Quantitative Studies of the Internalization of Iron Oxide Nanoparticles by Mesenchymal Stem Cells

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Introduction

Nanoparticles of iron oxide have been recently used to magnetically label cells *in vitro*. Small particles of iron oxide (SPIO, hydrodynamic diameter: 80-150 nm) and ultra small particles of iron oxide (USPIO, hydrodynamic diameter: 20-30 nm) were used in this context. Bone marrow mesenchymal stem cells (MSCs) have been magnetically labelled for a MRI monitoring of their migration after implantation in the injured rat brain (1). Our aim is to quantify the internalization of SPIO by MSCs, to study the mechanisms of internalization of SPIO by MSCs, and to compare the internalization of SPIO and of USPIO by MSCs. While stem cells magnetic labeling can be realized with nanoparticles of iron oxide pre-incubated with a lipofection or transfection agent (2-3), in our case, the internalization have been performed with the native particles.

Methods

MSCs were isolated from 8 weeks old rats femurs. After deep anesthesia with Nembutal, rats were sacrificed and their femurs were surgically extracted. Whole bone marrow extract was plated in 6-wells tissue culture dishes and 24 hours later, non-adherent cells were removed from the culture by replacing the medium, in order to isolate MSCs (4). MSCs were cultured during 12 to 15 days before the incubation with SPIO or USPIO. Wells contained 4 ml of Dulbecco's modified eagle medium supplemented with 10% FBS and 1% antibiotic-antimycotic solution. MSCs were incubated with SPIO at 0, 25, 50, 100 and 200 µg of iron/ml, or with USPIO at 100 and 200 µg of iron/ml during 48 hours in order to evaluate the capture of the particles as a function of the iron concentration in the incubation medium and of the particle size. MSCs were also incubated with SPIO at 100 µg of iron/ml during 3, 6, 9, 12, 24 and 48 hours to estimate the kinetic of internalization of SPIO by MSCs. Internalization mechanisms were inhibited by pre-incubation of the cells during 4 hours with cytochalasin B (to prevent actin microfilaments polymerization) or colchicine (to prevent microtubules polymerization) (5). Internalization of SPIO and USPIO by MSCs was evaluated by T_2-weighted MRI (4.7 T) and colorimetry after iron staining with Prussian blue on mineralized cells. For MRI, cells were resuspended in PCR tubes at a concentration of $5x10^5$ cells/ml in 2% gelatin and the following acquisition sequence was used: (TR=2000ms, TE=20ms, 16 echoes, NA=2, matrix = 256 x 256). PARAVISION software was used to measure T_2 values on the MR images.

Results

After incubation with SPIO (increasing incubation concentrations and increasing incubation times) , R_2 values obtained from the T_2-weighted MR images and iron concentration follow the same trend (Fig. 1a & 1b). USPIO are less internalized than SPIO by MSCs (Fig. 1c), and both cytochalasin B and colchicin have an inhibitory effect on the internalization of SPIO by MSCs (Fig. 1d).

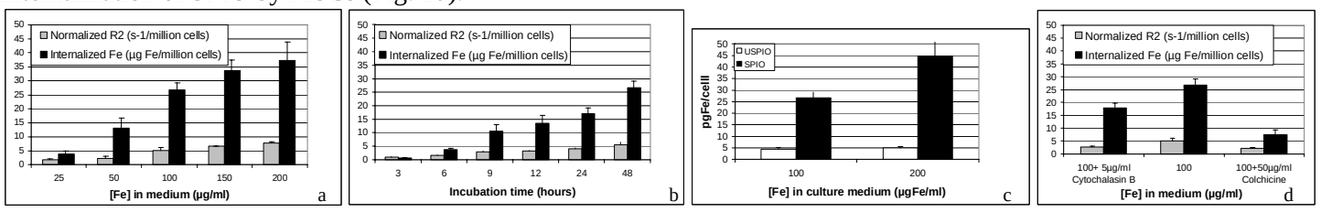

Fig.1. Normalized R_2 values (4.7 T) and amount of iron internalized by MSCs incubated with different iron concentrations (a) or during different times with 100 µg Fe/ml (b). Amount of iron internalized by MSCs incubated with 100 and 200 µgFe/ml SPIO or USPIO (c). Normalized R_2 values (4.7 T) and amount of iron internalized by MSCs incubated with 100 µgFe/ml, pre-incubated with cytochalasin B or colchicine (d).

Discussion and conclusions

MRI and colorimetry allow for the quantification of internalized iron. A decrease of the normalized R_2 value of cells incubated with 100 µgFe/ml is observed in the presence of colchicine and cytochalasin B, suggesting that at least two processes are involved in the endocytosis of SPIO by MSCs. The first onedepends on actin microfilaments polymerization, like macropinocytosis, and the other one depends on microtubules polymerization, like pinocytosis. Size also seems to be an important parameter for the capture of iron oxide nanoparticles by MSCs, since USPIO are less internalized than SPIO.

References

1. Jendelova P. et al., Magn Reson. Med., 50, 767, 2003, **2**. Frank J.A. et al., Radiology, 228(2), 480-7, 2003, **3**. Hoehn M. et al., Proc Natl Acad Sci U S A., 99(25), 16267, 2002, **4.** Prockop D.J., Science, 276(5309), 71, 1997, **5.** Fleige G. et al., Invest Radiol., 37(9), 482, 2002