ANTIMICROBIAL AGENTS

Potential of the effects of chlorhexidine diacetate and cetylpyridinium chloride on mycobacteria by ethambutol

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Summary. Ethambutol enhanced the effects of chlorhexidine diacetate and cetylpyridinium chloride against *Mycobacterium avium*, *M. bovis* BCG, *M. fortuitum* and *M. phlei*. The findings show that it is possible to increase the susceptibility of mycobacteria to agents that normally exhibit poor activity against these organisms because of their reduced cellular penetration.

Introduction

The antiseptics chlorhexidine diacetate and cetylpyridinium chloride have poor mycobactericidal activity,¹ probably because the cell wall acts as a barrier to their penetration into the cell. The search for new antibacterial compounds with low toxicity and activity at low concentration has intensified because of the restrictions imposed on the use of aldehydes as disinfectants and the increasing prevalence of mycobacterial infections in AIDS patients and the homeless. This study was undertaken to determine whether it is possible to increase the activity of the two antiseptics in the presence of ethambutol, an anti-tubercular agent that enhances the activity of a wide range of drugs against mycobacteria.² Some preliminary observations have been reported previously.³

Materials and methods

Mycobacterium fortuitum ATCC 6841, *M. bovis* BCG (NCO 5692), *M. phlei* NCTC 8151 and laboratory isolate 3906 of *M. avium* were grown at 37°C on Löwenstein-Jensen slopes.

Minimum inhibitory concentrations (MICs) of chlorhexidine diacetate (Sigma), cetylpyridinium chloride (BDH) and ethambutol (Lederle) were estimated in Middlebrook 7H9 liquid medium. Ten-ml volumes of medium, containing the desired concentrations of biocides, were inoculated with 20 µl of a

test culture, prepared by mixing a loopful of bacteria from a culture on a Löwenstein-Jensen slope in a 7-ml screw-capped bottle with 0.75 ml of phosphate buffer and two small nails, and stirring until an even suspension was obtained. Presence or absence of visible growth was noted after incubation at 37°C for 10 days. MICs of test biocides were also assessed in the presence of ethambutol (0.5–10 mg/L).

To study the effects of pre-treatment with ethambutol, heavy suspensions of cells were exposed to ethambutol, 1 or 12.5 mg/L in 10 ml of sterile distilled water and held at room temperature for 20 min, 60 min or 24 h. The suspensions were washed twice by centrifugation and used to prepare inocula for MIC determinations as described above. Control suspensions were subjected to the same procedure but without ethambutol.

Possible interactions between ethambutol and biocides were also examined in the Bactec 460 radiometric system in which ¹⁴C-labelled CO₂ is generated by the metabolism of a ¹⁴C-labelled substrate in Middlebrook 7H12 medium. Various concentrations of the chemicals were added to this medium and the growth (radiometric “growth index”) of the organisms at 37°C was recorded daily for up to 30 days.

Results

MICs of the test compounds for the four strains of mycobacteria are shown in the table.

Pre-treatment of a washed suspension of *M. bovis* BCG or *M. avium* 3906 with ethambutol 1 mg/L, or *M. fortuitum* ATCC 6841 or *M. phlei* NCTC 8151

Figure. Effect of ethambutol and biocides, alone and in combination on the growth of *M. avium* 3906 at 37°C as measured radiometrically. **a:** ●, control; ◆, ethambutol 1; □, chlorhexidine 10; △, chlorhexidine 25; ○, chlorhexidine 50; ◇, ethambutol 1 plus chlorhexidine 1. **b:** ●, control; ◆, ethambutol 0.1; □, chlorhexidine 10; △, chlorhexidine 25; ○, chlorhexidine 50; ◇, ethambutol 0.1 plus chlorhexidine 1; ▽, ethambutol 0.1 plus chlorhexidine 2.5. **c:** ●, control; ◆, ethambutol 0.5; ◇, cetylpyridinium 5; □, cetylpyridinium 10; ●, ethambutol 0.5 plus cetylpyridinium 5; ▲, ethambutol 0.5 plus cetylpyridinium 10. **d:** ●, control; ◆, ethambutol 0.1; □, cetylpyridinium 10; △, cetylpyridinium 25; ○, cetylpyridinium 50; ▲, ethambutol 0.1 plus cetylpyridinium 10; ▽, ethambutol 0.1 plus cetylpyridinium 25; ×, ethambutol 0.1 plus cetylpyridinium 50. All concentrations in mg/L.

with ethambutol 12.5 mg/L, had no effect on the MICs of chlorhexidine or cetylpyridinium.

MICs of chlorhexidine for *M. fortuitum* and *M. phlei* were slightly reduced in medium containing ethambutol 10 mg/L. In the presence of ethambutol 1 mg/L the MIC of chlorhexidine for the BCG strain of *M. bovis* decreased from 2.5–5 mg/L to 1 mg/L. The MIC of cetylpyridinium for *M. avium* fell from

50 mg/L to 10 mg/L in the presence of ethambutol 0.5 mg/L.

In radiometric experiments, chlorhexidine alone at concentrations of 10, 25 and 50 mg/L or ethambutol alone at a concentration of 1 mg/L slightly reduced the rate of growth of *M. avium*. A combination of chlorhexidine 1 mg/L and ethambutol 1 mg/L completely inhibited growth (figure a).

Table. Inhibitory concentrations of test compounds, alone and in combination, against four strains of mycobacteria

Test strain	MIC (mg/L)					
	ETH	CHA	CHA + ETH (1 mg/L)	CHA + ETH (10 mg/L)	CPC	CPC + ETH (0.5 mg/L)
<i>M. fortuitum</i> ATCC 6841	25	1–2.5	NT	0.5	25–50	NT
<i>M. bovis</i> NCO 5692	2.5	2.5–5	1	NT	10–25	NT
<i>M. phlei</i> NCTC 8151	25	0.5–1	NT	< 0.5	5–10	NT
<i>M. avium</i> 3906	5	50	1	NT	25–50	10

ETH, ethambutol; CHA, chlorhexidine diacetate; CPC, cetylpyridinium chloride; NT, not tested.

When the experiment was repeated with ethambutol at a lower concentration of 0.1 mg/L, the drug alone did not reduce the growth rate of *M. avium* but it greatly enhanced the effect of chlorhexidine. Ethambutol 0.1 mg/L with chlorhexidine 2.5 mg/L completely suppressed growth for 7 days (figure b).

In similar experiments with cetylpyridinium chloride, ethambutol 0.5 mg/L (figure c) but not 0.1 mg/L (figure d) enhanced inhibition of growth by the biocide. Growth was completely inhibited by ethambutol 0.5 mg/L with cetylpyridinium 10 mg/L or by ethambutol 0.1 mg/L with cetylpyridinium 50 mg/L, although the quaternary ammonium compound alone was inhibitory at this concentration.

Discussion

Chlorhexidine and cetylpyridinium chloride have little mycobacterial activity,¹ but low concentrations are mycobacteristatic (table). This suggests that the compounds traverse the mycobacterial cell wall in sufficient concentration to inhibit growth but not to kill the organisms. It is likely that the cytoplasmic membrane is the target site of chlorhexidine and cetylpyridinium in mycobacteria as it is in other bacteria, although evidence is lacking.

References

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