Experimental Model of the Ascending Urinary Tract Infection of Mice with *Pseudomonas aeruginosa*

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**SUMMARY**

We could make the experimental model of the ascending urinary tract infection of mice with *Pseudomonas aeruginosa*. Microorganisms were infected into bladder of mouse after surgical insertion of a small glass bead and urethra was ligated to stop urinary flow for two hours. A number of microorganisms was found in kidneys and bladders of all mice even 35 days after the infection. Histopathological studies indicated that characteristic pyelitis was induced in all mice by the method described in this paper.

**INTRODUCTION**

Urinary tract infection in man is caused by either hematogenous or ascending infection with such bacteria as *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and so on. An experimental hematogenous pyelonephritis can be easily induced in animals by the intravenous injection of such microorganism (1-6). Many studies were reported from other laboratories (7-12) attempting to make ascending pyelonephritis in experimental animals. However, their reports showed that one third or more of the infected
animals were naturally cured in a short time after the infection and no microorganisms were detected in both kidneys and bladders of the infected animals. This paper deals with the method to make the model of the ascending urinary tract infection of mice with *Pseudomonas aeruginosa*.

**MATERIALS AND METHODS**

*Animals.* Six-week old female mice of ICR/JCL strain, weighing 28-30 g, were used. Mice were out-bred at Japan Clea Co., Tokyo.

*Bacteria.* *Pseudomonas aeruginosa* GN409 was isolated from a patient with urinary tract infection. Mouse was injected intraperitoneally with 0.5 ml of bacterial suspension (1 x 10^8 cells/ml). One day after the injection, mouse was killed by cervical dislocation, and kidney was aseptically removed, suspended in physiological saline and homogenized with universal homogenizer for 1 min. The homogenate was spread on a heart infusion agar plate. After 18 hrs of incubation at 37°C, three colonies were randomly picked up and identified as *P. aeruginosa* by ordinal procedure. One of the colonies was used for the same passage. After successive two passages, the bacteria was stored in ampules by freeze-drying and used for further experiments. Brain-heart infusion (BHI) broth was used for liquid culture. Bacterial concentration was determined photometrically at 530 nm.

*Ascending urinary tract infection.* Mice were anesthesized with ether. A 1 cm incision was made on the abdominal midline and the bladder was pulled out through the incision. The apex of the bladder was opened with fine siccors and one glass bead (2mm in
diameter and 1 mm high) was inserted into the bladder. The bladder was closed by silk suture. The penis was ligated by stainless-steel clip to stop urination and 0.05 ml of the bacterial cell suspension was injected into the bladder using 1/5 gauge needle. The peritoneum, muscles and skin were sutured with silk. After 2 hrs of injection with bacteria, the clip of penis was set free.

*Number of bacteria in kidney.* Mice were killed after the infection by cervical dislocation, and their kidneys were removed and weighed. Both kidneys were cut into halves and each half was weighed. Half of each kidney was homogenized for 1 min in 10 ml of BHI broth. The homogenate was immediately diluted with physiological saline and the number of bacteria was counted on an agar plate. The remaining half of each kidney was fixed with 10% formalin and used for histopathological study.

*Number of bacteria in bladder.* Bladder was removed from a mouse and weighed. The bladder was cut into small pieces with scissors and homogenized for 1 min in 10 ml BHI broth. The number of bacteria was counted as described above.

*Number of bacteria in urine.* A 0.005 ml sample of urine was obtained from the bladder and suspended in 2 ml of broth. The number of bacteria was counted on an agar plate. As control of each experiment, mice were injected physiological saline instead of bacterial cell suspension.

**RESULTS**

*Number of bacterial cells in kidney, urine and bladder.*

Various numbers of *P. aeruginosa* GN409 were ino-
culated into bladders of mice. When $2.5 \times 10^8$ bacterial cells were inoculated into bladder, large abscesses were observed in the kidney and urine became turbid on day 3 after the infection, resulting in the death of all mice 4 days after the infection. When $7 \times 10^6$ bacteria were inoculated into the bladder, the number of bacteria in the kidney decreased rapidly and abscess was not found in all kidneys, although the number of bacteria in urine and bladder did not decrease at day 7 of the infection. In contrast, the number of bacterial cells in urine, bladder and kidney was almost constant during 7 days after the infection with $1.6 \times 10^8$ bacterial cells (Table 1). From these results, $1-1.6 \times 10^8$ bacterial cells were used for the infection into bladder in the following experiments.

Daily changes of bacterial cell number in bladder, urine and kidney was examined after infection with $1 \times 10^8$ bacterial cells into bladder. As shown in Table 2, the number of bacteria in kidney, bladder and urine reached maximum on day 3 after the infection and constant number of bacterial cells was observed even 35 days after the infection.

*Histopathological changes.*

Kidneys obtained at appropriate days after the infection were fixed with 10% formalin and stained with hematoxyllin-eosin solution. Fig. 1 shows the histological changes of kidney on day 14 after the infection with $1.1 \times 10^8$ bacterial cells. Pathological changes were observed only at pelvis but not in glomerular site. Oedema and polymorphonuclear leukocyte exudation (ocasionally necrosis or abscess) were
observed at pelvic site. No lesion or a little hypertrophy was observed in tubules. These pathological findings showed that inoculation of bacteria into bladder could induce exudative inflammation of pelvis (pyelitis).

**DISCUSSION**

It is important to make the experimental model of animal infection for studying infectious states in human beings and for discovering effective therapeutic methods.

Most of urinary tract infections in man are caused by *Escherichia coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa*. Main routes of urinary tract infections are known to be both hematogenous and ascending routes.

An experimental model of hematogenous pyelonephritis was accompanied by intravenous injection of microorganisms (1-6) but ascending pyelonephritis has not yet been accomplished. It was reported that ascending urinary tract infection in guinea pigs, rats or rabbits by either injection of bacteria into bladder which had been inserted with foreign body such as zinc disc, steel cylinder or glass bead (7-11), or ligature of urethra to stop urinary flow (12). However, 30-50% of animals in these experiments cured no bacteria was detected in kidneys.

We succeed in secure induction of ascending urinary tract infection in ICR mice with *Pseudomonas aeruginosa* by both surgical insertion of a glass bead and ligation of urethra, and microorganisms were detected in all kidneys and bladders even 35 days after the infection. The method described in this
paper will be available to study mechanisms of genesis of pyelonephritis and to study therapeutical efforts.

REFERENCES


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Fig. 1. Pathological changes in kidney.
Kidney was obtained 14 days after the infection with 1.1 x 10^8 cells of Pseudomonas aeruginosa.
Table 1. Number of bacteria in kidney, urine and bladder after infection

<table>
<thead>
<tr>
<th>No. of bacteria injected into bladder</th>
<th>Days after infection</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>3 day</td>
<td>7 day</td>
</tr>
<tr>
<td></td>
<td>Kidney&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Urine&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2.5 x 10&lt;sup&gt;8&lt;/sup&gt;</td>
<td>7.8</td>
<td>11.0</td>
</tr>
<tr>
<td>1.6 x 10&lt;sup&gt;8&lt;/sup&gt;</td>
<td>6.8</td>
<td>8.4</td>
</tr>
<tr>
<td>7.0 x 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>3.6</td>
<td>6.4</td>
</tr>
</tbody>
</table>

Five mice received an infection with 0.05 ml sample of bacterial cell suspension into bladder as described in Methods.

<sup>a</sup> and <sup>c</sup> Log of a mean number of bacteria in 1 g of each organ.

<sup>b</sup> Log of a mean number of bacteria in urine (ml).

<sup>nd</sup> Not done because mice died before 7 days after infection.

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Table 2. Number of bacteria in bladder, urine and kidney of infected mice

<table>
<thead>
<tr>
<th>Organs and urine</th>
<th>Time after infection</th>
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<tbody>
<tr>
<td></td>
<td>2 hours</td>
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<tr>
<td>Bladder&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.8 ± 0.7</td>
</tr>
<tr>
<td>Urine&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.2 ± 0.7</td>
</tr>
<tr>
<td>Kidney&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.2 ± 0.8</td>
</tr>
</tbody>
</table>

Six mice received an infection with a 0.05 ml sample of bacterial cell suspension (2.2 x 10<sup>9</sup> cells/ml) into bladder as described in Methods.

<sup>a</sup>, <sup>b</sup> and <sup>c</sup> See footnotes of Table 1.