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Photo-stimulation of persistent luminescence nanoparticles enhances cancer cells death

Gonzalo Ramírez-García[a,b,c,d,e,f], Minerva Martínez-Alfaro[b], Fanny d'Orlyé[c,d,e,f], Fethi Bedioui[c,d,e,f], Nathalie Mignet[c,d,e,f], Anne Varenne[c,d,e,f], Silvia Gutiérrez-Granados[a,*], Cyrille Richard[c,d,e,f,**]

[a] Departamento de Química, Universidad de Guanajuato, 36050, Guanajuato, Mexico
[b] Departamento de Farmacia, Universidad de Guanajuato, 36050, Guanajuato, Mexico
[c] Chimie ParisTech, PSL Research University, Unité de Technologies Chimiques et Biologiques pour la Santé, 75005, Paris, France
[d] INSERM, Unité de Technologies Chimiques et Biologiques pour la Santé (U 1022), 75006, Paris, France
[e] CNRS, Unité de Technologies Chimiques et Biologiques pour la Santé UMR 8258, 75006 Paris, France
[f] Université Paris Descartes, Sorbonne Paris Cité, Unité de Technologies Chimiques et Biologiques pour la Santé, 75006 Paris, France

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ABSTRACT

Persistent luminescence nanoparticles made of $ZnGa_{1.995}Cr_{0.005}O_4$ (ZGO-NPs) are innovative nanomaterials that emit photons during long periods of time after the end of the excitation, allowing their use as diagnosis probes for *in vivo* optical imaging. During the excitation process, a part of the energy is stored in traps to further emit photons over long time. However, we observed in this study that some of the energy reduces molecular oxygen to produce reactive oxygen species (ROS). Following this observation, theoxidative stress induction and cytotoxic effects of these NPs were investigated on human breast cancer cells. The results indicate that ROS production was stimulated by exposition of the hydroxylated ZGO-NPs to UV or visible light, and the oxidative stress induced in cells after internalization can be directly correlated to their dose-dependent inhibition of cell viability. On the contrary, PEGylated ZGO—NPs were not uptaken by cells and have no effect on the production of ROS. Thus, the cell viability was not altered by these nanoparticles. This study reveals the importance of considering light irradiation and surface coating of luminescent nanoparticles toxicity which open new perspectives for their use in photodynamic therapy.

1. Introduction

Nanoparticles have great potential for cancer therapy and diagnosis since they can allow the *in vivo* detection and monitoring of this pathology (Brigger et al., 2012; Fukumori and Ichikawa, 2006). Among the different imaging techniques available in preclinic, optic is an interesting one since it is not expensive, easy to use and allow doing real-time imaging. However, optical imaging is limited in sensitivity due to autofluorescence and light scattering in deep biological tissues. For these reasons more sensitive tools are necessary (Coll, 2011).

Persistent luminescence is an optical phenomenon in which light emission remains for extended periods of time (minutes to hours) after the irradiation source is switched off (le Masne de Chermont et al., 2009; Maldiney et al., 2011a). This property allows overcoming the main disadvantage of conventional optical markers such as quantum-dots and organic dyes responsible of non negligible autofluorescence signals, providing many conveniences for optical imaging (Palner et al., 2015; Richard et al., 2012).

To overcome this disadvantage, our group pioneered the use of persistent luminescent nanoparticles (PLNPs). A series of PLNPs have been developed and applied in the biomedical field as

Abbreviations: APTES, (3 Aminopropyl)triethoxysilane; DLS, Dynamic light scattering; DMF, *N,N*-Dimethylformamide; LDE, Laser doppler electrophoresis; NOx, Nitric oxide metabolites; PEG, Polyethylene glycol; PLNPs, Persistent luminescence nanoparticles; POx, Aqueous peroxides; ROS, Reactive oxygen species; ZGO-NPs, $ZnGa_{1.995}Cr_{0.005}O_4$ persistent luminescence nanoparticles; ZGO-OH, Hydroxyl-functionalized ZGO-NPs; ZGO-PEG, PEGylated ZGO-NPs.

* Corresponding author at: Unité de Technologies Chimiques et Biologiques pour la Santé (UTCBS), CNRS UMR8258, 75006, Paris, France
** Corresponding author at: Universidad de Guanajuato, Departamento de Química, Pueblito de Rocha s/n, Guanajuato 36040, Mexico.
E-mail addresses: gutigs@ugto.mx (S. Gutiérrez-Granados), cyrille.richard@parisdescartes.fr (C. Richard).

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contrast agents for near infra-red *in vivo* imaging (Lecuyer et al., 2016; Maldiney et al., 2014a, 2012a, 2015, 2012b, 2013; Teston et al., 2015). Among them, the innovative chromium-doped zinc gallate $ZnGa_{1.995}Cr_{0.005}O_4$ nanoparticles (ZGO-NPs) have denoted attractive optical properties due to the long persistent luminescence activated by UV and its capacity to be re-activated *in vivo* through living tissues using highly penetrating low-energy orange/red photons (Maldiney et al., 2014b). Due to its photonic emission at the region of the tissue transparency window, this material has demonstrated its application for optical imaging of vascularization, tumors and grafted cells with high sensitivity (Maldiney et al., 2014b).

The complexity and great diversity of new engineered nanomaterials and their properties imply the understanding of their complete mechanisms of nanoparticle toxicity, which are far from being comprehended (Djurišić et al., 2015). Actually, the side effects of most nanoparticles, including PLNPs (in living organisms) are not well known, and exhaustive toxicological studies are paramount (Hofmann-Amtenbrink et al., 2015).

One of the most important cause of nanoparticle toxicity is frequently attributed to reactive oxygen species (ROS) production, and the resulting oxidative stress (Soenen et al., 2011). In the literature, the most reported ways for ROS production by nanoparticles are the followings (Pisanic et al., 2009; Soenen et al., 2011): *(i)* direct generation of ROS as a result of nanoparticles exposition to an acidic environment (such in the lysosomes) (Jain et al., 2008; Stroh et al., 2004), *(ii)* alteration of subcellular functions by interaction of the nanoparticles with organelles such as mitochondria (Soto et al., 2007), *(iii)* interaction of nanoparticles with the redox active proteins such as NADPH oxidase, and *(iv)* interaction of the nanoparticles with cell surface receptors and activation of intracellular signaling pathways (Pisanic et al., 2009. In all these cases, the ROS production depends on direct interactions between cells and nanoparticles.

In recent studies it has been demonstrated that the irradiation of some metal oxide nanoparticles by light enhances ROS production. This property has been applied principally in photocatalytic antimicrobial treatments for water disinfection (Brunet et al., 2009; Li et al., 2012; Xiao et al., 2015). In these cases, the nanoparticle acts as photosensitizer (Levy, 1994; Zhang et al., 2015): upon irradiation, the activated nanoparticle transfers its excited-state energy to surrounding oxygen, resulting in ROS production, such as singlet oxygen ($^1O_2$), hydroxyl radical ($^\bullet OH$) or superoxide radical ($^\bullet O_2^-$), which can to be cytotoxic in a well localized area (Kochevar and Redmond, 2000; Kolarova et al., 2008; Macdonald and Dougherty, 2001).

In this work, we evaluated the cytotoxic effect of ZGO-NPs on two cancer cell lines, MCF-7 and MDA-MB-231 derived from human breast cancer model. This study was focused on the evaluation of ROS production by photo-stimulated and non photo-stimulated nanoparticles with different coating, *in vitro* and in contact with cells, in order to reveal a possible mechanism of cancer cell death induced by exposure to ZGO-NPs.

2. Materials and methods

2.1. Synthesis and functionalization of $ZnGa_{1.995}Cr_{0.005}O_4$ nanoparticles

$ZnGa_{1.995}Cr_{0.005}O_4$ nanoparticles were synthesized and functionalized according to a previously reported method (Maldiney et al., 2014b). After their synthesis, the ZGO-NPs were first hydroxylated (ZGO-OH) in aqueous 5 mM NaOH media. As an intermediary, aminosilane groups were covalently attached to hydroxylated nanoparticles by adding (3-aminopropyl) triethoxysilane (APTES) purchased from Sigma-Aldrich in DMF. Finally, pegylated nanoparticles (ZGO-PEG) were obtained by reacting aminosilaned nanoparticles with methoxy-polyethylene glycol N-hydroxysuccinimide (5000 Da) (MeO-PEG_{5kDa}-NHS, from Iris Biotech GmbH). Nanoparticles were carefully washed with water before *in vivo* experiments.

2.2. Nanoparticle characterization

Nanoparticle size and zeta potential were characterized before administration to culture media. Dynamic light Scattering (DLS) and Laser Doppler Electrophoresis (LDE) measurements were performed with a Nano ZS Zetasizer instrument (Malvern Instruments, Southborough, MA). Further information about photophysical, chemical and colloidal properties of $ZnGa_{1.995}Cr_{0.005}O_4$ nanoparticles has been published in our previous works (Maldiney et al., 2014b; Ramirez-Garcia et al., 2015).

2.3. Superoxide production by isolated nanoparticles

The determination of $^\bullet O_2^-$ produced by nanoparticles was carried out by suspending ZGO-OH or ZGO-PEG in 100 μM XTT (2,3-bis(2-methoxy-4-nitro-5-sulfophehyl)-2H-tetrazolium-5-carboxanilide) as indicator (Brunet et al., 2009; Li et al., 2012) (purchased from Sigma-Aldrich). Nanoparticles concentration was 5.0 mg/L. After UV or visible light irradiation (λ=254 nm) or visible light (20W fluorescent lamp, from Philips) for different periods of time, a sample of the orange-colored XTT-formazan (the product resulting from the reduction of XTT by $^\bullet O_2^-$) was measured into a quartz vial with a Varian, Cary 50 UV–vis spectrophotometer at 470 nm. The experiments were also carried out in the dark to determine the background $^\bullet O_2^-$ production without photostimulation. A control consisted in the XTT solution without nanoparticles was irradiated under the same conditions in parallel.

2.4. Experiments with breast cancer cells

2.4.1. Culture cell

MCF-7 and MDA-MB-231 human breast cancer cells were routinely maintained in DMEM culture media without phenol red, supplemented at 10% with fetal bovine serum and 1% of penicillin/streptomycin. Cells were grown and maintained in cell culture 24 well plates at 37◦C in a 5% CO_2 humidified incubator.

2.4.2. Nanoparticle treatment and administration

Nanoparticles were reserved in darkness 1 week before use, and manipulation was performed in safelight conditions to avoid excitation by natural visible light. The effects of 0, 0.01, 0.05, 0.25 and 0.50 mg/mL ZGO-NPs concentrations in breast cancer cell toxicity were tested on this study. To do so, suspensions of ZGO-NPs were prepared using the culture media and dispersed for 1 min by using a sonication cube to prevent aggregation. A parallel set of cells was treated with pre-irradiated nanoparticles, which were exposed to UV-lamp (UVP, inc. Model UVGL-25) at 254 nm wavelength during 5 min just before use in culture cell media.

2.4.3. Cell viability test

Cell viability was measured by the Trypan Blue viability test (Strober, 2001) by using a TC20 Automated Cell Counter from BioRad. The percentage of viable cells was calculated as follows (Eq. (1)):

$$viable cells(\%) = \frac{\text{total number of viable cells per mL of aliquot}}{\text{total number of cells per mL of aliquot}} \text{x}100 \quad (1)$$