activity and PGE₂ secretion from IEC, whereas no such effect was observed upon stimulation with the TLR2 ligand LTA (45). Interestingly, bacterial DNA motifs seem to downregulate induced COX-2 promoter activity and PGE₂ secretion. In contrast, the transcription of COX-2 in macrophages has previously been shown to be induced by CpG via the TLR9/MyD88, with p38 being a further downstream signaling element (46). Although, for example, SW480 cells have been shown to constitutively express TLR4 as well as TLR associated adaptor molecules and to be clearly responsive to LPS (30,47), cells utilized in this study were transiently transfected with plasmids coding for full length TLR2, TLR4, and MD-2 in order to rule out ineffective regulation of induced COX-2 expression via the TLRs due to low expression levels of these receptors. However, also following overexpression of the pattern recognition receptors, no significant regulation of induced COX-2 expression was observed, making a major contribution of TLRs in the observed regulatory effect unlikely. Concluding from these observations, the regulation of induced
COX-2 expression is more likely mediated by factors other than a distinct TLR ligand such as metabolic products released by living probiotic bacteria as it has been previously reported. Results from this study suggest, furthermore, that the putative factor is heat sensitive because the regulatory potential was abolished following heat treatment. Heat-sensitive factors have previously been associated with probiotics mediated regulation of cytokine expression in IEC. However, a specific factor has so far not been identified.

This has also been suggested in a previous report on the effect of probiotics on the basal expression of COX enzymes in Caco-2 cells (39) identifying butyrate and propionate as essential metabolic products in the regulation of COX-1 and COX-2 expression. Just recently the first two bacterial proteins with beneficial effects on intestinal homeostasis have been purified from Lactobacillus rhamnosus GG (37). However, significantly different capacities in the regulation of cytokine production have been described not only for different probiotics strains but also for sub species of these probiotics strains. Therefore, individual organisms should be carefully evaluated for their therapeutic capacity. Furthermore, it obviously needs to be confirmed whether the observed effects are unique to the cell lines used or are also characteristic for an in vivo situation.

In summary, given the pathophysiological role of abnormally high levels of COX-2 and its putative involvement in intestinal inflammation and carcinogenesis, specific probiotics strains might be considered as a therapeutic alternative in the regulation of this enzyme in the intestinal epithelium. Furthermore, a combination of a standard NSAR therapy with probiotic organisms might decrease the dose of the NSAR needed, and the probiotic organisms might potentially alleviate the side effects of a NSAR therapy. Although data from this study provide further insight into the interaction of the intestinal flora and intestinal epithelial cells, additional characterization of suitable organisms and mechanisms involved is needed before such treatment can be considered for broad clinical application.

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