A COMPARATIVE STUDY OF THE BACTERICIDAL MECHANISMS OF CHLORHEXIDINE AND ALEXIDINE

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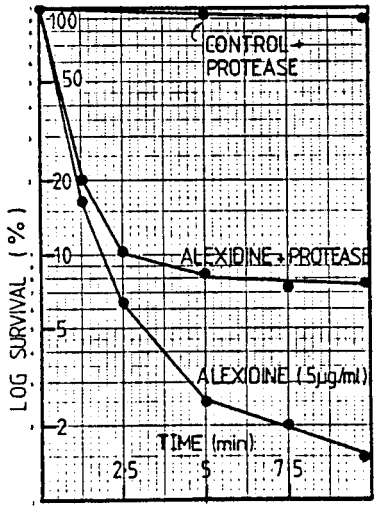
Bisbiguanides, such as chlorhexidine, are widely employed as antiseptics and as preservatives of pharmaceutical and cosmetic products. Their mechanism of action is thought to involve interaction with lipid components of the cytoplasmic and outer membranes of bacterial cells, causing permeability change, loss of osmoregulatory control and leakage of key cytoplasmic constituents (Hugo & Longworth 1964). In spite of a large number of published investigations, the mechanisms of action at a molecular level are poorly understood. Polymeric biguanides, however, are thought to bind specifically to acidic phospholipids, causing segregation and domain formation within the membrane (Ikeda *et al* 1984, Broxton *et al* 1984). Correlation of bisbiguanide resistance and cell-envelope acidic phospholipid content suggests similar mechanisms for chlorhexidine.

In this study the bactericidal activity and mechanism of action has been investigated for the bisbiguanides, chlorhexidine and alexidine. These have 4-Cl phenyl and 2-ethylhexyl end-groups respectively. Washed suspensions of *Escherichia coli* **ATCC 8739** ($2x10^7$ cells /ml) were exposed, at 35°C, to the agents (2-10µg/ml), for a 10min period, during which time aliquots (0.2ml) were removed to Lecithin-Tween 80 broth. Futher serial dilutions were made in Lecithin-Tween 80 broth. Ammounts (0.2ml) were transferred to the surfaces of triplicate predried nutrient agar plates and viable counts made after incubation at 35° for 16h. Plots of log-survival *versus* time were biphasic. Initial phases were extremely rapid, complete within 3min and in both cases directly proportional to drug concentration. Concentration exponents calculated from these data gave identical activities (D=1min,5µg/ml) and exponents (ca. 1.9) for the two compounds. Viability remained unaffected during the second phase of chlorhexidine exposure, but decreased steadily ,(D=15min), for alexidine treated cells. Rate of inactivation during the second phase of alexidine treatment were unaffected by concentration. Determinations were repeated at a variety of temperature (22-45°) with fixed concentrations of the two agents (5µg/ml) and temperature coefficients were calculated. For the initial inactivation phase these were similar for the two agents (Chlorhexidine Q_{10}=1.1, Alexidine Q_{10}=1.3). The secondary-rate for alexidine treatment, however, decreased with increasing temperature (Q_{10}=0.6). These data suggested that alexidine, but not chlorhexidine, had initiated an enzyme-mediated lytis of treated cells. Such action might be mediated through activation and release of envelope associated autolysins. The hypothesis was tested by incorporating a protease enzyme (0.05units/ml) in the neutralising broth and recovery agar. Inactivation during the second phase of killing by alexidine, in such systems, was at a much reduced rate (D=60min),with the overall levels of kill being significantly less (Figure). The results support the hypothesis of autolysin release by alexidine but not chlorhexidine treatment.



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