Electronic Supplementary Information (ESI)

Autophagic Stress; A New Cellular Response to Nanoparticles. Could it be a New Strategy for Inhibition of Liver Cancer Cell Invasion and Metastasis?

Chalermchai Pilapong [a,b,c,]*Thipjutha Phatruengdet,[a] and Saowalak Krungchanuchat [a]

[a] Center of Excellence for Molecular Imaging (CEMI), Department of Radiologic Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai 50200, Thailand

[b] AMS cancer research unit, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai 50200, Thailand

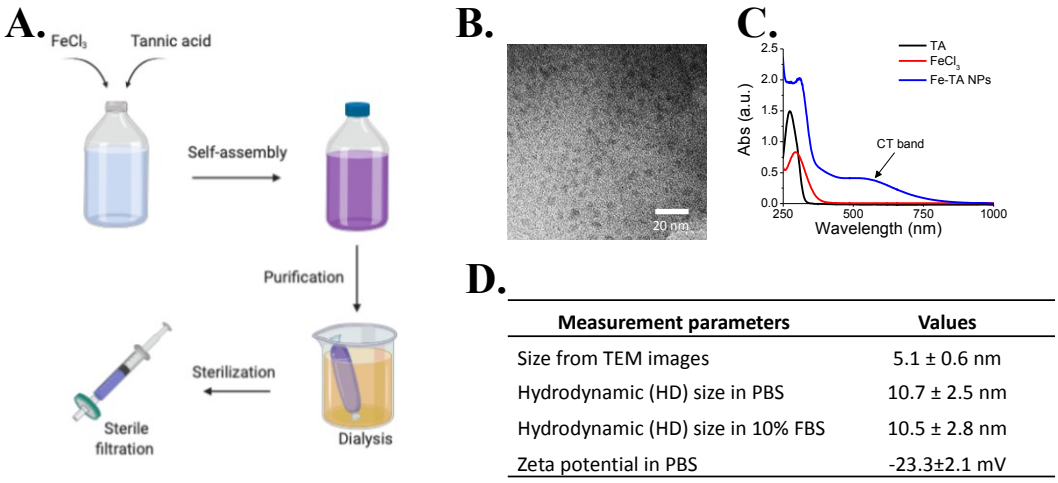
[c] Materials Science Research Center, Faculty of Science, Chiang Mai University, Chiang Mai, 50200, Thailand

*Corresponding author.
Email: chalermchai.pilapong@cmu.ac.th

Experiment

Inhibition study

The effect of organic anion transporter (OAT) inhibitors on cellular uptake of Fe-TA NPs was determined by measuring intracellular iron content and compared with control cells. In typical, HepG2.2.15 cells were seeded in 24-well plates (5 × 10^4 cells/well). when the cells were approximately 80% confluent, the cells were then co-treated with 100 uM Fe–TA NPs and 10 uM OAT inhibitors, cyclosporine A (CsA)[1] and quercetin(Que)[2,3] for 24 hours. After washing, the cells were detached by trypsinization and centrifuged at 7000 rpm for 1 min. The resulting cell pellets were lysed with a mixed acid solution (1:1 of HCl:HNO3) for 30 min at 60 °C. After that, the lysed cells were made to spin down, and the supernatants were collected. The iron-containing supernatants were mixed with an excess amount of KSCN. Finally, the iron content was determined by measuring the absorbance of the iron-thiocyanate complex at 470 nm.



Measurement parameters	Values
Size from TEM images	5.1 ± 0.6 nm
Hydrodynamic (HD) size in PBS	10.7 ± 2.5 nm
Hydrodynamic (HD) size in 10% FBS	10.5 ± 2.8 nm
Zeta potential in PBS	-23.3±2.1 mV

Figure S1. (a) Schematic illustration of key steps for preparation of Fe-TA NPs, (b) TEM image of Fe-TA NPs, (c) UV-Vis spectra of the precursors ($FeCl_3$ and tannic acid) and Fe-TA NPs. Charge transfer (CT) band found in Fe-TA NPs spectrum confirmed the existence of Fe-TA NPs. (d) Typical characteristics of Fe-TA NPs (Hydrodynamic diameter (HD) was measured after being incubated in PBS and 10%FBS for 1 h).

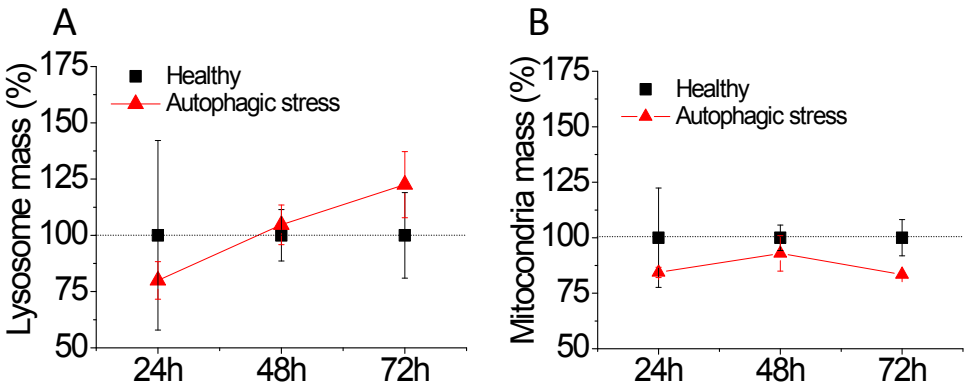


Figure S2. Flow cytometry analysis of (A) lysosome mass and (B) mitochondria mass

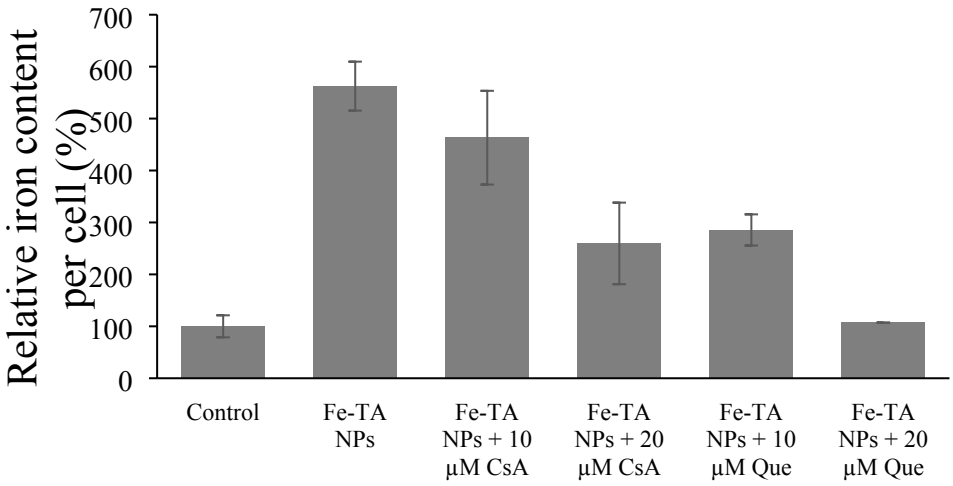


Figure S3. The relative intracellular iron content of HepG2.2.15 cells treated with Fe–TA NPs at 37 °C with/without OAT inhibitors. (CsA is cyclosporin A, Que is quercetin)

References

1. R. Amundsen, H. Christensen, B. Zabihyan and A. Asberg, *Drug Metab. Dispos*., 2010, **38**, 1499-504.
2. L. X. Wu, C. X. Guo, W. Q. Chen, J. Yu, Q. Qu, Y. Chen, Z. R. Tan, G. Wang, L. Fan, Q. Li, W. Zhang and H. H. Zhou, *Br. J. Clin. Pharmacol*., 2012, **73**, 750-7.
3. G. An, X. Wang, M. E. Morris, *Drug Metab. Dispos*., 2014, 42, 1357-66.