Spatiotemporal pattern of USPIO enhancement in experimental stroke lesions depends on the type of ischemic injury: a 9,4 T MRI study

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Introduction

Heterogeneity of stroke pathophysiology is regarded as one of the main obstacles for establishing new therapeutic approaches. New methods are required to obtain additional pathophysiological information *in vivo*.

Focal cerebral ischemia elicits a profound inflammatory response which essentially contributes to the functional and morphological sequelae of stroke. Conventional MRI fails to visualize postischemic inflammation (3). USPIO (ultra small superparamagnetic iron oxide) particles are taken up by circulating monocytes/macrophages and can be detected by MRI, enabling the visualization of cellular inflammation in subacute stroke(2). We investigated two stroke models that distinguish divergent stroke pathogenesis and pathophysiology. Photothrombosis (PT) is a model of permanent microvascular occlusion. Infarction is completed within hours and lacks a penumbra. By contrast, transient middle cerebral artery occlusion(tMCAO) represents a macrovascular pathology that leads to a gradual ischemic tissue damage with a substantial zone of tissue at risk. Reperfusion might augment the inflammatory response(1).

This study compared the influx of USPIO-marked macrophages in these two different infarct models, PT and MCAO, at an early time point after stroke (two days) and at the time of maximum macrophage infiltration (7 days). We made use of high magnetic field (9.4T), high resolution, MRI, for the sensitive detection of USPIO effects *in vivo*. MRI signal loss was matched with iron deposition and macrophages accumulation on tissue sections obtained from the same animal after scanning.

Methods

Animal experiments

All ischemia experiments were performed in male Wistar rats weighing ~300g (n= 2-3) per group and time point. For induction of PT, the photosensitive dye rose bengal was injected intravenously, and the brain was illuminated for 15 minutes. Activation of the dye within the light cone leads to thrombosis of microvessels and a circumscribed cortical infarction. For tMCAO, a nylon monofilament was advanced intraluminally from the carotid bifurcation towards the offspring of the middle cerebral artery (MCA). The filament was left in place to induce ischemia for 45 minutes and then withdrawn to allow reperfusion. Ferumoxtran (Sinerem®) was kindly provided by Guerbet (Roissy CDG Cedex, France). Animals received intravenous injections of either USPIO (300µmol Fe/kg body weight) or saline 24 hours before scanning.

MRI acquisition

In both ischemia models, MRI was performed at either 2 days or 7 days after ischemia induction. All experiments were performed using a Varian UnityInova console, interfaced to a 9.4T magnet equipped with gradients of 270mT/m and 200µs rise time. A home-built animal handling system was employed, including a heating pad and a 4cm diameter surface coil. The MRI measurement protocol consisted of a 2D gradient echo and a 2D spin echo sequence, both with long echo times, to enhance the contrast between USPIO-affected regions and normal tissue, and a 3D gradient echo sequence with short echo and repetition time, for whole brain coverage with isotropic resolution. The measurement parameters were: (i)2D GE: TR=500ms, TE=18ms, α=20°, 256x256, FOV=30x30mm^2, 10 slices of 0.5mm each, 4av;(ii) 2D SE: TR=1000ms, TE=24ms, α/β=90°/180°, 256x256 pixels, FOV=30x30mm^2, 4 averages ;(iii) 3D GE: TR=50 ms, TE=2.5ms, α=15°, 256x256, FOV=56x28x28mm^3, 4 averages. The total measurement time per animal was 9+17+6=32 minutes for the above measurement protocol, and 10 minutes for setup scans (shimming, RF power calibration, localizer).

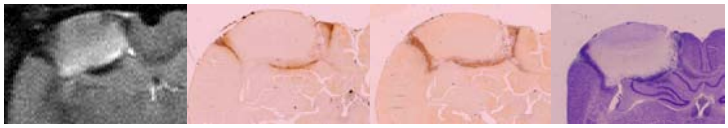
Histological assessment

After MRI acquisition the brains were processed by standard fixation, embedding, and sectioning, and slices corresponding to the MRI coronal sections were stained to depict: the infarction by cresyl violet histology; macrophages by immunohistochemistry against mab ED1; and iron with enhanced Prussian blue staining (4).

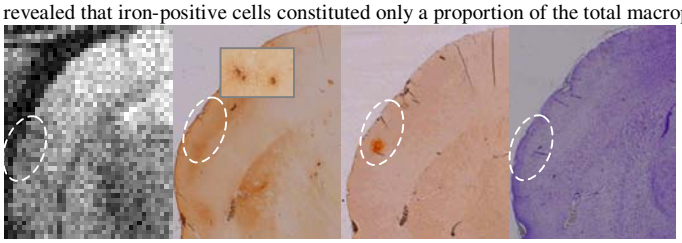
Results and discussion

USPIO detection in the photothrombosis model

Two days after induction of PT, MRI did not show any signal change in USPIO-injected animals suggestive of iron accumulation in the infarct area. Likewise, histochemical iron staining performed on tissue sections obtained from the same animals after scanning did not reveal iron deposition Immunohistochemically, a scattered infiltrate of macrophages were detected in the outer parts of the infarct.



Seven days after onset of ischemia (fig.1), T2 signal loss could be detected in both spin echo and gradient echo images. In PT, signal loss was found in a rim-like distribution in the outer parts of the infarct (left) which closely corresponded to the pattern of iron deposition (left middle) and ED1+ macrophage infiltration (right middle) detected histologically at this time point. Comparison of Prussian blue stained tissue sections with those stained immunohistochemically for ED1 revealed that iron-positive cells constituted only a proportion of the total macrophage infiltrate present in the infarct border zone (fig.1).



USPIO detection after MCAO

Two days after induction of MCAO, some animals showed spotted signal hypointensities in the MR images (fig.2), which corresponded to iron deposition histochemically (circled areas in fig.2). High magnification confirmed cellular deposition of iron. This contrasted to the results two days after PT.

Seven days after tMCAO, iron-dependent signal changes were more pronounced. Spotted or polygonal iron deposition patterns were seen in subcortical and cortical areas of the infarct, localised predominantly on the outer parts of the infarct (not shown).

Conclusions

USPIO enable the visualisation of the macrophage influx from the circulation. At the early stage of 2 days after ischemia iron deposition could be only detected in the transient MCAO model whereas in permanent ischemia induced via PT iron-positive cells did not appear before 7 days after the insult. Taken together the findings suggest that inflammatory responses detected by USPIO enhanced MRI depend on the type of ischemia. USPIO-enhanced MRI is therefore capable to noninvasively detect this pathophysiological heterogeneity and might be a promising tool for targeting anti-inflammatory therapy in stroke.

Acknowledgement

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References

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