Detection limits of Very Small Iron Oxide Nanoparticles in labeled cells: a quantitative evaluation of histochemistry and MR-relaxometry

G. Klug[1], J. Bremicker[1], T. Kampf[2], E. Bauer[1], T. Basse-Lüsebrink[2], M. Weber[3], U. Gbureck[4], U. Nöth[3], P. M. Jakob[2], and W. R. Bauer[1]

[1]Medizinische Klinik und Poliklinik I, Universitätsklinikum Würzburg, Würzburg, Germany, [2]Experimentelle Physik 5, Universität Würzburg, Würzburg, Germany, [3]Orthopädisches Zentrum für Muskuloskelettale Forschung, Orthopädische Klinik König-Ludwig Haus, Würzburg, Germany, [4]Abteilung für Funktionswerkstoffe der Medizin und der Zahnheilkunde, Universitätsklinikum Würzburg, Würzburg, Germany

Background: Prussian-Blue (PB) histochemistry is a standard method to proof intracellular accumulation of iron, which is also valid for Iron Oxide Nanoparticles (IONs). Schroeter et al. demonstrated a lack of sensitivity of PB for IONs in experimental brain ischemia and proposed an improved method which relies on the enhancement of PB with diaminobenzidine (PB+DAB) [1]. With regard to the growing interest in MR-cell-tracking based on ION-labeled cells a determination of detection limits of IONs by means of MRI and histochemistry remains crucial.

Purpose: We investigated the relation between intracellular iron content (pg/cell) and the capability of histochemistry and MRI to detect *in vitro* ION-labeled cells.

Methods: Immortalized peritoneal mouse macrophages ($2*10^7$/6ml medium) were incubated with Very Small Iron Oxide Nanoparticles (VSOP C-200, Ferropharm, Teltow, Germany) at increasing concentrations (n=10) ranging from 0 to 200 µg/ml for 4 hours. In order to determine iron content per cell (pg/cell) inductively coupled plasma mass spectrometry (ICP-MS) was performed. Furthermore cellular uptake of VSOP was verified by transmission electron microscopy (TEM).
Histological slides (n=6) were prepared from cells of each labeling-protocol for PB- and DAB-PB-staining in order to evaluate their sensitivity. Cells were counted in 3 high-power fields (magnification: 100x) and the percentage of apparently stained cells was recorded. Furthermore, $2*10^6$ cells from the same protocols were homogeneously dispensed in 0.5ml of 0.5% agarose gel for MR-relaxometry.
Additionally, the impact of cell number on MR detection limits was investigated on $2x10^5$ to $8x10^6$ identically prepared cells. MR measurements were performed on a 7 Tesla MR-scanner (Bruker Biospin GmbH, Rheinstetten, Germany). The MR-protocol contained Multi-Spin-Echo-Sequences (CPMG-echo-train, TR: 4.5 [ms], TE: 5 [ms], 250 echoes) for T2 determination. T2* values were determined spectroscopically by fitting the FID after a single pulse excitation. Subsequently the change in relaxation rates induced by VSOP uptake (ΔR2, ΔR2*) was plotted against the number of iron labeled cells. All data are presented as mean ± SD.

Results: After labeling, trypanblue exclusion test showed more than 90% of viable cells. With rising VSOP concentrations used for cell-labeling cellular iron content increased from 0.26±0.06 to 3.26±0.7 pg/cell (Fig. 1. A). TEM confirmed the vesicular uptake of VSOP.
Histological slides showed a higher percentage of iron-positive cells with increasing intracellular iron content determined by ICP-MS. The enhancement of PB by DAB lead to a higher sensitivity for intracellular iron (Fig. 1. B). Nevertheless at iron contents below 2.13 pg/cell both staining methods showed less than 25% iron positive cells. On TEM images and ICP-MS, however, intracellular iron was detected at concentrations as low as 0.26 pg/cell whereas PB and DAB-PB revealed only 4% and 5% iron-positive cells (Fig. 2.).
The T2 and T2* shortening effects of VSOP were positively correlated with intracellular iron content (Fig. 3. A). Even at 0.26 pg/cell a significant change of relaxation rates was observed. ΔR2* and ΔR2 increased with ascending number of cells in the phantom. At low cell number, ΔR2* was up to 25-times ΔR2 (Fig. 3. B).

Conclusion: We have shown that MRI is able to detect even low amounts of intracellular VSOP that histochemistry fails to detect. Our results suggest that for the histological validation of MR-cell-tracking studies with VSOP at least 2.13 pg/cell should be achieved.

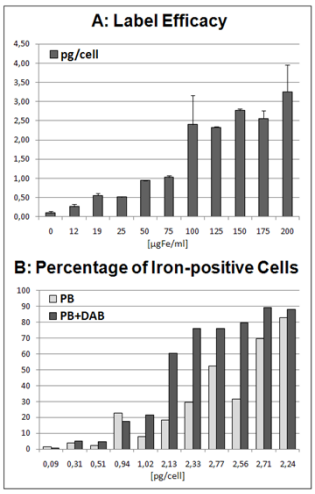

Fig. 1. A: Intracellular iron content (pg/cell) after labeling plotted against the label concentration (µgFe/ml).
B: Comparison of PB and PB-DAB.

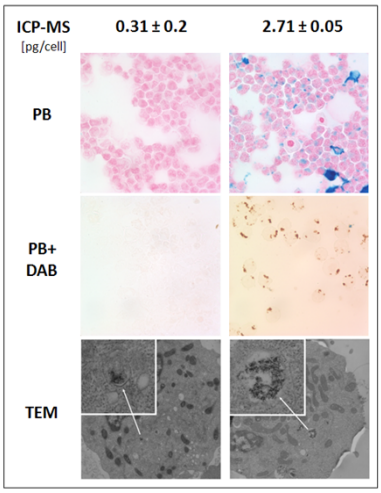

Fig. 2: Examples of high-power fields used for the determination of PB-positive (upper row, blue staining) and PB-DAB- positive (middle row, brown staining) cells. TEM (lower row) confirmed vesicular iron at low and high intracellular iron content (ICP-MS).

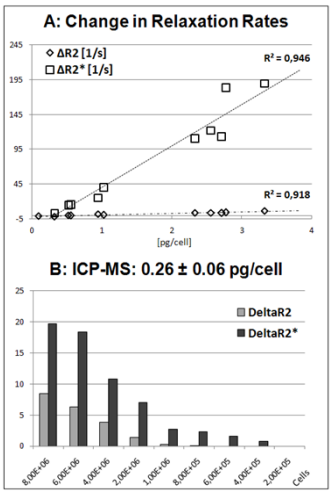

Fig. 3. A: Scatterplot showing the correlation of intracellular iron content with the change of relaxation rates.
B: At identical iron content the change in R2 and R2* decreased with the absolute cell number in the phantom.

References: [1] M.Schroeter et al.; *MRM, V. 52, pp.403-406 (2004)*