Cytotoxic Effect of Chitosan-Gold Nanoparticles on Two Cell Lines in Culture

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ABSTRACT

Among metallic nanoparticles gold possess several unique properties. It has been argued that AuNPs have significant toxicity both *in vitro* and *in vivo* but that coating may partially prevent harmful effects. Chitosan is a natural polysaccharide derivative of chitin known to have immunoenhancing effects, antitumor, antifungal and antimicrobial activities. The aim of this study was to investigate the cytotoxic effect of chitosan-AuNPs on C26 (murine colon carcinoma) and HeLa (human cervix carcinoma) cell lines. C26 and HeLa cells were exposed to 10 and 60 nm sized chitosan-AuNPs at five different concentrations (5, 10, 25, 50 and 100 µg/ml). After 24 h of incubation, cytotoxicity was assessed by the MTT colorimetric method and IC50 values were calculated. In C26 cells 60 nm particles were more toxic than 10 nm particles. On the other hand in HeLa cells the situation was reversed and 10 nm particles had the most harmful effect at a concentration 2.5 times smaller than that of 60 nm particles. Our results could suggest that chitosan-AuNPs have an antiproliferative effect on C26 and HeLa cell lines but that this depends on cell type and is influenced by particle size and concentration.

Keywords: C26, chitosan-Au NPs, HeLa, antiproliferative

INTRODUCTION

According to a recently suggested definition of the European Union nanotechnologies involve particles shorter than 100 nm at least in one dimension (BSI-PAS71, 2005). They are called 'intended' particles because they are intentionally produced in order to fulfil specific functions or gain characteristics in science, technology, medicine, industries and many other applications (Kreyling *et al.*, 2006; Pop *et al.*, 2015).

Because of its chemical inertness, gold has been used internally in humans for the past 50 years, from teeth to implants to radioactive gold used in cancer treatment (Mfhlen and Beller, 1979; Rosenberg *et al.*, 1985).

The fact that amine (Selvakannan *et al.*, 2003) and thiol (Templeton *et al.*, 2000) groups

bind strongly to AuNPs has enabled their surface modification with amino acids (Selvakannan *et al.*, 2004; Joshi *et al.*, 2004), proteins (Phadtare *et al.*, 2003) and DNA (Rosi *et al.*, 2006). This has lead to important biomedical applications (reviewed by Shukla *et al.*, 2005). Nanotechnology has broad applications in fighting cancer and can be employed in molecular imaging, molecular diagnosis, targeted therapy and bioinformatics (Cai *et al.*, 2008).

If clinical applications for NPs are to be developed their biocompatibility is crucial (Shukla *et al.*, 2005) and further studies of their toxicity are required. It has been argued that naked AuNPs have significant toxicity both *in vitro* and *in vivo*, while appropriate coating with chitosan

or PEG may partially prevent their harmful effects (Jokerstet *et al.*, 2011; Sabella *et al.*, 2011).

Chitosan is a natural polysaccharide derivative of chitin with biodegradable, biocompatible, non-toxic characteristics (Qi *et al.*, 2005) and it is known to have immunoenhancing effects, antitumor, antifungal and antimicrobial activities (Roller and Covill, 1999; Wang *et al.*, 2011; Zheng *et al.*, 2003). Beside these, it can act as a stabilizing agent and can be used to tailor nanocomposite properties and also to provide long-term stability to the nanoparticles by preventing particle agglomeration (Chen *et al.*, 2013). Bhumkar *et al.* (2007) developed a new strategy to exploit the use of chitosan as a reducing agent for the production of AuNPs. Interestingly, chitosan-AuNPs were bactericidal against antibiotic resistant strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Regiel-Futyra *et al.*, 2015).

In vivo and *in vitro* studies regarding cytotoxicity of nanoparticles have contradictory results and there is still not enough information on their effects. In this context, we investigated the cytotoxic effect of chitosan-AuNPs on C26 (murine colon carcinoma) and HeLa (human cervical carcinoma) cell lines.

MATERIALS AND METHODS

Characterization of chitosan gold nanoparticles

Two solutions of chitosan-gold nanoparticles (chitosan-AuNPs) were graciously provided by the National Institute for Research and Development of Isotopic and Molecular Technologies Cluj-Napoca. Nanoparticles were characterized by absorbance reading at 300-800 nm using a V-630 UV-Vis Spectrophotometer in order to assess their size.

Cell lines and cell culture

Murine colon carcinoma cell line C26 (Cell Lines Service GmbH, Eppelheim, Germany) was maintained in RPMI-1640 medium without antibiotics but supplemented with 1 mM glutamine and 10% foetal bovine serum while for human tumour cervical HeLa (ATCC CCL-2) MEM medium supplemented with the same reagents was used.

MTT cell proliferation assay

Cytotoxicity was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra-

zolium bromide (MTT) colorimetric method. C26 and HeLa cells were seeded at a concentration of $1 \cdot 10^6$ / ml into 96-well microplates and kept in their respective media for 24 hours. Afterwards, cells were exposed for 24 hours to media containing characterised chitosan-AuNPs from the two solutions at five different concentrations: 5, 10, 25, 50 and 100 µg/ml using 5 wells for each concentration. Chitosan-Au NPs had been sterilized by filtration through 0.22 µm pores. The media containing chitosan-AuNPs was eliminated and cells were washed with 150 µl phosphate buffered saline (PBS) which was then replaced with 150 µl MTT solution in PBS (0.5 mg/ml). The resulting formazan particles were solubilized with 200 µl dimethyl sulfoxide. The absorbance was read with the HT Synergy microplate plate reader (BioTek Instruments, USA) at 550 nm and expressed relative to the background absorbance read at 630 nm. Experiments were repeated 3 times. Results were expressed as percentages of antiproliferative activity with respect to an untreated control and based on them IC50 values were calculated using the Excel program (Microsoft Corporation, USA).

RESULTS AND DISCUSSIONS

Characterization of chitosan-AuNPs

Absorbance measurements indicated that particle diameter was 10 nm for the first solution while the second contained 60 nm sized particles.

Cell proliferation

We found that chitosan-AuNPs decreased cell proliferation in a dose dependent manner (Fig. 1). As shown by the IC50 values in C26 cells the antiproliferative effect was higher for 60 nm nanoparticles (25.70 µg/ml) than for 10 nm nanoparticles (36.70 µg/ml).

The antiproliferative effect was higher in C26 cells than in HeLa cells for both types of chitosan-AuNPs. For HeLa cells the situation was different, with the 10 nm nanoparticles having a more powerful antiproliferative activity (IC50: 43.36 µg/ml) than the 60 nm nanoparticles (IC50: 107.43 µg/ml).

Cell morphology was visibly altered in both lines (Fig. 2). Characteristic changes such as vacuolation, cytoplasmic granulations and cell detachment were apparent after a 24 hour exposure and became worse as concentration increased.

Fig. 1. Antiproliferative effect of chitosan-AuNPs in C26 cells (a) and HeLa cells (b)

In vivo, Au-NPs sized 10-15 nm have been localised in all tissues including blood, liver, lung, spleen, kidney and heart of mice while particles between 200-250 nm showed very minute presence in organs including blood, brain and spleen (reviewed by Fratoddi *et al*, 2014). Stefan *et al.* (2013) have demonstrated that smaller sized chitosan-AuNPs (12 nm) show protective effects against toxicity induced by lipopolysaccharides in Wistar rats.

In vitro, cytotoxicity of AuNPs has been studied on various cell lines such as human skin fibroblasts (HeLa), human leukemia (K562), human hepatocarcinoma (HepG2), human breast carcinoma (SK-BR-3) (Fratoddi *et al*, 2014).

Chitosan-AuNPs have been shown to induce cytotoxic effects on neural and non-neural cell types (Gao *et al.*, 2012) but also to inhibit proliferation of human gastric carcinoma cell line MGC803 (Qi *et al.*, 2005) while Choi *et al.* (2015)

Fig. 2. Morphology of C26 cells treated with 60 nm chitosan-AuNPs (a, b) and HeLa cells treated with 10 nm chitosan-AuNPs (c,d).

found that these appeared to provoke cell death in human lung carcinoma cells, line THP.

In the cell Au-NPs have been found free in the cytoplasm but also encased in a membrane-bound vacuole, possibly an endosome or lysosome by Murdock (2010) and inside lysosomes and the reticuloendothelial network by Shukla *et al.* (2005).

Mironava *et al.* (2010) found that differently sized AuNPs: 45 nm at a concentration of 20 µg/ml and 13 nm at 142 µg/ml were sequestered inside large vacuoles without showing nuclei penetration. On the other hand Pan *et al.* (2007) reported that 1 and 4 nm gold nanoparticles were the most cytotoxic toward connective tissue mouse fibroblasts (L929 mouse), cervical carcinoma epithelial cells (HeLa), mouse macrophages (J774A1) and melanoma cells (SK-Mel-28) (IC50 approximately 30-46 µg/ml), whereas 15 nm AuNPs were not toxic at concentrations up to 100-fold higher (up to 6300 µg/ml). Conversely, Hondroulis *et al.* (2010) observed no difference in cytotoxicity of 10 and 100 nm AuNPs and Connor *et al.* (2005) found no cytotoxic effects for 18 nm AuNPs up to a 100 µM concentration. AuNPs have been reported as being nontoxic and biocompatible with mouse macrophages (RAW264.7 cell line) (Shukla *et al.*, 2005). In contrast, 10 nm AuNPs coated with chitosan were found to be cytotoxic to human acute monocytic leukemia line THP-1 (Boyles *et al.*, 2015). Chitosan coated AuNPs have been shown to promote bone formation (Choi *et al.*, 2015) and proposed as carriers for insulin delivery (Bhumkar *et al.*, 2007). Our research shows that chitosan-AuNPs have an antiproliferative effect on C26 and HeLa cell lines. In murine colon carcinoma (C26) cells larger particles (60 nm) were more toxic than smaller ones (10 nm). On the other hand in human cervical carcinoma (HeLa) cells the situation was reversed and 10 nm particles did the most had the most detrimental effect at a concentration 2.5 times smaller than that of 60 nm particles.

CONCLUSION

Given the contradictory nature of results in the gold nanoparticle field and the recent use of chitosan-gold nanocomposites our aim was to investigate their cytotoxicity in murine colon carcinoma and human cervical carcinoma cells. Results of this research could suggest that the

antiproliferative effect of chitosan-AuNPs depends on cell type and is influenced by particle size and concentration.

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