CELL WALL SYNTHESIS IN *STAPHYLOCOCCUS AUREUS* IN THE PRESENCE OF PROTEIN SYNTHESIS INHIBITORY AGENTS

TETRACYCLINE AND AMINOGLYCOSIDE ANTIBIOTICS

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Abstract

Morphological changes of *Staphylococcus aureus*, exposed to protein synthesis inhibitory antibiotics such as tetracyclines and aminoglycosides were studied by electron-microscopic observation using ultra-thin sections. Cell wall thickening of *S. aureus* was observed by these antibiotics, and abnormal cell division was remarkably noticed with tetracyclines. With clinically isolated *S. aureus*, resistant to each antibiotic, the phenomena of cell wall thickening and abnormal cell division were not observed when exposed to that antibiotic.

In previous publication[1,2,3], we reported that the cell wall of *S. aureus* became so thickened and often accompanied with the formation of multilayers, and the electron-density of cytoplasm was increased following exposure to protein synthesis inhibitory antibiotics such as lincomycins and macrolides, and these phenomena were not observed with clinically isolated resistant strains. On the other hand, this phenomenon of cell wall thickening was also confirmed in biochemical study[4]. This time we investigated electron-microscopically the morphological changes of *S. aureus* treated with tetracycline and aminoglycoside antibiotics.

Materials and Methods

As the test organism, *Staphylococcus aureus* 209-P JC and clinically isolated three strains of *Staphylococcus aureus*; (*S. aureus* No. 8, resistant to tetracycline, *S. aureus* No. 12, resistant to streptomycin, *S. aureus* No. 7, resistant to both streptomycin and kanamycin) were used. Cells were preincubated in Tryptosoya Broth (TSB, Nissan) for 18 hours, and diluted by Heart Infusion Broth (HIB, Nissan), and mixed with Heart Infusion Agar (HIA, Nissan) to make plates containing about 10^6 cells/ml. Cylinders were placed on the plates, then tetracycline (TC; Lederle (Japan) Ltd.), minocycline (MINO, Lederle (Japan) Ltd.), streptomycin (SM, Meiji Seika Kaisha, Ltd.) and kanamycin (KM, Meiji Seika Kaisha, Ltd.), (10 mcg/ml respectively) were poured into each cylinder. The plates were cultivated for about 24 hours at 37°C to make the inhibitory circle, and the boundary of the circle was cut out to obtain agar blocks. With resistant strains the inhibitory circle was not produced, so the agar just outside the cylinder was cut out. Agar blocks were fixed with 1% OsO_4 dissolved in KELLENBERGER buffer[5] at room temperature for 18 hours. After the fixation, blocks were stained with 0.5% of uranyl acetate and dehydrated by alcohol series, then embedded in epoxy resin[6]. Ultra-thin sections, obtained by ultra-microtome (LKB), were stained with uranyl acetate and lead citrate[7], then observed by electron microscope AKASHI S-500.

Results and Discussion

Fig. 1 shows the electron-micrograph of intact *S. aureus* 209-P JC. Following exposure to tetracycline antibiotics the phenomena of cell wall thickening, abnormal cell division and the increase of electron-density in cytoplasm were observed (Fig. 2, 3). With aminoglycoside antibiotics such as SM, KM the phenomenon of peripheral cell wall thickening was also observed, but abnormal cell division or the increase of electron-density in cytoplasm were not noticed (Fig. 4, 5). In the case of clinically isolated strain which shows resistance to TC, morphological changes such as in sensitive strain were not noticed with following exposure to TC (Fig. 6), but typical phenomena with tetracycline antibiotics were noticed when treated with MINO (Fig. 7). The relation between the cross resistance in aminoglycoside antibiotics and the phenomenon of cell wall thickening was also the same in the case of tetracyclines (Fig. 8, 9).

The results obtained in this experiment are

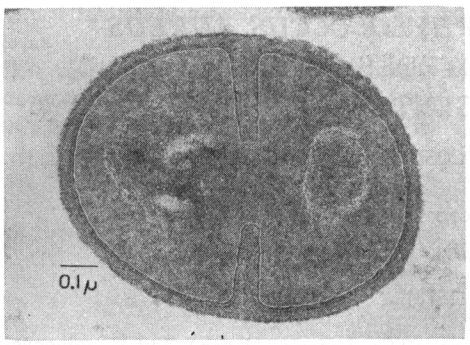

Fig. 1. Intact *S. aureus* 209-P JC. ×119,000
Nuclear division is finished and cross walls growing are observed.

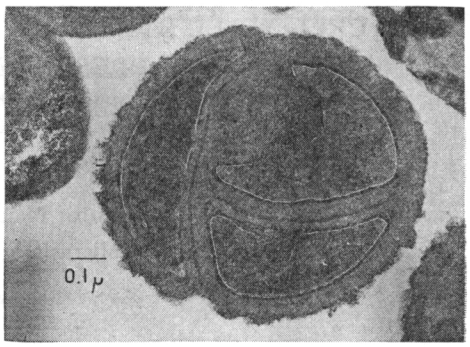

Fig. 2. *S. aureus* 209-P JC, treated with TC. ×114,000
Cell walls are thickened and normal binary division is disrupted. Electron-density in cytoplasm is increased.

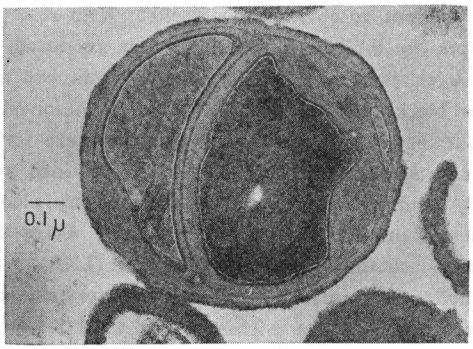

Fig. 3. *S. aureus* 209-P JC, treated with MINO. ×117,000
Peripheral cell wall is thickened and layers are seen in it. Electron-density in cytoplasm is increased.

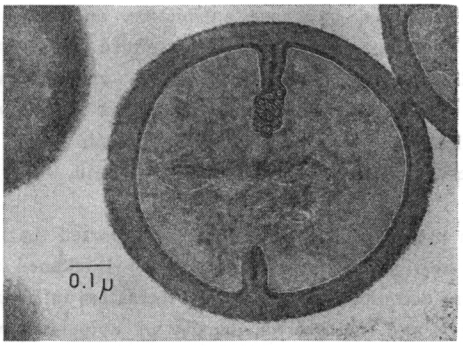

Fig. 4. *S. aureus* 209-P JC, treated with SM. ×131,000
Peripheral cell wall is thickened but the thickness of cross wall is not so changed compared with intact cell.

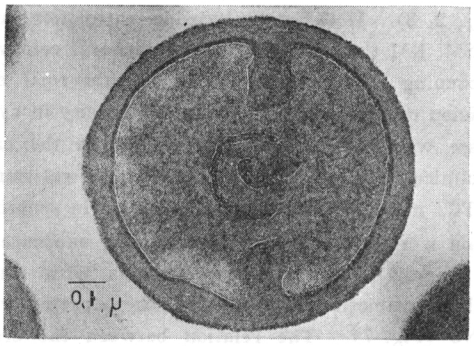

Fig. 5. *S. aureus* 209-P JC, treated with KM. ×120,000
The degree of cell wall thickening is about the same as in the case of SM.

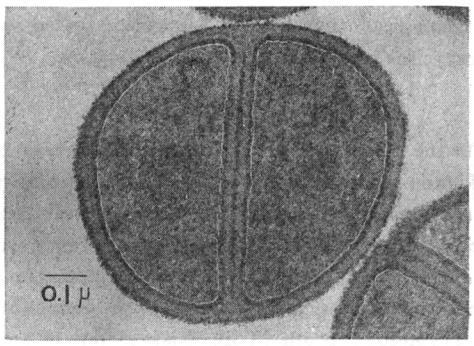

Fig. 6. *S. aureus* No. 39, treated with TC. ×139,000
Morphological changes observed in sensitive strain are not seen.

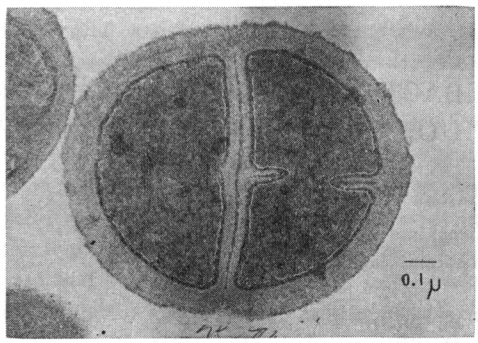

Fig. 7. *S. aureus* No. 39, treated with MINO. ×107,000
The phenomena of cell wall thickening, abnormal cell division and the increase of electron-density in cytoplasm which are characteristic of tetracycline antibiotics are observed.

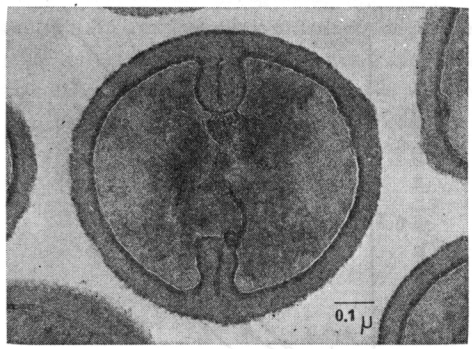

Fig. 9. *S. aureus* No. 12, treated with KM. ×131,000
Peripheral cell wall is thickened and mesosomes in contact with the slightly thickened cross wall of growing state are seen.

summarized as follows: cell wall of *S. aureus* became thickened following exposure to tetracycline and aminoglycoside antibiotics. With tetracylines, abnormal cell division and increased electron-density in cytoplasm were not noticed. In this study, the degree of cell wall thickening of cells treated with tetracyclines or aminoglycosides was not so extreme compared with the cells treated with macrolide antibiotics. Relation between the cross resistance of drugs and the phenomenon of cell wall thickening was the same as in the case of macrolides.

Acknowledgment

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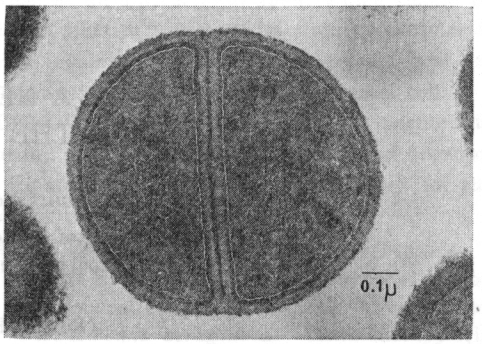

Fig. 8. *S. aureus* No. 12, treated with SM. ×117,000
Cell wall thickening is not seen.

References

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