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ASSESSMENT OF GOLD NANOPARTICLE EFFECT ON PROSTATE CANCER LNCaP CELLS

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In recent years gold nanoparticles (AuNPs) have received considerable attention for various biomedical applications including diagnostics and targeted drug delivery. However, more research is still needed to characterize such aspects of their use in clinical oncology as permeability, retention and functional effect on tumor cells. *Aims*: This study was designed to describe the effect of non-functionalized AuNPs on LNCaP prostate cancer cells growth. *Material and Methods*: LNCaP cells were cultured in RPMI-1640 medium containing AuNPs covered by polyvinylpyrrolidone of average size 26.4 nm (10.0 μg/ml). Counts of cells were calculated and their morphology was examined. *Results*: AuNPs conglomerates have been visualized in cultured cells. After 4-day incubation in presence of AuNPs significant retardation of LNCaP cells growth was observed both in 5α-dihydrotestosterone stimulated and non-stimulated cultures. No morphological changes of live LNCaP cells were seen in any experiment. *Conclusion*: Given absence of morphological changes in live cells and dribble and relatively constant numbers of dead cells, it was concluded that inhibitory effect of AuNPs on LNCaP cells growth was caused by alterations of proliferation.
***Key Words*: prostate cancer, LNCaP, gold nanoparticles, 5α-dihydrotestosterone.**

Prostate adenocarcinoma (prostate cancer — PCa) is one of the most common cancer that affects 12–17% of male population in the developed countries [1, 2]. PCa case incidence and mortality increased during past decades. In the year of 2012, PCa in Ukraine was diagnosed as the second malignancy in human male population (after lung cancer) and the most common oncourological disease [3].

PCa represents a classical model of hormone-dependent malignancy. In the most cases, growth of PCa is promoted by testosterone and other androgens. For this reason, advanced PCa is quite successfully treated by surgical or pharmacological (androgen receptor antagonists, hypothalamic luteinizing hormone — releasing hormone analogues, estrogens and other drugs) androgen ablation. However, castration and hormonal therapy exert only palliative effect, and usually after remission the tumor relapses due to refractoriness to drugs. Some patients initially demonstrate refractoriness to castration or other modalities of systemic endocrine therapy. Possible mechanisms of hormone resistance include androgen receptor (AR) gene mutations and amplification, ligand-independent AR signaling, changed interplay between AR and growth factors, intratumoral conversion of adrenal androgens to testosterone, and so on. Alternative treatments are needed for aggressive localized PCa and hormone-refractory metastatic disease.

Modern nanotechnologies provide the new possibilities in biology and medicine [4, 5]. In the recent decades, nanotechnology has brought new hopes in malignant tumors diagnosis and targeted therapy. Among various nanoparticles, the gold ones (AuNPs) have received considerable appreciation by investigators and clinicians due to their unique properties [6–8]. They have inert core, permeate through the cell membrane, can be functionalized easily, and demonstrate chemical stability and surface plasmon oscillation. Because of advanced technology of chemical synthesis, a large range of AuNPs with different size, shape and optical properties are available. Currently, the AuNPs are already applied or likely to be applied in the foreseeable future for bio-nano-analytics, diagnostic, photothermal therapy and targeted drug delivery [9–13]. In the meantime, however, clinical trials of AuNPs as antitumor agents are rather limited, and much more basic research is still needed to facilitate their transition to clinical oncology.

Earlier we observed suppressive effect of AuNPs on human PCa xenograft growth in mouse model [14]. Exploration of their effects on PCa cells *in vitro* could bring new information on the peculiarities of interaction between AuNPs and tumor cells. There was shown a cytotoxic influence of AuNPs on androgen-refractory PCa PC-3 cell line [15]. Cytotoxic activity of AuNPs conjugated with non-steroidal antiandrogens has been demonstrated *in vitro* toward hormone-insensitive PCa cell line [16]. Incorporation of AuNPs conjugated with glutamate carboxypeptidase inhibitor, prostate-specific membrane antigen, into LNCaP and PC-3 cells was observed [17], but their effects on the cells have not been studied. In this article, we represent the results of exploration of the effects of simple, non-functionalized AuNPs on androgen-sensitive PCa LNCaP cells growing freely or stimulated with 5α-dihydrotestosterone (DHT).

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Abbreviations used: AR – androgen receptor; DHT – 5α-dihydrotestosterone; AuNPs – gold nanoparticles; PVP – polyvinylpyrrolidone; PCa – prostate cancer.

MATERIALS AND METHODS

AuNPs. AuNPs were prepared using citric techniques of reduction of chloroauric acid [18], and kindly provided to us by the Research Institute for Nanotechnological Industry, Open International University of Human Development "Ukraine".

There was used ethanol dispersion of spherical AuNPs covered by polyvinylpyrrolidone (PVP) in order to prevent aggregation. The estimation of AuNPs parameters was carried out with laser correlation spectroscopy (Zetasizer-3, Malvern Instruments Ltd, UK).

Cells and experimental protocol. Human PCa cell line LNCaP was provided by R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of Sciences of Ukraine, Kyiv. Three series of experiments with similar design were carried out. Cells were cultured on 24 × 24 mm square glass cover slips placed in Petri dishes filled with culture medium of the following composition: RPMI-1640 medium (Sigma), 10% fetal bovine serum (Sigma), 50.000 IU/l penicillin and 50 mg/l streptomycin. Cell cultures were maintained in the thermostat at 37 °C in a 95% air — 5% CO_2 humidified atmosphere. Culture medium was changed daily.

After cells' density reached 50–60% of cover slip's surface, the cultures were separated onto 5 groups each consisting of 3–5 cover slips. The culture medium in the first group (control) contained ethanol, and the second one (PVP group) contained PVP-ethanol solution just to make sure it doesn't affect cells. The third group (AuNPs group) was exposed to AuNPs (10 μg/ml) alone, the fourth group (DHT group) contained DHT (10^{-4} M) alone, and the fifth group (AuNPs + DHT group) was supplemented with AuNPs (10 μg/ml) plus DHT (10^{-4} M). Ethanol concentration in culture medium of all experimental and control groups did not exceed 1%. After 4-days of culturing with daily replacement of respective medium, cells from each group were detached from cover slips using 0.25% trypsin-EDTA in 0.5 ml medium, suspended in additional 0.5 ml medium and centrifuged at 3200 rpm. The cells were resuspended in 1.0 ml medium, stained with Trypan Blue in order to discriminate live and dead cells, and the numbers of live and dead cells were calculated in Goryaev chamber.

Morphological studies. Part of cover slips was used for morphological studies. The cells were fixed in 4% paraformaldehide, then stained with Ferrum hematoxylin and underwent examination using the microscope Leika DME (Leika Microsystems, Germany).

Data analysis and statistics. Each experiment was repeated 4–6 times. Accretion percentage of cell counts in relation to initial seeding was calculated, and results were averaged and expressed as mean ± s.e.m. (standard error of the mean). The results were compared with those of appropriate controls. Student's *t*-test was used for statistical analysis, with $p < 0.05$ considered significant.

RESULTS AND DISCUSSION

AuNPs size measurements. It has been estimated that the colloidal solution contained nanoparticles with sizes ranging from 15.3 to 30.9 nm (90% of total number) and the prevalence of 21.8 nm one (42% of total number) (Fig. 1). The average size of nanoparticles was estimated as 26.4 nm.

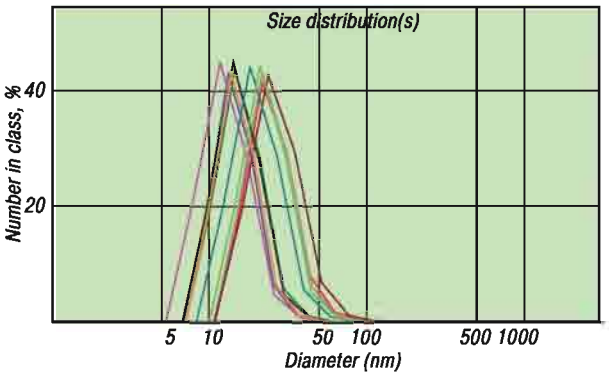

Fig. 1. AuNPs size (diameter) and number distribution by laser correlation spectroscopy

LNCaP cells growth. During 4-day-growth period, a total count of control LNCaP cells (i.e., cultured in the presence of ethanol and/or PVP) increased 7–21-fold. The relative numbers of dead cells against initial seeding increased only 2-fold, and was similar in all control and experimental groups. Given a total numbers of cultivated cells and numbers of live and dead cells in the end of the experiments, PVP in appropriate amounts did not affect the culture growth versus the vehicle control ($p > 0.05$) (Fig. 2).

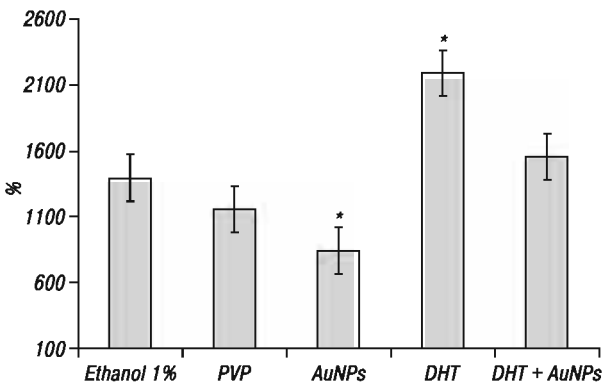

Fig. 2. Effect of AuNPs on percentage of total counts of LNCaP cells after 4-day cultivation in RPMI 1640 medium in relation to initial seeding (M ± m). Footnotes: Each bar represents an average data from 3 experiments. *$p < 0.05$ in comparison with ethanol control

Our preliminary *in vitro* experiments with various AuNPs concentrations of 0.1; 1.0 and 10.0 μg/ml had shown that only 10.0 μg/ml of AuNPs was effective in affecting LNCaP cells growth. In this study, after 4-day incubation of LNCaP cells in presence of AuNPs (10.0 μg/ml) we observed significant suppression of cells' growth. AuNPs decreased the total cells count on average by 40% and live cells count by 43% compared to the control, and both decreases were statistically significant ($p < 0.02$).

Supplementing culture medium with DHT stimulated LNCaP cells growth by increasing total cells count

on average by 57% and live cells count by 61% compared to the control. Addition of AuNPs in the presence of DHT dramatically reduced stimulatory effect of DHT on LNCaP cells growth by 80% and 77% when counted by the accretion of total and live cells, respectively, and brought total cell counts to control level ($p > 0.05$).

Cell morphology. Dark violet increments, supposedly aggregates of nanoparticles, were clearly seen in cells cultivated with AuNPs. No morphological changes of live LNCaP cells were seen in any experiment (Fig. 3).

In summary, it must be emphasized that we used androgen-sensitive LNCaP cell line derived from the human PCa lymph node metastasis to study the effects of non-functionalized AuNPs on cells' growth.

There is some discrepancy in published works concerning cytotoxicity of AuNPs toward eukaryotic cells. Cytotoxicity has been reported by a few authors [19, 20], meanwhile others did not observe it [21]. Cytotoxity of AuNPs both *in vitro* and *in vivo* strongly depends on particles' size, shape, dose, surface composition

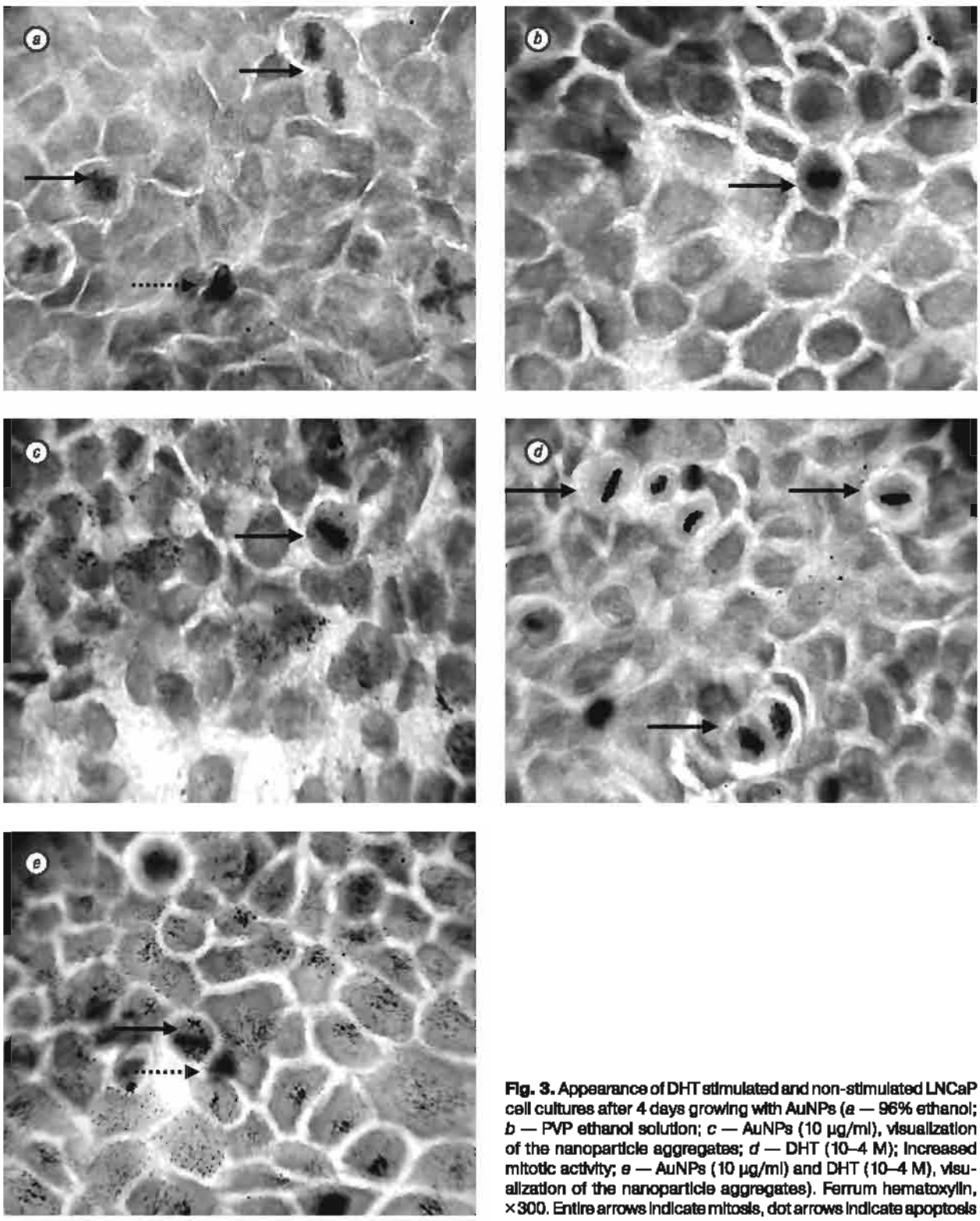

Fig. 3. Appearance of DHT stimulated and non-stimulated LNCaP cell cultures after 4 days growing with AuNPs (*a* — 96% ethanol; *b* — PVP ethanol solution; *c* — AuNPs (10 μg/ml), visualization of the nanoparticle aggregates; *d* — DHT (10–4 M); Increased mitotic activity; *e* — AuNPs (10 μg/ml) and DHT (10–4 M), visualization of the nanoparticle aggregates). Ferrum hematoxylin, ×300. Entire arrows indicate mitosis, dot arrows indicate apoptosis

and other features [15, 22–24]. Similarly to other metallic nanoparticles, AuNPs interact with cellular membrane, mitochondria, nucleus, resulting in damaging DNA and organelles, oxidative stress, apoptosis.

In this study, we explored relatively homogenous population of spherical AuNPs with average size of about 26 nm. By being added to the culture medium at concentration 10.0 μg/ml, these AuNPs inhibited non-stimulated LNCaP cell line growth significantly. The ratios of dead cells in the control and AuNPs-treated cultures relative to the initially seeded cells were minor and remained quite constant, whereas the relative numbers of live cells in the presence of AuNPs decreased dramatically. Suppression of cell growth by AuNPs in DHT stimulated culture was quite noticeable. Taken into consideration that DHT binds to nuclear AR, which interacts with specific regions of DNA followed by expression of growth factors and stimulation of mitosis, it can be assumed that AuNPs interfere with androgen and/or growth factor signaling in the affected cancerous cells. Morphology of live cells did not change, and numbers of visualized apoptotic cells were similar in control and experimental groups. From these observations, we have concluded, that AuNPs suppressed cell proliferation rather than caused apoptosis or necrosis.

AuNPs conglomerates have been visualized in cultured cells, which is in line with reported data on the ability of nanoparticles with 30 nm diameter or less to be endocytosed by cells [15, 20, 25, 26]. It was shown in human histiocyte-rich lymphoma U937 cell line that 20–30 nm AuNPs have a greater cell accumulation than that of nanoparticles of other size with prevalent localization in the lysosomes [11, 20]. 3–5 nm AuNPs penetrate nucleus easily. AuNPs capacity to damage DNA deserves special attention in terms of their safety. It was shown that even 10–20 nm AuNPs can damage DNA structure in CHO-K1 cell line, meanwhile 30 nm and 45 nm AuNPs were safe [27]. Genotoxity might be a cause of suppressive influence of AuNPs on LNCaP cell growth in our experiments.

One possible mechanism of AuNPs induced inhibition of LNCaP cell growth is modulation of the cell membrane enzyme activity. Though gold *per se* is known to be inert metal, AuNPs possess high catalytic properties [5]. For instance, it had been reported their ability to modulate *in vitro* the U937 tumor cell membrane Na^+, K^+-ATPase activity [20]. Since Na^+, K^+ -ATPase activity is required for adequate regulation of cell volume, which undergoes significant disturbances during cell-cycle progression, one might suggest that impaired cell volume regulation may in part be responsible for the anti-proliferative action of AuNPs. However, biochemical mechanisms underlying effect of AuNPs on cancerous cells are still poorly investigated and understood.

There is a hope that in the future AuNPs could earn a place in the treatment of PCa, however, much work for this is to be addressed.

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