NOTE

AN ELECTRON-MICROSCOPIC STUDY OF ANTAGONISM OF CEPHALEXIN AND ERYTHROMYCIN IN STAPHYLOCOCCI

TAKESHI NISHINO and SHOZO NAKAZAWA

Department of Microbiology, Kyoto College of Pharmacy, Kyoto, Japan

(Received for publication December 28, 1972)

Since the antagonism between chloramphenicol and penicillin in streptococci was first described in 1951 by JAWETZ *et al.*,[1] numerous reports have been published concerning antagonistic effects among various chemotherapeutic agents.[2,3,4] In our previous paper[5,6] we presented the results of the morphological investigation by electron microscope on *Staphylococcus aureus* exposed to cephalexin and macrolide antibiotics. The present study was undertaken to observe at cellular levels with an electron-microscope antagonistic effects on staphylococcal organisms of cephalexin (CEX), an antibiotic known to interfere with cell wall formation in the bacteria, and erythromycin (EM), an antibiotic which inhibits protein synthesis in them.

Staphylococcus aureus strain FDA 209 P was cultured in heart infusion broth (Nissan) at 37°C. When the bacterial cultures were entering the logarithmic phase of growth, they were added to the incubation medium. The antibiotics, each alone or both in combination, were added at the same time as the bacteria to give the following concentrations: cephalexin, 20 mcg/ml; erythromycin, 1.0 mcg/ml. After the antibiotics were added, sufficient time was allowed for them to permeate the cells. The cultures were measured to determine the numbers of growing bacteria and then centrifuged at 3,000 r.p.m. for 5 minutes to collect them. These cells were fixed in 1% osmium tetroxide for 16 hours according to the method of KELLENBERGER *et al.*,[7] dehydrated with an alcohol series and then embedded by the method of LUET *et al.*[8] Ultrathin sections were prepared by an LKB microtome (Sweden) with a diamond knife and then subjected to double staining with uranyl acetate and lead citrate for subsequent observations with an Akashi S-500 electron-microscope.

The determination of the numbers of growing bacteria demonstrated that the concurrent treatment with both CEX and EM resulted in marked antagonism between the two drugs in their effects on growth curves of *S. aureus* (Fig. 1).

Without either antibiotic, the viable cell number increased during 4 hours. With EM alone, it remained constant but with CEX alone the count rapidly decreased after 4 hours. However, when incubated in the presence of both EM and CEX together, the count remained constant during the entire 4-hour period, thus showing the antagonistic action of the compounds to each other. The antibiotic effect was not entirely vitiated when in combination, for the numbers of *S. aureus* did not increase but remained stationary, similar to the result of treatment with EM (Fig. 1). The electron microscopic observations revealed that the cells allowed to grow in the presence of CEX 20 mcg /ml alone had their protoplasts formed in almost all cases (Fig. 3) and morphological changes associated with protoplast formation were most pronounced at 4 hours after treatment with the drug. The cells treated with 1 mcg/ml EM

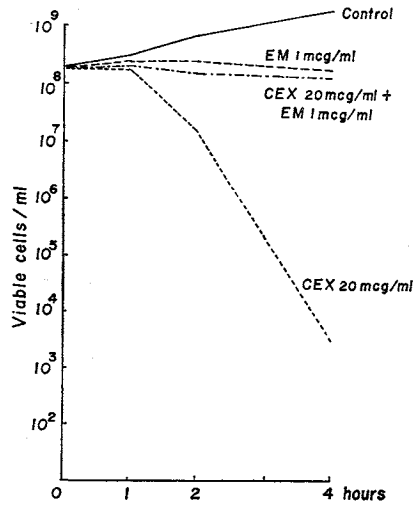

Fig. 1. Growth inhibition of *Staphylococcus aureus* by cephalexin and erythromycin.

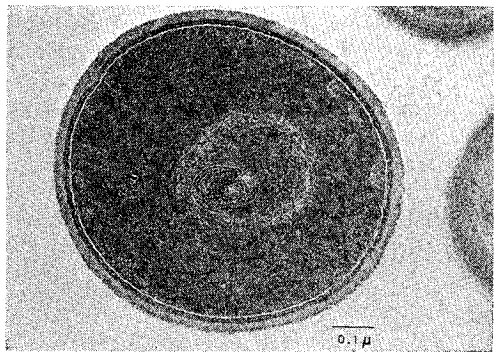

Fig. 2. Ultrathin section of intact *Staph. aureus* FDA 209 P.

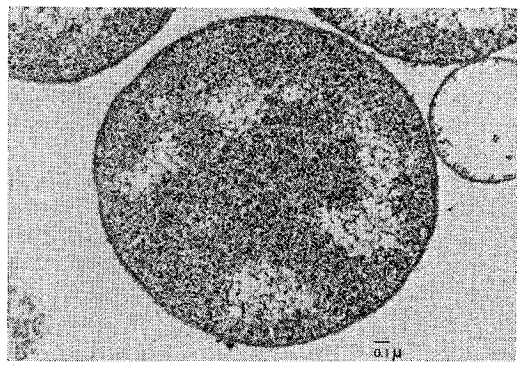

Fig. 3. Section of protoplasts in *Staph. aureus* 4 hours after treated with 20 mcg/ml cephalexin.

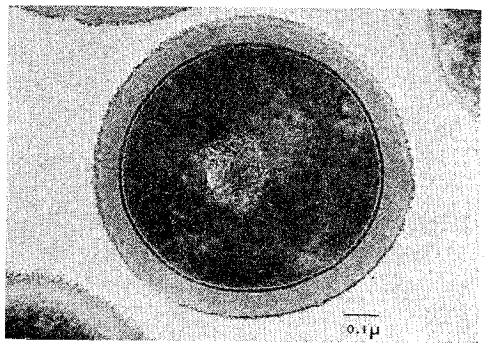

Fig. 4. Section of *Staph. aureus* 4 hours after treatment with 1 mcg/ml erythromycin. Note markedly thickened cell walls.

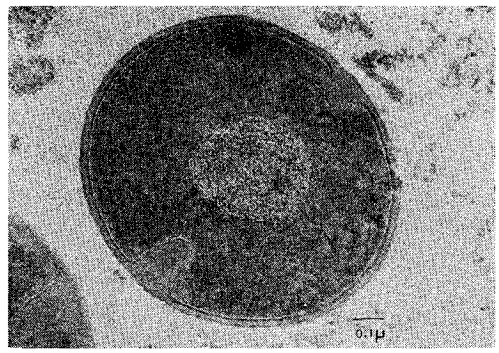

Fig. 5. Section of *Staph. aureus* 4 hours after treated with both cephalexin and erythromycin. Swollen separating walls are evident while no cell wall thickening is noted.

alone, on the other hand, developed markedly thickened cell walls (Fig. 4). In the presence of both CEX and EM, however, neither protoplast formation nor cell wall thickening did occur, but swollen separating walls were observed in the cells (Fig. 5).

In general, bacteria are known to produce autolytic enzymes that attack mucopeptides. The antagonism observed between bactericidal antibiotics, such as penicillin and cephalosporin, and bacteriostatic antibiotics, like chloramphenicol and tetracycline, is considered to be due to the inhibition of bacterial autolytic enzyme production by the latter. This blocks the lysis of cell walls. The present study was an attempt at visual analysis of these phenomena with the use of an electron-microscope. When CEX was allowed to act alone, bacterial cells turned to protoplasts that contained no cell walls. When both CEX and EM were allowed to act together, however, no protoplast formation was observed. This morphological finding suggests that the bacterial production of autolytic enzymes, which is considered responsible for the loss of the cell wall, is inhibited when both antibiotics act on *S. aureus*. As far as the authors know this is perhaps the first visual evidence of an antibiotic antagonism studied with an electron-microscope.

References

1) JAWETZ, E., J. B. GUNNISON, R. S. SPECK & V. R. COLEMAN: Studies on antibiotic synergism and antagonism. The interference of chloramphenicol with the action of penicillin. Arch. Internal Med. 87 : 349~359, 1951
2) BARTELL, P.: Interference of aureomycin with the activity of penicillin *in vivo*. J. Infect. Dis. 90 : 273~278, 1952
3) FLEK, S. D. & G. R. F. HILSON: Combined agar diffusion and replica plating techniques in the study of antibacterial substances. J. Clin. Path. 7 : 37~44, 1954

4) ROGERS, H. J.: Killing of staphylococci by penicillins. Nature 213 : 31~33, 1967
5) NISHINO, T. & S. NAKAZAWA: Morphological changes in *Staphylococcus aureus* and *Escherichia coli* exposed to cephalexin. Japan. J. Microbiol. 16 : 83~94, 1972
6) NAKAZAWA, S., M. NAKAO, E. KITANAKA & K. OCHIAI: Cell wall synthesis in *Staphylococcus aureus* in the presence of protein synthesis inhibitory agents. I. Lincomycin, clindamycin and macrolide antibiotics. Japan. J. Microbiol. 16 : 403~413, 1972
7) KELLENBERGER, E., A. RYTER & J. SECHAUD: Electron microscope study of DNA-containing plasms. II. Vegetative and mature phage DNA as compared with normal bacterial nucleotide in different physiological states. J. Biophys. Biochem. Cytol. 4 : 671~678, 1958
8) LUFT, J. H.: Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol. 9 : 409~414, 1961