Effect of cholera toxin on the human jejunum

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Abstract

In order to develop a model for secretory diarrhoea and to confirm the in vitro effects of cholera toxin in man in vivo the effect of intrajejunally administered cholera toxin was investigated in healthy volunteers. An intestinal perfusion technique with an occluding balloon proximal to the infusion site was used. The jejunum was perfused under steady state conditions with a plasma like electrolyte solution containing polyethylene glycol as a non-absorbable volume marker. After two control periods of one hour each, during which water was absorbed at a rate of 104 (14) (mean (SEM), n=15) and 94 (15) ml/30 cm/h, respectively, three different doses of cholera toxin (6.25 µg, 12.5 µg, 25 µg) were administered by bolus into the lumen of the jejunum. Cholera toxin reduced absorption of water and electrolytes progressively over four hours and induced secretion in a dose dependent fashion. In the fourth hour net secretion amounted to 22 (23), 36 (24), and 88 (40) ml/30 cm/h (each n=five) with doses of 6.25, 12.5, and 25 µg cholera toxin, respectively. The movement of sodium, chloride, and bicarbonate paralleled water movement. Our results suggest that cholera toxin may serve as a secretory model in the human jejunum which might allow testing of new antisecretory agents.

(Gut 1992; 33: 1174-1178)

Secretory diarrhoea is an important health problem particularly in developing countries. In order to test new antisecretory agents human models for intestinal secretion need to be established. Because infection with *Vibrio cholerae* is an important cause of diarrhoea, we decided to use cholera toxin as intestinal secretagogue. Cholera toxin is the enterotoxin of *Vibrio cholerae* and while causing secretion does not impair glucose dependent sodium absorption in the intestine and does not alter mucosal histology.¹⁻³ Earlier studies with cholera toxin in man revealed an increase in stool frequency and

volume, and intestinal secretion after administration of various preparations of cholera toxin.^{4,5} The results of these studies, however, were too variable to lead to the use of cholera toxin as a secretory model. The aims of the present study were to characterise the dose response relationship for cholera toxin after intrajejunal administration, to develop a standardised model of intestinal secretion using an intestinal perfusion technique, and to expand our information on the action of cholera toxin on the human jejunum.

Methods

SUBJECTS

Fifteen healthy volunteers (nine men, six women, mean age 28 years, range 22-38) gave written informed consent to participate. None of the subjects was taking any medication. A medical history, electrocardiograph, blood pressure, and standard laboratory findings were obtained before the experiment and were found to be negative or normal. Haematocrit was also obtained 24 hours after the experiment. The study was approved by the Ethics Committee of the University of Graz School of Medicine.

INTESTINAL PERFUSION

For intestinal perfusion a tube assembly with a proximal occluding balloon was used.⁶⁻⁸ The assembly with an outer diameter of 6 mm and a mercury bag at the tip was placed in the jejunum (infusion site at the ligament of Treitz). The correct position of the tube was confirmed by fluoroscopy. Because in animal studies digestive juices may destroy cholera toxin an occluding balloon proximal to the infusion site was used in order to avoid degradation of cholera toxin by pancreatic enzymes⁹ (E Beubler, unpublished observation). The sampling site was 30 cm distal to the infusion site. A fourth lumen enabled removal of endogenous secretions proximal to the occluding balloon. Perfusion was started in the morning after a 12-16 hour fast. A plasma like electrolyte solution (composition in mmol/l: Na⁺ 135, K⁺ 5, Cl⁻ 110, HCO₃⁻ 30, and 2 g/l polyethylene glycol, mean molecular weight 3350, as a non-absorbable volume marker) was perfused at 10 ml/minute using a peristaltic pump. The solution was bubbled with a mixture of 95% O₂ and 5% CO₂. A 30 minute equilibration period, during which the samples were discarded was followed by six hours of collection. Collection was performed by hand aspiration at a rate of 1.5 ml/minute using plastic syringes. Collected samples were pooled for each hour. After two control periods of one hour each, 6.25 µg, 12.5 µg, or 25 µg purified cholera toxin (Sigma, Deisenhofen, FRG) was administered intrajejunally as a bolus without interrupt-

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Accepted for publication 20 January 1992

TABLE 1 Effect of various doses of cholera toxin (CT) on net jejunal water movement (ml/30 cm/h). Cholera toxin was administered as a bolus after two hours of control perfusion

Time (h) after CT	Intrajejunal dose of CT		
	6.25 µg	12.5 µg	25 µg
Control	-54 (24)	-118 (12)	-135 (26)
Control	-38 (22)	-104 (12)	-139 (19)
1	-39 (21)	-98 (11)	-131 (22)
2	-37 (18)	-64 (12)*	-120 (34)*
3	-17 (24)	+4 (24)*	+44 (49)*
4	+22 (23)*†	+36 (24)*†	+88 (40)*†

Quade test: *significantly different from controls, p<0.05; †significantly different from all periods up to second hour after cholera toxin, p<0.05. Values are means (SEM), n=five, (-) denotes absorption, (+) denotes secretion.

ing the intestinal perfusion. The perfusion was continued for another four hours. The transmural potential difference was measured between a NaCl filled subcutaneous cannula on one forearm and the luminal contents by means of calomel electrodes, using agar bridges between the cannula and the perfusion tube.

During the entire study the subjects electrocardiograph was displayed on a monitor. Pulse and blood pressure were measured every 20 minutes. After the experiment subjects were asked to drink sufficient liquids and to record frequency of bowel movements and to collect all stools for 24 hours.

ANALYSIS OF SAMPLES AND CALCULATIONS

Intestinal samples were analysed for Na⁺, Cl⁻, K⁺, and HCO₃⁻ (as total CO₂) using an automated analyser (Synchron Clinical System CX3, Beckman, USA). Polyethylene glycol was determined according to the method of Hyden.¹⁰ Absorption and secretion rates in the test segment were calculated from the perfusion rate and the changes in polyethylene glycol and electrolyte concentrations.¹¹

STATISTICAL ANALYSIS

Results are expressed as means (SEM). Statistical analysis was performed using either the parameter free Quade test or the Kruskal-Wallis H-test as was appropriate. A p value of less than 0.05 was regarded as significant.

Results

EFFECTS OF CHOLERA TOXIN ON NET WATER AND ION TRANSPORT

During the two control periods absorption of water (104 (14) and 94 (15) ml/30 cm/h, n=15, respectively) and electrolytes was recorded in all subjects except in one who secreted. Jejunal secretion is, however, known to occasionally occur in healthy subjects.¹² The secretory response to cholera toxin developed gradually. The first changes of net water and ion transport were observed after the second or third hour after administration of cholera toxin. The maximal effect during the four hour experiment occurred in the fourth hour after administration of cholera toxin (Tables I-V).

Cholera toxin caused dose dependent changes

TABLE II Effect of various doses of cholera toxin (CT) on net jejunal Na⁺ transport (mmol/30 cm/h). Cholera toxin was administered after two hours of control perfusion

Time (h) after CT	Intrajejunal dose of CT		
	6.25 µg	12.5 µg	25 µg
Control	-6.3 (3.8)	-14.6 (1.3)	-16.4 (3.3)
Control	-4.2 (3.2)	-13.2 (1.8)	-16.5 (2.4)
1	-4.1 (3.0)	-11.6 (1.4)	-15.1 (2.8)
2	-4.0 (3.0)	-7.8 (1.8)*	-7.6 (4.4)*
3	-0.2 (4.1)	+2.5 (3.0)*	-11.2 (7.0)*
4	+5.7 (3.8)*†	+6.8 (3.4)*†	+17.0 (5.7)*†

Quade test: *significantly different from controls, p<0.05; †significantly different from all periods up to second hour after cholera toxin, p<0.05. Values are means (SEM), n=five, (-) denotes absorption, (+) denotes secretion.

TABLE III Effect of various doses of cholera toxin (CT) on net jejunal Cl⁻ transport (mmol/30 cm/h). Cholera toxin was administered as a bolus after two hours of control perfusion

Time (h) after CT	Intrajejunal dose of CT		
	6.25 µg	12.5 µg	25 µg
Control	-1.3 (2.9)	-5.7 (1.4)	-7.8 (2.9)
Control	+0.6 (2.1)	-4.8 (1.5)	-7.6 (2.3)
1	+0.7 (1.6)	-5.1 (0.9)	-6.9 (2.0)
2	+0.9 (2.3)	-3.3 (1.1)	-2.3 (2.4)*
3	+2.9 (2.8)*	+2.3 (1.1)*	+8.7 (5.3)*
4	+6.8 (2.5)*†	+4.8 (1.8)*†	+14.6 (4.8)*†

Quade test: *significantly different from controls, p<0.05; †significantly different from all periods up to second hour after cholera toxin, p<0.05. Values are means (SEM), n=five, (-) denotes absorption, (+) denotes secretion.

TABLE IV Effect of various doses of cholera toxin (CT) on net jejunal K⁺ transport (mmol/30 cm/h). Cholera toxin was administered as a bolus after two hours of control perfusion

Time (h) after CT	Intrajejunal dose of CT		
	6.25 µg	12.5 µg	25 µg
Control	-0.34 (0.13)	-0.76 (0.04)	-0.76 (0.11)
Control	-0.30 (0.10)	-0.72 (0.07)	-0.82 (0.10)
1	-0.30 (0.11)	-0.62 (0.06)	-0.76 (0.14)
2	-0.36 (0.11)	-0.48 (0.07)*	-0.56 (0.21)*
3	-0.30 (0.13)	-0.16 (0.12)*	+0.04 (0.31)*
4	-0.14 (0.10)	-0.06 (0.11)*†	+0.20 (0.26)*

Quade test: *significantly different from controls, p<0.05; †significantly different from all periods up to second hour after cholera toxin, p<0.05. Values are means (SEM), n=five, (-) denotes absorption, (+) denotes secretion.

TABLE V Effect of various doses of cholera toxin (CT) on net jejunal HCO₃⁻ movement (mmol/30 cm/h). Cholera toxin was administered as a bolus after two hours of control perfusion

Time (h) after CT	Intrajejunal dose of CT		
	6.25 µg	12.5 µg	25 µg
Control	-4.6 (0.7)	-8.8 (0.4)	-8.8 (1.3)
Control	-4.0 (1.0)	-8.2 (0.5)	-8.9 (1.3)
1	-4.1 (1.1)	-7.0 (1.0)	-8.5 (2.0)
2	-3.9 (1.0)	-4.2 (1.4)*	-5.3 (2.5)
3	-2.1 (1.6)	-0.2 (2.1)*	+1.2 (3.2)*
4	-0.1 (1.6)*†	+1.8 (1.8)*†	+2.5 (2.3)*†

Quade test: *significantly different from controls, p<0.05; †significantly different from all periods up to second hour after cholera toxin, p<0.05. Values are means (SEM), n=five, (-) denotes absorption, (+) denotes secretion.

TABLE VI Effect of various doses of cholera toxin (CT) on stool frequency and weight (g) within 24 hours after the experiment

	Intrajejunal dose of CT		
	6.25 µg	12.5 µg	25 µg
Frequency of liquid bowel movements	5 (1) [5]	8 (3) [5]	6 (2) [5]
Stool weight (g)	1000 (300) [5]	2017 (109) [3]	1528 (405) [5]

Values are mean (SEM), number of subjects from which results are available are given in square brackets.

in net fluid and ion transport (Figure). Measurements of water as well as of Na⁺ and Cl⁻ revealed significant changes toward secretion induced by all three doses of cholera toxin (Tables I-III). Net movement of water was significantly differ-

Dose response relationship for intrajejunal cholera toxin (CT) on Δ water and ion movement (ml or mmol/30 cm/h) during the fourth hour after administration of the enterotoxin. (Δ : net movement in the fourth hour minus basal movement; mean (SEM), five).

*significantly different from 6.25 μ g, $p < 0.05$; **significantly different from 12.5 μ g, $p < 0.05$.

ent from zero (secretion) when the 25 μ g dose of cholera toxin was administered. Net K⁺ transport was not changed by the lowest dose of cholera toxin (6.25 μ g). Net K⁺ absorption was significantly decreased by 12.5 μ g cholera toxin, and only the highest dose (25 μ g) caused net secretion (Table IV). HCO₃⁻ absorption was reduced by 6.25 μ g cholera toxin and changed to secretion by the higher doses (Table V). Cholera toxin caused no change in potential difference in any of the groups.

CLINICAL EFFECTS

Blood pressure and heart rate did not change during the course of the studies. Cholera toxin did not cause nausea or vomiting. Stool volume and frequency (Table VI) increased after all doses of cholera toxin to a higher degree than might be expected after intestinal perfusion studies using balanced electrolyte solutions. Only one subject who had received 6.25 μ g cholera toxin observed no increase in stool frequency, however, in this case only decreased absorption and not secretion was observed in the test segment. In two subjects receiving 25 μ g cholera toxin and in one receiving 12.5 μ g cholera toxin liquid bowel movements continued up to 48 hours but caused no serious inconvenience. In the other subjects liquid bowel movements stopped after 24 hours. After 24 hours haematocrit values were within the normal range (44 (1)%).

Discussion

In this study we developed a model for intestinal secretion in man using cholera toxin as a secretagogue and measured the changes in jejunal water and electrolyte movement in response to cholera toxin. Our experiments showed a significant dose dependent change from absorption toward secretion of water and electrolytes. We found a maximum of fluid secretion of 88 ml/30 cm/h with a dose of 25 μ g cholera toxin four hours after exposure to the toxin. Jejunal perfusion studies in patients with acute cholera studied by others showed a wide range of net fluid secretion from 2 to 326 ml/30 cm/h with corresponding movements of Na⁺, K⁺, Cl⁻, and HCO₃⁻ into the lumen.¹³ Thus, in the present study we induced secretion in the test segment which lies well within the range of secretion observed with naturally occurring cholera.

When 'cholera' is considered for experiments in healthy subjects the most important question to answer concerns safety. As opposed to natural cholera we used a single dose of purified cholera toxin but did not administer *Vibrio cholerae*, which would produce cholera toxin for several days. Thus, the expected time for intestinal secretion was limited. Moreover, only a very small segment of jejunum was exposed to the full dose of cholera toxin and because of the rapid binding of the toxin to the mucosa it was presumed that cholera toxin was not available in the distal small bowel to induce secretion. Some liquid bowel movements are expected after many

types of intestinal perfusion studies and in most of the subjects in this study liquid bowel movements had stopped after 24 hours. Only in three subjects liquid bowel movements continued up to 48 hours, but in no subject did this cause any serious inconvenience. During the perfusion studies we observed no change in blood pressure or heart rate. After the end of the study the volunteers were asked to drink 1.5–2 l of an oral rehydration solution over the next 24 hours, but most of the subjects preferred other liquids. A normal haematocrit after 24 hours was present in all subjects and indicated either sufficient oral liquid intake or insignificant fluid loss. We conclude that a bolus of cholera toxin of 6.25 to 25 µg can be used safely as an intestinal secretagogue when the described precautions are being taken.

In addition to the secretion of Na^+ and Cl^- we found marked secretion of HCO_3^- into the jejunum, thus confirming previous *in vivo* studies in animals.^{2,14–18} In experimental animals cholera toxin induces marked secretion of fluid and electrolytes. Where HCO_3^- was also measured net secretion was observed.^{2,14–16} This is in contrast with *in vitro* studies in ileal mucosa using short circuit current conditions where no significant changes of HCO_3^- fluxes were found.¹⁹ To our knowledge there are no *in vitro* studies using jejunal mucosa. The different findings *in vitro* and *in vivo* underline the importance of *in vivo* studies in man.

Normally HCO_3^- is absorbed by the jejunum. The absorption is thought to result from a Na^+/H^+ exchange mechanism whereby H^+ is secreted into the lumen in exchange for Na^+ .²⁰ Thus, in addition to the Cl^- and Na^+ transport mechanisms the putative jejunal Na^+/H^+ exchange may also be inhibited by cholera toxin. The secretion of HCO_3^- is of clinical importance as the high amount of HCO_3^- in cholera stools may lead to metabolic acidosis when diarrhoea continues for several days. Potential difference did not change during the observed effects in ion transport in our study. Although a change to lumen negativity would be expected with active anion secretion our results are in accordance with those of Sachar, who found marked accumulation of water and electrolytes in the intestine of patients with cholera without alteration of the transmural potential difference in the small bowel.²¹

We found no correlation between net secretion of water and electrolytes in the test segment and stool weight while liquid bowel movements were present after the experiment. Such a lack of correlation was also observed by Levin after oral administration of 5 µg purified cholera toxin to healthy volunteers.⁵ The amount of diarrhoea in acute cholera infection is also very variable. Many cases are mild and cannot be distinguished from viral gastroenteritis. When cholera toxin is used as secretagogue a variable response in stool volume should thus be expected.

In our experiments the secretory effect of cholera toxin began in the second hour after administration of the toxin. The observed maximal effect was reached in the fourth hour although we do not know whether secretion would still have been higher in the fifth or

sixth hour. For reasons of compliance it was not possible to plan for longer experiments (our experiments lasted approximately eight hours from start to end). This delayed effect of cholera toxin is well known from animal experiments.^{1,16,17,22} It might be explained by the fact that the toxin is effective from the luminal side and binds irreversibly to a particular membrane receptor. It probably takes time to enter the cell and to activate a cascade of intracellular mechanisms leading to secretion of water and electrolytes. Carpenter assumed that increased fluid secretion began shortly after toxin administration but that net fluid production was not observed until the rate of secretion exceeded the rate of absorption.²² The secretory effect of cholera toxin in our study probably continued for hours as evidenced by the stool output after the experiment. Recovery occurs when the affected intestinal epithelial cells on the villi are replaced by new enterocytes moving up from the crypts.³

The late onset and long duration of secretion and the induction of significant HCO_3^- secretion are the most important differences between the effect of cholera toxin and other secretagogues such as vasoactive intestinal polypeptide or prostaglandin. Intravenously administered vasoactive intestinal polypeptide instantly changed absorption to secretion and the effect stopped after the end of the infusion. No subject developed diarrhoea, and even at the highest dose tested of 400 pmol vasoactive intestinal polypeptide/kg/h only reduced absorption or a low rate of secretion of water and electrolytes was observed.^{23,24} There was only a slight but statistically not significant decrease in HCO_3^- absorption caused by vasoactive intestinal polypeptide.²³ This is in contrast to our results where cholera toxin induced marked secretion of HCO_3^- . Intrajejunally administered prostaglandin E_1 at a dose of 0.9 µg/kg/min induced marked secretion of water and electrolytes beginning after 30 minutes and ending within 60 minutes after prostaglandin infusion.²⁵ A dose of 5 µmol/l prostaglandin E_2 tested in our laboratory induced secretion of water, Na^+ and Cl^- similar to 25 µg cholera toxin, but without affecting HCO_3^- absorption.²⁶ One might speculate that HCO_3^- secretion is mainly initiated by certain agents such as toxins from the luminal side, whereas hormones or prostaglandins, which normally act from the basolateral membrane of enterocytes, do not significantly affect HCO_3^- transport in man *in vivo*. Another marked difference between the effects of cholera toxin and vasoactive intestinal polypeptide or prostaglandins is that secretion stopped promptly after prostaglandin or vasoactive intestinal polypeptide infusion. The latency of the response to cholera toxin as well as its prolonged effect depend on its mode of action as mentioned above. In contrast with cholera toxin, vasoactive intestinal polypeptide and prostaglandins are bound reversibly, and act only as long as their levels in plasma or in the intestinal lumen are raised.

In conclusion, our study shows a significant, dose dependent change from absorption to secretion of water and electrolytes in the human

jejunum induced by cholera toxin. This model is expected to lend itself to study the effectiveness of antisecretory agents in the human intestine.

Supported by Austrian Scientific Research Foundation Grant No P7741. The authors thank Ms S Hugill and Mr K Fürstauer for their excellent technical assistance.

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