Superparamagnetic Iron Oxide Nanocomposites and Autophagy Response from Macrophage Cells

Rongrong Jin [1], Changqiang Wu [1], Yang Li [1], Hua Ai [1, 2*]
1 National Engineering Research Center for Biomaterials, Sichuan University, PR China
2 Department of Radiology, West China Hospital, Sichuan University, PR China, huaai@scu.edu.cn

Statement of Purpose:

Superparamagnetic iron oxide nanoparticles (SPION) are excellent MR contrast agents, which are widely used in disease diagnosis and therapy. Polymer micelle based SPION nanosystem is one of common forms for biomedical applications (Fig. 1a). Once uptake by biological cells, SPION probes can improve imaging contrast and lead to better diagnosis. We synthesized N-Alkyl-PEI functionalized iron oxide nanocomposites (N-Alkyl-PEI/SPIO) for gene delivery and cell labeling [1, 2]. And it has been included in Molecular Imaging and Contrast Agent Database (MICAD). Recently, nanoparticles are considered as a new class of autophagy activator (Fig. 1b) [3]. For iron oxide base nanoparticles, their ability to induce cell autophagy is still under investigation. In this study, we try to control the surface properties of nanoparticles and thus to adjust their induced cellular autophagy.

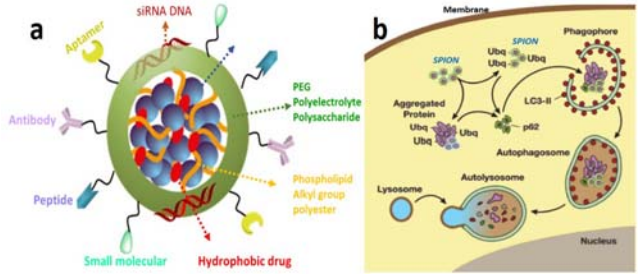

Fig1 Schematic illustration of the polymer micelle composite system of surface-engineered SPION (a) and its possible mechanisms of inducing autophagy (b).

Material and Methods:

1) N-Alkyl-PEI2k/SPIO nanoparticles were fabricated following a previously published protocol and modified with different levels of lactose (Fig 2); 2) The cytotoxicity of nanoparticles was evaluated using a fluormetric DNA assay in murine macrophage cell line Raw264.7; 3) Data were analyzed using Microsoft Excel for Student's paired *t*-test; $p<0.05$ was considered statistically significant; 4. Nanocomposites induced autophagy flux change including LC3 transformation and P62 downregulation were detected by western blot.

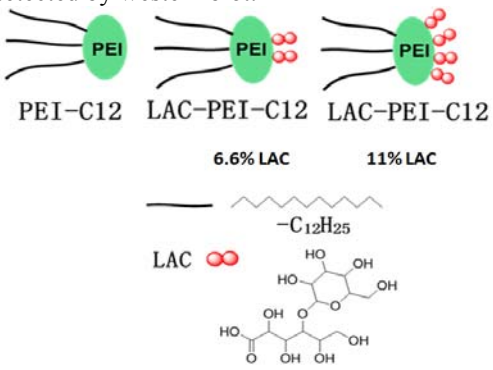

Fig 2. Schematic illustration of nanocomposite structure

Results:

Macrophages are the most important cells for ingesting foreign materials *in vivo*. So we chose cell line RAW264.7 for this preliminary study. As shown in Fig. 3a, PEI/SPIO composites could induce cytotoxicity in a dose dependent manner and lactose modification could reduce this phenomenon. At an iron concentration of 15 ug/ml, PEI/SPIO with 11% lactose did not induce cytotoxicity compared to 6% and unmodified ones. PEI/SPIO nanocomposites could also induce dose-depended autophagy in macrophage cell line, while with the higher lactose modification, PEI-Lac/SPIO showed lower LC3 transformation (Fig. 3b). But there was not P62 alteration in the processes, probably because of participation of p62-independent autophagy signaling pathway. The data indicated that lactose modification could reduce nanoparticles induced autophagy.

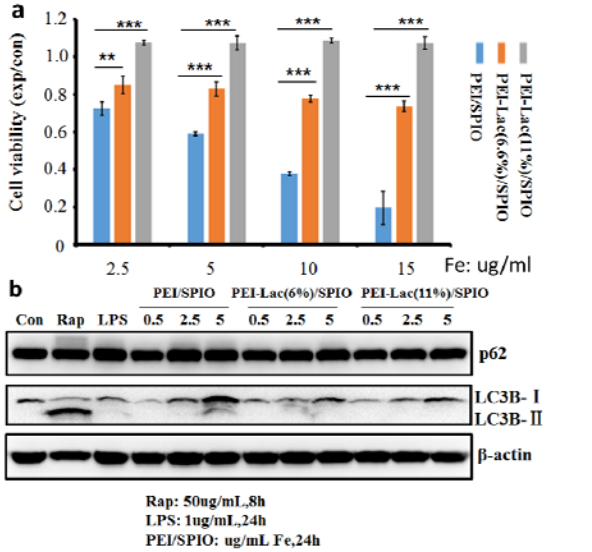

Fig 3. Lactose modification reduced nanocomposites induced cytotoxicity by downregulating autophagy. a) RAW264.7 cell was treated with nanocomposites with different conjugated levels of lactose for 24h. Cell viability was measured by floumetric DNA assay. b) Nanocomposites treated cells lysis were collected after 24h and autophagy related genes LC3 and p62 were detected by western blot. β-actin were detected as loading control.

Conclusions:

N-Alkyl-PEI2k/SPIO nanoparticles modified with lactose showed less cytotoxicity than the unmodified ones. And this modification can reduce autophagy which might be one of key reasons of cytotoxicity. It can be considered as a sensitive tool to sense early cytotoxicity.

References:

1. Liu G. et al., Biomaterials, 2011; 32: 528-537.
2. Liu G. et al., Small, 2011; 7: 2742-2749.
3. Stern et al. Particle and Fibre Toxicology 2012; 9: 20