Increased jejunal prostaglandin E₂ concentrations in patients with acute cholera

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SUMMARY Supraphysiologic doses of prostaglandins (PGs) mimic the effect of cholera toxin and cAMP in the small intestine, but not all observations are explicable in terms of the theory that links PGs to cAMP. Because no data exist on endogenous PGs in human cholera we measured PGE₂ concentrations in jejunal fluids and fasting intestinal flow rates of PGE₂ during slow marker perfusion of proximal jejunum in nine patients with high purging cholera. Nine patients in the recovery phase of cholera or other watery diarrhoeas served as controls. In acute cholera PGE₂ concentrations were significantly (p<0.001) raised (172-1435 (n=9) vs 60-270 (n=9) pg/ml) and negatively correlated (r=0.71; p<0.05) to the time following onset of diarrhoea. Also fasting jejunal flow rates of PGE₂ were significantly (p<0.005) increased (0.77-8.22 (n=7) vs 0.21-0.92 (n=6) ng/min), and positively correlated (r=0.84; p<0.01) to stool output (2.9-9.5 ml/min). By extrapolation, at normal stool output fasting jejunal flow rates of PGE₂ equalled those measured during convalescence. The results support the notion that PGs, in addition to cAMP, may play a pathophysiologic role in human cholera. As the ratio between the medians of the highest values measured during the acute phase of cholera and in late convalescence was at least 15, local intestinal PGE₂ formation in full blown cholera should result in mucosal PGE₂ concentrations above those required for a maximal secretory response. This observation might explain why conventional doses of aspirin and indomethacin had no significant antidiarrhoeal effect in clinical trials.

The diarrhoea of cholera is considered to result from stimulation of a cAMP-mediated active secretory mechanism. Although the secretory events caused by prostaglandins (PGs), primarily of the E type, are similar in many respects to those caused by cholera toxin it has been generally accepted that their role in intestinal secretion is secondary, rather than primary. Most studies, however, do not distinguish between physiologic and supraphysiologic concentrations of PGs although intestinal secretion secondary to an increase in mucosal cAMP can be obtained only with supraphysiologic concentrations of exogenous PGE₂. In contrast, evidence of secretory effects is observed with concentrations 100-1000 times lower than those required to affect the adenylatecyclase activity, provided that artificial in vitro PG formation is suppressed by indomethacin. Furthermore, anti-inflammatory compounds, such as aspirin and indomethacin given in therapeutic doses, prevent the secretory effects of cholera toxin in experimental animals, probably by inhibiting PG biosynthesis. Besides inhibiting secretagogue-stimulated secretion aspirin and indomethacin also promote absorption in the small intestine.

In an attempt to explain why treatment with aspirin or indomethacin had no significant anti-diarrhoeal effect on patients with cholera in controlled clinical trials performed at The International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDRB; unpublished observations) we decided to measure the increase, if any, in fasting jejunal flow rates of PGE₂ during secretPHYLOGENIC stimulus – that is, PGE₂ concentrations × flow rate of fluid – in patients with acute cholera. Although the amount of PGs in the intestinal fluids represents an overflow from the mucosal cells we measured PGE₂ released into the lumen, rather than its concentration in mucosal...
biopsies, to avoid influence of artificial in vitro PG formation and interference by PGs originating in endothelial cells or platelets.  

A slow marker perfusion technique was used for determination of the fasting jejunal flow rate of PGE₂ in patients with acute cholera. The results were compared with those obtained in patients during late convalescence of cholera and other watery diarrhoeas.

**Methods**

**Patients**

**Acute cholera.**

Adult male patients presenting to the ICDDR,B treatment centre were eligible for the study if they met the following criteria: (a) onset of watery diarrhoea less than 24 hours before admission, (b) *V. cholerae* in the stool as judged by dark-field microscopy, (c) moderate to severe dehydration, and (d) no history of current antibiotic use. Patients who gave their informed and signed consent were admitted to the study which was conducted in accordance with the Helsinki Declaration II and approved by the Institutional Ethical Committee of ICDDR,B.

After admission intravenous rehydration was started with a solution containing sodium 133 mmol/l, potassium 13 mmol/l, chloride 98 mmol/l, and acetate 48 mmol/l. During the study period patients were not given any medication and nothing by mouth was allowed. Stool losses in hospital were replaced with equal volumes of intravenous fluid.

A diagnosis of cholera was established by a positive bacteriologic culture. A fresh faecal specimen was cultured for *Salmonellae*, *Shigellae*, *Vibrios*, and *Campylobacter jejuni*. *Vibrio cholerae* 01 was identified by colony appearance on taurocholate-tellurite gelatine agar, by biochemical characterisation, and by agglutination with polyvalent anti-serum against *V. cholerae* 01. The 0-forms were identified with mono-specific antisera. The El Tor and the classical biotypes were distinguished by their susceptibility to polymyxin B (50 U), to Mukerjee’s group IV cholera-phage, and to chicken erythrocyte agglutination.

Nine patients who fulfilled the criteria for entry were included in the study. All had a positive culture for *V. cholerae* 01. Eight of these patients had classical strains and one had an El Tor strain. Five patients had an infection with the Ogawa serotype and four with the Inaba serotype.

**Late convalescence**

Nine patients were studied in the convalescent phase of their diarrhoeal disease 9–12 days after the acute episode. Three out of the nine patients, who were studied in the acute phase, returned to the hospital for follow up investigation. Three patients had suffered from cholera 10–12 days earlier, but could not be studied in the acute phase. Two patients had recovered from an infection with *V. cholerae* non-01 and one patient had had a culture negative diarrhoeal episode.

**Perfusion technique**

On the day of admission, after initial rehydration, a double-lumen polyvinyl perfusion tube with a terminal rubber bag containing 1 ml of metallic mercury was passed by the nose into the duodenum. The perfusion tube consisted of an aspiration port 25 cm distal to the infusion port, which was positioned beyond the ligament of Treitz, under fluoroscopic control. Fasting jejunal fluids were obtained by siphonage from the aspiration port before constant flow perfusion (0-5 ml/min) with saline, containing sodium-sulfobromophthalein (BSP) (The Vitarine Co, Inc, New York, NY) 15 mg/100 ml as a non-absorbable marker. The perfusion solution entered the jejunal lumen at the infusion port and after a 60 min equilibration period two successive 15 minute samples were obtained from the aspiration port. All samples of jejunal fluids were immediately stored at –20°C for analysis.

Aspiration of fasting jejunal fluids was carried out in all patients, and slow marker perfusion in seven patients with acute cholera and in six patients studied in the convalescent phase of their disease.

**Analytical procedures**

BSP was determined by the method of Seligson and Marino.  

**Calculations and statistics**

The fasting jejunal flow rate was calculated from the infusion rate (0·5 ml/min) multiplied by the ratio of BSP concentration in the perfusion solution to the mean concentration in the two aspirated samples.

The Wilcoxon’s rank sum test for unpaired data was applied to the experimental observations for statistical comparison and regression analysis was made by the method of least squares. A p value less than 0·05 was taken to indicate statistical significance.

**Results**

Patients with acute cholera were studied 17–36
hours after the onset of diarrhoea. Over the eight hours before aspiration of fasting jejunal fluids and slow marker perfusion purging rates were high in all patients and ranged from 175–570 ml per hour (median, 415 ml/h), corresponding to 4·2–13·7 litres per day (Table).

The medians and the ranges of PGE₂ concentrations in jejunal fluids aspirated before and during the perfusion procedure were almost identical (Table), but a significant (p<0·05) negative correlation (r=−0·71; p<0·05) was observed between the means of all values obtained in each patient and the time following onset of diarrhoea (Fig. 1).

The medians of the lowest and the highest values (490 and 990 pg/ml) obtained in patients with acute cholera were significantly (p<0·001) raised compared with the corresponding values (115 and 190 pg/ml) observed in the convalescent phase of cholera and other diarrhoeal diseases (Table). A significant (p<0·01) difference was observed even between the median of the lowest values obtained in patients during the acute phase of cholera and that of the highest values registered during late convalescence.

Jejunal perfusions were accomplished in seven of the nine patients with acute cholera, and control perfusions were carried out in six patients (Table). Marker perfusion was not successful in some patients because of power failure during steady state perfusion or insufficient recovery at the aspiration port and in two cases the test tubes were broken on arrival in Denmark. The fasting jejunal flow rate of PGE₂—which is PGE₂ concentration × flow rate—varied from 0·7–8·2 ng/min in acute cholera and from 0·2–0·9 ng/min in late convalescence. Again the medians of the lowest and the highest values observed in each of the two groups of patients differed markedly. Also the median of the lowest values in acute cholera was significantly (p<0·01) raised compared with that of the highest values in controls.

Figure 2 illustrates that there was a significant positive correlation (r=0·84; p<0·01) between the means of values obtained in each patient and the stool output during eight hours before jejunal perfusion. By extrapolation, at normal stool output the fasting jejunal flow rate of PGE₂ was similar to values measured by the perfusion technique in convalescent patients.

Table  PGE₂ concentrations in fasting jejunal fluids, fasting jejunal flow rates, and stool output in acute cholera and in convalescent phase of cholera and other watery diarrhoeas

<table>
<thead>
<tr>
<th></th>
<th>Preperfusion</th>
<th>Min*</th>
<th>Max*</th>
<th>Flow rate (ml/min)</th>
<th>PGE₂ × flow rate (ng/min)</th>
<th>Stool output (ml/min)</th>
</tr>
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<tr>
<td><strong>Acute cholera</strong></td>
<td></td>
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</tr>
<tr>
<td>Median</td>
<td>483</td>
<td>400</td>
<td>990</td>
<td>5·2</td>
<td>2·11</td>
<td>5·17</td>
</tr>
<tr>
<td>Range</td>
<td>175–1435</td>
<td>172–885</td>
<td>421–1435</td>
<td>3·5–6·9</td>
<td>0·77–4·60</td>
<td>2·00–8·22</td>
</tr>
<tr>
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<td>(9)</td>
<td>(9)</td>
<td>(9)</td>
<td>(7)</td>
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<tr>
<td></td>
<td>p&lt;0·01</td>
<td></td>
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<tr>
<td><strong>Convalescence</strong></td>
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<tr>
<td>Median</td>
<td>155</td>
<td>115</td>
<td>190</td>
<td>3·2</td>
<td>0·24</td>
<td>0·38</td>
</tr>
<tr>
<td>Range</td>
<td>60–270</td>
<td>60–270</td>
<td>100–270</td>
<td>1·8–4·2</td>
<td>0·21–0·92</td>
<td>0·22–0·92</td>
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<tr>
<td>No of patients</td>
<td>(9)</td>
<td>(9)</td>
<td>(9)</td>
<td>(6)</td>
<td>(6)</td>
<td>(6)</td>
</tr>
<tr>
<td>p less than</td>
<td>0·002</td>
<td>0·001</td>
<td>0·0005</td>
<td>0·02</td>
<td>0·005</td>
<td>0·005</td>
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* Min and max denote median of lowest and highest values, respectively, measured before and during slow marker perfusion.
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Discussion

Both cholera toxin and prostaglandins of the E type added to isolated sheets of intestinal mucosa stimulate secretion and increase mucosal adenylate-cyclase activity and cAMP concentration.¹ ³ ⁴ The demonstration of the named in vitro effects by supraphysiologic concentrations of exogenous PGs,³ ⁴ however, adds little to the understanding of the pathophysiologic role of PGs in patients infected with V cholerae.⁶ ¹³ ¹⁶ Because the literature contains no data on endogenous PGs in patients with cholera we found it of interest to compare intestinal PGE₂ concentrations in the acute phase of the disease with those obtained in late convalescence of cholera and other watery diarrheas. The relevance of measuring endogenous PGs was further emphasised by recent experiments,²¹ which provide support to the view that cholera toxin induces secretion, in part at least, by activation of intramurally located nervous reflex mechanisms, involving the release of 5-hydroxy-tryptamine which may lead to hydrolysis of membrane phospholipids and release of arachidonic acid and its metabolites.¹³ ¹⁶ ²²

In the present study we measured PGE₂ concentra-
tions in the jejunal fluids simultaneously with the jejunal flow rate to allow evaluation of the amount of PGE₂ released by the mucosa.²² Our observations that not only jejunal flow rates of PGE₂ but also the concentrations of PGE₂ in the jejunal fluids are markedly raised in acute cholera suggest a pathophysiologic role of PGs in cholera toxin induced secretion. This impression is strengthened by the demonstration of a significant inverse correlation between PGE₂ concentrations and the time following onset of diarrhoea. Even more impressive is the significant correlation between jejunal flow rate of PGE₂ and stool output (Fig. 2).

Indirect evidence of a primary role for PGs in cholera toxin induced secretion would be that treatment with PG synthetase inhibitors effectively reduced intestinal PG formation and fluid loss in acute cholera. Such an effect has not been shown. Controlled clinical trials conducted at ICDDR,B to study the antisecretory effect of anti-inflammatory drugs were unable to reveal a significant reduction of fluid loss in 22 and 20 adult cholera patients treated with aspirin (25 mg/kg/day; Islam A et al, unpublished data) and indomethacin (initially 150 mg and 50 mg after eight hours; Rabinii GH et al, unpublished observations), respectively. Thus the only clinical studies on the effect of aspirin and indomethacin performed in patients with cholera conflict with numerous experimental studies showing that anti-inflammatory agents inhibit the secretory effects of cholera toxin in vitro.⁷ ⁹ ¹¹–¹² ¹⁴–¹⁵ although a primary role for PGs in the action of the toxin has been questioned.³ ⁵ ²⁴–²⁵ Probability levels in accepting double-blind clinical trials with a low number of patients as negative should be appreciated, however, and indirect information derived from studies based on inhibition of PG biosynthesis should be critically evaluated, because cyclooxygenase inhibitors may interact with PGs at multiple sites in the PG cascade, whose specific end-product may have oppositely directed effects or have other effects than suppression of arachidonic acid metabolism.¹⁶

It is of interest, nevertheless, that soluble aspirin (25 mg/kg/day), given by mouth in a double blind trial to a mixed group of 31 infants and children with the clinical diagnosis of gastroenteritis, significantly reduced intestinal fluid loss and enhanced weight gain.²⁶ Similarly, indomethacin (4-5 mg/kg/day) administered orally to 270 infants and children with diarrhoea in an open study was reported to have a beneficial effect on fluid loss.²⁷

The PGE₂ concentrations in jejunal fluid from convalescent patients in the present study were low and comparable with those previously obtained in Scandinavian healthy volunteers (99% confidence
limits, 5–205 pg/ml; n=22). Also measurements of jejunal flow rates of fluid in acute cholera and convalescent patients were similar to those previously reported by Banwell and coworkers. Finally, PGE2 concentrations measured in stool water from five patients in the present study with acute cholera were within the normal range observed in 10 Scandinavian healthy volunteers (103–188 pg/ml). In previous studies on the role of PGs in diarrhoeal diseases, we have shown a positive correlation between PGE2 of the intestinal fluids and stool volume in patients with secretory diarrhoea, sensitive to indomethacin treatment, and that luminal PGE2 concentrations appear to be independent of the diarrhoea per se, as shown by subnormal values in – for example, pancreatic cholera and disaccharidase intolerance.

The lack of a significant clinical effect of aspirin and indomethacin on fluid loss in cholera patients might be because of insufficient suppression of an extremely high local intestinal PG production. Our findings of increased luminal PGE2 concentrations in the present study are inconclusive because PGE2 in the jejunal fluids merely reflects an overflow from the intestinal mucosa. Nevertheless, it appears reasonable to assume that the jejunal flow rate of PGE2 parallels the local production and thus mucosal PGE2 concentrations. A ratio of at least 15 (5,17/0.38; see Table) between the medians of the highest values of jejunal flow rates of PGE2 measured in the acute phase and during late convalescence, respectively, means that PG formation in acute cholera may result in mucosal concentrations higher than those required for a maximal secretory response, even following conventional doses of aspirin and indomethacin, because the slope of the dose-response curve occurs within 1-5 decades.

Although our data are correlative in nature they suggest a role of PGs, in addition to cAMP, in human cholera. It may be speculated that both cAMP and PGs are intracellular mediators for the stimulus secretion coupling via intracellular free Ca. Cyclic nucleotides could act by mobilising stored Ca within the epithelial cells and PGs by increasing Ca gating across the basolateral membrane. This idea of a final common pathway of diarrhoea production would imply a more holistic approach to symptomatic treatment of cholera with anti diarrhoeal drugs.

We wish to thank Anne Rasmussen and Lene Pernson for their valuable technical assistance and Rigmor Petersen for secretarial assistance. This Study was financially supported by The International Centre for Diarrhoeal Disease Research, Bangladesh, and was funded in part by Janssen Pharmaceutical Ltd. and The Smith, Kline & French’s Fund for Nordic Gastroenterologists.

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