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EFFECT OF SILVER NANOCOMPOSITES TREATMENT ON FEMALE REPRODUCTIVE FUNCTION

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ABSTRACT

Recently, it has been proved that polymers with a dextran core and grafted polyacrylamide chains (D-g-PAA) were effective nanoplatform for creation of nanocomposites for chemotherapy and photodynamic therapy. Also, these polymers can be like a matrix for stable silver sol synthesis, that gives confidence in the prospect of silver-based nanosystems for biomedical application. An animal studies are becoming more relevant to determine the effect of the treatment of nanosilver on female reproductive function in particular. In this work for the first time we have obtained data about the influence of star-like D70-g-PAA polymers on somatic, germenative cells, embryos and live newborns (pups) in mammals, which had not previously been investigated. No significant changes in meiotic maturation of such ovarian oocytes *in vitro*, the number of living cells of FEO and the number of such cells with morphological signs of apoptosis and necrosis, pre- and post-implantation mortality rates of embryos and the number of live newborns (pups) have been established under conditions of one-time treatment with D70g-PAA and D70-g-PAA(PE) at doses of 0,39 mg/kg and 3,90 mg/kg and AgNPs/D-g-PAA and AgNPs/D-g-PAA(PE) at doses of 0,20 mg/kg and 2,00 mg/kg. The effect of AgNPs/D-g-PAA(PE) at a dose of 2,00 mg/kg on oocytes is inhibitory (reduces the number of oocytes in the ovary). The inhibitory effect of such polymer D70-g-PAA (PE) at a dose of 3,90 mg/kg on oocytes was also established. However, there is no additional effect of AgNPs/D70-g-PAA(PE) on oocytes compared to the effect of the polymer itself (D70-g-PAA(PE). The inhibitory effect of AgNPs/D70-g-PAA and AgNPs/D70-g-PAA(PE) on cells of ILN has been established. However, the effect of D70-g-PAA and D70-g-PAA(PE) on cells of ILN is not suppressive. An additional effect of AgNPs/D70-g-PAA(PE) (2.00 mg/kg) on cells of ILN was also compared with the effect of D70-g-PAA(PE) polymer itself.

KEYWORDS: dextran-polyacrylamide polymers, pre- and post-implantation mortality, meiotic maturation of oocytes, apoptosis, necrosis.

INTRODUCTION

Silver nanoparticles (AgNPs) play an important role in nanosciences and nanotechnologies, especially in nanomedicine, owing to their extraordinary properties, including chemical stability, conductivity, catalytic activity, antibacterial, antifungal, antiviral and anti-inflammatory activity.

Due to their cytotoxic potential, AgNPs have been extensively investigated, in particular in cancer studies.[1] However, metal nanoparticles tend to aggregate through the large surface energy. Different types of stabilizing agents were used to prevent aggregation of nanoparticles,[2,3] these include polymers.

Nowadays, the use of nanotechnology to create new high-performance biomedical nanocomposites proves to be one of the most relevant research topics. Due to the structural features and manageability of the intramolecular structure, branched polymer systems are interesting objects of basic research as well as promising functional materials of the new generation. Such polymer systems are characterized by a more compact structure and, therefore, a higher concentration of functional groups compared to linear analogs of close molecular weight.

Recently, it has been proved that polymers with a dextran core and grafted polyacrylamide chains (D-g-PAA) were effective nanoplatform for creation of nanocomposites for chemotherapy and photodynamic therapy.[4,5]

Also, these polymers can be like a matrix for stable silver sol synthesis,[6] that gives confidence in the

prospect of silver-based nanosystems for biomedical application. An animal studies are becoming more relevant to determine the effect of the treatment of nanosilver on female reproductive function in particular.

This study aim is to evaluate the effect of a one-time treatment of different doses of AgNPs/D-g-PAA nanocomposite on reproductive function, namely on 1) the number of oocytes isolated from one ovary and the meiotic maturation of such ovarian oocytes *in vitro*; 2) the indicators of cell viability of the follicular environment of oocytes cells (FEO) and the cells of inguinal lymph nodes (ILN); 3) the pre- and post-implantation mortality rates and the number of live newborns (pups) in female mice.

MATERIALS AND METHODS

***Polymer matrix*.** As a nanocarrier we used a branched copolymer obtained by grafting polyacrylamide (PAA) chains onto dextran (M_w=7×10^4, g×mol^{-1}) backbone using a ceric-ion-reduce initiation method.[6] This redox process initiates free radical sites exclusively on the polysaccharide backbone, thus preventing from the formation of homopolymer polyacrylamde.

The detail of synthesis, identifications and analysis of internal polymer structure were described in.[7] The theoretical number of grafting sites per polysaccharide backbone for the sample we used as polymer nanocarrier in the present work was equal to 5, and the related dextran-graft-polyacrylamide copolymer was referred as D70-*g*-PAA. The choice of this copolymer among the series of the branched samples synthesized based on our previous research.

The D70-*g*-PAA copolymer was saponified by alkaline hydrolysis using NaOH to obtain branched polyelectrolyte Dextran-graft-(Polyacrylamide-co-Polyacrylic acid) referred as D70-*g*-PAA(PE) throughout.[7] The degree of saponification of carbamide groups to carboxylate ones onto PAA-granted chains determined by potentiometric titration was equal to 43 %.

The molecular parameters of non-charged and charged polymer matrix is shown in the Table 1, namely M_w - the weight average molecular weight; M_n - the number average molecular weight; R_g - the radius of gyration; M_w/M_n – the polydispersity of polymer samples.

Table 1: Molecular parameters of D-PAA polymer matrices.

Sample	M_w×10^{-6}, g×mol^{-1}	R_g, nm	M_w/M_n
D70-g-PAA	1,43	64	1,98
D70-g-PAA(PE)	1,43	-	1,98

AgNPs/Polymer nanosystem synthesis. AgNPs were synthesized by reduction of Ag precursor ($AgNO_3$) dissolved in polymer solution. 2 ml of a 0,1M $AgNO_3$ aqueous solution was added to 5 ml of aqueous polymer solution (C=1×10^{-3} g×cm^{-3}) and stirred during 20 min. Then, 2 ml of 0,1M aqueous solution of $NaBH_4$ was added. The final aqueous solution was stirred during 30 min. It turned reddish brown, thus the formation of AgNPs was indicated.

The identification of nanoparticles and the determination of their sizes were carried out using a Phillips CM12 high resolution transmission electron microscope (TEM) (Amsterdam, Netherlands). The samples were prepared by spraying the sol onto a copper grid. The TEM images were analyzed using the ImageJ program which allows to calculate a diameter (d) of nanoparticles from their geometric characteristics, namely, from the area S occupied by a particle in the image, either by their long d_1 or short d_i diameters (if a particle is not spherical). In this case, $d = [d_1 + d_2]/2$. For each nanosystem, several images were analyzed.

The TEM investigation of silver sols has shown that NPs synthesized in uncharged D70-g-PAA polymer matrix had the size of 8—15 nm (Fig. 1, *a*). The silver nanoparticles size distribution obtained by the computer analysis of the TEM images is presented in Fig. 1, *b*.

The silver sol synthesized in charged polymer matrix differ from the sols synthesized in non-charged matrix. Figs. 1, *c*, *d*. In can be observed the AgNPs with a size of 10—15 nm (*A*), as in the sols synthesized in the uncharged polymer matrix, AgNPs with a size of 2—5 nm also appeared (Fig. 1, *d*).

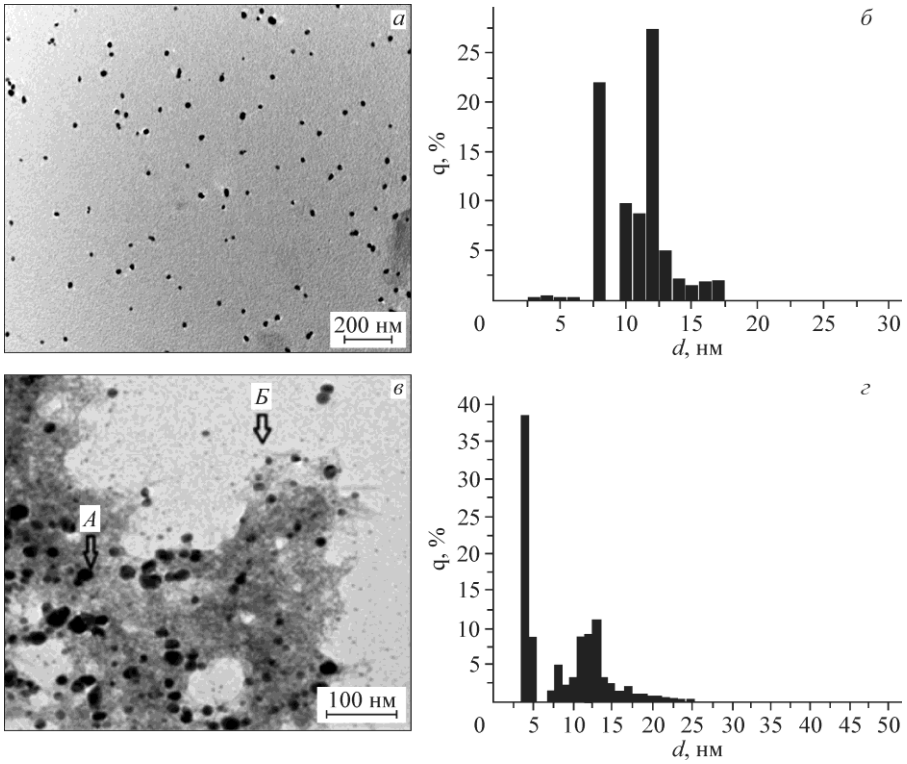

Fig. 1: TEM images of silver NPs and histograms of the NP size distribution in the sols synthesized in D70-g-PAA (*a*, *b*) and D70-g-PAA(PE) (*c*, *d*) polymer matrices.

TEM images (Fig. 1) demonstrated the differences in the sols obtained in the solutions of star-like uncharged and charged polymers and it can be explained by the chemical nature of both polymer matrices.

The interaction of silver ions with the anionic (charged) polymer matrix takes place with both carbamide (as in uncharged polymers) and carboxylated groups. Obviously, on the carbamide groups of charged matrices the same particles form as in the uncharged matrices (Fig. 1, *a*), particles formed on the carboxylated groups are smaller (Fig. 1, *c*).

Thus, the study of silver sols synthesized in branched polymer matrices of various natures has shown that AgNPs are spherical irrespective of the matrix nature. However, in the uncharged polymer matrix the particles with a size of 10—15 nm predominantly formed. In the charged polymer matrices the particles with a size less than 4 nm also observed.

***The treatment of substances was carried out in the following way*:** D70-g-PAA (0,39 and 3,90 mg/kg), D70-g-PAA(PE) (0,39 and 3,90 mg/kg), AgNPs/D70-g-PAA (0,20 and 2,00 mg/kg), AgNPs/D70-g-PAA(PE) (0,20 and 2,00 mg/kg), saline solution were introduced intravenously (in the tail vein) once a day.

Animals. Experiments (two series) have been conducted on 99 (45+54) females and 18 male white laboratory mice (weighing 20-22 g) in compliance with all requirements for work with laboratory animals (International European Convention for the Protection of Vertebrate Animals, Strasbourg, 1986). After the experiments, anesthetized by Nembutal animals were exterminated by cutting the spinal cord. The objective status of the animals (appearance, overall motor activity, need for food and water, and body weight) were evaluated before and during the experiment.

In the first series of experiments, animals were divided into the following groups treated with: I – physiological solution (0,3 mL) – control (N=5); II – D70-g-PAA (at a dose of 0,39 mg/kg) (N=5); III – D70-g-PAA at a dose of 3,90 mg/kg (N=5); IV - AgNPs/D70g-PAA at a dose of 0,20 mg/kg (N=5); V – AgNPs/70D-g-PAA at a dose of 2,00 mg/kg (N=5); VI –D70-g-PAA(PE) at a dose of 0,39 mg/kg (N=5); VII – D70-g-PAA(PE) at a dose of 3,90 mg/kg (N=5); VIII –AgNPs/D70-g-PAA(PE) at a dose of 0,20 mg/kg (N=5); IX – AgNPs/D70-g-PAA(PE) at a dose of 2,00 mg/kg (N=5); N is the number of animals in the group.

The sampling of the experimental material (ovaries and ILN) was performed under anesthetic anesthesia the day after administration. The animals were removed from the experiment by cutting the spinal cord under anesthetic anesthesia, following the rules of euthanasia.

In the second series of experiments, animals were divided in to the same groups: I – physiological solution (0,3 mL) – control (N=6); II – D70-g-PAA (at a dose of 0,39 mg/kg) (N=6); III – D70-g-PAA at a dose of 3,90 mg/kg (N=6); IV - AgNPs/D70-g-PAA at a dose of 0,20 mg/kg (N=6); V – AgNPs/D70-g-PAA at a dose of 2,00 mg/kg (N=6); VI – D70-g-PAA(PE) at a dose of 0,39

mg/kg (N=6); VII – D70-g-PAA(PE) at a dose of 3,90 mg/kg (N=6); VIII –AgNPs/D70g--PAA(PE) at a dose of 0,20 mg/kg (N=6); IX – AgNPs/D70-g-PAA(PE) at a dose of 2,00 mg/kg (N=6); N is the number of animals in the group.

On the third day after the treatment, males were planted to the females in a ratio of 1:3 (male/females). Coupling and subsequent manipulation of embryos were performed according to the method by Mank (1990). Sampling of experimental material (ovaries, tubes, and uterus) was performed under anesthetic anesthesia for 10/11 days after replanting. The experiment was completed on day 24 after replanting the male with birth in control and experimental animals live newborns (pups).

***Oocytes cultivation*.** The oocytes were mechanically isolated from the ovaries of mice in a non-enzymatic way (without cumulus cells) and units/one ovaries (pieces on one ovary) were counted. Then oocytes from one group were collected and distributed into separate chambers 10-20 oocytes each. All control and experimental oocytes were cultured under the same conditions (a sterile box, cameras with 0,4 ml culture medium DME and 15 mM HEPES, Ca^{2+} concentration of 1,71 mM, temperature 37° C, duration 20 hours). Morphological study of oocytes was performed under a microscope MBS-10 after 2 hours of cultivation (% of total): the oocytes which restored the meiotic maturation (resumption meiosis) and were at metaphase I stage (germinal vesicle break-down) and after 20 hours of cultivation oocytes with the first polar body (were at metaphase II stage) and oocytes with atypical morphology (unevenly granulated cytoplasm and fragmentation characteristics of the latter) were counted.

Method of color fluorescent dyes. The apoptotic and necrotic death of FEO cells and cells of inguinal lymph nodes (ILN) was estimated by morphological characteristics using the method of *in vivo* dual-color fluorescent dye nucleic acids Hoechst 33342 and propidium iodide. At least 400 cells were evaluated using a fluorescence microscope LUAMAM I-1 (Russia) with an x85 water-immersion lens.

***Embryonic mortality in mice*.** The pre- and post-implantation death rates were calculated by the formulas: ((C-A+B) /C)•100% and (B/(A+B))•100%, respectively. Counted: A - the number of live embryos, B - the number of sites of resorption (the number of dead embryos), C - the number of yellow bodies of pregnancy.

Statistical analysis. For the statistical analysis of the results the software package Graph Pad Prism version 5.00 for Windows (Graph Pad Software, San Diego California, USA) have been used. The verification of the received data on normality of distribution was carried out on the Kolmogorov-Smirnov test. For a normal distribution, the statistical processing of the results when comparing the two data groups was performed using Student's t test, with more data groups using a single-factor ANOVA analysis, followed by a comparison of mean values between the groups according to the Newman-Keuls post hoc test; $p < 0.05$ was considered statistically significant.

Ethical approval. The study was approved by Bogomoletz Instytute of Physiology Ethical Committee, Kyiv, Ukraine.

RESULTS

Effect of one-time treatment with AgNPs/D70-g-PAA and AgNPs/D70g-PAA(PE) on number of oocytes isolated from one ovary. No significant changes in the number of oocytes isolated from one ovary were established under conditions of AgNPs/D70-g-PAA treatment (0,20 mg/kg and 2,00 mg/kg), respectively, 12,72±0,41 and 12,34±0,43 pieces, as well as D70-g-PAA treatment (0,39 mg/kg and 3,90 mg/kg), respectively, 13,17±0,31 and 12,88±0,35 pieces compared with such values in the control 13,57±0,57 pieces.

The number of oocytes isolated from one ovary were established under conditions of AgNPs/D70-g-PAA(PE) (0,20 mg/kg and 2,00 mg/kg), respectively, 11,92±0,41 and 9,47±0,32 pieces, as well as D70-g-PAA(PE) treatment (0,39 mg/kg and 3,90 mg/kg), respectively, 12,17±0,31 and 10,08±0,37 pieces compared with such values in the control 13,57±0,57 pieces.

Thus, under conditions of one-time treatment with AgNPs/D70-g-PAA(PE) at a dose of 2,00 mg/kg it was obtained the decrese ($p<0.05$, n=6) of number of oocytes isolated from one ovary compared to this value under the conditions of treatment with AgNPs/D70-g-PAA at a dose of 2,00 mg/kg. Under conditions of one-time treatment with D-PAA(PE) a dose of 3,90 mg/kg it was obtained the decrese ($p<0.05$, n=6) of number of oocytes isolated from one ovary compared to this value given under conditions of treatment with D-PAA at doses of 3,90 mg/kg.

Effect of one-time treatment with AgNPs/D70-g-PAA and AgNPs/D70-g-PAA(PE) on oocytes meiotic maturation in vitro. No significant changes in *in vitro* meiotic maturation of oocytes were established under conditions of AgNPs/D70-g-PAA (0,20 mg/kg and 2,00 mg/kg), respectively, resumed meiosis: 81,11±2,14% and 75,25±3,24%, formed first polar body: 62,40±0,73% and 60,62±3,12%, as well as D70-g-PAA treatment (0,39 mg/kg and 3,90 mg/kg), respectively, resumed meiosis: 77,71±2,13% and 79,24±1,43%; formed first polar body: 57,09±0,58% and 60,05±2,37% compared with such values in the control 81,05±1,06% and 60,98±1,31%.

No significant changes in *in vitro* meiotic maturation of oocytes were established under conditions of AgNPs/D70-g-PAA(PE) (0,20 mg/kg and 2,00 mg/kg), respectively, resumed meiosis: 75,41 ± 2,48% and 72,65

± 3,43%, formed first polar body: 55,74±2,34% and 55,27±3,23%, as well as, D70-g-PAA(PE) treatment (0,39 mg/kg and 3,90 mg/kg), respectively, resumed meiosis: 77,43±1,74% and 75,27±2,34%; formed first polar body: 58,03±1,73%, and 57,23±1,38% compared with such values in the control 81,05±1,06% and 60,98±1,31%.

Effect of one-time treatment with AgNPs/D70-g-PAA and AgNPs/D70-g-PAA(PE) on cells of FEO. Data about the number of cells of FEO with morphological signs of apoptosis and necrosis under conditions of one-time treatment with AgNPs/D70-g-PAA and AgNPs/D70-g-PAA(PE) are presented in Table 2.

Table 2: The number of cells of FEO with morphological signs of apoptosis and necrosis under conditions of one-time treatment with AgNPs/D70-g-PAA and AgNPs/D70-g-PAA(PE).

Group of animals	Dose mg/kg	The number of cells of FEO, %		
		Living	Apoptotic	Necrotic
Control		89,19±1,62	8,44±1,21	2,38±0,88
AgNPs/D70-g-PAA	0,20	87,58±1,56	8,58±0,97	3,83±0,93
AgNPs/D70-g-PAA	2,00	82,33±1,54	14,08±1,24	3,58±0,58
D70-g-PAA	0,39	86,75±1,21	9,83±1,29	3,42±0,58
D70-g-PAA	3,90	86,92±1,50	9,25±1,29	3,83±0,61
AgNPs/D70-g-PAA(PE)	0,20	83,24±3,47	12,64±1,83	4,12±0,67
AgNPs/D70-g-PAA(PE)	2,00	83,37±3,43	12,25±2,27	4,38±0,47
D70-g-PAA(PE)	0,39	85,52±2,28	10,21±1,73	4,27±0,53
D70-g-PAA(PE)	3,90	85,72±2,83	9,56±0,93	4,72±0,92

There have been received no significant changes in viability of cells of FEO under conditions of one-time treatment with AgNP/D70-g-PAA and AgNP/D70-g-PAA(PE) (0,20 mg/kg and 2,00 mg/kg), D70-g-PAA and D70-g-PAA(PE) (0,39 mg/kg and 3,90 mg/kg), compared with such values in control group.

Effect of one-time treatment with AgNPs/D70-g-PAA and AgNPs/D70-g-PAA(PE) on ILN cells. Data about the number of cells of ILN with morphological signs of apoptosis and necrosis under conditions of one-time treatment with AgNPs/D70-g-PAA and AgNPs/D70-g-PAA(PE) are presented in Table 3.

Table 3: The number of cells of ILN with morphological signs of apoptosis and necrosis under conditions of one-time treatment with AgNPs/D70-g-PAA and AgNPs/D70-g-PAA(PE).

Group of animals	Dose mg/kg	The number of cells of ILN, %		
		Living	Apoptotic	Necrotic
Control		85,75±1,13	10,75±1,22	3,5±0,65
AgNPs/D70-g-PAA	0,20	86,67±1,33	9,25±0,82	4,08±0,86
AgNP/D70-g-PAA	2,00	76,50±1,22*#	16,75±1,33*#	6,75±0,94
D70-g-PAA	0,39	86,75±1,08	9,67±1,17	3,58±0,80
D70-g-PAA	3,90	82,33±2,04	12,42±1,32	5,25±0,82
AgNPs/D70-g-PAA(PE)	0,20	82,31±1,83	12,51±0,92	5,18±0,89
AgNPs/D70-g-PAA(PE)	2,00	70,60±1,42*$@&	23,25±1,73*$@&	6,15±0,97
D70-g-PAA(PE)	0,39	83,58±1,11	11,33±1,33	5,14±0,82
D70-g-PAA(PE)	3,90	80,58±2,40	14,17±1,54	5,25±0,82

Note: * - $p<0.05$ - probability differences in the average group data with respect to these variables in the control group animals; # - $p<0,05$ - to these variables in the group animals under conditions of 0,20 mg/kg AgNPs/D70-g-PAA(PE) treatment; $ - $p<0,05$ - to these variables in the group animals under conditions of 0,20 mg/kg AgNPs/D70-g-PAA treatment; @ - $p<0,05$ - to these variables in the group animals under conditions of 0,20 mg/kg AgNPs/D70-g-PAA(PE) treatment; & - $p<0,05$ - to these variables in the group animals under conditions of 3,90 mg/kg D70-g-PAA(PE) treatment.

There has been received a decrease ($p<0.05$, n=6) in the number of:

1) living cells of ILN and an increase ($p<0.05$, n=6) in the number of apoptotic cells under the conditions of one-time treatment with AgNPs/D70-g-PAA (2,00 mg/kg), compared with the such values in control and under the conditions of treatment with AgNPs/D70-g-PAA (0,20 mg/kg);
2) living cells of ILN and an increase ($p<0.05$, n=6) in the number of apoptotic cells under the conditions of one-time treatment with AgNPs/D70-g-PAA(PE) (2,00 mg/kg), compared with the such values in control and under the conditions of treatment with AgNPs/D70-g-PAA(PE) (0,20 mg/kg);
3) living cells of ILN and an increase ($p<0.05$, n=6) in the number of apoptotic cells under the conditions of one-time treatment with AgNPs/D70-g-PAA(PE) (2,00 mg/kg), compared with the such values in

control and under the conditions of treatment with AgNPs/D70-g-PAA (2,00 mg/kg).

Effect of one-time treatment with AgNPs/D70-g-PAA and AgNPs/D70-g-PAA(PE) on pre- and post-implantation embryonic mortality rates. No significant changes in pre- and post-implantation mortality rates of embryos were established under conditions of treatment of AgNPs/D70-g-PAA at doses of 0,20 mg/kg and 2,00 mg/kg, D70-g-PAA at doses of 0,39 mg/kg and 0,39 mg/kg and respectively, of pre-implantation mortality: 8,64±0,82% and 8,23±0,87%, as well as 7,36±0,24% and 8,16±0,81% and post implantation mortality: 5,63±0,54% and 5,77±0,87%, as well as 4,82±0,58% and 4,63±0,48%, compared with such values in control 8,76±0,74% and 5,38±0,37 %.

No significant changes in pre- and post-implantation mortality rates of embryos were established under conditions of treatment of AgNPs/D70g-PAA(PE) at doses of 0,20 mg/kg and 2,00 mg/kg, D70-g-PAA(PE) at doses of 0,39 mg/kg and 0,39 mg/kg and respectively, of pre-implantation mortality: 8,52±0,81% and 8,53±0,83%, as well as, 8,12±0,23% and 8,63±0,78% and postimplantation mortality: 5,66±0,73% and 5,79±0,83%, as well as, 5,32±0,59% and 5,81±0,47% compared with such values in control 8,76±0,74% and 5,38±0,37 %.

Number of live newborns (pups) under conditions of treatment with AgNPs/D70-g-PAA and AgNPs/D70-g-PAA(PE). No significant changes in the number of live pups were found under conditions of treatment with AgNPs/D70-g-PAA at doses of 0,20 mg/kg and 2,00 mg/kg and D70-g-PAA at doses of 0,39 mg/kg and 0,39 mg/kg, respectively, 7,33±0,33 pcs and 6,67±0,33 pcs, 7,00±0,00 pcs and 7,00±0,58 pcs; compared with such value in the control 6,67±0,33 pcs.

No significant changes in the number of live pups were found under conditions of treatment with AgNPs/D70-g-PAA(PE) at doses of 0,20 mg/kg and 2,00 mg/kg and D-PAA(PE) at doses of 0,39 mg/kg and 0,39 mg/kg, respectively, 6,00±0,48 pcs and 6,00±0,84 pcs; 6,12±0,42 pcs and 6,34±0,39 pcs, respectively, compared with such value in the control 6,67±0,33 pcs.

DISCUSSION

There is evidence of intravenous (IV) treatment of AgNPs. [8,9] So, after the treatment of AgNPs in different ways silver is found in the main organs.[10-12]

For all particle sizes, regardless of their coverage, the highest concentrations of silver were found in the spleen and liver, followed in lungs, kidneys, and in brain 24 hours after intravenous treatment. Most of the silver that reaches the blood is filtered by the liver and excreted through the bile (gall, choler).[13-15]

However, we assume that intravenous (IV) administration can still provide valuable information about *in vivo* behavior of AgNPs that overcomes major barriers (skin, lungs, gastrointestinal tract), and in clinical circulation for clinical purposes (for example: dressings, intravascular medical devices, for diagnostic purposes, for drug delivery).

Because this study was not intended to simulate human exposure scenarios to avoid limited systemic exposure due to cellular barriers present in the skin, gastrointestinal tract, and lungs, we used intravenous (IV) treatment to evaluate potential toxicity to the female reproductive system of both AgNPs in dextran-polyacrylamide polymers and such polymers separately (alone).

Branched polymer systems with a branched star-like structure of macromolecules can be used as nanocarrier or nanocontainer for the transport and release of water-soluble drugs or for the transport of DNA in genetic engineering. Nanocarriers based on the branched copolymers dextran-graft-polyacrylamide (D-PAA) were synthesized, characterized, and tested on phagocytic cells.[4] It has been shown that these nanocarriers are actively captured by phagocytic cells and that they are not cytotoxic. The polymer nanocarriers loaded with cisplatin at different concentrations from 0,1 to 10,0 мкg/mL provided adose-dependent decrease in viability of chronic myelogenous leukemia and histiocytic lymphoma cells. The lowest percentage of viable cells has been observed for lymphoma cells (22%). When the copolymers were conjugated to both nanosilver and cisplatin, such a nanosystem displayed less cytotoxic effect compared to the conjugates to dextran-polyacrylamide and cisplatin.[4]

We used the following doses of D70-g-PAA polymers: 0,39 mg/kg and 3,90 mg/kg. It has been accepted that a dose of 10 mg/kg body weight in mice is equivalent to a human dose of 0,81 mg/kg body weight, equivalent to about 50 mg for humans 60 kg, according to the principles of conversion of doses of animals to humans.[16] Following this rule 3,90 mg/kg D70-g-PAA (and D70-g-PAA(PE)) in mice is equivalent to a human dose of 0,3159 mg/kg body weight, equivalent to approximately 20 mg (18,954 mg) for humans 60 kg (or 22,113 mg for humans 70 kg) according to the principles of conversion of animal doses to humans.[16]

As mentioned above, dextran-polyacrylamide polymer matrixes (uncharged and charged form) were loaded with silver nanoparticles (AgNPs/D70-g-PAA). The size of such silver nanoparticles was discussed above.

Previously, we were used nanoparticles AgNPs - 30 nm (spherical, synthesized at the Ovcharenko Institute of biocolloidal chemistry NAS of Ukraine according to the original protocol (by chemical condensation). And it was shown that the ten-time AgNPs treatment (2 mg/kg and 4

mg/kg) results in inhibition of oocytes meiotic maturation in mice; a single- and five-time AgNPs treatment (2 mg/kg and 4 mg/kg) increases the number of apoptotic cells, while the ten-time AgNPs treatment results in an increase of the apoptotic and necrotic follicular cells surrounding oocytes.[17]

Also we received data that under conditions of ten-time administration of AgNPs (20 mg/kg), the inhibition of reproductive function in female females is observed: a decrease in the number and quality of ovarian oocytes is established. The reproductive function in experimental animals is restored 37 days after the last administration of AgNPs; there are no differences between the values of pre- and post-implantation mortality of embryos on the 33/34th day after male planting; in one of the three experimental group animals, 7 live pups were born on the 42/43rd day after the male fertilization (in animals of the control group during this period: twice (6±1 (n=6) live pups).[18]

Since we had previously used a dose of AgNPs (2 mg/kg), this time again we applied the same 2 mg/kg and 10 times smaller dose 0,20 mg/kg of AgNPs that were loaded into the respective polymer matrixes (D-PAA) at doses of 0,39 mg/kg and 3,90 mg/kg, respectively. In this work for the first time we have obtained data about the influence of star-like D70-g-PAA polymers on somatic, germenative cells, embryos and live newborns (pups) in mammals, which had not previously been investigated:

No significant changes in meiotic maturation of such ovarian oocytes *in vitro*, the number of living cells of FEO and the number of such cells with morphological signs of apoptosis and necrosis, pre- and post-implantation mortality rates of embryos and the number of live newborns (pups) have been established under conditions of one-time treatment with D70g-PAA and D70-g-PAA(PE) at doses of 0,39 mg/kg and 3,90 mg/kg and AgNPs/D-g-PAA and AgNPs/D-g-PAA(PE) at doses of 0,20 mg/kg and 2,00 mg/kg.

The effect of AgNPs/D-g-PAA(PE) at a dose of 2,00 mg/kg on oocytes is inhibitory (reduces the number of oocytes in the ovary). The inhibitory effect of such polymer D70-g-PAA (PE) at a dose of 3,90 mg/kg on oocytes was also established. However, there is no additional effect of AgNPs/D70-g-PAA(PE) on oocytes compared to the effect of the polymer itself (D70-g-PAA(PE).

The inhibitory effect of AgNPs/D70-g-PAA and AgNPs/D70-g-PAA(PE) on cells of ILN has been established. However, the effect of D70-g-PAA and D70-g-PAA(PE) on cells of ILN is not suppressive. An additional effect of AgNPs/D70-g-PAA(PE) (2.00 mg/kg) on cells of ILN was also compared with the effect of D70-g-PAA(PE) polymer itself.

Our findings are consistent with data about the effect of AgNPs on embryos and oocytes obtained previously.[17,19,20] The fact that we did not find differences in embryo mortality rates between groups confirms that the experiments were performed with concentrations of AgNPs below those that cause overt toxicity. And also agrees with,[21] that the toxicity of large-sized AgNPs and Ag^+ on embryos may be related to the toxicity of free Ag^+, and this is further evidence for the hypothesis that AgNPs toxicity is mainly related to the bioavailability of Ag^+. There is evidence that no effects of silver nanoparticles have been established on mouse embryos, unlike silver nanoparticles, the effect of a comparable concentration of Ag^+ ions led to an immediate delay in embryo development.[22]

Further studies are needed to establish the effect of such AgNPs/D70-g-PAA and AgNPs/D70-g-PAA(PE), and D70-g-PAA and D70-g-PAA(PE), depending on the route of administration, multiplicity and dose.

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CONCLUSIONS

In this work we have obtained new data about the influence of silver nanoparticles in star-like polymers with Dextran core and grafted Polyacrylamide chains (in uncharged and charged form) on somatic, germenative cells, embryos and live newborns (pups) in mammals, which had not previously been investigated:

1. No significant changes in meiotic maturation of such ovarian oocytes *in vitro*, the number of living cells of FEO and the number of such cells with morphological signs of apoptosis and necrosis, pre- and post-implantation mortality rates of embryos and the number of live newborns (pups) have been established under conditions of one-time treatment with AgNPs/D70-g-PAA.

2. The inhibitory effects of AgNPs/D70-g-PAA and AgNPs/D70-g-PAA(PE) on cells of ILN and AgNPs/D-g-PAA(PE) on oocytes have been established.

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