Synergy in the Antimicrobial Action of Penicillin and \(\beta\)-Chloro-d-Alanine In Vitro

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\(\beta\)-Chloro-d-alanine and penicillin G acted on early and late steps, respectively, in the biosynthesis of the bacterial cell wall. In combination these compounds showed a synergistic effect on the growth of *Salmonella typhimurium* and of *Escherichia coli* in vitro.

\(\beta\)-Chloro-d-alanine is an antimicrobial agent that has been suggested to act through inhibition of the pyridoxal-phosphate enzymes that catalyze the synthesis of d-alanine for the bacterial cell wall (8). Thus, d-glutamate-d-alanine transaminase and alanine racemase in crude extracts are irreversibly inhibited either directly by this chloroamino acid or indirectly by a compound formed from chloroalanine (6). However, it is possible that the chloroamino acid has other effects on the cell. During studies on the mode of action of \(\beta\)-chloro-d-alanine, we found that this inhibitor of the biosynthesis of an essential component of the bacterial cell wall acts in a synergistic fashion to inhibit bacterial growth when present with an inhibitor of the cross-linking step in cell wall synthesis, i.e., penicillin. In a separate study, \(\beta\)-chloro-d-alanine has been found to be bactericidal towards a penicillin-resistant strain of *Escherichia coli*.

The minimal inhibitory concentration (MIC) for \(\beta\)-chloro-d-alanine was variable and depended on growth media, growth phase, doubling time, and initial inoculum size. The minimal bactericidal concentration was 5- to 10-fold higher than the MIC. Low concentrations of \(\beta\)-chloro-d-alanine were found to be most effective in the early log phase of growth in minimal media. For *Salmonella typhimurium* strain LT-2, penicillin G (Eli Lilly Co.) had no antibacterial properties (Fig. 1A) at a concentration of 4.8 \(\mu\)g/ml (0.6 MIC). However, when \(\beta\)-chloro-d-alanine (Cyclo Chemical Co.) at a concentration of 4.8 \(\mu\)g/ml (<0.3 MIC) was present together with an ineffective concentration of penicillin G, there was a marked synergistic effect (Fig. 1B). Separate experiments with 9.6 \(\mu\)g of penicillin G per ml or 14.4 \(\mu\)g of \(\beta\)-chloro-d-alanine per ml showed no bactericidal effects.

In another series of experiments with *E. coli* W3110 (a K-12 wild type), in which viable cell counts were measured, penicillin G alone (12 \(\mu\)g/ml, 0.5 minimal bactericidal concentration) led to a slight bacteriostatic effect (Fig. 2A), but there was a synergistic effect when \(\beta\)-chloro-d-alanine (30 \(\mu\)g/ml, <0.5 MIC) was present together with penicillin G (Fig. 2B). A separate experiment with 60 \(\mu\)g of \(\beta\)-chloro-d-alanine per ml had no bactericidal effect.
Fig. 2. Effect of penicillin G on the number of viable cells of E. coli W3110 in the presence and absence of β-chloro-D-alanine. The experiment was done as described in Fig. 1, and serial dilutions (11) were plated on agar that contained the minimal medium described in Fig. 1. (A) Symbols: ● Number of colony-forming units in the presence of penicillin G at a final concentration of 12 μg/ml; ○, growth without penicillin. (B) Symbols: ○, Growth in the presence of β-chloro-D-alanine at a final concentration of 32 μg/ml; ●, number of viable cells in the presence of both penicillin G and β-chloro-D-alanine at a final concentration of 12 and 32 μg/ml, respectively. The initial time points (●) had the same number of cells for all experiments. The turbidity of the culture containing only penicillin G (A, ○) at any time point was not significantly different from that of the control culture (A, ●).

In a separate set of experiments, E. coli K390 (RTF-1 amp’ str’ cml’ sul”) was shown to be susceptible to β-chloro-D-alanine. By using a diffusion assay (9), we found that for this organism β-chloro-D-alanine (2.4 mg per disk) resulted in a zone of inhibition of 47 mm. For comparison, the zone of inhibition of the growth of E. coli K38 (wild type) was 41 mm with this compound (2.4 mg per disk). Strain K390 is resistant to penicillin G because it produces a β-lactamase that inactivates the antibiotic (7). With this strain there is no detectable zone of inhibition with penicillin G (0.1 mg per disk) whereas the wild type shows a zone of inhibition of 23 mm with penicillin (0.1 mg per disk).

Synergy between penicillin and other antimicrobial agents has been well documented (1, 3–5, 10, 12–14; N. E. Allen and J. K. Epp, Abstr. Annu. Meet. Am. Soc., Microbiol. 1975, A58, p. 10), but the second agent inhibits the activity or the synthesis of the β-lactamase that would otherwise inactivate penicillin G, and penicillin G is thus able to function normally. Alternatively, penicillin G alters the permeability barrier for the second agent. The synergy between β-chloro-D-alanine and penicillin G described here appears to be unique in that these antimicrobial agents act on opposite ends of the biosynthetic pathway for the bacterial cell wall.

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LITERATURE CITED


