Morphological and functional effects of 16,16-dimethyl-prostaglandin-E₂ on mucosal adaptation after massive distal small bowel resection in the rat

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SUMMARY The ability of 16,16-dimethyl-prostaglandin-E₂ (PGE) to augment mucosal adaptation 14 days after a 70% distal small bowel resection in the rat was evaluated. In resected (R) and sham operated (S) animals, subcutaneous PGE 75 mg/kg, 2×/day, induced significant ($p<0.05$) increases in mucosal protein, DNA, and disaccharidase concentrations per centimetre of bowel. The respective per cent increases in the residual proximal small intestine compared with their respective untreated controls were: protein, R=60%, S=66%; DNA, R=69%, S=29%; maltase, R=57%, S=5%. The uptake of leucine by intestinal rings was significantly higher (50%) in the PGE treated group at a concentration of 2 mmol/l of substrate, while the uptake of glucose was similar in both groups. Although mucosal mass was increased by PGE, crypt cell production was similar in all groups. The drug appears to be an effective agent in stimulating morphological and functional adaptation after massive distal small bowel resection.

The key element in determining the prognosis of short bowel syndrome is induction of substantial villus hyperplasia in the remaining small bowel, resulting in a gradual increase in nutrient absorption, which eventually obviates the need for parenteral nutrition.¹⁻¹¹ Previous studies have shown that administration of 16,16-dimethyl-prostaglandin-E₂ (PGE) to rats results in mucosal hyperplasia.¹² This response, however, appears limited to the proximal small intestine, primarily the duodenum. In our rat model, we have shown morphologic augmentation of mucosal hyperplasia primarily in the duodenum after jejunal resection.¹³ We did not, however, determine if these changes altered mucosal function. Because distal small bowel resections are most common in the clinical setting, we investigated whether or not PGE could augment morphological and functional adaptation after ileal resection in the rat.

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Received for publication 3 March 1988.

Methods

RATS

This study was carried out on 40 180 g male Sprague-Dawley rats (Sasco, Omaha, NE). Twenty of the 40 animals were subjected to 70% jejunoileal resection. Twenty four hours before surgery all animals received unflavoured Vivonex to reduce intraluminal content. Each animal was sedated with pentobarbital, 50 mg/kg and a midline abdominal incision was made. The small intestine was isolated and the bowel was divided 5 cm proximal to the ileocaecal valve. The total length of bowel was determined by measuring the total length of jejunum and ileum in representative animals of this age and weight, and a segment equal to 70% of this value was measured proximally from the initial enterotomy. The bowel was divided again at this point in the distal jejunum, all mesenteric vessels supplying the segment to be resected were ligated with 5-0 silk, and the distal jejunum and most of the ileum were removed.

The midjejunum was then anastomosed to the remaining 5 cm segment of ileum using single, interrupted 6/0 silk sutures. The remaining 20 animals underwent sham operation consisting of complete division of the bowel 5 cm proximal to the ileocaecal valve with subsequent reanastomosis.

Animals were fed Vivonex *ad libitum* for 24 hours after surgery and then were assigned to specific groups for pair feeding. Ten PGE treated resected animals were paired with 10 PGE treated sham operated animals. All PGE treated animals received the first injection of PGE just before surgery. PGE was administered subcutaneously in isotonic saline, at a dose of 75 $\mu\text{g/kg}$ body weight after pilot studies suggested this dose was efficacious and well tolerated. Solutions were freshly made each day. Ten untreated resected animals were likewise paired with 10 untreated sham operated animals. Pair feeding was carried out using powdered diet (Wayne Lab-blox) so that each animal received similar amounts of food during the course of the experiment. Spillage of food and amount of food intake were monitored. Animals consumed 24 ± 3 g chow per day. Weights were determined every other day.

On postoperative day 14, animals received an ip dose of vincristine (2 mg/kg) in normal saline and killed using cervical dislocation two hours later. Vincristine is a commonly used agent in inducing metaphase arrest for determination of crypt cell production. The abdomen was opened and the remaining small intestine from the ligament of Treitz to the anastomosis was removed from the resected animals, and corresponding segments were removed from the sham operated animals. The bowel was divided into proximal and distal segments and everted on a glass rod. The length and weight of each segment were determined. The proximal 5 cm of both segments was then removed, washed in iced saline, and cut longitudinally. Mucosa was scraped free from underlying serosa using a glass slide. Mucosal weight was determined. Mucosal samples were subsequently homogenised in deionized water, 25 mg mucosa/ml H_2O . Total mucosal protein content was measured according to the method of Lowry *et al.*¹⁴ Mucosal DNA was extracted by the method of Munro and Fleck¹⁵ and assayed according to the method of Burton¹⁶ as modified by Giles and Myers.¹⁷ Maltase activity was measured according to the method of Dahlquist¹⁸ and expressed as μmol glucose produced per minute per milligram protein.

The remaining intestine was then cut into rings 0.5 cm in length for *in vitro* determination of glucose and leucine uptake. Two additional 1/2 cm segments were saved to measure crypt cell production.

Mucosal uptake of glucose and leucine was determined according to a modification of the technique

of Sallee *et al.*¹⁹ originally described for use with intestinal sacs. Once cut, rings were suspended by long threads into iced oxygenated Krebs Ringers bicarbonate buffer. One ring from each segment of each animal was then placed into each container of 20 ml oxygenated Krebs Ringer bicarbonate buffer at 37°C for three minutes. Each 20 ml buffer contained one of the following concentrations of glucose (1, 2, 5, 10, 20, 40 mmol/l) or leucine (0.5, 1, 2, 5, 10, 20 mmol/l). Two microcuries ^{14}C glucose or leucine was added as well as 6 μCi of ^3H polyethylene glycol 4000 as an adherent fluid marker.

Rings were removed from the medium after exactly three minutes, plunged into iced saline to stop transport, and oven dried overnight at 93°C in a preweighed container. Tissue was then solubilised in 0.8 ml 0.75 N sodium hydroxide and an aliquot was taken for protein determination. The remainder of the homogenate was neutralised with acetic acid, and counted after addition of scintillation cocktail.

Uptake of both substances (J value) was calculated according to Sallee *et al.*¹⁹ expressed as substrate absorbed per minute per milligram protein, and uptake at each concentration was compared between groups by two way analysis of variance for each segment.

Crypt cell production was determined using a microdissection procedure similar to that of Wimber²⁰ and Clark.²¹ Segments were fixed in cold Carnoy's solution for 24 hours, and rehydrated by gentle rinsing for 10 minutes each in decreasing concentrations of alcohol (70, 50, and 30%). The tissue was then hydrolysed for five minutes in 1.0 N HCl at 60°C, and stopped by immersing in ice cold Schiff's reagent. After incubating in cold Schiff's reagent for two hours in the dark, the stained tissue was transferred to 15% acetic acid under a dissecting microscope and crypts were dissected free, placed on slides, and gently squashed as described by Wimber *et al.*¹⁹ Total number of mitotic cells per crypt was then counted under a light microscope to determine crypt cell production. A minimum of 30 crypts per segment were counted.

All data were compared between the four groups of animals using two way analysis of variance for each of the two segments.

Results

All animals were checked daily for any adverse effects or diarrhoea; none were observed. Starting weight in all groups was 180 ± 9 g; final weights were 261 ± 10 g in sham treated animals, 279 ± 17 g in sham placebo animals, 258 ± 13 in resected treated animals, and 247 ± 24 in resected placebo animals.

Mucosal weight values are shown in Figure 1. In

Fig. 1 Mucosal weight values expressed as gram mucosa per centimetre of bowel. Resected animals are labelled R, sham operated are labelled S. Solid bars indicate animals treated with 16,16-dimethyl-PGE₂, and hatched bars indicate untreated controls. Standard deviations are shown atop each bar. Asterisks above bars indicate treated animals differ from untreated controls by at least $p < 0.05$.

Fig. 3 Mucosal DNA per centimetre of bowel. Resected animals are labelled R, sham operated are labelled S. Solid bars indicate animals treated with 16,16-dimethyl-PGE₂, and hatched bars indicate untreated controls. Standard deviations are shown atop each bar. Asterisks above bars indicate treated animals differ from untreated controls by at least $p < 0.05$.

Fig. 2 Mucosal protein per centimetre of bowel. Resected animals are labelled R, sham operated are labelled S. Solid bars indicate animals treated with 16,16-dimethyl-PGE₂, and hatched bars indicate untreated controls. Standard deviations are shown atop each bar. Asterisks above bars indicate treated animals differ from untreated controls by at least $p < 0.05$.

the proximal segment (Proximal jejunum from ligament of Treitz to be halfway to the anastomosis), PGE increased mucosal mass in both resected and sham operated animals. In the distal segment (mid and distal jejunum extending proximal from the anastomosis halfway to the ligament of Treitz), resected animals showed increased mucosal mass when compared with sham operated animals but no augmentation of this increase was induced by the drug.

Mucosal protein is shown in Figure 2. Results per centimetre bowel paralleled mucosal weight data, with PGE induction of mucosal protein in shams and PGE augmentation of the normal increase in resected animals, but only in the proximal segment.

Mucosal DNA values per centimetre bowel are shown in Figure 3. PGE administration resulted in an increase in DNA concentrations in both resected and sham operated animals in the proximal but not in the distal segment. When data were expressed per milligram protein (Fig. 4) no differences were observed in the proximal bowel of resected animals. Changes in mucosal mass induced by PGE in these animals were therefore those of hyperplasia (increase in cell number) and not hypertrophy (increase in cell mass).

Fig. 4 Mucosal DNA expressed per milligram mucosal protein. Resected animals are labelled R, sham operated animals are labelled S. Solid bars indicate animals treated with 16,16-dimethyl- PGE_2 , and hatched bars indicate untreated controls. Standard deviations are shown on top each bar. Asterisks above bars indicate treated animals differ from untreated controls by at least $p < 0.05$.

Fig. 5 Mucosal maltase concentrations expressed as μmoles glucose generated per centimetre of intestine per minute. Resected animals are labelled R, sham operated animals are labelled S. Solid bars indicate animals treated with 16,16-dimethyl- PGE_2 , and hatched bars indicate untreated controls. Standard deviations are shown on top each bar. Asterisks above bars indicate treated animals differ from untreated controls by at least $p < 0.05$.

Mucosal maltase concentrations per centimetre bowel are shown in Figure 5. In the proximal segment, maltase activity was increased in resected animals given the drug when compared with untreated resected animals. These changes were not induced in sham operated animals. In the distal segment, the drug enhanced maltase concentrations in resected animals. In untreated animals, however, maltase concentrations were not altered.

Leucine uptake by intestinal rings in the proximal segment of resected animals is shown in Figure 6. Uptake was significantly greater in treated animals at the concentrations of 2, 5, and 20 mmol/l. No difference was noted at 10 mmol/l, probably because of the large variation in values obtained. Apparent K_m and J_{max} for leucine in the proximal segment of resected groups are shown in Table 1. Leucine uptake did not differ significantly between the two groups of resected animals in the distal segment.

Glucose uptake at various concentrations by intestinal rings in the proximal segment of resected groups is shown in Figure 7. Glucose uptake did not differ at any concentration between PGE_2 treated and untreated resected animals. Apparent K_m and J_{max} of glucose in the proximal segment of resected

animals is shown in Table 1. As no significant hyperplasia occurred in the distal segment, uptake data for that segment are not shown.

Fig. 6 Leucine uptake by intestinal rings in the proximal segment of resected animals. Treated refers to animals treated with 16,16-dimethyl- PGE_2 . Standard deviations are shown for each point. Asterisks above each point indicate values at that point differ between untreated and treated groups by at least $p < 0.05$.

Table 1 Apparent K_m and J_{max} of leucine in the proximal jejunum of PGE treated and untreated control resected rats

	Leucine K_m	Leucine J_{max}	Glucose K_m	Glucose J_{max}
PGE treated	8.5	21.9	28.9	85.6
Untreated	3.8	10.4	27.8	85.6

Table 2 Crypt cell production rates expressed as metaphase per crypt (SE)

	Proximal	Distal
Resected - PGE treated	40 (4.1)	49 (4.8)
Resected - untreated	48 (3.8)	50 (5.2)
Sham operated - PGE treated	46 (1.4)	48 (2.4)
Sham operated - untreated	47 (3.1)	44 (2.8)

Crypt cell production rates for each group of animals in proximal and distal segments are shown in Table 2. No differences were noted between any of the groups.

Discussion

16,16-dimethyl-prostaglandin- E_2 effectively augmented mucosal hyperplasia in the proximal segment of small intestine in these animals after 70% distal small bowel resection. The changes observed were those of hyperplasia as changes in DNA paralleled those of protein and mucosal weight. Enhancement of mucosal mass in non-resected animals in the proximal small intestine were similar to that reported by others.¹²

The beneficial effects of this agent on mucosal mass

Fig. 7 Glucose uptake by intestinal rings in the proximal segment of the PGE treated and untreated resected animals. Standard deviations are shown for each point. No significant differences occurred at any of the points.

appeared to translate into improved digestive and absorptive function in the proximal bowel. Mucosal maltase concentrations were increased per centimetre bowel in proximal and distal segments in resected animals treated with the drug. Leucine uptake likewise was greater at several concentrations in resected animals treated with PGE than in untreated animals. As this uptake was expressed per gram mucosa, uptake per centimetre bowel would be increased to a greater level, given the increased mucosal mass induced by PGE in treated animals. Although glucose uptake per gram mucosa did not increase in treated resected animals, this finding is of major significance in light of greater mucosal mass per centimetre of bowel in the treated resected animals, as it indicates net increased glucose uptake. Transport per gram mucosa of glucose and amino acids is usually decreased in hyperplastic mucosa suggesting that cells undergoing hyperplastic growth are less mature or do not allow transport as well as 'normal' homeostatic cells.²¹ Unlike the hyperplasia associated with surgery alone, PGE effectively augmented both mucosal mass and net absorption. This hyperplastic effect occurred in proximal jejunum only, as in the sham operated animals.

A complete study of the effect of this agent on the cell life cycle is beyond the scope of this study. Our study of crypt cell production suggests that PGE acts by prolonging cell survival rather than increasing cell production. Previous studies using a different synthetic prostaglandin, 15(R)-15-methyl-PGE₂, in gastric mucosa have suggested increased cell lifespan as the mechanism by which this similar agent increases mucosal mass in that organ.^{23,24} In man, 15(R)-15-methyl-PGE₂ results in increased thickness of the gastric mucosa, most likely by retarding senescence and exfoliation of epithelial cells.²⁵ In order to further define this possibility, more studies of cell migration and turnover at multiple times postoperatively would be required. Most likely, changes in rate of cell production would be observed at two to four days after surgery. As our study of crypt cell production was done at a time when previous studies suggest that the adaptation process is nearly complete, it is possible that measurement of this factor at an earlier time postoperatively would have yielded different results.

The exact mechanism by which PGE augments mucosal hyperplasia in our animals is unclear. Prostaglandins are known to have wide ranging effects on the gastrointestinal tract, many of which could contribute to this effect.²⁶⁻³³ 16,16-dimethyl-PGE₂ is a synthetic prostaglandin having more biological activity than most naturally occurring analogues. When a given tissue is in a proliferative state, either hyperplastic or neoplastic, the presence

of high quantities of prostaglandins is a common occurrence. This increased concentration of tissue prostaglandins is thought to be a contributing factor to cell proliferation.^{34,36} Likewise, hyperplastic and neoplastic tissues in a steady state do not contain excess prostaglandins. Drug induced inhibition of prostaglandin synthesis inhibits the proliferative state in carcinoma tissue,³⁷ and also inhibits to some degree the mucosal adaptation process in resected rats.³⁸ Prostaglandins may therefore play a role in mediating the high cell turnover rate after resection.

Of interest is the fact that the augmentation of mucosal adaptation in our study occurred predominantly in the proximal segment of the remaining small intestine distal to the anastomosis. This raises the question of whether gastric antisecretory activity or perhaps alteration in pancreatic secretory activity or enzyme production might have influenced the adaptation process. In addition, the agent might have caused release of a trophic hormone specific for the proximal small intestine, as hyperplasia was noted in resected and sham operated animals.

The ability of PGE to augment mucosal adaptation suggests that certain prostaglandins might be useful in the treatment of short bowel syndrome. As one of their common side effects is diarrhoea, their usefulness may be limited. It is interesting, however, that the increased mucosal mass in the proximal small intestine was associated with increased uptake of glucose and leucine. In untreated resected animals, uptake of these substances does not increase uniformly after surgery alone.²² Prostaglandins may, therefore, have significant therapeutic potential in enhancing mucosal function as well as mucosal mass in the short bowel situation. Prolonged studies as well as studies defining the effects of discontinuing the drug would be required to better evaluate this potential. Further investigation into this and other potential therapeutic agents in augmenting mucosal hyperplasia in short bowel syndrome is thereby warranted.

This study was partially funded by a grant from Travenol Laboratories. PGE was provided by Upjohn Laboratories.

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