

# Indomethacin decreases jejunal fluid secretion in addition to luminal release of prostaglandin E<sub>2</sub> in patients with acute cholera

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## Abstract

Human cholera is associated with an increased luminal release of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), but whether inhibition of increased PGE<sub>2</sub> synthesis will reduce or control intestinal secretion is uncertain. 'Steady state' perfusions (10 ml/minute) in 12 patients with acute cholera, and repeat perfusions in nine of these patients during the convalescent phase were therefore performed using the triple lumen technique. The proximal jejunum was perfused with isotonic saline containing sodium-sulphobromophthalein as a non-absorbable marker. After intravenous administration of indomethacin (1.0 mg/kg) the jejunal net transfer of fluid and the jejunal flow rate of PGE<sub>2</sub> were determined in 30 minute periods for 120 minutes after a 120 minute control period. Indomethacin decreased net fluid secretion (2.1 (0.3-4.2) v 4.5 (2.5-8.4) ml/hour×cm; medians, Q<sub>50</sub> ranges, p<0.01) and the jejunal flow rate of PGE<sub>2</sub> (1.5 (1.2-2.7) v 2.2 (1.4-4.9) ng/minute, p<0.05). The results of similar perfusion studies in 22 patients with acute cholera, used to establish the spontaneous time related change in fluid secretion, showed no significant change in net fluid transfer (3.5 (2.2-6.2) to 3.5 (2.6-11.6) ml/hour×cm, p>0.25) over 240 minutes. These data provide further evidence in favour of the hypothesis that prostaglandins have a role in the cholera toxin induced intestinal fluid secretion in man.

The diarrhoea caused by *Vibria cholerae* is generally considered to rely solely on a cyclic adenosine monophosphate mediated active secretory mechanism activated by an enterotoxin, which has convincingly been shown to stimulate active fluid production from small intestinal loops of experimental animals.<sup>1</sup> As early as 1971 it was suggested that cholera enterotoxin may cause diarrhoea by stimulating prostaglandin synthesis,<sup>2</sup> since non-steroidal anti-inflammatory compounds such as aspirin and indomethacin prevent the secretory effects of cholera enterotoxin in animal experiments.<sup>3-5</sup> A recent study in cholera patients has shown appreciably increased concentrations of luminal prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), which were negatively correlated with the time after the onset of diarrhoea and positively correlated with the stool output.<sup>6</sup> On the other hand, controlled clinical trials on the efficacy of non-steroidal anti-inflammatory compounds have shown no beneficial effects on the diarrhoea of acute cholera.<sup>7</sup> These observations may be explained by increased local intestinal PGE<sub>2</sub> production in

severe cholera resulting in mucosal PGE<sub>2</sub> concentrations above those required for a maximal secretory response<sup>8</sup> or, alternatively, by a maximal cholera toxin induced stimulation of the secretory system, which cannot be further enhanced by increasing the production of prostaglandins.<sup>1</sup> Since it still remains to be shown that inhibition of increased prostaglandin synthesis in human cholera will reduce or control intestinal secretion, we performed 'steady state' perfusions of the jejunum in 12 patients with acute cholera to clarify whether indomethacin, in addition to inhibition of prostaglandin synthesis, would reduce fluid secretion in human cholera.

## Methods

### PATIENTS

Men (median age 36 years, range 25-43) presenting to the International Centre for Diarrhoeal Disease Research, Bangladesh (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) a purging rate above 5 ml/kg/hour, (c) *V cholerae* in the stool as judged by dark field microscopy, (d) moderate to severe dehydration, and (e) no history of current antibiotic use. Patients who gave informed written 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.

On admission to hospital, 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 or anything to eat or drink. Stool losses in hospital were replaced with equal volumes of intravenous fluid.

A diagnosis of cholera was established by a positive bacteriological culture. A fresh faecal specimen was cultured for *Salmonella*, *Shigella*, *Vibrios*, and *Campylobacter jejuni*. *V cholerae* 01 was identified by colony appearance on taurocholate-tellurite gelatine agar, by biochemical characterisation, and by agglutination with polyvalent anti-serum against *V cholera* 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.

Twelve patients who fulfilled the criteria for entry were included in the study. All had a positive culture for *V cholerae* 01. Two of these

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patients had classical strains and 10 had an El Tor strain. Nine patients had an infection with the Ogawa serotype and three with the Inaba serotype. Nine of the patients returned to the hospital for study in the convalescent phase a fortnight after the acute episode.

#### EXPERIMENTAL DESIGN

On the day of admission, after initial rehydration, 'steady state' perfusions were carried out as previously described.<sup>9,10</sup> The jejunum was intubated with a triple lumen radiopaque polyvinyl tube, the position of which was checked fluoroscopically before and after the perfusion. The infusion port was placed at the ligament of Treitz. The mixing segment was 15 cm and the test segment was 30 cm. Another tube was positioned in the antrum of the stomach and this was emptied continuously. The jejunum was perfused with isotonic saline – that is, 154 mmol/l NaCl – at a rate of 10 ml/minute using a peristaltic pump (Criticon Inc 1980, Tampa, FL, USA) for infusion and sodium-sulphobromophthalein as a non-absorbable marker (BSP; Sigma Biochemical Co, St Louis, MO, USA) at concentrations of 750 mg/l and 150 mg/l in the acute and in the convalescent phases, respectively. Fluid entering and leaving the test segment was collected by free siphonage. Recoveries of BSP at the proximal and the distal sampling ports were 12% (9–17%) and 11% (6–32%) respectively in acute cholera and 25% (19–30%) and 20% (14–33%) respectively in convalescence. Transit time was not measured because it has been shown previously that it does not differ in acute cholera and convalescence.<sup>10</sup> The same staging time of 10 minutes was employed, therefore, in acute and convalescent studies.

After a 60 minute equilibration period samples were collected every 30 minutes. The 'steady state' of perfusions was established by a low dispersion of the results<sup>11</sup> (less than 50% of net fluid transfer within single subjects based on consecutive 30 minute periods). The SD was generally 20–30% (range 13–50%), but in the convalescent phase net absorption/secretion approached zero ml/hour×cm, which is why the relative SD exceeded 50% in those having net transfer rates less than 0.7 ml/hour×cm.

After an 120 minute control period an intravenous bolus injection of indomethacin (Dumex, Copenhagen, Denmark), 1.0 mg/kg, was infused into a cubital vein over 10 minutes and sampling was continued for another 120 minute period. Because of the lag phase of drug response, and

because no equilibration period was interposed between the experimental periods, the results of the first 30 minute collection after administration of indomethacin were added to those obtained in the previous period for calculation of the average transport/flow rate in that period.

#### ANALYTICAL PROCEDURES

Sodium-sulphobromophthalein was determined by the method of Seligson and Marino<sup>12</sup> and net fluid transfer was measured as previously described in detail.<sup>9,10</sup> The values were given in ml/hour×cm. Negative values represent absorption and positive values secretion.

PGE<sub>2</sub> concentrations in aspirates were measured by a radioimmunological method<sup>13</sup> validated by gas chromatography mass spectrometry,<sup>14</sup> as previously described in detail. The jejunal flow rate of PGE<sub>2</sub> – that is, the amount of PGE<sub>2</sub> passing a cross section of the jejunum per minute – was calculated as the concentration of PGE<sub>2</sub> in the aspirate from the proximal aspiration port times the flow rate of fluid passing this port.<sup>15</sup> Transepithelial secretion rates of PGE<sub>2</sub> are not given because the differences between the amounts passing the proximal and the distal ports were negligible and not significantly different from zero ( $p > 0.05$ ). The values of PGE<sub>2</sub> are given as ng/minute or ng/ml.

#### STATISTICAL ANALYSIS

The results are given as medians with Q<sub>50</sub> ranges and were analysed by Wilcoxon's test for paired variates. Differences were considered significant at  $p < 0.05$  (2 $\alpha$ ).

#### Results

##### PATIENTS

Patients with acute cholera were studied 22–43 hours after the onset of diarrhoea. During the four hours before starting the perfusion studies, the purging rates were high in all patients and ranged from 485–1150 ml/hour (median, 735 ml/hour), corresponding to 11.6–27.6 l/day. Indomethacin did not significantly change the purging rates, which ranged from 400–1635 ml/hour (median, 725 ml/hour), corresponding to 9.6–39.2 l/day, in the four hour period after injection of indomethacin.

##### FLUID TRANSFER

Fluid was secreted into the test segment in all but one patient with acute cholera. Indomethacin transiently decreased net fluid secretion from +4.5 to +2.1 ml/cm×h ( $p < 0.01$ , see Table). The response was most pronounced in the second and the third 30 minute period and reduced in the fourth 30 minute period after administration of indomethacin.

The results from similar studies in 21 patients with acute cholera (Van Loon FPL *et al*, unpublished observations) were used to establish the spontaneous time related change in fluid secretion. No change in net fluid transfer was observed over a 240 minute study period (+3.5,

Net transfer of fluid, jejunal prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) concentrations, and jejunal flow rates (JFR) of PGE<sub>2</sub> in acute cholera before and after the administration of intravenous indomethacin (1 mg/kg) and in the convalescent phase

	No	Net fluid transfer (ml/cm×h)	PGE <sub>2</sub> (ng/ml)	JFR of PGE <sub>2</sub> (ng/min)
Acute cholera	12	+4.5 (+2.5, +8.4)	0.17 (0.11, 0.35)	2.2 (1.4, 4.9)
Acute cholera plus indomethacin	12	+2.1 (+0.3, +4.2)†	0.10 (0.08, 0.22)*	1.5 (1.2, 2.7)*
Convalescent phase	9	-0.4 (-1.2, +0.3)*‡	0.10 (0.07, 0.16)*	1.1 (0.8, 1.7)*

+ Denotes secretion, – denotes absorption.

\* $p < 0.05$  and † $p < 0.01$  compared with acute cholera.

‡ $p < 0.05$  compared with acute cholera plus indomethacin.

+2.2 to +6.2 v +3.5, +2.6 to +11.6 ml/cm×h, n=21, p>0.25).

During convalescence net fluid absorption (-0.4 ml/cm×h, see Table) was observed.

#### FLOW RATE OF PGE<sub>2</sub>

The median concentration of PGE<sub>2</sub> in aspirates from the jejunum was 0.17 ng/ml and, similarly, the jejunal flow rate of PGE<sub>2</sub> was 2.2 ng/minute in the acute phase of cholera. Indomethacin significantly decreased the concentration of PGE<sub>2</sub> (0.10 ng/ml, p<0.05) and the jejunal flow rate of PGE<sub>2</sub> (1.5 ng/minute, p<0.05, see Table) towards the level observed in late convalescence (0.10 ng/ml and 1.1 ng/minute, respectively).

#### Discussion

This study suggests a role for prostaglandins in the signal-transduction mechanism leading to intestinal secretion in acute cholera, because indomethacin significantly reduced intestinal secretion of fluid, in addition to luminal PGE<sub>2</sub> concentrations and the jejunal flow rate of PGE<sub>2</sub> in patients with acute cholera. Thus, the study confirms the observations that cholera enterotoxin increases the release of PGE<sub>2</sub> into the intestinal lumen in both experimental animals and man.<sup>6,16</sup>

In this study we measured PGE<sub>2</sub> because it is the most abundant arachidonic acid metabolite in the gastrointestinal mucosa.<sup>17</sup> The luminal release of PGE<sub>2</sub> was determined, rather than biopsy specimen contents or the synthetic capacity of mucosal biopsy specimens, because this approach is considered relatively atraumatic and minimises non-specific PGE<sub>2</sub> formation.<sup>8</sup> Although luminal PGE<sub>2</sub> probably has no specific function of its own, it provides a reliable index of the balance between mucosal synthesis and degradation in vivo.

The observations of the increased luminal PGE<sub>2</sub> concentrations and jejunal flow rate of PGE<sub>2</sub> in acute cholera suggest that PGE<sub>2</sub> release is flow-independent, thus reflecting increased mucosal PGE<sub>2</sub> synthesis. The values for the jejunal flow rate of PGE<sub>2</sub> in the acute phase of cholera were similar to those previously reported, using a 'slow marker' perfusion technique, while those observed during convalescence were higher than those reported in the previous study.<sup>6</sup> This discrepancy may be explained by PGE<sub>2</sub> formation as a result of distension of the intestinal lumen during 'steady state' perfusion (10 ml/minute v 0.5 ml/minute). Any possible artifactual PGE<sub>2</sub> formation was, however, too low to mask the changes caused by *V. cholerae*.

The observation that indomethacin transiently reduced intestinal fluid secretion, in the absence of an effect on purging rates, agrees with previous results of controlled clinical trials showing no beneficial clinical effects of indomethacin.<sup>7</sup> The lack of clinical efficacy of indomethacin in acute cholera may be explained by

the relatively low conventional dosage of indomethacin permitted in order to avoid side effects of the drug. Thus a partial, but prolonged, inhibition of cholera enterotoxin induced intestinal secretion has been shown in rat experiments using a 10 fold higher dose of indomethacin.<sup>16</sup>

We would tentatively conclude, therefore, that the present study provides further evidence favouring the hypothesis that PGE<sub>2</sub> has a role in intestinal secretion of human cholera.

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- Rabbani GH, Greenough III WB. Cholera. In: Leberthal E, Duffey ME, eds. *Textbook of secretory diarrhea*. New York: Raven Press, 1990: 233-53.
- Bennett A. Cholera and prostaglandins. *Nature* 1971; 231: 536.
- Jacoby HI, Marshall CH. Antagonism of cholera enterotoxin by anti-inflammatory agents in the rat. *Nature* 1972; 235: 163-4.
- Gots RE, Formal SB, Bianella RA. Indomethacin inhibition of *Salmonella typhimurium*, *Shigella flexneri*, and cholera-mediated rabbit ileal secretion. *J Infect Dis* 1974; 130: 280-4.
- Wald A, Gotterer GS, Rajendra GR, Turjman NA, Hendrix TR. Effect of indomethacin on cholera-induced fluid movement, unidirectional sodium fluxes, and intestinal cAMP. *Gastroenterology* 1977; 72: 106-10.
- Speelman P, Rabbani GH, Bukhave K, Rask-Madsen J. Increased jejunal PGE<sub>2</sub> concentrations in patients with acute cholera. *Gut* 1985; 26: 188-93.
- Rabbani GH, Butler T. Indomethacin and chloroquine fail to inhibit fluid loss in cholera. *Gastroenterology* 1985; 89: 1035-7.
- Rask-Madsen J, Bukhave K. The difficulties of establishing the pathophysiological role of prostaglandins in secretion. In: Skadhauge E, Heintze K, eds. *Intestinal absorption and secretion*. Lancaster: MTP Press, 1984: 453-67.
- Cooper H, Levitan R, Fordtran JS, Ingelfinger FJ. A method for studying absorption of water and solute from the human small intestine. *Gastroenterology* 1966; 50: 1-7.
- Banwell JG, Pierce NF, Mitra RC, Brigham KL, Caranasos GJ, Keimowitz RI, et al. Intestinal fluid and electrolyte transport in human cholera. *J Clin Invest* 1970; 49: 183-95.
- Rask-Madsen J. Simultaneous measurement of electrical polarization and electrolyte transport by the entire normal and inflamed human colon during in vivo perfusion. *Scand J Gastroenterol* 1973; 8: 327-36.
- Seligson D, Marino J. Sulfbromophtalein (BSP) in serum. *Standard methods of clinical chemistry*. New York: Academic Press, 1958: 186-91.
- Bukhave K, Rask-Madsen J. Prostaglandin E<sub>2</sub> in jejunal fluids and its potential diagnostic value for selecting patients with indomethacin-sensitive diarrhoea. *Eur J Clin Invest* 1981; 11: 191-7.
- Bukhave K, Green K, Rask-Madsen J. Comparison of radioimmunological determinations with gas chromatography mass spectrometry dosage. A study of PGE<sub>2</sub> and PGE<sub>2α</sub> in gastrointestinal fluids. *Biomed Mass Spectrom* 1983; 10: 265-8.
- Munck LK, Mertz-Nielsen A, Westh H, Bukhave K, Beubler E, Rask-Madsen J. Prostaglandin E<sub>2</sub> is a mediator of 5-hydroxytryptamine induced water and electrolyte secretion in the human jejunum. *Gut* 1988; 29: 1337-41.
- Beubler E, Kollar G, Saria A, Bukhave K, Rask-Madsen J. Involvement of 5-hydroxytryptamine, prostaglandin E<sub>2</sub>, and cyclic adenosine monophosphate in cholera toxin-induced fluid secretion in the small intestine of the rat in vivo. *Gastroenterology* 1989; 96: 368-76.
- Aly A, Johansson C, Slezak P, Green K. Bioconversion of arachidonic acid in the human gastrointestinal tract. *Biochem Med* 1984; 31: 319-23.