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Ultrastructural Changes in the Glomerular Filtration Barrier of the Kidneys in Rats Following Acute Paraoxon Poisoning

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ABSTRACT

BACKGROUND: Paraoxon is an organophosphate compound, chronic poisoning by which has various manifestations. Individuals exposed to organophosphates often develop glomerular and/or tubular sclerosis. Ultrastructural changes in the glomerular filtration barrier (GFB) of the kidneys in experimentally induced acute paraoxon poisoning have not been described in available scientific publications, underscoring the relevance of this study.

AIM: This study aimed to identify ultrastructural changes in the glomerular filtration barrier of the kidneys in rats following acute paraoxon poisoning in sublethal doses.

METHODS: Kidney fragments were obtained from male outbred albino rats (*Rattus norvegicus*) 1, 3, and 7 days after paraoxon poisoning. Three modes of paraoxon exposure were used. Immunohistochemical, electron microscopy, and morphometric analyses were performed on the kidney tissue, followed by statistical analysis of the results.

RESULTS: Ultrastructural changes in the GFB were observed in all modes of paraoxon exposure. Endothelial cells of glomerular capillaries showed an increased diameter of fenestrations, accompanied by a decrease in their density. Podocytes exhibited changes in the size of the foot processes and a reduction in the number of third-order extensions that normally adhere to the glomerular basement membrane. All three experimental groups showed an increase in the thickness of the glomerular basement membrane on day 7 after poisoning. The most pronounced morphological changes in GBF structures following paraoxon exposure were observed in the endothelial cells of the glomerular capillaries.

CONCLUSION: Acute paraoxon poisoning leads to ultrastructural changes in the GFB in rat kidneys. The data obtained contribute to understanding the mechanisms of glomerulosclerosis development following chronic exposure to this toxin.

Keywords: transmission electron microscopy; paraoxon; nephrotoxicity; glomerular filtration barrier; morphometry.

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Ультраструктурные изменения гломерулярного фильтрационного барьера почек у крыс при остром отравлении параоксоном

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АННОТАЦИЯ

Обоснование. Параоксон — фосфорорганическое соединение, хроническое отравление которым имеет различные проявления. У лиц, контактирующих с фосфорорганическими соединениями, нередко выявляются гломерулярный или тубулярный склероз, а также их сочетание. Описание ультраструктурных изменений гломерулярного фильтрационного барьера (ГФБ) почек при экспериментальном моделировании острого отравления параоксоном на данный момент в доступной литературе отсутствует, что подчёркивает актуальность данного исследования.

Цель — выявить ультраструктурные изменения гломерулярного фильтрационного барьера почек у крыс при остром отравлении сублетальными дозами параоксона.

Методы. Фрагменты почек получены от самцов белых беспородных крыс *Rattus norvegicus* спустя 1, 3 и 7 суток после отравления параоксоном. В работе использовали три режима воздействия параоксона. Проведены иммуногистохимическое, электронно-микроскопическое и морфометрическое исследования почек, а также статистический анализ результатов.

Результаты. Ультраструктурные изменения ГФБ почек наблюдаются во всех режимах воздействия параоксона. В эндотелиальных клетках клубочковых капилляров почечных телец увеличивается диаметр фенестр при одновременном уменьшении частоты их расположения. В подоцитах обнаружено изменение размеров подошвенной части цитоподий и сокращение количества отростков III порядка, примыкающих к гломерулярной базальной мембране. У животных всех трёх опытных групп увеличивается толщина гломерулярной базальной мембраны на 7 сутки после острого отравления. Наиболее выраженные морфологические изменения структур ГФБ после воздействия параоксона наблюдали в эндотелиальных клетках клубочковых капилляров.

Заключение. Острое отравление параоксоном вызывает ультраструктурные изменения ГФБ почек у крыс. Полученные данные способствуют пониманию механизмов развития гломерулосклероза при хроническом воздействии токсиканта.

Ключевые слова: трансмиссионная электронная микроскопия; параоксон; нефротоксичность; гломерулярный фильтрационный барьер почек; морфометрия.

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大鼠急性对氧磷中毒时肾小球滤过屏障的超微结构变化

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摘要

论证。对氧磷是一种有机磷化合物，其慢性中毒表现多样。与有机磷化合物接触的人群中常发现肾小球或肾小管硬化，或两者兼有。目前在现有文献中尚无关于实验性急性对氧磷中毒模型下肾小球滤过屏障 (glomerular filtration barrier, GFB) 超微结构变化的描述，这突显了本研究的现实意义。

目的。揭示大鼠在亚致死剂量的急性对氧磷中毒情况下肾小球滤过屏障的超微结构变化。

方法。在对氧磷中毒后第1、3和7天获取无品系白化雄性大鼠 (*Rattus norvegicus*) 肾脏标本。实验中采用了三种对氧磷暴露方式。对肾脏组织进行免疫组化、透射电子显微镜和形态计量学研究，并进行了统计学分析。

结果。在所有对氧磷暴露方式下均观察到GBF的超微结构改变。在肾小球毛细血管内皮细胞中，孔径直径增加，而孔的分布频率降低。足细胞表现为足突基底部尺寸的改变，以及与肾小球基底膜相邻的三级突起数量减少。在所有三组实验动物中，中毒后第7天肾小球基底膜厚度增加。在对氧磷作用后，GFB结构中最显著的形态学变化出现在肾小球毛细血管的内皮细胞中。

结论。急性对氧磷中毒会引起大鼠GFB的超微结构改变。这些数据有助于理解慢性毒物暴露条件下肾小球硬化的发生机制。

关键词：透射电子显微镜；对氧磷；肾毒性；肾小球滤过屏障；形态计量学。

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BACKGROUND

A number of studies have addressed the nephrotoxicity of organophosphorus compounds (OPs), including investigations of chronic kidney disease of unknown etiology in humans and data obtained from experimental animals [1–3]. However, the results of these studies provide only indirect evidence of the role of OPs in the development of renal condition. Histological analyses of renal tissue under acute and chronic exposure to OPs have revealed tubular epithelial injury, interstitial fibrosis, and pronounced glomerulosclerosis associated with mild to moderate damage of tubular epithelial cells (TECs) in the proximal and distal tubules of the nephron [4–6]. OPs are widely used worldwide, including in agricultural applications, primarily as insecticides and acaricides. Noncompliance with safety procedures when handling this class of compounds allows OPs to penetrate the human body through the skin and mucous membranes [7]. Parathion is classified by the World Health Organization as an extremely hazardous organic pollutant of the highest toxicity class [8]. Exposure to parathion results in irreversible inhibition of acetylcholinesterase in the synaptic cleft. The subsequent excessive stimulation of muscarinic and nicotinic cholinergic receptors is the primary mechanism of toxicity for most OPs. Parathion metabolites, one of which is paraoxon [8], are excreted from the body via the kidneys.

The structure responsible for plasma filtration is the glomerular filtration barrier (GFB). It consists of fenestrated endothelial cells, podocytes, and the glomerular basement membrane (GBM) [9, 10]. Podocytes are epithelial cells with branched foot processes of various orders [9, 10]. The components of the GFB are functionally interconnected through cross-talk between the VEGFA–eNOS and NO–ET-1 signaling pathways, which ensure coordinated function of all GFB elements [11, 12]. A tubuloglomerular feedback mechanism has also been described in the nephron, linking distal TEC and the macula densa [13]. For effective filtration and maintenance of selective GFB permeability, the fenestrations of endothelial cells are covered with a glycocalyx, and the tertiary foot processes of podocytes are interconnected by slit diaphragms, whose main structural protein is nephrin. The selective permeability of the GBM depends on the molecular size of the filtered particles [14, 15]. Ultrastructural analysis of the GBM in mice following administration of gold-labeled albumin demonstrated that nanoparticles of different molecular weights show variable abilities to traverse the dense lamina of the GBM. Only particles with molecular weight smaller than 66 kDa pass through the GBM and accumulate adjacent to the podocyte foot processes [16].

Given the extremely small size of all components of the GFB, ultrastructural examination is essential for detecting alterations induced by acute or chronic exposure to OPs. Currently, the most vulnerable nephron component to OP-induced damage is considered to be the TEC population [4–6].

The choice of laboratory animal species is critical when modeling acute OP intoxication. OP exposure has been studied in rodents, primates, pigs, aquatic organisms, and representatives of the phylum *Platyhelminthes*, using various routes of administration, including intraperitoneal, intravenous, intramuscular, subcutaneous, dermal, inhalation, and oral [17–19]. Guinea pigs, nonhuman primates, and humans show low activity of plasma carboxylesterase, which metabolizes and inactivates OPs, thereby reducing the extent of their damaging effects on urinary tract cells [17]. Rats remain the most commonly used laboratory animals in toxicological studies involving OPs [17, 20, 21]. However, a key physiological feature of rats is their high carboxylesterase activity, which can stoichiometrically bind OPs entering the bloodstream [22]. To address this, several rat models of OP poisoning described in the scientific data include a step involving plasma carboxylesterase activity neutralization [23]. Such models involve the use of specific inhibitors, such as CBDP (2-(*o*-cresyl)-4H-1,3,2-benzodioxaphosphorin-2-oxide), or repeated administration of the tested OP to achieve neutralization of carboxylesterase activity, thereby partially aligning the obtained data with the effects of poisoning in humans.

Considering that the choice of an appropriate experimental model for studying acute and chronic OP toxicity requires careful attention to species-specific features of laboratory animals, and taking into account the importance of understanding ultrastructural changes in the GFB under acute toxic exposure, the present study is of particular relevance.

The work aimed to identify ultrastructural changes in the glomerular filtration barrier of the kidneys in rats following acute paraoxon poisoning in sublethal doses.

METHODS

Study Design

The study was based on biological material (rat kidney tissues) obtained from a prospective, controlled, randomized toxicological experiment.

Study Setting

Histological and ultrastructural examinations of rat kidneys were performed at the I.M. Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences and the S.M. Kirov Military Medical Academy.

Eligibility Criteria

The study included kidney samples from all surviving male rats weighing 250–370 g after acute exposure to a sublethal dose of paraoxon. The animals were housed in standard T2-type plastic cages at a temperature of +20–22 °C. They were provided with standard pellet feed Chara (Laboratorkorm, Russia) and had free access to drinking water.

Study Duration

Renal cortical tissue samples were obtained from animals following decapitation using a laboratory rodent guillotine (Open Science Research and Production Company, Russia) at 1, 3, and 7 days after paraoxon administration. The study was conducted from 2019 to 2023. Biological material from animals of the three poisoning models was collected sequentially, whereas the morphological examination was performed simultaneously.

Intervention

Kidney tissue samples were obtained from rats of control group and from three experimental groups of animals exposed to paraoxon toxicity: group M1 (poisoning model 1), single administration of paraoxon ($n = 15$); group M2 (poisoning model 2), double administration of paraoxon with a 1-hour interval ($n = 15$); group M3 (poisoning model 3), sequential administration of the carboxylesterase inhibitor CBDP and paraoxon with a 1-hour interval ($n = 15$). Animals of the control group received 1 mL of 0.9% NaCl. Paraoxon doses corresponded to the LD₅₀ values. All substances—paraoxon (Paraoxon-ethyl, D9286, Sigma-Aldrich, USA), CBDP (2-(*o*-cresyl)-4H-1,3,2-benzodioxaphosphorin-2-oxide, Research Institute of Hygiene, Occupational Pathology, and Human Ecology, Russia), and 0.9% NaCl—were administered subcutaneously. The groups of animals and administered doses are summarized in Table 1 and have been described previously [23]. Tissue samples were collected at 1, 3, and 7 days after paraoxon exposure. Immunohistochemical examination, transmission electron microscopy, and morphometric analysis were performed.

For immunohistochemical analysis, kidney cortex fragments were fixed in 10% neutral buffered formalin (pH 7.2–7.4), dehydrated, embedded in paraffin, and sectioned at a thickness of 5 μ m. Antigen retrieval was performed by heat-induced epitope unmasking in citrate buffer (pH 6.0). Primary antibodies against nephrin (rabbit polyclonal, 1:100 dilution; PRS2265-100UG, Sigma-Aldrich, USA) and collagen type IV (rabbit polyclonal, 1:100 dilution; SAB4500382, Sigma-Aldrich, USA) were applied. Deparaffinized sections were incubated with primary antibodies overnight at +4 °C in a humid chamber. Antigen visualization was performed using the Reveal Biotin-Free Polivalent DAB Detection System (Spring Bioscience Co., USA) according to the manufacturer’s instructions. Secondary antibodies conjugated with horseradish peroxidase were included in the detection kit.

For ultrastructural analysis, kidney cortex fragments were fixed in 2.5% glutaraldehyde in phosphate-buffered saline (pH 7.2–7.4), post-fixed in 2% osmium tetroxide (OsO₄) in the same buffer (pH 7.2–7.4), and embedded in Araldite resin (EMS, USA). Sections 80 nm thick were contrasted with lead citrate and 1% aqueous uranyl acetate (Serva, Germany). Electron micrographs were obtained using a Merlin transmission electron microscope (Zeiss, Germany) at magnifications of $\times 1,000$, $\times 5,000$, and $\times 8,000$.

Main Study Outcome

To identify ultrastructural changes in the GFB of rat kidneys following exposure to a sublethal dose of paraoxon, compared with the control group.

Group Analysis

No special criteria were applied for group allocation. Morphological analysis was performed on cortical kidney fragments obtained from animals in the three paraoxon poisoning models (M1, M2, and M3) described previously [23].

Paraoxon was administered subcutaneously at a dose corresponding to LD₅₀. Control animals received subcutaneous injections of 1 mL of 0.9% NaCl. The specific carboxylesterase inhibitor CBDP was also administered subcutaneously.

Outcomes Registration

Results of the immunohistochemical analysis were evaluated visually using a three-point semiquantitative scale and expressed as percentages. Morphometric assessment of GFB structures was performed according to the method described by Bgatova and Taskaeva [24] on electron micrographs of four renal corpuscles per animal. The following parameters were measured: the diameter and number of endothelial fenestrations in glomerular capillaries over a 2 μ m segment; the thickness of the GBM; the number and width of podocyte third-order processes along a 2 μ m GBM segment; and the height of TEC in proximal and distal tubules of the nephron.

Statistical Analysis

The sample size was not pre-calculated. The study included cortical kidney tissue fragments from all rats that survived exposure to sublethal doses of paraoxon across the three poisoning models.

Quantitative data were analyzed using GraphPad Prism 5.0 (GraphPad Software Inc., USA). The Kolmogorov–Smirnov test was used to verify the normal distribution of quantitative

Table 1. Modeling of acute paraoxon poisoning in sublethal doses in rats

Exposure	Control	Model 1	Model 2	Model 3
Carboxylesterase inhibition	–	–	Paraoxon, 110 μ g/kg	CBDP, 3.3 mg/kg
Administered compound	0.9% NaCl	Paraoxon, 250 μ g/kg	Paraoxon, 150 μ g/kg	Paraoxon, 150 μ g/kg

Note: CBDP, specific carboxylesterase inhibitor, 2-(*o*-cresyl)-4H-1,3,2-benzodioxaphosphorin-2-oxide.

variables. One-way ANOVA with Bonferroni correction was applied. Differences were considered significant at $p < 0.05$. Results are presented as $M \pm SD$, where M denotes the arithmetic mean and SD the standard deviation.

RESULTS

Study Objects

The survival rate of animals in all three paraoxon poisoning models was 50%, corresponding to the administered toxicant dose equal to LD_{50} .

Primary Results

In all experimental groups, one day after paraoxon administration, rats exhibited TEC alterations in the proximal tubules of the nephron, including microvilli damage, TEC desquamation, and the presence of cellular debris within the tubular lumen. By day 7 after intoxication, these changes were no longer observed. Renal corpuscles showed glomerular capillary loops with well-developed profiles and open capillary lumina, with no detectable debris within Bowman's capsule. At the ultrastructural level, changes in the components of the GFB were detected in rats across all experimental groups. Morphometric characteristics of GFB elements are summarized in Table 2.

The number of endothelial fenestrations decreased compared to controls in group M3 one day after intoxication and in groups M2 and M3 seven days after intoxication. At the same time, the diameter of fenestrations increased in group M1 at days 1 and 7 after paraoxon exposure and in group M2 on day 7 after acute poisoning. In group M3, this parameter did not differ significantly from control values (Table 2).

An increase in GBM thickness was noted in model M3 on day 1 after exposure; however, no difference from the control group was observed by day 7. In contrast, in models M1 and M2, GBM thickening was evident only on day 7 after paraoxon administration (Table 2).

The mean number of podocyte processes per 2 μm membrane segment increased only in model M2 one day after intoxication; in other experimental groups, no significant differences from controls were detected. The width of podocyte foot process bases was significantly greater in models M2 and M3 at day 7 after poisoning (Table 2). No changes were found in model M1 at these time points.

The height of TECs in both proximal and distal tubules of nephron differed from control values at both 1 and 7 days after paraoxon exposure. In the proximal tubules, TEC height increased in group M3 at both time points. In the distal tubules, TEC height decreased in group M1 on day 1 and increased in group M2 on day 7 compared with the control group (Table 2). In addition, hypertrophy of TECs in the distal tubules was observed in models M2 and M3, whereas in model M1, transient flattening of the cells was noted (Table 2).

The principal alterations in the structure of the GBM following paraoxon poisoning were similar among all experimental groups. In groups M1, M2, and M3, the basement membrane exhibited irregular thickness with rarefied areas adjacent to endothelial cells, as well as regions of GBM duplication. However, no disruption of GBM integrity or presence of osmiophilic electron-dense deposits was observed (see Fig. 1). The slit diaphragms connecting the tertiary podocyte processes remained intact in both the control and all experimental groups (Fig. 1).

Table 2. Morphometric parameters of glomerular filtration barrier elements in the control and experimental groups

Parameter	Day	Control	Model 1	Model 2	Model 3
Number of endothelial fenestrae per 2 μm segment, n	1	10.00 $\pm$ 1.90	9.66 $\pm$ 1.70	9.78 $\pm$ 1.50	6.55 $\pm$ 1.10***
	7	10.00 $\pm$ 1.90	9.00 $\pm$ 2.20	7.45 $\pm$ 1.60***	7.71 $\pm$ 2.10*
Diameter of fenestrae, μm	1	0.08 $\pm$ 0.02	0.12 $\pm$ 0.03***	0.08 $\pm$ 0.02	0.10 $\pm$ 0.02
	7	0.08 $\pm$ 0.02	0.10 $\pm$ 0.02*	0.10 $\pm$ 0.03*	0.09 $\pm$ 0.02
Thickness of the glomerular basement membrane, μm	1	0.18 $\pm$ 0.02	0.18 $\pm$ 0.02	0.17 $\pm$ 0.06	0.20 $\pm$ 0.05**
	7	0.18 $\pm$ 0.02	0.21 $\pm$ 0.05***	0.22 $\pm$ 0.09***	0.18 $\pm$ 0.05
Number of podocyte foot processes per 2 μm segment, n	1	5.93 $\pm$ 1.80	7.20 $\pm$ 1.90	7.40 $\pm$ 1.30*	6.70 $\pm$ 1.80
	7	6.07 $\pm$ 1.80	6.29 $\pm$ 1.50	6.04 $\pm$ 1.50	6.71 $\pm$ 1.50
Width of the basal portion of podocyte foot processes, μm	1	0.20 $\pm$ 0.09	0.221 $\pm$ 0.13	0.20 $\pm$ 0.09	0.25 $\pm$ 0.15
	7	0.19 $\pm$ 0.07	0.170 $\pm$ 0.07	0.27 $\pm$ 0.15***	0.27 $\pm$ 0.18**
Height of tubular epithelial cells in proximal tubules, μm	1	11.52 $\pm$ 2.90	12.70 $\pm$ 4.30	12.07 $\pm$ 2.80	13.44 $\pm$ 3.80*
	7	11.52 $\pm$ 2.90	10.61 $\pm$ 3.10	11.71 $\pm$ 2.80	13.56 $\pm$ 2.80*
Height of tubular epithelial cells in distal tubules, μm	1	8.72 $\pm$ 2.40	6.02 $\pm$ 2.10***	8.59 $\pm$ 2.30	9.59 $\pm$ 2.20
	7	8.72 $\pm$ 2.40	7.23 $\pm$ 3.30	10.43 $\pm$ 2.90*	8.57 $\pm$ 2.60

Note: Data are presented as $M \pm SD$, where M is the mean and SD is the standard deviation; * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ compared with the control.

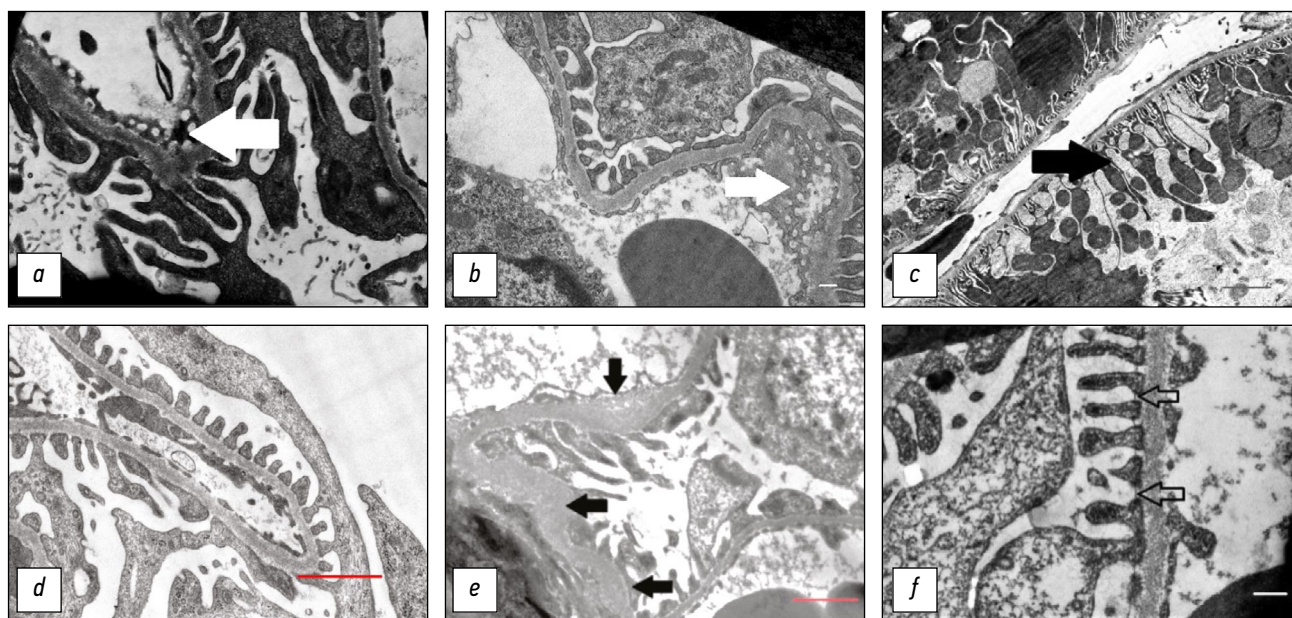


Fig. 1. Ultrastructure of the renal glomerular filtration barrier in three paraoxon poisoning models: *a*, fenestrations of endothelial cells in the control group on day 3 of the experiment; *b*, fenestrations of endothelial cells in a rat after a single paraoxon administration (model 1); *c*, disruption of the basal labyrinth structure of tubular endothelial cells; *d*, structure of the glomerular basement membrane in the control group; *e*, structural changes in the glomerular basement membrane 7 days after paraoxon exposure (model 2); *f*, structural changes in the glomerular basement membrane 7 days after paraoxon exposure (model 3). Arrows indicate: black, structural changes in the glomerular basement membrane beneath endothelial cells; white, representative areas of endothelial fenestrations; unfilled, slit diaphragms. Transmission electron microscopy, scale bars: *a*, *b*, 200 nm; *c*, 2 μ m; *d*–*f*, 1 μ m.

Additional Study Outcomes

No abnormalities in the localization of nephrin or type IV collagen were detected following acute paraoxon intoxication at sublethal doses (see Fig. 2). The intensity of the immunohistochemical reaction was visually assessed as strong positive staining in both the control and experimental groups.

DISCUSSION

Summary of Primary Results

Acute paraoxon poisoning in all three experimental models induced ultrastructural alterations in the components of the GFB, including endothelial cells of the glomerular capillary network, the GBM, and podocytes. The identified structural alterations were not detected in histological specimens examined by light microscopy, which highlights the need to employ ultrastructural methods in studies of paraoxon-induced nephrotoxicity.

Discussion of Primary Results

This study demonstrated ultrastructural alterations in the GFB of rat kidneys following acute paraoxon intoxication under three different exposure regimens. In all experimental groups, the GFB components exhibited similar structural changes, which is consistent with scientific data describing comparable morphologic manifestations in septic, toxic, and hypoxic renal injury models [25].

Restoration of TEC integrity in proximal tubules was observed by day 7 after paraoxon exposure, in agreement with previous reports describing TEC recovery in cases of unintentional human poisoning [6]. It is well established that nephron TECs possess the capacity for regeneration, proliferation, and migration [26, 27].

Immunohistochemical analysis using antibodies against nephrin and type IV collagen revealed no differences in staining intensity between renal tissue samples of the control group and those from the three experimental groups (three paraoxon-exposure models). Nephrin was localized within podocyte cytoplasm, whereas ultrastructural examination clearly demonstrated slit diaphragms in which nephrin acts as a linker protein between adjacent podocyte processes. The slit diaphragm proteins maintain the actin cytoskeleton of podocyte foot processes in a physiological state, essential for preventing proteinuria [2]. Podocytes are epithelial cells incapable of proliferation; however, their actin cytoskeleton retains contractile properties [2]. Damaged podocytes undergo hypertrophic changes and cytoskeletal remodeling of foot processes to compensate for the uncovered areas of the GBM [2].

Disturbances in renal microcirculation caused by acute OP poisoning imply a hypoxic impact on kidney cells [28]. Cellular injury resulting from ischemia–reperfusion, as well as abnormal levels of various circulating factors during OP intoxication, can lead to endothelial dysfunction [12]. The muscarinic receptor AChM1R (acetylcholine muscarinic receptor M1) has been reported to mediate structural

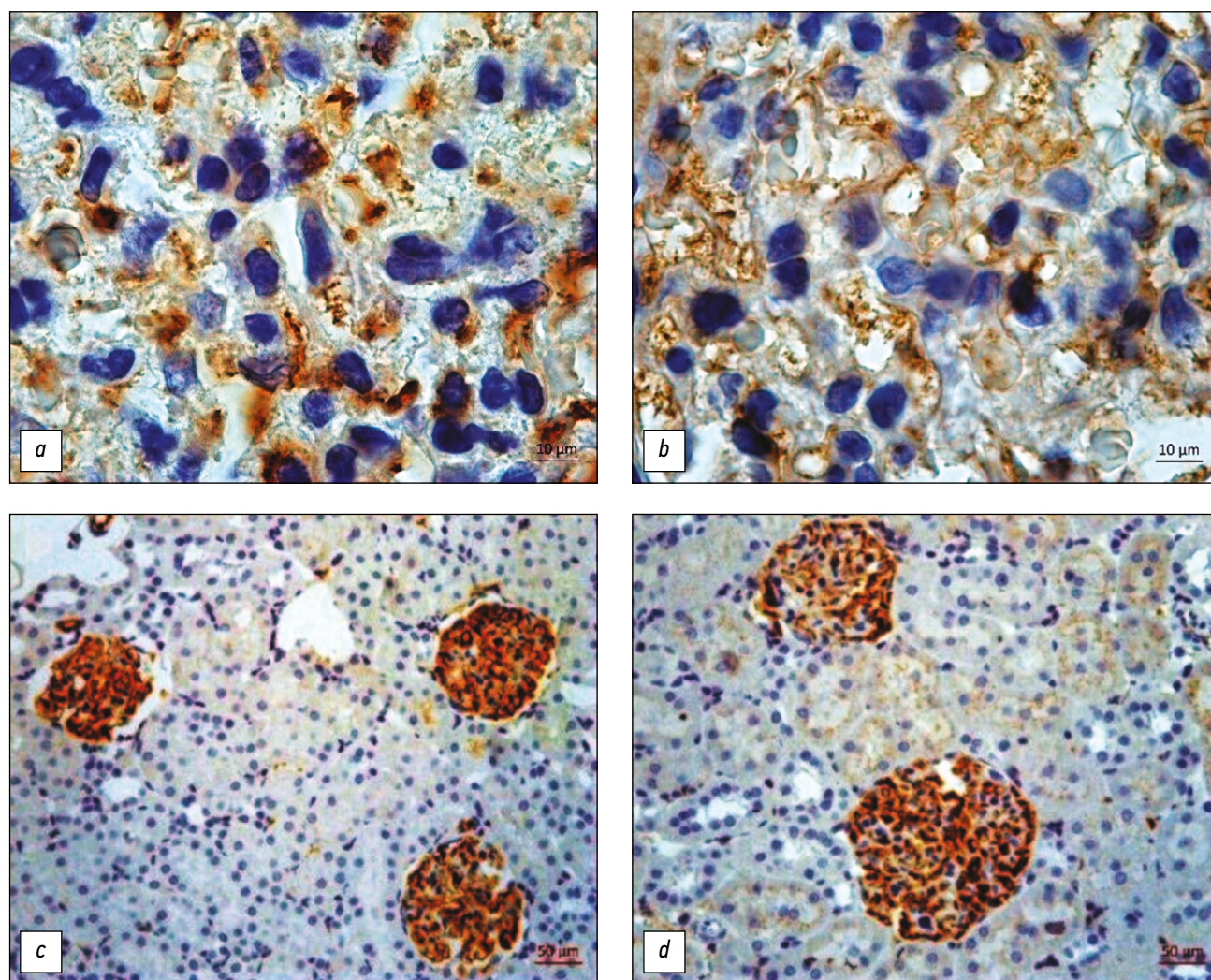


Fig. 2. Capillaries of the glomerular tuft in intracortical nephrons of rats: *a, c*, control group; *b, d*, after paraoxon exposure (model 1). Immunohistochemical staining with antibodies to: *a, b*, nephrin, 3 days after paraoxon exposure; *c, d*, type IV collagen, 7 days after paraoxon exposure; counterstained with Mayer hematoxylin. Magnification: *a, b*, $\times 1000$; *c, d*, $\times 400$.

alterations observed in the endothelial cells of glomerular capillaries [29]. In the present study, the most pronounced alterations were found in the endothelial cell architecture, including changes in fenestration diameter and number, detected across all experimental models on day 7 following paraoxon exposure. Fig. 3 schematically illustrates the GFB elements undergoing structural modification on day 7 after toxicant exposure.

The findings indicate that paraoxon exposure induces interrelated alterations among the GFB components. On day 7, a decrease in the number and an increase in the diameter of endothelial fenestrae were accompanied by thickening of the GBM, widening of podocyte foot processes, and an increase in their number. In model M3, one day after paraoxon administration, the reduction in fenestrae number and the increase in their diameter were associated with thickening of the GBM and widening of the basal portion of podocyte processes. In model M1, which lacked the carboxylesterase inhibition step, only an increase in fenestrae diameter was observed one day after exposure.

The changes of GBM structural changes differed among the experimental groups. Sequential GBM thickening was observed following single (group M1) and double (group M2) paraoxon administration. In contrast, in the group receiving CBDP (group M3), the thickening of the basement membrane noted 1 day after toxicant exposure had resolved by day 7. It may be assumed that the increased intensity of transglomerular transport in group M3 leads to transient protein accumulation within the GBM at day 1. By day 7, podocytes appear to remove the excess protein from the membrane, resulting in decreased GBM thickness [16]. In addition to altered selective permeability and protein accumulation within the GBM, abnormal synthesis of membrane components may occur as a consequence of acute toxic stress and metabolic uncoupling between podocytes and endothelial cells [30]. Along with impaired fenestral selectivity and macromolecular accumulation in intercellular signaling between GFB components are also likely [11, 31]. In models M2 and M3, on day 7 after

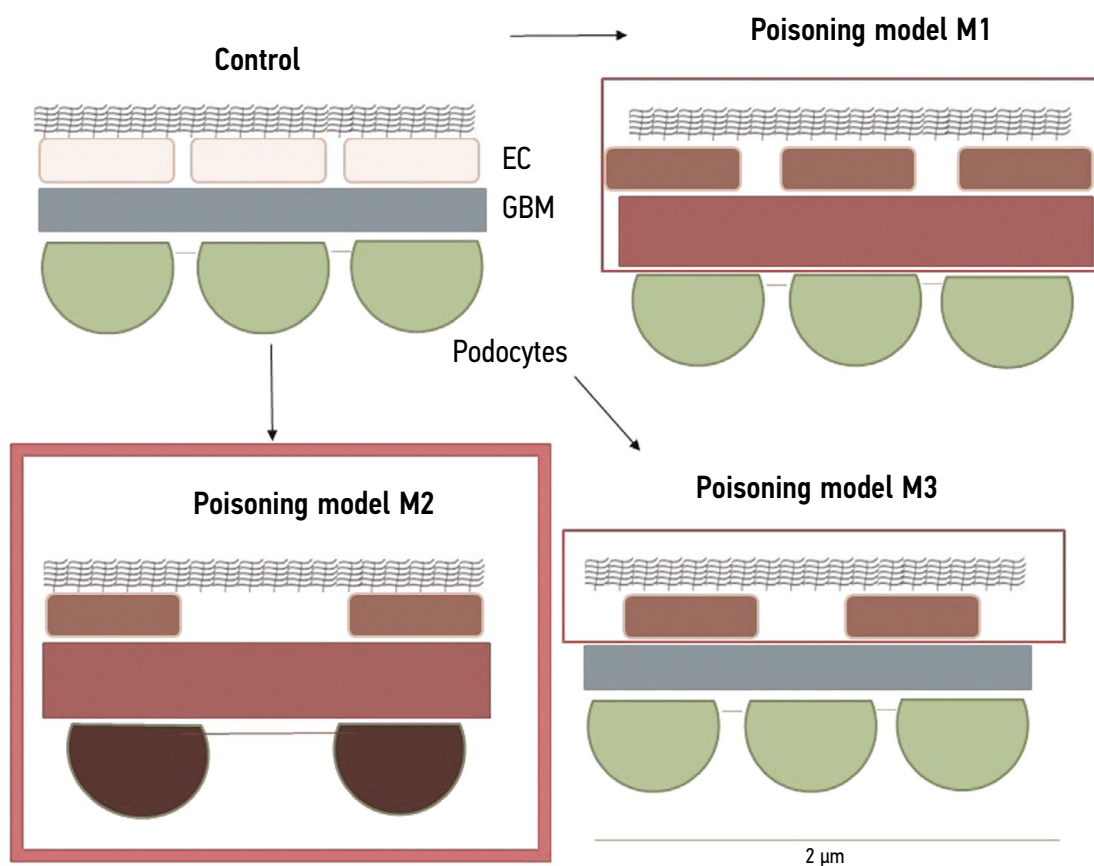


Fig. 3. Schematic representation of the renal glomerular filtration barrier in three experimental paraoxon poisoning models: framed areas indicate components of the glomerular filtration barrier in which changes were observed 7 days after acute paraoxon exposure. The thicker frame in model M2 highlights the severity of changes involving all components of the glomerular filtration barrier.

paraoxon administration, widening of the basal portions of podocyte processes was accompanied by GBM thickening. A possible mechanism underlying these alterations may involve actin microfilament reorganization within podocytes to compensate for the uncovered regions of the GBM. Cross-talk between endothelial cells and podocytes via the VEGFA–eNOS and NO–ET-1 signaling pathways enables the glomerular endothelium to initiate podocyte cytoskeletal remodeling through endothelin-1 (ET-1) release [11, 12].

Structural alterations in TECs of the distal tubules were detected on day 7 following paraoxon exposure in models M2 and M3. According to published data, the principal mechanism underlying distal tubular cell hypertrophy is an increase in Na^+ concentration in the tubular fluid [32]. Damage to the brush border of proximal TECs observed on day 1 after exposure, and the associated impairment of active ion transport, are likely responsible for the subsequent rise in Na^+ concentration in the tubular fluid [27].

Study Limitations

This study focused on the morphological changes in rat kidney tissues, aiming to identify alterations in the GFB resulting from paraoxon exposure. All GFB changes described in this work correspond to the early post-exposure period (days 1, 3, and 7 after poisoning).

CONCLUSION

Acute paraoxon poisoning induces ultrastructural alterations in GFB components in rats, specifically in the endothelial cells of glomerular capillaries, the GBM, and podocytes. These changes are not detectable by light microscopy, highlighting the necessity of ultrastructural examination of the kidneys when assessing OP-induced nephrotoxicity.

Endothelial cells of the glomerular capillary network are the most susceptible to acute paraoxon exposure, with the most pronounced alterations observed in rats following double administration of the toxicant (group M2). The findings suggest that this experimental model most accurately reflects the processes associated with the acute toxic effects of OPs. The use of this model in rodents may be valuable for assessing potential health risks in humans exposed to OPs. The ultrastructural alterations in GFB components observed in this study indicate that chronic OP poisoning may lead to decreased renal functional activity due to damage to renal corpuscles.

ADDITIONAL INFORMATION

Author contributions: M.O. Sokolova: investigation, data curation, formal analysis, writing—original draft; V.E. Sobolev: supervision,

investigation, writing—review & editing. All the authors approved the version of the manuscript to be published and agreed to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

Вклад авторов. М.О. Соколова — проведение исследования, работа с данными, анализ данных, написание черновика рукописи; В.Е. Соболев

— руководство исследованием, проведение исследования, пересмотр и редактирование рукописи. Все авторы одобрили рукопись (версию для публикации), а также согласились нести ответственность за все аспекты настоящей работы, гарантируя надлежащее рассмотрение и решение вопросов, связанных с точностью и добросовестностью любой её части.

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Этическая экспертиза. Эксперименты по моделированию интоксикации у крыс получили одобрение Этического комитета Института эволюционной физиологии и биохимии имени И.М. Сеченова РАН (протокол № 13-k-a от 15 февраля 2018 года).

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Раскрытие интересов. Авторы заявляют об отсутствии отношений, деятельности и интересов за последние три года, связанных с третьими лицами (коммерческими и некоммерческими организациями), интересы которых могут быть затронуты содержанием статьи.

Оригинальность. При проведении исследования и создании настоящей статьи авторы не использовали ранее полученные и опубликованные сведения (данные, текст, иллюстрации).

Доступ к данным. Все данные, полученные в настоящем исследовании, представлены в статье.

Генеративный искусственный интеллект. При создании настоящей статьи технологии генеративного искусственного интеллекта не использовались.

Рассмотрение и рецензирование. Настоящая работа подана в журнал в инициативном порядке, рассмотрена и опубликована вне очереди. В рецензировании участвовали два внешних рецензента и научный редактор издания.

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