International Journal of Pharmaceutics 532 (2017) 686–695

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Long-term toxicological effects of persistent luminescence nanoparticles after intravenous injection in mice

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ARTICLE INFO

Article history:
Received 3 May 2017
Received in revised form 5 July 2017
Accepted 6 July 2017
Available online 10 July 2017

Keywords:
Persistent luminescence nanoparticles
PEG functionalization
nanoprobes
chronic toxicology
optical imaging

ABSTRACT

The $ZnGa_{1.995}Cr_{0.005}O_4$ persistent luminescence nanoparticles offer the promise of revolutionary tools for biological imaging with applications such as cell tracking or tumor detection. They can be re-excited through living tissues by visible photons, allowing observations without any time constraints and avoiding the undesirable auto-fluorescence signals observed when fluorescent probes are used. Despite all these advantages, their uses demand extensive toxicological evaluation and control. With this purpose, mice were injected with a single intravenous administration of hydroxylated or PEGylated persistent luminescence nanoparticles at different concentrations and then a set of standard tests were carried out 1 day, 1 month and 6 months after the administration. High concentrations of hydroxylated nanoparticles generate structural alterations at histology level, endoplasmic reticulum damage and oxidative stress in liver, as well as rising in white blood cells counts. A mechanism involving the endoplasmic reticulum damage could be the responsible of the observed injuries in case of ZGO-OH. On the contrary, no toxicological effects related to PEGylated nanoprobes treatment were noted during our *in vivo* experiments, denoting the protective effect of PEG-functionalization and thereby, their potential as biocompatible *in vivo* diagnostic probes.

1. INTRODUCTION

Optical imaging is a rapidly developing field of research aimed at noninvasively interrogating animals for disease progression, evaluating the effects of a drug, assessing the pharmacokinetic behavior, or identifying molecular biomarkers of diseases (Byrne et al., 2013). The use of non-invasive luminescent systems as tools for tagging pathologies in animal models is of great interest in biomedical research (Ntziachristos, 2006). Some fluorescent probes, such as quantum dots (Byers and Hitchman, 2011; Ji et al., 2014; Smith et al., 2008), metal nanoparticles (Nam et al., 2013; Pan et al., 2011; Ricciardi et al., 2014), fluorescent proteins (Xenopoulos et al., 2012; Yang et al., 2012) and organic fluorophores (Lemasters and Ramshesh, 2007; Ptaszek, 2013)

Abbreviations: APTES, (3 Aminopropyl)triethoxysilane; DAPI, 4′,6-diamidino-2-phenylindole; DLS, dynamic light scattering; DMF, N,N-Dimethylformamide; DMSO, dimethyl sulfoxide; LDE, laser doppler electrophoresis; MeO-PEG_{5kDa}-NHS, methoxypolyethylene glycol N-hydroxysuccinimide (5000 Dalton); NOx, nitric oxide metabolites; PEG, polyethylene glycol; PLNPs, persistent luminescence nanoparticles; POx, aqueous peroxides; ROS, reactive oxygen species; TEM, transmission electron microscopy; ZGO-NPs, $ZnGa_{1.995}Cr_{0.005}O_4$ persistent luminescence nanoparticles; ZGO-OH, hydroxyl-functionalized nanoparticles ZGO-NPs; ZGO-NH_2, aminosilane-functionalized ZGO-NPs; ZGO-PEG, PEGylated ZGO-NPs.

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http://dx.doi.org/10.1016/j.ijpharm.2017.07.015
0378-5173/

have been used in optical imaging. Luminescent bioimaging based on nanoprobes have shown to be a highly selective, sensitive and non-invasive powerful tool for visualizing *in vivo* or *in vitro* molecular events (Niu et al., 2014). However, fast photobleaching, poor signal-to-noise ratio (Wu et al., 2011) and toxicity can limit their practical applications (Soenen et al., 2014; Soenen et al., 2011).

Persistent luminescence nanoparticles (PLNPs) are innovative materials in which the excitation energy can be stored in traps before to be slowly released by thermal activation producing photonic emission which can last for several hours (Van den Eeckhout et al., 2013; Van den Eeckhout et al., 2010). Their biomedical applications for *in vivo* imaging have been barely suggested for the first time in 2007 (Le Masne de Chermont et al., 2007). Contrary to classical fluorescent probes that need to be constantly excited to produce a signal, persistent luminescence nanoparticles can be excited and then to emit light for a long period of time (Le Masne de Chermont et al., 2007; Maldiney et al., 2012a; Maldiney et al., 2012b). The main advantage of persistent luminescence nanoparticles for biological imaging consist in the detection and imaging without concomitant external illumination, avoiding auto-fluorescence signals produced by the tissues, resulting in high target to background ratio images (Lecuyer et al., 2016; Viana et al., 2016, Sharma et al., 2017).

The recently reported $ZnGa_{1.995}Cr_{0.005}O_4$ persistent luminescence nanoparticles (ZGO-NPs) represent a new generation of optical nanoprobes, whose persistent luminescence can be activated before administration on live systems, as well as *in vivo* through living tissues (Maldiney et al., 2014a, 2014b). This unique *in situ* re-activation in the therapeutic window, contrary to the previously reported in the literature PLNPs only excitable *ex vivo* with UV light (Le Masne de Chermont et al., 2007; Maldiney et al., 2011a), results in the ability to make observations of the probe, whenever wanted, without any time constraints, opening new perspectives for a great variety of diagnosis applications (Maldiney et al., 2014a, 2014b).

The surface functionalization of this kind of nanoparticles is necessary to increase their selectivity for imaging (Chan et al., 2014), to enhance its biocompatibility, avoid or delay their capture by the reticulo-endothelial system cells, and for various biomedical applications (Maldiney et al., 2011b, Teston et al., 2015; Maldiney et al., 2015a; Maldiney et al., 2015b). For example, aminosilane functionalized ZGO-NPs have been applied to monitor labeled cells, while PEGylation of ZGO-NPs was applied to increase their permanence in blood circulation and thereby permitting the *in vivo* passive tumor targeting (Maldiney et al., 2014a, 2014b).

Because the same properties that are desirable and potentially useful for a biomedical perspective can also give rise to unexpected and hazardous toxicities, systematic toxicological studies are essential to design adequate nanomaterials for optical imaging or biomedical applications in order to avoid their adverse effects on health (Gnach et al., 2015; Ye et al., 2012).

The safe use of inorganic NPs for biomedical applications is a challenge (Jaque et al., 2016). However, the assessment of NPs safety is difficult due to a wide diversity of configurations. Regardless of the intrinsic differences between the various types of NPs, several mechanisms by which they can affect homeostasis at the cellular level can be mentioned: oxidative stress, alteration of cell morphology and cytoskeleton, deregulation of the intracellular signaling pathways, release of toxic components of the nanoparticles and interactions with biomolecules (Nel et al., 2006; Soenen et al., 2011). In the present work, we evaluated the safety/

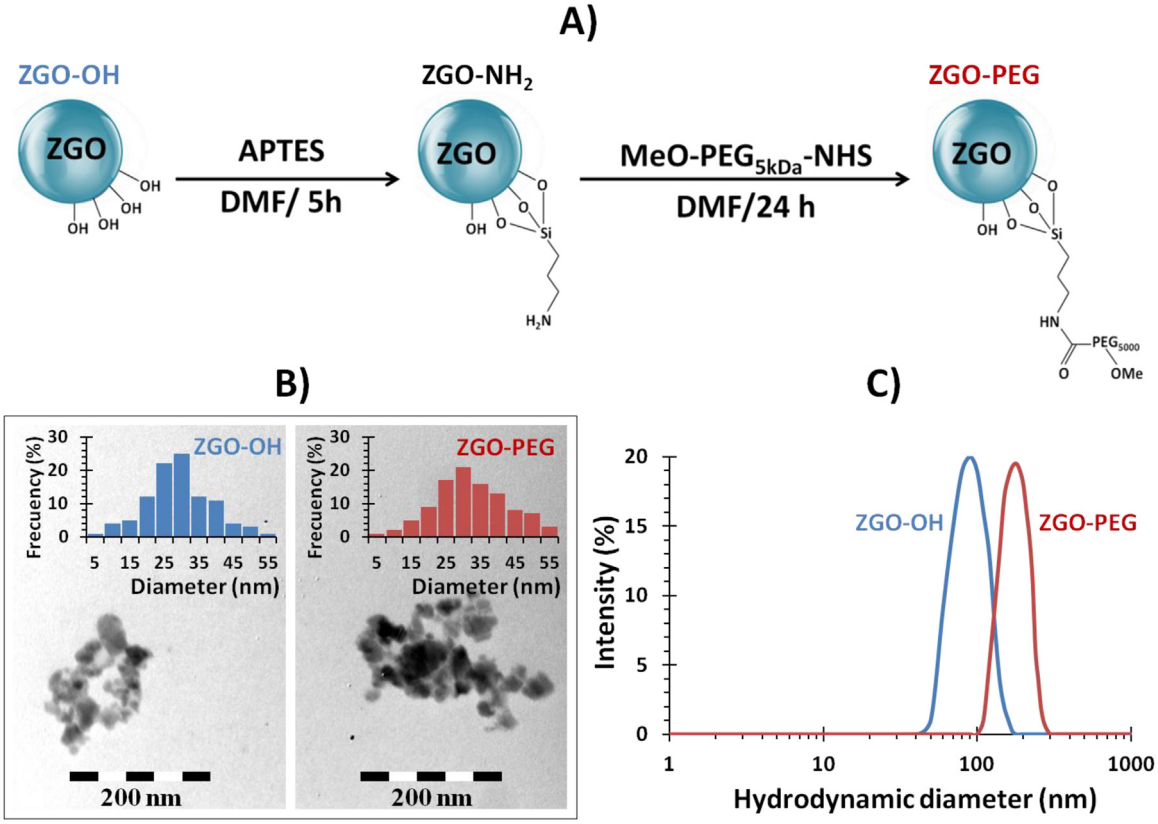

Fig. 1. A) Sequential surface functionalization of $ZnGa_{1.995}Cr_{0.005}O_4$nanoparticles. B) TEM images of the ZGO-OH and ZGO-PEG NPs. The corresponding histograms for size distributions are shown in the inset figures. C) DLS profiles indicating the hydrodynamic diameter of ZGO-OH and ZGO-PEG pH 7.4 phosphate buffer 30 mM. The PDI values were lower than 0.1.