Toxicology Letters 247 (2016) 29–34

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Size of silver nanoparticles determines proliferation ability of human circulating lymphocytes *in vitro*

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HIGHLIGHTS

- CBMN test represents a good model for studying genotoxicity and cytotostasis of nanoparticles.
- Inhibition of cell proliferation by AgNPs depends on size of the particles.
- AgNPs sized 2 nm stimulate cell proliferation enhancing ILGF1-1.

ARTICLE INFO

Article history:
Received 16 November 2015
Received in revised form 9 February 2016
Accepted 11 February 2016
Available online 15 February 2016

Keywords:
AgNPs
Size
DNA damages
Cell stasis
Human primary cells

ABSTRACT

In this work we present biological effects of silver nanoparticles (AgNPs) synthesized by picosecond laser ablation of silver in deionized water. We examined induction of chromosomal aberrations, lymphocyte micronuclei, appearance and recovery of double strand breaks (DSBs) of DNA, cell proliferation potential, concentration of lipid peroxidation products and insulin-like growth factor 1 (ILGF-1). We found that AgNPs sized from 3 nm to 8 nm induce cell cytostasis, which is accompanied with its clastogenic action on DNA, while AgNPs, sized 2 nm behaves contrary stimulating cell proliferation by enhancing ILGF-1 concentration.

1. Introduction

Nanotechnology is rapidly growing discipline, bringing wide range of newly synthesized nanoparticles with potential application in many fields of industry and medicine such as chemistry, micro/nano-electronics, optics and biomedicine. Among nanoparticles, silver nanoparticles (AgNPs) present chemically stable ones with well-known antibacterial and antifungal activity (Krutyakov et al., 2008, Wyatt et al., 1990, Kamikawa et al., 2014). Up to date, biological effects of AgNPs were examined using different cell lines, *in vitro* and *in vivo*. Summarised results show that AgNPs acts as excellent disinfectant, but also induce toxic changes in normal cells via increasing of ROS, disruption of mitochondrial function or by inducing necrosis. (Carlson et al., 2008; Uygur et al., 2009; Yen et al., 2009; Kim et al., 2012; Völker et al., 2013; Faedmaleki et al., 2014; Lee et al., 2014; Urbańska et al., 2015).

In review paper of Wong and Liu (2010) almost all aspects of AgNPs synthesis, biological actions and application in clinical medicine were discussed. Observation reported in this work of increased growth rate of keratinocytes treated with AgNPs was in contrast to most previously reported findings recording inhibitory effects of AgNPs (Barnea et al., 2010). Authors discuss this finding as potential influence of laboratory conditions, medium used for cell harvesting, etc. The same research group studied effects of AgNPs *in vivo* and found that AgNPs promote proliferation and migration of keratinocytes, underlying the complexity of causal mechanism leading to this outcome (Liu et al., 2010). Our previous work demonstrated that AgNPs display deteriorating effect on DNA (Stašić et al., 2014). In this study, we investigate cytotoxicity of AgNPs using primary human cells: circulating lymphocytes (Ly), red blood cells (RBC) and fibroblasts.

Ly were treated with different types (sizes) and concentrations of AgNps, and afterwards chromosomal aberrations, micronuclei formation, proliferation rate of treated lymphocytes and

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http://dx.doi.org/10.1016/j.toxlet.2016.02.007
0378-4274/

Table 1
Concentration of AgNPs.

Ag NPs type	Ag NPs mass concentration (μg/ml)[a]	Ag NPs particle concentration (particles/ml)
A	5.6	4.7×10^{12}
B	11.8	2.7×10^{14}
C	13.9	1.2×10^{13}
D	38.9	5.1×10^{14}
E	76.7	4.3×10^{14}

[a] μg/ml concentration is equal to ppm (parts per million) for aqueous solution.

morphology of erythrocytes were analysed. Concentrations of AgNPs, which showed clastogenic properties in whole blood model system, were further tested on fibroblasts employing γ-phosphorylation assays aiming to evaluate ability of AgNPs to induce DSBs of DNA. Acceleration of proliferation of lymphocytes treated with AgNPs sized 2 nm, was analysed further. We found that increased concentration of ILGF-1 in treated samples is associated with acceleration of cell proliferation. This finding is of importance in wound healing, where stimulation of cell proliferation is desired action; however this characteristic can present an adverse effect for common use of AgNPs containing particles sized 2 nm due to increase of inflammation which can drive pathogenesis of huge number of medical disorders.

2. Material and methods

2.1. Characteristics of AgNPs

AgNPs were synthesized by picosecond laser ablation of silver in deionized water: laser parameters were: wavelength 1064 nm, pulse duration 150 ps, near fundamental operation mode. Obtained Ag NPs were spherically shaped and their size distribution monitoring was done by dynamic light scattering (DLS) technique using a ZetasizerNano ZS (Malvern, Worcestershire, UK) with a 633 nm He–Ne laser and 173° detection optics (backscatter detection). Five types of particles were obtained, and their size distributions with corresponding concentrations are given in Table 1 and Fig. 1. The synthesized AgNPs showed negative zeta potential of −28 mV. UV/VIS characteristic peak is at 400 nm.

2.2. Samples

Blood and skin biopsy samples were obtained from three healthy male non-smoking volunteers undergoing plastic surgery who signed informed consent in accordance with the current Health and Ethical regulations in Serbia (Law of health care, 2005). For each type of AgNPs type 6 different concentrations were examined employing micronucleus test (Fenech, 2000). For further experiments, optimal three concentrations (listed in Table 1) were examined.

2.3. Lymphocyte cultures

Aliquots of heparinized whole blood (0.3 mL) were placed in cultures containing 2,7 mL of PB-max karyotyping medium (Life Science, USA). Different concentration of newly synthesized silver nanoparticles were put into the cultures and harvested within the cells. All cultures were set up in triplicate: one set serves for micronucleus test, the second for chromosome aberration analysis whereas third was used for evaluation of cytotoxicity using analysis of erythrocyte parameters (number, morphology and concentration of haemoglobin). Along with it concentration of ILGF-1 and lipid peroxidation products were analysed. Untreated cell cultures served as a control.

2.4. Chromosome aberration assay

For chromosome aberration analysis whole blood lymphocytes were harvested for 48 h in the presence of above mentioned concentration of AgNPs afterwards cells were spin down, treated with hypotonic solution (0,56% KCl) 10 min on 37 °C and fixed in McKarnoy fixative (Methanol:Acetic acid:3:1), three times. Metaphases spreads were stained in 10% buffered Giemsa (pH 6.8). After staining 1000 metaphases were scored for chromosome and chromatid aberrations. Each aberration was recorded, slides were unstained and G-banded using 0,25% trypsin (Life Science). Chromosomal aberrations were analysed according to criteria for analysis of fragile sites (Mrasek et al., 2010).

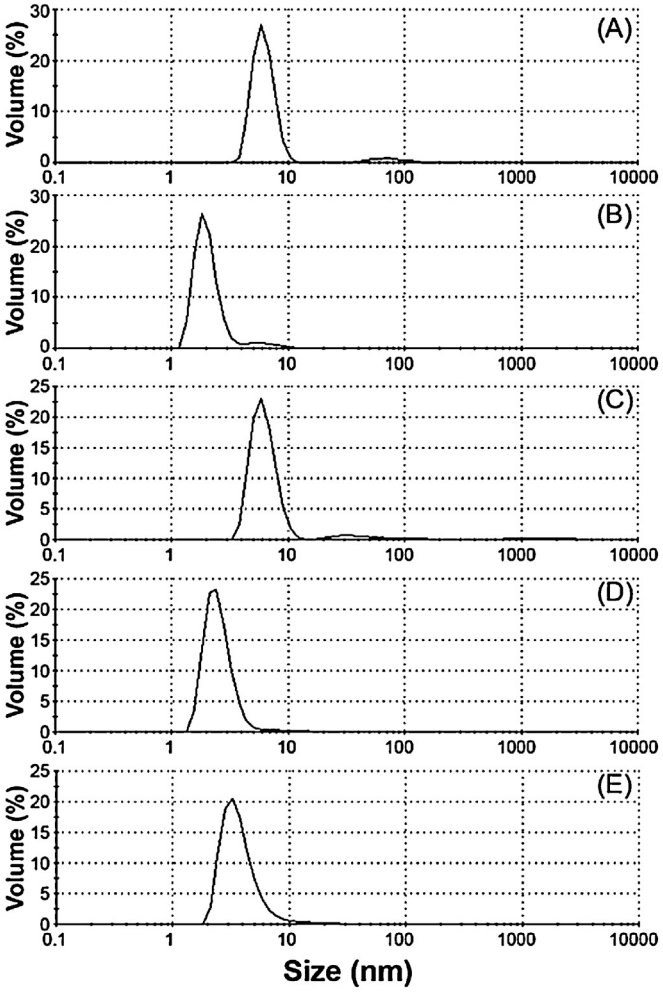

Fig. 1. Size distribution of AgNPs.