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Research Article

Intraoperative application of photodynamic therapy as a method of inactivation of uropathogenic flora (pilot study)

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ABSTRACT

BACKGROUND: The predictor of experimental work on animals on the use of antimicrobial photodynamic therapy to neutralize antibiotic-resistant strains of microorganisms was the analysis of the microbial landscape of patients' urine and swabbings from the working surfaces of objects of the urological hospital.

AIM: The aim of the study is to study the possibility of intraoperative photodynamic inactivation of uropathogenic microorganisms.

MATERIALS AND METHODS: Analysis of the species specificity of microorganisms was performed based on the results of urine cultures of patients in a urological hospital and external introduction infections over the past 10 years. The experimental part of the work was carried out on 7 animals (pigs). Photodynamic therapy was performed intraoperatively by local injection into the animals' renal pelvis of the photosensitizer photoditazine in physiological solution with the addition of the nonionic surfactant Triton X-100 to a concentration of 10%. To study the damaging effect on the tissue of the pelvis, a histological study of animal kidneys was performed. To assess the biocidal effect on uropathogenic bacteria introduced into the renal pelvis, we used suspensions of daily test cultures (1×10^8 CFU/ml) of microorganisms most often found in the stones of patients with urolithiasis.

RESULTS: *Escherichia coli*, *Enterobacter cloacae*, *Staphylococcus epidermidis*, *Enterococcus faecalis* and *Klebsiella pneumonia* were the most frequently present in urine over 10 years. The safety of using photodynamic therapy in the renal pelvis of animals was comprehensively studied. It was found that the accumulation of the photosensitizer by the cells of the lining epithelium of the renal pelvis did not occur; the photosensitizer solution in the renal pelvis was not heated during photodynamic therapy. Histological examination established the absence of significant damage to the epithelium of the renal pelvis of the animals under the influence of various irradiation modes. The analysis of the bactericidal activity of the method used showed that photodynamic therapy leads to the death of 99.9% of *E. coli* and 99% of *S. aureus*.

CONCLUSIONS: The experiment established that intraoperative photodynamic therapy is an effective and safe method of inactivating uropathogenic microorganisms, which allows it to be considered as an alternative to antibiotic therapy.

Keywords: photodynamic therapy; inactivation of uropathogenic flora; antibacterial PDT; biocidal effect of PDT; prevention of postoperative complications.

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Научная статья

Интраоперационное применение фотодинамической терапии как метода инактивации уропатогенной флоры (пилотное исследование)

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АННОТАЦИЯ

Актуальность. Предиктором экспериментальных работ на животных по применению антимикробной фотодинамической терапии с целью обезвреживания антибиотикорезистентных штаммов микроорганизмов стал анализ микробного пейзажа мочи пациентов и смывов с рабочих поверхностей объектов урологического стационара.

Цель — изучение возможности интраоперационной фотодинамической инактивации уропатогенных микроорганизмов в эксперименте.

Материалы и методы. Анализ видовой специфичности микроорганизмов выполнен по результатам посевов мочи пациентов урологического стационара и заносов инфекций за последние 10 лет. Экспериментальная часть работы выполнена на 7 животных (свиньи). Фотодинамическую терапию проводили интраоперационно путем местного введения животным в лоханку почки фотосенсибилизатора фотодитазин в 0,9 % растворе натрия хлорида с добавлением неионного поверхностно-активного вещества Тритон Х-100 до концентрации 10 %. Для изучения повреждающего эффекта на ткани лоханки выполнено гистологическое исследование почек животных. Для оценки биоцидного эффекта на уропатогенные бактерии, введенные в лоханку почки, использовали суспензии суточных тест-культур ($1 \cdot 10^8$ КОЕ/мл) микроорганизмов, наиболее часто встречающиеся в конкрементах пациентов с мочекаменной болезнью.

Результаты. Наиболее часто в моче больных урологического стационара присутствовали *Escherichia coli*, *Enterobacter cloacae*, *Staphylococcus epidermidis*, *Enterococcus faecalis* и *Klebsiella pneumonia*. Комплексно исследована безопасность применения фотодинамической терапии в лоханке почки животных. Установлено, что накопления фотосенсибилизатора клетками выстилающего эпителия лоханок почек не происходило, раствор фотосенсибилизатора в лоханке почки в процессе фотодинамической терапии не нагревался. При гистологическом исследовании установлено отсутствие значительных повреждений эпителия лоханки почки животных при воздействии различных режимов облучения. Анализ бактерицидной активности используемой методики показал, что фотодинамическая терапия приводит к гибели 99,9 % *E. coli* и 99 % *S. aureus*.

Заключение. В эксперименте установлено, что интраоперационная фотодинамическая терапия является эффективным и безопасным методом инактивации уропатогенных микроорганизмов, что позволяет рассматривать ее в качестве альтернативы антибиотикотерапии.

Ключевые слова: фотодинамическая терапия; инактивация уропатогенной флоры; антибактериальная ФДТ; биоцидный эффект ФДТ; профилактика послеоперационных осложнений.

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BACKGROUND

In modern urology, endoscopic techniques, including intraluminal surgery, are rapidly developing, which offer advantages such as low trauma and shorter patient recovery periods. However, the complex design of the equipment and instruments used, labor-intensive process after use, and human factors at all stages of work are risk factors for postoperative complications. Other risk factors for urinary infection include the need for drainage of urinary organs, such as the introduction of a foreign body, and the need to visualize the surgical field, which is achieved by fluid irrigation. These can increase the pressure in the system with pyelointerstitial reflux. In addition, preoperative and/or intraoperative antibiotic prophylaxis may be ineffective because of uropathogens resistant to antibiotics. According to the literature, between 30% [1] and 51% [2] of urinary stones are infected. Contemporary lithotripsy principles involve crushing concretions into small fragments, which are then removed through small-diameter accesses. However, in infection-related stones, small fragmentation inevitably releases large amounts of toxins and bacteria contained in the concrement [3]. Postoperative complications can be serious, such as systemic inflammatory response syndrome (SIRS), pyelonephritis, and urosepsis. The incidence of postoperative SIRS can reach 27.4%, whereas urosepsis can occur in up to 7.9% of cases [4–6]. Importantly, postoperative fever can occur even if the preoperative urine culture is sterile and prophylactic antibiotic therapy is administered because the source of infection may be the bacteria present in the nodule [7–9]. In the urological departments of medical and preventive institutions in the Russian Federation, as well as in many other countries worldwide, nosocomial infections (healthcare-associated infections [HCAIs]) are a significant problem. Despite adherence to sanitary, hygienic, and antiepidemic policies in medical institutions, the frequency of HCAIs remains high [10].

The prevention of postoperative infectious and inflammatory complications is a pressing issue. An area of scientific research is exploring the potential of photodynamic therapy (PDT), which is traditionally used to treat patients with cancer, for the treatment of localized bacterial infections, including antibiotic-resistant strains [11]. Furthermore, PDT [12] has been used in experimental and clinical studies to treat purulent wounds and purulent-septic complications of ENT organs. A previous study showed the promising potential of PDT application in dentistry [13]. Antibacterial PDT is a promising technology for combating chronic infections [14–17]. Our research group previously presented an analysis of the effects of PDT on the urinary "planktonic" microflora in patients with nephrolithiasis (NL) [18]. However, currently, no data exist on the use of PDT for preventing infectious complications in urological practice, specifically for treating NL.

This study analyzed the microbial landscape of patients' urine and washes from various surfaces in a regional urology hospital over 10 years and the sensitivity of isolated microorganisms to antibiotics. The results of this study suggest the need for new methods of antibacterial therapy. To this end, the safety of the intraoperative use of antibacterial PDT was evaluated.

This *study aimed* to investigate the possibility of intraoperative photodynamic inactivation of uropathogenic microorganisms. The study objectives were as follows: (1) analyze the microflora of patients' urine and environmental surfaces in the urological clinic and (2) evaluate the biocidal effect on uropathogenic bacteria and the potential tissue damage caused by intraoperative photodynamic exposure on the animal kidney pelvis.

MATERIALS AND METHODS

Analysis of microbial species specificity

In this study, a retrospective analysis of observational data on the urine microbial landscape of patients in the urology clinic of a large hospital (120 beds) from 2010 to 2019 was conducted. This study investigated the results of washes from different working surfaces of environmental objects in the clinic, including hands, linen, overalls, medical instruments, furnishings, medical equipment, and dressing tables. The primary uropathogens found in native urine samples from patients with complicated NL were also analyzed. This included patients with established bacteriuria, long-term urostomies, recurrent NL, and episodes of exacerbation of infectious-inflammatory processes.

Working with animals

The pelvic ducts of seven 4- to 4.5-month-old Landrace pigs, weighing 40–45 kg, were exposed to analyze the response of the pelvic tissue to PDT. In addition, four kidneys were obtained from different pigs that had not been exposed to serve as normal controls. The pigs were anesthetized during surgical interventions using a mixture of Zoletil and Rometar. The surgical area was disinfected twice using a 0.5% aqueous–alcoholic chlorhexidine bigluconate solution. Access to the kidney was achieved through an oblique incision in the subcostal region. The kidney and upper third of the ureter were isolated retroperitoneally. A soft tourniquet was used to clamp the ureter, and a catheter was inserted into the pelvis through an incision in the upper third.

The Ethical Committee of the Volga Region Research Medical University of the Ministry of Health of Russia approved all animal studies (Minutes No. 13, dated July 7, 2021), which were conducted in compliance with international legal acts on laboratory animal work.

Assessment of photosensitizer accumulation in kidney tissues

The study used 5 mg/mL photoditazine in a 0.9% sodium chloride solution supplemented with the non-ionic surfactant Triton X 100 to a concentration of 10%. The solution was injected through a catheter into the renal pelvis and left for 10 min. Subsequently, the pelvis was washed with 0.9% sodium chloride solution, and the kidney was removed. The accumulation of photosensitizer in kidney tissues was evaluated by measuring the fluorescence intensity using an IVIS surface fluorescence imaging unit.

In vivo PDT

Fifteen minutes after filling the pelvis with the photosensitizer solution, a light guide with a cylindrical diffuser, 5 mm in length at the end (Polironik LLC), connected to the Latus-K laser (Actus LLC), was introduced into the pelvis. The renal pelvic cavity was irradiated in continuous or pulsed modes with an output power of 150 and 300 mW and a wavelength of 662 nm. After irradiation, the ureter was sutured using an atraumatic polyglycolide suture, and the wound was closed layer by layer. The skin was treated with antiseptic and covered with an aseptic sticker. Histological examination of renal tissues was performed 3 h, 1 day, and 3 days after PDT. The temperature in the pelvis was measured using a portable multifunctional multimeter equipped with a thermocouple to exclude thermal effects during irradiation.

Antibacterial PDT *in vivo*

The microorganisms most frequently found in patient stones, *Escherichia coli* and *Staphylococcus aureus*, were used to evaluate the effectiveness of intraoperative PDT [18]. Microorganisms ($1 \cdot 10^8$ colony forming units [CFU]/mL) were injected into the kidney pelvis and incubated for 15 min, which corresponded to the average duration of crushing a single stone in the kidney pelvis in humans. Photoditazine was injected into the pelvis and irradiated for 10 min with an output power of 300 mW. The kidney was then removed, and washes were taken from the inner surface of the pelvis. The number of grown colonies was counted 24 h after seeding. The efficiency of PDT was estimated by the value of logarithmic reduction of CFU, where 1, 2, 3, 4, 5, and 7 represent 90%, 99%, 99.9%, 99.99%, 99.999%, and 99.9999% of dead microorganisms, respectively.

Biochemical blood analysis

Blood was collected from the vein located on the outer surface of the auricle of the animals before surgery and on the day of withdrawal from the study. The collection was performed using vacuum tubes containing ethylenediaminetetraacetic acid and vacuum tubes containing a coagulation activator. Biochemical blood tests, including urea, creatinine, and C-reactive protein concentrations, were performed using photometric tests on an automatic biochemical analyzer HumaStar 600 (Human, Germany). Blood samples were analyzed for cystatin C using the immunoturbodimetric method. Cystatin C is a reliable and sensitive marker of the glomerular filtration rate (GFR) and a cysteine protease inhibitor that is independent of the patient's sex and age characteristics [19]. General blood analysis was performed using a Mindray 6800 analyzer (Mindray, China).

Histological examination

After the animals were removed from the study, their kidneys were collected in a buffered formalin solution. The samples were washed after 48 h, and the sections of the pelvis were dissected. The preparations were fixed in 10% formalin solution, embedded in paraffin blocks, and 5 μm thick slices were prepared using a Leica CM 2000R microtome (Germany). Then, the slices were stained with hematoxylin and eosin using the standard method and studied light-optically (Leica DM 1000, ×40–400) with photofixation of images (Leica DFC290).

Statistical analysis

The data obtained were subjected to statistical analysis using SPSS Statistica version 26. The normality of the parameter samples was determined using the Kolmogorov–Smirnov test. For samples that did not meet the normality criterion, their general characteristics are presented as the quartile range, i. e., *Me* [Q_1; Q_3], where *Me* is the median (50%), Q_1 is 25%, and Q_3 is 75%. The standard deviation of σf% percentages was calculated using the following formula:

$$\sigma_f\% = \sqrt{f \cdot (1-f) / n},$$

where f is the percentage and n is the total number of sample items.

RESULTS

Observational data on the microflora of urology clinic patients

The analysis showed that NL was the most common pathology among patients treated, with percentages of 37.2% (1351/3626) in 2010, 41.7% (1293/3101) in 2013, 43.2% (1435/3318) in 2016, and 43.3% (1458/3363) in 2019. This represents an increase of 5.8% over 10 years. Most patients admitted had stones in the ureters (N20.1 [57.9%]), followed by the kidneys (N20.0 [22.1%]), and both kidneys and ureters (N20.2 [20%]). The mean age was 47.0 ± 0.8 years for men and 53.1 ± 0.9 years for women. Most patients received surgical treatment at the clinic, with surgical activity ranging from 77% to 80% over the 5-year interval, specifically 77.2% in 2021

(2509/3248). Among patients with NL, 52.5% ± 1.3% were treated surgically and 46.5% ± 1.3% were treated conservatively. Endoscopic procedures were performed in 35.2%, shock wave lithotripsy in 14.4%, and open surgery in 3.9% of the cases.

In the last decade, urologists have increasingly committed to urinary drainage after surgery, resulting in a clear prevalence of two-stage interventions. Consequently, in 2019, 95.1% (467/491) of the patients underwent endoscopic surgeries for NL and had various drains (such as nephrostomies, stents, ureteral and urethral catheters, and cystostomies) placed, which can contribute to hospital infections.

Table 1 presents the results of a 10-year observational analysis of the urine microflora of patients in the urology clinic. Data are presented as relative indices, indicating the detection rate per 1000 urine samples. This study traced the long-term dynamics of the microbial landscape of patients' urine, considering HCAIs and other infections. In addition, washes collected from environmental surfaces in the urological clinic were analyzed for 2010–2019. The most commonly found bacteria in urine samples were *Escherichia coli, Enterobacter cloacae*, and *Staphylococcus epidermidis*. *Enterococcus faecalis, Staphylococcus saprophyticus, Acinetobacter baumannii*, and *Klebsiella pneumoniae* were also widely distributed. The data obtained were consistent with the results of the analysis of microorganisms found on the working surfaces of the urological clinic. Over 10 years, the abundance of *Escherichia, Enterobacter*, and *Enterococcus* genera increased. In a previous study, multidrug resistance was found in most microorganisms isolated from stones of patients with NL [18]. The constant flow of patients passing through a urologic hospital and the use of endoscopic manipulations and surgeries, which are frequently accompanied by drainage of the urinary system, can lead to microbial contamination of the premises in a urologic clinic.

The factors listed above became predictors in experimental animal studies on the use of antimicrobial PDT in urology to neutralize antibiotic-resistant strains of microorganisms.

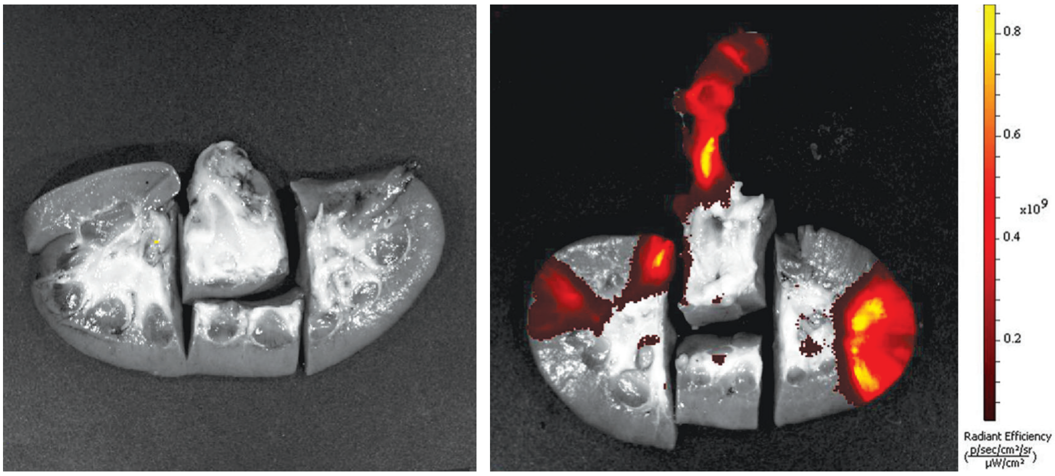
Safety assessment of antimicrobial PDT in an animal model

The pig renal pelvis volume was measured by injecting fluid into it while clamping the pelvic–ureter segment. The normal volume was 1.1 ± 0.1 cm^3. The accumulation of photoditazine by kidney tissues was evaluated, and no photosensitizer was found in the area of pelvic fluorescence (Fig. 1). The fluorescence signal detected in the ureter and on the lateral kidney surfaces was likely caused by the incomplete removal of the photoditazine solution from the organ during sample preparation. The histological study revealed that the normal pelvis was lined with epithelium consisting of 5–6 layers of cells with smooth contours of the outer layer. Underneath, loose connective tissue and some thin-walled vessels were observed. No changes in the state of the lining epithelium were observed after exposure to the photosensitizer (Fig. 2).

Then, the state of kidney tissues following PDT was evaluated. The results showed that irradiation of the pelvic cavity containing photoditazine with laser radiation at output powers of 150 and 300 mW resulted in only a focal increase in intercellular contacts of the surface

Table 1. Dynamics of changes in the species composition of microorganisms isolated from urine (detectability per 1000 samples)
Таблица 1. Динамика изменений видового состава микроорганизмов, выделенных из мочи (выявляемость на 1000 образцов)

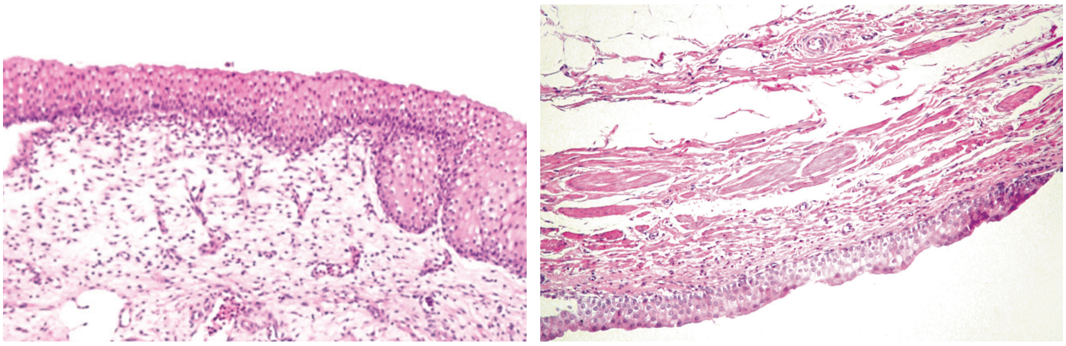
Name of the microorganism	2010	2013	2016	2019	Over 10 years, 50% [Q_1; Q_3]
Escherichia coli	102.8	77.4	116.9	137.9	105.2 [82.7; 123.8]
Enterobacter cloacae	49.3	70.9	99.4	95.7	75.2 [58.4; 95.3]
Staphylococcus epidermidis	96.9	33.8	70.2	62.1	62.8 [46.6; 75.8]
Enterococcus faecalis	40.7	20.6	64.4	89.5	43.2 [34; 63.44]
Staphylococcus saprophyticus	29.6	21.6	10.5	26.7	23.6 [11.8; 28.8]
Acinetobacter baumannii	16.1	9.6	0	5.9	15.5 [10; 17.1]
Klebsiella pneumoniae	11.5	16.7	0	8.3	13.9 [8.3; 17.9]
Pseudomonas aeruginosa	10.5	6.1	8.4	9.2	8.2 [7.1; 9.4]
Candida spp.	9.5	3.2	7.5	11.0	6.3 [4; 9]
Staphylococcus aureus	2.6	2.9	6.9	10.4	3.4 [3; 6.5]
Proteus mirabilis	6.9	3.2	4.5	0	3.7 [2; 4.7]
Citrobacter freundii	3.3	1.9	0.6	0	1.7 [0.1; 1.9]



a *b*

Fig. 1. Longitudinal section on the cavity of the pelvis of the control kidney (*a*) and the kidney after 10 minutes of exposure to photoditazine solution (*b*). Images are macrophotographs with superimposed fluorescence signal

Рис. 1. Продольный разрез на полость лоханки контрольной почки (*a*) и почки после 10-минутной экспозиции раствора фотодитазина (*b*). Изображения представляют собой макрофотографии с наложенным сигналом флуоресценции



a *b*

Fig. 2. Assessment of the condition of the control kidney's pelvis tissues (*a*) and after 10 minutes of exposure to a solution of photoditazin 10 % with Triton X-100, 5 vol.% (*b*). Stained with hematoxylin and eosin. ×100 magnification

Рис. 2. Оценка состояния тканей лоханок контрольной почки (*a*) и после 10-минутной экспозиции раствора фотодитазина 10 % с Triton X-100, 5 об.% (*b*). Окраска гематоксилином и эозином. Увел. ×100

layer of the urothelium when exposed to 150 mW in pulse mode (Fig. 3 *a*). When using the continuous mode with the same power, loosening and desquamation of the surface layer of cells occur, along with an increase in intercellular contacts of the surface layer of the urothelium (Fig. 3, *b*). Upon exposure to 300 mW in the pulse mode, the contours of the urothelium surface cells were damaged, and single small foci of desquamation were observed (Fig. 3, *c*). Continuous exposure with a power of 300 mW resulted in the formation of areas of pronounced loosening of cells because of the destruction of intercellular contacts and multiple merging areas of destruction of the outer rows of the covering urothelium. In some places, the thickness of the preserved rows is 2–3 cells. Thus, the superficial layer of the mucous membrane was determined to have incurred insignificant damage under the influence of 300 mW, whereas the underlying tissue remained unaffected (Fig. 3 *d*).

Determination of the temperature regimen for photodynamic irradiation

During the study, the researchers measured the temperature of the photoditazine solution in the pelvic cavity while being irradiated by a laser in the continuous mode with a power of 300 mW for 20 min. The results showed no increase in the temperature in the pelvis, regardless of whether irrigation was present or not. Irrigation was performed through the nephrostomy at an intra-channel pressure of no more than 30 cm of water.

Determination of microflora in the pelvic contents after photodynamic irradiation

A suspension of daily test cultures ($1 \cdot 10^8$ CFU/mL) was injected into the pig kidney pelvis, and PDT was performed after that. Washes from the inner surface of the kidney pelvis were sown on meat-peptone agar,

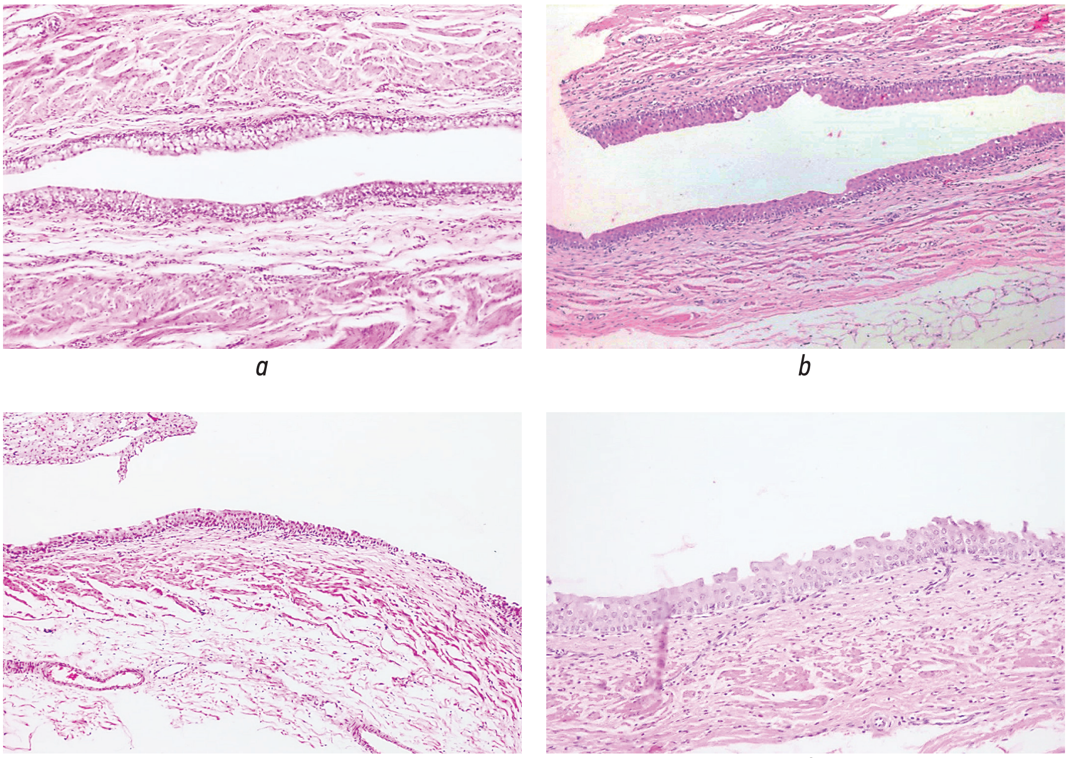


Fig. 3. Histological preparations: *a* — power density 150 mW/cm^2, pulse mode; *b* — power density 150 mW/cm^2, continuous mode; *c* — power density 300 mW/cm^2, pulse mode; *d* — power density 300 mW/cm^2, continuous mode; staining — hematoxylin and eosin, ×100 magnification

Рис. 3. Гистологические препараты: *a* — плотность мощности 150 мВт/см^2, импульсный режим; *b* — плотность мощности 150 мВт/см^2, непрерывный режим; *c* — плотность мощности 300 мВт/см^2, импульсный режим; *d* — плотность мощности 300 мВт/см^2, непрерывный режим; окраска гематоксилином и эозином, увел. ×100

Table 2. Blood parameters of pigs after photodynamic therapy (abbreviated version)

Таблица 2. Показатели крови свиней после фотодинамической терапии (сокращенный вариант)

Blood count	Reference	Pig 1		Pig 2	
		Day 0	Day 1	Day 0	Day 3
Creatinine, μmol/L	69.60–207.70	115.20	221.30	138.50	139.2
Urea, mmol/L	3.70–6.40	2.05	7.69	4.13	3.79
Leukocytes, 10^9/L	11.00–22.00	32.1	30.40	17.30	20.40
ESR, mm/h	2.00–9.00	8.0	3.0	2.0	34.00
Cystatin C (mg/L)	–*	0.44	0.37(↓)	0.52	0.37(↓)

*No reference value has been established for cystatin C levels in pigs. For humans, the reference range is 0.61–0.95 mg/L.

and PDT was found to kill 99.9% of *E. coli* and 99% of *S. aureus*, indicating the technique's effective bactericidal activity.

Assessment of the renal status

Biochemical blood analysis conducted 1 day after surgery revealed slight increases in creatinine and urea levels, which exceeded the acceptable reference interval (Table 2). No adverse effect on GFR was observed, and the cystatin C level decreased postoperatively.

DISCUSSION

A retrospective analysis of microbiological urine cultures from patients at the urology clinic between 2010 and 2019 revealed an increase in the number of patients with positive urine cultures. *E. coli, E. cloacae, S. epidermidis, E. faecalis*, and *K. pneumoniae* are most frequently present in urine. These microorganisms were also isolated from the urinary stones of patients in a previous study [18]. In addition, most isolated microorganisms exhibited resistance to multiple drugs, confirming a global trend [20]. Preoperative antibiotic therapy is ineffective against resistant microorganism strains. Furthermore, during surgery, these microorganisms can spread from the stone when it is crushed using instruments. This finding is supported by the similarity in the species composition of microorganisms found in urine and on the working surfaces of urologic hospitals. The specificity of urological operations that require urinary tract drainage and the presence of human factors may serve as additional sources of antibiotic-resistant strains. PDT may serve as an alternative to antibiotic therapy. Unlike antibiotics, PDT targets multiple areas within the bacterial cell and eliminates the possibility of resistance development [21].

Previous studies have shown the effectiveness of antibacterial PDT against various microorganisms. The research team optimized the PDT procedure for Gram-negative uropathogenic microorganisms and tested it on infected urine from patients [18]. This study comprehensively examined the safety of applying the developed technique to animals. Cells of the epithelium lining the renal pelvic ducts do not accumulate photoditazine after a 10-min exposure to the photosensitizer. The results suggest no significant tissue damage during photodynamic action on the renal pelvic tissue. The peculiar structure of the urothelium provides a barrier function that prevents the penetration of various substances, ions, and water into the kidney tissue [22]. The temperature change during PDT was evaluated because of the high-power densities of laser radiation used in the developed technique. The photosensitizer solution administered to the renal pelvis was not heated, which ruled out thermal damage to the organ tissues. A histological examination was conducted to evaluate the condition of the renal pelvic tissues after PDT. The lack of significant damage to the kidney pelvis epithelium in animals subjected to different irradiation regimes is likely due to the absence of photosensitizer in the epithelial cells.

A biochemical blood analysis was conducted to evaluate the functional state of the kidneys and the organism as a whole. Determining GFR values is widely accepted for diagnosing and monitoring renal dysfunction [23, 24]. Cystatin C is produced by all nuclear cells in the body at a constant rate. It is freely filtered into the renal tubules, almost completely reabsorbed, and destroyed in the renal tubules. Therefore, an increase in its serum level indicates a decrease in GFR and may signal the preclinical phase of renal dysfunction [23]. In this study, intraoperative PDT did not affect the renal GFR of the animal, and the cystatin C level decreased compared with the initial level.

Thus, this study demonstrates the safety of antibacterial PDT and the possibility of complementing traditional antibiotic therapy in the surgical treatment of NL. An advantage of using this technique is that the simultaneous intraoperative therapeutic action may allow for an antimicrobial effect in a very short time.

ADDITIONAL INFORMATION

Authors' contribution. Thereby, all authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study. Personal contribution of each authors: O.S. Streltsova — concept development, collection of biological material, analysis of the obtained data, writing the text of the manuscript; A.E. Antonyan — collection of biological material, analysis of the obtained data, writing the text of the manuscript; V.V. Elagin — concept development, analysis of the data obtained, editing the manuscript text; N.I. Ignatova — development of the concept, analysis of the data obtained, editing the manuscript text; K.E. Yunusova — preparation and analysis of histological material; T.R. Zhilyaeva-Fomina — preparation and analysis of material on epidemiological observational study; V.F. Lazukin — statistical processing of the material; V.A. Kamensky — general supervision of the study.

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