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Experimental evaluation of the influence of gold nanoparticles and cerium dioxide on normal and malignant cells and tissues

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Abstract

Presented are the results of experimental studies of the effect of gold nanoparticles (AuNP) on reproductive organs of male rats, human cells and tissues of androgen-dependent prostate cancer (PCa), as well as effect of cerium dioxide nanoparticles (CeO_2NP) on the testes and fertility in aging male rats. Presence of polydisperse colloidal solution of AuNP (10-50 nm) in the LNCaP culture medium at a final concentration of 10 μg/ml inhibited cell growth, while the monodisperse solution of AuNP (20 nm) had no effect. The polydisperse colloidal AuNP solution arrested the growth of human PCa xenografts in mice, when administered parenterally at a dose range of 0.64-6.4 μg/kg b.w. The selectivity of the the nanopreparations effect on the prostate malignant epithelium is confirmed by its destruction and a decrease in the epithelial-stromal ratio on histological preparations of the xenografts. There was no significant damaging effect of poly- and monodisperse AuNP solutions on the testes and accessory sexual glands of rats when administered for up to two weeks.

The stimulating effect of administered orally a low dose of CeO2NP (1 mg/kg b.w.) on testosterone secretion and spermatogenesis, proliferative and secretory processes in the prostate of aging male rats was found. However, fertility of animals reduced in comparison with the control group due to immaturity of a part of the spermatozoa. The mechanisms of the stimulating or damaging effects of metal nanoparticles and their salts on normal and malignant tissues require further research in order to evaluate their therapeutic potential and toxicity.

Keywords: gold nanoparticles, cerium dioxide nanoparticles, reproductive system, aging, prostate cancer

The expansion of nanomaterials and their commercial use in various directions have exacerbated discussions of their potential dangers to human health and environment, as well as possibilities of therapeutic administration. In many countries, government programs of nanotechnology development were approved with toxicological and biomedicine research included [1].

Nanotechnology has opened up new promising prospects in biology and medicine. Nanomaterials are characterized not only by more obvious biological activity, but in some cases, significant toxicity. They possess a high permeability through biological barriers, including blood-brain barrier and cell membranes, and can accumulate in the tissues [2-4].

Among the variety of metal nanoparticles, gold, silver, copper, iron and cerium dioxide deserve special attention. There is growing body of experimental studies of their properties and application for medical purposes [5].

In particular, there is information of the attempts of AuNP using in diagnosis and treatment of cancer in order to improve the results of photo- and radiation therapy [6-8]. It has been shown that AuNP demonstrate a high antioxidant activity, exert a neurotrophic and neuroprotective effects, increase the lifespan of micro- and macroorganisms [9-11]. Nevertheless, despite numerous data on the use of AuNP and CeO_2NP in biology and experimental medicine, evidence for the effects of these nanometals on the reproductive system and reproductive processes during aging is extremely limited.

The article summarizes the main results of research that we have obtained in recent years.

Biological effects of AuNP

Two preparations of the AuNP were assessed:

1) Aqueous alcoholic polydisperse colloidal solution of the AuNP stabilized by polyvinylpyrrolidone with an average size of 26.4 nm with predominance of particles of 21.8 nm (42.5% of the total amount of the nanoparticles) that was kindly provided by Research Institute for Nanotechnological Industry, Open International University of Human Development “Ukraina”.

2) Aqueous monodisperse colloidal solution of the AuNP of 20 nm, that were synthesized at the F.D. Ovcharenko Institute of Biocolloid Chemistry, National Academy of Sciences of Ukraine.

AuNP were prepared with citric reduction techniques using chloroauric acid [12]. The estimation of AuNP parameters was carried out by laser correlation spectroscopy (Zetasizer-3, Malvern Instruments Ltd, UK).

Study of effects of the polydisperse colloidal solution of AuNP in a concentration of 10 μg/ml showed inhibition of LNCaP cell growth. The total number of cells decreased on average by 40% ($p<0.05$) compared to control group. Presence of 5α-dihydrotestosterone (DHT) in culture medium stimulated LNCaP cell growth by an average of 57% ($p<0.05$), which confirms androgen-sensitivity of this cell line. Adding gold nanoparticles caused weakened stimulating effect DHT (an average by 45%) compared to DHT stimulated culture [13].

Unlike the polydisperse solution, the total number of cultivated cells when have been exposed to monodisperse AuNP solution in a final concentration of 10 μg/ml did not differ from that of the control group.

Thus, it was revealed the difference of the effects of polydisperse and

monodisperse solutions of AuNP regarding their ability to inhibit proliferation of LNCaP cell line. This is probably due to different dimensional characteristics of the investigated solutions that interact differently with cells.

When adsorbed on the surface or penetrating through the membrane, nanoparticles can change surface tension and other properties of the membrane, which, in turn, can affect its function, cell mitotic activity, etc. [14, 15]. When using a polydisperse solution of AuNP, particles of different size were accumulated in several compartments of the cell, which led to inhibition of their proliferation. According to the literature, the contact of cells with nanoparticles with a

size of 10-20 nm leads to their accumulation mainly in vacuoles. Nanoparticles of 30-45 nm accumulate mostly in lysosomes. The cytotoxicity of AuNP *in vivo* and *in vitro* depends on the size, shape, dose and surface modification of the particles [16-18].

Microscopic examination of hematoxylin-stained LNCaP cells revealed dark projections of aggregated nanoparticles on the membranes or inside the cells. When using both trailed solutions of gold nanoparticles, there were neither significant death cases nor alteration of the morphology of LNCaP cells. It seems that the decrease in the number of cells in culture under the influence of polydisperse colloidal solution of AuNP was due to inhibition of proliferation.

To test the possible anticancer potential of polydisperse colloidal solution of AuNP with relation to hormone-dependent PCa, their effect was investigated on the human PCa xenografts inoculated under renal capsule in mice. The dependence of tumor growth on androgens was confirmed by inhibition of xenograft weight gain in the group of castrated recipient animals.

The polydisperse colloidal solution of AuNP in the dose range of 0.64 - 6.4 μg/kg b.w. led to arrest of the xenograft growth. In all experimental groups, the final weight of xenografts differed significantly from that in the control group.

The next step of the study was to determine which structural elements of grafts were affected by AuNP. To this end, we studied the ratio of epithelial and connective tissue in xenografts by computer analysis after staining of histological specimens by Mallory [19].

Subcutaneous administration of a solution of AuNP at a dose of 0.64 μg/kg b.w. did no change epithelial-stromal ratio compared with the control group. We observed such a trend at a dose of AuNP 1.7 μg/kg and a significant decrease in the rate at a dose of 6.4 μg/kg. Therefore, the predominant effect of AuNP on malignant prostate epithelium has been shown [20].

It was appropriate to conduct histological examination of the xenografts that could confirm the presence of cytotoxic effect of AuNP on the malignant epithelium. We observed moderate vacuolization of the cytoplasm in the epithelial cells under exposition to AuNP at a dose of 0.64 μg/kg. The presence of vacuolization may indicate metabolic disorders of cells. The vacuolization of the cytoplasm increased significantly, and the cells themselves appeared damaged and disorganized at a dose of 1.7 μg/kg. The nuclei were smaller, more hyperchromic compared to those in the group of animals that received AuNP at a dose of 0.64

μg/kg. When using of AuNP in a dose of 6.4 μg/kg, even more epithelial cells had hyperchromic nuclei, some of the cells had pyknotic nuclei, and cytoplasm was reduced. Thus, *in vivo* AuNP showed dose-dependent cytotoxic effect on malignant prostatic epithelium.

We hypothesized that inhibition of tumor growth happens due to decreased testicular testosterone (T) production in recipient mice because of impairment of the testes. Earlier it was reported damaging effect of AuNP of 2-5 nm on the testes of mice [21]. However, in our experiments, the weight of the testes did not differ in the control and experimental groups, and histological examination of the gonads did not reveal pathological changes in the spermatogenic epithelium and morphological structure of Leydig cells that produce T. Thus, the antitumor effect of AuNP was due to the effect of the preparation on tumor tissue, rather than a deficiency of endogenous T.

The affinity of AuNP for tumor cells can be explained by several reasons, in particular, the selective accumulation of nanoparticles in tumors due to impaired permeability of cell membrane, changes in electrochemical properties of nanoparticles in contact with these cells, their catalytic properties in relation to biochemical processes on the surface and inside cells [22].

The prospects for the practical use of metal nanoparticles and their salts and conjugates as drugs are related to the results of the study of their toxicity to normal organs and tissues. Novel nanomaterials and nanotechnologies is a big challenge to human health and the environment. This also applies to the use of nanoparticles, which have a large relative surface because of small size, and hence high reaction activity and ability to accumulate in the body [23, 24].

The toxicity of AuNP is still under discussion. It is needed to study the accumulation of nanoparticles in various organs and tissues to assess the safety of their use. After intravenous infusion of AuNP, their maximum accumulation occurs in the liver and spleen, and after oral administration, they accumulate mainly in the kidneys [3].

We investigated the effects of poly- and monodisperse AuNP solutions on the morphology of the testes and the prostate ventral lobe of normal adult male rats. When using polydisperse colloidal solution of AuNP in a dose of 5 mg/kg subcutaneously during one week, no damaging effect on the organs of the reproductive system were revealed. The levels of T in blood plasma, the concentration of sperm in the washing from the epididymis, the weights of the testes and accessory sexual glands did not differ in control and experimental groups, but

some decrease of seminal vesicles weight was noted in the group of animals that received the AuNP solution.

Subcutaneous introduction of monodisperse colloidal AuNP solution at a dose of 0.3 mg/kg for two weeks also did not show the negative effect on the studied organs weights except of ventral prostate. The blood plasma T levels, the concentration of sperm in the washing from the epididymis did not response. However, the weight of ventral prostate significantly reduced, and degenerative and atrophic changes of acinar epithelium and distinct inflammatory reaction in the connective tissue were detected.

We did not observe harmful effect in the testes, supposedly due to haematotesticular barrier [25].

Reported data on the effect of AuNP on the gonads of laboratory animals are quite controversial. AuNP with a size of 2.5 nm induced chromosomal mutations in early first-order spermatocytes, but the authors did not note violations of the structural organization of the spermatogenic epithelium and spermatogenesis cycle [26]. Another study describes a negative effect of spherical shaped AuNP with a diameter of 5 nm on the reproductive function in male rats, which manifested itself in teratozoospermia, sperm agglutination and reduced fertility [27].

AuNP of 5 nm and 20 nm accumulate in the testis of rats when injected during one week. In addition, there is a decrease in the expression of the cell proliferation factor at the level of transition of germ cells from spermatogonia to first-order spermatocytes, and, as a consequence, an incomplete cycle of spermatogenesis is possible [28]. But this assumption was not confirmed by the results of our experiment, probably due to the limited time of introduction of AuNP relative to the duration of the cycle of spermatogenesis in rats (48 days). Similarly, the introduction of AuNP during two weeks did not reveal impairment of the testes.

As mentioned above, harmful effects on the gonads were found for AuNP of very small size - 2.5 nm and 5 nm. Therefore, it should be taken into account that such nanoparticles penetrate through the cell and nuclear membranes almost without hindrance, and they exert genotoxic effect [21].

Dystrophy and atrophy changes in epithelium of the ventral prostate exposed to AuNP monodisperse solution for two week could have been caused by a decrease in the secretion of testosterone. However, this assumption is highly unlikely because the size and histological structure of Leydig cells and their number remained normal. This also was confirmed by

hormonal studies: testosterone level in blood plasma of rats that received AuNP solution did change.

It is known that the ventral prostate of immature or castrated rats is very susceptible to the androgen stimulation. This leads to intensive vascular growth, rebuilding of quantitative and qualitative composition of the secretory epithelium. We hypothesized that effect of nanoparticles on the ventral prostate and other accessory gonads may be more pronounced under conditions of exogenous testosterone stimulation. Therefore, testing the effect of AuNP on the ventral prostate stimulated with exogenous T was performed on immature gonadectomized rats.

The study has demonstrated that AuNP do not interfere with androgenic stimulation of the ventral prostate. However, the signs of inflammation process were observed which is consistent with reported data [29-31].

The absence of dystrophic and atrophic changes in the acinar epithelium of the ventral prostate of gonadectomized immature animals, in contrast to adult ones, may be due to the fact that testosterone stimulation overcomes the possible damaging effect of AuNP.

In most cases, the introduction of nanoparticles causes a systemic inflammatory reaction, which is accompanied by dystrophic changes in the liver, kidney, lung, spleen. At the same time the signs of activation of proliferation and differentiation of immunocompetent cells were noted [31]. In our work, it was shown that inflammatory process also occurs in the ventral prostate and they led to atrophic changes in the epithelium.

Biological effects of CeO_2NP

Citrate CeO_2NP colloid dispersion stabilized with polyacrylic acid that contained particles with a size of 2-3 nm [9] was used for studying the effect of CeO_2NP on reproductive system in aging male rats [32].

Aging is accompanied with involutive changes in the testes and a decrease in their hormonal activity. In our research on rats aged 18 months, the blood plasma T levels was 2.64 ± 0.40 nmol/l, that was approximately twice less than that in males aged 6 months. After 10 days of oral administration of CeO_2NP solution at a dose of 1 mg/kg b.w., T level in aging animals was significantly increased ($p < 0,05$), but under conditions of administration of a much higher dose of CeO_2NP (100 mg/kg) did not change significantly compared to control.

One of the signs of testicular steroidogenesis is the morphology of Leydig cells. With the introduction of a low dose of CeO_2NP (1 mg/kg), changes in the testicular structure in aging rats were observed, namely, an increase in the number of activated Leydig cells. This is the most likely cause of increased T level in blood plasma. Activation of Leydig cells was evidenced by the presence of enlarged normochromic nuclei with enlightened nucleolus. The cytoplasm of these cells had an increased volume and was often vacuolated at the periphery.

In males exposed to a high dose of CeO_2NP (100 mg/kg), the number of activated Leydig cells was significantly less compared to that of a low dose of CeO_2NP. Otherwise, under the conditions of introduction of CeO_2NP at a dose of 100 mg / kg, the same age-related changes in Leydig cells morphology were observed as in control animals.

The concentration of sperm in the washing of the epididymis after 10 days of administration of CeO2NP at a dose of 1 mg/kg significantly increased ($p < 0.05$), and at a dose of 100 mg/kg, it had a tendency to increase compared to the control.

Changes in sperm structure of 18-month-old rats after application of CeO2NP were characterized by “softening” of their tail, that led to reduced motility and, presumably, impaired fertilization. A fertility study of males that received CeO_2NP at a dose of 1 mg/kg showed twice reduction of the pregnancy index that may be due to the presence of immature sperm in the seminal liquid.

Thus, this study showed a stimulating effect of a low dose of CeO2NP (1 mg/kg) on testicular hormonal function and spermatogenesis in aging male rats. The mechanisms of the stimulating effect of CeO_2NP on the reproductive system of aging animals require further study.

CONCLUSION

Nanoparticles of metals and their salts can have both stimulating or destructive effects on organs and tissues, what should be taken into account when studying their therapeutic potential and toxicity.

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