Transport of nutrients into the renal brush border membrane vesicles as marker in evaluating the role of antipili antibodies in modulation of ascending pyelonephritis in rats

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1. SUMMARY

The uptake of D-glucose, L-aspartate, L-lysine and L-proline was investigated in renal brush border membrane (BBM) vesicles prepared from control, infected or passively-immunized-infected rats. Except L-aspartate, a progressive decrease in the uptake of these nutrients in both infected and immunized-infected groups during the course of infection was observed, but the changes were less apparent in immunized-infected rats than in non-immunized ones. The uptake of L-aspartate was increased in vesicles from early stages of infection but decreased in those from later stages. Also in L-aspartate uptake, the changes were smaller in immunized animals. The uptake of nutrients was detectable earlier than were histopathological alterations of both kidneys. The observations demonstrated that uptake of D-glucose and amino acids in the kidneys is disturbed prior to appearance of histopathological lesions and thus can be used for early detection of the disease. The data also demonstrate that antipili antibodies afford partial protection against ascending pyelonephritis.

2. INTRODUCTION

Most upper urinary tract infections (UTIs) in infants and children are caused by Escherichia coli and are known to be notorious for recurrence [1]. After exposure to Escherichia coli, an individual develops antibodies against 0 antigen and pili, while antibodies to K antigen appear less frequently [2–4]. As antibodies against certain 0 antigens cross-react with renal parenchyma [5], immunization with 0 antigens should be avoided. Immunization with pili may prove useful for immune prophylaxis of UTI as antipili antibodies block adherence to uroepithelial cells, a prerequisite for UTI [3].

To test the role of antipili antibodies in prevention of ascending pyelonephritis mortality rate, number of colony forming units in the kidney or...
urine and histopathological changes have been used as markers for the detection of the disease [3–6]. These markers detect the disease when it is fully developed and irreversible. There is a need for a sensitive marker which could detect the disease when fully grown lesions are not developed and the disease still is reversible. We tried to solve this problem by analysing the uptake of D-glucose and amino acids in renal brush border membrane (BBM) vesicles, as a possible marker for the detection of the disease. Uptake of nutrients is altered as early as 3 days postinfection [7–8]. To the best of our knowledge, this is the first report of exploring the role of antipili antibodies in modulation of ascending pyelonephritis by using uptake of nutrients into BBM vesicles as a marker for the disease.

3. MATERIALS AND METHODS

3.1. Bacterial strain

Escherichia coli serotype 06K13, a wild type strain isolated from a patient of acute UTI, was used to induce infection. The bacteria were grown in tryptic soy broth.

3.2. Immunization of rats

Pili were purified as described by Dodd and Eisenstein [9]. Pili agglutinated human erythrocytes even in the presence of 2.5% mannose but not guinea pig erythrocytes, indicating that they were mannose-resistant and probably P-pili [10]. Their purity was checked by SDS-PAGE and immunodouble diffusion [11,12]. Antipili antiserum was raised in rabbits as described by Silverblatt and Cohen [3]. Rabbits were injected intradermally with 200 μg of pili emulsified in complete Freund's adjuvant. 6 and 7 weeks later, an additional 200 μg pili in saline were administered intravenously. The animals were bled one week after the last injection, and the sera were heated at 56°C for 30 min. Rats were injected with 0.5 ml of the rabbit antipili antiserum 24 h before the infection. Control rats received 0.5 ml of normal rabbit serum.

3.3. Antipili antibodies determination

The levels of antipili antibodies were determined by the method of enzyme-linked immunosorbent assay [13].

3.4. Pyelonephritis

Pyelonephritis was produced as described earlier [7,8]. In brief, after partial ligation of the left ureter, 0.4 ml of inoculum with 10⁶ bacteria or saline without bacteria was injected into the bladder. Sham operated animals received 0.4 ml of sterile broth and no ligation of the ureter was done. In another group, 10⁶ bacteria were injected into the bladder, but no constriction of the ureter was done. The animals were sacrificed 0 h, 6 h, 12 h, 1 day, 2 days, 3 days, 4 days, 7 days or 14 days postinfection.

3.5. Preparation of BBM vesicles

The BBM vesicles from renal cortex were prepared and their quality checked as described by Turner and Moran [14]. The final preparation was suspended in the reconstitution buffer (300 mM mannitol, 1 mM Tris-Hepes, pH 7.5) to a protein concentration of 10–15 mg/ml.

3.6. Transport studies

Uptake of D-glucose, L-aspartate, L-lysine and L-proline was determined by the Millipore filtration technique of Hopfer et al. [15]. In brief, 10 μl of membrane vesicles (60 μg protein) were incubated at 20°C in the incubation medium having 50 μM D-glucose, L-aspartate, L-lysine or L-proline and 0.2 μCi of the 14C labelled respective substrate. The reaction was stopped by addition of 5 ml stopping buffer (150 mM NaCl, 1 mM Tris-Hepes, pH 7.5). The mixture was filtered through 0.45 μm Millipore filter. The filter was air dried and radioactivity was counted in LKB-1215 Rackbeta Liquid Scintillation Counter.

3.7. Histopathological studies

For the evaluation of renal lesions coronal section of each kidney was evaluated as described earlier [16].

3.8. Statistical analysis

Student's t test was used to compare various groups.
4. RESULTS

Uptake of D-glucose, L-aspartate, L-lysine and L-proline was determined in renal BBM vesicles from control, infected and immunized-infected rats. No significant difference in the uptake of nutrients was observed in the group in which constriction of the ureter was not done but only intravesical infection was given. Also no change was found in the group in which constriction of the ureter was done without an infection (data not shown). The uptake of nutrients was different in unobstructed-infected and obstructed-infected kidneys, while no difference was noted in right and left kidneys of sham operated animals. No significant difference in the uptake of nutrients could be observed by 2 days postinfection (data not shown). Afterwards, there was a significant difference in the uptake of these nutrients in both kidneys. The changes were more pronounced in the obstructed-infected (left) kidneys than unobstructed-infected kidneys (Table 1).

Table 1 shows the uptake of D-glucose, L-aspartate, L-lysine and L-proline in BBM vesicles. The uptake of D-glucose, L-lysine and L-proline was significantly decreased ($P < 0.05$) in the infected group during the course of infection. When the animals were immunized with antipili antiserum, a significant decrease ($P < 0.05$) in the uptake of these nutrients was still observed, but it was smaller than in the infected group. The uptake of L-aspartate increased ($P < 0.05$) in early stages and decreased ($P < 0.05$) in later stages of the infection. Also in L-aspartate uptake the changes were less pronounced in the immunized-infected animals.

Pyelonephritis was confirmed histopathologically at day 7 postinfection in obstructed-infected kidney and at day 14 postinfection in unobstructed-infected kidney. Immunized-infected animals, when sacrificed at days 7 and 14 postinfection, had histopathological alterations in the obstructed kidney, but the changes were less pronounced than in the infected group. Also, the unobstructed kidney from immunized-infected animals showed somewhat lesser histopathological changes than the ones from the infected group.

The antibody activity against the pili in rabbit serum was determined. Standardization of antibody was carried out by addition of various dilu-

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Group</th>
<th>Right kidney (3)</th>
<th>Left kidney (3)</th>
<th>Right kidney (4)</th>
<th>Left kidney (4)</th>
<th>Right kidney (7)</th>
<th>Left kidney (7)</th>
<th>Right kidney (14)</th>
<th>Left kidney (14)</th>
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<tr>
<td>D-glucose</td>
<td>Control</td>
<td>67 ± 3.5</td>
<td>69 ± 4.3</td>
<td>70 ± 4.0</td>
<td>63 ± 7.0</td>
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<tr>
<td></td>
<td>Infected</td>
<td>60 ± 3.0 *</td>
<td>41 ± 2.2 c</td>
<td>59 ± 3.6 a</td>
<td>38 ± 2.5 c</td>
<td>46 ± 3.2 c</td>
<td>38 ± 1.7 c</td>
<td>32 ± 1.6 c</td>
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<tr>
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<td>Immunized</td>
<td>64 ± 2.9</td>
<td>60 ± 4.5 b, *</td>
<td>59 ± 5.5 a</td>
<td>54 ± 5.9 b, *</td>
<td>49 ± 3.3 c</td>
<td>43 ± 3.5 c, *</td>
<td>37 ± 3.9 c, *</td>
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<td>L-aspartate</td>
<td>Control</td>
<td>82 ± 3.4</td>
<td>80 ± 4.6</td>
<td>84 ± 5.9</td>
<td>78 ± 10.0</td>
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<td>86 ± 8.4</td>
<td>101 ± 9.1 b</td>
<td>120 ± 10 c</td>
<td>223 ± 18 c</td>
<td>86 ± 4.6</td>
<td>79 ± 7.2</td>
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<td>84 ± 6.7</td>
<td>82 ± 3.7, *</td>
<td>109 ± 9.9 b</td>
<td>165 ± 18 c, *</td>
<td>92 ± 10</td>
<td>76 ± 10</td>
<td>66 ± 4.6</td>
<td>59 ± 5.9 a</td>
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<td>114 ± 9.4</td>
<td>109 ± 11.1</td>
<td>99 ± 13.0</td>
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<td>Infected</td>
<td>101 ± 9.5</td>
<td>81 ± 3.9 c</td>
<td>100 ± 11, *</td>
<td>76 ± 3.6 c</td>
<td>90 ± 4.7, *</td>
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<td>60 ± 3.1 c</td>
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<td>105 ± 11</td>
<td>98 ± 8.6, *</td>
<td>99 ± 5.5 a</td>
<td>78 ± 7.0 b, *</td>
<td>97 ± 7.0</td>
<td>93 ± 7.3 *</td>
<td>67 ± 3.1 c, *</td>
<td>64 ± 7.4 c, *</td>
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<td>L-proline</td>
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<td>113 ± 11</td>
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<td>103 ± 5.8</td>
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<td>85 ± 6.1 b</td>
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<td>67 ± 6.6 c</td>
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<tr>
<td></td>
<td>Immunized</td>
<td>109 ± 11</td>
<td>102 ± 5.9, *</td>
<td>99 ± 10</td>
<td>106 ± 8.4 *</td>
<td>99 ± 7.0 *</td>
<td>93 ± 10, *</td>
<td>66 ± 5.2 c</td>
<td>66 ± 6.8 c</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.D. of four determinations.

$^d$ Days postinfection in parentheses.

$^a P < 0.05$, $^b P < 0.01$, $^c P < 0.001$ as compared to control.

$^* P < 0.05$ as compared to infected group.
tions of pooled negative sera and pooled positive sera (immunized sera) of rabbits to various concentrations of antigen and antirabbit immunoglobulin Horse Radish Peroxidase (HRP) conjugate. The optical densities were measured for each dilution of antigen and antibody in the checkerboard system. Best differentiation was observed with 2 μg/ml of pili antigen, one in one thousand dilution of antiserum and one in two thousand dilution of HRP conjugate. O.D. was measured at 490 nm. The five fold increase (0.81 ± 0.05) was observed in terms of O.D. as compared to that in pre-immunized sera (0.16 ± 0.009).

5. DISCUSSION

In the present study uptake of D-glucose, L-aspartate, L-lysine and L-proline were used as markers for the early detection of pyelonephritis and to describe the effect of antipili antibodies in modulation of ascending pyelonephritis in rats.

Uptake of D-glucose and amino acids was found to be altered in both unobstructed-infected and obstructed-infected kidneys but the changes were more pronounced in the obstructed-infected kidney. Also the group in which constriction of the ureter was not done, did not show any change. These observations are in agreement with earlier histopathological, immunological and physiological reports [6–8,17,18].

The uptake of D-glucose and amino acids was observed to be significantly altered at 3 days postinfection and onwards in both kidneys. However, histopathological changes appeared 7 or 14 days postinfection. This demonstrates that physiological changes are seen much earlier than histopathological changes. These findings are in agreement with our earlier reports [7,8].

The progressive decrease in the uptake of D-glucose, L-lysine and L-proline in both kidneys of the infected and the immunized-infected groups demonstrate a degree of malfunctioning in the uptake of these nutrients [7,8]. However, the significant difference in the uptake of these nutrients in the infected and the immunized-infected groups at different stages of the infection (Table 1) demonstrates that antipili antibodies afford partial protection against ascending pyelonephritis [11,12]. The increase in the uptake of L-aspartate at early stages of the infection may reflect differences in the carrier systems for individual amino acids [19]. These observations are in agreement with our earlier reports [7,8]. The significant difference in the uptake of L-aspartate between infected and immunized-infected groups again demonstrates the protective effect of antipili antibodies.

In conclusion our data demonstrate that, after infection of the kidney, uptake of D-glucose and L-amino acids are altered earlier than histopathological changes become apparent and that they can be used as a sensitive marker for early stages of pyelonephritis. The data reveals that antipili antibodies can to some extent, prevent these alterations during the pathogenesis of experimental pyelonephritis in a rat ascending model.

REFERENCES


