Loperamide Inhibits the Enhanced Intestinal Glucose Absorption of Cystic Fibrosis *In Vitro*

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ABSTRACT. Enhanced Na^+-linked nutrient absorption has been demonstrated in the cystic fibrosis bowel and may contribute to the dehydration of the luminal contents. The ability of loperamide to inhibit glucose transport was therefore assessed in jejunal biopsy specimens from children with cystic fibrosis by measuring the increased short-circuit current associated with active glucose absorption using a mini-Ussing chamber technique. The presence of loperamide in the luminal solution reduced the rise in short-circuit current induced by glucose over the range of concentrations tested (2.5 to 40 $mmol \cdot L^{-1}$). This finding suggests that the enhanced Na^+ absorption, which is a feature of cystic fibrosis, is amenable to treatment and can be restored to normal levels. This result may have therapeutic implications for gastrointestinal function and may be applicable to other tissues where similar therapeutic approaches to reduce Na^+ and thus water absorption are being pursued. (*Pediatr Res* 35: 354–356, 1994)

Abbreviations

CF, cystic fibrosis
SCC, short-circuit current

CF is a multiorgan disease that is characterized by abnormal ion transport across epithelia (1). It is now well established that, in CF, epithelia that normally secrete Cl^-, *e.g.* those lining the intestine and the airways, fail to do so, (1–7) and it has been suggested that this abnormality is responsible for the excessive dehydration of the luminal contents. The recent discovery of the CF gene (8) and evidence that its product, CF transmembrane conductance regulator, can act as a Cl^- channel (9) has led to emphasis being placed on this aspect of epithelial ion transport dysfunction in CF. However, failure of Cl^- secretion is not the only abnormality present in CF epithelia. Increased Na^+ absorption has also been reported in both the airway (10) and the intestine, (4–6) and this increased absorption is accompanied in the small intestine by enhanced Na^+-linked nutrient absorption (11). This increased absorption will also contribute to luminal dehydration and indeed may prove to be of greater clinical significance than impaired Cl^- secretion, particularly in the airway that is capable of a normal response to Ca^{2+}-mediated secretagogues, only failing to secrete Cl^- when challenged by cyclic AMP–mediated agonists (12). Thus, therapy directed at reducing the enhanced absorption in CF may prove, in the short-term at least, more effective than attempts to activate secretion.

The antidiarrheal agent loperamide inhibits Na^+-linked nutrient absorption in rat small intestine, (13) and the aim of the

present study was to determine whether this agent could reduce the enhanced absorption in the CF bowel.

MATERIALS AND METHODS

Patients. Peroral jejunal biopsy specimens were obtained from 11 children with CF who were undergoing investigation for chronic diarrhea that was unresponsive to changes in pancreatic enzyme supplementation and that was not associated with evidence of enteropathy. The diagnosis of CF was confirmed by at least two successive abnormal sweat tests, and a genetic analysis was performed to identify the defect in the CF gene product. Five of the control group of six patients and three of the test group of five patients were ΔF_{508} homozygotes. The other three children were ΔF_{508} heterozygotes, two with undefined mutations (one control, one test), both of whom had meconium ileus at diagnosis, and one with the R553X mutation (test group). All children were pancreatic insufficient. The mean age of the control group was 8.3 y (range, 0.4 to 18.5 y), and the mean age of the test group was 4.3 y (range, 1.0 to 9.0 y). Intestinal morphology was normal in all cases.

Experimental procedure. The electrical activity of biopsy specimens was measured as described previously (3). Each tissue was mounted as a sheet (exposed area, 3 mm^2) in a mini-Ussing chamber and incubated at 37°C with Krebs bicarbonate saline gassed with 95% O_2/5% CO_2. The serosal solution contained 10 $mmol \cdot L^{-1}$ glucose and the mucosal solution equimolar mannitol. The potential difference was measured with salt bridge electrodes connected by way of calomel half cells to a differential input electrometer whose output was displayed on a chart recorder (Linseis L6512, Selb, Germany). Current was applied across the tissue by conductive plastic electrodes. Tissue resistance was determined from the change in potential difference induced by a 10-μA current pulse, corrected for the fluid resistance. The SCC was then calculated from potential difference and resistance measurements with Ohm's law. After 10 min of stabilization, potential difference and resistance were measured at 1-min intervals. Each tissue was initially challenged with acetylcholine (10^{-3} $mol \cdot L^{-1}$ in serosal solution), which was added after 5-min basal readings. The serosal solution was replaced, and the tissue was allowed to restabilize. In the control tissues, glucose was added sequentially to the mucosal solutions to give concentrations of 2.5 to 40 $mmol \cdot L^{-1}$, and the rise in SCC was taken as the difference between the maximum value achieved at each concentration and the value immediately before the start of the glucose application. The mucosal solution was then replaced, and the procedure was repeated with equimolar concentrations of mannitol to assess the effect of the increased osmolarity resulting from the mucosal addition of glucose. In the test tissues, the effects of 10 $mmol \cdot L^{-1}$ concentrations of glucose and mannitol were tested before loperamide was added. After washing out, loperamide (1.9×10^{-4} $mol \cdot L^{-1}$) was added to the mucosal solution, followed 5 min later by serial additions of glucose as in the control experiments. The mucosal solution was then re-

placed, and the procedure was repeated with mannitol in the presence of loperamide. The experimental protocol is illustrated in Figure 1, which shows data from a control tissue and a test tissue.

To assess the rise in SCC associated with active glucose absorption, the change in SCC induced by each concentration of mannitol was subtracted from the increase in SCC caused by the same concentration of glucose.

Expression of results. Results are expressed as mean values ± 1 SEM of the mean of the number of observations indicated, and the significance of loperamide action was assessed by an unpaired t test.

Chemicals. Mannitol was obtained from May & Baker Ltd. (Dagenham, UK); glucose was obtained from BDH Chemicals Ltd. (Poole, UK); and acetylcholine was obtained from Sigma Chemical Company Ltd. (Poole, UK).

RESULTS

The increase in SCC associated with active glucose absorption in jejunal biopsy specimens from children with CF was greater than that observed in non-CF tissues. When data from all the biopsy specimens we studied were analyzed, the rise in SCC induced by 10 mmol·L^{-1} glucose was 44.1 ± 4.6 (n = 30) $\mu A \cdot cm^{-2}$ in CF tissues compared with 24.3 ± 3.1 (n = 48) $\mu A \cdot cm^{-2}$

Fig. 1. SCC generated by a control (a) and a test (b) jejunal biopsy specimen from children with CF. The following additions were made at the points indicated by the arrows: A, 10^{-3} mol·L^{-1} acetylcholine; G, 10 mmol·L^{-1} glucose; M, 10 mmol·L^{-1} mannitol; L, 1.9 × 10^{-4} mol·L^{-1} loperamide; $G(2.5-40)$, serial additions of glucose to give luminal concentrations of 2.5, 5, 10, 15, 20, 25, 30, 35, and 40 mmol·L^{-1}; $M(2.5-40)$, serial additions of mannitol to give luminal concentrations of 2.5, 5, 10, 15, 20, 25, 30, 35, and 40 mmol·L^{-1}.

in non-CF tissues (p < 0.001), confirming, with an increased number of patients, previous observations (11).

In the CF tissues used in the present study, the basal SCC was similar in the control (n = 6) and test (n = 5) groups (control, 11.8 ± 9.2 $\mu A \cdot cm^{-2}$; test, 5.8 ± 3.3 $\mu A \cdot cm^{-2}$, p > 0.05) and no rise in SCC occurred when acetylcholine was added (control, −0.5 ± 0.4 $\mu A \cdot cm^{-2}$; test, −1.2 ± 1.1 $\mu A \cdot cm^{-2}$, p > 0.05). This finding compares with a mean increase of 37.3 ± 4.2 (n = 48) $\mu A \cdot cm^{-2}$ observed in biopsy specimens from children without CF.

The effects of glucose and mannitol on the SCC generated by CF intestine are illustrated in Figure 1. In the test group the changes in SCC induced by 10 mmol·L^{-1} glucose (34.1 ± 5.9 $\mu A \cdot cm^{-2}$) and 10 mmol·L^{-1} mannitol (−8.6 ± 0.7 $\mu A \cdot cm^{-2}$) were not significantly different from those observed in the control group (glucose, 39.9 ± 2.9 $\mu A \cdot cm^{-2}$; mannitol, −13.7 ± 3.6 $\mu A \cdot cm^{-2}$, p > 0.05 in both cases). The effect of glucose, corrected for its osmotic action, is expressed as a percentage of the response to 10 mmol·L^{-1} glucose in the absence of loperamide to avoid any bias from the fact that the glucose-dependent rise in SCC was slightly lower in the test group. Changes in SCC induced by glucose in the control group are similarly related to the 10 mmol·L^{-1} value.

Loperamide (1.9 × 10^{-4} mol·L^{-1}) reduced the basal SCC of the five test tissues by 6.2 ± 0.7 $\mu A \cdot cm^{-2}$ but had no effect on the decrease in SCC induced by mannitol. In the five test tissues, 10 mmol·L^{-1} mannitol reduced the SCC by 8.6 ± 0.7 $\mu A \cdot cm^{-2}$ when loperamide was absent and by 6.6 ± 1.8 $\mu A \cdot cm^{-2}$ when it was present (p > 0.05). A comparison of control and test groups demonstrated that loperamide did not alter the relationship between the fall in SCC and the mannitol concentration (control, 12.1 ± 2.5 $\mu A \cdot cm^{-2}$/10 mmol·L^{-1}; test + loperamide, 6.0 ± 1.3 $\mu A \cdot cm^{-2}$/10 mmol·L^{-1}, p > 0.05).

Loperamide reduced the rise in SCC associated with the active absorption of glucose to values that were 66.2 ± 0.9% of those observed at the same concentrations in control tissues (Fig. 2). The degree of inhibition did not alter with the concentration of glucose.

The apparent transport constant and the V_{max} were calculated with both the Eadie-Hofstee plot (14) and the direct linear plot (15). These two methods produced values that did not differ
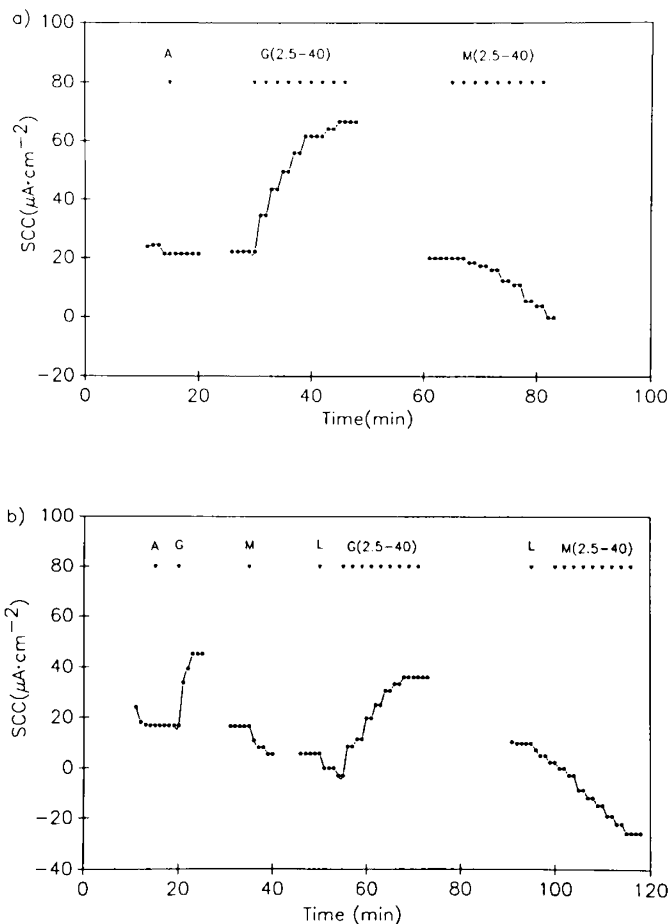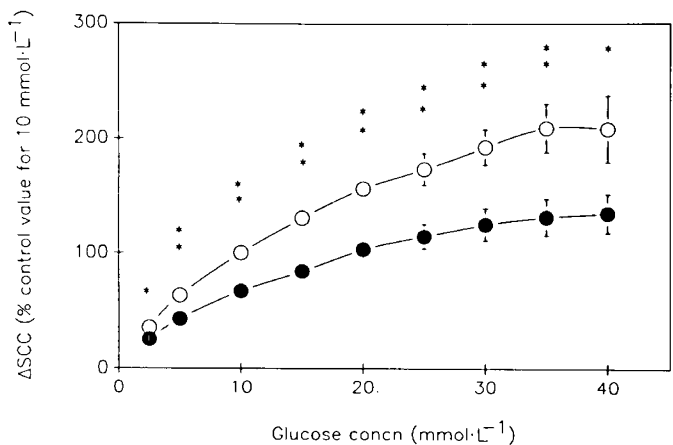

Fig. 2. Effect of loperamide (1.9 × 10^{-4} mol·L^{-1} in mucosal solution) on the rise in SCC associated with active glucose absorption in jejunal biopsy specimens from children with CF. Glucose was added to the mucosal solution to give the concentrations indicated, and the osmotic component of the response was assessed with equimolar concentrations of mannitol. The effect of glucose, corrected for its osmotic action, is expressed as a percentage of the SCC change induced by 10 mmol·L^{-1} glucose in the absence of loperamide. Each point represents the mean ± SE of the mean of six control (O) and five test (●) tissues, and the significance of loperamide action was determined with an unpaired t test. *, p < 0.05; **, p < 0.01.

significantly ($p > 0.05$ for both indices). The kinetic analysis revealed that loperamide reduced the V_{max} without affecting the transport constant (Table 1).

DISCUSSION

The increased electrical activity induced by glucose is directly related to its rate of active absorption (16). Thus, an enhanced response to glucose in jejunal biopsy specimens from patients with CF (11) suggests that active glucose absorption by the intestine is increased in the disease, a finding supported by *in vivo* perfusion studies demonstrating greater glucose uptake by the jejunum of patients with CF (17). A limited number of flux determinations performed on larger intestinal sheets suggest that basal Na^+ absorption may also be increased in CF (4-6).

The basis of the increased absorption in CF is unclear. It has been suggested that the lack of normal Cl^- channel activity in the luminal membrane of CF enterocytes could cause a hyperpolarization that would provide a greater driving force for Na^+ and nutrient entry (11). Such an explanation cannot, however, account for enhanced absorption by the CF airway where direct measurement of the apical membrane potential has revealed a decrease, rather than an increase, in CF tissues (18).

Net fluid movement across epithelia is determined by net solute transport, which in turn depends on the balance between absorptive and secretory processes. Hence, an enhanced absorption, particularly if coupled to impaired secretion, will lead to a dehydration of the luminal contents, which may contribute to clinical symptoms. This situation can be improved either by activating secretory mechanisms or by reducing absorption, and the present study reveals that loperamide, an agent currently in clinical use, can inhibit absorptive processes in the intestine (Fig. 2).

The ability of loperamide to inhibit intestinal absorption has been established with tissue from the rat (13). Loperamide in the mucosal solution was found to reduce the Na^+-linked absorption of nutrients determined both directly and as an increase in transintestinal electrical activity. Kinetic analysis of the data demonstrated that this effect resulted from a decrease in V_{max}, and, because loperamide was much less effective when the Na^+ gradient was reduced, it was suggested that it could interfere with the interaction of Na^+ with its site on the nutrient cotransporter, preventing it from using the energy from the Na^+ gradient. In jejunal biopsy specimens from patients with CF, the inhibitory actions of loperamide similarly result in a reduced V_{max} (Table 1), which is consistent with an action limiting the energy available to the cotransporter.

In addition to its actions on nutrient transport, loperamide also reduced the basal SCC in the CF jejunal biopsy specimens.

Table 1. *Influence of loperamide (1.9 $\times$ 10^{-4} mol·L^{-1} in mucosal solution) on the kinetics of the rise in SCC associated with active glucose absorption in jejunal biopsy specimens from children with CF**

	Control ($n = 6$)	Loperamide ($n = 5$)	p value
Transport constant (mmol·L^{-1})	27.8 ± 4.5	19.4 ± 3.1	>0.05
V_{max} (μA·cm^{-2})	195.0 ± 20.3	82.0 ± 6.5	<0.001

* Transport constant and V_{max} values were determined with the Eadie-Hofstee plot, and an unpaired t test was used to assess the significance of loperamide action.

This action may represent an inhibition of basal Na^+ transport, as has been observed in rat small intestine (13).

In the normal intestine, loperamide, in addition to its antiabsorptive actions, is a potent inhibitor of Cl^- secretion, (19) and this inhibition contributes to its effectiveness as an antidiarrheal agent. However, because the CF intestine lacks the ability to secrete Cl^- (2-7), this aspect of loperamide action will not be of consequence to CF patients.

Enhanced Na^+ absorption in CF is a feature not only of the intestine but also of the airway (10). The possibility that reducing this aspect of ion transport dysfunction in CF might alleviate clinical symptoms has been tested by the administration of amiloride, an inhibitor of Na^+ transport, in aerosol form to patients with CF. The regime was found to slow the rate of deterioration of respiratory function in a small number of adult patients with established chronic lung disease (20). The present study extends the range of agents that inhibit the enhanced absorption of CF and suggests an alternative approach to treatment of some of the symptoms of the disease.

REFERENCES

1. Welsh MJ, Fick RB 1987 Cystic fibrosis. J Clin Invest 80:1523-1526
2. Taylor CJ, Baxter PS, Hardcastle J, Hardcastle PT 1987 Absence of secretory response in jejunal biopsy samples from children with cystic fibrosis. Lancet 2:107-108
3. Taylor CJ, Baxter PS, Hardcastle J, Hardcastle PT 1988 Failure to induce secretion in jejunal biopsies from children with cystic fibrosis. Gut 29:957-962
4. Berschneider HM, Knowles MR, Azizkhan RG, Boucher RC, Tobey NA, Orlando RC, Powell DW 1988 Altered intestinal electrolyte transport in cystic fibrosis. FASEB J 2:2625-2629
5. Goldstein JL, Nash NT, Al-Bazzaz F, Layden TJ, Rao MC 1988 Rectum has abnormal ion transport but normal cAMP-binding proteins in cystic fibrosis. Am J Physiol 254:C719-C724
6. O'Loughlin EV, Hunt DM, Gaskin KJ, Stiel D, Bruzuszcak IM, Martin HCO, Bambach C, Smith R 1991 Abnormal epithelial transport in cystic fibrosis jejunum. Am J Physiol 260:G758-G763
7. Hardcastle J, Hardcastle PT, Taylor CJ, Goldhill J 1991 Failure of cholinergic stimulation to induce a secretory response from the rectal mucosa in cystic fibrosis. Gut 32:1035-1039
8. Kerem B-S, Rommens JM, Buchanan JA, Markiewicz D, Cox TK, Chakravarti A, Buchwald M, Tsui L-C 1989 Identification of the cystic fibrosis gene: genetic analysis. Science 245:1073-1080
9. Tabcharani JA, Chang X-B, Riordan JR, Hanrahan JW 1991 Phosphorylation-regulated Cl$^-$ channel on CHO cells stably expressing the cystic fibrosis gene. Nature 352:628-631
10. Boucher RC, Stutts MJ, Knowles MR, Cantley L, Gatzy JT 1986 Na$^+$ transport in cystic fibrosis respiratory epithelia. J Clin Invest 78:1245-1252
11. Baxter P, Goldhill J, Hardcastle J, Hardcastle PT, Taylor CJ 1990 Enhanced intestinal glucose and alanine transport in cystic fibrosis. Gut 31:817-820
12. Boucher RC, Cheng EHC, Paradiso AM, Stutts MJ, Knowles MR, Earp HS 1989 Chloride secretory response of cystic fibrosis human airway epithelia. J Clin Invest 84:1424-1431
13. Hardcastle J, Hardcastle PT, Cookson J 1986 Inhibitory actions of loperamide on absorptive processes in rat small intestine. Gut 27:686-694
14. Engel PC 1981 Enzyme kinetics, 2nd Ed. Chapman and Hall Ltd., London, pp 14-25
15. Levin RJ 1979 Fundamental concepts of structure and function of the intestinal epithelium. In: Duthie HL, Wormsley KG (eds) Scientific Basis of Gastroenterology. Churchill Livingstone, Edinburgh, pp 308-337
16. Luppa D, Hartenstein H, Müller F 1987 Relation between microvilli membrane potential and glucose transport capacity of rat small intestine. Biomed Biochim Acta 46:341-348
17. Frase LL, Strickland AD, Kachel GW, Krejs GJ 1985 Enhanced glucose absorption in the jejunum of patients with cystic fibrosis. Gastroenterology 88:478-484
18. Boucher RC, Cotton CU, Gatzy JT, Knowles MR, Yankaskas JR 1988 Evidence for reduced Cl$^-$ and increased Na$^+$ permeability in cystic fibrosis human primary cell cultures. J Physiol (Lond) 405:77-103
19. Hardcastle J, Hardcastle PT, Read NW, Redfern JS 1981 The action of loperamide in inhibiting prostaglandin-induced intestinal secretion in the rat. Br J Pharmacol 74:563-569
20. Knowles MR, Church NL, Waltner WE, Yankaskas JR, Gilligan P, King M, Edwards LJ, Helms RW, Boucher RC 1990 A pilot study of aerosolized amiloride for the treatment of lung disease in cystic fibrosis. N Engl J Med 322:1189-1194