Activity of Cefixime against *Helicobacter pylori* and Affinities for the Penicillin-Binding Proteins

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Cefixime induced the formation of rounded cells from the spiral bacillary form of *Helicobacter pylori* at the MIC or less. Three main penicillin-binding proteins, called A, B and C, were separated from *H. pylori*. Cefixime had the strongest affinity to penicillin-binding protein B. The binding of cefixime to this protein may induce the formation of rounded *H. pylori* cells.

The pathogenesis of *Helicobacter pylori* in patients with peptic ulcers is not understood, although the organism is frequently found in the gastric mucosa of these patients (2, 4, 10, 11, 15, 16). Its susceptibility in vitro to various antimicrobial agents suggests a possibility of concomitant therapy with antulcer drugs, and so in some clinics, both drugs are being used concomitantly (B. J. Marshall, K. R. Dye, M. Plankey, H. F. Frierson, S. R. Hoffman, and R. L. Guerrant, Am. J. Gastroenterol. 83:1035, 1988; J. P. Papp, Am. J. Gastroenterol. 83:1075, 1088; D. Y. Graham, G. M. Lew, and P. A. Michaleutz, Gastroenterology 96:181, 1989; T. Oh, S. Saeki, T. Nakajima, H. Okano, M. Miyamoto, and A. Tanaka, Gastroenterology 96:373, 1989; C. D’Morian, G. Coghlan, D. McKenna, D. Gilligan, and A. Tobin, Gut 29:1439–1440, 1988). It was recently reported that cefixime, which is an oral cephalosporin with a broad and potent spectrum against gram-negative bacteria (7), showed good activity against *H. pylori* (3). However, the mode of action of this drug against *H. pylori* has not been elucidated. In the present study, the penicillin-binding protein (PBP) binding pattern of cefixime was examined in *H. pylori* and subsequently correlated with its observed effects on bacterial morphology.

*H. pylori* strain FP1532 was isolated from a gastric biopsy specimen of a patient at the Center for Adult Disease, Osaka, Japan, who was diagnosed endoscopically as having a gastric ulcer. This strain was a motile, gram-negative, spiral or curved bacillus and was identified by its biological properties; it was positive for oxidase, urease, and catalase (6). The MIC of cefixime (Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan) was determined by the standard agar dilution method by using brucella agar (BBB Microbiology Systems, Cockeysville, Md.) supplemented with 5% defibrinated horse blood (5).

Strain FP1532 showed vigorous growth in brucella broth supplemented with 1% starch. This strain was cultured at a dilution of 1:10 with fresh brucella broth supplemented with 1% starch containing appropriate concentrations of cefixime in a carbon dioxide incubator. After exposure to the drug for 5 h, the cells were removed and prefixed for 3 h with 1.5% glutaraldehyde in the buffer described by Kellenberger et al. (8) and postfixed for 16 h with 1% osmium tetroxide. The fixed cells were hydrated with a graded series of ethanol. The specimens were dried to a critical point, coated with gold, and examined with a scanning electron microscope (S-450; Hitachi Co., Ltd., Tokyo, Japan).

Strain FP1532 was streaked onto brucella agar supplemented with 1% starch and cultured at 37°C for 2 days in a carbon dioxide incubator. The cells were suspended in brucella broth supplemented with 1% starch and adjusted to an *A*₅₆₅₀ of 0.1 and was grown at 37°C for about 12 h, that is, until the turbidity reached an *A*₅₆₅₀ of 0.3. Then, the cells were harvested, washed once, and resuspended in ice-cold 50 mM sodium phosphate buffer (pH 7.0). After disruption with an Insonator model 200M (Kubota Medical Appliance Supply, Tokyo, Japan), the membranes were pelleted by centrifugation at 10,000 × g and stored at −80°C at a protein concentration of 10 mg/ml. Protein was assayed by a previously described method (9). The yield from membranes isolated from a 2-liter culture was 3 to 5 mg of protein. The membranes were incubated (300 μg of protein) with the appropriate amount of cefixime (freshly prepared aqueous solution; final concentrations, 0, 0.06, 0.25, 1, 4, 16, and 64 μg/ml) at 30°C for 10 min in a total volume of 30 μl. A 5-μl volume of ¹⁴C-labeled penicillin G (3.12 mg/ml; specific activity, 156 μCi/mg; Amersham Corp.) was added, and incubation was continued for another 10 min. The reaction was stopped by adding 3 μl of 20% Sarkosyl NL97 containing an excess of nonradioactive penicillin (30 mg/ml), and the samples were maintained at room temperature for 20 min. A 20-μl volume of sample dilution buffer (12) and 10 μl of 2-mercaptoethanol were added, and the entire sample was boiled for 2 min and subjected to slab gel electrophoresis by using sodium dodecyl sulfate-polyacrylamide followed by fluorography as described previously (12). The densities of the PBP bands were measured on a chromato scanner (CS-910; Shimadzu Corp., Kyoto, Japan).

Figure 1 shows some representative scanning electron micrographs of strain FP1532 after exposure for 5 h to cefixime at its MIC or less (MIC of the drug, 0.5 μg/ml). The untreated control showed spiral bacillary profiles with bluntly rounded ends. Cefixime induced the formation of rounded cells in strain FP1532 at concentrations of greater than 0.13 μg/ml.
The interaction of cefixime with the PBPs of strain FP1532 was investigated (Fig. 2). At least three PBPs, called A, B, and C, were separated from H. pylori. The affinities of cefixime for these PBPs were estimated by measuring the competition of unlabeled cefixime and ^14^C-labeled penicillin G for their binding. Cefixime had the strongest affinity to PBP B, with a 50% inhibitory concentration (I_{50}) of 0.074 µg/ml, followed by PBP A, with an I_{50} of 1.5 µg/ml, and PBP C, with an I_{50} of 2.5 µg/ml.

Low concentrations of cefixime caused filamentation of Escherichia coli, which is a prelude to bacteriolysis at high concentrations (7). That is PBP 3 was inhibited by low concentrations, and PBPs 1A and 1B were inhibited by high concentrations (13). However, cefixime induced the formation of rounded H. pylori at low concentrations (one-fourth the MIC). We conclude that the primary target of cefixime is PBP B and that binding of the drug to this protein may induce morphological changes which eventually lead to the formation of rounded cells. On the other hand, the inhibition of all three PBPs may be necessary to obtain lysis, because bacteriolysis was only induced by high concentrations (greater than 2 µg/ml) (data not shown). Armstrong et al. (1) reported that ampicillin, penicillin G, and cephalaxin caused H. pylori to bulge and induced in the organism dumbbell-like profiles. The mode of action of these drugs against H. pylori must fundamentally be the same as that of cefixime. The rounded shape taken on by the cells after incubation with cefixime closely resembled the ovoid cells observed when E.

FIG. 1. Scanning electron micrographs of H. pylori FP1532 exposed for 5 h to nothing (A), cefixime at 0.13 µg/ml (B), and cefixime at 0.5 µg/ml (C). Bars, 2 µm.

FIG. 2. Competition of cefixime for the PBPs in isolated membranes of H. pylori. Cell membranes were isolated from strain FP1532 and reacted with concentrations of cefixime for 15 min at 37°C before adding saturating ^14^C-labeled penicillin G. Lane E, E. coli NIH; JC2; lane P, control; lane 1, 0.06 µg of cefixime per ml; lanes 2, 3, 4, 5, and 6, 0.25, 1, 4, 16, and 64 µg of cefixime per ml, respectively.

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coli was exposed to aminocillin (mecillinam), a drug with especially strong affinity to PBP 2 (14).

LITERATURE CITED


