Sodium Salicylate and Bile Acid-induced Colonic Secretion in the Rat

SAMUEL A. KOCOSHIS, M.D.
and CATARINA TSE WONG, M.S.

Department of Pediatrics,
University of Pittsburgh School of Medicine,
and Children's Hospital of Pittsburgh,
Pittsburgh, PA 15213

ABSTRACT

Previous studies by us suggested that luminally administered sodium salicylate blocks dihydroxy-bile acid-induced colonic secretion in the rat. In the present study, an in vivo rat cecal loop technique is employed to compare the effects of luminally administered and parenterally administered sodium salicylate upon chenodeoxycholic acid-induced colonic secretion. In our experiment, inoculation of four mM chenodeoxycholic acid into the rat cecum produced net secretion of water and sodium which was not reversed by preincubation of this bile acid with eight mM of sodium salicylate. Similarly, an intravenous bolus of either five mg of sodium salicylate per kg of body weight or 50 mg of sodium salicylate per kg of body weight failed to block salt or water secretion. Furthermore, 30 minute incubation of chenodeoxycholic acid with sodium salicylate produced neither reduction of in vitro aqueous bile acid concentration nor inhibition of ex vivo bile acid-facilitated hypotonic red cell hemolysis. These data suggest that sodium salicylate fails to sequester bile acids from aqueous solution and fails to block bile acid-mediated colonic secretion in the rat.

Introduction

Orally or rectally administered salicylates have been employed as anti-diarrheal agents in a number of clinical settings. Sulfasalazine and analogues of five-amino-salicylic acid are efficacious in the therapy of ulcerative or granulomatous colitis. Investigators have found aspirin to be of benefit for both toxigenic and non-toxigenic diarrhea. Another agent, bismuth subsalicylate, has enjoyed success in the prophylaxis against travelers' diarrhea and in the treatment of viral or nonspecific diarrhea.

Bismuth subsalicylate's mode of action is only incompletely understood, but it has previously been shown by us that one of its potentially salutary effects is amelioration of bile acid mediated colonic secretion. Even though in vitro bile acid adsorption by bismuth subsalicylate and by sodium salicylate (which appears in significant quantities within

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* Send reprint requests to Samuel A. Kocoshis, M.D., Children's Hospital of Pittsburgh, 3705 Fifth Avenue, Pittsburgh, PA 15213.
the intestinal lumen following dissociation of bismuth subsalicylate) have been demonstrated, no direct evidence has been found by us that bile acid adsorption contributes significantly to anti-diarrheal properties of bismuth subsalicylate nor even that the adsorption demonstrated by ultracentrifugation experiments has any physiological significance at all.16

An equally attractive hypothesis is that any salt of salicylate will act systemically to blunt the mucosal secretory response following intraluminal application of bile acids. Indeed, in a previous study, it was shown by us that a 30 minute in vitro incubation of chenodeoxycholic acid with sodium salicylate followed by instillation of the mixture into the rat cecum seemed to blunt the colonic secretory response to chenodeoxycholic acid.10

The present experiment was conducted in an attempt to confirm our previous data regarding sodium salicylate and to test the hypothesis that parenterally administered sodium salicylate blocks bile acid mediated in vivo colonic secretion as well.

Materials and Methods

Cecal Pouch Technique

An in vivo rat cecal closed loop technique was employed which was described in detail previously.10 Under sodium pentobarbital (45 mg per kg) anesthesia, non-fasted, male Sprague-Dawley rats weighing 250 to 350 g underwent surgical isolation of the cecum. After the cecum was cleansed with 154 mM NaCl and emptied of fluid, three ml of each infusate were instilled, and the cecum sutured closed. Three hours afterwards, the cecum was re-entered so that intraluminal fluid could be retrieved for measurement of fluid and electrolyte movement and salicylate concentration. Fluid movement (expressed as µl per min per g dry weight of cecum) was determined by measuring changes in polyethylene glycol 4000 concentration according to the method of Malawer and Powell.12 Positive values represented net absorption and negative values net secretion into the lumen. Recovery of PEG 4000 was noted to be 96.4 ± 6.8 percent by measurement of [14C] PEG 4000 (0.2 µCi per ml) with a liquid scintillation counter.* Sodium concentrations before and after incubations were measured by a direct reading flame photometer.† Net sodium movement was expressed as µEq per min per g dry weight of cecum with positive values representing absorption and negative values secretion. Animals, still under pentobarbital anesthesia, were subsequently sacrificed humanely by exsanguination and ceca were removed, desiccated, and weighed. Five animals each had been infused with test or control inocula.

All solutions were adjusted to a final pH of 7.4 and osmolality of 298 mOsm per kg (equivalent to physiologic saline). Experimental groups received the following inocula: group 1, 154 mM NaCl; group 2, four mM chenodeoxycholic acid in saline; group 3, four mM chenodeoxycholic acid which had been incubated for 30 minutes in a 37°C rocker bath with 1.3 mg per ml (8.0 mM) of powdered sodium salicylate (99.5 percent pure by high performance liquid chromatography); group 4, four mM chenodeoxycholic acid immediately following a 5 mg per kg intravenous bolus of sodium salicylate (99.5 percent pure by high performance liquid chromatography); group 5, four mM chenodeoxycholic acid immediately following a 50 mg per kg intravenous sodium salicylate bolus.

The intracecal sodium salicylate dose was chosen to approximate the peak

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* Packard Instruments, Downers Grove, IL.
† Instrumentation Laboratory Inc, Lexington, MA.
‡ Fisher Laboratories, Springfield, NJ.
SODIUM SALICYLATE AND BILE-ACID COLONIC SECRETION IN THE RAT

The 50 mg per kg intravenous sodium salicylate bolus was chosen to approximate serum salicylate levels achievable when recommended oral antidiarrheal doses of acetylsalicylic acid are employed.

Means and standard deviations of water and sodium movement were calculated for each group of animals tested, and differences between experimental groups and control groups were analyzed for significance by Student's t test for the means.

FACILITATION OF HYPOTONIC HEMOLYSIS

This experiment was conducted by the method of Seeman.15 Fresh human red blood cells (RBC) were washed twice with 154 mM NaCl and 10 mM sodium phosphate (pH 7.0) and centrifuged at 1800 rpm for 10 minutes. One ml of the packed cells was combined with 15 ml of the buffered saline to make a stock red blood cell (RBC) solution. The following hypotonic solutions were prepared: (1) 66.5 mM NaCl buffered to pH 7.0 with 10 mM sodium phosphate; (2) buffered 66.5 mM NaCl combined with 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, and 1.6 mM chenodeoxycholic acid; (3) buffered 66.5 mM NaCl

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§ Procter and Gamble, Cincinnati, OH (unpublished data).
† Millipore Corp., Milford, MA.
¶ Kratos Analytical Co, Ramsey, NJ.
I Saline
II 4mM chenodeoxycholic acid
III 4mM chenodeoxycholic acid + i.v. salicylate (5mg/kg)
IV 4mM chenodeoxycholic acid + i.v. salicylate (50mg/kg)
V 4mM chenodeoxycholic acid + intracecal 8mM sodium salicylate

**Figure 2.** Water movement in rat cecum after 180 minute inoculation with normal saline, four mM chenodeoxycholic acid, four mM chenodeoxycholic acid after five mg per kg body weight intravenous bolus of sodium salicylate, four mM chenodeoxycholic acid after 50 mg per kg body weight intravenous bolus of sodium salicylate, or four mM chenodeoxycholic acid plus eight mM intracecal sodium salicylate.

combined with 0.9, 1.0, 1.1, and 1.2 mM chenodeoxycholic acid preincubated with 8 mM sodium salicylate; (4) buffered 66.5 mM NaCl combined with 1.2 mM chenodeoxycholic acid and preincubated with 3.3 mg per ml or 33.0 mg per ml bismuth subsalicylate; and (5) buffered 66.5 mM NaCl combined with 1.2 mM chenodeoxycholic acid and 3.3 mg per ml or 33.0 mg per ml cholestyramine. The hemolysis induced by each was compared with that induced by deionized water.

To measure hemolysis, 0.5 ml of RBC was added to 7.5 ml of each hypotonic solution. Test tubes were inverted to ensure mixing, incubated for exactly five minutes at ambient temperature, then centrifuged at 1800 rpm for five minutes. The optical density of the supernatant was then read at 543 nm with a Carl Zeiss, model PMQII spectrophotometer.* The optical density after incubation of RBC with deionized water was operationally defined as 100 percent hemolysis. Optical densities after incubation with the other solutions were divided by the optical density after incubation with deionized water and multiplied by 100 percent to calculate the percent hemolysis each solution had induced. Incubations were performed in triplicate.

Separate 1.2 mM chenodeoxycholic acid solutions were incubated in buffered NaCl, in buffered NaCl plus 3.3 or 33.0 mg per ml bismuth subsalicylate, in buffered NaCl plus 3.3 or 33.0 mg per ml cholestyramine, or in buffered NaCl plus 8 mM sodium salicylate for 30 minutes at 37°C and then centrifuged at 50,000 × g. The chenodeoxycholic acid concentration in the supernatant was measured by gas-liquid chromatography after derivatization as previously described in detail.8

**Results**

**Water and Sodium Movement**

Net absorption of water (figure 2) and of sodium (figure 3) were observed when saline alone was present in the inoculum. The four mM chenodeoxycholic acid inoculum induced profound secretion of both water (figure 2) and sodium (figure 3) into the pouches. Preincubation of four

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* Carl Zeiss Instruments, Thornwood, NY.
mM chenodeoxycholic acid with sodium salicylate and subsequent inoculation into ceca led to net water (figure 2) and sodium (figure 3) secretions which were comparable to those seen following inoculation of the control animals. Similarly, treatment of animals with low dose (five mg per kg) and high dose (50 mg per kg) intravenous sodium salicylate failed to abolish chenodeoxycholic acid-induced fluid (figure 2) and electrolyte (figure 3) secretion into the lumen. The magnitude of secretion was unrelated to the intravenous salicylate dose employed. A low salicylate level (0.03 ± 0.001 mg per ml) was present within cecal contents at the termination of the four mM chenodeoxycholic acid inoculation which had followed the 50 mg per kg intravenous salicylate bolus.

**Facilitated Hemolysis and Bile Acid Adsorption**

When a 66.5 mM NaCl solution at pH 7.0 was used to incubate the red cells, 14 percent lysis occurred compared with hemolysis induced by deionized water. Progressively increasing chenodeoxycholic acid concentrations were added to the NaCl solution, and decreasing hemolysis was observed until concentrations of chenodeoxycholic acid reached 0.8 mM. At this concentration, virtually no hemolysis was observed. Above this concentration, the percent of hemolysis progressively increased, finally reaching 100 percent at concentrations of 1.2 mM or greater (figure 4). When chenodeoxycholic acid solutions of 1.2 mM or greater were incubated with eight mM sodium salicylate and the hemolysis tested, the percentage of hemolysis was identical to that produced by equivalent concentrations of chenodeoxycholic acid not incubated with sodium salicylate (figure 4). However, when 1.2 mM chenodeoxycholic acid was incubated with bismuth subsalicylate or cholestyramine (table I), which were then removed by centrifugation, the resultant supernatants produced less hemolysis than the control solution of chenodeoxycholic acid.

**Discussion**

In this experiment it was not possible to reproduce our previous finding that

![Figure 3. Sodium movement in rat cecum after 180 minute inoculation with normal saline, four mM chenodeoxycholic acid, four mM chenodeoxycholic acid after five mg per kg body weight intravenous bolus of sodium salicylate, four mM chenodeoxycholic acid after 50 mg per kg body weight intravenous bolus of sodium salicylate or four mM chenodeoxycholic acid plus eight mM intracecal sodium salicylate.](image-url)
sodium salicylate blocks bile acid-induced colonic secretion, even when a large dose of salicylate is delivered directly into the cecal lumen. In our original experiment, the effects were tested of bismuth subsalicylate and sodium salicylate upon four mM chenodeoxycholic acid-induced cecal water and salt movement. Five experimental animals were employed for each agent, but bile acid mixtures had been stored for over 48 hours before being centrifuged and inoculated into each cecum. It is quite possible that some non-specific precipitation of chenodeoxycholic acid owing to alteration in temperature or pH may have occurred and been mistakenly interpreted as sequestration. If such an event occurred, it is likely that those inocula contained too low a concentration of chenodeoxycholic acid to exert a secretory effect.

The authors do not believe that sequestration of chenodeoxycholic acid by bismuth subsalicylate occurred in a similar fashion since it was repeatedly demonstrated at physiologic pH and temperature and because not only inhibition of chenodeoxycholic acid-induced colonic secretion but also chenodeoxycholic acid-induced red blood cell hemolysis were demonstrated. Having demonstrated in our current experiment neither inhibition of chenodeoxycholic acid-

**TABLE I**

Effect Upon Bile Acid Sequestration and Subsequent Red Blood Cell Hemolysis After In Vitro Bile Acid Incubation With Anti-secretory Candidates

<table>
<thead>
<tr>
<th>Bile Acid + Anti-secretory Candidate</th>
<th>Percent Sequestration of Chenodeoxycholic Acid</th>
<th>Percent Hemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2 mM chenodeoxycholic acid</td>
<td>—</td>
<td>95.2</td>
</tr>
<tr>
<td>1.2 mM chenodeoxycholic acid + 3.3 mg/ml bismuth subsalicylate</td>
<td>18.7</td>
<td>5.6</td>
</tr>
<tr>
<td>1.2 mM chenodeoxycholic acid + 33.0 mg/ml bismuth subsalicylate</td>
<td>64.9</td>
<td>4.7</td>
</tr>
<tr>
<td>1.2 mM chenodeoxycholic acid + 3.3 mg/ml cholestyramine</td>
<td>100.0</td>
<td>28.5</td>
</tr>
<tr>
<td>1.2 mM chenodeoxycholic acid + 33.0 mg/ml cholestyramine</td>
<td>100.0</td>
<td>28.5</td>
</tr>
<tr>
<td>1.2 mM chenodeoxycholic acid + 8 mM sodium salicylate</td>
<td>0</td>
<td>98</td>
</tr>
</tbody>
</table>
facilitated hemolysis nor a fall in aqueous chenodeoxycholic acid concentration after incubation with a luminally achievable dose of sodium salicylate, the authors believe that no true sequestration of bile acid from aqueous solution occurred.

Furthermore, neither luminal nor high-dose parenteral sodium salicylate attenuated the colonic response to chenodeoxycholic acid, suggesting that the bismuth salt of salicylate and luminal bile acid-sequestering properties not shared by other salicylates.

These findings are generally in agreement with those of Rampton et al\textsuperscript{13} who found that bile acid-induced colonic secretion occurred in experimental animals despite pretreatment with a parenteral cyclooxygenase inhibitor (indomethacin). Because Rampton et al\textsuperscript{13} also demonstrated that inhibition of prostaglandin synthesis bears little, if any, relationship to inhibition of fluid secretion, and because sodium salicylate’s ability to suppress cyclooxygenase is rather low,\textsuperscript{1} it was chosen not to measure prostaglandin levels within the cecal lumen or the cecal mucosa. However, by demonstrating similar effects of luminally delivered and parenterally delivered sodium salicylate, it has been shown by us that the potential efficacy of bismuth subsalicylate in treating bile acid mediated diarrhea is a unique property not shared by all salicylates.

In most clinical settings, deoxycholic acid, rather than chenodeoxycholic acid, induces diarrhea following ileal resection, but chenodeoxycholic acid was chosen as our secretagogue for specific reasons. Though chenodeoxycholic acid and deoxycholic acid possess very similar effects upon colonic mucosa,\textsuperscript{6} a slightly greater magnitude of colonic secretion at the concentration (four mM) of bile acid inoculated was observed during our experiments. This phenomenon may occur because deoxycholic acid has a greater propensity than chenodeoxycholic acid for gel formation,\textsuperscript{6} thereby inducing less secretion than induced by chenodeoxycholic acid. Furthermore, deoxycholic acid, a better detergent than chenodeoxycholic acid, is more likely to inactivate cyclic adenosine monophosphate (AMP)\textsuperscript{16} and blunt the colonic secretory response.

Other investigators have observed that aspirin applied to the mucosal surface of jejunum will partially block the secretory effect of cholera toxin, a cyclic AMP inducer,\textsuperscript{5} or that sodium salicylate administered parenterally appears to block Escherchia coli heat-stable toxin, a cyclic guanosine 3′5′-monophosphate (GMP) inducing jejunal secretagogue.\textsuperscript{18} While salicylates may counteract small intestinal secretagogues, our data suggest that salicylates, apart from bismuth subsalicylate, have no protective effect against chenodeoxycholic acid-induced secretion in the rat cecum. Any potential efficacy of bismuth subsalicylate in treating bile acid-mediated diarrhea appears not to be shared by sodium salicylate.

References


