Toxicology Letters 226 (2014) 303–313

Contents lists available at ScienceDirect

Toxicology Letters

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

The role of reactive oxygen species in the genotoxicity of surface-modified magnetite nanoparticles

Monika Mesárošová[a], Katarína Kozics[a], Andrea Bábelová[a], Eva Regendová[a], Michal Pastorek[a], Dominika Vnuková[b], Barbora Buliaková[a], Filip Rázga[b], Alena Gábelová[a,*]

[a] Cancer Research Institute, SAS, Vlárska 7, 833 91 Bratislava, Slovakia
[b] Polymer Institute, SAS, Dúbravská cesta 9, 845 41 Bratislava, Slovakia

HIGHLIGHTS

- Magnetite nanoparticles (MNPs) induced variable levels of iROS in human lung cell lines.
- No oxidative damage to DNA was detected in MNP-treated human lung cell lines.
- No substantial changes in the TAC, iGSH or GPx activity were found in either of the cell lines.
- Oxidative stress generation plays, at most, only a marginal role in MNP genotoxicity.

ARTICLE INFO

Article history:
Received 23 July 2013
Received in revised form 26 February 2014
Accepted 27 February 2014
Available online 12 March 2014

Keywords:
Magnetite nanoparticles
Human lung cells
Reactive oxygen species
Oxidative DNA damage
Antioxidant enzymes

ABSTRACT

The generation of reactive oxygen species (ROS) has been proposed as the underlying mechanism involved in the genotoxicity of iron oxide nanoparticles. The data published to date are, however, inconsistent, and the mechanism underlying ROS formation has not been completely elucidated. Here, we investigated the capacity of several surface-modified magnetite nanoparticles (MNPs) to generate ROS in A549 human lung adenocarcinoma epithelial cells and HEL 12469 human embryonic lung fibroblasts. All MNPs, regardless of the coating, induced significant levels of DNA breakage in A549 cells but not in HEL 12469 cells. Under the same treatment conditions, variable low levels of intracellular ROS were detected in both A549 and HEL 12469 cells, but compared with control treatment, none of the coated MNPs produced any significant increase in oxidative damage to DNA in either of these cell lines. Indeed, no significant changes in the total antioxidant capacity and intracellular glutathione levels were observed in MNPs-treated human lung cell lines regardless of surface coating. In line with these results, none of the surface-modified MNPs increased significantly the GPx activity in A549 cells and the SOD activity in HEL 12469 cells. The GPx activity was significantly increased only in SO-Fe_3O_4-treated HEL 12469 cells. The SOD activity was significantly increased in SO-PEG-PLGA-Fe_3O_4-treated A549 cells but significantly decreased in SO-Fe_3O_4-treated A549 cells. Our data indicate that oxidative stress plays, at most, only a marginal role in the genotoxicity of surface-modified MNPs considered in this study in human lung cells.

© 2014 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Magnetic iron oxide nanoparticles, including γ-Fe_2O_3 (maghemite), α-Fe_2O_3 (hematite) and Fe_3O_4 (magnetite), are increasingly being investigated for use in a variety of biomedical applications, both diagnostic and therapeutic (Corchero and Villaverde, 2009; Wahajuddin and Arora, 2012). Because iron metabolism is well controlled and excess iron is efficiently removed from the body (Jomova and Valko, 2011), iron oxide nanoparticles are considered to be biocompatible and nontoxic.

Magnetite is one of the most frequently used forms of iron oxide in nanoparticles. Magnetite nanoparticles (MNPs) have great potential as magnetic resonance imaging (MRI) contrast agents (Singh et al., 2009; Triantafyllou et al., 2013), heating mediators in hyperthermia-based cancer therapy (Jordan et al., 2001) and

* Corresponding author at: Department of Genetics, Laboratory of Mutagenesis and Carcinogenesis, Cancer Research Institute, Slovak Academy of Sciences, Vlárska 7, 833 91 Bratislava, Slovakia. Tel.: +421 2 59327512; fax: +421 2 59327250.
E-mail address: alena.gabelova@savba.sk (A. Gábelová).

http://dx.doi.org/10.1016/j.toxlet.2014.02.025
0378-4274/© 2014 Elsevier Ireland Ltd. All rights reserved.

nanocarriers in targeted drug/gene delivery (Duguet et al., 2006). Moreover, the superparamagnetic properties of MNPs allow the delivery and trapping of drug-loaded MNPs in the target site *via* an external magnetic field (Marszall, 2011). The coating of MNPs with synthetic and natural chemical moieties minimizes hydrophobic interactions, thus enhancing their desirable properties, such as colloid stability and internalization efficiency, and enabling their functionalization with ligands, drugs, genes or antibodies that enhance target-specific interactions with tumor cells, thus increasing their therapeutic benefit (Babic et al., 2008; Gupta and Gupta, 2005). MNPs are also being utilized for a plethora of biotechnological applications, including enzyme immobilization, targeted cell/macromolecule separation and purification or magnetofection (Corchero and Villaverde, 2009).

Although the benefit of MNPs is obvious, the impact of MNPs on basic cellular processes such as the cell cycle, cell signaling, apoptosis, oxidative stress and inflammation has not been sufficiently explored (Singh et al., 2009, 2010). MNPs have already been approved as contrast agents for MRI analysis (Duncan and Gaspar, 2011). In addition, they are frequently being utilized in cellular therapy such for cell labeling and sorting (Andreas et al., 2012; Elias and Tsourkas, 2009; Marek et al., 2008). Therefore, a thorough investigation of the impact of MNPs on diploid cells and gaining an understanding of nanoparticle–cell interactions are necessary. The number of studies of MNP bio-safety in non-tumor (diploid cells) and tissues is, however, limited. Several studies have shown that MNPs can cause serious damage to healthy cells. Strong cytotoxicity, the disruption of cytoskeletal structures, apoptosis, and oxidative stress have been detected in both human and mammalian diploid cells treated with MNPs (Buyukhatipoglu and Clyne, 2011; Guichard et al., 2012; Hanini et al., 2011; Mesarosova et al., 2012; Wu et al., 2010).

The generation of reactive oxygen species (ROS) has been proposed as the underlying mechanism involved in the genotoxicity of metal oxide nanoparticles, particularly iron oxide nanoparticles. Several mechanisms have been proposed for ROS generation. Iron ions released into the cytosol due to lysosomal enzymatic degradation can participate in the Fenton reaction, producing the hydroxyl radicals (Valko et al., 2006), or the particle surface *per se* may act as a catalyst (Klein et al., 2012). Alternatively, ROS formation may occur due to MNP-mediated damage to the mitochondrial membrane (Sioutas et al., 2005), or MNPs may interact with NADPH oxidase in the plasma membrane during entry into the cell (Bedard and Krause, 2007). Although free iron ions are thought to be stored in the iron-storage proteins ferritin and hemosiderin and progressively re-used (Elias and Tsourkas, 2009), the iron-binding capacity of cellular ferritin is limited (Ganz, 2003). Efforts to achieve the maximum therapeutic effect can lead to iron overload at the target site and the disruption of iron homeostasis, promoting ROS generation. However, cells possess an effective inherent antioxidant defense system composed of non-enzymatic and enzymatic antioxidants; therefore, the contribution of MNP-mediated oxidative stress to the toxicity of MNPs is poorly understood (Soenen et al., 2011). The particle size and surface chemistry have been shown to have great importance in the ROS-mediated activity of MNPs (Hong et al., 2011; Hoskins et al., 2012). Bare iron oxide nanoparticles might be significantly more toxic than coated nanoparticles because the surface iron ions are more efficient inducers of ROS production (Voinov et al., 2011) while the coating may function as a barrier and attenuate the potential toxic effects (Auffan et al., 2006). Data publishing until now are, however, controversial. The bare MNPs were shown to induce ROS generation in human diploid and tumor cells (Choi et al., 2009; Hoskins et al., 2012; Karlsson et al., 2009; Zhu et al., 2008), rat lung epithelial cells (Ramesh et al., 2012), and Chinese hamster ovary cells (Kawanishi et al., 2013) but not in Syrian hamster embryo cells (Guichard et al., 2012), Cos-1 cells (Magdolenova et al., 2012) and human lung adenocarcinoma epithelial A549 cells (Kain et al., 2012; Konczol et al., 2011). On the other hand, variable levels of ROS formation was detected in human and mammalian cell lines treated with surface-modified MNPs. The MNPs-mediated ROS generation was dependent on surface coating (Guichard et al., 2012; Hong et al., 2011; Konczol et al., 2011; Liu et al., 2011; Magdolenova et al., 2012; Sharma et al., 2014) and cell type (Guadagnini et al., 2013; Liu et al., 2011). The citrate-coated MNPs did not induce any ROS formation in L-929 cells in contrast to tetraethyl orthosilicate- and (3-aminopropyl)trimethoxysilane-modified MNPs (Hong et al., 2011). A dose-dependent increase in ROS formation was determined in SH-SY5Y cells treated with polyethyleneimine coated MNPs (MNP-PEI), however, coating the MNP-PEI particles with polyethylene glycol (MNP-PEI PEG) resulted in ROS production consistent with the control cells (Hoskins et al., 2012). The sodium oleate coated MNPs induced ROS generation in Cos-1 cells (Magdolenova et al., 2012) and in 16HBE cells but not in A549 cells (Guadagnini et al., 2013). Accordingly, increased ROS production was determined in several human and mammalian cell lines but not in HeLa cells after treatment with DMSA-coated MNPs (Liu et al., 2011).

The goals of this study were as follows: (i) to investigate the role of ROS generation in the genotoxicity of coated MNPs, (ii) to compare the sensitivities of human lung cancer and diploid cells to MNP treatment, and (iii) to assess the contribution of the surface chemistry of MNPs to oxidative stress and ROS generation. Magnetic nanoparticles with a 7.6 nm magnetite core and different hydrophilic shells were characterized in depth using different physicochemical assays (Mesarosova et al., 2012). The MNPs used in this study were coated with the following: (i) sodium oleate (SO prevents aggregation and makes MNPs stable; SO-Fe_3O_4,), (ii) SO + polyethylene glycol (PEG reduces interactions with plasma proteins and thus minimizes MNP internalization and clearance by macrophages; SO-PEG-Fe_3O_4), and (iii) SO + PEG + poly[lactide-*co*-glycolic acid] (PLGA prevents degradation and aids in the regulation of drug release from nanoparticles; SO-PEG-PLGA-Fe_3O_4). DNA breakage, oxidative damage to the DNA, the intracellular ROS levels and the activities of antioxidant enzymes (superoxide dismutase (SOD) and glutathione peroxidase (GPx)) were assessed in both A549 and HEL 12469 cells under control and MNP-exposed conditions.

2. Materials and methods

2.1. Chemicals

Poly(lactide-*co*-glycolic acid) [PLGA, D,L-lactide to glycolide ratio 85:15, M_w = 50–75 kDa], Pluronic F68, poly(ethylene glycol) [PEG, 1 kDa, M_w = 1000], 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFH-DA, CAS 4091-99-0), ethidium bromide (EtBr, CAS 1239-45-8), low-melting-point (LMP) agarose, Triton X-100, HEPES, propidium iodide (PI, CAS 25535-16-4), and hydrogen peroxide (H_2O_2) were purchased from Sigma–Aldrich (Lambda Life, Slovakia). Sodium oleate was purchased from Riedel-de Haën (Hannover, Germany), formamidopyrimidine-DNA glycosylase/AP nuclease (FPG) was purchased from Biolabs (BioTech, Slovakia), and methanol and glycine were purchased from SERVA (BioTech, Slovakia). (R)-1-[(10-Chloro-4-oxo-3-phenyl-4H-benzo(a)quinolizin-1-yl)carbonyl]-2-pyrrolidine-methanol (Ro 19-8022; RO) was provided by Hoffmann – LaRoche AG (Basel, Switzerland), and the RANSOD kit was purchased from Randox Laboratories (Crumlin, UK). Culture media, fetal bovine serum (FBS), antibiotics and other chemicals used for cell cultivation were purchased from GIBCO (KRD Ltd., Slovakia). All other chemicals and solvents were of analytical grade.

2.2. Magnetite nanoparticles

The spherical magnetic iron oxide (Fe_3O_4) nanoparticles with a 7.6 nm magnetite core and different hydrophilic shells were kindly provided by Dr. M. Timko, PhD, Institute of Experimental Physics, SAS, Košice, Slovakia. Three types of magnetite nanoparticles (MNPs) were used in these experiments: (i) MNPs coated with sodium oleate (SO-Fe_3O_4), (ii) MNPs coated with SO + polyethylene glycol (SO-PEG-Fe_3O_4), and (iii) MNPs coated with SO + PEG + poly[lactide-*co*-glycolic acid], PLGA (SO-PEG-PLGA-Fe_3O_4). The physico-chemical characteristics of the surface-

Download English Version:

https://daneshyari.com/en/article/5860321

Download Persian Version:

https://daneshyari.com/article/5860321

Daneshyari.com