

STUDY ON MECHANISM OF ANTIBACTERIAL ACTION OF AMOXYCILLIN. I

Rapid Bacterial Action and its Microscopic Observation

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Bactericidal activities of amoxycillin and ampicillin were examined and compared by using *Escherichia coli* as test organisms. Amoxycillin showed a more rapid bactericidal action than ampicillin in experiment of the effect on the growth curve with biophotometer and viable cell counts. Observation of the bactericidal picture with phase-contrast microscope confirmed the rapid bactericidal action of amoxycillin and revealed that amoxycillin destroys organisms in different manner from ampicillin. These suggest some difference in the action mode between amoxycillin and ampicillin.

Amoxycillin^{1,2)} is a semi-synthetic penicillin for oral use developed in 1970 at Beecham Research Laboratories in England. Like ampicillin, it has broad antibacterial spectrum.

Amoxycillin has such a chemical structure as a hydroxyl group is introduced into benzene nucleus of ampicillin at para position. It is stable in acid, like ampicillin, and absorbed well, blood concentration of amoxycillin after oral administration in a certain dose being about twice as high as that of ampicillin administered in as much dose³⁻⁶⁾.

The effect of amoxycillin against gram-positive and -negative organisms is nearly equal with that of ampicillin^{1,2)}. According to ROLINSON *et al.*²⁾, there was no difference in bactericidal activities between amoxycillin and ampicillin. We found, however, in detailed investigation of the activities of amoxycillin and ampicillin against *Escherichia coli* strains through biophotometer and by counting of viable cells, that amoxycillin produces greater bactericidal effect than ampicillin in an initial stage. The experiments and results are described below.

Materials

1. Test antibiotics

Amoxycillin (Beecham Research Laboratories, potency : 830 mg/g)

Ampicillin (Kyowa Hakko Kogyo Co., Ltd., potency : 837 mg/g)

2. Test strains

Escherichia coli NIHJC-2, *Escherichia coli* O-55

and *Escherichia coli* K-12.

3. Biophotometer

Jasco/Jouan biophotometer, Model BLO-LOG, made in France.

4. Phase-contrast microscope

Inverted microscope, Model MD, made by Nippon Bunko Kogyo Co., Ltd.

Methods

1. Effect on growth curve

a) Investigation of bactericidal activity by biophotometer

One loopful of test organisms were inoculated into tryptsoya bouillon (8 ml, pH 7.2) and were allowed to stand overnight at 37°C. The seed culture thus prepared was diluted 10⁵ times with heart infusion medium (pH 7.2), distributed to cells of biophotometer, and incubated at 37°C on shaker. The drugs were added when bacterial growth reached mid-logarithmic phase (approx. 60% in terms of T%).

b) Investigation of bactericidal activity by determination of the number of viable cells

Samples of culture used for the investigation a) were successively diluted suitably at certain time intervals, mixed into heart infusion agar and incubated overnight at 37°C. The number of colonies formed on the agar.

2. Inspection of morphological change by phase-contrast microscope

One loopful of *Escherichia coli* NIHJC-2 was inoculated into tryptsoya bouillon (8 ml, pH 7.2) and was allowed to stand overnight at 37°C. This seed culture was inoculated into heart infusion bouillon (8 ml, pH 7.2) to the level of 2.5% and incubated at 37°C on shaker. When bacterial growth reached mid-logarithmic phase, a loopful of the culture was taken up onto a coverglass, which was then placed on a slide glass covered with a layer of heart infusion agar approx. 0.3 mm thick. Both glasses were sealed with paraffin. Observation was carried out with phase-contrast microscope provided with a 37°C-thermostat.

Results

1. Bactericidal activity

Under the present conditions, *Escherichia coli* NIHJC-2 enters logarithmic after about 2 hours of lag phase (Fig. 1).

The drugs were added approximately 3 hours after the start of culture (about 60% in terms of T%, corresponding to mid-logarithmic phase). In case of addition of amoxycillin 6.25 mcg (MIC), absorbance kept rising to reach the peak 30 minutes

Fig. 1 Bactericidal activity of AB-PC and amoxycillin against *E. coli* NIHJC-2

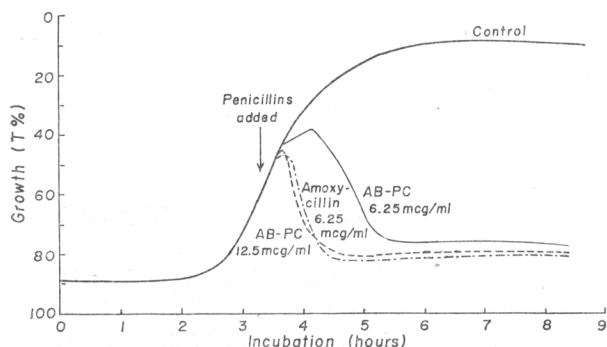
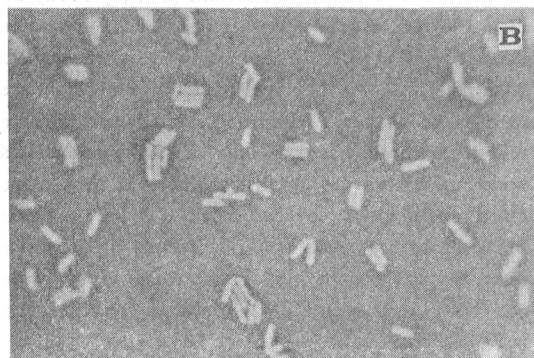
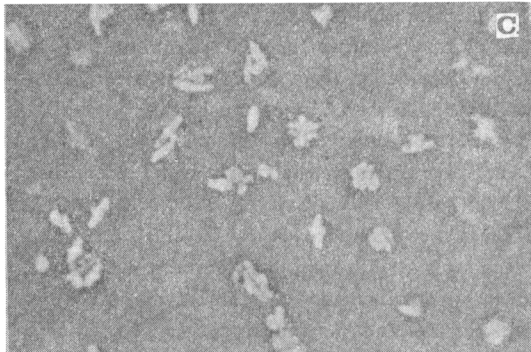
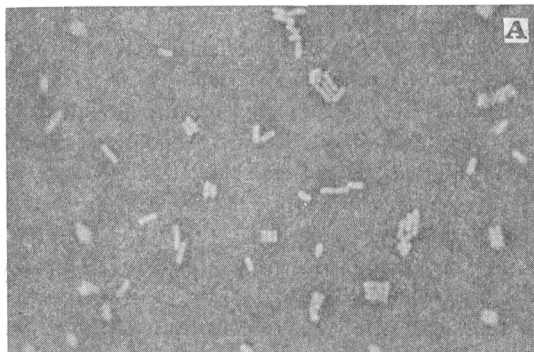


Photo 1 Morphological changes in *Escherichia coli* exposed to amoxycillin

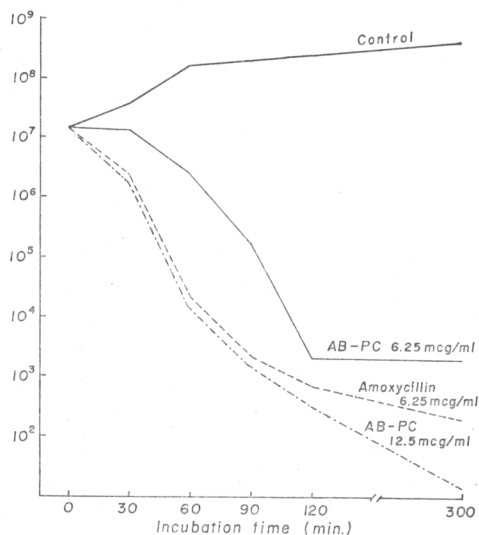
A) View of logarithmic-phase *Escherichia coli* NIHJC-2 in normal state.

B), C), D) 30 min., 60 min., 90 min. after treatment with amoxycillin 6.25 mcg/ml respectively.



after addition, then decreased rapidly until the 60 th minute and remained constant thereafter.

Fig. 2 Bactericidal activity of AB-PC and amoxycillin against *E. coli* NIHJC-2



In case of addition of ampicillin 6.25 mcg (MIC), absorbance kept rising to reach the peak 60 minutes after addition, then decreased rapidly until the 120 th minute and remained constant thereafter.

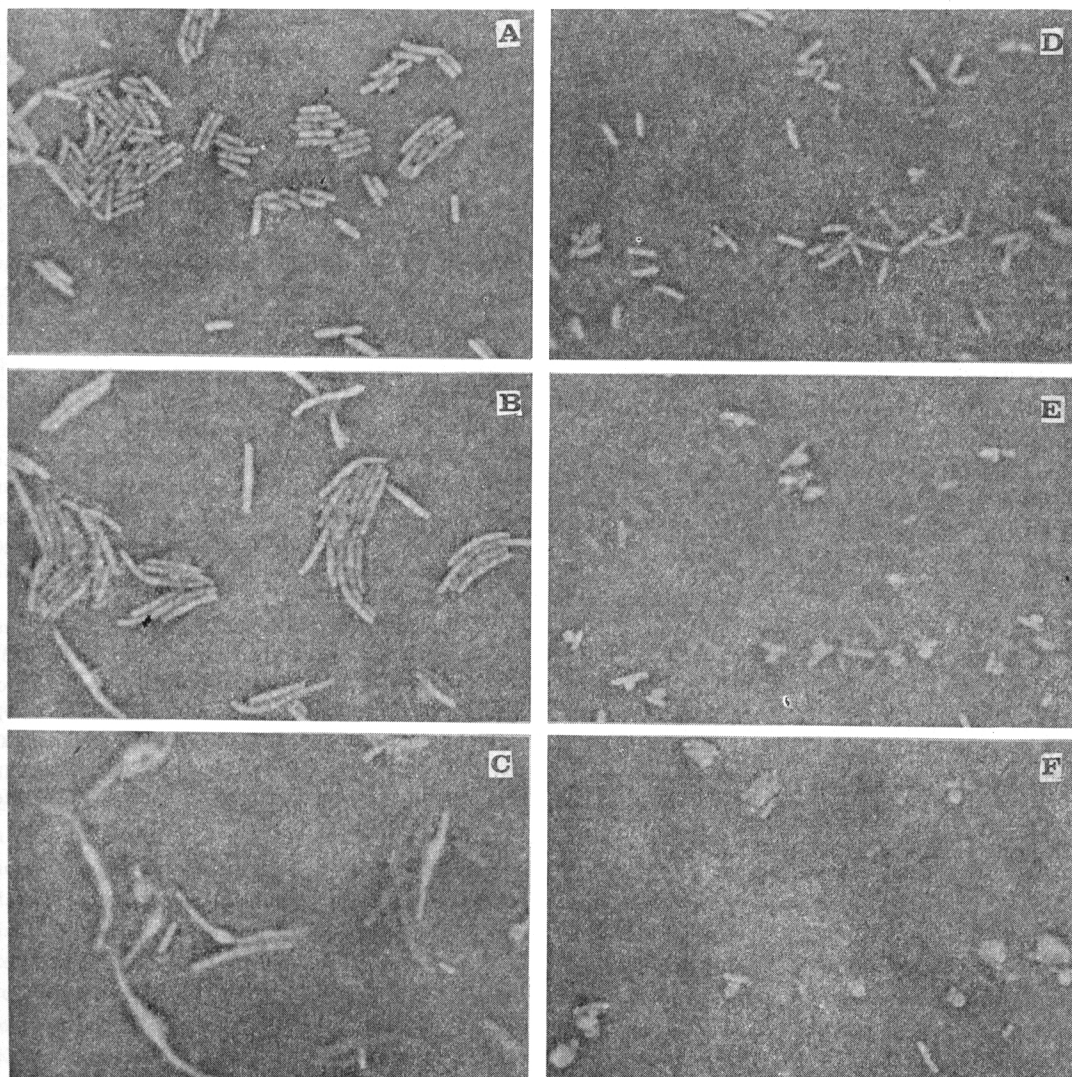
In case of addition of ampicillin 12.5 mcg (2 MIC), the bactericidal activity curve was almost identical with that obtained from addition of amoxycillin 6.25 mcg. The same phenomenon was observed also when other *Escherichia coli* strains such as *Escherichia coli* O-55 and *Escherichia coli* K-12 were used.

The bactericidal activity revealed in the above experiment was confirmed by counting of viable cells (Fig. 2). In case of addition of amoxycillin 6.25 mcg/ml, the number of organisms began to decrease slightly 30 minutes after addition, rapidly decreased after about 60 minutes, was decreasing gradually after 90 minutes, and reached several hundreds/ml of medium after 120 minutes. In case of addition of ampicillin 6.25 mcg/ml, it remained nearly constant for 30 minutes after addition, decreased slightly after 60 minutes, decreased rapid-

Photo 2 Morphological changes in *Escherichia coli* exposed to ampicillin

A),B),C) 30 min., 60 min., 90 min. after treatment with ampicillin 6.25 mcg/ml respectively.

D),E),F) 30 min., 60 min., 90 min. after treatment with ampicillin 12.5 mcg/ml respectively.



ly after about 90 minutes, reached several thousands/ml of medium after 120 minutes and remained constant until after 300 minutes. In case of addition of ampicillin 12.5 mcg/ml, the curve for live bacteria count was similar to that obtained from addition of amoxycillin 6.25 mcg/ml.

2. Morphological survey

The effects of amoxycillin and ampicillin on *Escherichia coli* NIHJC-2 were observed morphologically with phase-contrast microscope. The results were showed in Photo 1 and Photo 2. At 30 minutes after treatment with amoxycillin 6.25 mcg/ml (MIC: minimum inhibitory concentration), most of the cells were normal, and spheroplast was formed in the center of some cells (presumably just before division), and some had undergone lysis (Photo 1B). At 60 minutes after treatment with the drug, most of the cells were spindle-formed, and ensuing violent lysis was demonstrated (Photo 1C). Furthermore, at 90 minutes after treatment, bacteriolysis was nearly completed, and took place by sudden rupture (Photo 1D). On the other hand, at 30 minutes after treatment with ampicillin 6.25 mcg/ml (MIC), elongation was noticed (Photo 2A). At 60 minutes after treatment with the drug, most of the cells were rapidly elongated, and spheroplast was formed in the center of some of the elongated cells, and bacteriolysis was seen here and there (Photo 2B). At 90 minutes after treatment, elongation was arrested almost everywhere, and formation of spheroplast, bulging of the center of the cell, and ensuing violent lysis were demonstrated, but the outer form of the cell remained after lysis (Photo 2C). With exposure to ampicillin 12.5 mcg/ml (2 MIC), at 30 minutes after treatment with the drug, most of the cells were normal, spheroplast was formed in the center of some cells (presumably just before division), and some had undergone lysis (Photo 2D). At 60 minutes after treatment, formation of spheroplast was advanced in most of the cells, and ensuing violent lysis was demonstrated (Photo 2B). At 90 minutes after treatment, lysis was finished almost everywhere, but the outer form of the cell remained after lysis in most of the

cells, and some were in a state of fusion (Photo 2F).

Discussion

From the effect of amoxycillin on the growth curve for *Escherichia coli* and the bactericidal picture by phase-contrast microscope, it became clear that amoxycillin shows a more rapid bactericidal action than ampicillin in destroying bacteria and achieves bacteriolysis in different process from that employed by ampicillin.

Further study by biochemical approach seems necessary to clarify if amoxycillin plays some role in altering permeability of the surface of *Escherichia coli* cell, by having such a chemical structures as a hydroxyl group is introduced into ampicillin's benzene nucleus at para position, or if it has a different antibacterial action mode from ampicillin.

Acknowledgement

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