Prevention of Emergence of Bacterial Resistance with the Combination of Sulphamethoxazole and Trimethoprim

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An in vitro technique is described for testing the proposition that combining two antibiotics prevents the emergence of bacterial resistance to either. The model was applied to testing the combination of sulphamethoxazole and trimethoprim (co-trimoxazole). Under the test conditions when a doubly sensitive organism was exposed to sulphamethoxazole or trimethoprim alone, resistance to sulphamethoxazole or trimethoprim readily emerged. When exposed to both drugs together, the doubly sensitive organism was eliminated from the cultures without emergence of bacterial resistance to either drug. A sulphamethoxazole-resistant organism exposed to trimethoprim alone or to sulphamethoxazole plus trimethoprim developed trimethoprim resistance with equal facility. The experiments show that the use of sulphamethoxazole-trimethoprim prevents the emergence of bacterial resistance among doubly-sensitive strains.

At the time of its release for clinical use one of the claims made for co-trimoxazole, a fixed ratio combination of sulphamethoxazole and trimethoprim, was that its use would prevent the emergence of bacterial resistance to either component (1). This seemed a reasonable claim at the time, but was made on slight evidence (2).

The traditional experiments to test this may be simplified thus. A single drug (A) is added to a modest inoculum of bacteria in a test tube of broth and incubated, resulting in the emergence of a population of A-resistant bacteria. Similarly, a bacterial population exposed to drug B becomes B-resistant. When a culture of similar bacteria is exposed to A and B simultaneously, resistant organisms do not emerge and the bacteria are eliminated. The experiment is simple to perform but the conditions differ from those in human infections where large numbers of bacteria may be involved. Such bacteria are provided with fresh nutrients, their metabolic waste products are removed, and they are exposed to fluctuating cycles of antibiotic concentrations repeated many times in the space of a week.

We have developed an in vitro model which attempts to reproduce these features of human bacterial infection. Using this technique we have shown that rifampicin plus trimethoprim prevents the emergence of bacterial resistance (3). We now report the findings of the application of our experimental model to the use of co-trimoxazole.

Materials and Methods

Initially two organisms were used, both wild strains of Escherichia coli recently isolated from urinary tract infections. One strain was sensitive to sulphamethoxazole and trimethoprim (MIC 0.5 µg/ml and 0.15 µg/ml respectively), and the other strain was resistant to sulphamethoxazole and sensitive to trimethoprim (MIC 3,000 µg/ml and 0.6 µg/ml respectively). Experiments were performed which reflected the changes in antibiotic concentration to be expected in the blood of a patient being treated every 12 h with co-trimoxazole (4). The organism was cultured overnight in 20 ml Wellcotest broth in a universal container shaken in a water bath at 37 ºC. At the beginning of the experiment and every 6 h thereafter the broth was centrifuged for 10 min at 2,500 rpm, discarded and the organism washed before being resuspended in a
Table 1: Results of exposure of a susceptible strain (S) and a resistant strain (R) of *Escherichia coli* to sulpha-methoxazole (SMZ) and trimethoprim (TMP).

<table>
<thead>
<tr>
<th>Organism</th>
<th>Experiment</th>
<th>SMZ (µg/ml)</th>
<th>TMP and SMZ (µg/ml)</th>
<th>Time (h) for 1000-fold increase in number of organisms resistant to SMZ TMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain S</td>
<td>1</td>
<td>50</td>
<td>20</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>50</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>Strain R</td>
<td>4</td>
<td>-</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>50</td>
<td>2</td>
<td>20</td>
</tr>
</tbody>
</table>

*MICs for Strain S: SMZ 0.5 µg/ml, TMP 0.15 µg/ml
MICs for Strain R: SMZ 3,000 µg/ml, TMP 0.6 µg/ml

Experiments were stopped when either the count of antibiotic-resistant bacterial/ml of broth had increased more than 1,000-fold (i.e. from less than 50 organisms/ml to more than 50,000 organisms/ml), or the bacteria had been eliminated.

A further series of experiments six strains of doubly-sensitive *Escherichia coli*, including the original strain, were subjected to similar test procedures.

Results

The results of five experiments are shown in Table 1. In Experiments 1, 2, 4 and 5 the bacterial population showed a 1,000-fold increase in the number of resistant cells at times ranging from 36 to 78 h. In Experiment 3 the bacterial population was eliminated in 66 h. By two passes after 66 h it was no longer possible to recover the organism from Experiment 3 by incubation in large volumes of antibiotic-free broth.

Repetition of Experiment 3 resulted in bacterial elimination after 66 h when the same strain was used and after 60, 60, 60, 60, and 78 h respectively when five other doubly-sensitive urinary strains of *Escherichia coli* were used. In no instance did a doubly-sensitive strain develop resistance to sulpha-methoxazole or trimethoprim when exposed to both drugs simultaneously.

The MIC of sulpha-methoxazole for the doubly-sensitive *Escherichia coli* strain rose from 0.5 µg/ml to 32–64 µg/ml during the exposure