Iron oxide labeling of primary rat monocytes using Magneto Electroporation

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Introduction

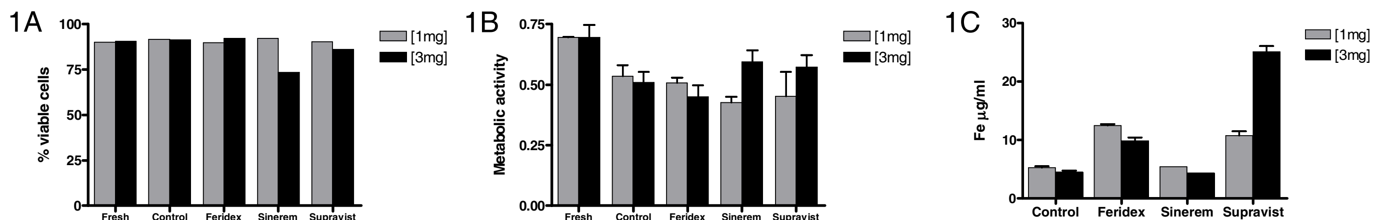
In multiple sclerosis (MS), infiltration of monocytes in the central nervous system contributes to ongoing inflammation and lesion development. Cellular MRI of monocytes will help to develop and monitor therapeutic strategies aimed at the inhibition of cell entry in to the central nervous system. In this view the development of (ultra small) superparamagnetic particles of iron oxide ((U)SPIO) extended the use of MRI to study cell dynamics in vivo. In earlier studies concerning monocyte infiltration in vivo, USPIOs are administered intravenously and it is believed that cellular uptake occurs in circulation [1]. However, a-specific labeling and the presence of extra-cellular iron may contribute to cellular MR signal. In vitro labeling of monocytes and subsequent transfusion of labeled cells can overcome these issues. The success of cellular MRI using iron oxide labeled monocytes is highly dependent on iron oxide size [2] and labeling strategy. MR tracking experiments require an effective and fast labeling procedure without affecting cell function. An alternative labeling technique, magneto electroporation (MEP), proven to achieve endosomal labeling within a few milliseconds in stem cells [3], may be an ideal method. During electroporation there is a temporarily loss of cell membrane integrity which drives the uptake of contrast agents present in the medium. The purpose of this study was to evaluate and optimize MEP for monocyte labeling with respect to different iron oxides and monocyte cell function.

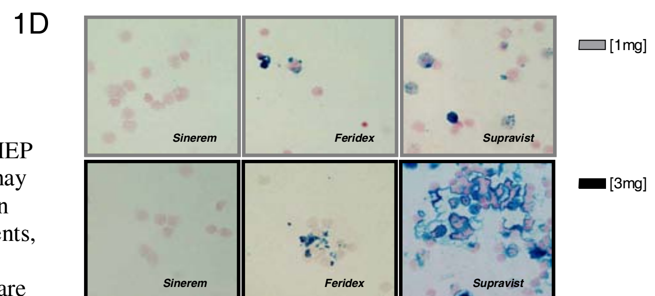
Material and Methods

Peripheral blood mononuclear cells (PBMC) were freshly isolated from Lewis Hannover rats by perfusion. Monocytes were purified from the PBMCs by negative selection using fluorescence activated cell sorting. Subsequently, monocytes were electroporated under a variety of conditions (60 – 200V; 1 – 10 pulses) and in the presence of 1 and 3 mg Fe/ml iron oxide in the medium. To assess the effect of particle size and surface coating we used the following iron oxides; Supravist (ionic, 26nm; Schering AG, Germany), Feridex (150nm; Berlex Imaging, USA) and Sinerem (30nm; Guerbet, France). Following MEP labeling, the presence of intracellular iron was microscopically analyzed by Prussian blue staining and quantified by a spectrophotometric Ferrozin assay. Cell viability was assessed by trypan blue exclusion and mitochondrial metabolic rate was determined 24h after electroporation using an MTS assay as an indicator of cellular toxicity [3].

Results

The MEP procedure was optimized for monocyte labeling: 5 pulses of 100V and 5ms with an inter pulse delay of 100ms. Our results show that MEP does not affect cell viability (1A) at both iron concentrations as compared to freshly isolated cells and slightly decreases metabolic activity (1B). Interestingly, MEP at high concentrations of either Sinerem or Supravist results in a less pronounced effect on metabolic activity. Quantification of intracellular iron (1C) and histochemical staining (1D, iron in blue and nuclei in red) of labeled monocytes revealed that MEP using Sinerem does not result in cellular labeling. MEP with Feridex showed intracellular labeling and extracellular iron clustering without significant increase at a higher iron concentration. In contrast, MEP in the presence of Supravist (1mg) resulted in efficiently labeled monocytes, the iron distributed as small blue dots throughout the cytoplasm. At 3mg a massive iron uptake was observed which was higher than all other conditions.





Discussion

This study shows that MEP can label freshly isolated monocytes without affecting cell viability and metabolic activity. Interestingly, the success of MEP is particle dependent. Supravist showed the most promising results, which may be explained by its ionic surface properties. In comparison to conventional in vitro labeling methods like simple incubation and the use of transfection agents, MEP is ultra fast, requires no additional compounds and results in a high amount of intracellular iron oxide. Most likely, monocytes labeled by MEP are a valuable tool in the field of cellular MRI and study monocyte infiltration longitudinally in animal models for MS.

References

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