Materials Science & Engineering C 93 (2018) 465–471

Contents lists available at ScienceDirect

Materials Science & Engineering C

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

Effect of silver nanoparticles in the induction of apoptosis on human hepatocellular carcinoma (HepG2) cell line

Elham Ahmadian[a,b,c,1], Solmaz Maleki Dizaj[a,1], Elaheh Rahimpour[d], Amir Hasanzadeh[b], Aziz Eftekhari[b,*], Hasan Hosain zadegan[b,**], Jamal Halajzadeh[b], Hale Ahmadian[e]

[a] Dental and Periodontal Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[b] Maragheh University of Medical Sciences, Maragheh, Iran
[c] Students' Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran
[d] Food and Drug Safety Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[e] Chemical Engineering, Urmia University, Urmia, Iran

ARTICLE INFO

Keywords:
Metal nanoparticles
Reactive oxygen species
Silver nanoparticle
Cancer
Hepatocellular carcinoma
Nanomedicine

ABSTRACT

Silver nanoparticles (Ag NPs) serve numerous chief functions in cosmetics, engineering, textile, food technology and medicine. These nanoparticles are also utilized in the pharmaceutical industry particularly in the production of novel antimicrobial agents. However, despite the various studies of Ag NPs induced toxicity, there is a lack of information concerning cellular toxicity mechanisms of these nanoparticles on human cells. In the current project, we investigate the anti-cancer effects of Ag NPs in HepG2 (liver hepatocellular adenocarcinoma) cells. The mean particle size and morphology for the prepared nanoparticles were determined by dynamic light scattering (DLS) and transmission electron microscopy (TEM), respectively. Cell viability, reactive oxygen species (ROS) formation, cytochrome *c* amount and expression level of BAX/CASP 3/CASP 8/CASP 9 were assayed in HepG2 cells after incubation with Ag NPs. The prepared nanoparticles showed the mean particle size of 30.71 nm with polydispersity index (PDI) of 0.21. Our results revealed decreased cell viability in a concentration-dependent manner and the IC50 of 75 μg/mL for Ag NPs. Ag NPs cytotoxicity was associated with induction of ROS and cell apoptosis in HepG2 cell line. According to our findings, Ag NPs could be considered as potential chemotherapeutic agents in the treatment of liver hepatocellular carcinoma.

1. Introduction

Numerous studies have been conducted on nano-scaled advanced materials (generally smaller than 100 nm) in nanomedicine area so far [1]. Nano-pharmacology is a rather novel division of pharmacology which explores interaction of a nanomaterials in living systems at cellular levels [2,3]. Nano-pharmacology which is an innovative improvement in modern medicine offers important benefits to deliver nanomaterials for treating a wide range of human diseases in empirical and clinical research [3–5]. According to the reports, silver nanoparticles (Ag NPs) are well known for their antimicrobial effects [6], they also display extraordinarily physical, biochemical and biological properties which make them appropriate agents in wound healing and contraceptive use as well as cosmetics, textile and food technology appliance [7]. From the pharmacological point of view, many attempts have been made to assess the anti-cancer activity of Ag NPs with optimistic future directions [8]. Such a hopeful option offer outstanding advantages to the field of medicine since small sized nanomaterials would successfully decline the monetary losses in cancer theranostic. Besides, targeted delivery of nano-silver could benefit cancer patients with increased efficacy. Preceding experiments have demonstrated that the mechanism of nano-silver cytotoxicity involves disruption of the mitochondrial electron transferring chain resulting generation of ROS and interruption of ATP synthesis, which in turn causes DNA damage [9]. In addition, according to some in vivo and in vitro experiments silver nanoparticles induce genotoxicity [10].

Cancer, as a major public health problem, is the second leading cause of death in the world [11]. According to world health

* Correspondence to: A. Eftekhari, Department of Pharmacology and Toxicology, Maragheh University of Medical Sciences, PO Box: 78151-55158, 8 Maragheh, Iran.
** Correspondence to: H. Hosainzadegan, Department of Microbiology, Maragheh University of Medical Sciences, PO Box: 78151-55158, Maragheh, Iran.
E-mail addresses: ftekhari@ymail.com (A. Eftekhari), hasanhosainy122@yahoo.com (H. Hosain zadegan).
[1] The authors contributed equally to this work.

https://doi.org/10.1016/j.msec.2018.08.027
Received 23 November 2017; Received in revised form 14 July 2018; Accepted 8 August 2018
Available online 09 August 2018
0928-4931/

organization (WHO) report, there is an estimation of > 21 million cancer cases and 13 million death in worldwide [12]. Hepatocellular carcinoma (HCC) has been previously reported to be the third most common cause of death from cancer [13,14]. Although surgical resection is considered as the standard curative treatment of HCC, the majority of patients are not candidates for this type of treatment mainly due to the advanced tumor extension at the first diagnosis and/or insufficient liver functionality reservoirs [15]. In addition, chemotherapy regimes in suitable candidates have often been restricted because of major organ damages, inefficient drugs and poor prognosis [16]. Thus, search for novel anticancer agents or regimes with higher efficacy and minimal side effects is continued. The present study aims to investigate the potential cytotoxicity mechanisms of silver nanoparticles in liver hepatocellular carcinoma cell line (HepG2).

2. Methods and materials

2.1. Chemicals

RPMI-1640, penicillin and streptomycin and trypsin-EDTA solutions, L-glutamine, phosphate buffered saline (PBS), and fetal bovine serum (FBS) were obtained from Gibco Life Technologies Ltd. 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyltetra-zoplium bromide (MTT) and 2′,7′dichlorofluorescin diacetate (DCFH-DA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other materials were analytical/cell culture tested grade.

2.2. Synthesis of Ag NPs

Ag NPs were prepared by following the previously reported method [17] with some modification. Briefly, 50 mL of 2.0×10^{-4} mol L^{-1} $AgNO_3$ was transferred into a 100 mL beaker and heated for 2 min at 30 °C. 2.0 mL of 1% sodium citrate aqueous solution was added to it with vigorous stirring. Then its temperature fixed at 80 °C. After 10 min, 10 μL of freshly prepared ice-cold $NaBH_4$ solution (0.1 mol L^{-1}) was added to the solution. The colloid was stirred for another 30 min. As a result, a clear yellow solution was obtained. It was transferred to a 100 mL volumetric flask and diluted to the mark with distilled water. Centrifugation was performed at 12,000 rpm for 30 min and then the nanoparticles were lyophilized and dry powder was used for the analytical sections.

2.3. Characterization of Ag NPs

The mean particle size and zeta potential measurements were performed using DLS instrument (Malvern, United Kingdom) and completely particle free ultra-pure water at room temperature. At least three independent samples were taken. TEM image of the prepared nanoparticles was achieved using LEO 906E TEM (Zeiss, Germany).

2.4. Cell culture

HepG2 (liver hepatocellular adenocarcinoma) line was obtained from Pasture Institute, Tehran, Iran. Cells were cultured in RPMI medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL of penicillin and 100 μg/mL of streptomycin and were incubated in humidified atmosphere containing 5% CO_2 at 37 °C according to the guidebook of the cell line bank.

2.5. Determination of cell proliferation

The effects of Ag NPs on cell proliferation of HepG2 cells were assessed by MTT assay [18]. Cells, with seeding density 1.2×10^4 cells/well, were seeded in 96-well microplate containing 200 μL RPMI growth medium. The cells were then treated with various concentrations of nano-silver (0, 25, 50, 75 and 100 μg/mL) over different incubation time points (24, 48, and 72 h). After treatment time point, cells (treated/untreated control cells) were incubated with 150 μL fresh medium plus with 50 μL MTT solutions (2 mg/mL in PBS) for 4 h at 37 °C. After incubation time, the MTT contained medium was removed and the mixture of 200 μL DMSO and 25 μL mL Sorenson's glycine buffer (0.1 M glycine, 0.1 M NaCl, pH 10.5) were added to each well and were again incubated for 30 min at 37 °C. The absorbance of plates was measured at 570 nm using a microplate reader (Biotek, ELx800, USA).

2.6. Determination of ROS formation

The non-fluorescent dye, 2′,7′dichlorofluorescin diacetate (DCFH-DA), is oxidized to fluorescent dichlorofluorescein (DCF) by hydroperoxides, was utilized to determine relative levels of cellular ROS generation [19]. Cells (3×10^4 cells/well) were treated with nano-silver (0–100 μg/mL) for 24 h at 37 °C. Also ROS formation in nano-silver–-treated (200 μM) cells were measured in the presence of *N*-acetyl cysteine (NAC) as a potent well-known antioxidant. All treated/untreated control cells were detached by trypsin-EDTA (0.25% trypsin and 0.02% EDTA) and were washed by PBS (0.1 M, pH 7.2) and then were incubated in FBS free culture medium containing 50 μM dye for 30 min. The washing process was performed once more and then the cell suspensions were centrifuged at 412*g* for 10 min. The supernatant was removed and cell plates were dissolved with 1% Triton X100. Fluorescence changes were measured using a Jasco R_FP-750 spectrofluorometer (Jasco Corporation, Tokyo, Japan) with excitation and emission wavelengths of 485 and 530 nm, respectively [20].

2.7. Determination of cytochrome c amount

Enzyme linked immunosorbent assay (ELISA) kit (Cytochrome C ELISA Kit, Human - Thermo Fisher Scientific, Waltham, MA, USA) was used to quantified cytosolic cytochrome *c*. The cells in the density of 5×10^5 were lysed in lysis buffer according to the manufacturer's user instructions and samples were centrifuged at 500*g*, room temperature for 10 min. The supernatants and cytochrome *c* conjugate were added to the 96 well microplates coated with human cytochrome *c* monoclonal antibody. The absorbance of samples was measured at 450 nm in a microplate reader (Biotek, ELx800, USA). A standard curve was prepared by plotting the absorbance values of diluted solutions of a human cytochrome *c* standard and the cytochrome *c* concentration was expressed as ng/mL.

2.8. Real time

All untreated/treated cells were washed with phosphate buffer saline (pH 7.2) then 1 mL ice cold RNX-plus solution (SinaClone, Iran) was added into cells according the kit user instruments.

Complementary DNA (cDNA) was synthesized using PrimeScript RT Reagent kit (Takara Bio Inc., Tokyo, Japan). The cDNA synthesis was confirmed by usual agarose gel electrophoresis method, then cDNAs were stored at −20 °C for Real-Time PCR experiments.

Each Real-Time PCR experiment, triplicate for each sample, was subjected to ABI-step I plus (Applied Biosystems, Forster City, CA, USA) instrument.

Three gene-specific sets of primers for Bax (F 5′-CCCGAGAGGTCT TTTTCCGAG-3′ and R 5′-CCAGACCATAGCACACTCGG-3′), caspase 3 (F: 5′-TGCCTGTAACTTGAGAGTAGATGG-3′ and R: 5′-CTTCACTTTCT TACTTGGCGATGG-3′), caspase 8 (F: 5′-GACAGAGCTTCTTCGAGA CAC-3′ and R: 5′-GCTCGGGCATACAGGCAAAT-3′), caspase 9 (F: 5′-CTCAGACCAGAGATTCGCAAAC-3′ and R: 5′-GCATTTCCCCTCAAA CTCTCAA-3′) and housekeeping gene GAPDH (F 5′-AAGCTCATTTCCT GGTATGACAACG-3′ and R 5′-TCTTCCTCTTGTGCTCTTGCTGG-3′) were used.

The relative expression is commonly used where the expression of