Effect of Chlorpromazine on the Secretory Activity of 
Escherichia coli Heat-Stable Enterotoxin

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The effect of chlorpromazine on the net intestinal accumulation of fluid induced by Escherichia coli heat-stable (ST) enterotoxin in an infant mouse model was examined. Chlorpromazine, when administered with ST enterotoxin, caused a highly significant decrease in net intestinal fluid accumulation. The inhibition of ST activity was dose dependent with various concentrations of chlorpromazine (P < 0.001). A significant inhibition of toxic activity was also observed when chlorpromazine was administered before (P < 0.02) or after (P < 0.05) ST enterotoxin challenge. No significant differences in fluid accumulation were observed between control mice treated with buffer alone and those treated with only chlorpromazine. These data indicate that chlorpromazine markedly decreases the net intestinal fluid accumulation induced by E. coli ST enterotoxin. Further studies on the potential use of chlorpromazine in both the prophylaxis and the treatment of diarrheal diseases appear warranted.

Two distinct extracellular enterotoxins, heat labile (LT) and heat stable (ST), are elaborated by enterotoxigenic Escherichia coli. Both LT (10, 23) and ST (10, 24) enterotoxins are capable of inducing a catastrophic diarrheal illness in human subjects. The LT enterotoxin is biologically and immunologically similar to the enterotoxin produced by Vibrio cholerae. Numerous studies have demonstrated that both cholera enterotoxin (CT) and LT enterotoxin elicit a dose-dependent activation of adenylate cyclase (EC 4.6.1.1.), which converts adenosine 5'-triphosphate to adenosine 3',5'-cyclic monophosphate (cAMP), and hence elevates intracellular cAMP levels (1, 7, 15, 21, 25, 26). cAMP presumably regulates ion and water transport across the intestinal mucosa by inhibiting coupled sodium and chloride absorption by villus cells and by stimulating secretion of anions from crypt cells (6, 11, 14). In this regard, Holmgren et al. (11) reported that chlorpromazine reversed CT- and LT-induced fluid accumulation in mice. Thus, it has been suggested that chlorpromazine reverses the secretory effect of CT and LT enterotoxins by inhibiting adenylate cyclase activity (11, 17). More recently, Rabbani et al. (22) reported the ability of chlorpromazine to reduce fluid loss in cholera patients.

In contrast to CT and LT, ST does not stimulate intracellular cAMP levels, but produces a rapid elevation of intracellular guanosine 3',5'-cyclic monophosphate (cGMP) concentrations (9, 12, 19). The present study examines the effect of chlorpromazine on net intestinal fluid accumulation induced by E. coli ST enterotoxin in an infant mouse model before, during, and after initiation of the toxic response.

MATERIALS AND METHODS

Microorganism. E. coli PB-122-B1 (serotype O80:H9) was obtained from Doyle Evans (University of Texas Medical School, Houston, Tex.). This strain was originally isolated from a case of traveler's diarrhea in Mexico and has maintained stability of the ST enterotoxin plasmid in the laboratory (5). Stock cultures of the organism were lyophilized in sterile fetal calf serum and stored at −70°C until required.

Toxin production. A lyophilized culture of the microorganism was reconstituted and inoculated onto a Trypticase soy agar slant (Difco Laboratories, Detroit, Mich.). After 18 h of incubation at 37°C, the slant was harvested with 1.0 ml of the synthetic medium of Alderete and Robertson (2), and 0.1 ml was used to inoculate a pre-culture of 50 ml of synthetic medium in a 250-ml Erlenmeyer flask. The pre-culture was then incubated for 18 h at 37°C and used as an inoculum for toxin production. Two-liter Erlenmeyer flasks, each containing 400 ml of synthetic medium (2), were inoculated with 50 ml of the seed culture, resulting in an initial optical density (at 540 nm) of 0.05. The organism was grown for 8 h in a platform shaker (160 rpm) at 37°C. The culture was then centrifuged at 16,000 × g for 1 h, and the supernatant was filter-sterilized (0.45 μm; Millipore Corp., Bedford, Mass.). The sterile supernatant was partially purified by collecting the filtrate from an Amicon DM-5 ultrafiltration membrane (Amicon Corp., Lexington, Mass.) and dialyzing over an Amicon UM-2 ultrafiltration membrane (nominal molecular weight cut-off = 1,000). The UM-2 retentate was then twice dialyzed with an equal volume (10 ml) of 0.005 M potassium phosphate.
buffer (pH 7.0), filter sterilized (0.45 μm), and assayed for toxic activity in infant mice.

**Infant mouse assay.** The infant mouse model of Dean et al. (4) was routinely used to assay for toxic activity. Briefly, infant mice (2 to 4 days of age) were randomly separated into groups of four and injected percutaneously into the stomach with a 0.1-ml sample containing 0.02% Niagara Sky Blue. After 3 h of incubation at 23°C, each animal was killed by decapitation, and the entire intestinal tract was removed and weighed. The ratio of intestinal to remaining body weight was used as a measure of the amount of toxic activity as described elsewhere (4). If dye was not present in the stomach at death, the animal was discarded.

A dose-response titration curve was constructed as previously reported (18) from dilutions of the concentrated UM-2 retentate. A dilution giving a toxic response in the linear portion of the titration curve was routinely used. In all subsequent drug studies this was referred to as a standard toxin dilution. The standard toxin dilution gave a ratio of approximately 0.100 when mixed with an equal volume of 0.005 M potassium phosphate buffer (PPB) at pH 7.0.

**Simultaneous treatment with chlorpromazine.** A stock solution (200 μg) of chlorpromazine hydrochloride (Smith Kline & French Laboratories, Philadelphia, Pa.) was suspended in PPB (pH 7.0) to specified concentrations. An equal volume of the standard toxin dilution was then added to each drug concentration. The solution was blended in a Vortex mixer and immediately injected into infant mice to assay for toxic activity (see above).

**Pre- and posttreatment with chlorpromazine.** Chlorpromazine (100 μg in 50 μl of PPB) was administered percutaneously into the stomach of each infant mouse 30 min before or 30 min after challenge with the standard toxin dilution. Control mice were injected with 50 μl of PPB 30 min before or 30 min after challenge with the same standard toxin dilution. All mice were killed 3 h after toxin challenge, and the ratio of intestinal to body weight was determined. The ability of ST enterotoxin to induce detectable intestinal fluid accumulation 30 min postchallenge was determined by killing groups of mice 30 min after toxin injection.

**RESULTS**

The simultaneous injection of infant mice with various concentrations of chlorpromazine and the standard toxin dilution resulted in a significant inhibition of net intestinal fluid accumulation (Fig. 1). In the absence of chlorpromazine, titration of the standard toxin dilution gave a response of 0.101 ± 0.009 (data not shown). This response, in the presence of various concentrations of chlorpromazine, was significantly diminished and dose dependent from 25 to 100 μg of drug. The difference between toxin alone and 50 or 100 μg of chlorpromazine was significant (P < 0.001), whereas drug concentrations of 5 and 10 μg showed no significant reduction of fluid accumulation. Furthermore, no significant difference was noted between the buffer control and the standard toxin dilution containing 100 μg of chlorpromazine, thus indicating a complete reversal of the effect of ST enterotoxin at this concentration. Neither chlorpromazine alone nor buffer (PPB) caused the accumulation of intestinal fluid (see below).

Both pre- and posttreatment of infant mice with 100 μg of chlorpromazine resulted in the partial inhibition of net intestinal fluid accumulation. When 100 μg of chlorpromazine was injected 30 min before administration of the standard toxin dilution, there was a significant (P < 0.02) decrease in fluid accumulation when compared with control groups of mice receiving buffer 30 min before challenge with the standard toxin dilution (Table 1). However, the pre- and posttreatment of infant mice with chlorpromazine resulted in a modest reduction of fluid accumulation when compared to simultaneous treatment. A significant (P < 0.02) amount of fluid accumulation was observed 30 min after challenge with ST enterotoxin when compared with the buffer control (Table 1). Therefore, this time interval was selected to test the ability of chlorpromazine to reverse the net intestinal accumulation of fluid after initiation of the toxic response. Groups of mice were injected with the standard toxin dilution. After 30 min, chlorpromazine (100 μg) was administered. After incubation, a significant decrease (P < 0.05) in net intestinal fluid accumulation was observed. Animals injected with either buffer or chlorpromazine (100 μg) alone showed no significant difference in the ratio of intestinal weight to body weight.
DISCUSSION

This investigation demonstrates the ability of chlorpromazine to inhibit the net intestinal fluid accumulation of infant mice induced by E. coli ST enterotoxin. This inhibition was dose dependent from 25 to 100 µg when the drug was administered simultaneously with toxin. The administration of chlorpromazine 30 min before or 30 min after the initiation of intestinal fluid accumulation resulted in a significant, but modest, inhibition of the toxic response. Mice killed 30 min after toxin challenge showed that a significant amount of fluid had already accumulated before administration of the drug. Gianella and Drake (9) have shown a significant accumulation of intestinal fluid as early as 60 min after challenge with ST enterotoxin. Recent studies, using E. coli or cholera heat-labile enterotoxins (11, 17, 22), or viable E. coli (16) in other animal systems, have used lower dosages of chlorpromazine. Whether E. coli ST-induced fluid secretion is more resistant to the inhibitory effect(s) of chlorpromazine than E. coli or cholera enterotoxins or viable E. coli is not known. Further studies, using equivalent toxic units, will have to be performed in a comparable animal system to quantitate the effect of chlorpromazine on E. coli enterotoxins, cholera enterotoxin, and viable E. coli.

It has been demonstrated that chlorpromazine has an inhibitory effect on adenylate cyclase (13, 20, 27). However, Holmgren et al. (11) reported that chlorpromazine inhibits intestinal fluid secretion mediated by dibutyryl-cAMP, a finding which indicates that the primary action of this drug may be targeted at a point subsequent to the stimulation of adenylate cyclase. Since ST enterotoxin mediates intestinal fluid accumu-

**Table 1. Effect of pre- and posttreatment of infant mice with chlorpromazine (CPZ) 30 min before or 30 min after ST enterotoxin challenge**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Drug concn (µg)</th>
<th>Ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPZ 30 min before</td>
<td>100</td>
<td>0.084 ± 0.011</td>
</tr>
<tr>
<td>CPZ 30 min after</td>
<td>100</td>
<td>0.099 ± 0.005</td>
</tr>
<tr>
<td>Toxin alone</td>
<td>None</td>
<td>0.108 ± 0.061</td>
</tr>
<tr>
<td>CPZ alone</td>
<td>100</td>
<td>0.864 ± 0.006</td>
</tr>
<tr>
<td>Buffer alone</td>
<td>None</td>
<td>0.067 ± 0.003</td>
</tr>
</tbody>
</table>

* Ratios represent the mean intestinal weight/body weight ± standard deviation. All P values were determined by Student's t test. Differences between pre- and posttreatment and toxin alone (see text for details) were significant at P < 0.02 and P < 0.05, respectively; no significant difference was observed between CPZ alone and buffer alone. The ratio of toxin alone 30 min after challenge was 0.077 ± 0.006; this value was significantly (P < 0.05) higher than buffer alone.

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**LITERATURE CITED**


