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Research Article

Experimental Evaluation of the Therapeutic Efficacy of Oxidized Dextran in a Model of Spermatogenesis Disorders in Rats Under the Action of the Endotoxin *Escherichia coli*

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Abstract

Rationale for the Study: The relevance of the study is due to a significant increase in the proportion of male fertility.

Objective of the Study: To evaluate the therapeutic efficacy of parenteral administration of a 2% solution of oxidized dextran in an *in vivo* model imitating impaired spermatogenesis after bacterial and viral infections, including COVID-19.

Materials and Methods: Studies performed on rats of the Wistar line. Spermatogenesis disorders were caused by intraperitoneal administration of the lipopolysaccharide *Escherichia coli* (LPS). In the experimental group, after 30 minutes after intraperitoneal administration of LPS, a 2% solution of oxidized dextran was administered intraperitoneally. After 3 days, the animals were euthanized with an overdose of ether anesthesia and standard histological sections of the testes were prepared, followed by a morphometric assessment of the results.

Results with Data: Parenteral administration of a 2% solution of oxidized dextran can significantly reduce all morphological manifestations of spermatogenesis disorders caused by the introduction of LPS: numerical density (Nai) of spermatozoa in the lumen of the tubule, early and late spermatids, spermatocytes, spermatogoniums, Sertoli cells, Leydig cells, as well as the numerical density (Nai) of blood vessels the peritubular zone of the testes, the volume density (Vv) of the interstitium between the convoluted seminiferous tubules and the volume density (Vv) of cell infiltration.

Conclusion: Oxidized dextran can be a very promising therapeutic and prophylactic drug for disorders of spermatogenesis caused by bacterial and viral infections, including COVID-19.

Keywords: Oxidized Dextran; Disorders of Spermatogenesis; Male Fertility

Currently, according to WHO, male infertility is approaching 50% of all infertile marriages. At the same time, in the structure of the causes of male infertility, the infectious factor is at least 15% [1]. It should be noted that this percentage refers mainly to infections for which correlations with spermatogenesis disorders have been accurately established. First of all, these are

chronically urogenital infections. and (chlamydia, microplasmosis, ureaplasmosis, trichomoniasis and candidiasis. Very often they form a mix of infection with traditional pathogens, staphylococci, streptococci, proteus, etc. A distinctive feature of such mixed pathogenic cultures is the formation of biofilms of polysaccharides that surround microorganisms and make them particularly resistant to antibiotics. Obviously, the sanitation of all infectious foci is one of the primary tasks for the treatment of male infertility. However, there are other reasons disorders of spermatogenesis, which are not directly related to any diagnosed chronic urogenital infectious process. We are talking about the consequences of viral infections. It has been established that the papilloma virus, influenza virus, SARS virus, herpes virus, hepatitis virus and SARS-CoV-2 coronavirus, which causes COVID-19, are pathogenetic factors of spermatogenesis disorders, even at the stage of long-term consequences of previous infections [2]. The mechanism of spermatogenesis disorders in viral diseases is mainly based on a powerful inflammatory reaction of a systemic nature with elements of microcirculation disorders, microthromboses, fibrosis in tissues and hormonal testicular dysbalnsa. As an experimental model of spermatogenesis disorders in viral infections, we used a model with intraperitoneal administration of lipopolysaccharide (endotoxin) *Escherichia coli* [3]. By the nature of the systemic immunological reaction, this model most accurately corresponds to viral diseases, including COVID-19 [4-8]. This model has been used in the development of drugs to treat COVID-19 and prevent pulmonary fibrosis [9,10]. At the same time, this model reflects the main pathogenetic mechanisms of the influence of chronic urogenital infections on spermatogenesis through autoimmune reactions characteristic of the chronic inflammatory process in violation of the hematotesticular barrier.

Materials and Methods

The study was performed on sexually mature individuals of male rats of the Wistar line weighing 180-200 g. The animals were divided into 3 study groups of 5 animals:

- Group 1 - male rats with a single intraperitoneal administration of 2 ml of 0.9% NaCl solution,
- Group 2 - male rats with a single intraperitoneal administration of lipopolysaccharide (LPS) *E. coli* at the rate of 50 μg/kg body weight,
- Group 3 - male rats with a single intraperitoneal administration of LPS *E. coli* at the rate of 50 μg/kg of body weight and intraperitoneal administration of 2 ml of 2% solution of oxidized dextran.

Animals were removed from the experiment by an overdose of ether anesthesia for 3 days after the introduction of the tested substances in compliance with the principles of humanity of the European Community Directives (86/609/EEC) and the Declaration of Helsinki and in accordance with the requirements of the rules for conducting work using experimental animals.

The object of histological examination were fragments of the testes of rats of the Wistar line, which were fixed in a neutral 10% buffered formalin solution with subsequent sample preparation, manufacture of paraffin blocks and histological glasses with sections of testicular tissue stained with hematoxylin and eosin.

Visualization of structural changes in the testes was carried out by direct light microscopy at an increase of x200. Morphometric evaluation of histological structures was carried out in transverse sections of convoluted seminiferous tubules with the most advanced stages of spermatogenesis in 10 arbitrarily taken fields of vision at an increase of x400 using a morphometric grid at 100 points with a sparing of $3.64x10^4$ μm^2. At the same time, the numerical density (Nai) of spermatozoa in the lumen of the tubule, early and late spermatids, spermatocytes, spermatogoniums, Sertoli cells, Leydig cells was counted, as well as the numerical density (Nai) of the vessels of the peritubular zone of the testes, the volume density (Vv) of the interstitium between the convoluted seminiferous tubules and the volume density (Vv) of the vessels of the peritubular zone of the testes, the volume density (Vv) of the interstitium between the convoluted seminiferous tubules and the volume density (Vv)) cell infiltration.

The study of histological samples was carried out using a digital laboratory light optical immersion fluorescence polarization phase microscope Olympus CX43 (Japan) with software for image analysis and photoarchy. Photography of preparations was carried out digital camera MOTICAM S6, (Japan) with subsequent image processing.

In addition, thendex of maturation of spermatogenic cells was also determined - the ratio of the sum of young (spermatogony,

spermatocytes) and mature forms (spermatids, spermatozoa) of the spermatogenic epithelium.

Statistical processing of the obtained data was carried out using the programs Statistica and Excel. Assessment of the reliability of statistical differences between the indicatorsof analytical parameters was also carried outusing the Student's t-criterion. Differences in the p ri level of significance of $p \leq 0.05$ were considered reliable.

Results of the Study

During survey microscopy of cross-sections of the testes of white rats in all groups of the study, the histoarchitectonics of the organ is not changed. In part of the histological sections, there are fragments of the herringbone shell and represented by a dense unformed connective tissue, and the sleep area of the transverse and tangential sections of the testes is occupied by convoluted seminiferous tubules, predominantly rounded or elliptical in shape. The seminiferous tubules are separated from each other by a thin layer of interstitial connective tissue with blood vessels surrounded by Leydig cells.

Histological examination of sections of the testes of animals of group 1 revealed focal destructive changes in the form of areas of moderate dystrophy of the spermatogenic epithelium of the convoluted seminiferous tubules with an accumulation in the lumen of the secret without an admixture of spermatozoa. The thickness of the spermatogenic epithelium in a number of convoluted tubules is uneven. In areas of slight hypotrophy, a decrease in the number of layers and the formation of single degenerative forms spermatogenic cells are large cells with polymorphic hyperchromic nuclei. At the same time, the epithelium itself is represented mainly by basally located Sertoli cells, spermatogoniums and spermatocytes. Visually, the content of spermatids in the wall of the seminal convoluted tubules is reduced. Between the tubules, thin layers of interstitial connective tissue with focal fullness of blood vessels were visualized. These signs violations of hemocirculation were accompanied by the formation of focal weakly pronounced edema of the interstitium. At the same time, cellular infiltration of interstitial tissue and walls of convoluted seminiferous tubules was not detected (Figure 1 a, b).

Figure 1: (a, b) – Fragment of the convoluted seminiferous tubule of the Wistar line rat after a single intraperitoneal administration of 0.9% of the NaCl solution: toleshchina of the spermatogenic epithelium in a number of convoluted tubules is uneven with the formation of single degenerative forms of spermatogenic cells and the accumulation of secretion in the lumen without sperm impurity; m with tubules thin layers of interstitial connective tissue, focal fullness of blood vessels. Staining with hematoxylin and eosin. Increase x 200.

In animals of group 2, after the introduction of LPS *E. coli*, histological examination revealed focal moderate edema of the intercanal interstitium in areas of moderate focal fullness of blood vessels (Figure 2 a, b).

Figure 2: a, b - Fragment of the convoluted seminiferous tubule of a rat of the Wistar line after a single intraperitoneal administration of LPS *E. Coli*: a, b - focal moderate fullness of blood vessels, moderately pronounced edema of the intercanal interstitium; unevenness of the oles of the spermatogenic epithelium in the convoluted tubules, with focal moderate and weak dystrophy of spermatogenic epithelium cells. Coloration with hematoxylin and eosin. Increase x 200.

In the spermatogenic epithelium, parts of the convoluted seminiferous tubules revealed destructive changes of a focal nature in the form of areas of moderate dystrophy and foci of micronecrosis with an accumulation in the lumen of the tubules of secretion, spermatozoa in the lumen of these tubules were not detected (Figure 2 a, b, 3).

Figure 3: Fragment of the convoluted seminiferous tubule of a rat of the Wistar line after a single intraperitoneal administration of LPS E. Coli: degenerative changes in the spermatogenic epithelium mainly due to damage to spermatids and spermatocytes. In a number of cells, the nucleus is hyperchromic, the cytoplasm is vacuolized; focal desquamation of the epithelium with impaired intercellular connections between Sertoli cells, spermatids and spermatocytes. In the lumen of convoluted seminal tubules a small amount of secretion; focal perivascular moderately pronounced edema. Coloration with hematoxylin and eosin. Enlargement x 400.

The thickness of the spermatogenic epithelium in the convoluted tubules is uneven. Hypotrophy of the spermatogenic epithelium was manifested by a decrease in the number of layers and the formation of dystrophically altered spermatogenic cells, mainly spermatocytes (Figure 2a, b, 3).

Along with areas of hypotrophy of the spermatogenic epithelium in single tubules, areas of meiotic activity of spermatocytes were identified (Figure 4).

Figure 4: Fragment of the convoluted seminiferous tubule of a rat of the Wistar line after a single intraperitoneal administration of LPS E. Coli: along with degenerative changes in spermatogenic epithelium cells in a number of convoluted seminiferous tubules of spermatogenic epithelium cells with signs of mitotic activity; focal expansion of the zones of intercanal interstitium due to focal cell infiltration by macrophage cells. Coloration with hematoxylin and eosin. Magnification x 400.

Sertoli cells are located along the basement membrane, the content of sprematocytes is reduced, in comparison with the indicators in the 1st group of the study by 16%. The numerical density of spermatogonium is reduced, in comparison with the indicators in animals of the 1st group by 20% (Table 1). Visually, the content of spermatids in the epithelium of the seminal convoluted tubules is reduced, which with morphometry is manifested by a decrease in this indicator in comparison with the values of the same indicator of group 1 by 25%. The numerical density of spermatozoa in animals of this group was less than in animals in group 1 by 20%. Between the tubules, the expansion of the interstitium layers was visualized due to the formation of focal perivascular weakly and moderately pronounced edema of the interstitium in areas of moderately pronounced vascular fullness (Figure 3). At the same time, the indicator of the numerical density of Leydig cells in animals of group 2 was slightly lower in comparison with the value of a similar indicator in animals of group 1 (Table 1). Also,

the focal expansion of the zones of the intercanal interstitium may be due to the formation of areas of cellular infiltration by cells of the macrophage type (Figure 4). This may not be due to true cellular infiltration, but to an increase in the number of activated Leydig cells in the intercanal interstitium (Table 1). For accurate verification of the histophenotype of cells in the composition of cell infiltrates, their immunohistochemical typing is necessary.

In animals of group 3, the number of seminal convoluted tubules did not significantly differ from the values of this indicator in animals of comparison groups (Table 1). During histological examination, focal weakly and moderately pronounced perivascular edema of the intercanal interstitium was detected.

The thickness of the spermatogenic epithelium in individual convoluted tubules is uneven. In the areas of hypotrophy of the spermatogenic epithelium of these tubules, a decrease in the number of layers and foci of the formation of destructive changes in the spermatogenic epithelium, mainly spermatocytes, were revealed. Signs of spermatogenesis in the seminal convoluted tubules were preserved. Along with areas of hypotrophy of the spermatogenic epithelium in single tubules, areas of meiotic activity were observed. spermatocytes (Figure 5 a, b).

Figure 5: a, b - Fragment of a convoluted seminiferous tubule of a rat of the Wistar line after a single intraperitoneal administration of LPS E. Coli and intraperitoneal administration of a solution of 0.9% NaCl: a - in the part of the tubules focal destructive changes in the spermatogenic epithelium, mainly due to dystrophic changes in spermatocytes; b - areas of meiotic activity in the cells of the spermatogenic epithelium; spermatogenesis in most convoluted spermatogenic tubules is preserved. Staining with hematoxylin and eosin. Magnification x 400.

Sertoli cells are located along the basement membrane, their number does not significantly differ from the values of this indicator in groups 1 and 2 (Table 1). The indicator of the numerical density of spermatocytes and spermatogonium in animals of this group was greater by 20% than the values of similar indicators in group 2, while no significant differences from indicators in group 2 were revealed (Table 1). At the same time, areas of mitotic activity of spermatocytes in the epithelium of part of the seminal convoluted tubules were visualized. The numerical density of spermatids in the epithelium of the seminal convoluted tubules of animals of group 3 was greater than in animals of group 2 by 10.3% and reliable did not differ from the indicator in animals of group 1. The numerical density of spermatozoa in animals of this group was unreliably less than in animals of group 1 and not significantly greater than in animals of group 2 (Table 1).

Between the tubules, the expansion of the interstitium layers was visualized due to the formation of foci of perivascular weakly and moderately pronounced edema in the areas of fullness of blood vessels and fociof cellular infiltration of cells of macrophage type (Figure 6).

Figure 6: Fragment of the convoluted seminiferous tubule of a Wistar rat after a single intraperitoneal administration of LPS E. coli and intraperitoneal administration of an oxidized dextran solution: focal moderately pronounced expansion of the intercanal interstitium due to weakly and moderately pronounced perivascular edema, focal weak and moderate cell infiltration by cells of macrophage type; signs of spermatogenesis in the convoluted seminiferous tubules are preserved. Coloration with hematoxylin and eosin. Magnification x 400.

In animals of this group, there was not a significant predominance of the volumetric density of the intercanal interstitium in comparison with the indicators in the comparison groups (Table 1). Perhaps these changes are due to more pronounced cellular infiltration, which is confirmed by morphometry data - the volumetric density of cell infiltration of the intercanal interstitium is 17% greater than in animals of group 2. They also revealed the predominance of the numerical density of Leydig cells by 22.5% and 19%, compared with indicators in groups 2 and 1 of animals, respectively. For accurate verification of the histophenotype of these cells in the composition of infiltrates, their immunohistochemical typing is necessary.

Options	Study Groups		
	1 group	Group 2	Group 3
Numerical density of convoluted seminiferous tubules, Nai	30,83 ± 1,37	29,94 ± 1,86	32,98 ± 1,86
Numerical density of Sertoli cells, Nai	20,89 ± 1,26	17.52 ± 1.24^{pp}	18,87 ± 1,45
Numerical density of Leydig cells, Nai	11,23 ± 1,39	10,75 ± 1,15	13.87 ± 1.47^{b}
Numerical density of spermatocytes, Nai	38,74 ± 1,26	29.80 ± 1.25^{a}	37.29 ± 1.47^{b}
Numerical density of spermatogonium, Nai	48,97 ± 1,35	39,71 ± 1,53	49,34 ± 1,38
Numerical density of spermatids, Nai	33,14 ± 1,52	24.81 ± 1.46^{pp}	27.79 ± 1.74^{a}
Numerical density of spermatozoa in the lumen of the tubule, Nai	300,87 ± 15,23	239.71 ± 11.35^{pp}	299.64 ± 13.32^{b}
Numerical density of vessels of the peritubular zone of the testes, Nai	7,98 ± 0,62	9,28 ± 0,74	11.21 ± 1.23^{a}
Volumetric density of intercanal interstitial tissue, Vv	8,25 ± 0,87	12.74 ± 1.24^{a}	10.47 ± 1.2^{b}
Bulk density of cell infiltration interstitium between convoluted seminiferous tubules, Vv	___	5,64 ± 0,32	7.12 ± 0.46^{b}

Table 1: Structural changes in the testes of white rats after intravenous administration of LPS E. and their prevention intravenously by administering a 2% solution of oxidized dextran (M ± m).

Note: α - the reliability of differences in the values of similar parameters from the indicators of group 1; β is the reliability of differences in the values of similar parameters between the indicators of groups 2 and 3. At $p \leq 0.05$.

Discussion

Evaluating the histological picture integrally for all 3 groups of animals and the morphometric data presented in Table 1, it can be unequivocally stated that 3 days after the intarperitoneal administration of LPS *E. coli* in rats there is a clear tendency to reduce all the main parameters of spermatogenesis in combination with elements of aseptic inflammation in the form of swelling of the interstitium, full-bloodedness and cellular infiltration. These changes are moderate due to that the experiment used a low dose of LPS E. witholi, which does not lead to fatal disorders of spermatogenesis, but allows you to assess the dynamics of the pathological process when it is corrected with oxidized dextran. So in rats of group 3, which immediately after intraperitoneal administration of LPS E. witholi, a 2% solution of oxidized dextran was administered in comparison with animals of group 2, all spermatogenesis indicators were higher. This trend is most clearly manifested in the indicator of the numerical density of spermatozoa in the lumen of the tubule, which in group 3 of animals is almost close to the value of the indicator in group 1 and is significantly higher than in animals of group 2. It should be noted that that in animals of group 3, in comparison with animals of group 2, the morphological manifestations of hemocirculatory disorders in the form of interstitium edema are less pronounced. However, the indicators of the volumetric density of cellular infiltration of the interstitium between the convoluted seminiferous tubules and the numerical density of the vessels of the intercanal zone of the testes in animals

of group 3, on the contrary, are slightly higher than in animals of group 2, which may indicate an improvement in local blood flow and activation of residentx testicular macrophages, in response to induced intraperitoneal administration of LPS E. Such dynamics may indicate that oxidized dextran under experimental conditions exhibits a moderate anti-inflammatory effect, which was previously studied in detail on various models in vivo. It should be noted that in this model in vivo violation of spermatogenesis is not direct, but to a greater extent, indirect, due to an acute immunological reaction to intraperitoneal administration of LPS *E. coli* by type of systemic aseptic inflammation. Taking into account the previous data we obtained in an in vivo model simulating COVID-19-induced lung damage by injecting LPS E into the upper respiratory tract. witholi, we believe that inraperitoneal administration of LPS E. witholi can serve as an adequate model of impaired spermatogenesis after viral diseases, including spermatogenesis disorders caused by COVID-19. This is supported by the fact that the mechanism of damaging action of LPS *E. coli* is associated not only with the development of local peritoneal reactions in response to direct damage, but also possibly an indirect, secondary damaging effect in the formation of a systemic immunological response associated with the formation of a „cytokine storm".

Findings

The experimental data obtained suggest the possibility of using oxidized dextran as a promising means of preventing and correcting spermatogenesis disorders caused by the action of infectious agents of bacterial and/or viral etiology. However, the mechanisms for the implementation of the positive protective properties of oxidized dextran in relation to spermatogenesis disorders caused by damage to the testicular tissue by pathological agents of infectious etiology need further comprehensive detailed study.

Bibliography

1. Brunner RJ., *et al*. "Review of Guidelines for the Evaluation and Treatment of Leukocytospermia in Male Infertility". *The World Journal of Men's Health* 37.2 (2019): 128-137.

2. Adamyan LV., *et al*. "Influence of COVID-19 and other viral infections on male fertility (literature review)". *Problems of Reproduction* 26.6 (2020): 77-82.

3. Poplavskaya YuA. "Narrushion of spermatogenesis of male white rats under the influence of lipopolysaccharides of gram-negative bacteria (*E. coli* and *S. marcescens*) as a cause of reproductive losses". Abstract of the dissertation for the degree of Candidate of Biological Sciences. Minsk (2015).

4. Wanpeng Yu., *et al*. "Estrogen promotes Leydig cell engulfment by macrophages in male infertility". *Journal of Clinical Investigation* 124.6 (2014): 2709-2721.

5. Wang M., *et al*. "Characterization of the Micro-Environment of the Testis that Shapes the Phenotype and Function of Testicular Macrophages". *The Journal of Immunology* 198 (2017): 4327-4340.

6. De Falco., *et al*. "Macrophages Contribute to the Spermatogonial Niche in the Adult Testis". *Cell Reports* 12 (2015): 1107-1119.

7. Kalashnikova SA and Polyakova LV. "And the use of bacterial lipopolysaccharide for modeling pathological processes in medical and biological research (literature review)". V*estnik of New Medical Technologies* 24.2 (2017): 209-219.

8. Nikiforova LR., *et al*. "Review of preclinical models of sepsis and septic shock". *Laboratory Animals for Scientific Research* 4 (2021): 17-28.

9. Patent for the invention of the Russian Federation No. 2728938. „The use of dalargin for the production of means of treatment and prevention of pneumonia". publ. 03.08.2020, Bulb. No. 22.

10. Troitsky AV., *et al*. "Prospects for the use of oxidized dextran for the prevention of viral pneumonia and pulmonary fibrosis". *Medical Alliance* 9.4 (2021): 45-53.