INFECTION AND IMMUNITY, Feb. 1982, p. 471–475
0019-9567/82/020471-05$02.00/0

Vol. 35, No. 2

Berberine Inhibits Intestinal Secretory Response of *Vibrio cholerae* and *Escherichia coli* Enterotoxins

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Received 3 August 1981/Accepted 14 October 1981

Berberine, an alkaloid from the plant *Berberis aristata*, which has been known since ancient times as an antidiarrheal medication in India and China, inhibited by approximately 70% the secretory responses of the heat-labile enterotoxins of *Vibrio cholerae* and *Escherichia coli* in the rabbit ligated intestinal loop model. The drug was effective when given either before or after enterotoxin binding and when given either intraluminally or parenterally; it did not inhibit the stimulation of adenylate cyclase by cholera enterotoxin and caused no histological damage to intestinal mucosa. Berberine also markedly inhibited the secretory response of *E. coli* heat-stable enterotoxin in the infant mouse model. Although the mechanism of action of the drug is not yet known, these data provide a rationale for its apparent clinical usefulness in treating acute diarrheal disease.

The alkaloid berberine (Fig. 1) is derived from the roots and bark of the plant *Berberis aristata*, a spiny, deciduous, evergreen shrub with yellow flowers (berberry bush), extracts of which have been used as antidiarrheal medication in the practice of Auyverdic medicine in India and in the traditional medicine of China for the past 3,000 years. As one of several indigenous antidiarrheal plant extracts being studied in India by Dutta et al. approximately 25 years ago, it alone was found to significantly reduce the severity of *Vibrio cholerae* infection in the infant rabbit model (7). Since then, the drug has been investigated widely by Indian scientists and has been found to have activity against a broad array of infectious agents such as selected bacteria (including *V. cholerae* but not *Escherichia coli* [4]), protozoa (23), fungi (24), and leishmania (17). Furthermore, it has been used with reported success to treat a number of specific acute diarrheal diseases including cholera (14) and giardiasis (11) and is marketed pharmaceutically in India.

More recently, berberine has been shown by Akhter et al. (2) and Sabir et al. (19) to specifically inhibit the action of *V. cholerae* enterotoxin when administered before or simultaneously with the enterotoxin in animal models. We report that berberine significantly: (i) inhibits the secretory response of both *V. cholerae* and *E. coli* heat-labile enterotoxins (LT) in the rabbit ligated intestinal loop model even when administered after the enterotoxin has bound to intestinal mucosa; and (ii) also inhibits the secretory response of the heat-stable enterotoxin of *E. coli* in the infant mouse model.

MATERIALS AND METHODS

Berberine. Pure berberine sulfate (BS) (molecular weight, 384.4) obtained from Sigma Chemical Co. (St. Louis, Mo.) was dissolved in distilled water. Solutions of BS had an original pH of 2.8; this was adjusted to pH 7.4 with NaOH before use in the animal models. The maximum solubility of BS in water is approximately 10 mg/ml; the solution of which contains 60 mosmol/liter.

Enterotoxin preparations. *V. cholerae* crude enterotoxin (CT) was of the standard 001 lot (National Institutes of Health, Bethesda, Md.) (20). *E. coli* LT was a lyophilized crude culture filtrate of *E. coli* 408-3 (20), which produces both LT and heat-stable enterotoxin (ST). *E. coli* ST was the crude culture filtrate of *E. coli* 35897 (a human strain originally isolated in Bangladesh), which only produces ST.

Intestinal loop assay. Ligated intestinal loops (ca. 10 cm) made in adult rabbits (5) were injected with 1-ml volumes of test materials (either CT or berberine) dissolved in distilled water.

The rabbits were sacrificed 18 h after the injection unless otherwise specified. The intestinal loops were measured, and volume/length ratios were determined.

Statistics. Comparisons were made with the *t* test; means ± 1 standard error of the mean are given unless otherwise stated.

Infant mouse assay. The infant mouse model (6, 16) was used; increasing concentrations of BS were added to a standard amount of ST diluted to a concentration (1:8 of the crude filtrate) which would still produce a near-maximum secretory response in this model. BS and ST were mixed immediately before being injected percutaneously into the stomachs of the infant mice; the mice were sacrificed at 4 h, and the gut/remaining body carcass ratios were determined as a measure of intestinal fluid secretion. Ratios above 0.083 have been shown to indicate intestinal secretion in this model (16).

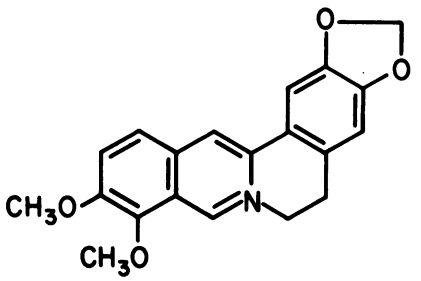

FIG. 1. Structural formula of berberine.

Histological studies. Full-thickness biopsies of adult rabbit intestinal loops were taken (in 10% buffered Formalin) at 1, 4, and 18 h after injection of the loops with 10 mg of BS, 1 mg of CT, or a mixture of the two. Light microscopic examination was done on coded specimens.

Assays for cAMP. Full-thickness biopsies of adult rabbit intestinal loops were immediately frozen in liquid nitrogen; cyclic AMP (cAMP) was assayed by the procedure of Steiner et al. (22). Protein was assayed by the Lowry method (15).

RESULTS

After preliminary experiments which demonstrated that both the pharmaceutical preparation (Berberal, Alembic Chemical Works Co., Ltd., Baroda, India) and pure BS inhibited the secretory response of CT when given either immediately before or 15 min after the enterotoxin injections, a dose-response curve was established with a single standard CT challenge (the minimum amount needed to produce a near-maximum secretory response) and increasing concentrations of BS injected 15 min after the enterotoxin injection. The results (Fig. 2) indicate that greater inhibition of the secretory response occurred with increasing amounts of BS to the point of maximum solubility. Loops injected with BS alone had no fluid accumulation at 18 h.

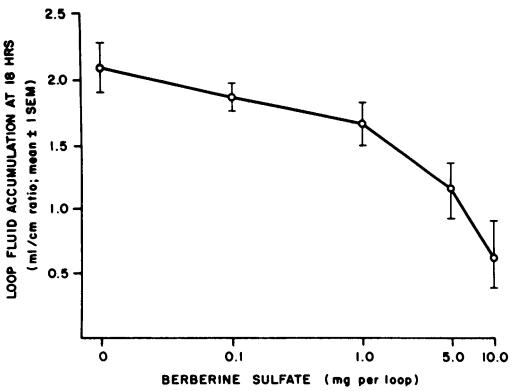

FIG. 2. Effect of BS on the secretory response to a standard challenge of *V. cholerae* CT (1 mg per loop) in the rabbit intestinal loop model (n = six rabbits, with one loop at each concentration in each rabbit). BS was injected 15 min after CT. The differences from the control challenge are significant ($P < 0.01$) at 5 and 10 mg of BS.

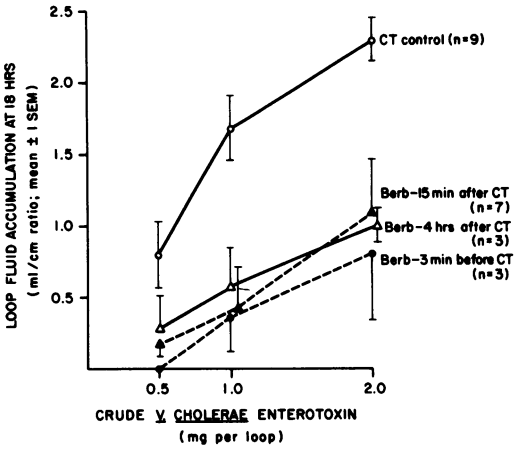

FIG. 3. Effect of BS (10 mg per loop) injected at different intervals in relation to cholera CT on the secretory response curve. BS + CT curves for all three time intervals were significantly different from the CT control ($P < 0.02$).

We then injected the maximum effective soluble dose of BS, 10 mg/ml, at different time intervals both before and after the injection of increasing amounts of CT. The results (Fig. 3) indicate that the inhibition of secretion by BS given immediately before CT ($P < 0.02$) was similar to the inhibition of secretion by BS given up to 4 h after CT ($P < 0.01$). Identical inhibitory responses were obtained with berberine hydrochloride (Sigma Chemical Co., molecular weight, 371.8) rather than the sulfate (data not shown).

To determine whether BS inhibited the activation of adenylate cyclase by CT (21), intestinal loops in eight rabbits were injected first with 1 mg of CT and after 3 h injected again with either 10 mg of BS or normal saline; 1 h later, full-thickness biopsies of the loops were taken for cAMP determinations. The results (Fig. 4) indicate that no inhibition of the CT-stimulated cAMP production by BS was found.

No identifiable changes secondary to BS were seen in the histological sections.

To determine whether the inhibitory effect of BS required direct mucosal contact, we injected BS (20 mg/kg) intraperitoneally 15 min after CT only was injected into the intestinal loops. A significant (~50%) inhibition of secretion was also found (1 mg of CT [controls], mean ratios of 1.69 ± 0.11, n = 9; 1 mg of CT, followed by BS given intraperitoneally, mean ratios of 0.83 ±

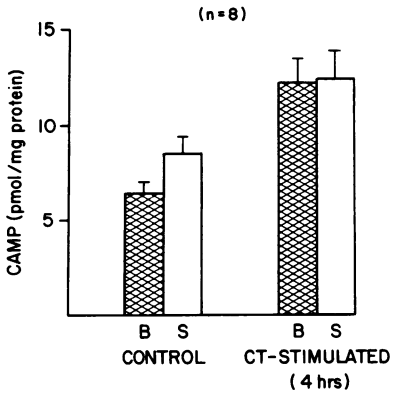

FIG. 4. Effect of berberine on the concentration of cAMP after stimulation by cholera CT. B, Berberine injection; S, saline injection. Control loops were injected only with either B or S; CT-stimulated loops, injected with 1 mg of CT, were injected with either B or S 3 h later, and biopsied 1 h later. Means plus 95% confidence limits are shown (difference between control and CT-stimulated loops, $P < 0.05$).

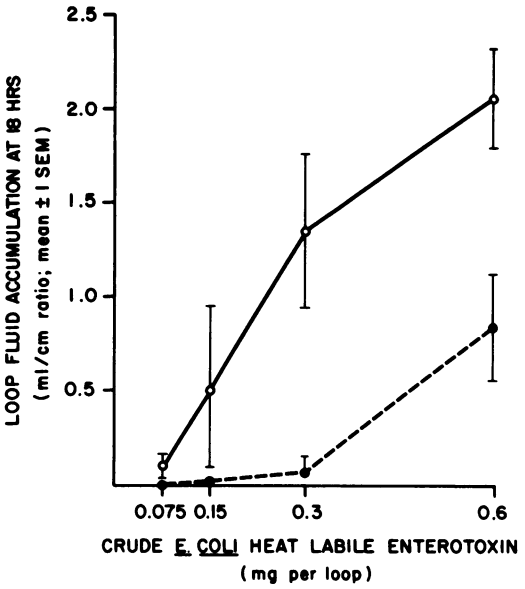

FIG. 5. Effect of BS (●) ($n = 4$) on the secretory responses to *E. coli* LT (○) ($n = 4$) in the rabbit intestinal loop model. BS was injected 15 min after LT. Secretion in all loops containing BS was significantly less ($P < 0.01$–0.05) than control loops.

0.32, $n = 12$, $P < 0.025$), indicating that direct mucosal contact was not a prerequisite for BS activity. When 20 mg of BS per kg (10 mg per loop; four loops per rabbit; four rabbits) was injected alone into intestinal loops immediately adjacent to loops injected only with CT, however, no inhibition of secretion was found, suggesting that the absorption of BS from the intestinal tract was not adequate to inhibit CT-induced secretion in an adjacent segment of bowel.

The effect of BS on LT-induced secretion is shown in Fig. 5; a similar degree of inhibition was found. Although the crude culture filtrate of *E. coli* 408-3 contains both LT and ST, only LT produces a secretory response in the 18-h rabbit loop (8).

High BS concentrations (10 mg per loop) produced a small but definite secretory response in the rabbit ligated intestinal loop model at 4 h (mean ratio of 0.24 ± 0.025, $n = 11$). This secretory response was transient, however, and was not seen at 1, 7, or 18 h after injection ($n = 42$). Because of this observation, the ligated intestinal loop could not be used efficiently in the evaluation of the effect of BS on ST. Therefore, we used the infant mouse model for studies of ST-induced secretion (Fig. 6). BS significantly inhibited the secretory response of ST at concentrations of 0.05 and 0.1 mg per mouse (ca. 25 and 50 mg/kg, respectively). BS alone at 0.1 mg per mouse elicited no secretory response in this model (0.069 ± 0.003, $n = 5$).

DISCUSSION

These results indicate that berberine: (i) inhibits the intestinal secretory response induced by *V. cholerae* and *E. coli* LTs, both of which act through the stimulation of adenylate cyclase (21, 9) although they do not interfere with this stimulation or cause any structural damage to the intestinal mucosa; and (ii) also inhibits the secretion induced by *E. coli* ST, which acts through activation of guanylate cyclase (10). This antisecretory effect is therefore not dependent upon enterotoxin type. It is possible that berberine

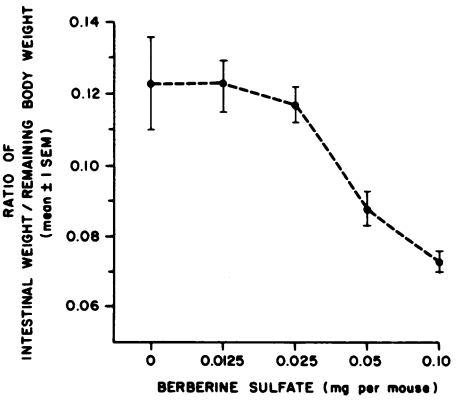

FIG. 6. Effect of BS on the dose-response curve to a standard challenge of *E. coli* ST (1:8 dilution of culture filtrate) in the infant mouse model. BS was injected at the same time as the culture filtrate. Secretion was significantly less ($P < 0.01$) in mice receiving 0.05 and 0.1 mg of BS.

may act at a biochemical step after cyclase activation or it may be acting nonspecifically to enhance intestinal absorption. It has been reported, however, that BS does not inhibit osmotically induced intestinal secretion (3).

Most important from a clinical standpoint, the inhibition of secretion by BS occurs both before and after CT and LT are bound to intestinal mucosa. Although a number of agents, such as charcoal or gangliosides (12), can bind CT and LT and thus prevent secretion, few agents can modify the course of secretion once the toxins are bound. Only two, chlorpromazine (13) and nicotinic acid (25), have been shown to inhibit the activation of adenylate cyclase and intestinal secretion in animal models; chlorpromazine has also been shown in humans to diminish the diarrheal output in cholera (18). Chlorpromazine has also recently been shown to inhibit the intestinal secretion due to ST in the infant mouse model (1). Other agents (12) such as salicylates, indomethacin, steroids, and ethycrynic acid have been shown to inhibit enterotoxin-induced intestinal secretion in animal models by different postulated mechanisms, but none has yet demonstrated clinical usefulness in diarrheal disease therapy. It is also of clinical importance that berberine has not been reported to have significant side effects at the doses used clinically in humans (5 to 10 mg/kg per day orally [14,11]), whereas the other potentially useful drugs all have at least some undesirable side effects (12).

These results thus provide a firm rationale for the reported clinical effectiveness of an ancient antidiarrheal medication made from the extracts of the common berberry bush and used in areas of the world where diarrheal disease due to *V. cholerae* and enterotoxigenic *E. coli* (probably also ancient diseases) have occurred with great regularity and severity. The drug warrants further study, to define both its mechanism of action and its clinical usefulness.

ACKNOWLEDGMENTS

We thank John Yardley, Department of Pathology, The Johns Hopkins University School of Medicine, for examining the intestinal mucosal sections and Bertram Sacktor and C. Tony Liang, The Gerontology Research Center, National Institute of Aging, for performing the cAMP assays.

This study was supported by a contract (DADA 17-73-C-3055) with the U.S. Army Medical Research and Development Command.

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