PRELIMINARY REPORT

Effects of Short-Chain Fatty Acids on Human Rectosigmoid Mucosal Colonocyte Brush-Border Enzymes

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Short-chain fatty acids produced by bacterial fermentation of dietary fiber may provide a tonic stimulus to colonocyte differentiation that contributes to the protective effect of fiber against colorectal malignancy. Since brush-border enzymes are common markers of colonocytic differentiation, we compared the effects of equimolar (10 mmol/L) concentrations of the three most common short-chain fatty acids, acetate, butyrate, and propionate, on the alkaline phosphatase and dipeptidyl dipeptidase specific activity of human colonic mucosal biopsies obtained from normal volunteers. Only butyrate significantly stimulated alkaline phosphatase specific activity (50.4% ± 18.6%, $P < .05$). Short-chain fatty acid stimulation of dipeptidyl dipeptidase did not achieve statistical significance. Fibers yielding high colonic butyrate levels could have different effects on human colonic mucosal differentiation.

HIGH-FIBER DIETS reduce the risk of colon cancer both in animal models and in humans.[1,2] One potential mechanism for this effect is the fermentation of dietary fiber to short-chain fatty acids within the colonic lumen, where total concentrations may exceed 100 mmol/L.[3] Studies in established colonocyte lines demonstrate that one short-chain fatty acid, butyrate, stimulates colonocytic differentiation and inhibits proliferation and motility.[4-10]

Although the colonic lumenal ratio of acetate to propionate to butyrate is approximately 6:3:1, the production and ratio of short-chain fatty acids within the colon is complex and depends critically on the colonic microbial population, which may contain microbes preferentially metabolizing to butyrate or propionate or acetate, as well as the location in the colon from which samples are taken and the resistance of the fiber to fermentation. Readily fermentable fibers such as oat bran are rapidly fermented in the right colon, while more resistant fibers such as wheat germ can increase short-chain fatty acid levels in the left colon, as well.[11-13]

However, it is clear that various fibers exert different effects on the colonic mucosa,[14-18] while short-chain fatty acids modulate proliferation and differentiation with different potency in established cell lines.[4,6,19] The differential potency of short-chain fatty acids in primary human colonocytes is less clear. We therefore treated normal human colonic mucosa ex vivo with equimolar acetate, butyrate, and propionate, and assayed alkaline phosphatase, a marker of colonocytic differentiation,[4,8] as well as dipeptidyl dipeptidase, expressed by colonocytes but more characteristic of enterocytic differentiation.[20,21]

SUBJECTS AND METHODS

Seven asymptomatic men underwent screening colonoscopy to exclude colonic malignancy. In each case, eight 5-mm rectosigmoid mucosal biopsies were washed in 37°C oxygenated L15 medium, and two biopsies each were incubated for 6 hours in sterile 37°C oxygenated L15 medium with 100 U/mL penicillin G and 0.1 mg/mL streptomycin or medium additionally supplemented with 10 mmol/L acetate, butyrate, or propionate.

Each tissue was lysed on ice in Dulbecco's phosphate-buffered saline containing 0.5% Triton X-100 and 0.35 mol/L NaCl and assayed for protein (Bicinchoninic Acid [BCA]; Pierce, Rockford, IL). Alkaline phosphatase and dipeptidyl dipeptidase were assayed in triplicate by synthetic substrate digestion and spectrophotometric quantitation of the reaction product.[4] Enzyme activity was calculated by interpolation against simultaneously assayed standard curves, and specific activity was calculated as the ratio of activity to protein in each sample. Data from duplicate tissues were averaged and expressed as a percentage of control values (unsupplemented samples) before statistical analysis by unpaired t test.

RESULTS AND DISCUSSION

Seven men were studied (mean age, 68.7 ± 3.3 years). Butyrate significantly increased alkaline phosphatase activity (50.4% ± 18.6%, $P < .05$), while neither acetate nor propionate achieved a statistically significant effect (Fig 1). All short-chain fatty acids tended to increase dipeptidyl dipeptidase by about 6%, but this was not statistically significant (Fig 1). The concentrations of short-chain fatty acids used are actually slightly lower than those found in the colonic lumen in vivo,[3] and were chosen both for consistency with previous cell culture studies[4] and to approximate the level of supplementation or alteration likely to be engendered by a change in dietary fiber composition.[11,15-18]

Alkaline phosphatase is frequently used in studies of colonocytic differentiation. Colonocytic mucosal alkaline phosphatase specific activity is one to two orders of magnitude higher than that of alkaline phosphatase in the small bowel mucosa.[22,23] Indeed, stimulation of alkaline phosphatase by butyrate has previously been described in established colonic cell lines.[4,8,24] By contrast, dipeptidyl dipeptidase, although expressed by colonocytes, is more typical of an enterocytic phenotype.[20,21] The more substantial stimulation of alkaline phosphatase observed here may therefore be consistent with butyrate promotion of a differentiation marker more characteristic of colonocytes. However, the level of alkaline phosphatase is also higher in the colon during the developmental period, so these results leave open the question of whether butyrate is inducing a

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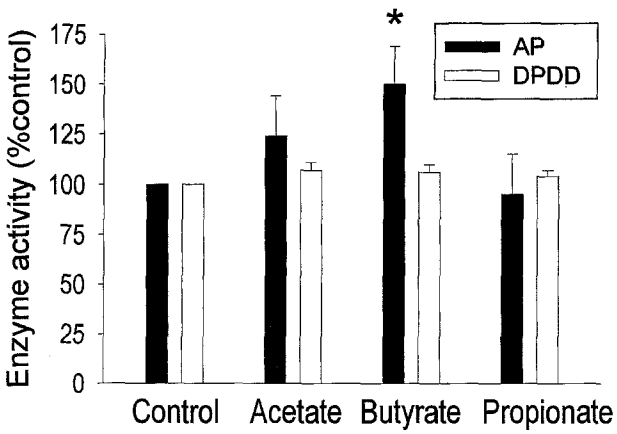

Fig 1. Effect of 10 mmol/L acetate, butyrate, and propionate supplementation on colonic mucosal alkaline phosphatase (AP) and dipeptidyl dipeptidase (DPDD) as compared with control mucosa maintained in warmed oxygenated L15 medium without short-chain fatty acids.

phenotype characteristic of the mature villus tip cell or of the fetal colonocyte.

The present study found a 50% increase in alkaline phosphatase with butyrate treatment. Although some in vitro studies have demonstrated much higher butyrate responses,[6,8] these have largely been in poorly or moderately well-differentiated cell lines in which basal alkaline phosphatase levels are low. The present results are more consistent with the results we previously observed using the same alkaline phosphatase assay in differentiated human Caco-2 cells.[4] In addition, although such cell-culture studies have generally involved treatment for 24 hours or more, the present study used only a short 6-hour treatment to maintain colonocytic viability. Longer treatment could have stimulated alkaline phosphatase more substantially. Furthermore, from a functional point of view, alkaline phosphatase is a catalytic enzyme, so a 50% increase in activity could be consistent with an effect on cell biology.

Although differential effects of various fibers have been described in animals and effects of various short-chain fatty acids have been explored in cell culture, these studies, to our knowledge, represent the first report of the effects of short-chain fatty acids on brush-border enzyme activity in human colonic mucosal biopsies ex vivo. These data suggest that the most common short-chain fatty acids are not equipotent in promoting at least one colonocytic differentiation marker in normal human colonic mucosa. The differences between ex vivo brush-border enzyme expression and in vivo differentiation are multiple and obvious. Furthermore, prolonged treatment could yield different results. Nevertheless, this model suggests that variations in the proportion of butyrate produced with colonic fermentation could contribute to differences in the effects of fiber on colonocytes in vivo.

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