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BACTERIAL CONTAMINATION OF THE INTACT KIDNEY IN COURSE OF THE ACUTE UNILATERAL KIDNEY INFLAMMATION IN RATS: DIFFERENT TIME FRAMES AFTER NEPHRECTOMY ON THE AFFECTED SIDE

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Summary. *Exceptionally clinically important is clear understanding of the pathophysiological mechanisms for localization and migration of pathologic microorganisms towards the intact kidney in course of the unilateral inflammatory process. The study aimed to determine the intact kidney's pathogenic species composition and numerosity in course of rat acute unilateral kidney inflammation, different time frames after the affected organ removal. The experiment was carried out on 54 mature nonlinear white rats Rattus Norwegicus. In all animals an acute unilateral kidney inflammation has been modeled. For modeling of the acute unilateral kidney inflammation in experimental rats, an* E.Coli *strain was used. The pathogen was previously isolated from urine in patients with the diagnosed urinary tract infection. In order to determine the affected kidney influence on the intact organ microbial contamination, nephrectomy on the affected side was performed on the 10th, 14th and 21st days. Bacterial concentrations in the residual kidney tissue were determined 4, 7 and 14 days after nephrectomy. Control group constituted animals without nephrectomy. Scores were compared to the respective ones of the intact kidney in control group, on the same day of experiment. Reliable changes of almost all microbiologic contamination scores were detected after removal of the affected organ. Infection of the inoculated kidney spread onto intact contralateral organ by the hematogenous and/or lymphogenous routes and persisted there for some specific time. The nature of the infection in the contralateral kidney changed between the 10th and 14th days after inoculation – the agent apparently penetrated the kidney tissue and developed inflammation. Removal of the inoculated kidney timely, before the infection of the intact organ has progressed to its invasive stage, facilitated complete elimination of the pathogen in the contralateral kidney by the 28th day.*

Key words: *kidney inflammation, pyelonephritis, contralateral kidney, intact kidney, nephrectomy, bacterial contamination,* Escherichia Coli.

Introduction. Acute kidney inflammation constitutes presence of pathogenic or opportunistic flora in the kidney tissue as its perquisite [3, 7, 11]. Therefore, studying qualitative and quantitative composition of microorganisms causing the disease is of primary importance in determining the treatment strategy [2, 4, 13]. According to the global data, population-specific composition of etiologic microorganisms is constantly changing [1]. Differences in etiologic microflora could be found in different parts of the world too [12]. Moreover, the composition of microflora in acute kidney inflammation may vary even within the same country [1, 12]. Nowadays of high interest are special features of adaptation and behavior of pathogenic and opportunistic flora in a single organism [8, 10]. Especially clinically important is clear understanding of the pathophysiological mechanisms of localization and migration of microorganisms

towards the intact kidney (in case of unilateral process) and other organs, as well as conditions necessary for that [15]. Formation of microbial associates under certain conditions is ambiguous too [9]. Not clear remains the identification of the most mutually affine microorganisms and impact of surgical trauma on the opportunistic flora migration towards the inflammation site [6].

Objectives. The study aimed to determine the intact kidney's pathogenic species composition and numerosity in course of a rat acute unilateral kidney inflammation, different time frames after the affected organ removal.

Materials and Methods. The experiment was carried out on 42 mature nonlinear white rats Rattus Norwegicus. All of those were male, aged 18-20 weeks, of 180-205g weight. In all animals an acute unilateral kidney inflammation has been modeled. The core group consisted of 24 rats; it has been divided into three subgroups: I (n=12) – a nephrectomy on the affected side was performed on the 10th day of the experiment; II (n=8) – a nephrectomy on the affected side was performed on the 14th day; III (n=4) – a nephrectomy on the affected side was performed on the 21st day. Bacterial contamination of the residual kidney was determined using routine methods on the 14th (Ia, n=4), 21st (Ib, n=4; IIa, n=4) and 28th days (Ic, n=4; IIb, n=4; III, n=4). The control group consisted of 18 animals, no nephrectomy was made. The intact kidney bacterial contamination was studied on the 14th (C_1, n=6), 21st (C_2, n=6) and 28th day (C_3, n=6). Routine statistical methods were used for calculation purposes.

For modeling of the acute unilateral kidney inflammation in experimental rats, an *E. Coli* strain was used. The pathogen was previously isolated from urine in patients with the diagnosed urinary tract infection. The strain had a number of features letting him be easily distinguished from other *E. Coli* strains (autologous) which could get into the research material (kidney tissue) from the intestine of animals by the hematogenous, lymphogenous or ascending routes [5, 9]. The strain we used was lactose negative and had the ability to grow on Simmons' medium. Based on the combination of other biochemical tests it was confidently identified as *E.Coli* [14]. A 4.05-6.55 • 10^7 per 1 ml colony-forming units (CFU) suspension was prepared. 0.1 ml per 100g rat mass was injected in kidney parenchyma unilaterally.

Results and discussion. According to the microbiologic data, injection of the *E.Coli* suspension in experimental animals' renal parenchyma created infectious process not only in the inoculated kidney, but in contralateral organ too. This happens obviously due to the spread of the inoculated pathogen onto healthy kidney by the hematogenous and (or) lymphogenous routes.

Removal of the affected organ 10 days after inoculation resulted in a slight decrease in the concentration of the pathogen in the intact kidney on the 14th day (4 days after surgery) comparing with non-operated animals (Table 1). On the 21st day (11 days after surgery) the difference between operated and non-operated animals has grown, whereas on the 28th day (14 days after surgery) almost no bacteria were isolated from the intact kidney. The nephrectomy resulted in the elimination of infection of the contralateral kidney. This is quite an unexpected result, as large quantities of the introduced *E.Coli* strain were identified in the contralateral kidney tissue in non-operated rats at all days of experiment. As a possible explanation for this we suppose a difference in the nature of infectious process in the inoculated and intact kidneys.

Most likely it is the microtrauma that causes immediate development of an inflammatory process in the inoculated kidney after injection of the etiologic strain. Other explanations may include: local immunity of the intact kidney, lower tissue concentrations of the pathogenic bacteria, or gradual insemination of the contralateral organ. For some reason an infectious agent persists in the intact kidney without evoking definite signs of inflammation. Only evaluation of the histological data may confirm or reject this assumption.

After the removal of the inoculated kidney on a later term – 14 days after infection was injected – the release rate of *E.Coli* strain from the contralateral kidney has decreased. 7 days after surgery (day 21) amount of pathogen in the contralateral kidney was just slightly lower compared to the same period in non-operated animals (Table 2). 14 days after nephrectomy (28th day) the quantitate data of identified bacteria in the contralateral kidney of experimental animals showed lowering, but the variety of pathogenic strains remained similar to that of non-operated animals. Finally, the group of animals operated on the 21st day, showed seven days after surgery almost no difference to the kidney parenchymal contamination scores of those without nephrectomy (Table 2).

This suggests that the nature of infection in the contralateral kidney changed over time. At the beginning, pathogen persisted in the kidney (possibly in the urine, not

Table 1.

Intact kidney tissue bacterial concentrations isolated from control group (no nephrectomy) and animals receiving nephrectomy on the affected side on the 10th day (residual kidney), lg CFU (x ± Sx)

Isolated bacteria	14th day		21st day		28th day	
	Control, C_1 (n=6)	Residual, Ia (n=4)	Control, C_2 (n=6)	Residual, Ib (n=4)	Control, C_3 (n=6)	Residual, Ic (n=4)
E. Coli (inoculated strain)	3.75±0.38	3.28±0.34 p<0.05	3.58±0.38	1.78±1.22 p<0.01	3.74±0.46	n.i. p<0.001
E. Coli (autologous strain)	2.70±2.10	2.31±1.59	2.81±2.21	1.50±1.73	2.60±2.07	0.58±1.17
E. Faecalis	2.01±2.24	1.65±1.91	1.72±1.90	0.65±1.30	1.70±1.96	n.i.
P. Mirabilis	n.i.	n.i.	n.i.	n.i.	1.58±1.79	n.i.

Note: *p* value specified where applicable; n.i. – not isolated.

Table 2.

Intact kidney tissue bacterial concentrations isolated from control group (no nephrectomy) and animals receiving nephrectomy on the affected side on the 14th and 21st days (residual kidney), lg CFU (x ± Sx)

Isolated bacteria	Nephrectomy on the 14th day				Nephrectomy on the 21st day	
	21st day		28th day		28th day	
	Control, C2 (n=6)	Residual, IIa (n=4)	Control, C3 (n=6)	Residual, IIb (n=4)	Control, C3 (n=6)	Residual,IIIc (n=4)
E. Coli (inoculated strain)	3.58±0.38	3.30±0.35	3.74±0.46	1.47±1.70 p<0.01	3.74±0.46	3.64±0.39
E. Coli (autologous strain)	2.81±2.21	2.22±1.49	2.60±2.07	1.42±1.64	2.60±2.07	2.69±1.85
E. Faecalis	1.72±1.90	1.48±1.71	1.70±1.96	0.53±1.06	1.70±1.96	1.65±1.92
P. Mirabilis	n.i.	n.i.	1.58±1.79	0.98±1.95	1.58±1.79	1.87±2.18

Note: *p* value specified where applicable; n.i. – not isolated.

in the parenchyma itself) without multiplying or causing any inflammation. Thus it was easily eliminated from the body after the permanent source of infection removal – inoculated kidney. Over time, a penetration of the pathogen (invasion) in the renal tissue occured, and the inflammation developed.

In this case, the pathogen in the contralateral kidney was not eliminated even after nephrectomy on the affected side. Therefore, a critical milestone for the transition of persistent infection into the invasive stage was period between 10th and 14th days after inoculation of bacteria in rats.

Conclusions

1. Infection of the inoculated kidney spread onto intact contralateral organ by the hematogenous and/or lymphogenous routes and persisted there for some specific time.
2. The nature of the infection in the contralateral kidney changed between the 10th and 14th days after inoculation – the agent apparently penetrated the kidney tissue and developed inflammation.
3. Removal of the inoculated kidney timely, before the infection of the intact organ has progressed to its invasive stage, facilitated complete elimination of the pathogen in the contralateral kidney by the 28th day.

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КОНТАМІНАЦІЯ КОНТРЛАТЕРАЛЬНОЇ НИРКИ В РІЗНІ ТЕРМІНИ ПІСЛЯ ВИДАЛЕННЯ УРАЖЕНОГО ОРГАНА ЗА ГОСТРОГО ОДНОБІЧНОГО ЗАПАЛЕННЯ НИРОК У ЩУРІВ

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Резюме. Виняткове клінічне значення має чітке розуміння патофізіологічних механізмів локалізації (нерозповсюдження) та міграції мікроорганізмів в інтактну нирку за умов однобічного запального процесу. Дослідження переслідувало мету визначити видовий популяційний склад та кількісні характеристики патологічної мікробіоти в тканині інтактної нирки за однобічного запального процесу нирок у різні терміни після видалення ураженого органа. Експеримент здійснено на 54 статевозрілих нелінійних білих щурах Rattus Norwegicus. Всім тваринам моделювали гострий однобічний запальний процес нирок. Для моделювання запального процесу в нирках дослідних щурів був використаний штам *E.Coli*, виділений з сечі хворої з діагностованою інфекцією сечової системи. З метою визначення ступеню впливу ураженої нирки на мікробну контамінацію інтакного органа, проводили нефректомію з ураженого боку на 10-ту, 14-ту та 21-шу добу експерименту. Концентрації збудників у тканині резидуальної нирки визначали через 4, 7 та 14 діб після видалення ураженого органа. Тваринам контрольної групи також моделювали гостре однобічне запалення нирок, проте нефректомію з ураженої сторони не проводили. Показники порівнювали з аналогічними в інтактної нирки на таку ж добу експерименту у щурів контрольної групи. Визначено, що після видалення ураженої нирки у всіх підгруп тварин змінювалися практично всі показники контамінованості контрлатерального органа по відношенню до таких, де нефректомія не проводилася. Інфекція з інокульованої нирки гематогенним або/і лімфогенним шляхами потрапляла у інтактний контрлатеральний орган і певний час персистувала в ньому. Між 10-ю і 14-ю добою після інокуляції характер інфекційного процесу у контрлатеральному органі змінювався – збудник проникав у тканини нирки спричиняв запальний процес в останній. Видалення інокульованої нирки до переходу інфекційного процесу у контрлатеральній нирці в інвазивну стадію сприяло повній елімінації збудника з контрлатерального органу.

Ключові слова: запалення нирок, пієлонефрит, контрлатеральна нирка, інтактна нирка, нефректомія, контамінація, *Escherichia Coli.*

КОНТАМИНАЦИЯ КОНТРАЛАТЕРАЛЬНОЙ ПОЧКИ В РАЗНЫЕ ТЕРМИНЫ ПОСЛЕ УДАЛЕНИЯ ПОРАЖЕННОГО ОРГАНА ПРИ ОСТРОМ ОДНОСТОРОННЕМ ВОСПАЛЕНИИ ПОЧЕК У КРЫС

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Резюме. Исключительное клиническое значение имеет четкое понимание патофизиологических механизмов локализации (нераспространении) и миграции микроорганизмов в интактную почку в условиях одностороннего воспалительного процесса. Исследование преследовало цель определить видовой популяционный состав и количественные характеристики патологической микробиоты в ткани интактной почки при одностороннем воспалительном процессе почек в разные сроки после удаления пораженного органа. Эксперимент проводился на 54 половозрелых нелинейных белых крысах Rattus Norwegicus. Всем животным моделировали острый односторонний воспалительный процесс почек. Для моделирования воспалительного процесса в почках исследуемых крыс был использован штамм *E.Coli*, выделенный из мочи больного с диагностированной инфекцией мочевой системы. С целью определения степени влияния пораженной почки на контаминацию контралатерального органа, проводили нефрэктомию с пораженной стороны на 10-е, 14-е и 21-е сутки эксперимента. Концентрации возбудителей оставшейся почки определяли через 4, 7 и 14 суток после удаления пораженного органа. Животным контрольной группы также моделировали острое одностороннее воспаление почек, однако нефрэктомию с пораженной стороны не проводили. Показатели сравнивали с аналогичными в интактной почке в те же сутки эксперимента у крыс контрольной группы. Определено, что после удаления пораженной почки у всех подгрупп животных изменялись практически все показатели контаминации контралатерального органа по отношению к таким, где нефрэктомия не проводилась. Инфекция из инокулированной почки гематогенным и/или лимфогенным путем попадала в интактный контралатеральный орган и некоторое время в нем персистировала. Между 10-ми и 14-ми сутками после инокуляции характер инфекционного процесса в контралатеральном органе менялся – возбудитель, по-видимому, проникал в ткани почки и вызывал воспалительный процесс в органе. Раннее удаление пораженного органа, до перехода инфекционного процесса в контралатеральной почке в инвазивную стадию, способствовало полной элиминации возбудителя из контралатерального органа.

Ключевые слова: воспаление почек, пиелонефрит, контралатеральная почка, интактная почка, нефрэктомия, контаминация, *Escherichia Coli.*