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Mitotic activity of cultured human lymphocytes treated with gold nanoparticles

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Abstract:

Potential health and environmental effects of nanoparticles need to be thoroughly assessed before their widespread commercialization. The present investigation was planned with the aims to determine the effects of gold nanoparticles (GNPs) on blast (BI) and mitotic (MI) indices of cultured lymphocytes. The results revealed that BI (50.3 ± 2.3, 30.2 ± 1.9, 10.5 ± 0.7 and 0.0%, respectively) and MI (70.1 ± 2.9, 20.4 ± 1.1, 5.3 ± 0.1 and 0.0%, respectively) showed a gradual decreased percentage as the concentration of GNPs was increased from 0.085 to 0.66 µg/mL, and the difference was significant compared to control culture (81.6 ± 2.5 and 90.2 ± 3.7%, respectively). A maximum inhibition of BI and MI was occurred at the concentration 0.66 µg/mL. In conclusion, GNPs can be considered as a growth inhibiting agent, but it is too early to reach a final conclusion in this regard and further investigations are required to elucidate the biochemical mechanisms involved in inhibiting the mitotic activity of lymphocytes.

Key word: *gold nanoparticles, mitotic index, blast index, lymphocytes*

Introduction:

Nanoparticles have a wide range of applications in the field of biomedicine. They can be employed to deliver medicines, in addition to their diagnostic and therapeutic potentials. This is reasoned by the fact of their nanoscaled size [1].An important group of these particles is gold nanoparticles (GNPs). They have abundant use in the field of biotechnology and biomedicine, because they have a large surface bio-conjugation with molecular probe properties and, they are also presented with many optical properties that are mainly concerned with a localized plasmon resonance (PR) [2]. Hence, GNPs have a wide range of applications in diagnosis and treatment of several diseases, such as cancer and microbial infections [3]. In cancer, GNPs are emerging as promising agents for therapy of cancer and are being suggested as drug carriers, photothermal agents and radiosensitisers, which consequence in a growth inhibition of cancer cells [4,5].

The growth and proliferation rate of cancer cells can be measured by a determination of mitotic index (MI), which is expressed as a percentage of cells in metaphase among dividing cells; therefore, it is an important cytogenetic test that is employed in in vivo and in vitro analyses of proliferating cells. Accordingly, the MI is used to examine compounds that may inhibit or induce mitotic progression. It is also an important prognostic factor predicting both overall survival and response to therapy, especially in cancer patients [6]. The main factors that determine MI are proportion of cell population that participates in the whole cycle of interphase leading to division, relative lengths of interphase and recognizable mitotic stages [7].

The present investigation was planned with the aims to determine the effects of GNPs on the mitotic activity of cultured lymphocytes.

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Materials and Methods:

Gold nanoparticles

Colloidal GNPs were purchased from China Company and their concentration was determined by using atomic absorption microscopy with flame (AAMF). Ultra violet visible (UV-Vis) spectroscopy was used to characterize surface plasmon resonance (SPR) through union space apparatus, and it was around 514nm. Atomic force microscopy (AFM) tech-

nique was used to characterize the size and morphology of GNPs through scanning probe microscope.

Culture establishment

The whole blood culturing method was adopted as a previously described [8]. Blood samples (5 mL) were drawn from health adult volunteers in heparinized tubes (10 IU/ml). Five culture tubes were set-up, each contained 5 mL of culture medium (RPMI-1640) supplemented with 10% of heat-inactivated fetal calf serum. An aliquot (0.5 mL) of blood was added to each test tube, followed by 0.3 mL of phytohaemagglutinin. Then, 0.2 ml of GNPs solutions was added to tubes number 2, 3, 4 and 5, at concentrations 0.085, 0.17, 0.33 and 0.66 µg/mL, respectively, while tube number 1 was considered as a control (GNPs-free culture). The tubes were incubated in CO2-incubator for 72 hours at 37ºC, with a gentle shaking every 12 hours. At the hour 71 of incubation, 0.1 mL of colchicine was added to each tube and the incubation continued for a further hour. After the incubation period, the tubes were centrifuged for 10 minutes at 1500 rpm. The supernatant was discarded, while precipitated cells were gently suspended in 5 mL of warmed hypotonic KCl (0.075M), and the tubes were incubated for 40 minutes at 37°C. After incubation, the tubes were centrifuged for 10 minutes at 1500 rpm, and the supernatant was discarded. Then, few drops of freshly prepared fixative solution (absolute methanol:glacial acetic acid in ratio 3:1) were added drop-wise with a gentle mixing, and the volume was made-up to 5 mL with fixative. The tubes were centrifuged for 10 minutes at 1500 rpm, and the supernatant was discarded. The fixation was repeated 2-3 times or until the precipitate was white. Finally, the cells were re-suspended in 2 ml of the fixative and stored at -20°C, for at least 30 minutes. All cultures were set-up in duplicate.

Slide preparation and staining

The cell suspension was centrifuged for 10 minutes at 1500 rpm, and the supernatant was discarded. The cells were re-suspended in an appropriate amount of the fixative to make thinly cloudy suspension. By using Pasteur pipette, 3-4 drops of the cells suspension were dropped from a height of about 30 cm on grease-free slides and allowed for air-drying at room temperature. The slides were stained with a freshly made Giemsa stain.

Analysis of BI and MI

The slides were microscopically examined (10X), and at least 1000 cells were inspected to determine the BI and MI, which were given as a percentage of total cells [9]

Statistical analysis

The data were given as mean ± standard error (SE), and a significant difference was assessed by analysis of variance (ANOVA) followed by Duncan test. A p-value ≤ 0.05 was considered significant. These analyses were carried out using the statistical package SPSS version 13.0.

Results and Discussion:

Characterization of gold nanoparticles

The results AAMF showed that the concentration of colloidal GNPs was equal to 20 µg/ml. The assessment by AFM revealed that the shape of GNPs was spherical with some aggregation and the size was about 65nm (Figures 1 and 2).

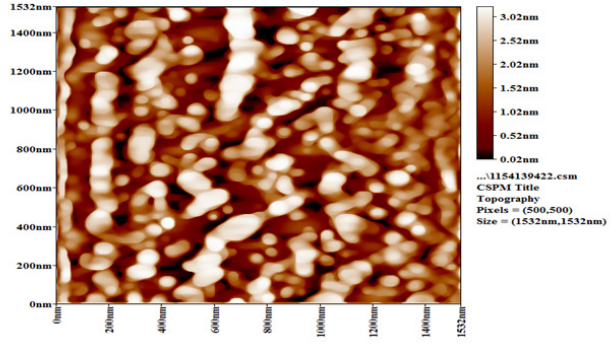

Figure (1): The distribution of GNPs as single particles with some agreggates. In addition, the particle have a sphericle shape.

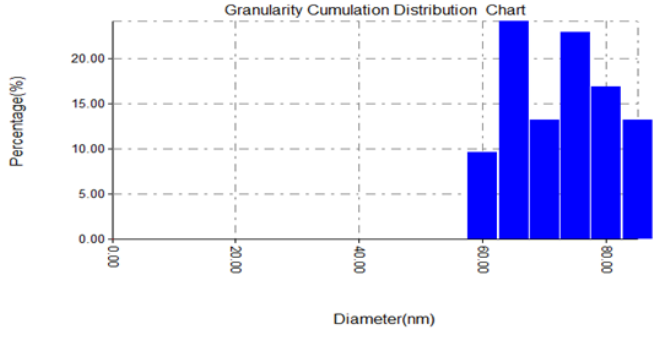

Figure (2): Percentage distribution of GNPs by AFM. The GNPs size is in the range 60-85nm and the higher rate is equal to 65nm.

Effects of GNPs on BI and MI

The BI (50.3 ± 2.3, 30.2 ± 1.9, 10.5 ± 0.7 and 0.0%, respectively) and MI (70.1 ± 2.9, 20.4 ± 1.1, 5.3 ± 0.1 and 0.0%, respectively) showed a gradual decreased percentage as the concentration of GNPs was increased from 0.085 to 0.66 µg/mL, and the difference was significant compared to control culture (81.6 ± 2.5 and 90.2 ± 3.7%, respectively). As shown in table 1, a maximum inhibition of BI and MI was occurred at the concentration 0.66 µg/mL.

Table 1. Blast and mitotic indices of cultured lymphocytes treated with different concentrations of gold nanoparticles.

Gold nanoparticles Concentration (µg/mL)	Mean ± SE (%)*	
	Blast Index	Mitotic Index
0 (Control)	81.6 ± 2.5^{A}	90.2 ± 3.7^{A}
0.085	50.3 ± 2.3^{B}	70.1 ± 2.9^{B}
0.17	30.2 ± 10.5^{C}	20.4 ± 1.1^{C}
0.33	10.5 ± 0.7D	5.3 ± 0.1^{D}
0.66	0.0	0.0

*Different superscript letters represent significant difference between means in each column

The results of present study demonstrated that GNPs inhibited the BI and MI in a concentration-dependent manner. This inhibition could be due to a slower progression of cells from S (DNA synthesis) phase to M (mitosis) phase of the cell cycle as a result of exposure to GNPs. The impairment in cell cycle progression, which was associated with GPNs, may suggest that these particles were cytotoxic to the cultured lymphocytes. In agreement with such suggestion, it has been demonstrated that nanoparticles can impair stages of cell division and cause chromatin bridges, stickiness of chromosomes, disturbed metaphase, multiple chromosomal breaks and cell disintegration [10]. The statistical analysis of BI and MI demonstrated a significant variation between the concentrations of GNPs, and a complete inhibition of mitotic activity was observed at the dose 0.66 µg/mL. Accordingly, the concentration of these particles is a critical parameter in determining the cytotoxicity [4]. A further investigation suggested that the variation in MI was directly proportional to GNPs concentration and inversely related to their size [11].

Some investigators demonstrated that when lymphocytes were exposure to GNPs, the particles can penetrate these cells to stabilize in cytoplasm. As lymphocytes have no phagocytosis activity, the only mechanism is the direct penetration of GNPs into the cytoplasm of these cells [12]. When GNPs penetrate the cytoplasm, a disturbance of cell cycle can occur by a mechanism that may mimic colchicine effects on spindle fibers. Equally important, GNPs may interfere with the formation of DNA, RNA and some key cellular proteins involved in cell division [13]. In this context, it has been presented that GNPs are cytotoxic during the S phase of the cell cycle, and they have a greater toxic effect on rapidly dividing cells (such as malignant and myeloid cells, gastrointestinal and oral mucosa). These cells replicate their DNA more frequently, and thus inhibiting their growth and proliferation by GNPs may suggest a therapeutic strategy based on these particles [14].

In conclusion, GNPs can be considered as a growth inhibiting agent, but it is too early to reach a final conclusion in this regard and further investigations are required to elucidate the biochemical mechanisms involved in inhibiting the mitotic activity of lymphocytes.

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