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Influence of Berberine Sulfate on Synthesis and Expression of Pap Fimbrial Adhesin in Uropathogenic *Escherichia coli*

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We investigated the influence of berberine sulfate, an ancient Chinese antibiotic, upon the adhesion of uropathogenic *Escherichia coli* to erythrocytes and epithelial cells. Although berberine sulfate in increasing concentrations had no effect on bacterial growth or on the synthesis of major outer membrane proteins of the *E. coli* organisms, it increasingly blocked adhesion. The decreased adhesion was accompanied by a reduction in the synthesis of fimbrial subunits and in the expression of assembled fimbriae. These results suggest that the anti-infectious activity of berberine sulfate in *E. coli*-induced urinary tract infections may be mediated by the selective suppression of the synthesis and assembly of fimbriae by uropathogenic organisms.

Berberine sulfate is an alkaloid derived from the plant *Berberine aristata*, which has been used for the past 3,000 years as an antimicrobial medication in China and in the Indian subcontinent. Although berberine has been demonstrated to reduce the infectivity of bacteria, fungi, and protozoa in both animals and humans (1, 8), very little is known about its mode of action. In the first step of the infective process, pathogenic microorganisms adhere to host mucosal or epithelial surfaces. Inhibition of microbial adherence invariably results in the abortion of such infections (2).

In this study, we examined the effect of berberine sulfate on the adhesive properties of a uropathogenic strain of *Escherichia coli*. We show that berberine has little effect on the rate of bacterial growth but markedly reduces bacterial adherence and that the reduction in adherence is related to the loss of the synthesis and expression of Pap fimbriae on the surface of the berberine-treated bacteria.

E. coli CI6 was a Pap-fimbriated clinical isolate from a urinary tract infection; the strain was kindly provided by Itzhak Ofek (Sackler School of Medicine, Tel Aviv, Israel). Experiments to determine the MIC of berberine on *E. coli* CI6 were done as follows. The strain was grown at 37°C for 18 h in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) with shaking in the presence of increasing concentrations of berberine (Sigma Chemical Co., St. Louis, Mo.). Bacterial growth was determined by measuring the optical density of each culture at periodic intervals. We previously found a good correlation between optical density readings at 550 nm and the number of viable organisms (data not shown). The growth of *E. coli* CI6 was remarkably resistant to berberine; concentrations of berberine sulfate as high as 300 μg/ml reduced growth by only 10% (Table 1).

To examine the effect of berberine treatment on bacterial adherence, we tested the ability of *E. coli* grown in various concentrations of berberine to hemagglutinate human erythrocytes and to attach to human uroepithelial cells. Hemagglutination assays were performed as described previously (3). Berberine reduced the hemagglutinating power of the bacteria in a dose-related fashion, and bacteria exposed to 300 μg of berberine per ml lost all hemagglutinating ability (Table 1). In vitro adherence tests were performed as described by Svanborg-Eden et al. with uroepithelial cells collected from fresh human female urine (9). Berberine inhibited the adherence of *E. coli* CI6 to the uroepithelial cells in a dose-dependent manner (Table 1). These results indicate that berberine sulfate exerts an inhibitory effect on the adhesive properties of uropathogenic *E. coli* CI6.

Since Pap fimbriae have been shown to mediate the adherence of uropathogenic *E. coli* cells, it was of interest to determine if the decrease in the adhesive properties of the organisms was related to a concomitant decrease in the number of cell surface fimbriae. *E. coli* CI6 cells cultured in the absence of berberine were heavily fimbriated (Fig. 1A). In contrast, the number of cell surface fimbriae was markedly reduced in organisms exposed to berberine; at berberine concentrations of 200 μg/ml and above, no fimbriae were seen on the bacterial cell surface (Fig. 1B). In addition, the flagella appeared to be disrupted on many bacteria (Fig. 1B). When *E. coli* CI6 in the stationary phase of growth was incubated in saline containing 300 μg of berberine per ml for 6 h at 37°C, no reduction in cell surface fimbriae was observed. These results suggest that berberine exerts its effect only on growing organisms.

The absence of fimbrial structures on the surface of berberine-treated bacteria could be the result of inhibition of fimbrial protein synthesis or inhibition of the assembly of fimbrial subunits into the filamentous structure. To determine the mode of action of berberine on *E. coli* CI6, we assayed total fimbrial protein in berberine-treated and untreated *E. coli* cells by inhibition of the enzyme-linked immunosorbent assay reaction as described previously (7). Briefly, the amount of fimbrial protein synthesized was quantitated by measuring the capacity of identical amounts of sodium dodecyl sulfate (SDS)-solubilized bacterial fractions to inhibit the reaction of anti-Pap-fimbria antibodies with isolated Pap fimbriae adsorbed to microdilution trays. Lysates of *E. coli* CI6 grown in 300 μg of berberine per ml lost their ability to inhibit the enzyme-linked immunosorbent assay reaction (Fig. 2). These results were confirmed in Western blots (immunoblots) of SDS extracts of the berberine-treated bacteria. A corresponding decrease in the production of bacterial fimbrial protein was detected (Fig. 3).

We also considered the possibility that berberine caused the shedding of fimbrial filaments. However, an examination of the spent culture concentrate from berberine-treated bacteria revealed no increase in the amount of free fimbriae as compared with the amount in the culture concentrate from untreated controls (data not shown).

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TABLE 1. Effect of berberine on growth, hemagglutination, and adherence to uroepithelial cells of Pap-fimbriated *E. coli*[a]

Berberine concn (μg/ml)	% Inhibition of growth[b]	% Loss of hemagglutination ability[c]	% Loss of adherence ability[d]
0 (Control)	0	0	0
50	0	50	41 ± 0.7
100	2	75	76 ± 1.5
200	7	93	86 ± 3.1
300	10	100	90 ± 2.6

[a] The bacteria were grown in the indicated concentrations of berberine for 18 h at 37°C, and the optical density at 550 nm was measured to determine growth. The bacteria were washed, and hemagglutination ability and adherence ability were determined as described in the text.

[b] Expressed as [100 − (optical density at 550 nm in the presence of berberine/optical density at 550 nm of the control)] × 100.

[c] Expressed as [100 − (hemagglutination titer of berberine-treated bacteria/hemagglutination titer of control bacteria)] × 100.

[d] Expressed as [100 − (number of berberine-treated bacteria per epithelial cell/number of control bacteria per epithelial cell in the absence of berberine) × 100] ± standard error of the mean (n = 3).

Because berberine appeared to inhibit the synthesis of Pap fimbriae, it was of interest to see if the drug inhibited the synthesis of other major proteins. Aliquots of berberine-treated bacterial suspensions were adjusted to the same A_{550}, solubilized in electrophoresis sample buffer, and subjected to SDS-polyacrylamide gel electrophoresis (6). No significant difference between the protein profiles of treated and untreated organisms was observed (Fig. 4). Fimbrial proteins were not detected on the gel because they were present in relatively minor amounts as compared with the other proteins. These findings suggest that berberine affects the expression of Pap fimbrial proteins but not in any major way the expression of the other proteins of *E. coli* CI6. These findings are in agreement with those of previous reports that this alkaloid had no effect on the growth of *E. coli* (1).

The action of berberine on *E. coli* appears to be specifically directed at the expression of the Pap fimbriae; in experiments similar to those described above, we found that berberine failed to block the expression of type 1 fimbriae in

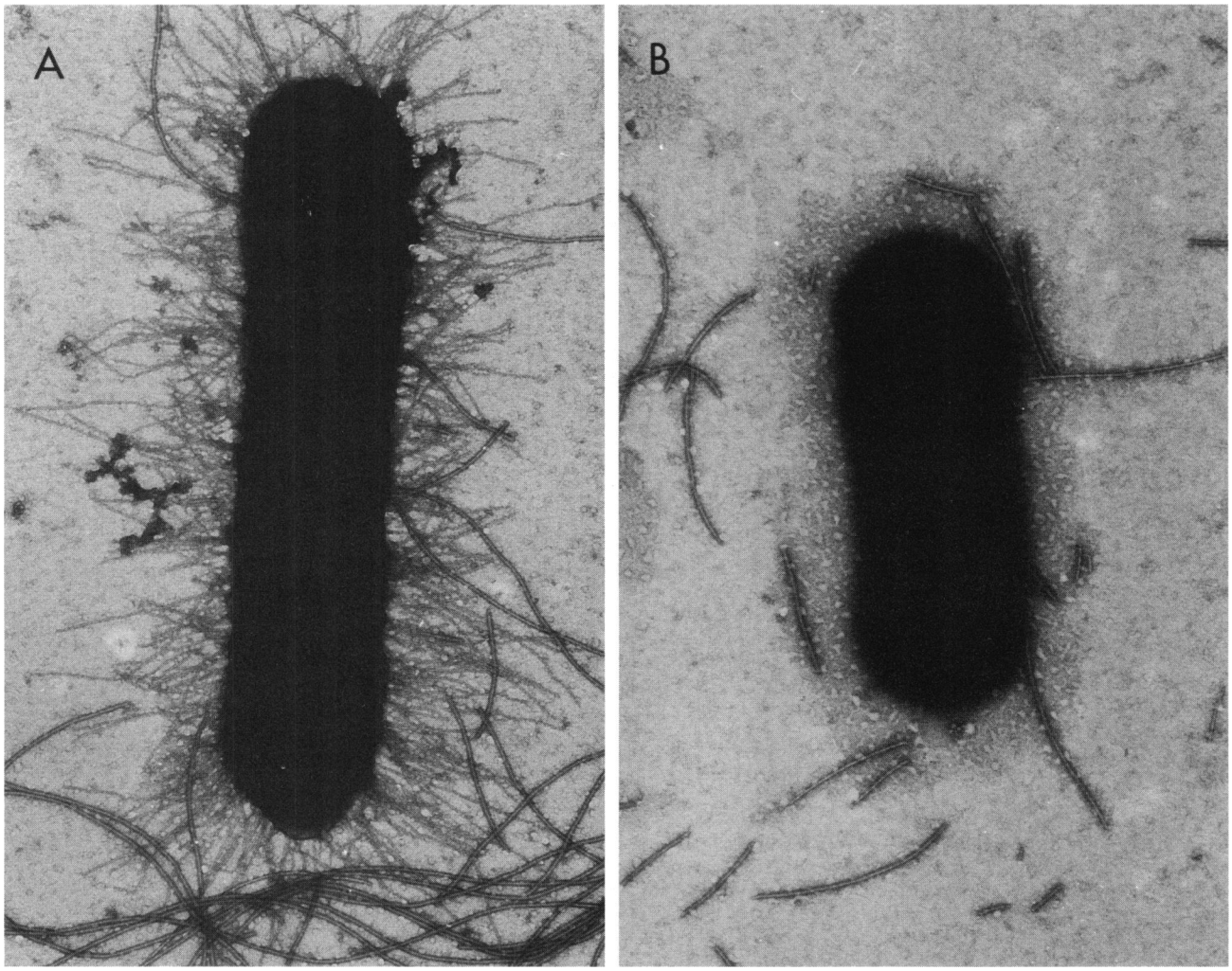

FIG. 1. (A) Electron micrograph of *E. coli* CI6 grown in brain heart infusion broth in the absence of berberine for 18 h. The bacteria were heavily fimbriated and flagellated. (B) Electron micrograph of *E. coli* CI6 grown in brain heart infusion broth in the presence of 200 μg of berberine per ml for 18 h. No fimbriae were seen on the surface of the bacteria, and the flagella appeared to be disrupted. Magnification, ×33,200.

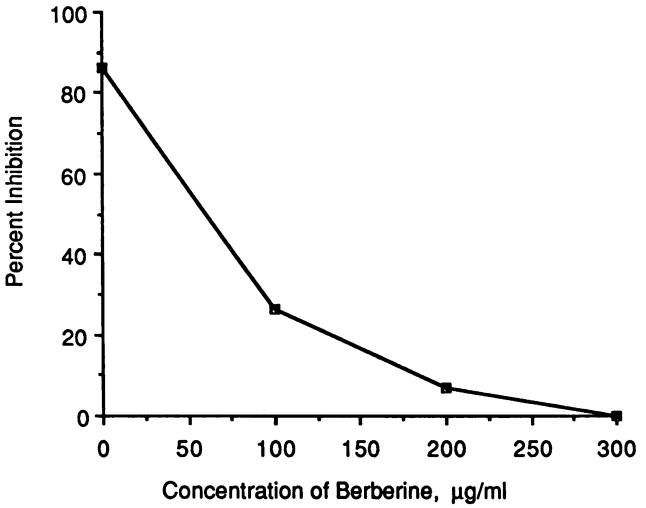

FIG. 2. Inhibition of the enzyme-linked immunosorbent assay reaction of anti-Pap-fimbria antibodies with isolated Pap fimbriae. SDS extracts of *E. coli* CI6 cultured in increasing concentrations of berberine sulfate were used as inhibitors. The inhibition was expressed as a percentage of the inhibition achieved by extracts from untreated controls.

another uropathogenic strain of *E. coli*, CI5 (data not shown).

In summary, our data suggest that berberine specifically blocks the synthesis and assembly of Pap fimbriae on the surface of *E. coli* cells without affecting the growth of the bacteria. Pap fimbriae are characterized by their ability to mediate the adherence of *E. coli* to Gal-Gal residues on the uroepithelium of the urinary bladder and the kidneys (4, 5). The apparent ability of berberine to specifically inhibit *E. coli* Pap fimbria expression in vitro may explain in part why some traditional Chinese medications which contain berberine are effective in the prevention and treatment of urinary tract infections. Further studies are required to determine whether berberine sulfate may be useful in the treatment of urinary tract infections caused by Pap-fimbriated *E. coli*.

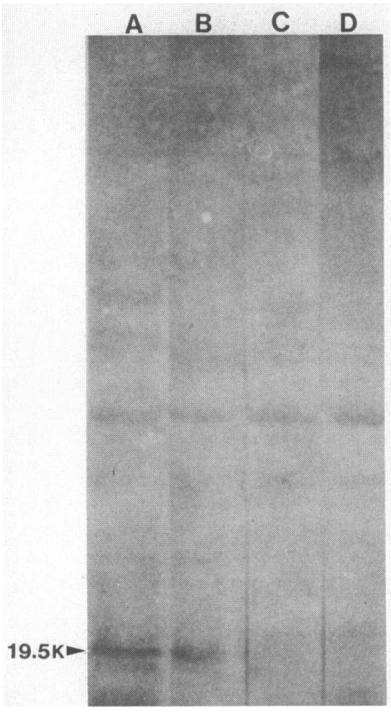

FIG. 3. Western blot analysis of SDS extracts of *E. coli* cultured in different concentrations of berberine and reacted with antibody against Pap fimbriae. (A) Control *E. coli*, no berberine; (B) berberine at 100 μg/ml; (C) berberine at 200 μg/ml; (D) berberine at 300 μg/ml. The 19.5-kilodalton fimbrial protein is indicated by an arrow. The additional (34-kilodalton) band seen on the blot is that of an unknown bacterial protein which cross-reacts with the anti-Pap-fimbria antibody. Note that the synthesis of this protein was unaffected by berberine.

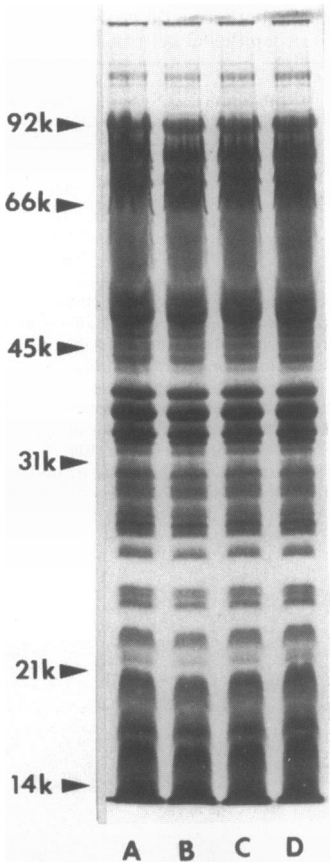

FIG. 4. SDS-polyacrylamide gel electrophoresis analysis of Pap-fimbriated *E. coli* cultured in media containing increasing concentrations of berberine. (A) Control bacteria, no berberine; (B) berberine at 50 μg/ml; (C) berberine at 100 μg/ml; (D) berberine at 200 μg/ml. The positions of the standard proteins (in kilodaltons) are indicated on the left.

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