In vitro efficacy of ciprofloxacin alone and in combination with amoxycillin against Salmonella typhi isolates

S Mandal¹, Manisha (Deb) Mandal & N K Pal

Department of Bacteriology and Serology, Calcutta School of Tropical Medicine, C. R. Avenue, Kolkata 700 073, India

Received 18 November 2002; revised 16 January 2003

Combined effect of ciprofloxacin (Ci) and amoxycillin (Ax) has been studied in vitro against 12 clinical isolates of S. typhi that showed Ci minimum inhibitory concentration (MIC) of ≥1 µg/ml. By agar dilution method, MIC values of Ax were 10-16 µg/ml for 11 isolates and 0.5 µg/ml for the remaining one isolate. The isolates, when treated with Ci and Ax in combination, showed fractional inhibitory concentration (FIC) of 0.004-0.256 µg/ml for Ci, FIC of Ax ranged from 0.5-4 µg/spot, except for a single isolate that showed Ax FIC of 0.25 µg/ml. Thus Ci was more efficacious in combination with Ax against S. typhi than Ci alone. The antibiotic combination exhibited an additive effect for all the isolates showing FIC index 0.504-0.832.

Combination effect of antimicrobial agents appears to be of particular interest in the treatment of infection caused by microorganisms resistant to clinically achievable concentration of single drug, and also in order to reduce the chances of emergence of drug resistance. Ciprofloxacin (Ci) was the drug of choice, over the period of last one decade, for the treatment of enteric fever due to infection of multidrug resistant (MDR) S. typhi¹. But treatment failure of enteric fever with Ci-therapy² as well as enteric fever epidemic due to Ci resistant S. typhi³, which frequently showed resistance to conventional antityphoid antibiotics (ampicillin/Ax, chloramphenicol and cotrimoxazole)⁴, is a cause of concern. This in turn leaves few therapeutical alternatives, viz cephaporphins of third generation⁵. In this communication we, therefore, evaluated the in vitro efficacy of Ci in combination with Ax against S. typhi isolates.

Strains—Twelve S. typhi isolates, which were unresponsive to Ci during treatment, were studied. The isolates were obtained by culturing blood collected from suspected enteric fever patients attending Calcutta School of Tropical Medicine (India) for treatment during 2000-2001. MICs of Ci for the isolates were 1-1.25 µg/ml.

Susceptibility to amoxycillin — Susceptibility of the isolates to Ax was checked by disc diffusion method⁶. The amount of Ax per disc was 10 µg. MIC values of Ax to the isolates were determined by agar dilution method⁷ using Mueller-Hinton agar plates. Each of the plates was divided into 12 equal sectors and inoculated with approximately 10⁴ CFU/spot. The plates were then incubated at 37°C for 24 hr. The concentrations of Ax used for the determination of MICs were 0.25-25 µg/ml.

Study of combined antimicrobial activity — Combined effect of Ci and Ax against the S. typhi isolates was studied by checkerboard agar dilution method⁸. In this method each test isolate was exposed to 8 different concentrations of Ci and Ax in combination. The concentrations of Ci for all isolates were 0.004, 0.008, 0.016, 0.032, 0.064, 0.128, 0.256 and 0.512 µg/ml. Concentrations of Ax were 0.25, 0.5, 1, 2, 4, 6, 8 and 9 µg/ml for 6 isolates that showed MIC value of 10 µg/ml and 0.5, 1, 2, 4, 6, 8, 10 and 12 µg/ml for another 5 isolates showing MIC value of 15-16 µg/ml. For the remaining one isolate with MIC value of 0.5 µg/ml, the concentrations of Ax used were 0.005, 0.0075, 0.0125, 0.025, 0.05, 0.075, 0.125 and 0.25 µg/ml. The inoculum size was approximately 10⁴ CFU/spot on Mueller-Hinton agar plates. After an incubation for 24 hr at 37°C, the FIC values were determined from the highest dilution of the antibiotic combination permitting no visible growth, and the FIC indices were calculated. Synergy was defined as the FIC index ≤ 0.5, addition as an FIC index 0.5-4 and antagonism as an FIC index >4 (Ref. 8).

Statistical analysis — Correlation coefficient between the activity of Ci by itself and in combination with Ax against S. typhi isolates was assessed with a simple regression analysis program. Test of significant difference from zero of the correlation coefficient

*Correspondent author: E-mail: samtropmed@rediffmail.com
was calculated at 5% level for 10 degrees of freedom using t-test and the difference was considered significant if the calculated value of t was greater than table value of t.

MIC and FIC of both Ax and Ci, and FIC index for 12 S. typhi isolates are shown in Table 1. MICs of Ax for 11 isolates ranged from 10-16 µg/ml, and that for remaining one isolate was 0.5 µg/ml. In combination with Ax, the isolates showed Ci FICs of 0.004-0.256 µg/ml. The FICs of Ax were 6-10 µg/ml for 11 isolates, while the remaining one showed Ax FIC of 0.25 µg/ml. FIC indices for the isolates were 0.504-0.832. Correlation coefficient between combined Ci-Ax and Ci alone was 0.44. The equation for regression is MIC of Ci = 0.6512 + 6.04 × FIC of Ci.

Eleven of the isolates, for which the MICs of Ax were 10-16 µg/ml, showed zone diameters of 14-15 mm around 10-µg Ax disc, while the rest one with Ax MIC of 0.5 µg/ml had 33 mm zone diameter of inhibition. This indicates that the isolates were towards the resistant range, except the later one that was highly susceptible to Ax.

Studies of combinations of fluoroquinolones and nonquinolone agents, especially β-lactams have most commonly shown indifferent or additive effects, occasionally synergism, and only rarely antagonism5,10. Huovinen et al.11 reported synergistic effect of Ci in combination with trimethoprim (Tr) against Escherichia coli. Here we reported the effect of a combination of Ci and Ax on bactericidal activity against 12 S. typhi isolates in vitro. We found strong additive effect of the drug combination for all the isolates tested.

The combination effect of β-lactams with other antibiotics against Staphylococcus aureus has been shown to be additive and/or synergistic12,13 and antagonistic14,15. Aritaka et al.16 reported both additive and antagonistic effect of seven β-lactam antibiotics in combination with vancomycin against Staph. aureus strains. Such contradictory observations regarding the combination effect of β-lactam and non-β-lactam antibiotics against Staph aureus may cause difficulty in selecting appropriate combination of antibiotic. However, our data suggests that the Ax-Ci combination is superior to Ci alone against S. typhi, and such finding may have important implications for treating patients infected with MDR S. typhi.

Ax-resistance among S. typhi isolates has been reported8. Emergence of S. typhi isolates causing treatment failure with Ci-therapy has also been reported8. The use of Ci-Ax combination may suppress the emergence of quinolone resistance since the current finding represents the first time that an antimicrobial, here Ci, has its activity significantly increased with Ax. This view is supported by very low FIC values of Ci (0.004-0.256 µg/ml) in combination with Ax, as found in the present study. The possible explanation of such effect is that Ax would affect the S. typhi cell wall, increasing the permeability as a consequence and thus facilitating the uptake of a second drug, Ci.

Table 1 — MIC and FIC values of Ax and Ci, and FIC index for S. typhi isolates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>MIC</th>
<th>FIC</th>
<th>FIC Index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ci</td>
<td>Ax</td>
<td>Ci</td>
</tr>
<tr>
<td>1</td>
<td>1.00</td>
<td>15</td>
<td>0.004</td>
</tr>
<tr>
<td>2</td>
<td>1.00</td>
<td>14</td>
<td>0.004</td>
</tr>
<tr>
<td>3</td>
<td>1.00</td>
<td>15</td>
<td>0.004</td>
</tr>
<tr>
<td>4</td>
<td>1.25</td>
<td>10</td>
<td>0.004</td>
</tr>
<tr>
<td>5</td>
<td>1.25</td>
<td>15</td>
<td>0.004</td>
</tr>
<tr>
<td>6</td>
<td>1.25</td>
<td>15</td>
<td>0.256</td>
</tr>
<tr>
<td>7</td>
<td>1.25</td>
<td>15</td>
<td>0.004</td>
</tr>
<tr>
<td>8</td>
<td>1.00</td>
<td>10</td>
<td>0.128</td>
</tr>
<tr>
<td>9</td>
<td>1.00</td>
<td>10</td>
<td>0.032</td>
</tr>
<tr>
<td>10</td>
<td>1.00</td>
<td>0.5</td>
<td>0.004</td>
</tr>
<tr>
<td>11</td>
<td>1.25</td>
<td>15</td>
<td>0.256</td>
</tr>
<tr>
<td>12</td>
<td>1.00</td>
<td>10</td>
<td>0.032</td>
</tr>
</tbody>
</table>

MIC = Minimum inhibitory concentration, FIC = Fractional inhibitory concentration, Ax = Amoxicillin, Ci = Ciprofloxacin

References

7 Miles R S & Amyes S G B. Laboratory control of antimicrobial therapy, in Mackie and McCartney Pratical Medical Microbiology, edited by J G Collee, A G Fraser, B P Marrian and A Simmons (Churchill Livingstone, New York) 1986, 151.
8 Leclercq R, Bingen E, Su, Q H, Lambert-Zechowski N, Courvalin P & Duval J. Effects of combinations of


