Influences of lactic acid, succinic acid and ammonia on epithelial cell proliferation and motility of the large bowel

Takashi Sakata[1] PhD, Hirofumi Ichikawa[2] MD, PhD and Akiko Inagaki[1] MSc

[1]*Department of Basic Sciences, Ishinomaki Senshu University, Ishinomaki, Japan*
[2]*Department of Surgery II, Tohoku University School of Medicine, Sendai, Japan*

Microbial breakdown of carbohydrates in the hindgut mainly produces short-chain fatty acids; however, it sometimes leads to the accumulation of lactic or succinic acid. Ammonia is a major product of the bacterial breakdown of nitrogenous substances in the large intestine. This review discusses the conditions leading to the production of lactic and succinic acids and the interaction between the metabolism of carbohydrates and that of nitrogenous compounds by hindgut bacteria. We briefly reviewed the absorption of lactic and succinic acids and of ammonia, and the contribution of lactic and succinic acids in the regulation of lumen pH. The influence of these metabolites on gut functions is different from that of short-chain fatty acids. Lactic acid and ammonia stimulate epithelial cell proliferation of the large bowel *in vivo* without increasing the epithelial cell number. Succinic acid inhibits the colonic epithelial cell proliferation in vivo and *in vitro*. Infusion studies *in vivo* indicated that both succinic acid and low pH reduced the hindgut motility, while short-chain fatty acids were necessary to maintain the normal motility. This suggests the importance of avoiding a pH level in the hindgut lumen that is too low, possibly by regulating the entrance rate of carbohydrates into the large intestine.

Key words: lactic acid, succinic acid, fatty acid, ammonia, cell division, intestine, pH.

Introduction

Microbial breakdown of carbohydrates in the large bowel mainly produces short-chain fatty acids.[1] Short-chain fatty acids influence various digestive functions.[2] However, information concerning the influences of other bacterial metabolites such as lactic acid, succinic acid and ammonia on functions of the digestive organs is scarce, in spite of the presence of these metabolites at concentrations similar to those of short-chain fatty acids under certain circumstances. This paper introduces recent information regarding the influence of these substances on epithelial cell division and motility of the large bowel.

Conditions leading to the production of lactic and succinic acids

Clinical conditions

There are people who have had their small bowel either partly or totally removed. Their faeces often contain a high concentration of lactic acid.[3] Accumulation of lactic acid in the large bowel sometimes leads to lactic acidosis in such subjects.[3] Succinic acid sometimes accumulates in the large bowel of patients suffering from ulcerative colitis.[4,5]

Substrates

Feeding of certain types of indigestible saccharides leads to the accumulation of lactic or succinic acid. We recently found that feeding a diet containing fructo-oligosaccharides (7.5% in a purified diet, w/w AIN 76) resulted in the accumulation of lactic acid in the cecal lumen of rats (Inagaki and Sakata, unpubl. data, 1999). The concentration of lactic acid (approximately 140 mmol/L) exceeded by far that of short-chain fatty acids (approximately 40 mmol/L in total) 5–11 h after the onset of feeding.

We found that meal-feeding rats a diet containing xylosylfructoside (3-hour feeding period, two times per day) or ileal infusion (three times per day) of this succharide resulted in the accumulation of succinic acid in the rat cecum, while the feeding of a diet containing the equivalent amount of either sucrose or galactosylsucrose did not result in the accumulation of succinic acid.[6]

A diet containing 10% galactosyloligosaccharide fed *ad libitum* increased cecal concentrations of lactic and succinic acids in ex-germfree rats innoculated with human fecal flora.[7]

Feeding of partially hydrolysed guar gum or its ileal infusion (three times per day) resulted in the accumulation of lactic acid in the rat cecum.[8]

A high concentration of succinic acid was observed in rats fed a diet containing resistant starch prepared from amylomaize.[9] Simultaneous feeding of protein resistant to autoenzymic digestion abolished the accumulation of succinic acid.[9]

Lumen pH

Low lumen pH in the hindgut seems to lead to the microbial production of lactic and succinic acids instead of short-chain fatty acids. Batch cultures using mixed pig cecal bacteria showed a marked influence of starting pH on the oprganic acids produced from the fermentation of various oligosaccharides by these bacteria.[10] Short-chain fatty acids are the

Correspondence address: Prof. Takashi Sakata, Department of Basic Sciences, Ishinomaki Senshu University, Minamisakai Shinmito 1, 986–8580 Ishinomaki, Japan.
Tel: 81 225 22 7713 (ext. 3112); Fax: 81 225 22 7746
Email: sakata@isenshu-u.ac.jp

main products when the initial pH is higher than 6.3. When the initial pH is lower than 6.0, lactic acid is produced. Succinic acid is also produced when the initial pH is lower than 5.0. Such effects of initial pH are common for raffinose, isomalto-oligosaccharides and xylosylfructoside.

Production of ammonia

Substrate

Ammonia can be produced by the degradation of nitrogenous materials such as dietary protein resistant to autoenzymic digestion, sloughed gut epithelial cells, digestive enzymes and mucin.[1] Urea that enters the large intestine either in the digestive secretions upstream or through the wall of the large bowel can be converted to ammonia by bacterial urease.[11]

Influence of energy supply to bacterial nitrogen metabolism

Bacteria in the large bowel use ammonia as a source of nitrogen for their protein synthesis. Thus, energy supply to bacteria in the large bowel can stimulate their proliferation (i.e. synthesis of protein for bacterial cell body and thereby consumption of ammonia). An increase in energy supply may also decrease the bacterial consumption of protein or other nitrogenous materials as energy substrates leading to the decrease in ammonia production from these materials. Actually, the addition of indigestible but fermentable carbohydrates to diets decreased urinary nitrogen excretion and increased fecal insoluble (very likely bacterial) nitrogen excretion.[11–15]

Absorption of lactic acid, succinic acid and ammonia from the large intestine

Lactic and succinic acids are absorbed through a mechanism that is different from that of short-chain fatty acids. Absorption of short-chain fatty acids accompanies the secretion of bicarbonate, while the absorption of lactic or succinic acid does not.[16] Lactic acid seems to be absorbed, at least partly, via a carrier mediated mechanism which depends on proton gradient.[17] Succinic acid is absorbed via a sodium-dependent mechanism common to di- and tricarboxylic acids.[18–21]

Lactic and succinic acids are absorbed more slowly than are short-chain fatty acids. This leads to an accumulation of these acids.[16] We found virtually no short-chain fatty acids in the rat cecum after the infusion of short-chain fatty acids (acetic acid 90, propionic acid 30 and n-butyric acid 30 mmol/L; 33 mL/day for 7 days) but found approximately 75 mmol/L lactic acid in the cecum after the infusion of this acid (total 150 mmol/L, 33 mL/day for 7 days).[22] This also suggests that the lumen concentration of a short-chain fatty acid is a poor indicator of the production rate of that acid in the large bowel.

Influence of lactic and succinic acids on lumen pH

It is interesting that lactic and succinic acids, but not short-chain fatty acids, are the major determinants of lumen pH in the large bowel.[6] Slow absorption of lactic and succinic acids and the lack of bicarbonate secretion against the absorption of these acids may be mainly responsible for the above correlation (i.e. the significant negative correlation between the lumen pH and concentrations of lactic and succinic acids in the large bowel). However, the rapid disappearance of short-chain fatty acids and bicarbonate secretion against short-chain fatty acids absorption should favour the maintenance of lumen pH.

Influence of lactic and succinic acids, and ammonia on the absorption of water and solutes

Short-chain fatty acids stimulate the absorption of water and sodium from the large intestine but lactic or succinic acids do not.[16] Thus, it is not likely that short-chain fatty acids induce diarrhea where the production of these acids is within the capacity of the large intestine to absorb them.[23] However, the absence of apparent co-transport of water or sodium with lactic or succinic acid leads to the potential for these acids to induce diarrhea. This mechanism could be responsible for the diarrhea in patients suffering from short-bowel syndrome or ulcerative colitis.

Ammonia increases the absorption of short-chain fatty acids and vice versa.[24] This is considered to be due to the provision of protons from ammonium cation to short-chain fatty acids-anions to make both more lipophilic.

Influence of lactic and succinic acids, and ammonia on epithelial cell kinetics and tissue mass

Short-chain fatty acids administered into the large bowel

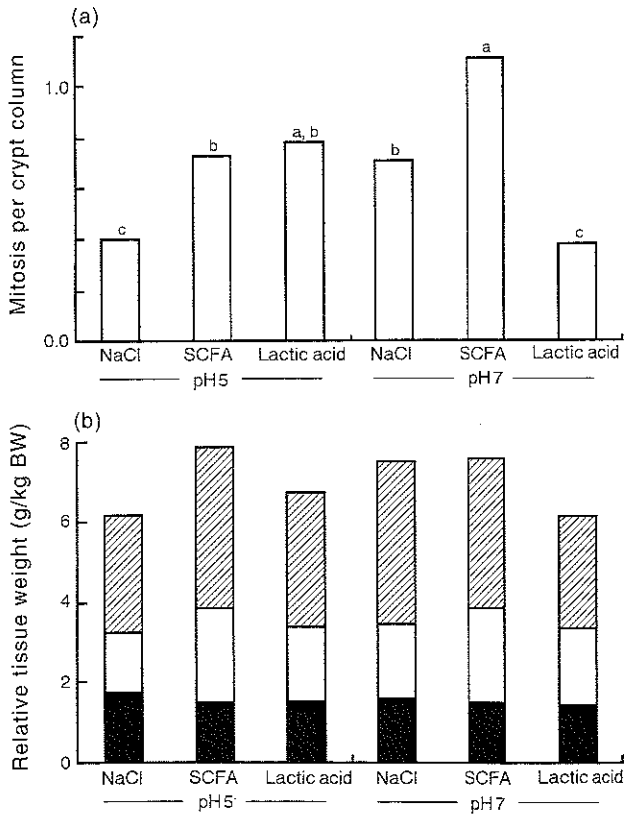

Figure 1. Cecal mitotic activity (a) and relative tissue weight (b) in rats intracecally infused with 150 mmol/L sodium chloride, a mixture of short-chain fatty acids (SCFA; acetic 90, propionic 30 and n-butyric 30 (mmol/L)) or 150 mmol/L L-lactic acid either at pH 5.0 or pH 7.0 at 33 mL/day for 8 days. NaCl, Sodium chloride; BW, bodyweight. (▨) Mucosa SCFA > Lac = NaCl; (□) submucosa SCFA > Lac = NaCl; (■) muscle, no variance. (a) Bars not bearing the same letter differ significantly ($P < 0.05$; (b) ANOVA for solute < 0.001; ANOVA for pH is not significant; 2-way ANOVA < 0.001.[22]

stimulate epithelial cell proliferation of the small and large intestine.[2] L-Lactic acid (Fig. 1)[22] or ammonia (Fig. 2) continuously infused into the isolated cecum or colon also stimulate the crypt cell production of the large bowel, but in a way different from that of short-chain fatty acids.[25]

Short-chain fatty acids increase the mucosal and submucosal tissue mass and crypt cell number; however, lactic acid or ammonia stimulates epithelial cell proliferation without increasing the gut tissue mass or crypt size.[22,25] Accordingly, short-chain fatty acids should accelerate the rate of cell production with less or no increase in the rate of cell death by sloughing and apoptosis. On the other hand, lactic acid and ammonia should increase the rate of cell death at the same magnitude as the rate of cell production.

Thus, short-chain fatty acids might be an important physiological factor for maintaining the normal epithelial cell number and thereby epithelial function, but lactic acid or ammonia might not. Such a discrepancy between the proliferative activity and epithelial cell number points out the danger of predicting one from the other; epithelial cell number, protein content or DNA content does not always reflect epithelial cell proliferation and *vice versa*. This suggests to us the necessity of re-evaluating earlier reports on the effects of dietary fibers and various lumen conditions.

The trophic effects of short-chain fatty acids, lactic acid and ammonia are rather complex. Trophic effects of short-chain fatty acids and lactic acid are pH-dependent, although in opposite manners. The trophic effect of short-chain fatty acids is stronger at pH 7.0 than at pH 5.0; however, that of lactic acid is significant at pH 5.0 but not at pH 7.0.[22] Epithelial cell proliferation was even inhibited by lactic acid infusion at pH 7.0, sometimes accompanying the loss of surface epithelial cells.[22] Thus, the changes in lumen pH can result in a decrease in epithelial proliferation and a loss of mucosal barrier when a certain amount of lactic acid exists in the lumen.

It is also interesting that the cecal epithelial cell proliferation was more active in rats infused with organic acid-free solution adjusted to pH 5.0 with hydrochloric acid than in those given acid-free solution at pH 7.0.[22] This implies that the trophic effect of short-chain fatty acids or lactic acid is not due to the effect of protons. Lumen acidification alone is not enough to exert a gut trophic effect. We need experiments to estimate the effect of lactic acid in the presence of short-chain fatty acids. For example, it could be established whether the additional lactic acid increases the cell production by its own trophic effect or reduces the cell proliferation by lowering lumen pH and thereby reduces the trophic effect of short-chain fatty acids.

The trophic effects of ammonia and short-chain fatty acids are not additive.[25] There was a negative interaction effect of ammonia and short-chain fatty acids on epithelial cell production.[25] Thus, the accumulation of ammonia in the hindgut lumen may attenuate the trophic effect of short-chain fatty acids.

Short-chain fatty acids in the hindgut lumen also stimulate epithelial cell proliferation in the small intestine.[2] However, we do not know if lactic acid or ammonia in the large bowel has such an effect on distant segments of the gastrointestinal tract.

Succinic acid infused into the isolated and defunctioned colon (75 mmol/L, pH 6.5, 24 mL/day for 8 days) reduced the crypt cell proliferation of the isolated colon (Fig. 3) (Inagaki *et al.*, unpubl. data, 1999). This might be responsible for the ulceration in ulcerative colitis, at least in part. This

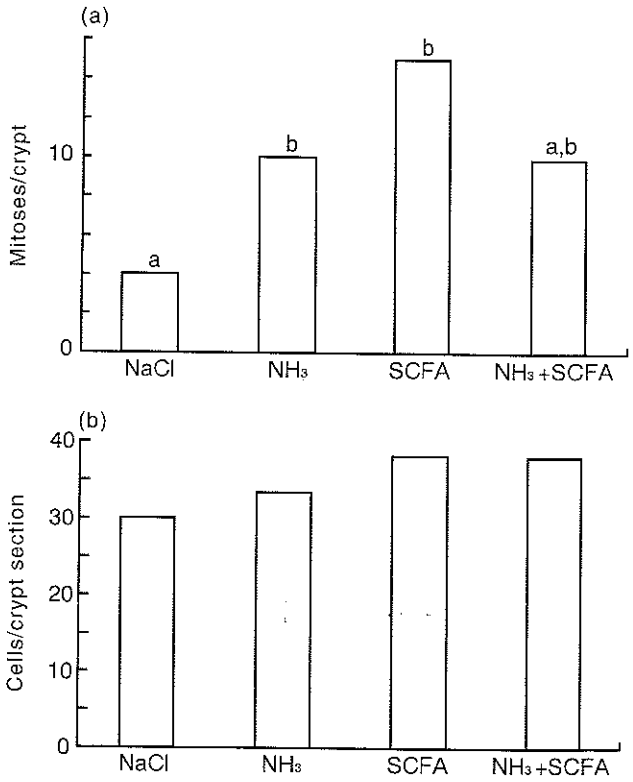

Figure 2. Cecal proliferative activity (a) and crypt size (b) in rats intracecally infused with a solution with or without a mixture of short-chain fatty acids (SCFA; acetic 70, propionic 35 and n-butyric 35 (mmol/L)) and with or without 75 mmol/L ammonia all adjusted to pH 7.2 at 24 mL/day for 7 days. NaCl, Sodium chloride; NH_3, ammonia. (a) Bars not bearing the same letter differ significantly ($P < 0.05$); (b) ANOVA for SCFA < 0.001; ANOVA for NH_3 is not significant; 2-way ANOVA is not significant.[25]

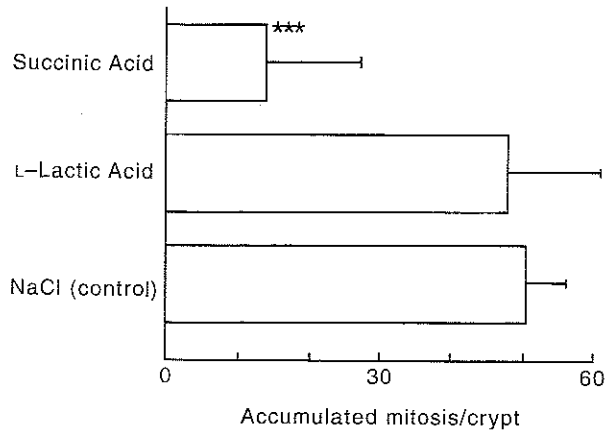

Figure 3. Colonic crypt cell proliferative activity in rats infused either 75 mmol/L succinic acid, 75 mmol/L L-lactic acid or 75 mmol/L sodium chloride (control) and all adjusted to pH 6.5 at 24 mL/day for 7 days. Metaphase figures were accumulated for 3 h by intraperitoneal injection of vincristine sulfate (1 mg/kg bodyweight). ***Different from other groups ($P < 0.001$).

negative effect of succinic acid on crypt cell production rate again agrees with the suggestion that proton concentration in the lumen alone cannot stimulate gut epithelial cell proliferation.

Influence of lactic and succinic acids, and ammonia on gut motility

Infusion of xylosylfructoside or partially hydrolysed guar gum into the ileum reduced motility ratio (duration of contraction period divided by the time length of the observation) of the cecum *in vivo*.[8] Because these conditions led to the luminal accumulation of succinic acid, Hoshi conducted a three-way experiment to study the effects of short-chain fatty acids, succinic acid and pH on rat cecal motility.[8] She infused solution with or without short-chain fatty acids at pH 7.0 or pH 5.5. Her results showed that low pH, lack of short-chain fatty acids or succinic acid reduced cecal motility ratio.

Thus, both succinic acid and low pH reduced the motility, while short-chain fatty acids were necessary to maintain motility. This implies that a large bowel condition that mainly produces succinic acid inhibits the large bowel motility by the action of succinic acid itself and through its pH-lowering effect.[6] This may partly be responsible for the atony of the proximal colon in patients suffering from ulcerative colitis. Such a reduced motility of the large intestine may increase the exposure of the large bowel mucosa to potentially carcinogenic substances.

Atonic conditions also reduce the mixing of gut contents leading to heterogeneous fermentation even in a single chamber. Such a situation may produce unusual metabolites such as formic acid. The diffusion of bacterial metabolites to the absorptive surface should decrease under such conditions. This should result in the increase in bacterial metabolites in the hindgut lumen, which may alter the bacterial metabolism (e.g. by lowering lumen pH). It is also likely that sulfur compounds such as hydrogen sulfide or methylmercaptan accumulate at the mucosal surface by microbial degradation of sulfur-containing amino acids in the hindgut mucin and colonic epithelial cells. This again suggests the danger of a too low lumen pH and the resultant accumulation of succinic acid.

Although we have not yet tested this, lactic acid could inhibit colonic motility through its pH-lowering effect.

Importance of flux rate of substrates into the large bowel

The above information suggest the importance of the entry rate of fermentable carbohydrates into the fermentation chamber (i.e. the large bowel).[23] If the flux rate of fermentable carbohydrates exceeds the capacity of the fermentation chamber to absorb microbially produced organic acids, then the excess acids remain in the lumen. This lowers the lumen pH, favoring the production of lactic and succinic acids.[10] Given that these acids have smaller pKa (acid dissociation constant) values (i.e. stronger acids) than do short-chain fatty acids[26] and that the absorption of lactic or succinic acid is slower than for short-chain fatty acids,[16] lumen pH, once lowered, may lead to the further accumulation of lactic or succinic acid and to a further lowering of lumen pH. Absorption of short-chain fatty acids accompanies the secretion of bicarbonate,[16] which should antagonize the pH-lowering effect of these acids. However, lactic or succinic acid does not stimulate the secretion of bicarbonate.[16] Accordingly, a too rapid entrance of carbohydrates into the large intestine can be harmful. This is actually the case in lactic acidosis in short-bowel patients.[3]

Necessity to control hindgut fermentation

Factors affecting the flux rate of carbohydrates into the large bowel should have a significant effect on the gut fermentation. Oligosaccharides or sugar alcohols ingested as a drink may reach the large bowel rapidly as a bolus, while the flux of such carbohydrates contained in a structured solid food may be slower and smoothed by intermittent gastric emptying. Further, food structure may reduce the available surface of fermentable materials for bacterial attack. Our study indicates that the entrance rate sometimes has a more significant effect than the chemical composition of fermentable carbohydrates.[10]

The effects of lactic or succinic acid are rather different from those of short-chain fatty acids; the former mostly hazardous. Thus, it is important to regulate the gut fermentation to produce short-chain fatty acids and to reduce the accumulation of lactic or succinic acid in the lumen. As the lumen pH seems to be a key issue for determining the final products of microbial breakdown of carbohydrates in the large bowel, we should pay more attention to intake rate, particle size of indigestible carbohydrates and the motility of the stomach and the small intestine in order to understand the influence of such carbohydrates and gut bacteria. Bicarbonate secretion from the upper digestive organs may also influence the hindgut fermentation. In this regard, the influence of pancreatic and small intestinal bicarbonate secretion on large bowel fermentation should be studied.

The ingestion of indigestible and fermentable carbohydrates often reduces the ammonia concentration in the large bowel lumen.[11–15] This reduction affects the host animal through the reduced effect of ammonia. However, such a reduction in ammonia concentration can potentially modulate effects of short-chain fatty acids.

The above discussion suggests the need for multifactorial experiments when working on the effects of bacterial metabolites on digestive organs.

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