Note

Inhibitory Effect of Succinic Acid on Epithelial Cell Proliferation of Colonic Mucosa in Rats

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Summary Microbial breakdown of carbohydrates in the large intestine mainly produces short-chain fatty acids (SCFA). SCFA stimulate epithelial cell proliferation of the digestive tract in vivo. Succinic acid sometimes accumulates in the colonic lumen. However, the effect of succinic acid on colonic epithelial cell proliferation is unknown. Thus, we planned to clarify the influence of succinic acid on colonic epithelial cell proliferation in vivo. We continuously administered infusate with or without succinic acid (100 mM) into the distal colon of rats for 6 d and measured accumulated mitosis per crypt of distal colon of these rats. Succinic acid infused into rat colons significantly inhibited colonic cell proliferation and reduced crypt size. These results clearly indicated the inhibitory effects of succinic acid on colonic epithelial cell proliferation in vivo.

Key Words succinic acid, short-chain fatty acids, large intestine, cell division

Microbial breakdown of carbohydrates in the large intestine mainly produces short-chain fatty acids (SCFA) such as acetic, propionic, and *n*-butyric acids (1, 2). SCFA stimulate epithelial cell proliferation of the large intestine in vivo (3, 4). Succinic acid, being an intermediate of bacterial breakdown of carbohydrates (5, 6), rarely exists in normal human feces. However, succinic acid sometimes accumulates in the large intestine of patients suffering from ulcerative colitis (7, 8). Nevertheless, the influence of succinic acid in the lumen of the large intestine is limited. Succinic acid inhibits the motility of the large intestine (9), and stimulates water secretion from the temporarily isolated small intestine (10). Succinate enemas into the rectum of mice, whose anal verge was glued, caused focal erosions of the mucosa and edema of the submucosa (11). However, the in vivo influence of succinic acid on the epithelial cell proliferation of the colon is unknown. It is important to study such an effect of succinic acid when we think of the healing of ulcers in the colon. Thus, we studied the influence of succinic acid on colonic epithelial cell proliferation in vivo.

Materials and Methods

Experimental animals. Twenty male Wistar rats at 8 wk of age (approximately 250 g body weight) were purchased from Funabashi Farm (Funabashi, Japan). They were housed in stainless-steel wire-mesh bottom cages (3 or 4 rats per cage) and maintained under a 12 h light: 12 h dark cycle (light from 0830 h). Rats had free

access to a non-purified diet containing (g/100 g) crude protein 27.2, crude fat 5.0, crude fiber 3.7, crude ash 7.7, NFE 48, minerals and vitamin mixture (CA-1, Clea Japan Inc., Tokyo, Japan) and water for 7 to 9 d prior to surgical operation.

Surgical operation. Rats were fasted overnight before surgery. After an intraperitoneal injection of sodium pentobarbital (30 mg/kg body weight), we performed a midline laparotomy. A silicon tube (Silascon Medical Grade Tube, Kaneka Medics, Osaka, Japan; inner diameter 0.5 mm; outer diameter 1.0 mm) was inserted into the most oral part of the distal colon near the entrance of the left branch of the middle colic artery and fixed with a purse string suture. The tube was pulled out through the laparotomized wound, fixed onto the rectus abdominis muscle with a suture and tunneled out subcutaneously to a skin opening at the midscapular region. Then, we attached a harness (Unique Medical Imada, Natori, Japan) mounted with a spring. The externalized tube was pulled through the coil and further connected to a swivel (IITC Inc., California). We transferred the rats to individual wire-mesh bottomed metabolic cages. The swivel was connected to a continuous infusion system including a peristaltic pump (Watson Marrow, Cornwall, England). We infused 150 mM NaCl solution into the distal colon at 30 mL/d for 5 d.

Infusion. After the surgery, rats were allowed free access to AIN-76 diet containing (g/100 g) milk casein 21.1, *dl*-methionine 0.3, cornstarch 15.8, sucrose 52.6, corn oil 5.2, choline bitartrate 0.2, AIN-76 vitamin mixture 1.1, mineral mixture 3.7 (Oriental Yeast, Japan) and water during the entire infusion period. We

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Table 1. Composition of experimental solutions. (mM)

	Control	L-Lactic	Succinic
NaCl	150	50	50
NaOH		100	100
L-Lactic acid		100	
Succinic acid			100

The pH of each solution was adjusted to 6.5 with 1 N HCl or 1 N NaOH.

randomly divided rats into three groups of 5 or 6 to infuse either 150 mM NaCl, 100 mM succinic acid or 100 mM lactic acid (Table 1) into the isolated distal colon at 30 mL/d for 6 d from the morning on the 5th post operative day. The pH of all infusates was adjusted to 6.5.

Accumulated mitoses per crypt. In order to arrest gut epithelial cells in mitosis at metaphase and thereby to amplify the difference in cell proliferation, we lightly anesthetized rats with diethylether and administered vincristine sulfate intraperitoneally at 1 mg/kg body weight between 8 and 9 h on the 6th day of colonic infusion (4). Rats were re-anesthetized with diethylether and killed with an overdose of sodium pentobarbital (more than 100 mg/kg body weight) into the inferior cervical vein 3 h after the administration of vincristine sulfate. The segments of the colon were removed and fixed in a mixture of acetic acid and ethyl alcohol (1 : 3, v/v) for at least 3 h. These tissue samples were transferred to 70% ethanol and stored until crypt dissection.

Fixed tissue pieces (approximately 3×3 mm) of the distal colon 1 cm aborad to the entrance of the left branch of the middle colic artery, i.e. the proximal end of the distal colon, were stained with Feulgen reaction en bloc (12). Crypts were dissected from these tissues under a stereo microscope and squashed onto a glass slide. Metaphase figures per crypt were counted in 20 randomly selected crypts per specimen (13). The mean metaphase frequency in 20 crypts was arbitrarily termed "accumulated mitoses per crypt" in the present study.

The residual segment of the distal colon was embedded in paraffin. A 3 µm-thick cross section was prepared from each specimen and stained with hematoxylin and eosin. We counted the number of epithelial cells on the right side of the axial crypt section (number of cells per crypt column) in 20 randomly chosen complete axial crypt sections per animal as a measure of crypt size (13).

Ethical consideration. The experimental plan of the present study was approved by the Animal Research and Animal Care Committee of the School of Medicine, Tohoku University. Any handling of animals was conducted according the guideline of the above committee under the surveillance of personnel of Animal Research Facility, School of Medicine, Tohoku University.

Statistics. The difference among means of experi-

Table 2. Accumulated mitoses per crypt and crypt size of the distal colon in rats infused with 150 mM NaCl (negative control), 100 mM L-lactic acid (positive control) or 100 mM succinic acid into the colon for 6 d. Means (SD), $n=5$.

	<i>n</i>	Accumulated mitoses per crypt (metaphase/crypt)	Crypt size (cells/crypt column)
Succinic acid	5	12.0 (2.4) ^b	23.4 (2.0) ^b
L-Lactic acid	6	20.0 (6.4) ^{ab}	29.0 (2.5) ^a
Control	6	25.5 (6.3) ^a	30.8 (3.2) ^a
ANOVA- <i>p</i>		<0.01	<0.001

Means in the same column not sharing a common superscript differ significantly by Scheffé's post hoc comparison ($p<0.05$).

mental groups was tested by analysis of variance (ANOVA) using Stat View 4.0 program (Abacus Concept Inc., Berkeley, CA) followed by Scheffé's post-hoc comparison. Difference between means was considered significant at $p<0.05$.

Result

No macroscopic abnormality of colonic mucosa was observed (data not shown). Both the accumulated mitoses per crypt and number of epithelial cells per crypt column (crypt size) of the colonic mucosa infused with succinic acid were significantly less than those infused with either NaCl or L-lactic acid (crypt size alone) (Table 2). Neither accumulated mitoses per crypt nor crypt size of rats infused with L-lactic acid differ from those of rats infused with NaCl (Table 2).

Discussion

Concentration of lactic or succinic acid in the colonic lumen of normal humans is at most 5 mM (14, 15). The lactic or succinic acid concentration in the infusate used in Experiment 1 (Table 1) was much higher than this value, though well within the concentration of these acids found in the colonic lumen of patients suffering from short-bowel syndrome or ulcerative colitis (15, 16). The infusion rate we used in Experiment 1 was higher than the entrance rate of contents into the colon. This was to keep the luminal concentration of succinic or L-lactic acid at the required levels. Such an abnormally fast infusion rate by itself has no serious effects on colonic mucosa (13).

In a previous study, L-lactic acid at pH 5.0 but not at pH 7.0 stimulated colonic epithelial cell proliferation in vivo (16). In the present study, succinic acid depressed colonic epithelial cell proliferation and reduced crypt size (Table 2). However, L-lactic acid changed neither the cell proliferation nor the crypt size at pH 6.5 (Table 2), basically agreeing with our previous in vivo result (16).

Ariake et al. (11) observed erosion of the mucosa when they instilled succinic acid into the colo-rectal segment with its anal verge glued. However, we did not

observe such an abnormality despite the higher dose of and longer exposure to succinic acid in the present study than Ariake et al. (11). We have no clear answer for the difference. However, obstruction of the normal passage of contents in Ariake et al. (11) could be responsible for the erosion.

The inhibitory effect of succinic acid on colonic epithelial cell proliferation (Table 2) may either cause or worsen ulcerative colitis. This may partly explain the cause of erosion by succinic acid in the previous study (11).

Anyhow, treatments that increase the production of succinic acid in these patients should be avoided. Low luminal pH inhibits production of SCFA while stimulating accumulation of lactic and succinic acids and further lowering of luminal pH (17). In this regard, it might be worth trying to maintain normal colonic pH by providing buffers in the colonic lumen artificially or by stimulating bicarbonate secretion from digestive organs.

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