Microbial Degradation Products Influence Colon Cancer Risk: the Butyrate Controversy1,2

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ABSTRACT All dietary fiber, by definition, escapes digestion in the small intestine and thus arrives relatively intact in the large intestine. Its fate in the large intestine depends upon the type of fiber and the colonic microflora. Highly fermentable fibers result in short chain fatty acids including butyrate, which is thought by some to be protective against colon cancer. However, not all studies support a chemopreventive effect for butyrate and the lack of agreement (particularly between in vivo and in vitro studies) on butyrate and colon cancer has been termed the “butyrate paradox.” There are a number of reasons for this discrepant effect including differences between the in vitro and in vivo environments, the timing of butyrate administration, the amount of butyrate administered, the source of butyrate (usually dietary fiber) as a potential confounder, and an interaction with dietary fat. Collectively, the studies suggest that the chemopreventive benefits of butyrate depend in part on amount, time of exposure with respect to the tumorigenic process, and the type of fat in the diet. J. Nutr. 134: 479–482, 2004.

KEY WORDS: • colon • butyrate • dietary fiber • omega 3 fatty acids

A recent National Academy of Sciences report defines dietary fiber as nondigestible carbohydrates and lignin that are intrinsic and intact in plants (1). This means that by definition, nearly 100% of dietary fiber reaches the colon. The fate of fiber in the colon depends primarily on the colonic microflora and the physico-chemical characteristics of the fiber itself. Fiber sources such as oat bran, pectin, and guar are highly fermented, whereas others such as cellulose and wheat bran may be poorly fermented (2–4). In general, foods rich in hemicelluloses and pectins, such as fruits and vegetables, contain fiber that is more completely fermented than foods rich in celluloses, such as cereals (2–4). The products of fiber fermentation include CO2, methane, other gases and short chain fatty acids (SCFA), the major anions in colonic contents. Butyrate, an SCFA fiber fermentation product, is thought to be chemopreventive by some, but not all studies show this beneficial effect against colon cancer development. This paper deals with potential reasons for the seemingly paradoxical effects of butyrate on colon physiology and colon carcinogenesis.

Fermentation of fiber in the colon and its significance to colon cancer

There is some debate in the literature as to whether the fermentability of a fiber plays a role in its protection/promotion of colon carcinogenesis (5,6). The poorly fermented fibers are good in vivo dilutors and may decrease the concentration of carcinogens, pro-carcinogens and tumor promoters in the fecal stream, thus reducing access of these substances to the colonic mucosa (7). Cummings performed a meta-analysis on ~100 studies of the effect of fiber on stool weight (8). The greater the stool weight, the better the fiber is at diluting fecal constituents. He showed a wide range of the contribution of dietary fiber to fecal weight (e.g., an increase of 5.7 g fecal bulk per g of wheat bran fed compared to 1.3 g/g of pectin in the diet). Also, in general, poorly fermented fibers may accelerate passage of the stool through the colon compared to highly fermented fibers (9,10), again affording less access of fecal constituents to the colonic mucosa. In contrast, highly fermented fibers produce greater amounts of SCFA than do fibers that are less fermented. Butyrate, an SCFA, is considered chemopreventive by some investigators. Others, however, have noted certain discrepancies in the literature as to the protective nature of butyrate against colon cancer (5,6). This paper discusses specific reasons as to why studies may differ as to whether or not butyrate is chemopreventive.
Evidence (pro and con) that butyrate (a fiber fermentation product) may protect against colon cancer

Butyrate has been documented by Roediger to be the primary aerobic fuel for colon cells (11). Butyrate has long been documented to decrease the growth of most human colon cancer cell lines by inhibiting cell proliferation and enhancing differentiation and apoptosis (12,13). Although some consider butyrate production to be a potential mechanism by which dietary fiber may protect against colon carcinogenesis, this hypothesis remains the subject of debate, due in part to apparent inconsistencies in the literature (5,6). For example, the first animal study to test the effects of butyrate against the development of experimentally-induced colon cancer found that administration of butyrate in the drinking water of rats actually increased the percentage of rats with colon tumors (85% of the rats consuming 1% butyrate had tumors compared to 50% of the rats with no added butyrate in the drinking water) (14). In a study designed to test two fibers with very different degrees of fermentability (i.e., oat bran that is highly fermentable compared to poorly fermented wheat bran) with respect to their protection/promotion of experimentally-induced colon cancer, we found that oat bran resulted in significantly higher (P < 0.001) levels of butyrate in both the proximal and distal colon compared to wheat bran (15). However oat bran also resulted in a greater number of colon tumors than did wheat bran (52% of rats fed oat bran had tumors compared to 27% of rats fed wheat bran; P < 0.021) (15). In an effort to separate out the effects of butyrate itself from the fiber substrate for butyrate production, Deschner et al. provided tributyryl in the diet of mice and found no protective effect of butyrate administration against colon tumors (16). Caderni et al. supplied butyrate to the colon in the form of slow-release pellets. Although they did show a higher amount of butyrate in rat colons in which the pellets had been provided, there was no benefit of butyrate administration with respect to aberrant crypt formation (17) (a precursor to colon tumors). A later study from the same laboratory tested the effect of the slow release butyrate pellets on azoxymethane-induced colon tumors in rats and found no protective effect of butyrate administration (18).

Reasons for the equivocal effects of butyrate

In vitro vs. in vivo experiments. One important reason why the effects of butyrate may be different when tested in vitro vs. in vivo is the very different conditions that exist in these two systems. For example, in vivo, colon epithelial cells are arranged in patterns called crypts and cell/cell communication is key to an individual cell's survival. Colon epithelial cells are born toward the bottom of the crypt and migrate up a crypt column where they may undergo several in transit divisions. In general, cells cease dividing 2/3 of the way up the crypt column and become fully differentiated. Toward the top of the crypt they may undergo apoptosis and be exfoliated in the fecal stream. In contrast, this pattern is not preserved in cell culture, which is one reason why it has been so difficult to maintain primary cultures of normal colon epithelial cells. Other potential "artifacts" of cell culture conditions include the maintenance of a relatively stable pH in a buffered system. Again, in vivo, the pH of the colon can vary greatly depending upon SCFA production, which lowers luminal pH (19,20). Because of the inability to maintain "normal" colon cells in culture, most in vitro experiments with butyrate are done in transformed cells. Since signal transduction processes may be different in transformed cells compared to their nontransformed counterparts, it is not surprising that a nutrient such as butyrate could have one effect in an in vitro system and a different effect in vivo.

Timing with respect to tumor development. The effect of butyrate on colon carcinogenesis may also depend upon the timing of butyrate administration in relationship to the stage of cancer development. For example, butyrate is a known inhibitor of histone deacetylase (21,22), which means that in its presence DNA would be in a more open or "vulnerable" form. This might be optimal if DNA damage had occurred and repair enzymes were necessary to approach the damaged DNA. Alternatively, in the presence of a carcinogen, a more open configuration of DNA would not be optimal. It is interesting to note the reports from the major prospective cohort studies on whether or not fiber is protective against colon carcinogenesis. Two of the major prospective studies, such as the Nurses' Health Study and the Health Professionals Followup Study, initially reported a protective effect of fiber against adenoma development (23,24), but later showed no such protection against colon cancer (25,26). Also, the three major clinical intervention trials, all of which used polyp recurrence as the endpoint, failed to show a protective effect against this surrogate marker for colon cancer (27–29). Some have suggested that fiber may be protective against the early stages of polyf formation, but not at the stage of transition of a polyp to a carcinoma. All of the individuals in the three clinical intervention trials already had adenomatous polyps.

Amount of butyrate. It is entirely possible that different concentrations of butyrate may result in very different physiological effects. For example, several laboratories have shown that low amounts of butyrate may stimulate cell proliferation while high amounts may inhibit it (30). In one report (30) colonic smooth muscle cells in primary culture were exposed to different molarities of butyrate. A low concentration of butyrate significantly stimulated cell proliferation whereas at higher levels of butyrate an inhibition of cell proliferation was observed. We (31) and others (32) have shown that there is a plateau for butyrate oxidation to CO₂. Once that plateau has been reached, higher concentrations of butyrate are redirected to ketone body production, lipid synthesis, and other synthetic events (31). This may account for the seemingly paradoxical effects of butyrate at different molarities. Up to the plateau for butyrate oxidation, addition of butyrate may stimulate cell proliferation as the cell reaches its maximum energy level. Above that level, there may not be an increase in cell proliferation as butyrate will be redirected to other pathways.

Fiber as a confounder. A number of studies ostensibly test the effect of butyrate on colon cancer development using dietary fiber as their source for butyrate production. However, the fiber source per se may have independent effects on colon tumorigenesis that are different from the effects of butyrate. Since signal transduction processes may be different in transformed cells compared to their nontransformed counterparts, it is not surprising that a nutrient such as butyrate could have one effect in an in vitro system and a different effect in vivo.

Interaction with dietary fat. A number of years ago we hypothesized that a diet containing fish oil and cellulose would be more protective against experimentally-induced colon cancer than one containing corn oil and pectin. Our rationale was that fish oil is high in omega 3 fatty acids, which a number of laboratories had shown were chemopreventive. and cellulose is an excellent bulking agent and thus would be a good in vivo dilutor of carcinogens and promoters such as bile acids. To our surprise, the most protective diet ended up being the combination of fish oil and pectin (a highly fermentable fiber) (33,34). Since that finding we have explored possible mechan-
In contrast, the percentage of cells in the crypt undergoing apoptosis decreases. As the amount of DNA adducts (O6 methyl-guanine) increases in the corn oil group, the apoptotic response (percentage of cells in the crypt undergoing apoptosis) decreases. In contrast, in the fish oil group as DNA damage or adduct level increases the apoptotic response increases. We have interpreted these data to mean that fish oil feeding enhances apoptotic removal of DNA damaged cells at the initiation stage of colon tumorigenesis. A similar effect of fish oil on apoptotic removal of colon cells is observed during the promotion stage of the tumorigenic process. Again, the combination of fish oil and pectin results in a greater number of apoptotic cells/crypt column throughout this promotion stage. We have two hypotheses as to how this particular combination of fish oil and pectin works together to upregulate colonic apoptosis. One involves inactivation of Cox-2, and the other concerns upregulation of apoptosis via a reactive oxygen species mechanism.

Mechanisms by which the combination of fish oil and pectin (a high butyrate producer) may protect against colon tumorigenesis. Cox-2 is inducibly expressed in tumor tissue. For example Cox-2 mRNA levels are markedly increased in 86% of human colorectal cancers. In turn, Cox-2 expression has been shown to downregulate apoptosis. When Tsuji and DuBois overexpressed Cox-2 in rat intestinal epithelial cells, those cells were resistant to butyrate-induced apoptosis. We have shown that Cox-2 expression is downregulated with fish oil feeding in rat colon cells compared to providing corn oil in the diet. A second mechanism by which the combination of fish oil and pectin may initiate apoptosis may involve production of reactive oxygen species. The mitochondria are considered the central executioners of apoptosis. We hypothesized that the fatty acids from fish oil would incorporate into the mitochondrial membrane and because they are highly unsaturated could be targets for reactive oxygen species generated as a normal part of the electron transport process. This in turn could trigger additional ROS leading to a decrease in mitochondrial membrane potential, release of cytochrome C into the cytosol and activation of caspase 3, initiating apoptosis. To test this hypothesis rats were provided with diets high in fish oil or corn oil (15% by weight), and measurements made of the fatty acid composition of mitochondrial phospholipids, reactive oxygen species, translocation of cytochrome C, and activation of caspase 3. We report that fatty acids from the fish oil diet did incorporate into mitochondrial phospholipids compared to corn oil, the unsaturation index; in the presence of butyrate, cytochrome C translocated to the mitochondria, and caspase 3 activity was upregulated. Interestingly, the mechanism appears to be an increase in reactive oxygen species with fish oil feeding. Recently we separated the effects of butyrate production from the fiber source by providing rats with slow-release butyrate pellets and evaluating their effect on aberrant crypt formation. In the absence of butyrate administration, corn oil and fish oil feeding resulted in similar numbers of high multiplicity aberrant crypts/rat. In stark contrast, when butyrate was administered there were 5 times as many aberrant crypts/rat in the corn oil group compared to rats receiving fish oil (P < 0.0006). This finding lends further support to our previous studies showing that the type of fat determines the efficacy of the fiber.

In summary, there is little doubt that the colonic microflora exert an effect on colon tumor development either directly or indirectly through their fermentation products, one of which is butyrate. However, the exact mechanism behind these physiological effects of butyrate, the timing of the effects, and the differential response at different levels of butyrate still remain to be determined.

LITERATURE CITED

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