ABSTRACT

The objective of the study was to create a model of acute hematogenous pyelonephritis in the rat without causing urinary retention by ligation of the ureter.

Mixed bacterial suspension containing $1.5 \times 10^6$ colony-forming units (CFU) of S. aureus and $3.0 \times 10^6$ CFU of E. coli was inoculated in the caudal vein at a dose of 0.5 ml/kg. Control animals received the same amount of saline solution. Pyelonephritis was confirmed by lab urine tests and histopathological study of the kidneys.

Infected animals initially developed sepsis with a significant increase of leukocytes and C-reactive protein in the blood. Originally only bacteriuria was found in the urine of experimental animals, but later, in the course of the development of pyelonephritis (12–18 days), leucocyturia and active leukocytes (glitter cells) were also available in urine. The levels of β-2 microglobulin in the urine of infected animals ($4.02 \pm 0.04 \text{ mmol/l}$ on day 16 and $4.18 \pm 0.07 \text{ mmol/l}$ on day 18) were significantly highly increased ($p < 0.0001$) in comparison with the value of the control group ($0.088 \pm 0.005 \text{ mmol/l}$). In the early days the histopathological examination of the kidneys established erythrocyte stasis. Later leukocyte infiltrates were observed in the interstitial tissue around the kidney tubules, glomeruli and vascular walls, and inflammatory cell infiltration and degenerative changes were present in the epithelium of the canaliculi.

Combined hematogenous infection with S. aureus and E. coli led to the development of pyelonephritis in rats. The pathology in the kidney tubules was confirmed by histopathological study and by the elevated levels of β-2 microglobulin and the presence of active leukocytes in urine.

Key words: hematogenous pyelonephritis, rats, Staphylococcus aureus, Escherichia coli

INTRODUCTION

Bacterial pyelonephritis belongs to the kidney diseases that characteristically have only the interstitial tissue affected, usually manifested with inflammatory-toxic changes. Infectious agents reach the renal parenchyma most frequently (95-97%) ascendently starting from the urinary tract and less frequently (3-5%) descendently from the blood (acute hematogenous pyelonephritis). Escherichia coli (E. coli) is the most common uropathogen, while the most common cause of hematogenous pyelonephritis is Staphylococcus aureus (S. aureus).

Experimental models of pyelonephritis are induced by inoculation of bacteria directly into the renal parenchyma$^{1-4}$, or into the urinary bladder followed by temporary occlusion of the urethra for urinary retention$^5$, or into the caudal vein after prior unilateral ligation of one of the ureters for urinary retention$^6,7$.

Based on literature data that staphylococci are a predisposing factor for coli-pyelonephritis$^8$, the purpose of this study was to create an experimental model of hematogenous pyelonephritis without the invasive procedure – ligation of the ureter. For this purpose combined inoculation of E. coli and S. aureus was performed in the caudal vein and clinical laboratory parameters were monitored: leukocyte count and C-reactive protein (CRP) in blood, β-2 microglobulin and active leukocytes (glitter cells) in urine. Renal pathology was confirmed by histo-
pathological study. The advantage of such a model over the invasive models with urinary retention is that it avoids the surgery-induced traumas and contamination.

MATERIAL AND METHODS

ANIMALS AND EXPERIMENTAL PROCEDURE

Forty-eight male Wistar rats were used in the study. The animals (200-250 g) were housed in standard conditions at a temperature of 22-25°C and on a 12-h light-dark cycle (light 7:00-19:00) with ad libitum access to food and drinking water. All procedures concerning animal treatment and experimentation were in accordance with the national and international laws (EEC Council Directive 86/609, IL 358, 1, December 12, 1987; NIH Guide for Care and Use of Laboratory Animals, NIH publication no. 85-23, 1985).

The rats were divided into two groups: control (n = 12) and experimental (n = 36). Using intravenous cannulation of the caudal vein the animals were injected with mixed bacterial culture at a dose of 0.5ml/100 g body weight that was obtained in vitro and containing S. Aureus (ATCC 25922) $1.5 \times 10^6$ colony-forming units (CFU) and E. coli (ATCC 25923) $3.0 \times 10^6$ CFU. The same amount of saline solution was injected to the control group.

Microbiological urine study of the experimental animals was carried out 24 hours after the injection of the bacterial suspension. Urine was collected by holding the rat and placing a sterile test tube under the urethral opening. Only animals with bacteriuria were included in the experiment from this point on. From day 2 to day 8 two experimental animals were decapitated under diethyl ether narcosis every other day. From day 10 to day 18 five experimental animals were decapitated every other day.

BLOOD TESTS

In the blood of the experimental animals leukocyte count was performed (hematology counter Sysmex FS 3000) and the level of C-reactive protein (CRP) was measured immunoturbidimetrically (Hitachi 920) before injecting the bacterial suspension and 24 hours after the injection. Blood smear (Giemsa staining) was made for differential leukocyte count and detection of toxic granulations in leukocytes.

URINE TESTS

From day 12 to day 18 urine was obtained from the experimental animals that were to be decapitated (n = 5) as previously described. A sediment was prepared from the urine that was routinely tested – native preparation (microscope Zeiss 10×40) and was further treated for visualization of the active leukocytes. The active leukocytes were microscopically examined (light microscope Zeiss) using a method our own modification for native staining with methyleneblau (1% solution); the results were documented by an Olympus microphotocamera. This staining modification is a simple, fast, inexpensive and reliable method, alternative to the phase contrast microscopy of native sediment. Urine of control animals (n = 12) on day 16 and of experimental animals on days 16 (n = 5) and 18 (n = 5) was tested for β-2 microglobulin level (immunoturbidimetric method with Roche Diagnostics test, Hitachi 940).

HISTOPATHOLOGICAL STUDY

Kidneys, heart, liver, lungs and spleen of decapitated animals were fixed in 10% neutral buffered formalin. Fixed tissues were embedded in paraffin, sliced, placed on microscope slides and stained with hematoxylin-eosin (H&E). Light-microscopic histopathological examination was performed (microscope Zeiss) and the results were documented by an Olympus microphotocamera.

STATISTICS

Results are presented as mean ± standard error. Groups were compared using the Student’s t-test. The value of p < 0.05 was considered statistically significant. The GraphPad Prism statistical software was used.

RESULTS

BLOOD TESTS

The blood leukocyte count before the injection of the bacterial suspension was 6.76 ± 0.19\times10^9/l, and 24 hours after the injection there was a highly significant increase of the leukocyte count to 22.41 ± 1.56\times10^9/l (p < 0.0001). Blood smear from the experimental animals 24 hours after the treatment showed elevated levels of segmented granulocytes and in most of them toxic granulations were found.

The level of CRP in the blood before administration of the bacterial suspension was 1.26 ± 0.036 mg/l, and 24 hours after the injection there was a highly significant increase of this index to 16.07 ± 1.09 mg/l (p < 0.0001).

URINE TESTS

The microbiological analysis of urine at 24 hours showed that bacteria were present in all animals treated with the bacterial suspension except for three (they were eliminated from the experiment).
The bacteria in the urine were those that caused the sepsis. In 68% of the animals the bacteriuria reached significant values (≥ 10^5 CFU/ml). The sediment examination at this stage showed neither leukocyturia nor any other pathological changes.

Leukocyturia was found in the urine of the experimental animals between days 12 and 18. Leukocytes were found that were poorly stained with methylene blue and the phase contrast imaging showed chaotically moving particles (Brownian motion) in their cytoplasm, which is characteristic of the so called active or live leukocytes (glitter cells) (Fig. 1).

The level of β-2 microglobulin in the urine of control animals (n = 12) on day 16 was 0.088 ± 0.005 mmol/l, while for the experimental animals on day 16 it was 4.02 ± 0.04 mmol/l (n = 5), and on day 18 – 4.18 ± 0.07 mmol/l (n = 5). These levels of β-2 microglobulin in experimental animals were significantly higher (p < 0.0001) compared with the control value.

**Histopathological study**

Histopathological study of the kidneys in the early experimental days revealed erythrocyte stasis in the interlobar veins (Fig. 2). Inflammatory infiltrates...
Figure 3a. Inflammatory infiltrate in the interstitial tissue, around the kidney tubules, the glomeruli and the vessel walls. H&E staining, magnification 10×40.

Figure 3b. Inflammatory infiltrate of segmented leukocytes and macrophages in the interstitial tissue, kidney tubules and glomeruli. H&E staining, magnification 10×100.

Figure 4. Inflammatory cell infiltration and degenerative changes in the epithelium of a renal canaliculus. Segmented leukocytes and bacteria in the lumen of the canaliculus. H&E staining, magnification 10×100.
of segmented leukocytes and macrophages were observed later in the interstitial tissue around the kidney tubules, glomeruli and vascular walls (Figs 3a, 3b). Inflammatory cell infiltration and degenerative changes were found in the epithelium of the canaliculi (Fig. 4). The myocardium had intact myofibres, and the interstitium and the vessels inside it had no morphological changes. The examination of the liver showed stasis in the vessels, parenchymal degeneration of hepatocytes, intact lobules and portal spaces without inflammatory infiltrations. In the lungs there was alveolar focal edema, while the alveolar barriers and the interstitium had no histological changes. The spleen had intact white pulp and stasis in the vessels.

**DISCUSSION**

The increase in the blood leukocyte count and CRP confirmed that the animals developed sepsis. Development of pyelonephritis is facilitated by factors leading to retention and reproduction of microbes in the kidney. In models of hematogenous pyelonephritis with E. coli, accompanied by urinary retention induced by ligation of the ureter, there is ascending penetration of bacteria. In this case, after passing through the glomeruli, bacteria reach the tubular lumen where they reproduce and penetrate the interstitium through the tubular epithelium. The mechanism of development of pyelonephritis in combined infection with E. coli and S. aureus is different. It is known that S. aureus produces coagulase that affects the conversion of fibrinogen into fibrin thus creating favourable conditions for the slowing down of the blood flow in the renal veins with possible full stasis. This enables concentration of microbes in the renal vessels. Urine sediment tests at this stage of development of pyelonephritis may detect only bacteria. Venous stasis and parenchymal swelling increase the renal pressure and disrupt tissue trophism. This results in the reduction of tissue resistance to infection and intensification of the penetration of microorganisms from the vessels into interstitial tissue vessels where they quickly proliferate. With the advance of the inflammatory process a leukocyte shaft is formed around the foci of microbial clusters. Leukocyte infiltration in renal interstitial tissue leads to destruction of renal tubules in the area of the inflammatory infiltrate. Segmented neutrophil granulocytes and bacteria penetrate into the lumen of the canaliculi which end up in urine tubular destruction. At this stage of development of acute pyelonephritis there is a rich finding in the sediment, where leucocyturia and bacteriuria are detected. Active leukocytes (glitter cells) are found which swell due to the urine hypotonicity and their cytoplasmic granules are in constant Brownian movement. As a result, cells are glittering, especially under phase contrast imaging. This type of neutrophils are found in upper urinary tract infections (pyelonephritis) and polymicrobial infections. Pathology in the proximal tubules of the kidney is also demonstrated with increased levels of β-2 microglobulin in urine. β-2 microglobulin is produced constantly by all nuclear-bearing cells. The small molecular mass (11,800 D) enables it to freely pass through the glomerular membrane but under normal conditions it is reabsorbed 99.9% in the proximal tubules. β-2 microglobulinuria is one of the best and most sensitive tests to specify the status of the proximal tubules of the kidney.

**CONCLUSIONS**

Combined hematogenous infection with S. aureus and E. coli leads to the development of pyelonephritis in the rat which is evidenced by histopathological and clinical laboratory test results. Leukocyturia is absent in the first days of acute hematogenous pyelonephritis. It appears later – after microorganisms enter the kidney during the development of acute purulent-inflammatory changes in the parenchyma. The detection of active leukocytes in urine sediment proves the presence of infectious and inflammatory process in the kidney. Pathology in the kidney tubules is demonstrated also with increased levels of β-2 microglobulin in urine.

**REFERENCES**


МОДЕЛЬ ОСТРОГО ЭКСПЕРИМЕНТАЛЬНОГО ГЕМАТОГЕННОГО ПИЕЛОНЕФРИТА У КРЫС

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РЕЗЮМЕ

Исследование ставит себе целью создать модель острового гематогенного пиелонефрита у крыс посредством лигирования уретры, не вызывая задержки мочи.

В копчиковую вену введена смешанная бактериальная суспензия в дозе 0.5 ml/kg, содержащая S. aureus 1.5 x 10^6 колониобразующие единицы (КОЕ) и E. coli 3.0x10^6 КОЕ. Контрольным животным введено такое же количество физиологического раствора. Пиелонефрит доказан с помощью клинико-лабораторного исследования мочи и гистопатологического исследования почек.

У зараженных животных первоначально разивается септическое состояние, сопровождающееся сильным повышением лейкоцитов и С-реактивного протеина в крови. В моче подопытных животных установлена только бактериурия, а позже в ходе развития пиелонефрита (12 – 18-ый день) в моче наблюдается лейкоцитурия и активные лейкоциты (блестящие клетки). Уровни β-2 микроглобулина в моче зараженных животных (4.02 ± 0.04 mmol/l на 16-ый день и 4.18 ± 0.07 mmol/l на 18-ый день) статистически значимо повышены (p < 0.0001) по сравнению со стоимостью контрольной группы (0.088 ± .005 mmol/l). Гистопатологическое исследование почек в первые дни показывает эритроцитарный стаз. Позже в интерстициальной ткани около почечных канальцев, гломерулов и сосудистых стенок наблюдаются лейкоцитарные инфильтраты, а в эпителии канальцев – воспалительная клеточная инфильтрация и дегенеративные изменения.

Комбинированное гематогенное введение S. aureus и E. Coli приводит к развитию пиелонефрита у крыс. Патология в почечных канальцах объективизирована как гистопатологически, так и повышенными уровнями β-2 микроглобулина и наличием активных лейкоцитов в моче.