

BACTERIOLOGICAL STUDIES ON THE
COMBINED ACTION OF
AMINOGLYCOSIDE ANTIBIOTICS AND
SYNTHETIC PENICILLINS AGAINST
PSEUDOMONAS AERUGINOSA

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One of the recent therapeutical problems to the medical profession is that of the intractable infection due to *Pseudomonas aeruginosa*. The conventional therapy for this infection uses aminoglycoside antibiotics such as gentamicin (GM) and 3'4'-dideoxykanamycin B (DKB) or synthetic penicillins such as sulbenicillin (SB-PC) and carbenicillin (CB-PC). These antibiotics, however, have defects in that, when used in high doses for a prolonged period, they are likely to cause high renal toxicity and give rise to the emergence of resistant strains. In an effort to minimize these defects, GM and CB-PC have been used in combination. Such combination therapy, however, has produced controversial results: some investigators argue that the two antibiotics are synergistic¹⁻⁵⁾ while others maintain that GM is inactivated by CB-PC.⁶⁻⁸⁾ The present studies have been carried out in an attempt to clarify this controversial point both *in vitro* and *in vivo*.

Materials and Methods

Minimum Inhibitory Concentration (MIC): SB-PC and GM, alone and in combination and diluted by the checker board titration method, were incubated for 18 hours at 37°C to determine the MIC for 3 strains of *P. aeruginosa*: No. 12, Nc-5, and IFO-3445.

Bactericidal Effect: To examine their effect on the growth of *P. aeruginosa*, SB-PC and GM, alone and in combination, were added to the culture of *P. aeruginosa* No. 12 in heart infusion broth (Nissan) when the bacterial growth was in its logarithmic growth phase. For the addition of the two antibiotics, three different methods were employed: (1) two drugs simultaneously, (2) SB-PC first, followed

by GM after a 2-hour interval, and (3) the reverse of 2. At given intervals after each addition, the bacterial suspension was sampled for viable cell count by Biophotometer (JASCO BIO-LOG II).

Electron Microscopic Study: Four hours after exposure to either or both antibiotics, the bacterial suspension as prepared in Bactericidal Effect Study was centrifuged for 5 minutes at 3,000 rpm to collect organisms. The collected organisms were fixed by the KELLENBERGER and RYTER method,⁹⁾ dehydrated with an alcohol series and embedded by the method of LUFT *et al.*¹⁰⁾ Ultrathin sections were prepared with an LKB microtome (Sweden) and then double-stained with uranyl acetate and lead citrate for subsequent examination with an Akashi S-500 electron microscope.

In Vivo Study: *P. aeruginosa* No. 12 and Nc-5, preincubated in Tryptosoya broth ((Nissan), were each transferred to heart infusion broth and incubated for 12 hours at 37°C while stirring. This bacterial suspension was diluted to 10⁻⁴, mixed with an equal amount of 10% mucin, and then inoculated into mice intraperitoneally. SB-PC and GM were given subcutaneously by the following three different methods: 1) a single dose of either antibiotic 2 hours after inoculation, 2) a single combined dose of the two antibiotics 2 hours after inoculation, and 3) one dose each of the two antibiotics, one 2 hours, the other 4 hours, respectively, after inoculation.

Results

MIC

The combination of SB-PC and GM, as tested

Fig. 1. Combination effect of SB-PC and GM on *P. aeruginosa* No. 12 by checker board titration method.

| | | GM (mcg/ml) | | | | | | | | | SB-PC alone |
|----------------|------|-------------|------|------|------|------|------|-------|-------|-------|-------------|
| | | 3.12 | 1.56 | 0.78 | 0.39 | 0.19 | 0.09 | 0.045 | 0.022 | 0.011 | |
| SB-PC (mcg/ml) | 400 | - | - | - | - | - | - | - | - | - | - |
| | 200 | - | - | - | - | - | - | - | - | - | - |
| | 100 | - | - | - | - | - | - | - | - | - | - |
| | 50 | - | - | - | - | - | - | - | - | - | - |
| | 25 | - | - | - | - | - | - | - | - | - | + |
| | 12.5 | - | - | - | - | - | - | - | - | - | + |
| | 6.25 | - | - | - | - | - | - | - | + | + | + |
| | 3.12 | - | - | - | - | - | + | + | + | + | + |
| | 1.56 | - | - | - | - | - | + | + | + | + | + |
| GM (mcg/ml) | - | - | - | - | - | + | + | + | + | + | |

by the checker board titration method, showed synergism against each of the 3 strains tested of *P. aeruginosa*, especially notably in No. 12 (Fig. 1).

Bactericidal Effect

The bactericidal effect was strongest when the culture of *P. aeruginosa* was exposed to SB-PC and GM simultaneously. The second strongest was the combination of SB-PC first and GM later. When GM was added to the culture ahead of SB-PC, the effect was about equal to that shown by either drug alone (Fig. 2).

Electron Microscopic Study

While an ultrathin section of normal *P. aeruginosa* No. 12 shows distinctly cell wall, cytoplasmic membrane, and a nucleus (Fig. 3), the cell exposed to SB-PC alone is extremely prolonged in a filamentous form (Fig. 4); the

Fig. 3. An ultrathin section of normal *P. aeruginosa* No. 12.

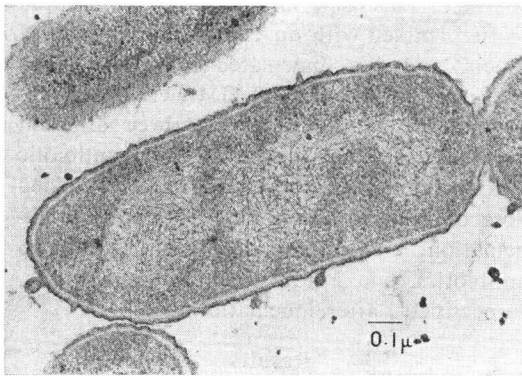


Fig. 5. Section of *P. aeruginosa* No. 12, 6 hours after exposure to GM. The cell wall is perforated (arrow).

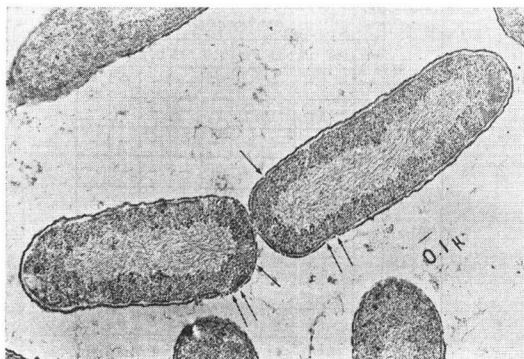


Fig. 2. Bactericidal effect of SB-PC and GM, alone and in combination, on *P. aeruginosa* No. 12.

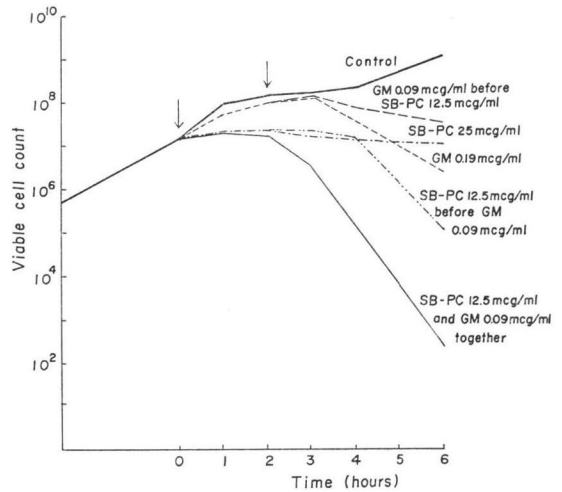


Fig. 4. Section of *P. aeruginosa* No. 12, 6 hours after exposure to SB-PC. Note markedly filamentous cell.

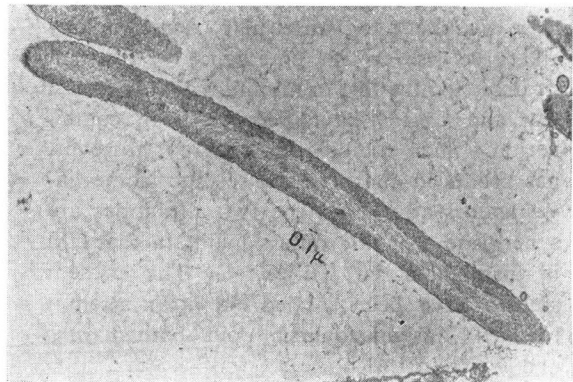
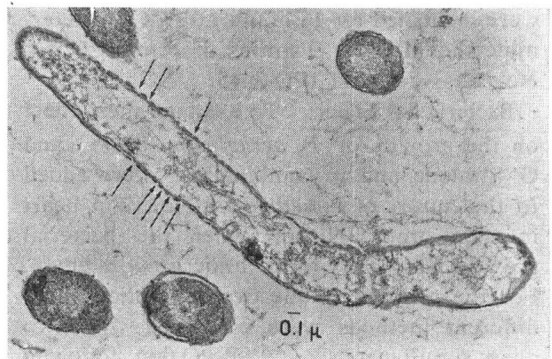


Fig. 6. Section of *P. aeruginosa* No. 12, 6 hours after exposure to SB-PC and GM simultaneously. The surface layer of the cell is damaged (arrow); the degree of cellular damage is much severer than that by each drug alone.



cell exposed to GM alone has perforations in its cell wall (Fig. 5); and the cell exposed to SB-PC and GM simultaneously is prolonged in form, although not so markedly as the one exposed to SB-PC alone, and damaged in its surface layer, and the cellular damage is more notable than in the ones exposed to either drug alone (Fig. 6).

In Vivo Study

The therapeutic effect on infections experimentally induced in mice with *P. aeruginosa* No. 12 and Nc-5 was generally better when SB-PC and GM were used in combination than when each was used alone. While none of the mice treated with either drug alone survived the infection, the simultaneous administration of SB-PC and GM attained the best survival rate of 70%. The second best survival rate (50%) was obtained with the administration of SB-PC first and GM later. The combination was weakest (survival rate: 20%) when GM was given before SB-PC (Fig. 7).

Discussion

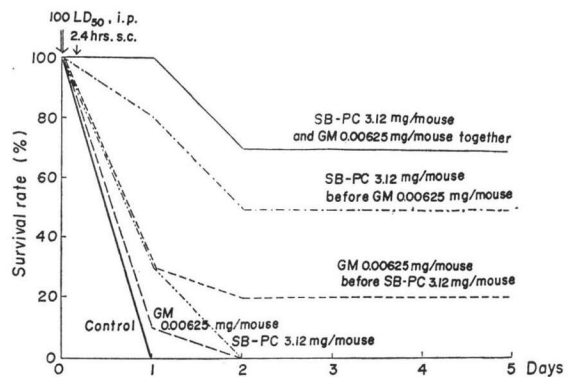
Synergism has been observed both *in vitro* and *in vivo* in our present study not only in the combination of SB-PC and GM as illustrated above but in other combinations between aminoglycoside antibiotics and synthetic penicillins such as SB-PC plus DKB, CB-PC plus GM, and CB-PC plus DKB.

Different degrees of synergism observed with different sequence of exposure or administration is considered attributable to the mechanism of action of each antibiotic composing the combination. Further studies are under way in an effort to elucidate the cause of such difference in synergism.

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Fig. 7. Protecting effect of SB-PC and GM, alone and in combination, against experimental infections of *P. aeruginosa* No. 12 in mice.



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