ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1986, p. 659–663
0066-4804/86/110659-05$02.00/0

Vol. 30, No. 5

# Modulation of Bacteriolysis by Cooperative Effects of Penicillin-Binding Proteins 1a and 3 in *Escherichia coli*

E. TUOMANEN,* K. GILBERT, AND A. TOMASZ
*The Rockefeller University, New York, New York 10021*

Received 16 June 1986/Accepted 28 August 1986

***Escherichia coli*** **characteristically lyses upon treatment with most beta-lactam antimicrobial agents. In contrast, an investigational aminothiazole cephem, CGP 31523A, produced a new combination of antibacterial effects: it was highly bactericidal without causing cell wall degradation or lysis. Killing was associated with the formation of vacuolated filaments. Because the compound bound to penicillin-binding proteins (PBPs) 1a and 3, we investigated the role of PBP 3 in modulation of lysis caused by inhibition of PBP 1a. A temperature-sensitive mutant with a nonfunctional PBP 3 lysed when treated with CGP 31523A. The combination of a PBP 1 inhibitor (cefsulodin) and a PBP 3 inhibitor (aztreonam) also caused filamentation and death without lysis of wild-type cells over a narrow concentration range. We conclude that cooperative effects between PBPs in *E. coli* can lead to a dissociation of bacterial killing and lysis.**

At least nine penicillin-binding proteins (PBPs) have been detected in *Escherichia coli* by radiolabeled benzylpenicillin (12). Using beta-lactam antimicrobial agents of high affinity for a single PBP (in the appropriate concentration range), it has been demonstrated that the inhibition of PBP 1b (2, 14) (or 1a plus 1b [13, 18]) is associated with rapid killing and lysis (7), whereas inhibition of PBP 2 produces round cells and inhibition of PBP 3 produces filaments (12). Most beta-lactams bind to several PBPs, and it has been suggested that the resultant antibacterial effect is the sum of the individual inhibition effects and is in some cases even synergistic (4, 8, 11, 16). These findings suggested it might be possible to demonstrate that PBPs cooperate with one another. Such cooperative effects might include both up- and down-modulation of lysis or killing. For instance, inhibition of single PBPs which alone do not cause lysis (e.g., PBPs 2 and 3) may actually down-modulate lysis or killing when these PBPs are inhibited in combination with the lytic PBP 1b. Such interactions between PBPs would be consistent with the findings that (i) mutants lacking PBP 1a, 1b, or 2 have strikingly different morphologic responses to inhibition of PBP 3 by furazlocillin (8), and (ii) inhibition of PBP 1a can interfere with lysis induced by inhibition of PBP 1b (4).

We now report that an investigational cephalosporin, CGP 31523A, which binds to PBPs 1a and 3, produces a new and unique combination of antibacterial effects in *E. coli*: the compound is highly bactericidal without causing degradation of cell walls or lysis of bacterial filaments. This finding led us to investigate the effect of inhibition of the nonlytic PBP 3 on lysis induced by inhibition of PBP 1a. We describe what appears to be the modulation of lysis but not lethality when inhibition of PBP 3 occurs simultaneously with inhibition of PBP 1a. Since bacteriolysis may not always be an advantageous accompaniment to bactericidal activity at all sites of infection in vivo (14a, 15), the cooperation beween PBP 1a and 3 may be important to exploit when considering improvements in drug design.

* Corresponding author.

## MATERIALS AND METHODS

**Bacterial strains and culture conditions.** *E. coli* W7 (*dap*, *lys*) was cultured in M9 minimal salts medium supplemented with 40 μg of lysine, 4 μg of diaminopimelic acid, and 2 μg of glucose per ml (5). The organisms were grown with aeration in a 37°C water bath. *E. coli* KN 126 and its temperature-sensitive cell division mutant, Sp 63 (PBP 3 temperature sensitive), were kindly provided by B. Spratt (11) and used in one set of experiments to investigate the mechanism of a proposed interaction between PBPs 1a and 3. Cultures were grown as previously described (11) in Penassay broth (antibiotic medium no. 3; Difco Laboratories, Detroit, Mich.) at both the permissive (30°C) and the nonpermissive (42°C) temperature.

**Characterization of bactericidal and bacteriolytic activity.** The new aminothiazole cephem CGP 31523A (7-β[(2,5)-2-(2-aminothiazol-4-yl)-2,4-formamido-acetamido]-3-[(1,2,3-thiadiazol-5-yl-thiomethyl)]-3-cephem-4-carboxylic acid) was provided by O. Zak and R. Scartazzini of CIBA-GEIGY Ltd., Basel, Switzerland (17). The beta-lactam antimicrobial agents cephaloridine, cefsulodin, and aztreonam were from commercial sources.

The MICs of the beta-lactam antimicrobial agents were determined by the tube dilution method at a cell concentration of $10^5$/ml after 24 h of incubation at 37°C for *E. coli* W7 or at 30 and 42°C for both strain Sp 63 and KN 126. Values for Sp 63 and KN 126 were the same and did not vary with temperature. Determinations for strain W7 and KN 126, respectively, were as follows: CGP 31523A, 0.001 and 0.6 μg/ml; cephaloridine, 2 and 5 μg/ml; cephalexin, 5 and 10 μg/ml; aztreonam, 0.005 and 0.8 μg/ml; and cefsulodin, 20 and 24 μg/ml.

The bactericidal and bacteriolytic effects of CGP 31523A were determined for the concentration range 0.5 to 1,000× the MIC. At 0 time, the drug was added to mid-logarithmic-phase *E. coli*. Culture turbidity was monitored by recording the optical density at 620 nm in a spectrophotometer (Sequoia-Turner, Mountainview, Calif.). At regular intervals after the addition of drug, the cell viability (CFU) was determined by plating on Penassay broth agar plates for strains KN 126 and Sp 63 or on nutrient agar plates supple-

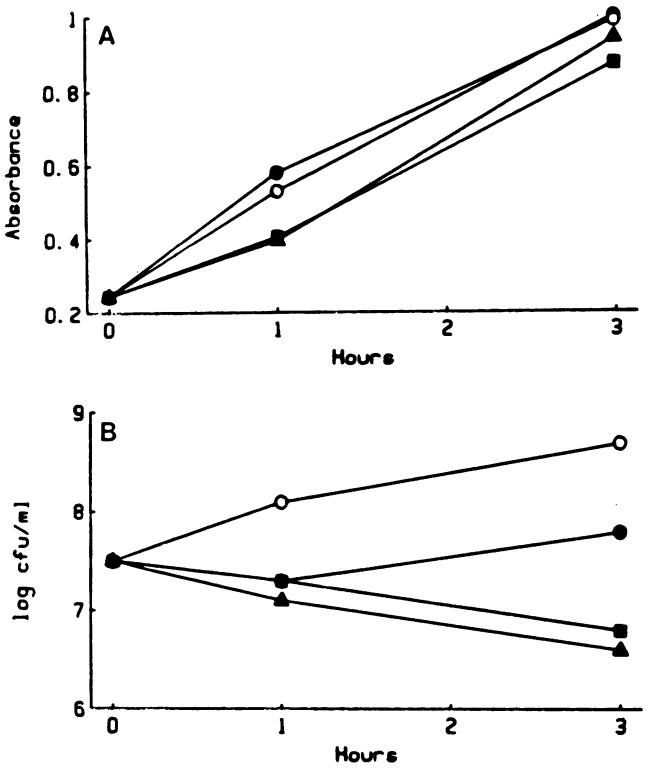


FIG. 1. Dissociation of bacteriolytic and bactericidal activity of CGP 31523A. *E. coli* W7 was treated with 0 (○), 1 (●), 10 (■), or 100 (▲) × the MIC of CGP 31523A. Culture turbidity (A) and viability (B) were monitored over 3 h.

mented with 40 μg of lysine, 4 μg of diaminopimelic acid, and 2 μg of glucose per ml for strain W7. Light microscopy was performed on a Zeiss Research microscope. Electron micrographs were done as described previously (3).

*E. coli* mutant strain Sp 63, with a thermosensitive PBP 3, was used to study the role of PBP 3 inactivation in the simultaneous inhibition of PBPs 1 and 3. The Sp 63 mutant grew as rods at 30°C (permissive temperature) and as unseptated filaments at 42°C (nonpermissive temperature). Overnight cultures grown at 30 and 42°C for both Sp 63 and parent KN 126 were diluted 1:100 in fresh Penassay broth. At mid-logarithmic phase, 2× the MICs of CGP 31523A (1.25 μg/ml) and cephaloridine (10.0 μg/ml) were added to Sp 63 and KN 126 at both the permissive and nonpermissive temperatures. At regular intervals after drug additions, the turbidity of the cultures was monitored, samples for CFUs were taken, and the morphology of the cultures was recorded.

To study the bactericidal effect of inhibition of PBP 3 in conjunction with PBP 1a, combinations of various concentrations of two beta-lactam antimicrobial agents which selectively bind to either PBP 3 (aztreonam) or PBP 1a (cefsulodin) were tested. Exponentially growing *E. coli* was exposed to the simultaneous addition of 100× the MIC of aztreonam and 0.01 to 2× the MIC of cefsulodin. In one experiment, the addition of aztreonam preceded the addition of cefsulodin by 60 min. At several times after drug addition, optical density was recorded and samples for CFU determination were taken (as described above).

**Assay of wall degradation.** The degree of wall degradation was quantitated during the killing of *E. coli* W7 by CGP 31523A and the combination of aztreonam and cefsulodin. *E. coli* W7 was grown in M9 medium (5) supplemented with glycerol (4 μg/ml) instead of glucose and 1 μg (1 μCi/ml) of [$^3$H]*N*-acetyl-D-glucosamine (3.4 Ci/mmol; Amersham, Inc., Amersham, United Kingdom) per ml. After 2 h, the culture was filtered into fresh M9 medium supplemented with glycerol and incubated for 30 min at 37°C. Immediately after the 30-min chase, aliquots of the culture were exposed to CGP 31523A. To determine cell-wall-associated radiolabel, duplicate serial 200-μl samples were remove from the culture and frozen. Radioactivity associated with boiling 5% sodium-dodecyl-sulfate-precipitable material (cell wall) was determined as previously described (9).

**Assay of PBPs in cell membranes and whole cells.** In vitro labeling of PBPs in strain W7 cell membranes was performed as described by Spratt (12). Briefly, in the competition assay, CGP 31523A (0.1 to 1,000× the MIC) was added to bacterial membranes (200 μg of protein) and incubated for 15 min at 30°C. [$^3$H]benzylpenicillin (ethylpiperidium salt; 25.4 Ci/mmol; Merck Sharp & Dohme, Rahway, N.J.), at a final concentration of 2.5 μg/ml, was added to the sample for an additional 10 min at 30°C. The samples were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the PBPs were detected by fluorography as previously described (12). PBP band density was measured on a Quick Scan Junior scanning densitometer (Helena Laboratories, Beaumont, Tex.).

The assay of PBPs in whole cells was performed essentially as described by Broome-Smith and Spratt (1). Specifically, in the competition assay, 1 ml of mid-logarithmic-phase culture was incubated for 15 min at 30°C with the specified concentrations of drugs followed by the addition of [$^3$H]penicillin at 1.25 μg/ml (final concentration) for 10 min at 30°C. The whole cell samples were processed for sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

## RESULTS

**Dissociation of bactericidal and bacteriolytic activity of CGP 31523A.** CGP 31523A demonstrated a striking dissociation of killing and lytic activities in that effective killing was accompanied by a steady increase in culture turbidity with time (Fig. 1). Upon overnight incubation with the drug, the viable titers of the cultures decreased 2.5 logs at 10× the MIC and >3 logs at 100× the MIC. Thus, in degree of killing, CGP 31523A approached the effectiveness of such beta-lactam agents as cephaloridine or ampicillin (7). On the other hand, the killing effects by the cephalosporin differed profoundly from that of the latter drugs in that no detectable lysis accompanied the bactericidal effect of CGP 31523A. In fact, lysis did not occur until the drug concentration exceeded 1,000× the MIC. As shown in Fig. 2, the apparent discrepancy between increased culture turbidity and decreased viability could be explained on the basis of the morphological changes associated with treatment of *E. coli* with this agent. As the drug concentration was increased, the cells formed long, phase-dense filaments, the length of which increased with the length of incubation (Fig. 2B). As bactericidal activity progressed, localized vacuoles appeared in the filaments, but no disruption of the cell wall was found (Fig. 2C and D). The lack of lysis was confirmed in cell wall degradation studies. Only 15% of [$^3$H]*N*-acetyl-D-glucosamine was lost over 4 h from cells treated with 100× the MIC of CGP 31523A (1.5-log kill). In contrast, the eventual appearance of lysis at 1,000× the MIC of CGP 31523A was accompanied by a >75% loss of cell-wall-associated radio-

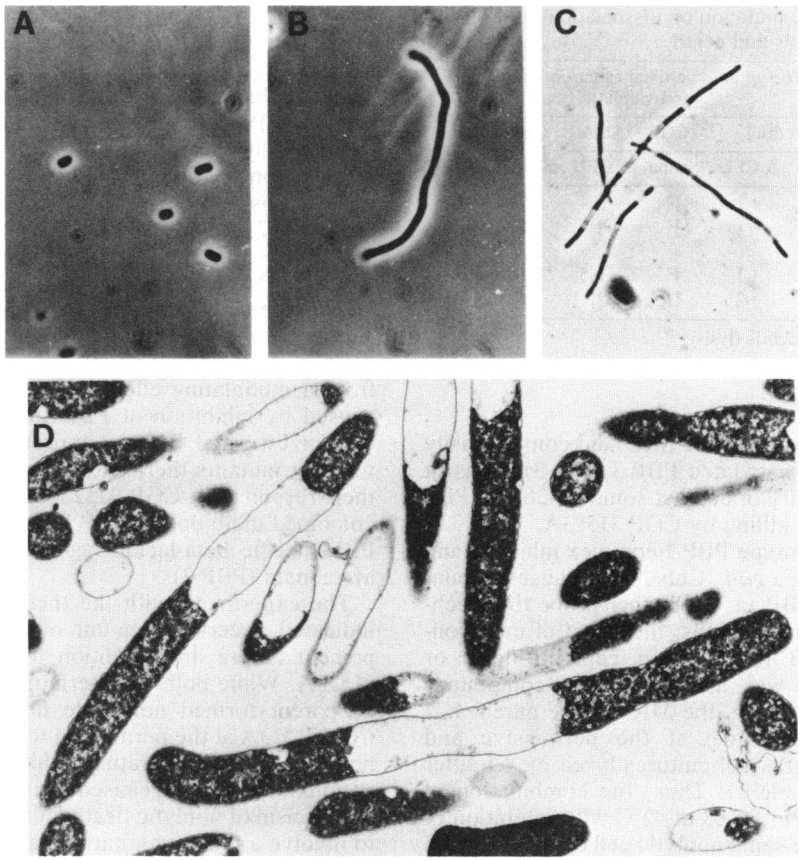


FIG. 2. Morphology of *E. coli* treated with bactericidal concentrations of CGP 31523A. *E. coli* W7 was treated with 10× the MIC of CGP 31523A, and morphology was monitored over time by light microscopy (A to C; magnification, × 100). The electron micrograph (D; magnification, × 14,625) corresponds to the light micrograph in panel C. Duration of treatment with CGP 31523A: A, 0 min; B, 1 h; C, 3 h.

label in 4 h (3.2-log kill). Thus, during the exposure of the cells to between 1 and 100× the MIC of this drug, cell-wall-associated radiolabel did not decrease as the cells lost viability.

The PBP pattern of CGP 31523A as determined in the competition assay with [$^3$H]penicillin is shown in Fig. 3. Highest affinity binding occurred to PBP 3 (50% inhibitory concentration [$I_{50}$], 0.01 μg/ml), followed by PBP 1a ($I_{50}$, 0.05 μg/ml). At 1,000× the MIC, the concentration at which lysis occurred in the presence of CGP 31523A, binding extended to PBP 1b. Thus, the range of CGP 31523A concentrations showing concurrent PBP 1a and 3 binding correlated with the range for optimal nonlytic bactericidal action with filament formation (10 to 100× the MIC).

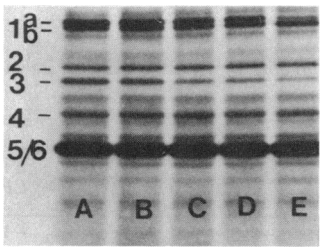


FIG. 3. PBP profile of CGP 31523A. *E. coli* W7 membrane preparations were exposed to the indicated concentrations of CGP 31523A. Lanes: A, no drug; B, 1 μg; C, 5 μg; D, 10 μg; E, 50 μg. [$^3$H]penicillin was then added, followed by electrophoresis and fluorography for 4 days as described in Materials and Methods.

**Alteration of lytic activity of beta-lactams in *E. coli* mutants with altered PBP 3.** The effect of mutational alteration of PBP 3 on the antibacterial activity of cephaloridine (PBP 1 specific) and CGP 31523A (PBP 1a and 3 specific) was studied to determine the relative role of PBPs 1a and 3 in nonlytic cell death observed in *E. coli* W7 treated with CGP 31523A. *E. coli* Sp 63, a thermosensitive PBP 3 mutant, and its parent strain, KN 126, were used in the study. Both strains grew as rods at 30°C and contained PBP 3 (11). At 42°C the phenotype of the parent was unchanged, whereas the mutant grew as a filament and binding of [$^3$H]penicillin to PBP 3 disappeared (11).

The results of treatment of *E. coli* KN 126 and Sp 63 at the permissive and nonpermissive temperatures with 2× the MIC (1.2 μg/ml) of CGP 31523A are shown in Table 1. The parent KN 126 strain, grown at 30 and 42°C in the presence of CGP 31523A, demonstrated the same filamentation with cell death as that observed with *E. coli* W7. The addition of CGP 31523A to mutant strain Sp 63 at the permissive temperature also resulted in nonlytic cell death of filaments. However, strain Sp 63 grown for several generations before the addition of antimicrobial agent at 42°C (nonfunctional PBP 3) lysed after 4 h in the presence of CGP 31523A. Strain Sp 63 grown at 42°C had no detectable PBP 3 by the fluorographic assay, and binding of CGP 31523A was limited to PBP 1a. In contrast, wild-type bacteria treated with CGP 31523A within the concentration range that produced the

TABLE 1. Effect of PBP 3 mutation on dissociation of bacterial lysis and death

| Strain | Temperature (°C) | PBP 3 | Effect of antimicrobial agent on cell shape[a] and viability[b] | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | No drug | | CGP 31523A | | Cephaloridine | |
| | | | Shape | Δ CFU | Shape | Δ CFU | Shape | Δ CFU |
| KN 126 | 30 | + | N | +1 | F | −2 | D | −1.5 |
| | 42 | + | N | +1 | F | −2 | D | −2 |
| Sp 63 | 30 | + | N | +1 | F | −1.5 | D | −1.5 |
| | 42 | − | F | +2 | D | −2 | D | −2 |

[a] N, Normal; F, filament; D, debris (lysis).
[b] Change in log CFU over 4 h.

nonlytic killing effect (2 to 100× the MIC) did contain easily detectable amounts of nonacylated PBP 3 (Fig. 3). Thus, it appears that the availability of at least some functional PBP 3 was critical to nonlytic killing by CGP 31523A.

Cephaloridine is a prototype PBP 1 complex inhibitor and usually causes lysis of *E. coli*. Cefsulodin causes similar effects by inhibition of PBP 1a. To further probe the mechanism of PBP 1a and 3 interactions, the effect of mutation-induced loss of PBP 3 function on cephaloridine- or cefsulodin-induced lysis was assessed. Both compounds produced the same effect at 2× the MIC on the parent KN 126 and mutant Sp 63 grown at the permissive and nonpermissive temperatures: all cultures lysed by 4 h after the addition of drug (Table 1). Thus, the combination of mutational loss of PBP 3 in Sp 63 at 42°C with inhibition of PBP 1 did not result in the same nonlytic cell death seen with the partial inhibition of PBP 3 plus 1a by CGP 31523A in these strains.

**Modulation of cefsulodin-induced lysis by aztreonam.** If the explanation of the nonlytic death associated with CGP 31523A involves modulation of lysis associated with inhibition of PBP 1a by simultaneous inhibition of PBP 3, then it appeared reasonable to test whether a PBP-3-specific inhibitor (aztreonam) could modulate the lytic activity of a PBP 1 inhibitor (cefsulodin). This appeared to be the case within a limited concentration range for the drug combination (Table 2). Aztreonam at 100× the MIC caused filamentation without death in *E. coli* W7, as expected for PBP 3 inhibition. Also as expected, cefsulodin alone caused lysis. When aztreonam was combined with low (sub-MIC) concentrations of cefsulodin, filamentation was accompanied by moderate killing without lysis. This effect could be amplified if aztreonam (0.1 μg/ml) was added to the cells 1 h before cefsulodin (12 μg/ml; 0.05× the MIC). Whereas the simultaneous addition of these agents led to an equal mixture of debris and vacuolated filaments (0.8-log kill), pretreatment with aztreonam resulted in a similar degree of killing, but >90% of the cells remained intact as vacuolated filaments and wall degradation decreased from 62 to 37% (Table 2).

## DISCUSSION

CGP 31523A is a cephalosporin which is known to cause filamentation of *E. coli* K-12 at low concentrations (≤100× the MIC), while at high concentrations (1,000× the MIC) it causes lysis (17). This is consistent with the PBP pattern of this compound, i.e., PBP 3 inhibition at concentrations causing filamentation ($I_{50}$, 0.015 μg/ml; 2× the MIC) and PBP 1a and b inhibition at concentrations leading to lysis (PBP 1b $I_{50}$, 4 μg/ml; 500× the MIC) (17). Our results confirmed these previous findings with the exception of the $I_{50}$ for PBP 1a, which we found to be 0.05 μg/ml, 100-fold lower than the $I_{50}$ for PBP 1b (i.e., the lytic concentration). The striking and unusual finding in these studies was made when the bactericidal activity of this compund was investigated. CGP 31523A is the first beta-lactam described which rapidly kills *E. coli* without cellular lysis. The complete dissociation of lysis and killing (>3-log killing during overnight exposure) of *E. coli* between 2 and 100× the MIC of CGP 31523A was accompanied by the formation of nonviable, phase-dense filaments containing localized vacuoles. No cell wall degradation accompanied cell death.

To understand how killing without lysis might occur in general, we investigated the mechanism of action of CGP 31523A. We reasoned that killing without lysis may arise from a modulating effect of PBP 3 inhibition on the lysis caused by inhibition of PBP 1a. This possible mechanism was investigated by two approaches: (i) determination of whether mutants thermosensitive for PBP 3 were altered in their response to CGP 31523A and (ii) reconstruction of a combined inhibition of PBP 1a and 3 using two separate but PBP-specific beta-lactam agents, cefsulodin (PBP 1a) and aztreonam (PBP 3).

The experiment with the thermosensitive PBP 3 mutant indicated a certain amount of functional PBP 3 must be present before drug addition for nonlytic killing by CGP 31523A. While both the thermosensitive PBP 3 mutant and its parent formed nonviable filaments when treated with CGP 31523A at the permissive temperature, treatment at the nonpermissive temperature (which leads to complete inactivation of PBP 3 [11]) caused lysis without filamentation. The mechanism of nonlytic death induced by CGP 31523A seems to involve a fine, quantitative balancing of the inhibitions of PBPs 3 and 1a. Extensive inhibition of PBPs 1a and 1b (by cephaloridine), together with the extensive inhibition of PBP 3 (in the thermosensitive mutant grown at 42°C), resulted in lysis and not in the nonlytic cidal activity. The appropriately balanced inhibition of PBPs 3 and 1a was successfully reproduced by the use of aztreonam (a PBP 3 inhibitor) in conjunction with cefsulodin (inhibition of PBP 1a at the concentrations used). In a sub-MIC range in which cefsulodin-induced lysis and killing were not evident, the combined effect of aztreonam and cefsulodin treatment was to change viable aztreonam-induced filaments to nonviable filaments with vacuoles. As the concentration of cefsulodin was raised, more vacuoles appeared in the filaments and greater killing was achieved. However, the concentration range in which killing without lysis was achieved was very narrow (i.e., between 0.5 and 1× the MIC). Finally, at higher concentrations of cefsulodin (above 2× the MIC), lysis

TABLE 2. Modulation of cefsulodin-induced lysis by aztreonam

| Amt of cefsulodin | Bactericidal and lytic effect when combined with: | | | | | |
|---|---|---|---|---|---|---|
| | No drug | | Aztreonam (0.1 μg/ml) | | Pretreatment with aztreonam (0.1 μg/ml for 1 h) | |
| | Morphology[a] | Δ log CFU[b] | Morphology[a] | Δ log CFU | Morphology | Δ log CFU |
| 2 × MIC | D | −2.5 | D | −3 | | |
| 0.5 × MIC | N | +0.5 | 50% D, 50% $F^V$ | −0.8 | $F^V$ | −1.1 |
| 0.01 × MIC | N | +1.0 | $F^V$ | −0.3 | | |
| None | N | +1.2 | F | +0.3 | | |

[a] D, Debris; N, normal rod; F, filament; $F^V$, filament with vacuole.
[b] Change in viability (log CFU/ml) over 4 h.

accompanied by cell wall degradation supervened (as was shown for this combination previously [4]).

The combined antibacterial effect of inhibition of several PBPs has been documented frequently (e.g., see references 4, 5, 10, and 16). Schmidt et al. described the effects of inhibition of PBP 3 in mutants lacking PBP 1a to include lysis not evident in parent strains treated with the same PBP 3 inhibitor (8). It is possible that the nonlytic killing described in this report represents an intermediate stage in the same type of synergistic effect as that observed by Schmidt et al. (8): the nonlytic killing effect may involve the induction of a peptidoglycan "nicking"-murein hydrolase activity similar to that envisioned to explain penicillin-induced nonlytic death in group A streptococci (6). Alternatively, the phenomenon described in this report may be an example for a down-modulation of lytic antibiotic effects as a consequence of an appropriately balanced inhibition of two PBPs. Whatever the mechanism, the final balance between lysis and production of nonviable, structurally intact filaments clearly depends on the relative degree of inhibition of PBPs 1a and 3. Interactions between PBPs which lead to modulation of lysis are important, because killing of bacteria without release of highly inflammatory autolytic cell wall degradation products may be an important avenue for the improvement of chemotherapy (14a, 15).

## ACKNOWLEDGMENTS

This work was supported in part by Public Health Service grant BRSG PHS RR07065 20 from the National Institutes of Health and the Irma T. Hirschl Fellowship to E.T.

We thank J. Suter for the electron microscopy and M. Geller for preparation of the manuscript.

## LITERATURE CITED

1. **Broome-Smith, J. K., and B. G. Spratt.** 1982. Deletion of the penicillin-binding protein 6 gene of *Escherichia coli*. J. Bacteriol. **152**:904–906.
2. **Chase, H. A., C. Fuller, and P. E. Reynolds.** 1981. The role of penicillin-binding proteins in the action of cephalosporins against *Escherichia coli* and *Salmonella typhimurium*. Eur. J. Biochem. **117**:301–310.
3. **Cozens, R. M., E. Tuomanen, W. Tosch, O. Zak, J. Suter, and A. Tomasz.** 1986. Evaluation of the bactericidal activity of β-lactam antibiotics on slowly growing bacteria cultured in the chemostat. Antimicrob. Agents Chemother. **29**:797–802.
4. **Curtis, N. A. C., R. L. Eisenstadt, K. A. Turner, and A. J. White.** 1985. Inhibition of penicillin-binding protein 3 of *Escherichia coli* K-12. Effects upon growth, viability and outer membrane barrier function. J. Antimicrob. Chemother. **16**: 287–296.
5. **Goodell, W., and A. Tomasz.** 1980. Alteration of *Escherichia coli* murein during amino acid starvation. J. Bacteriol. **144**:1009–1016.
6. **Horne, D., and A. Tomasz.** 1977. Tolerant response of *Streptococcus sanguis* to beta-lactams and other cell wall inhibitors. Antimicrob. Agents Chemother. **11**:888–896.
7. **Kitano, K., and A. Tomasz.** 1979. Triggering of autolytic cell wall degradation in *Escherichia coli* by beta-lactam antibiotics. Antimicrob. Agents Chemother. **16**:838–848.
8. **Schmidt, L. S., G. Botta, and J. T. Park.** 1981. Effects of furazlocillin, a β-lactam antibiotic which binds selectively to penicillin-binding protein 3, on *Escherichia coli* mutants deficient in other penicillin-binding proteins. J. Bacteriol. **145**:632–637.
9. **Schwarz, U., A. Ryter, A. Rambach, R. Hellion, and Y. Hirota.** 1975. Process of cellular division in *Escherichia coli*: differentiation of growth zones in the sacculus. J. Mol. Biol. **98**:749–759.
10. **Seeger, K., and E. Schrinner.** 1983. The interdependence of bacterial shape and simultaneous blocking of PBP 2 and 3 in *Escherichia coli* Sp 45 and *Escherichia coli* Sp 63, p. 481–486. *In* R. Hakenbeck, J. V. Holtje, and H. Labischinski (ed.), The target of penicillin. Walter de Gruyter & Co., Berlin.
11. **Spratt, B. G.** 1977. Temperature-sensitive cell division mutants of *Escherichia coli* with thermolabile penicillin-binding proteins. J. Bacteriol. **131**:293–305.
12. **Spratt, B. G.** 1977. Properties of the penicillin-binding proteins of *Escherichia coli* K12. Eur. J. Biochem. **72**:341–352.
13. **Suzuki, H., Y. Nishimura, and Y. Hirota.** 1978. On the process of cellular division of *Escherichia coli*: a series of mutants of *E. coli* altered in the penicillin-binding proteins. Proc. Natl. Acad. Sci. USA **75**:664–668.
14. **Tamaki, S., S. Nakajima, and M. Matsuhashi.** 1977. Thermosensitive mutation in *Escherichia coli* simultaneously causing defects in penicillin-binding protein 1Bs and in enzyme activity for peptidoglycan synthesis *in vitro*. Proc. Natl. Acad. Sci. USA **74**:5472–5476.

14a. **Tuomanen, E.** 1986. Phenotypic tolerance: the search for β-lactam antibiotics that kill nongrowing bacteria. Rev. Infect. Dis. **8**(Suppl. 3):279–291.

15. **Tuomanen, E., H. Liu, B. Hengstler, O. Zak, and A. Tomasz.** 1985. The induction of meningeal inflammation by components of the pneumococcal cell wall. J. Infect. Dis. **151**:859–868.
16. **Williamson, R., L. Gutmann, M. D. Kitzis, and J. F. Acar.** 1984. An evaluation of the bacteriolytic and biochemical properties of ceftiolene (42980 RP). J. Antimicrob. Chemother. **14**:581–593.
17. **Wise, R., C. Cross, and J. M. Andrews.** 1984. In vitro activity of CGP 31523A, a broad-spectrum cephalosporin, in comparison with those of other agents. Antimicrob. Agents Chemother. **26**:876–880.
18. **Yousif, S. Y., J. K. Broome-Smith, and B. G. Spratt.** 1985. Lysis of *Escherichia coli* by beta-lactam antibiotics: deletion analysis of the role of penicillin-binding proteins 1A and 1B. J. Gen. Microbiol. **131**:2839–2845.