Early stage investigations of USPIO-induced signal changes after focal cerebral ischemia in mice

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Rationale and objectives: The interest of ultrasmall superparamagnetic iron oxide particles (USPIO) for monitoring post-ischemic neuroinflammation with magnetic resonance imaging (MRI) has been recently highlighted [1]. Nevertheless, the interpretation of MR signal changes at the early stages of focal cerebral ischemia remains controversial [2]. Three hypothesis have been proposed: (1) intravascular iron particles [3], (2) USPIO-loaded phagocytes [4], based on the assumption that USPIO was primarily taken up by circulating phagocytes [1] and (3) intersticial USPIO, passively diffused across the damaged blood brain barrier (BBB) [5]. The aim of the present study was to specifically investigate these mechanisms in the first 24h post-ischemia.

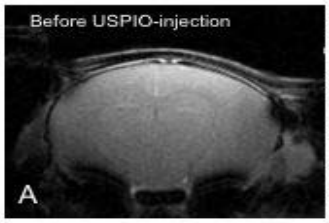
Methods: Cerebral infarctions were induced in 40 mice using permanent middle cerebral artery occlusion (pMCAO) by electrocoagulation. USPIO (Ferumoxtran-10 Guerbet, France, 2 mmol Fe/kg) were injected i.v. 5h post-injury. Mice were scanned before contrast agent injection and then either 1h (n=17) or 19h (n=23) after. Multiparametric MRI (diffusion-weighted, T2-weighted, gradient echo GE, T2 maps) was performed at 7T. Gadolinium (0.5 mmol/kg Dotarem, Guerbet) was administered to assess BBB integrity. Peripheral blood (withdrawn twice per mouse in 24h) was treated by EasyLyse™ (Dako) in order to isolate mononuclear cells for cytospin iron staining. After the last MR examination, mice were sacrificed for immunohistochemical analysis to depict: iron with Prussian blue staining, immunoglobulins deposits by immunohistochemistry against IgG and macrophages by immunochemistry against F4/80. For in vitro evaluation of cell labelling, free-floating monocytic cells were derived from bone-marrow cultures and incubated for 24h with USPIO (2mg Fe/mL) and analyzed by Prussian Blue staining.

Results: Four areas of signal changes after USPIO injection on GE T1-weighted imaging were observed at 6h and 24h post-injection: a marked signal drop-out of in the border zone of the lesion (Area 1), hypointense signal lines in the third ventricle and the two hippocampi region (Area II), a signal loss along the ipsilateral lateral ventricle (Area III), a hyperintense signal in the ipsilateral corpus callosum (Area IV) (Figure). Enhancement of the lesion indicated BBB disruption at the time of USPIO administration confirmed by histological immunoglobulin staining. On histology, iron staining was mostly associated to the vascular and the cerebrospinal fluid compartments. Only F4/80+cells in peripendymal region and in velum have already phagocytosed iron particules at these early stages post-ischemia. USPIO-loaded cells were not detected in the blood of ischemic mice within the first 24h. Cultured phagocytes incubated with USPIO did not show significant iron labelling.

Conclusion: Taken together these findings suggest that USPIO-induced MR signal changes before 24h are mainly caused by intravascular trapping and unspecific iron particles leakage due to breakdown of the barrier, rather than by peripheral phagocytes infiltration. These results strongly suggest that we must consider time-window dependent results interpretation of USPIO-related signal changes in experimental stroke models.

References: [1] Stroke 2007; 38; 642-645. [2] Stroke 2007; 38(5): e12; author reply e13. [3] J Cereb Blood Flow Metab 2005; 25(11); 1548-1555. [4] J Cereb Blood Flow Metab 2003; 23(11); 1356-1361. [5] Stroke 2007; 38(1); 131-137.

Correlation of early USPIO-induced MRI signal changes with histological distribution of USPIO after focal cerebral ischemia.



A-C: GRE sequence 5 hours (A: before USPIO) and 6 hours post-ischemia (B and C: 1h post i.v. injection of USPIO) in transverse slices through the mouse brain (Bregma -1,28 mm (A;B) and +0,74 mm (C) according to Franklin and Paxinos's atlas)). B and C: Note the hyposignal (arrows) at the periphery of the lesion (Area I), in the hippocampus formation (Area II) and along the ipsilateral lateral ventricle (III). Note the hypersignal in the corpus callosum (Area IV)

Area I-IV: Prussian blue staining for iron of the corresponding slices. Positive staining was observed in vessels in the perilesional (p) and necrotic zone (n) in Area I and in the lacunar-molecular layer (lm) of hippocampus in Area II. Iron staining was also positive: Area II in velum (arrow) bathed by CSF and Area III in peri-ependymal zona along the ipsilateral lateral ventricle (lv) and in choroid plexus (p). No iron staining was present in the Area IV cc:corpus callosum. dg: dentate gyrus.