Effects of Subinhibitory Concentrations of Antibiotics and Antibodies on the Adherence of Escherichia coli to Human Uroepithelial Cells In vitro

Maryam Jahanshahi, Saeed Azad, Bahram Aslanbeigi and Mohammad Rahbar
1Department of Microbiology, Faculty of Medicine, Tarbiat Modarres University, Tehran, Iran
2Department of Pathology, Khatamol Anbya Hospital, Tehran, Iran
3Department of Microbiology, Iranian Reference Health Laboratory, Tehran, Iran

Abstract: Uropathogenic Escherichia coli is an important cause of urinary tract infection. Adherence to uroepithelial cells is a first step for colonization of bacteria. The aim of this study was to determine effects of sub Minimum Inhibitory Concentrations (MICs) of antibiotics and antibodies on the adherence of this organism at in vitro condition. Seven strains of E. coli isolated from patients with acute pyelonephritis were subject of the study. MICs of trimethoprim, sulphamethoxazole, sulphadiazine and ampicillin for these strains were determined and effect of these antibiotics at the ½ and ¼ MIC on the adhesion of E. coli to human urinary tract epithelial cells were studied. Trimethoprim, sulphamethoxazole and sulphadiazine at ½ and ¼ of the MICs decreased the adherence of four out of five E. coli strains tested whereas combinations of the compounds did not potentiate the effect. Ampicillin caused a similar effect. Specific pili-antibodies as well as gamma globulin and milk inhibited the adherence but did not work synergistically with ampicillin. In conclusion, present preliminary investigation gives further support to the theory that the effect of sub inhibitory concentration of antibiotics on the growth of bacteria may prevent their attachment to urinary epithelial cells.

Key words: Sub MIC, uropathogenic E. coli, uroepithelial cells, ampicillin, trimethoprim, sulphadiazine, pili-antibodies

INTRODUCTION

Association between bacteria and mucosal surfaces is considered necessary for colonization (Jones, 1977). The microorganism may bind to different constituents of the mucosa. Binding to epithelial cells is defined as attachment ability to selectively attach to different epithelial surfaces is a determinant of the microflora in each ecological niche (Ellen and Gibbons, 1974). Adhesive capacity may also be essential for the pathogenesis of infection (Jones, 1977; Ellen and Gibbons, 1974; Svanborg-Eden et al., 1978a), as one of several virulence factors co-appearing on the infection strain. The biochemical basis of the adhesion process has been defined most extensively for E. coli strains causing Urinary Tract Infection (UTI) and possessing the ability to adhere to human urinary tract epithelial cells (Svanborg-Eden et al., 1979) and erythrocytes (Kallenius et al., 1980). Bacterial pili (fimbriae) have been found to bind to glycosphingolipid receptors on the membranes of epithelial cells (Leffler and Svanborg-Eden, 1980; Kallenius et al., 1980). Such ligand mediated adherence of the bacteria can be prevented by interference either with the pili or with the epithelial receptor structure. Specific bacterial antibodies can bring about an inhibition of the binding. On the mucous membranes particularly secretory IgA (SigA) but also IgG, possess such activities (Svanborg-Eden et al., 1978a). Analagous of the glycolipid receptor can inhibit attachment. There have previously demonstrated that sub-Inhibitory Concentrations (sub-MICs) of ampicillin and amoxycillin possess adherence-decreasing activities in vitro (Sandberg et al., 1979; Svanborg-Eden et al., 1978b; Vidya et al., 2005; Loudbeyre et al., 1993; Wojnicz and Jankowski, 2007). The aim of the present study was to investigate the effect of sub MICs of trimethoprim, sulphadiazine and sulphamethoxazole alone or in combination on the attachment of E. coli to uroepithelial cells. Furthermore, antibodies to isolated pili were used to evaluate the effect of ampicillin on the piliation of bacteria.

MATERIALS AND METHODS

E. coli strains possessing good adhesive capacity isolated from the urine of patients with acute urinary tract infection were used. The bacteria, kept in deep agar storage cultures were transferred to lactose-bromothymol blue agar plates.

Corresponding Author: Muhammad Rahbar, Department of Microbiology, Iranian Reference Health Laboratory, Tehran, Iran
Antibiotics and determination of MICs: Trimethoprim, sulphasemethoxazole, sulphadiazine and ampicillin were used. The Minimum Inhibitory Concentration (MIC) of each antibacterial agent for each *E. coli* strain was determined under conditions identical to those employed for growth of bacteria in the adhesion test system. Serial twofold dilutions of the compounds in antibiotic sensitive medium were inoculated with 10^6 bacteria from a 4 h broth culture. After incubation for 24 h at 37°C, the lowest concentration of antibiotic without visible turbidity was recorded and considered as the MIC.

The range of MICs of trimethoprim for the seven strains of *E. coli* tested was 0.3-1.25 μg mL⁻¹, of sulphasemethoxazole 50-500 μg mL⁻¹ and of sulphadiazine 100-600 μg mL⁻¹.

Adhesion testing: Adhesion testing was performed as previously described. Uroepithelial cells were obtained from the sediment of fresh urine from a non-bacteruric woman. The cells were washed, resuspended in phosphate-buffered saline (PBS; pH 7.1, 300 mosm/l) and quantitated by direct light microscopy using Burker chamber. To 10⁶ epithelial cells were added 10⁶ bacteria and PBS to total volume of 1 mL after incubation of bacteria and epithelial cells for 60 min. The number of bacteria attached to the cells was counted under a light microscope. Adhesion was expressed as the mean number of bacteria attached to 40 epithelial cells. In each experiment antibiotic-treated bacteria and identically handled controls were compared (Korhonen et al., 1980).

Treatment of bacteria with subinhibitory concentrations of antibiotics: Parts of one bacterial colony were transferred from a lactosebroth dextromethyl blue agar plate to 3 mL of ASM broth. After 2 h growth at 37 °C without shaking, the antibacterial agent suspended in ASM broth was added to a final concentration of one-half or one-fourth of the MIC. After incubation for 4 h at 37°C, the bacteria were harvested by centrifugation and used for adhesion testing as described above.

Viable counts: The number of bacteria registered by viable counts in the adhesion test tube agreed well with the number counted in the Burker chamber by direct light microscopy (Loudbeyre et al., 1993; Wojciecz and Jankowski, 2007; Korhonen et al., 1980).

Ampicillin and antibodies: One strain of *E.coli* with good adhesive properties, *E.coli* 3048, untreated or treated with Ampicillin at 1-8 of the MIC was used. The MIC of ampicillin for this strain was 6.25 μg mL⁻¹. Purified pili were obtained from the same strain as recently described by Korhonen et al. (1980) and Svanborg-Eden et al. (1976). Antiserum to the purified pili was obtained from immunized rabbits. The antiserum was specific for the pili and did not react with the O or K antigen of the strain.

After preincubation of bacteria for 30 min with 0.001, 0.01, 0.05 and 0.1 mL of anti-pili serum, epithelial cells and PBS were added to a total volume of 1 mL and adhesion testing was completed as described above. Bacteria exposed to preimmune serum served as a control. Commercial gamma globulin and pool of human breast-milk served as sources of IgG and IgA antibodies (Svanborg-Eden and Svernerholm, 1978).

RESULTS

All concentrations of one-half and one-fourth of their MICs, trimethoprim, sulphasemethoxazole and sulphadiazine decreased the adhesive capacity of 6-7 *E.coli* strains tested.

The combination of sub-MICs of trimethoprim and either of the sulphonamides did not decrease adherence further compared with each compound alone Table 1. Bacteria exposed to sub-MICs of ampicillin but not of the other antibacterial agents, became elongated, as studied by direct microscopy. The majority of the bacteria that retained their adhesive capacity in presence of ampicillin were not elongated.

Specific antipili antibodies (as well as gamma globulin and milk (data not shown)) inhibited adherence in a dose-response relationship. Pretreatment of bacteria with ampicillin at 1-8 of the MIC diminished the adherence

<table>
<thead>
<tr>
<th><em>E. coli</em> strain</th>
<th>Control</th>
<th>1/2</th>
<th>1/4</th>
<th>1/2</th>
<th>1/4</th>
<th>1/2</th>
<th>1/4</th>
<th>1/2</th>
<th>1/4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45</td>
<td>13</td>
<td>13</td>
<td>12</td>
<td>24</td>
<td>38</td>
<td>35</td>
<td>19</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>0</td>
<td>8</td>
<td>9</td>
<td>16</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>0</td>
<td>9</td>
<td>15</td>
<td>11</td>
<td>6</td>
<td>0</td>
<td>20</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>16</td>
<td>14</td>
<td>23</td>
<td>16</td>
<td>15</td>
<td>20</td>
<td>18</td>
<td>28</td>
</tr>
<tr>
<td>5</td>
<td>58</td>
<td>6</td>
<td>35</td>
<td>23</td>
<td>100</td>
<td>51</td>
<td>57</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>6</td>
<td>33</td>
<td>143</td>
<td>12</td>
<td>0</td>
<td>3</td>
<td>14</td>
<td>14</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>70</td>
<td>39</td>
<td>28</td>
<td>28</td>
<td>19</td>
<td>35</td>
<td>35</td>
<td>14</td>
<td>4</td>
</tr>
</tbody>
</table>
Fig. 1: Effect of anti-pili antibodies in hyperimmune rabbit serum on the attachment to human uroepithelial cells of E. coli 3048, either untreated (×) or treated (○) with one-eighth of the MIC of Ampicillin

further Fig. 1. No decrease in attachment could be demonstrated when bacteria were incubated with premune serum only

DISCUSSION

The ability of E. coli to attach to human urinary tract epithelial cells in vitro has been found to correlate with the type of UTI caused by the strains (Korhonen et al., 1980; Svanborg-Edén et al., 1976, Deschner, 1976). Thus, E. coli isolated from patients with acute pyelonephritis and acute cystitis adhere more efficiently than strains from patients with asymptomatic bacteriuria (Svanborg-Edén et al., 1976).

We have shown before that sub-MICs of ampicillin and amoxicillin suppress bacterial adherence to uroepithelial cells (Svanborg-Edén et al., 1978a, b; Sandberg et al., 1979). The underlying mechanism explaining this effect may be a distortion of the bacterial cell wall synthesis producing elongated forms of resulting in a lack of adhesion-mediating structures on the surface of the bacteria.

In the present study, using the same In vitro adherence model, one-half and one-fourth of the MIC of trimethoprim, sulphadiazine and sulphamethoxazole suppressed the adhesive ability of all except one of the E. coli strains tested. Both trimethoprim and sulphonamides are inhibitors of bacterial folate metabolism resulting in disturbances of DNA and RNA synthesis. This may cause deficient production of structures, possibly pili, involved in the adherence process.

Bacteria exposed to sub-MICs of ampicillin and nitrofurantoin became elongated but only ampicillin decreased attachment. This suggests that the altered morphology observed, per se is not responsible for the altered adhesive properties of the bacteria but may be due to unrelated injuries either within or on the microbial cells (Klainer and Perkins, 1972; Smellie et al., 1976).

The attachment of E. coli to human uroepithelial cells is inhibited by commercial gamma globulin, human breast-milk containing IgA antibody and IgG and SlgA fractions of urine from patients with acute pyelonephritis (Stamey and Condly, 1975). These antibodies are probably directed against various bacterial surface structures (Svanborg-Edén et al., 1978a). The adherence decreasing effect of commercial gamma globulin seems to be potentiated by sub-MICs of ampicillin (Svanborg-Edén et al., 1978b).

In this study, specific anti-pili antibodies decreased attachment in a dose-response relationship. A synergistic effect between ampicillin and anti-pili antibodies could not be demonstrated but the effects were additive. This finding may be explained by a lower density of pili present on bacteria after ampicillin treatment.

The clinical impact of these in vitro findings has not yet been elucidated. Excretion of small amounts of antimicrobials in urine or vaginal fluid can be obtained by low-dose prophylaxis (Cattel et al., 1976, Stamey et al., 1978). A marked reduction in E. coli carriage both in the stool and especially on the periurethral area, persisting over months has been demonstrated in patients on long-term low-dose prophylaxis with trimethoprim-sulphamethoxazole (Stamey et al., 1978).

Furthermore, a high percentage of women not prone to UTI and seldom colonized with enterobacteria periurethrally had Cervicovaginal Antibodies (CVA) against their predominant faecal strain of E. coli in contrast, a minority of non-bacteriuric, UTI-susceptible subjects had CVA against enterobacteriaceae colonizing the introitus.

This suggests that local antibody is one important determinant of bacterial adherence to periurethral cells, possibly by blocking bacterial adhesions (Cattel et al., 1976, Stamey et al., 1978). UTI-prone individuals lacking specific CVA may avoid being colonized with potentially uropathogenic bacteria by receiving low-dose prophylaxis with antimicrobial drugs.

CONCLUSION

The present preliminary investigation gives further support to the theory that the effect of sub inhibitory concentration of antibiotics on the growth of bacteria may prevent their attachment to urinary epithelial cells.
ACKNOWLEDGEMENT

The researchers are grateful to Khutamolanbia Hospital for financial support of this study. The researchers declare that there is no conflict of interest.

REFERENCES


