Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2022 Jan 27; 10(A):232-235.
https://doi.org/10.3889/oamjms.2022.8067
eISSN: 1857-9655
Category: *A - Basic Sciences*
Section: *Pathology*

The Model of Acute Obstructive Pyelonephritis for Studying Bacterial Translocation of *E. coli* from Gastroenteric Tract

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Edited by: Sinisa Stojanoski
Citation: Turgunov Y, Shakeyev K, Sharapatov Y, Lavrinenko A, Pronkin E. The Model of Acute Obstructive Pyelonephritis for Studying Bacterial Translocation of *E. coli* from Gastroenteric Tract. Open-Access Maced J Med Sci. 2022 Jan 27; 10(A):232-235. https://doi.org/10.3889/oamjms.2022.8067
Keywords: Acute pyelonephritis; Obstruction; Bacteria; Intestinal translocation
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Received: 24-Nov-2021
Revised: 29-Dec-2021
Accepted: 17-Jan-2022

Funding: This research did not receive any financial support
Competing Interest: The authors have declared that no competing interest exists

Abstract

BACKGROUND: The role of intestinal microflora translocation in the development of obstructive pyelonephritis has not been sufficiently studied. The urgency to develop a new model of acute obstructive pyelonephritis is due to the search for characteristics that are able to meet the criteria for reproducibility of microbial translocation from the intestine, the reversibility of the stages of the inflammatory process with further observation in the dynamics of development.

AIM: The aim of the given research is to develop a model of acute obstructive pyelonephritis to study the pathogenetic role of bacterial translocation of *Escherichia coli* (hereinafter *E. coli*) from the gastrointestinal tract (GIT).

METHODS: Twenty outbred male rabbits aged 3 months and weighing 3.0 ± 0.5 kg were used for the research. All the animals were randomly divided into two groups: Experimental (n = 10) and control (n = 10). In the experimental group, obstructive pyelonephritis was modeled by ligating the external opening of the urethra and injecting an antibiotic-resistant *E. coli* strain into the GIT using enteric capsules. In the control group, the strain was administered in the same way, but without forming a model of obstructive pyelonephritis. The animals were withdrawn from the experiment on the 3rd day by air embolism under general anesthesia. In both groups, the sizes of the kidney, pelvis, ureter, and the number of leukocytes in urine were assessed.

RESULTS: In the experimental group, there was an increase in the size of the kidney, pelvis, as well as ureter with some pronounced leukocyturia observed, which indicates the development of obstructive pyelonephritis. In the control group, only one animal had leukocyturia. The statistically significant differences were revealed between the groups in all studied parameters.

CONCLUSION: The results of this research demonstrated that the proposed model provided an opportunity to study the role of intestinal translocation of microorganisms in the development of acute obstructive pyelonephritis.

Introduction

Pyelonephritis accounts for 11.1% of the total number of diseases in the urinary system. The etiological role in the development of urinary tract infections in 80–90% of cases belongs to the microorganisms of the *Enterobacteriaceae* family. The Gram-positive coccal flora is found in 10–15% of cases [1].

In the pathogenesis of acute obstructive pyelonephritis, the leading role is played by the obstruction of the upper urinary tract caused by calculi, strictures, or compression of the ureter from outside. Even with timely urological intervention and adequate antimicrobial therapy, some complications and life-threatening conditions such as disseminated intravascular coagulopathy, multiple organ dysfunction, or septic shock can develop rapidly. It is, therefore, important to search for the predictors of severe conditions and death from acute obstructive pyelonephritis for the timely identification of patients in need of intensive care [2].

There are two main ways for the infection to penetrate into the urinary system, which are ascending and hematogenous. In the ascending way, the bacterial flora migrates from the lower urinary tract. The main factor here is urine stagnation in any segment of the urinary tract, which contributes to the rapid growth of microorganisms with the development of inflammation in the renal parenchyma [3], [4].

The interest of researchers has recently increased in the role of bacterial translocation of intestinal microflora in the development of various diseases, including acute pyelonephritis. In the experiments on animals, the highest level of intestinal microflora translocation was recorded in mesenteric lymph nodes, liver, spleen, lungs, and soft tissues [5], [6]. When pyelonephritis occurs, there is a direct dependence of the degree of intestinal-renal translocation on the concentration of bacteria and the duration of urinary tract obstruction. Once microorganisms

pass the immunological barriers and enter into the vascular bed, hematogenous dissemination occurs under certain conditions, which results in the infection of the renal tissue [7].

Nevertheless, the role of intestinal microflora translocation in the development of obstructive pyelonephritis has not been sufficiently studied.

The urgency to develop a new model of acute obstructive pyelonephritis is due to the search for characteristics that are able to meet the criteria for reproducibility of microbial translocation from the intestine, the reversibility of the stages of the inflammatory process with further observation in the dynamics of development. An important task of modeling was to observe the degree of purity in the experiment as well as to maintain the technical simplicity of execution and the survival of the animal after manipulation.

Methods

The experimental study was carried out on rabbits in the vivarium of Karaganda Medical University, Non-Commercial Joint Stock Company (hereinafter NJSC KMU). Maintenance, nutrition, and care of the animals before and after their removal from the experiment were done in accordance with the republican and international requirements for working with experimental animals [8], [9]. To study the model, 20 outbred male rabbits were used at the age of 6 months and 3.0 ± 0.5 kg in mass. All animals were kept under similar conditions: At 20–23° C, 70–75% of humidity, a 12 h light-dark cycle, as well as an appropriate diet. The design of this research was approved by the ethical commission at NJSC KMU (Minutes of meeting No. 10 dated March 16, 2020).

All manipulations with animals were performed under general anesthesia. As a preparation, ketamine was used intramuscularly at the dosage of 0.5 mg per kilogram of animal body weight.

The infectious agent was administered to the gastrointestinal tract (GIT) of rabbits using enteric capsules (gelatin capsules, size 2). The meat infusion agar was placed in the capsule (Obolensk, Russia) containing 0.5 of MCFARLAND bacterial suspension of *Escherichia coli strain* No. 49579, which is resistant to cefepime, ciprofloxacin, and tetracycline. This strain was used as a marker to confirm the translocation of the microorganism from the intestine to the renal parenchyma.

In the experimental group, the modeling was carried out as follows: After anesthesia, the animal was fixed in the prone position on the right side. The length of the gastric tube was measured from the level of the oral cavity to the xiphoid appendix. The gastric tube No. 20 was used as a probe as per Sharier. The probe lubricated with Vaseline oil was inserted through the mouth into the stomach cavity. The presence of the probe in the stomach was confirmed by the appearance of stomach contents in the syringe attached to the probe once the piston was pulled toward itself. A capsule with an infectious agent was placed into the lumen of the probe (Figure 1a), a syringe with 20 ml of 0.9% saline was attached to the end of the probe. The capsule was injected into the lumen of the stomach by means of compression under fluid pressure (Figure 1b).

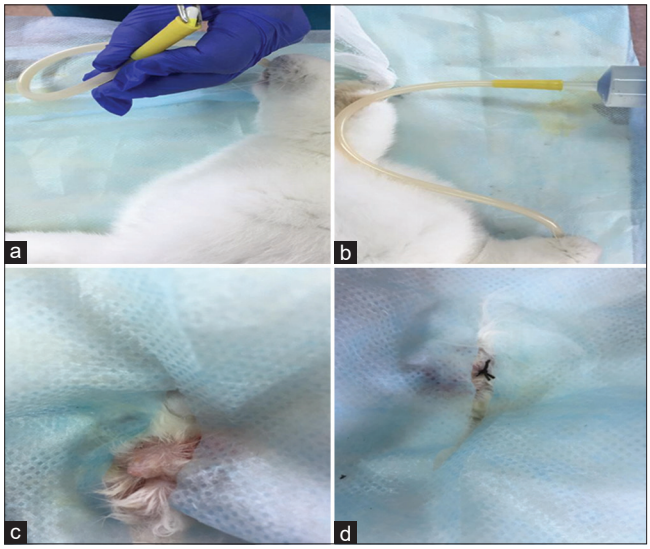

Figure 1: The sequence of modeling. The enteric capsules being administered into the tube (a); the enteric capsules being introduced into the stomach (b); the external opening of the urethra being determined (c); the external opening of the urethra being sutured (d)

At the second stage, the obstruction of the urinary tract was modeled [10]: After exposing the head of the penis from the preputial sheath (Figure 1c), the external opening of the urethra is sutured with a 3/0 thread (Figure 1d).

In the control group, the bacterial strain was administered in the same way, but without blocking the urethra.

Before the start of the experiment, urine was taken from animals of both groups by catheterization of the urinary bladder using a Nelaton No. 8 catheter as per Sharier.

In each group, animals were removed from the experiment on the 3rd day by air embolism under general anesthesia in accordance with international recommendations [11].

A previous pilot study was carried out to select the optimal time for withdrawing animals from the experiment, depending on the degree of development of obstructive pyelonephritis. Six rabbits were subjected to a model of acute pyelonephritis according to the above method. On the 1st, 3rd, and 5th days, two animals had been withdrawn from the experiment at each time. It was found that no changes were observed on the 1st day in laboratory samples of both animals. There were obvious macroscopic and laboratory signs of

obstructive pyelonephritis in both animals on the 3rd day. On the 5th day, two animals showed signs of bladder rupture. In a further study, all animals were withdrawn from the experiment on the 3rd day.

At the autopsy on the 3rd day, the sizes of the kidneys, pelvis, and ureter of the animals were assessed. The urine was taken from the bladder using a 5.0 ml syringe in the glass tubes for laboratory analysis.

The urine was analyzed in the collective use laboratory of NJSC KMU. The material was centrifuged for 5 min at 1500 rpm (OPn-3-02 Dostan centrifuge). Following that, the transparent top layer was merged, and a drop was transferred from the remaining sediment onto a glass slide, covered with a cover glass to carry out the microscopy of the urine sediment (Leica Microsystems GmbH microscope, Model-DM1000, Germany).

The statistical data were processed using the Statistica 8.1 software (Statsoft). For each quantitative indicator, the maximum and minimum values were determined, and the mean (M) and standard deviation (SD) were calculated. To determine the statistical significance of the differences between the groups for the studied parameters, the Mann–Whitney U-test was used for two independent groups. The differences in the groups were considered statistically significant at $p < 0.05$.

Results

Before developing a model and introducing an infectious agent, the leukocytes in the urine were not detected in the animals of both groups.

In the experimental group, the maximum number of leukocytes after being withdrawn from the experiment was 35 in the field of view, and the minimum number being 0 in the field of view was found in only one animal. The average value of leukocytes in the urine after the withdrawal from the experiment on the 3rd day in the experimental group was 19.30 ± 9.46 in the field of view.

In the control group, after the withdrawal from the experiment, the maximum number of leukocytes in the urine was 12 in the field of view. This was found in only one animal. In the rest of the animals, leukocytes in urine were not detected; the average value of leukocytes in the urine on the 3rd day in the control group was 1.20 ± 3.80 in the field of view.

When assessing the size of the kidney, ureter, and pelvis, significant differences were revealed among the groups. In the experimental group, on average, the length of the kidney was 1.5 times greater than the one in the control group, and the width of the kidney in the experimental group was 35% greater than in the control group. In the experimental group, an increase in the pelvis was noted by 81.0% in comparison with the control one, which indicates the development of hydronephrosis. In the experimental group, on average, the width of the ureter was 2.2 times greater than in the control group (Table 1).

Table 1: Estimated sizes of kidney, pelvis, ureter, and the number of leukocytes in the groups on the 3rd day of the experiment (M ± SD)

Parameters	Experimental group (n = 10)	Control group (n = 10)	z	p-value
Kidney length, cm	4.74 ± 0.61	3.10 ± 0.17	−3.87	0.0001
Kidney width, cm	3.51 ± 0.22	2.60 ± 0.17	−3.84	0.0001
Pelvis width, cm	0.31 ± 0.17	0.11 ± 0.03	−3.50	0.0001
Ureter width, cm	0.25 ± 0.09	0.11 ± 0.03	−3.82	0.0001
Leukocytes in urine (in the field of view)	19.30 ± 9.46	1.20 ± 3.80	−3.60	0.0001

z is the value of the Mann–Whitney U-test

Thus, it was found that when infected through the GIT with an uropathogenic culture of antibiotic-resistant *Escherichia coli* at the concentration of 10^8 CFU/ml in the experimental animals of the experimental group, pyelonephritis develops. This is evidenced by the level of leukocyturia in dynamics as well as by a significant increase in the size of the kidneys, pelvis, and ureter.

The results of the experiment showed that the developed model of acute obstructive pyelonephritis provides an opportunity to study the pathogenetic role of translocation of intestinal microflora into the organs of the urinary system.

Discussion

The experimental modeling of the pathological process in laboratory animals is a standard method for studying the main mechanisms of the development of various diseases, including obstructive pyelonephritis. There are several fundamentally different methods of modeling acute pyelonephritis, which provide some accurate reproduction of the pathological process.

The authors used *E. coli* as an infectious agent in an amount of 10^9 CFU/ml in a volume of 0.4 ml. The bacterial suspension of *E. coli* is injected into the bladder. Later, the external opening of the urethra is closed for 4 h. This provides the necessary reflux of the infected urine into the renal pelvis and the reproduction of obstructive pyelonephritis. This technique is a model to develop bilateral refluxogenic pyelonephritis [12].

The earlier reports describe a technique for simulating acute pyelonephritis by introducing the pathogen directly into the blood of animals. To implement this method, a 20–25% ethanol solution was injected once intravenously. After 8 h, 3 ml of *E. coli* at the concentration of 10^8 CFU/ml was intravenously injected. The disadvantage of this technique is the usage of a highly active toxic substance in modeling of acute pyelonephritis, which does not correspond to the clinical conditions to develop the disease [13].

In recent years, the modeling method of experimental obstructive pyelonephritis by open ligation of the ureter and subsequent introduction of a bacterial strain into the renal pelvis has been increasingly used [14]. In the given method, the authors pulled both ureters to the anterior abdominal wall, thereby causing partial obstruction. To create a complete obstruction of the ureter, a 3/0 thread was inserted under it. The ureter was ligated followed by introducing bacterial pathogen at the concentration into the renal pelvis [15].

With all of the above methods, the infectious agent is injected either into the blood or directly into the urinary tract, which limits the use of these models in studying the role of intestinal microflora translocation in the development of acute obstructive pyelonephritis.

The results of the study found that intestinal translocation plays an important role in the development of obstructive pyelonephritis, on day 3, the level of leukocytes and kidney size was more pronounced than in the control group.

Thus, the involvement of intestinal microflora translocation in the pathogenesis of acute obstructive pyelonephritis and the development of inflammatory changes in the kidneys is beyond doubt, but requires further investigation.

Conclusion

The model developed by us makes it possible to reproduce obstructive pyelonephritis in an experiment with the least aggressive surgical technique. The results of the research showed that this model of obstructive pyelonephritis differs from its analogs by the enteral administration of an infectious agent into the GIT, which makes it possible to study the pathogenetic mechanisms of the acute obstructive pyelonephritis development caused by translocation of intestinal microflora into the urinary tract.

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