The Interaction of Rifampicin in Association with Other Antimicrobial Drugs on *Mycobacterium Fortuitum* *in vitro*

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=국문초록=

*Mycobacterium Fortuitum*에 대한 rifampicin과 기타 항균제와의 상호작용에 관한 연구

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비결핵성 항산균증예가 점증하고 있는데다 이들의 치료도 매우 어려운 경우가 많아 크게 관심을 끌고 있다. 기존 항결핵제를 이용한 치료의 어려움은 주로 대부분의 비정형 항산균이 그러한 약제에 대해 높은 내성을 나타내고 있기 때문이다. 그러나 강력한 병합요법으로 성공적인 치료를 한 경우도 가끔 볼 수 있는데, 이는 아마도 약제간의 상승적 항균작용이 있기 때문이 아닌가 생각되어 *M. fortuitum*에 대한 rifampicin과 기타 항균제와의 상호작용을 관찰하였다. 그 결과 rifampicin과 ethambutol 또는 prothionamide 사이에는 강한 상승작용이 있어서, 각 약제 단독으로 약 56-88%의 생균수 감소를 일으킬 수 있는 농도로 서로 섞었을 때, 단독으로 처리했을 때 살아남은 균을 99.3% 이상 더 감소시킬 수 있었다. Rifampicin보다 ethambutol의 농도가 증가함에 따라 상승효과가 크게 증가하였다.

Rifampicin과 amikacin과의 상승작용은 크지 않았지만 후자의 항생제는 *M. fortuitum*에 대해 강한 항균력을 가지고 있었다. Rifampicin과 tetracycline 사이에는 다소의 상승효과가 관찰되었지만 시험된 다른 약제들에 비하면 매우 미약하여 균에 대해 독립적으로 작용한다고 사료된다. *M. fortuitum*은 prothionamide와 tetracycline에 대해서도 비교적 높은 농도이지만 감수성을 나타내고 있었다.

Introduction

The rising incidence of atypical mycobacterioses and the problems associated with their treatment attracted considerable attention in recent years (1,2). The various successes of treatment of atypical mycobacterial infection with proper combination of antituberculous drugs have been reported in many papers although almost all of pathogenic atypical

mycobacteria are resistant to the most of antituberculous drugs (1,2,3). This might suggest that the treatment of patients with atypical mycobacterioses need not necessarily depend upon the results of drug sensitivity test. Occasional favorable response to combined chemotherapy might be resulted from the synergistic effect of drugs used.

In this study, we have investigated *in vitro* interaction of rifampicin in association with other antimicrobial drugs on the growth of *Mycobacterium fortuitum*.

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Materials and Methods

Strain and inoculum preparation:

M. fortuitum KTM 29 was used in this study and the culture was maintained on Löwenstein-Jensen (L-J) medium. In order to obtain enough bacillary mass, successive subculture was made in Sauton's liquid medium in several generations.

Bacillary mass was homogenized in a screw-capped bottle (1/2 ounce) with 6 to 8 glass beads (3 mm diameter) and 0.5 ml of distilled water on Voltex mixer, and dispersed bacilli were suspended in sterile saline. After settling down the large clumps, the supernatant was transferred into a sterile cuvette and its turbidity was adjusted to have 88 to 90% transmittance at 650 nm. Bacterial suspension was then diluted to 10^{-4} , $1/2 \times 10^{-4}$, and $1/4 \times 10^{-4}$. Then 0.15 ml of each dilution inoculated on drug-free media. Media containing the various concentrations of drugs in combination or alone were inoculated with approximately 10^2 , 10^3 , 10^4 , 10^5 , and 10^6 viable cells. The medium used for this study was Kirchner semiliquid medium supplemented with 10 % horse serum.

Drugs and their concentrations tested:

In preliminary sensitivity tests with amikacin (AKC), cephalosporin, cycloserine, ethambutol (EMB), isoniazid, p-aminosalicylic acid, prothionamide (PTH), rifampicin (RFP), and tetracycline (TC), considerable sensitivity of a test strain was noticed only to amikacin, prothionamide, and tetracycline.

In order to determine drug concentrations which inhibit 20 to 90% of viable organisms, the proportional sensitivity tests with diluted inocula were performed using narrowly expanded ranges of drug concentrations. For the combination of rifampicin with amikacin, ethambutol, prothionamide and tetracycline, the following concentrations were prepared: rifampicin: 1.78, 3.56, 7.13 nM (equivalent to 1.5, 3, 6 mcg/ml); amikacin: 0.26, 0.39, 0.52 nM (equivalent to 0.2, 0.3, 0.4 mcg/ml); ethambutol: 9.79, 19.57, 39.14 nM (equivalent

to 2, 4, 8 mcg/ml); prothionamide: 4.44, 6.66, 8.88 nM (equivalent to 1.4, 2.4, 3.4 mcg/ml).

The proportional sensitivity of *M. fortuitum* was tested to those drug concentrations in combination or alone. Colony count of inoculated media was performed after 9 day incubation at 37°C.

Results

Of the various drugs tested, *M. fortuitum* KTM 29 was highly sensitive to amikacin and relatively sensitive to prothionamide and tetracycline, showing total growth inhibition of approximately 4.3×10^4 viable cells inoculated, at 1.3 nM of amikacin, 44.36 nM of prothionamide, and 10.4 nM of tetracycline.

Striking synergism was observed in combination of rifampicin and ethambutol or prothionamide as seen in figure 1 and 2. The 3.56 nM of rifampicin and 19.57 nM of ethambutol reduced viable units from log 6.377 to 6.021 or 5.592, but the association of both reduced up to log 2.586, indicating more than 99.9% reduction of viable cells which were survived by the treatment of either one drug alone. Increasing concentration of ethambutol in combination with rifampicin reduced much more viable cells than increasing concentrations of rifampicin. The combinations of 3.56 nM of rifampicin with 9.79 or with 19.57 nM of ethambutol reduced viable cells from log 6.377 to 4.909 or 2.586 respectively.

The 3.56 nM of rifampicin and 6.66 nM of prothionamide lowered viable units from log 6.377 to 6.021 and 5.458 respectively, but it was reduced to log 3.301 when combined both, showing more than 99.3% reduction of viable cells which were survived by the treatment of either one drug alone. Viable units could be reduced up to log 3.067 or to 2.971 with the combinations of 6.66 nM of prothionamide and 7.13 nM of rifampicin or of 8.88 nM of prothionamide and 3.56 nM of rifampicin as seen in figure 2. Synergistic effect between ethambutol and rifampicin was

Fig. 1. Interaction of rifampicin in association with ethambutol on *Mycobacterium fortuitum*. Numbers in a square represent log number of colony forming cells per ml of inoculum.

greater than that of prothionamide-rifampicin combinations. Ethambutol (19.57 nM)-rifampicin (3.56 nM) combination decreased viable units from log 6.377 to 2.586 and prothionamide (6.66 nM)-rifampicin (3.56 nM), to log 3.301; however, combination of all three drugs reduced viable units

up to 0.204 indicating further potentiation by this triple regimen.

Three concentrations of amikacin decreased viable units from log 6.182 to 5.553, 4.815 and 3.467 respectively, and in association with 3.56 nM of rifampicin, they reduced to log 4.602 or less

Fig. 2. Interaction of rifampicin in association with prothionamide on *Mycobacterium fortuitum*. Numbers in a square represent log number of colony forming cells per ml of inoculum.

than 2.000 as seen in figure 3. The association of 1.78 nM of rifampicin with 0.39 nM of amikacin reduced viable units from log 5.930 or 4.815 which were survived by the treatment of either one drug alone, to log 2.727, indicating more than 99.2% reduction.

Three concentrations (2.91, 4.99 and 7.07 nM)

of tetracycline reduced viable cells from log 6.182 to 5.926, 5.858, and 5.745 respectively. The combination of 1.78 nM of rifampicin with 4.99 nM of tetracycline decreased viable units to log 5.435 and 3.56 nM of rifampicin with each of three concentrations of tetracycline reduced to log 5.514, 5.125, and 4.924. The combination of 7.13 nM of

Fig. 3. Interaction of rifampicin in association with amikacin on *Mycobacterium fortuitum*. Numbers in a square represent log number of colony forming cells per ml of inoculum.

rifampicin with 4.99 nM of tetracycline reduced up to log 4.951 from 6.182 viable cells, but no total inhibition was observed. Highest reduction was noticed in the combination of 3.56 nM of rifampicin with 7.07 nM of tetracycline, showing less than 90% reduction of viable cells survived by treating with either one drug alone.

Discussion

The combined chemotherapy of antituberculous drugs is usual practice for the treatment of tuberculosis and it is aimed to rapid disappearance of the largest possible number of bacilli, probably

Fig. 4. Interaction of rifampicin in association with tetracycline on *Mycobacterium fortuitum*. Numbers in a square represent log number of colony forming cells per ml of inoculum.

due to multiple metabolic lesions caused, and to prevent the selective growth of resistant bacilli (4,5). Additional advantage in some combination of drugs is the sparing effect on concomitantly administered drug by competing with each other for inactivation in the body (6).

However, chemotherapeutic response of patients with nontuberculous mycobacterioses was variable and often produced poor results, presumably due to high resistance to most of antituberculous drugs (1,2,3). But some favorable response had been occasionally documented when patients were treated with proper combined chemotherapy of those including rifampicin, ethambutol, prothionamide, pyrazinamide, cycloserine or kanamycin. Favorable response could be expected if those regimens have synergistic interaction between drugs included. Strong synergism between rifampicin and ethambutol had been observed in tuberculosis (7,8) and kanamycin and ethambutol was very effective combination in murine atypical mycobacteriosis (9).

In this study we observed some interaction aspects of rifampicin with other antimicrobial drugs on *M. fortuitum* *in vitro*. To show clearcut interaction between drugs in the combinations, we used drug concentrations, which inhibited growth of 44 to 95% of cultivable cells. The results showed that rifampicin plus ethambutol or rifampicin plus prothionamide revealed striking synergism but much less prominent in the combination of rifampicin with amikacin even if amikacin was the most effective antibiotic against *M. fortuitum*. The association of rifampicin and ethambutol with concentrations which showed 56 to 84% growth inhibition suppressed growth of 99.9% of viable cells which were survived by the treatment of either one drug alone. The higher concentration of ethambutol in combinations, the greater synergism was observed. However, increased synergism with increasing concentrations of rifampicin was obtained when combined with 19.57 nM of ethambutol. Similar synergistic effect was obtained with the combination of 84% growth inhibitory concentra-

tion of rifampicin (1.78 nM) with 88% growth inhibitory concentration of prothionamide (6.66 nM), but it was less prominent than that of rifampicin-ethambutol combination. Tetracycline did not show apparent synergism with rifampicin but some additive effect could be obtained. The combination of rifampicin and tetracycline, which inhibited growth of approximately 50% of viable organisms, reduced 82 to 83% of viable cell units survived by the treatment of either one drug alone.

Summary

Selection of antimicrobial drugs effective on *M. fortuitum* and investigation on the interaction aspects of rifampicin with other antimicrobial drugs have been carried out using the drug concentrations which inhibited growth of 44 to 95% of cultivable cells. Total growth inhibition of approximately 4.3×10^4 viable bacilli inoculated was obtained with 1.3 nM of amikacin, 44.36 nM of prothionamide, and 10.4 nM of tetracycline. The association of 56% growth inhibitory concentration of rifampicin with 84% growth inhibitory concentration of ethambutol caused more than 99.9% reduction of viable cells which were survived by the treatment of either one drug alone. The similar synergistic effect was observed in the combination of rifampicin with prothionamide, but not much prominent with amikacin. Tetracycline seemed to act independently although some additive effect was clearly demonstrated.

REFERENCES

- 1) Wolinsky, E.: Nontuberculous mycobacteria and associated diseases. *Am Rev Resp Dis*, 119:107, 1979.
- 2) Davidson, P.T.: Treatment and long term follow-up of patients with atypical mycobacterial infections. *Bull IUAT*, 51:257, 1976.
- 3) Davidson, P.T.: The management of disease with atypical mycobacteria, *Clin Notes Resp Dis.*, 18:3, 1980.

- 4) Toman, K.: Tuberculosis. World Hlth Organization, pp 75-80, 1979.
- 5) Stead, W.W. and Bates, J.: Tuberculosis. In *Harrison's principles of internal medicine*, by G.W. Thorn, R.D. Adams, E. Braunwald, K.J. Isselbacher, R.G. Petersdorf. 9th ed. McGraw-Hill, pp 908-909, 1977.
- 6) Long E.R.: The chemistry and chemotherapy of tuberculosis. 3rd ed. Williams and Wilkins Co., Baltimore, p 351, 1952.
- 7) Trnka, L. and Tousek, J.: Interaction aspects of antimycobacterial drugs in the chemotherapy of tuberculosis. I. Role of individual antituberculous drugs in drug regimens used in the the chemotherapy of human tuberculosis. *Chemotherapy*, 20:165, 1974.
- 8) Trnka, L., Mison, P. and Staflove, S.: Interaction aspects of antimycobacterial drugs in the chemotherapy of tuberculosis. II. The role of rifampicin and other drugs in the dependent of independent action of drug associations *in vitro*. *Chemotherapy*, 20:82, 1974.
- 9) Sakamoto, T. and Goda, T.: Effectiveness of ethambutol on mice infected with atypical acid-fast bacilli. *Kekkaku*, 4:233, 1966.