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Original Article

Surface-modified magnetite nanoparticles act as aneugen-like spindle poison

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Abstract

Iron oxide nanoparticles are one of the most promising types of nanoparticles for biomedical applications, primarily in the context of nanomedicine-based diagnostics and therapy; hence, great attention should be paid to their bio-safety. Here, we investigate the ability of surface-modified magnetite nanoparticles (MNPs) to produce chromosome damage in human alveolar A549 cells. Compared to control cells, all the applied MNPs increased the level of micronuclei moderately but did not cause structural chromosomal aberrations in exposed cells. A rise in endoreplication, polyploid and multinuclear cells along with disruption of tubulin filaments, downregulation of Aurora protein kinases and p53 protein activation indicated the capacity of these MNPs to impair the chromosomal passenger complex and/or centrosome maturation. We suppose that surface-modified MNPs may act as aneugen-like spindle poisons via interference with tubulin polymerization. Further studies on experimental animals revealing mechanisms of therapeutic-aimed MNPs are required to confirm their suitability as potential anti-cancer drugs.
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Key words: Magnetite nanoparticles; Human A549 cells; Micronuclei; Chromosome aberrations; Microtubule dynamics

The increasing range of applications of iron oxide nanoparticles (IONPs), maghemite (i.e. γ-Fe_2O_3) or magnetite (i.e. Fe_3O_4) nanoparticles in biomedicine has triggered discussions about their genetic safety for human health. IONPs have already been approved as contrast agents for magnetic resonance imaging (MRI) and are promising heating mediators in hyperthermia-based cancer therapy, as well as nanovectors in targeted drug/gene delivery.[1,2] IONPs are frequently being utilized in cellular therapy for cell labeling and sorting[3] and in a plethora of biotechnological applications including enzyme immobilization, targeted cell/macromolecule separation and purification or magnetofection.[4] They are in the center of an intensive research aimed at combining the therapeutic and diagnostic functions within a single nanostructure, to build theranostic nanocarriers.[5] The benefit of IONPs in biomedicine is obvious; therefore, the fate of IONPs following their therapeutic or diagnostic application generates a necessity to thoroughly investigate their potential negative impact on human health.

The number of genotoxicity studies on IONPs is still limited. The most employed method used to evaluate the genotoxic potential of these nanoparticles is the single-cell gel electrophoresis called the comet assay.[6,7] The endpoint measured by this versatile and sensitive technique is DNA breakage, i.e. DNA strand breaks formed due to DNA damage and error free/prone DNA repair.[8] A single genotoxicity assay can neither objectively consider the bio-safety of IONPs nor disclose all kinds of DNA damage that the particles might cause.[9] Therefore, inclusion of additional standard methods to evaluate nanoparticle genotoxicity/bio-safety more comprehensively is of highest interest. Only a limited number of studies investigated the ability of IONPs to induce chromosomal damage, i.e. changes in chromosome structure or number.

The authors declare that they have no competing interests.

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Table 1
Basic physico-chemical properties of surface-modified magnetite nanoparticles.

	SO-MNPs	SO-PEG-MNPs	SO-PEG-PLGA-MNPs
Magnetite inner core diameter [nm]	7.60 ± 0.05	7.60 ± 0.08	7.60 ± 0.04
Particle size (D_H) diameter in H_2O [nm]	44.00 ± 4.04	76.00 ± 1.53	155.00 ± 3.51
I_s at 295 K [Am^2kg^{-1}]	7.70 ± 0.02	6.00 ± 0.08	0.88 ± 0.01
Zeta potential (ζ) [mV]	−41.80 ± 0.90	−42.30 ± 0.80	−50.00 ± 1.10
Particle size distribution and mean diameter in culture medium [nm]	Unimodal 245.0 ± 6.0 (100%)	Unimodal 289.0 ± 7.0 (100%)	Tri-modal 884.0 ± 311.0 (94.9%)
Zeta potential (ζ) in culture medium [mV]	−14.80 ± 1.00	−14.10 ± 0.90	−14.70 ± 1.20

Moreover, the published results from these genotoxicity studies are controversial. Enhanced level of micronuclei (MN) after exposure to magnetite nanoparticles (MNPs) was detected in human A549 cells[10] and in human lymphoblastoid MCL-5 cells treated with dextran-coated maghemite nanoparticles.[11] In contrast, neither uncoated maghemite nanoparticles nor bare or dextran-coated MNPs elevated the level of MN in MCL-5 cells under the same treatment conditions.[11] No increase in the number of MN was observed also using Syrian hamster embryo cells,[12] Chinese hamster lung cells,[13] human granulosa HLG-5 cells[14] and monkey kidney cell line CHS-20[15] after treatment with IONPs. Contradictory results were found also among studies evaluating the frequency of MN in experimental animals. Intravenous administration of polyaspartic acid-coated MNPs[16] and intraperitoneal exposure to MNPs[17] enhanced the levels of MN in bone marrow cells significantly. An increase in the MN formation was detected in female mice reticulocytes after intraperitoneal exposure to bare IONPs.[18] In contrast, no effect was observed after oral gavage of maghemite nanoparticles,[19] intraperitoneal injection of MNPs loaded with daunorubicin,[20] and maghemite encapsulated in albumin-based nanospheres.[21] Maghemite nanoparticles induced various structural chromosome aberrations (CA) in human lymphocytes[22] and in Chinese hamster ovary cells.[23] On the other hand, no structural chromosome changes were observed in human peripheral blood cells after exposure to polyacrylic acid-coated and non-coated MNPs,[24] PEG- and PEI-coated IONPs[25] and bare MNPs.[26]

Recently, we have shown that MNPs coated with sodium oleate (SO), SO and poly(ethylene glycol) (PEG), and SO-PEG and poly(lactide-*co*-glycolic acid) (PLGA) have induced DNA breakage in A549 cells. However, the generation of reactive oxygen species (ROS) played only a marginal role in MNPs genotoxicity in this case.[27] In order to better understand the mechanism(s) involved in toxicity of surface-modified MNPs and evaluate their bio-safety objectively, the aim of this study was to investigate their capacity to induce chromosomal damage in terms of structural CA and/or MN in A549 cells. While the CA assay allows an accurate identification of all different chromosome mutation types (chromatid- and chromosome-type), the MN test is able to identify both clastogenic and aneugenic events induced by xenobiotics.[28] MNPs used in this study were characterized in-depth employing various physical and chemical methods, and their stability in culture medium was analyzed by dynamic light scattering (DLS). Besides genotoxicity, we investigated also the impact of MNPs on mitotic spindle assembly by examining tubulin staining patterns, on the activation of Aurora protein kinases, as well as β-tubulin and p53 protein expression by Western blotting. The effect of MNPs on cell cycle progression was monitored by flow cytometry. The internalized amount of MNPs was quantified by atomic absorption spectrometry (AAS). Our results indicated that non-DNA-reactive mechanisms might underlie the genotoxicity of surface-modified MNPs in A549 cells.

Methods

Magnetite nanoparticles (MNPs)

The synthesis, coating and physico-chemical characteristics of spherical magnetic iron oxide (Fe_3O_4) nanoparticles with a 7.6 nm magnetite core and different hydrophilic shells have been already published.[27,29] In brief, three types of MNPs were used in this study: i. MNPs coated with sodium oleate (SO-MNPs, D_H = 44 nm), ii. MNPs coated with SO and poly(ethylene glycol) (SO-PEG-MNPs, D_H = 76 nm), and iii. MNPs coated with SO + PEG and poly(lactide-*co*-glycolic acid) (SO-PEG-PLGA-MNPs, D_H = 155 nm). The basic characteristics of used MNPs after preparation and in culture medium are shown in Table 1.

Dynamic light scattering (DLS)

Particle size distribution and zeta potential of surface-modified MNPs in culture medium at 37 °C were determined by DLS using Zetasizer Nano-ZS (Malvern Instruments, UK) equipped with a 4 mW helium/neon laser (λ = 633 nm) and a thermoelectric temperature controller. The characteristics of MNPs and culture medium have already been published elsewhere.[27]

Treatment of cells

Exponentially growing human lung adenocarcinoma epithelial A549 cells were exposed to different concentrations of surface-modified MNPs in Dulbecco's modified Eagle medium (DMEM) supplemented with 2% fetal bovine serum (FBS) and antibiotics (penicillin, 100 U/mL and streptomycin, 100 μg/mL) for 24 h. Based on the cytotoxicity of particular surface-modified MNPs in A549 cells determined by MTT (viability between 100% and 20%),[29] concentrations between 0.05 and 1.00 mM were chosen for the reported experiments. Identical mM concentrations of Fe_3O_4 were applied to expose A549 cells to equal amount of MNPs regardless of the surface coating. The concentrations of surface-modified MNPs expressed as the μg of Fe_3O_4 *per* surface dish and as the number of MNPs *per* surface dish are shown in Table S1. The cell treatment was finished by removing the medium and washing the cells twice with phosphate buffer saline (PBS).

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