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Применение мезенхимных стромальных клеток в комплексной терапии экспериментального туберкулеза половых органов

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Обоснование. Клеточная терапия — перспективное направление лечения заболеваний с преобладанием в патогенезе процессов воспаления и склероза, к которым относится генитальный туберкулез, характеризующийся развитием сальпингита с окклюзией маточных труб.

Цель — определение эффективности применения мезенхимных стволовых клеток вместе со специфической полихимиотерапией при экспериментальной туберкулезной инфекции женских половых органов.

Материалы и методы. Кролики-самки породы шиншилла (n = 27) были разделены на четыре группы. Первая группа (n = 6) — контрольная, здоровые животные; вторая (n = 7) — животные, зараженные генитальным туберкулезом, без последующего лечения; третья (n = 7) — животные, зараженные туберкулезом, лечение только противотуберкулезными препаратами; четвертая (n = 7) — животные, зараженные генитальным туберкулезом, лечение противотуберкулезными препаратами в сочетании с мезенхимными стволовыми клетками. Для моделирования генитального туберкулеза использовали культуру *M. tuberculosis Erdman*, которую вводили под серозную оболочку левой маточной трубы в количестве 10^7 КОЕ/0,2 мл. Для оценки эффективности лечения анализировали гематологические и биохимические показатели периферической крови, выполняли гистеросальпингографию, диагностическую лапароскопию и учитывали фагоцитарную активность перитонеальных макрофагов.

Результаты. У зараженных генитальным туберкулезом животных увеличивалось количество лейкоцитов, С-реактивного белка в периферической крови, отмечались отечность и окклюзия маточных труб. У кроликов, получавших лечение противотуберкулезными препаратами в сочетании с мезенхимными стволовыми клетками, уменьшался лейкоцитоз ($8,18 \pm 1,39 \cdot 10^9$/л против $9,32 \pm 1,36 \cdot 10^9$/л, $p < 0,05$) и снижался уровень С-реактивного белка ($1,1 \pm 0,8$ мг/л против $2,2 \pm 1,2$ мг/л, $p < 0,01$) в периферической крови в сравнении с кроликами, получавшими только противотуберкулезные препараты. В четвертой группе наблюдалось также усиление фагоцитарной активности макрофагов. У кроликов, получавших лечение мезенхимными стволовыми клетками, по данным гистеросальпингографии была подтверждена проходимость маточных труб. По данным гистологического исследования констатирована стабилизация спаечного процесса с преобладанием процессов репарации.

Заключение. Мезенхимные стволовые клетки способствуют развитию репаративных процессов в маточных трубах в комбинации с противотуберкулезными препаратами при лечении генитального туберкулеза у кроликов.

Ключевые слова: стволовые клетки; туберкулез; мезенхимные стромальные клетки; генитальный туберкулез; сальпингит; бесплодие.

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Mesenchymal stromal cells application for experimental genital tuberculosis combination therapy

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BACKGROUND: Cell therapy is a promising trend in treating characterized by inflammation and sclerosis diseases such as genital tuberculosis, marked by pelvic inflammatory disease and uterine tube occlusion.

AIM: Efficacy evaluation of combined mesenchymal stem cells and specific polychemotherapy treatment of the experimental genital tuberculosis.

MATERIALS AND METHODS: Chinchilla rabbits (n = 27) were divided on 4 groups. Group 1 (n = 6) comprised control group, healthy animals. Group 2 (n = 7) infected with genital tuberculosis, without treatment. Group 3 (n = 7) infected with genital tuberculosis, treated with anti-tuberculous drugs only. Group 4 (n = 7) infected with genital tuberculosis, treated with anti-tuberculous drugs and mesenchymal stem cells. Culture of *M. tuberculosis Erdman* in dose 10^7 CFU/0.2 ml was injected under the left uterine tube serosa for the genital tuberculosis modelling. For treatment efficacy evaluation following tests were used: full blood count, blood chemistry, hysterosalpingography, diagnostic laparoscopy, peritoneal macrophage phagocytic activity assessment.

RESULTS: Infected with genital tuberculosis animals had leukocytosis, elevated C-reactive protein, swelled and occluded uterine tubes. Treated with anti-tuberculous drugs and mesenchymal stem cells rabbits had lower white blood cell count ($8.18 \pm 1.39 \cdot 10^9$/L vs $9.32 \pm 1.36 \cdot 10^9$/L, $p < 0.05$) and C-reactive protein (1.1 ± 0.8 mg/L vs 2.2 ± 1.2 mg/L, $p < 0.01$) compared to animal treated with anti-tuberculous drugs only. Animals in group 4 had also increased peritoneal macrophage phagocytic activity. Treated with mesenchymal stem cells animals had unobstructed uterine tubes, stabilized adhesive process within small pelvis with reparative process prevalence.

CONCLUSIONS: Mesenchymal stem cells combined with anti-tuberculous drugs therapy favors reparative process in uterine tubes in genital tuberculosis.

Keywords: stem cells; tuberculosis; mesenchymal stromal cells; genital tuberculosis; salpingitis; infertility.

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BACKGROUND

Infertility affects over 15% of couples worldwide. Despite recent progress in the control of infertility with the use of assisted reproductive technologies, more than 80% of couples experience insurmountable impairment of fertility [1]. Thus, the search for new therapies is extremely relevant. Currently, to restore the structure and function of damaged tissues, cell therapy is actively used in various fields of medicine [2].

Genital organs are the most common extrapulmonary localization of a specific infection in women [3]. Tuberculosis of the female genital organs is a significant cause of chronic pelvic pain syndrome and infertility. The true prevalence of the disease cannot be analyzed, since the disease is asymptomatic in 11% of the patients [4]. According to many authors, the incidence of infertility in genital tuberculosis varies from 10% to 85% worldwide [5]. Cellular medicine, which uses the unique properties of progenitor cells having high biological activity, differentiation potential, and the ability to form colonies, is being developed [6, 7]. Upon entry to the body, stem cells can accumulate in a damaged organ and differentiate into cells that form its tissues; they can also activate dormant and suppressed cells. For example, mesenchymal stromal cells (MSCs) transplanted in an experimental model of premature ovarian failure have demonstrated their ability to remain in the ovarian tissue and participate in ovarian regeneration and generation of oocytes [8].

Stem cells secrete biologically active substances and implement a paracrine effect on the production of various enzymes, proteins, and cytokines, which activate cell proliferation, slow the process of apoptosis of functional cells, and promote the differentiation of progenitor cells into cells of damaged tissues. Stem cells have an immunosuppressive effect through cell contacts and the production of factors that inhibit the proliferation of natural killer cells and contribute to the restoration of intercellular signals. The efficiency of MSCs in cicatricial changes in the uterine cavity has been proven experimentally [9]. Data obtained on model animals and in clinical practice in a limited group of patients revealed the successful use of the cell product in the treatment of the uterine cavity synechia, namely, Asherman syndrome, in combination with adhesiolysis and hormonal therapy [10, 11]. MSCs are used in the comprehensive treatment of tuberculosis [12], multidrug-resistant and extensively drug-resistant tuberculosis [13], and pulmonary fibrosis [14].

The work aimed to investigate the effect of bone marrow MSCs in combination with anti-tuberculosis drugs (ATDs) on the course of experimental genital tuberculosis and the structural and functional characteristics of the fallopian tubes in model animals.

MATERIALS AND METHODS

The experiment was performed on 27 female chinchilla rabbits weighing 2.5–3.0 kg in a certified vivarium. The criteria for the inclusion of animals in the experiment were an increase in body weight during the quarantine period and the absence of visible disease symptoms. The efficiency of MSCs in combination with comprehensive specific chemotherapy was evaluated using a previously developed model of isolated tuberculosis of the female genital organs [15]. To simulate genital tuberculosis, a suspension of a standardized drug-susceptible virulent strain of *M. tuberculosis Erdman* (MTB) of the second generation was used, which was injected under the serous membrane of the left fallopian tube at a dose of 10^7 CFU/0.2 mL. Thirty days after infection contamination, the experimental animals were divided into the following groups: group 1 (n = 6) included intact animals, group 2 (n = 7) included rabbits infected without further treatment (infection control), group 3 (n = 7) included rabbits treated only with ATDs, and group 4 (n = 7) consisted of animals treated with ATDs in combination with bone marrow mesenchymal cells.

Anti-tuberculosis therapy was started with positive results of an intradermal test with recombinant tuberculosis allergen (RTA) 1 month after infection contamination using isoniazid (Moskhimfarmpreparaty im. N.A. Semashko, Russia; intramuscularly at 10 mg/kg), rifampicin (Macleods Pharmaceuticals LTD, India; intragastrically at 10 mg/kg), ethambutol (Lupin LTD, India; intragastrically at 20 mg/kg), and perchlozon (tioureidoiminomethylpyridinium perchlorate, Pharmasyntez, Russia; intragastrically at 15 mg/kg). Allogeneic MSCs were isolated and cultivated according to the standard method at the Center for Cell Technologies of the Institute of Cytology, Russian Academy of Sciences [16, 17]. Immunophenotyping of passage 3 cells was performed using Abcam monoclonal antibodies (USA) on an Epics XL flow cytometer (Beckman Coulter Inc., CA, USA). The relative counts of positive cells for $CD90^+$ and $CD105^+$ immunophenotypic markers characteristic of MSCs were 81% and 92%, respectively; the $CD45^+$ hematopoietic marker was absent. The stain PKH-26 (1 kit Lot 122k 0428 PKH26 RED Fluorescent cell linker mini kit, Sigma-Aldrich, USA) was used for intravital labeling. The stain was administered into the cells according to the standard procedure [18]. Cells in stain-containing tissues were detected by indirect immunofluorescence. Stained cells were identified using a Leica TCSSL confocal microscope (Zeiss, Germany). MSCs were transplanted once under the serous membrane of the left uterine horn at a concentration of 5 million/mL 2 months after the start of chemotherapy in group 4.

The control of the tuberculosis infection activity in experimental animals and evaluation of the treatment efficiency were performed as follows.

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1. Performing an intradermal test with RTA 30, 90, and 150 days after infection contamination. RTA was administered to experimental animals intradermally on the back in the zone of projection of the infected fallopian tube at a concentration of 2 μg/mL in 0.1 mL of isotonic sodium chloride solution. The test result was evaluated 72 h after RTA administration, determining the erythema diameter in millimeters.
2. Evaluation of hematological (Emerald, Abbot, USA) and biochemical (Synchron, Beckman Coulter, Inc.) peripheral blood parameters before infection and at 30, 90, and 150 days after MTB inoculation.
3. Confirmation of an isolated tuberculous process in the genitals. An isolated tuberculous process was evidenced by the absence of changes in the lung tissue according to the results of multislice computed tomography (Aquilion 32 tomograph, Canon Medical Systems Corp., USA) 30 days after infection.
4. Macroscopic assessment of the genital organs. Diagnostic laparoscopy was performed 30 days after MTB inoculation (n = 27) under combined general anesthesia (Zoletil at a dose of 25 mg/kg and Rometar 2%, 1.0 mL each intramuscularly).
5. Evaluation of the patency of the fallopian tubes based on hysterosalpingography results. It was performed 150 days after modeling tuberculous salpingitis immediately before euthanasia. During laparotomy, 1 mL of urografin at a dose of 75 mg/mL was injected into each horn. X-ray images were taken immediately after administration and then after 5 and 10 min.
6. Assessment of the phagocytic activity (PA) of peritoneal macrophages (pMs). PMs were obtained by flushing the abdominal cavity of rabbits with medium 199 containing 10% bovine serum and 5 IU/mL heparin. A cell suspension of pM (1×10^6) was placed on disposable plastic Petri dishes and incubated at +37°C for 1 h in an atmosphere of 5% CO_2. After removal of pM not attached to the monolayer, a suspension of yeast cells of the genus *Saccharomyces cerevisiae* (1×10^7 cells per dish) preliminarily opsonized with mouse serum was added. Counting was performed at 80-fold magnification. Based on the data obtained, the indicators of pM PA as the proportion of pMs involved in phagocytosis were calculated, including the phagocytic number as the average count of yeast cells absorbed by one pM, phagocytosis completion rate (PCR) as the count of yeast cells digested by pMs for 1.5 h of cultivation, and phagocytosis completion index as the ratio of the phagocytic number for 1 h of cultivation to the phagocytic number for 2.5 h of cultivation. Bacteriological studies were performed 150 days after infection contamination using the dosed inoculation of biopsy specimens or homogenates of the mucous membrane of the fallopian tube on a Löwenstein–Jensen dense egg-based medium by serial dilutions. The lower limit of the method sensitivity was 2×10^3 CFU of MTB. The pathomorphological study included necropsy, macroscopic examination, and histological examination of internal organs. Histological specimens were stained with hematoxylin and eosin, according to Ziehl–Neelsen.

Statistical processing of the material was performed using Microsoft Excel 2013 (Microsoft Corp., USA) and Statistica 7.0 for Windows (Stat Soft Inc., USA). Statistical processing of the results obtained was performed using parametric and nonparametric statistics. Descriptive statistics included the estimation of the arithmetic mean (M), mean error of the mean values (m) for attributes with a continuous distribution, and incidence of characteristics with discrete values. The critical significance level (p) for testing null hypotheses was taken as $p < 0.05$. To assess the treatment efficiency, the method of analysis of variance for dependent samples (ANOVA repeated) was used.

RESULTS

Thirty days after MTB inoculation, in response to intradermal administration of RTA, a negative test result was obtained in intact animals. A positive reaction (18.50 ± 1.49 mm erythema; $p < 0.0001$) was recorded in 21 model animals, after which the model animals were distributed into groups. In the peripheral blood of the infected rabbits, the level of C-reactive protein was increased (from 0.6 ± 0.6 mg/L to 10.8 ± 2.8 mg/L, $p < 0.001$), which confirmed the development of the inflammatory process. Multislice computed tomography of the lungs in model animals 30 days after infection contamination revealed that no animals had any specific changes in the lung tissue, which indicated the development of a local specific process in the genital organs. In the experiment, the control group developed tuberculous pansalpingitis with obliteration of the lumen of the fallopian tubes. The results of the hematological studies also confirmed the inflammatory activity; in addition, the leukocyte counts and erythrocyte sedimentation rate (ESR) exceeded significantly the indicators in the experimental groups during the entire experiment. At the final stage, the leukocyte counts were 1.4–1.6 times higher than data of groups 4 and 3 ($14.95 \pm 5.31 \times 10^9$/L versus $8.71 \pm 1.4 \times 10^9$/L and $8.97 \pm 0.62 \times 10^9$/L, $p < 0.0$; $13.2 \pm 5.63 \times 10^9$/L versus $8.18 \pm 1.39 \times 10^9$/L and $9.32 \pm 1.36 \times 10^9$/l, $p < 0.05$), whereas the ESR was 2.1–2.3 times higher in groups 4 and 3 ($p < 0.05$). In addition, in infected untreated animals, a consistently high level of C-reactive protein was registered throughout the follow-up period, which amounted to 13.75 ± 2.0 g/L by the end of the experiment compared with 2.2 ± 1.2 g/L in group 3 (ATD) and 1.1 ± 0.8 g/L in group 4 (ATD + MSC) ($p < 0.01$).

The abdominal cavity was examined over time. After 60 days, progressive swelling, severe hyperemia, and dilatation of the ampullar section of the fallopian tube on the side of the infected uterine horn were noted. MTB growth was noted in cultures of mucosal homogenates. During repeated revisions of the abdominal cavity of the model animals 150 days after infection contamination, loose and planar adhesions and obliteration of the fallopian tubes were revealed, and hysterosalpingography revealed their occlusion. Microscopic examination revealed severe necrotic changes in the infected fallopian tube wall of rabbits of group 2 after 150 days, most pronounced in the epithelium. The endometrium was rejected, and the horn walls were ectasic. The inner surface of the horn wall was represented by an epithelioid-macrophage bank without the formation of giant multinucleated cells, loosely associated with the lamina propria densely infiltrated by lymphocytes and neutrophils. The muscle layer was indurated, with symptoms of myocyte dystrophy. The perimetrium was thickened to some extent and slightly infiltrated with neutrophils and lymphocytes. An impairment of the entire epithelial layer integrity was detected, including the formation of ulcerative defects and erosions, in the bottom of which nuclear detritus and decaying leukocytes were determined. In addition, a typical granulomatous reaction with caseous-necrotic foci surrounded by an epithelioid cell bank was revealed, and accumulations of fibroblasts and lymphoid elements were located outside. Single Langhans cells were also detected there. Loosening, edema, focal lymphohistiocytic reaction, and necrobiotic changes were noted in the muscular and serous membranes. The tube lumen contained abundant necrotic masses representing cellular and nuclear detritus. Vascular disorders were also detected. In some places, the vessels were dilated, whereas in others, the lumen was distinctly narrowed and was slit-shaped following compression by the perivascular lymphohistiocytic infiltrate. When stained according to Ziehl–Neelsen, numerous mycobacteria were noted in necrotic masses.

After 60 days of treatment in group 3, cultures of homogenates of the mucous membrane of the fallopian tubes were found to be sterile. The average size of the erythema in the sample with RTA significantly decreased (to 4.9 ± 1.0 mm versus 21.0 ± 1.28 mm for 30 days of treatment, $p < 0.05$). Analysis of hematological data presented a significant decrease in the level of leukocytes from 14.95 ± 5.31 × 10^9/L in group 2 to 8.71 ± 1.4 × 10^9/L ($p < 0.05$), which was almost comparable to the findings of the intact group after 120 days of therapy (8.18 ± 1.39 × 10^9/L versus 7.67 ± 0.47 × 10^9/L). After 60 days of therapy, the level of C-reactive protein in the peripheral blood significantly decreased to 3.5 ± 1.3 mg/mL versus 13.7 ± 2 mg/mL in group 2 ($p < 0.01$), and its concentration decreased by 1.6 times by day 120 of the experiment. An endoscopic assessment of the abdominal cavity and small pelvis organs exhibited a moderate degree of adhesions, and no gross deformities of the fallopian tubes were found. During hysterosalpingography, partial patency of the fallopian tubes was noted. In group 3, no necrotic masses in the lumen of the tube were found, which indicated the restoration of its peristalsis and evacuation ability. The epithelial lining was determined over a larger extent, but in some areas, it was thinned, somewhat loosened, and consisted of flattened epithelial cells. The fusion of the mucous membrane folds and formation of rough papillary structures with fibrous stroma were noted. For the most part, the normal general cytological characteristics of epitheliocytes were also preserved. Maximum changes were detected in the submucosa and adjacent layer of circular muscle fibers. Alternation of hyperemic and ischemic foci was noted in the muscle layer, and perivascular lymphohistiocytic accumulations were detected between the bundles of muscle fibers. No circulatory disorders were detected in the tube wall. The serous membrane was thickened; loosening and fibrosis of subserous tissue were observed. Microscopic examination of an intact tube revealed small perivascular lymphoid accumulations and loosening of the subserous tissue in its wall.

On day 90 after infection contamination in group 4, the inflammatory activity decreased. On day 150 after infection, the erythema size decreased significantly by 7 times in response to RTA administration ($p < 0.05$), the level of C-reactive protein decreased significantly by 2 times (1.1 ± 0.8 mg/L vs. 2.2 ± 1.2 mg/L, $p < 0.01$), and the leukocyte count decreased by 1.2 times (8.18 ± 1.39 × 10^9/L vs. 9.32 ± 1.36 × 10^9/L, $p < 0.05$), compared with the group treated with ATD alone.

When assessing phagocytosis in the infection control group (group 2), phagocytic reactions were inhibited when compared with that in the intact group, that is, absorptive by 1.3 times in terms of PA (from 42.8% ± 0.71% to 33.67% ± 2.66%, $p < 0.05$) and digestive by 2.3 times (according to PCR, from 88.67 ± 14.0 yeast cells to 37.0 ± 4.48, $p < 0.01$). The use of an MSC-based cell product also led to a significant increase by 1.3 times in the absorptive capacity of the pMs by PA (up to 43.8% ± 3.01% versus 33.67% ± 2.66% in the infection control group, $p < 0.05$) and that by 1.8 times in digestive capacity according to PCR (up to 67.75 ± 5.14 yeast cells versus 37.0 ± 4.48, $p < 0.05$).

In group 4, during visual monitoring, a comparative assessment of the macroscopic criteria of a local inflammatory reaction indicated a clear tendency of the adhesive process stabilization, represented mainly by single, loose narrow adhesions without deformity of the anterior abdominal wall and fallopian tubes. No signs of fibrin organization were found. The alteration in the area of the infected fallopian tube proceeded less aggressively; the volume and relief of the ampullar section of the fallopian tube were preserved. During hysterosalpingography, the fallopian tubes were

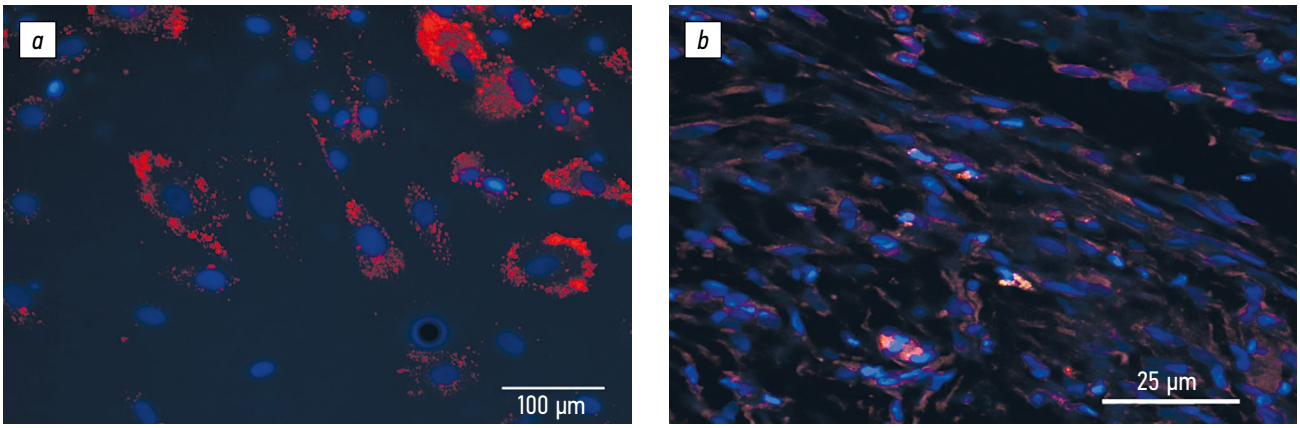

Figure. Walls of the fallopian tube of a female rabbit from group 4 (day 150 after mycobacteria inoculation), confocal microscopy, ×40; *a,* mesenchymal stem cells in a monolayer, *in vitro* staining with PKH-26 (scale bar: 100 μm); *b,* mesenchymal stem cells in fallopian tube cryosections (scale bar: 25 μm)

passable. The combination of specific therapy with MSC administration affected significantly the nature of fibrotic changes in the fallopian tube. The tube lumen was free; the microscopic structure of the normal folding of the mucous membrane was seen almost along its length. The epithelium was located on a fine fibrillar basis and was represented by secretory and ciliated cells with a clear predominance of the latter. In the deep layers of the submucosa, where significantly pronounced fibrotic changes and proliferation of myofibroblasts were noted in rabbits treated with ATD, in group 4, thin, loosely arranged collagen fibers and a gently basophilic cell-free matrix were revealed, against which fibroblasts and a few myofibroblasts were distinguished. Between the collagen fibers and noncellular matrix, the terminal sections of the glands without any dystrophic or atrophic signs were visible. Next to these epithelial structures in the submucosa, numerous pointed or polypoid processes of endothelial cells were noted, directed to similar processes of the endothelium of the nearest capillary and typical elements of maturing nonspecific granulation tissue in the form of small newly formed vessels with a thickened wall, juicy endothelium, and the same juicy adventitional cells. The blood filling of the submucosal vessels was normal. Accumulations of macrophages and plasma cells around small veins were noted. Acute vascular inflammatory changes were also not detected. Neither specific granulations nor mycobacteria were found. Thus, in rabbits that received MSCs during ATD, the final stage of tuberculous salpingitis was registered with a predominance of a regenerative reaction in the mucosa and submucosa of the fallopian tube without signs of excessive fibrosis.

Confocal microscopy of cryosections 120 days after MSC transplantation demonstrated the incorporation of labeled viable MSCs into different layers of the fallopian tube wall at the end of the experiment. Their viability was evidenced by fluorescent foci of red (MSC membrane labeled with PKH-26) and blue glow (nuclei labeled with DAPI stain) (Fig. 1).

DISCUSSION

Tuberculosis of the genital organs was modeled in 21 rabbits, and 6 animals were left intact. When MTB was injected into the wall of the fallopian tube in rabbits, severe tuberculous pansalpingitis occurred [19]. Isolated tuberculosis of the genital organs was confirmed by the absence of pathological changes in the lungs during multislice computed tomography of the chest cavity of rabbits 30 days after infection contamination and according to the results of a morphological study in experimental animals, obtained when the latter were sacrificed.

Animals of group 2 exhibited progression of the isolated tuberculous process in the form of persistent erythema by the end of the experiment in response to the intradermal test with RTA (24.5 mm versus 4.9 mm in group 3; 0.14 mm in group 4, $p < 0.01$). The leukocyte count and ESR exceeded significantly the indicators in the experimental groups during the experiment, and at the final stage, the leukocyte count was 1.4–1.6 times ($p < 0.05$) higher than the leukocyte count in groups 4 and 3, respectively, and the ESR was 2.1–2.3 times higher in groups 4 and 3 ($p < 0.05$).

The evaluation of the efficiency of phagocytosis of pMs in infection control rabbits revealed the inhibition of phagocytic reactions in two of the four studied parameters compared with the intact group, namely, absorptive by 1.3 times by PA (from 42.8% ± 0.71% to 33.67% ± 2.66%, $p < 0.05$) and digestive by 2.3 times (according to PCR, from 88.67 ± 14.0 yeast cells to 37.0 ± 4.48, $p < 0.01$). Mononuclear phagocytes, along with type 1 T-helper lymphocytes, are believed to be the most significant immune cells in the development of tuberculosis infection.

At 150 days after infection, in the infection control group, a pronounced cicatricial adhesive process in the genitals was detected, as well as the formation of hydrosalpinges in half of the cases. Hysterosalpingography revealed bilateral occlusion of the fallopian tubes. According to the results

of a morphological study, during Ziehl–Neelsen staining, acid-resistant forms of bacteria were found in the caseous masses of the affected uterine horn.

Group 3 presented a decrease in specific inflammation compared with the infection control group. After 60 days of treatment, MTB was not detected in cultures of homogenates of the mucous membrane of the fallopian tubes. The average size of the erythema in the sample with RTA significantly decreased to 4.9 ± 1.0 mm versus 21.0 ± 1.28 mm at day 30 of treatment ($p < 0.05$). The analysis of the findings of hematological studies of the peripheral blood after 60 days of therapy revealed a significant decrease in the leukocyte count from 14.95 ± 5.31 (10^9/L) in the control of infection to 8.71 ± 1.4 ($p < 0.05$) with the stabilization of this indicator to the completion of the treatment course (in 120 days), which was almost comparable to the indicators of the intact group. At 60 days after the start of the treatment course, the level of C-reactive protein in the peripheral blood decreased significantly to 3.5 ± 1.3 mg/mL versus 13.7 ± 2 mg/mL in the infection control group ($p < 0.01$). In the subsequent period of therapy, its level continued to decrease, and by day 120 of the experiment, this figure decreased by 1.6 times. Under the influence of anti-tuberculosis therapy, along with the activation of the reparative reaction, signs of excessive fibrosis appeared. The involution of tuberculous lesions of the fallopian tubes in rabbits receiving only etiotropic therapy was accompanied by pronounced fibrotic changes and multiple adhesions deforming the fallopian tubes and the abdominal wall.

The use of a specific MSC therapy made it possible to channel the inflammatory process toward a reparative reaction. In this case, a more or less complete restoration of the structural integrity of the tubes was combined with the normalization of physiological functions, particularly cellular immunity. The use of bone marrow MSCs was accompanied by a significant increase by 1.3 times in the absorptive capacity of pMs by PA (up to 43.8% ± 3.01% versus 33.67% ± 2.66% in the control of infection, $p < 0.05$) and digestive capacity by 1.8 times by PCR (up to 67.75 ± 5.14 yeast cells versus 37.0 ± 4.48, $p < 0.05$) almost to the level of intact animals. Experimental data have been obtained on the regulation by stromal cells of the production of systemic and local Th1/Th2 cytokines in the treatment of recurrent miscarriage in mice. Published data revealed that MSCs have the same bactericidal mechanisms as macrophages, and they act on pathogens with free oxygen radicals, NO molecules, and hydrolytic enzymes of lysosomes that merge with phagosomes. Autophagocytosis also plays a significant bactericidal role, which is intensified to a certain extent when MSCs are administered together with antituberculous, antitumor, and antiparasitic drugs [20]. On day 120 of anti-tuberculosis therapy in combination with MSCs in rabbits, no increase in MTB was registered, but there was a significant decrease in the size of erythema in response to intradermal administration of RTA by 7 times ($p < 0.05$), a significant decrease by 2 times in the level of C-reactive protein in the peripheral blood, and decrease in leukocytes by 1.2 times compared with the group treated with ATD alone.

The survival of MSCs in the tissues of the fallopian tube wall of recipient rabbits for 2 months after transplantation has been experimentally confirmed, that is, at the end of the experiment. Their viability was evidenced by fluorescent foci of red (MSC membrane labeled with PKH-26) and blue (nuclei labeled with DAPI stain).

The use of MSCs in combination with a specific therapy was accompanied by a decrease in the severity of fibrotic changes in the fallopian tubes. The epithelial layer was formed, the mucous membrane was represented by functioning secretory and ciliated cells, the submucosa had minimal signs of fibrotic changes, and no signs of atrophy were determined in the glandular structures. Between the collagen fibers and noncellular matrix, the terminal sections of the glands were visible without any dystrophic or atrophic signs. Thus, in rabbits treated with a combination of ATD with MSC administration, a regenerative reaction of the epithelium and subepithelial layer of the fallopian tubes was formed with almost complete restoration of muscle structures.

The effect of MSC cell therapy on the course of inflammation and fibrosis processes can most probably be associated with the anti-inflammatory effect of MSC revealed, according to the literature, which is manifested by the ability to reduce the infiltration of the inflammation focus by neutrophils and inhibit the production of pro-inflammatory cytokines [21]. Moreover, the restoration of the structural integrity of the fallopian tubes, noted in this study during the use of cell therapy, is possibly due to the ability of MSCs to accelerate tissue repair and regeneration. According to the literature, MSCs are multipotent and can migrate to the site of damage, fixate, differentiate, perform the function of replaced cells, and stimulate growth factors [22]. Divergent differentiation of MSCs toward osteoblasts, adipocytes, and nonphagocytic reticular cells and the regulatory effect of MSCs on the differentiation of endothelial cells, osteoclasts, and macrophages have been established [23]. The decrease in the inflammatory activity and prevention of the development of early fibrosis with MSC administration at the stage of reverse development of experimental tuberculosis of the genitals in rabbits (after 2 months of specific therapy) may be due to both the anti-inflammatory effects of MSCs and their stimulating effect on growth factors and differentiation of the main repair cells.

CONCLUSIONS

In laboratory animals (rabbits), inoculation with a mycobacterial suspension into the ampullar segment of the fallopian tube results in a typical tuberculous pansalpingitis,

proceeding according to the type of primary tuberculosis. Single local injection of bone marrow MSCs after 3 months of anti-tuberculosis therapy in rabbits with tuberculosis infection of the genital organs reduces the degree of sensitization and activity of a specific inflammatory process, prevents the deformity of the ampullar section of the fallopian tube, helps restore its structural and functional integrity, prevents early fibrosis, and provides re-epithelialization of the inner lining of the fallopian tube.

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