Proton beam activation of aluminum oxide nanoparticles and biodistribution studies in rodents.

Pérez-Campaña,C.;[a] *Gómez-Vallejo,V.*;[a] *Puigivila ,M.*;[a] *Martin,A.*;[a] *Calvo-Fernández,T.*;[a] *Moya,S.E.*;[b] *Ziolo,R.F.*;[c] *Reese,T.*;[a] *Llop,J.*[a]

[a]*Molecular Imaging Unit, CIC biomaGUNE, San Sebastian, Spain;* [b]*Biosurfaces Unit, CIC biomaGUNE, San Sebastian, Spain;* [c]*Advanced Materials Department, CIQA, Saltillo, Mexico.*

jllop@cicbiomagune.es

Introduction

Nanoparticles (NPs) have interesting and unique properties due to their small size and high surface area. Among all NPs, metal oxide (MO) ones are ubiquitously utilized in many industrial and societal sectors. This increased use has raised many concerns about potential risk for human health and the environment. However, the investigation of the potential toxicological effects and biological fate of the NPs after incorporation into biological systems is challenging, because they are extremely difficult to detect *in vivo.* One alternative to overcome this problem consists of incorporating a radionuclide, e.g. a positron emitter, into the NPs, which allows further *in vivo* detection of the NPs after administration into an organism. Recently, we have reported a methodology for the incorporation of the positron emitter ^{18}F into metal oxide NPs.[1] In continuation of our work, we present here an strategy for the activation of commercial aluminum oxide NPs. The generation of ^{13}N atoms in the crystal lattice of the NPs allowed the investigation of the biodistribution pattern using different administration routes.

Methods

Four commercially available aluminum oxide nanoparticles with nominal diameters of 10 nm (NS_{10nm}), 40 nm (NS_{40nm}), 150 nm (NS_{150nm}) and 10000 nm ($NS_{10\mu m}$) were irradiated with 16 MeV protons. After irradiation (beam intensity = 5 µA, integrated current = 0.6 µAh) the biodistribution pattern of the different NPs was investigated in rats using intravenous (IV, tail vein) and intra-arterial (IA, inner carotid) administration and Positron Emission Tomography (PET). Images were reconstructed, Volumes of Interest (VOIs) were manually drawn in the different organs and the uptake in each VOI was determined as % of injected dose per gram of tissue. In all cases, NPs were characterized before and after irradiation by Transmission Electron Microscopy (TEM) and Dynamic Light Scattering (DLS). Radiological characterization was also performed using coincidence detection.

Results

Radiological characterization of the NPs showed that >99% of the radioactivity was due to ^{13}N at the end of the proton irradiation. Average specific radioactivity values around 1.35 GBq/mg were obtained, independently of particle size. Morphological characterization and Z-potential values showed no significant differences between NPs before and after irradiation process. *In vivo* studies demonstrated that biodistribution patterns are size-dependent. Intravenous administration resulted in high accumulation in lungs and liver for bigger sized NPs (NS_{150nm} and $NS_{10\mu m}$) while smaller NPs accumulated mainly in the stomach (NS_{10nm} and NS_{40nm}) and in the liver (NS_{40nm}). For NS_{10nm} and NS_{40nm} NPs, high uptake was also observed in bladder, suggesting elimination via urine. For intra-arterial administration, high NP uptake was observed in the brain for $NS_{10\mu m}$ and NS_{150nm} NPs, probably due to capillary occlusion. Significant uptake was also observed in the same organ for NS_{40nm}, suggesting the formation of aggregates just before or after administration.

Conclusions

Direct irradiation of commercial Al_2O_3 NPs with 16 MeV protons allowed sufficient activation to perform *in vivo* biodistribution studies after IV and IA administration in rodents. The accumulation of NPs in the different organs could be determined up to 68 minutes post-administration. Although the residence time of NPs in the body was longer than the activation time due to the short half life of ^{13}N, valuable information concerning the biodistribution pattern during the first minutes post-administration could be obtained. The presented methodology can be applied to any commercially available metal oxide NP.

Acknowledgements:
The authors would like the 7th Framework Program for funding this work through the HINAMOX PROJECT Contract Agreement: NMP4-SL-2009-228825.

References

[1] Llop, J. *et al*, Analyst, **137** (2012) 4902-4906.