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Fabrication of adenosine 5′-triphosphate-capped silver nanoparticles: Enhanced cytotoxicity efficacy and targeting effect against tumor cells

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ABSTRACT

The aim of this study is to investigate the effect of different concentrations of adenosine 5′-triphosphate (ATP) as a stabilizing agent on the physicochemical and biological behavior of AgNPs. The results revealed that the size of distribution and dispersion of AgNPs were controlled by changes in the ratio of ATP and $AgNO_3$ in the presence of $NaBH_4$. The data of FT-IR and zeta potential values show that ATP molecules have been coordinated on the surface of nanoparticles through the adenine moiety of ATP. Cellular viability studies in osteosarcoma cells (Saos-2), breast cancer cells (MCF-7 and T47D), and leukemia cells (K562) demonstrated that ATP-capped silver nanoparticles (ATP@AgNPs) possess high antitumor efficacy compared with the naked ones. It was found that the cellular uptake of ATP@AgNPs occurs through P2X7 receptors and clathrin-mediated endocytosis. As the P2X7 receptor is overexpressed on the surface of many tumor cells; ATP on nanoparticle surface plays the role of a targeting molecule. Moreover, it was demonstrated that the cytotoxicity induced by ATP@AgNPs proceeds from the perturbation of intracellular oxidative status, leading to the induction of apoptosis. From these data, ATP, as "dispersant" and "complexant," improves the physicochemical properties of AgNPs and potentiates their application in cancer therapy.

1. Introduction

In recent years, the application of nanotechnology for cancer therapy that provides unique strategies for overcoming chemoresistance and cancer progression has received considerable attention [1,2]. Nanoparticles and metal nanoparticles in particular are invaluable versatile components with a variety of biomedical applications and are emerging as an attractive platform for cancer therapy in a wide variety of targets and clinical settings [3,4]. Among the metal nanoparticles, it has been estimated that silver nanoparticles have the highest degree of commercialization owing to their unique features such as impressive electrical, optical, and thermal properties. Such features render silver nanoparticles suitable for several applications such as conductors, chemical sensors, and catalysts [5,6]. However, in the biomedical field, the silver nanoparticle has gained more attention because of the advantage obtained from its antibacterial properties for the prevention of infections, plasmonic and metallic properties for diagnostics, and biocompatibility for drug delivery [7]. Moreover, substantial evidence has demonstrated the striking effects of silver nanoparticles against malignant cells. *In vitro* research studies employing a spectrum of cancer cell lines have revealed that silver nanoparticles drastically reduce tumor cell viability and drive cells to apoptosis [2,8,9]. The proposed mechanisms by which silver nanoparticles cause growth inhibition and cell death are interception of cell cycle progression and perturbation of the intracellular oxidative status [10–12]. Recently, *in vivo* studies on rats with Pliss lymphosarcoma and other studies on breast cancer xenograft-bearing mice also have indicated the high therapeutic index of silver nanoparticles [13–15].

In fact, the intrinsic properties of metal nanoparticles such as toxic manifestations and pharmacological behaviors can be tuned by the modulation of physicochemical properties of nanoparticles including size, shape, chemical composition, crystal structure, and surface area [16]. These parameters can be controlled by employing different types of chemical components to be used as stabilizing or capping agents, which are frequently used during synthesis. For instance, Li et al. used aniline as a stabilizer to control the morphology and particle size of silver nanoparticles using hydrazine and sodium citrate as reducing agents [17]. Recently, cationic surfactants as dispersants have gained much interest in preparing size- and shape-controlled silver nanoparticles [18,19]. Other components such as thiols, phosphines, amines, polymers, and plant or microbial extracts have been reported as protective agents to stabilize nanoparticles. However, most of these components do not have biocompatibility or purity, and these properties restrict their applications in the biological system [20–24]. Therefore,

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the application of biomolecules as stabilizing agents may pave the way for more therapeutic utilization of metal nanoparticles. In this study, adenosine 5′-triphosphate (ATP) as a purine nucleotide was used as a candidate stabilizing agent during the synthesis of silver nanoparticles because of its unique structure, nontoxic nature, and pharmacological features. ATP is a multifunctional nucleotide with major roles in the transfer of energy within the cell and in the modulation of allosteric enzymes activity. It also is a building block of nucleic acids and coenzymes [25,26]. Moreover, it has previously been recognized that extracellular adenine-based nucleotides play a role in cell signaling and that they affect not only the wealth of different cellular responses but also regulate fundamental pathophysiological processes such as tissue homeostasis, wound healing, neurodegeneration, immunity, inflammation, and cancer [27]. Advances in analytical methods for measuring extracellular ATP levels have revealed a high level of nucleotide in the tumor microenvironment (at least 10-fold) relative to healthy tissue [28]. It has been documented that high extracellular ATP levels allow a specific microenvironment for tumors, suppressing the immune responses and stimulating the growth of tumor cells [26]. Virtually, all tumor cell lines and many primary human tumors that have so far been investigated highly express P2 type of purinergic receptors, and they are more sensitive to ATP than normal cells [26]. Among purinergic receptors, P2X receptors are homo- or heterotrimeric ion channels that mediate transmembrane fluxes of mono- and divalent ions upon interaction with the extracellular ATP. These receptors are highly expressed by many different human tumor cell lines (for example, U937 and NB4 human acute myelogenous leukemia, Jurkat lymphoma, and A375 human melanoma) and by several primary human tumors (glioblastoma and prostatic cancer), demonstrating a correlation between the expression of P2X receptors and cancer progression [29]. In this line, it has been demonstrated that osteosarcoma cells such as Saos-2 cell line and breast cancer cells such as MCF-7 and T47D cell lines severely express P2X7 receptors at the level of mRNA and protein [30–32]. Therefore, targeting the P2X receptors in tumor cells would enhance the therapeutic index of nanoparticles in cancer patients. On the basis of this knowledge, it was conceptualized that capping silver nanoparticles with ATP not only has an impact on physicochemical properties and pharmacological effects of silver nanoparticles but also imparts an effective tumor-targeting property to them, which may eventually ameliorate numerous toxic side effects. Hence, the aim of this study is to investigate the effect of different concentrations of ATP on size, surface charges, shape, and cytotoxicity of silver nanoparticles against tumor cells and normal cells.

2. Materials and methods

2.1. Materials

Silver nitrate ($AgNO_3$), sodium borohydride ($NaBH_4$), acridine orange, and ethidium bromide were purchased from Merck. The cell culture medium (RPMI-1640) and penicillin–streptomycin were purchased from Biosera (France). Fetal bovine serum (FBS) was purchased from Gibco BRL (Life Technology, Paisley, Scotland). The cell line was obtained from the Pasteur Institute of Iran (Tehran, Iran). Methylthiazolyldiphenyl-tetrazolium bromide (MTT), propidium iodide, genistein, and adenosine 5′-triphosphate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 2′,7′-dichlorodihydrofluorescin diacetate (DCFH-DA) and KN-62 [(4-[(23)-2-[(5-isoquinolinsulphonyl)methylamino]-3-oxo-3-(4-phenyl-1-piperazinyl) propyl]phenyl isoquinoline sulphonic acid ester)] were purchased from Cayman Chemical Co. (Ann Arbor, MI, USA).

2.2. Synthesis of Ag nanoparticles

Silver nanoparticles (AgNPs) and ATP-capped silver nanoparticles (ATP@AgNPs) were prepared according to the following procedure. All glassware pieces were placed in chromic acid and were rinsed several times with water. $AgNO_3$ (3.41×10^{-2} M) was dissolved in 60 ml of deionized water, and then 461 µl of ATP solution at different concentrations (1.3 mM of (ATP@Ag (1)) and 10 mM of (ATP@Ag (2))) was added, and the solution was stirred vigorously for 5 min. The freshly prepared $NaBH_4$ aqueous solution (0.1 M, 1.2 ml) was then added to the stirred $AgNO_3$ solution dropwise and stirred for another 2 h. The resulting solution was aged for 7 days, and then it was centrifuged for 20 min to remove the excess amounts of stabilizing and reducing agents.

2.3. Characterization of synthesized nanoparticles

The crystal structure of the nanoparticles was analyzed by X-ray diffraction (XRD) using CuKa radiation ($\lambda = 0.154$ A°) at 40 kV and 30 mA, at a step size of 0.010° and step time of 1 s (EXPLORER, GNR, Italy). Fourier-transform infrared (FT-IR) spectra were recorded using a Nicolate Avatar 370 FT-IR Therma spectrometer. Transmission electron microscopy (TEM) was performed using a Leo 912 AB (120 kV) microscope (Zeiss, Germany). The morphology of fabricated samples was observed by field-emission SEM (MIRA3TESCAN-XMU) with a 20 kV accelerating voltage. Dynamic light scattering (DLS) and the surface charge of the nanoparticles were analyzed with a laser zetameter (Zeta compact, CAD instrumentation, France). UV–vis absorption spectra of the samples were recorded by a UV–vis spectrophotometer (Optizen 322 OUV, MECASYS, North Korea) in quartz cell.

2.4. Cell culture

Human osteosarcoma cell line (Saos-2), breast cancer cell lines (MCF-7 and T47D), and leukemia cell line (K562) were cultured in RPMI-1640 medium supplemented with FBS (10%, v/v), streptomycin (100 g/ml), and penicillin (100 U/ml). The cells were incubated under 5% CO_2 humidified atmosphere at 37 °C. Cell number and viability were assessed using a hemocytometer and the ability of the viable cells to exclude trypan blue. Human dermal fibroblast cell line (HDF) and human embryonic kidney cell line (HEK-293) were obtained from the Research Institute of Biotechnology (Ferdowsi University of Mashhad, Mashhad, Iran). The cells were cultured in DMEM (Dulbecco's modified Eagle's medium) supplemented with glucose (4500 mg/l), FBS (10%, v/v), streptomycin (100 g/ml), and penicillin (100 U/ml). The cells were incubated under 5% CO_2 humidified atmosphere at 37 °C.

2.5. Measurement of cell viability

MTT was used as an indicator of cell viability as determined by its mitochondria-dependent reduction to formazone. To evaluate the cytotoxicity of nanoparticles, cells were seeded at a density of 1×10^4 cells/well into 96-well tissue culture plates. After 24 h, the culture medium was replaced with medium containing 0.05–31.25 µg/ml nanoparticles and incubated at 37 °C for 48 h. Then, 10 µl of MTT (5 mg/ml) was added to each well, and the plate was incubated at 37 °C for 4 h. The culture supernatant was removed, and the formazone crystals were dissolved using DMSO. The mixture was agitated for 15 min at room temperature (RT). The absorbance was read at 570 nm using an ELISA reader (BioTek, ELX800, USA). Cell viability was expressed as the percentage of surviving cells in the treated sample relative to the control sample.

2.6. Acridine orange and ethidium bromide staining

For the detection of apoptotic cells, acridine orange (AO) and ethidium bromide (Et) dual staining was carried out. The cells were seeded at a density of 2×10^4 cells/well into 24-well tissue culture plates. After 24 h, the culture medium was replaced with the medium containing nanoparticles, and this was incubated at 37 °C for 48 h. After