Toxicology in Vitro xxx (2015) xxx–xxx

1

Contents lists available at ScienceDirect

Toxicology in Vitro

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

5
6

3 # Gold nanoparticles do not induce myotube cytotoxicity but increase the
4 susceptibility to cell death

7 Paulo Emílio Corrêa Leite [a,*], Mariana Rodrigues Pereira [c], Carlos Antonio do Nascimento Santos [a],
8 Andrea Porto Carreiro Campos [b], Ticiana Mota Esteves [a], José Mauro Granjeiro [a,c]

9 [a] Divisão de Biologia Celular e Bioengenharia, Diretoria de Metrologia Aplicada as Ciências da Vida (DIMAV), Brazil
10 [b] Divisão de Metrologia de Materiais (DIMAT), Instituto Nacional de Metrologia, Qualidade e Tecnologia (INMETRO), 25250-020 Duque de Caxias, RJ, Brazil
11 [c] Instituto de Biologia, Universidade Federal Fluminense, Niterói, RJ, Brazil

12
13

ARTICLE INFO

16 *Article history:*
17 Received 12 November 2014
18 Accepted 16 February 2015
19 Available online xxxx

20 *Keywords:*
21 Gold nanoparticle
22 PEG
23 Nanotoxicity
24 Bioengineering
25 Skeletal muscle
26 Inflammation
27

ABSTRACT

Gold nanoparticles (AuNP) have been widely used for many applications, including as biological carriers. A better understanding concerning AuNP safety on muscle cells is crucial, since it could be a potential tool in the nanomedicine field. Here, we describe the impact of polyethylene glycol-coated gold nanoparticles (PEG-AuNP) interaction with differentiated skeletal muscle C2C12 cells on cell viability, mitochondria function, cell signaling related to survival, cytokine levels and susceptibility to apoptosis. Intracellular localization of 4.5 nm PEG-AuNP diameter size was evidenced by STEM-in-SEM in myotube cells. Methods for cytotoxicity analysis showed that PEG-AuNP did not affect cell viability, but intracellular ATP levels and mitochondrial membrane potential increased. Phosphorylation of ERK was not altered but p-AKT levels reduced ($p < 0.01$). Pre-treatment of cells with PEG-AuNP followed by staurosporine induction increased the caspases-3/7 activity. Indeed, cytokines analysis revealed a sharp increase of IFN-γ and TGF-β1 levels after PEG-AuNP treatment, suggesting that inflammatory and fibrotic phenotypes process were activated. These data demonstrate that PEG-AuNP affect the myotube physiology leading these cells to be more susceptible to death stimuli in the presence of staurosporine. Altogether, these results present evidence that PEG-AuNP affect the susceptibility to apoptosis of muscle cells, contributing to development of safer strategies for intramuscular delivery. 29–43

© 2015 Elsevier Ltd. All rights reserved. 44
45
46

1. Introduction

Nowadays, the development of efficient and safe strategies for drug and gene delivery or the improvement of existing strategies is essential. Concerning toxicity effects of AuNP, many works reported their nontoxic, nonimmunogenic and biocompatible properties with relevance application in nanomedicine (Ghosh et al., 2013; Mieszawska et al., 2013; Connor et al., 2005). For this reason, AuNP are widely used in biomedical applications such as the diagnosis, cell labeling, drug and intracellular siRNA delivery, antitumor therapy medicine, biological sensors (Zhang et al., 2011), and photothermal conducting agent (You et al., 2014). This is possible due to their better cellular uptake, low cytotoxicity and possibility for functionalization (Papasani et al., 2012).

However, some studies demonstrated that AuNP cause cellular damage through indirect mechanisms. Several studies report toxicity, including induction of necrosis and apoptosis (Pan et al., 2007), oxidative stress, inflammation (Shukla et al., 2005), DNA damage and alterations in gene expression (Li et al., 2010). Then, there are conflicting reports describing whether AuNP are toxic or nontoxic to different cells under specific conditions.

Regarding AuNP effects on skeletal muscle cells viability, few data are available in the literature. Due to crescent interest of AuNP utilization for clinical application as DNA vaccine administration (Noh et al., 2007) and treatment of muscular disorders (Acharya and Hill, 2014) that involve intramuscular administration route, it is necessary better to evaluate their effects on skeletal muscle cells viability. Then, the murine C2C12 cell lineage is a good model because these cells can be differentiated and assume a multinucleated and striated myotube organization, as occurs in mammals. Previously, AuNP demonstrated efficient transfection of NADPH Oxidade 4 siRNA in C2C12 myoblast cell lineage, but cellular viability assays were not investigated (Acharya and Hill, 2014). In another study using the same cell lineage, AuNP carrying plasmid DNA encoding for murine IL-2 showed a high cell viability measured by MTT assay (Noh et al., 2007). In the same way, the survival of C2C12 cells treated with

* Corresponding author at: Av. Nossa Senhora das Graças 50, Laboratório de Biologia – Prédio 27, Duque de Caxias – Xerém, RJ 25250-020, Brazil. Tel.: +55 21 2145 3150; fax: +55 21 2679 9837.
E-mail address: leitepec@gmail.com (P.E.C. Leite).

http://dx.doi.org/10.1016/j.tiv.2015.02.010
0887-2333/© 2015 Elsevier Ltd. All rights reserved.

UNCORRECTED PROOF

polyethylene glycol (PEG)-coated gold nanorods was not affected through MTS assay quantification (Rayavarapu et al., 2010). However, other studies with a variety of cell types demonstrated through different approaches beyond the analysis of formazan reaction product (MTT and MTS assays) the toxic effects of AuNP (Chueh et al., 2014; Arvizo et al., 2013; Chuang et al., 2013; Yen et al., 2009; Pan et al., 2009).

In this sense, this study intends to contribute for a better understanding of the PEG-AuNP effects on muscle cells viability, due to the widely use of PEG for nanodelivery, its neutral charge and resistance characteristics for protein adsorption (Verma and Stellacci, 2010; Guo et al., 2010; Zhao et al., 2012). We investigated the effect of PEG-AuNP on the viability of C2C12 lineage through different methods, determining eventual modulation and production of factors and also the susceptibility of cells to death.

2. Methods

2.1. Nanoparticle and chemicals

Spherical monodisperse 5-kDa PEG-AuNP with 4.5 ± 0.6 nm were purchased from Nanocomposix (San Diego, CA, EUA), batch JMW1410, with 1 mg/mL Au mass concentration and 10^{15}-particles/mL in sterile MilliQ water.

Dulbecco's modified Eagle's medium (DMEM) and supplement fetal bovine serum (FBS) were purchased from Gibco BRL (Grand Island, NY, USA). XTT water soluble In Cytotox XTT-NR-CVDE (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) from Xenometrix, Aniara Co. (Mason, OH, USA). Calcein AM reagent of the Live-dead kit from Molecular Probes (Eugene, OR, USA). MTT water insoluble (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide), dimethyl sulfoxide (DMSO), methanol, paraformaldehyde (PFA), cell lysis buffer (CelLytic M), and protease inhibitor cocktail (I3786) from Sigma Chem. Co. (Saint Louis, MO, USA). Apo-ONE Homogeneous Caspase-3/7 Assay from Promega (Madison, WI, USA), and staurosporine from B-Bridge International, Inc. (Cupertino, CA, USA). All other reagents were of analytical grade.

2.2. Nanoparticle validation

PEG-AuNP were mixed with absolute ethanol, sonicated for 5 min and deposited on a lacey carbon support film suspended on a 300 mesh copper grid, air-dried and analyzed by Energy Dispersive X-ray Spectroscopy (EDS) in a probe-corrected FEI Titan 80–300 transmission electron microscope equipped with an EDAX EDS System operating at 300 kV in parallel and STEM modes. Hydrodynamic diameter and biodispersion were performed in all concentrations of PEG-AuNP utilized diluted in complete medium through dynamic light scattering (DLS) and zeta potential, in a Malvern Zetasizer Nano ZS apparatus (Malvern Instruments Ltd., Worcestershire, UK). Wavelength scanning absorbance at different concentrations diluted at 50 μL final volume in ultrapure water were done in Tecan Infinite 200PRO microplate reader (Tecan Group Ltd., Mannedorf, CH) ranging from 400 to 800 nm with 10 nm wavelength steps. Nanoparticles were incubated with the chemical reagents in culture medium to verify whether they could interact resulting in interference on colorimetric, fluorescence and luminescence methods.

2.3. Cell culture

Mouse myoblastoma C2C12 cells mycoplasma free (MycoAlert Mycoplasma Detection Kit – Lonza, Bazel, Switzerland) were maintained as subconfluent monolayer in complete DMEM according to ATCC Laboratories. C2C12 cells were cultivated with 10% FBS during 48 h for proliferation of cells. After, this medium was changed by fresh DMEM supplemented with 5% horse serum (HS) for C2C12 myotube differentiation during 72 h. Thus, medium was changed by a fresh DMEM 5% HS containing different concentrations of PEG-AuNP (10^{11}, 10^{12}, 10^{13} and 5×10^{13} particles/mL, or 0.1 μg/mL, 1 μg/mL, 10 μg/mL and 50 μg/mL, or 0.51 nM, 5.1 nM, 51 nM or 254 nM, respectively), that were previously diluted in room temperature and vigorously mixed for 1 min. After 24 h of treatment, C2C12 supernatants were collected and cells used in the experimental procedures.

The number of plated cells varied depending on the assay type as follow: 10^4 cells on each well of 96-well plates for analysis of different cellular parameters, or on 8-well Millicell EZ slide (Millipore Corp., Billerica, MA, USA) for images acquisition. For western blot analysis, 5×10^5 cells were plated on each well of 6-well plates. An experimental group was treated only with PEG (MPEG-SH-5,000, Laysan Bio Inc., Florida, USA) at 50 mg/mL considering that it is the maximum concentration possible if PEG was all packed and all dissociated from the AuNP surface. We did not detect effect mediated by PEG treatment in the assays. Positive control of cell death was done for all assays incubating cells with 70% methanol during 30 min at room temperature (RT), before starting both assays. Absorbances were measured in a Tecan Infinite 200PRO microplate reader at 570 nm, with reference reading at 690 nm. Intrinsic absorbance, fluorescence or luminescence of PEG-AuNP were measured without cells plus the respective reagents and subtracted of the experimental groups.

2.4. STEM-in-SEM (Scanning transmission electron microscopy mode in Scanning electron microscopy mode)

Cells were fixed with 2.5% glutaraldehyde in 0.1 M PBS pH 7.4 for 2 h, and rinsed with 0.1 M cacodylate buffer pH 7.4 for three cycles of 15 min. Cells were postfixed with 1% osmium tetroxide, 0.8% potassium ferrocyanide and 5 mM calcium chlorate in 0.1 M cacodylate buffer for 1.5 h. Then, three samples of three independent experiments were rinsed in the same cacodylate buffer, stained with 1% uranyl acetate overnight at 4 °C, dehydrated in a graded acetone series up to 100% acetone, and then embedded in epoxy resin Poly/Bed 812. Semi-thin sections (0.4 μm) were cut and stained with 0.1% toluidine blue for light microscopy. Ultrathin sections (60–70 nm) were cut on a Leica EM UC6 ultramicrotome (Leica Microsystems, Wetzlar, Germany). After staining with uranyl acetate and lead citrate, sections were observed with a FEI Magellan 400 XHR-SEM in STEM mode. The images that better represent the results were showed.

2.5. XTT and MTT assays

After PEG-AuNP exposure times previously described, cells were washed and followed by addition of 200 μL 5% FBS DMEM without phenol red

For XTT assay, the solution 1 (substrate) were diluted in the solution 2 (buffer) at 1:100 and protected from light. After mixing, 50 μL were added to each well followed by incubation for 90 min. Then, samples were homogenized and absorbances measured in the Tecan Infinite 200PRO microplate reader at 480 nm, with reference reading at 690 nm.

For MTT assay, 20 μL of 1.5 mg/mL MTT diluted in DMEM without phenol red were added to each well followed by incubation for 3 h. Then, samples were dissolved with 200 μL DMSO.

UNCORRECTED PROOF

Please cite this article in press as: Leite, P.E.C., et al. Gold nanoparticles do not induce myotube cytotoxicity but increase the susceptibility to cell death. Toxicol. in Vitro (2015), http://dx.doi.org/10.1016/j.tiv.2015.02.010

Download English Version:

https://daneshyari.com/en/article/5861655

Download Persian Version:

https://daneshyari.com/article/5861655

Daneshyari.com