Accumulation of Micron Sized Iron Oxide Particles (MPIOs) in Endothelin-1 Induced Focal Cortical Ischemia in Rats is Independent of Cell Migration

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INTRODUCTION: Endogenous labeling of stem/ progenitor cells via intracerebroventricular injection of micron-sized particles of iron oxide (MPIOs) has become standard methodology for MRI imaging of adult neurogenesis (1). While this method is well characterized in the naïve rodent brain, it has not been fully investigated in disease models. Here we describe methodological challenges that can confound data analysis when this technique is applied to a rat model of stroke, the Endothelin-1 (ET1) model of focal cortical ischemia. Our efforts intended to track MPIO-labeled endogenous neuroblast migration from the subventricular zone to the stroke area. The results suggest that MPIOs accumulation in stroke regions of ET1-treated brains involves two dynamic steps: an initial rapid cell independent mechanism, followed by slower MPIOs accumulation. While the latter may in part be attributable to cell dependent delivery of the particles, we suggest that the cell independent mechanism complicates the interpretation of the data. Strategies aimed at pre-labeling the stem cell niche reduced cell independent MPIOs accumulation, but failed to abolish it.

MATERIALS AND METHODS:

ET1 induced focal cortical ischemia: ET1 was infused into the somatosensory cortex (S1) of rats (2). Briefly, 600 pmol ET1 in 3 µL saline were infused over the course of 10 minutes using a 10 µL Hamilton syringe. Cortical coordinates from bregma were 4 mm (Medial-Lateral), 2 mm (Apical-Proximal) and 1.5 mm (Dorsal-Ventral). Saline control was infused into the contra-lateral hemisphere. Foot fault tests were performed to identify successful strokes.

Endogenous cell labeling: In vivo labeling was performed as previously described (1). Briefly, 20 µL green fluorescent-MPIOs (0.86 µm, 45% magnetite, styrene/divinylbenzene encapsulated, Bangs Laboratories) were stereotactically injected into the third ventricle at -0.8 mm (AP), -1.2 mm (ML), 4 mm (DV) from bregma. Variations in ET1 and MPIO injections were performed with some animals receiving MPIOs immediately following ET1 (n=5), some animals seven days following ET1 (n=20) and some animals two days before ET1 (n=8).

MRI experiments: MRI was performed at 11.7T. To confirm stroke, T_2 and ADC mapping experiments were performed using a multi-echo spin echo sequence with the following parameters: FOV 2.562 cm, matrix 128 X 64, 10 slices, 0.75 mm thickness. Additionally, for T_2 mapping: TR 10 s, 8 echo times evenly separated from 10 – 80 ms. For ADC mapping: TR 2000 ms, TE 25 ms, 5 b-values (0, 200, 500, 800, 1200 s/mm^2). To identify MPIO labeled cells, T_2* weighted imaging experiments were performed using a 3D gradient echo sequence: 100 µm isotropic voxel size, TR 30 ms, TE 8 ms. To probe BBB integrity, T_1 weighted Gd-DTPA (0.4 mmol/kg,) enhanced MRI were performed using a 3D gradient echo sequence with TR 10 ms, TE 1 ms, flip angle 30°.

Immunohistochemistry: At the end of the last MRI sessions, rats were sacrificed by perfusion, brains were processed for frozen sections and coronal sections (16 µm) were cut. Nissl staining was performed for general brain structure evaluation. Microglia staining was performed with rabbit anti-IBA1 (Wako 1:250) and secondary donkey anti-rabbit conjugated to Cy5 (Jackson; 1:250).

RESULTS and DISCUSSION:

ET1 induced focal cortical ischemia: Following ET1 infusion, significant increase in T_2 and reduction in ADC were observed in S1, p=0.006. Behavioral assessment revealed reduction in coordination of left foot usage at 1 day post ET1 infusion (p=0.00003 for ET1, p=0.1 for saline-control), confirming damage to S1. Nissl staining showed cell death in the ET1–treated area. (Figure 1)

Detection of MPIO-labeled Cells at stroke area: In an attempt to image migration patterns of SVZ derived-NPCs, MPIOs were injected intracerebroventricularly at 7 days post stroke onset and rats were scanned one week later. Dark contrast was detected in the RMS as early as 1 day post injection, corpus callosum (CC) and the rim of the infarct areas. Notably, dark contrast accumulated in the stroke region 1 week post MPIOs injection and a trajectory leading from the CC to the stroke site was detectable. While hypointensity was also observed at the saline-control site, it did not accumulate but rather diluted over time. Presence of MPIOs in the CC of the ET1 infused hemisphere was verified by fluorescence microscopy.

To further study early migratory rates, MPIOs were injected immediately after ET1/saline infusions. RMS migration was confirmed at 1 day post injection and dark contrast from CC towards the ischemic cortex was observed. Longitudinal imaging of these rats revealed continuous accumulation of the signal over four days.

Cell independent delivery of MPIOs to ischemic cortex: Cell dependent accumulation of MPIOs was expected to be selective to injured sites and to occur on a time scale of 24 hours, if carried by immunoglial cells or several days, if carried by NPCs. However, the robust detection of MPIO-induced dark contrast at the stroke site 1 day following injection of MPIOs suggested cell independent accumulation of particles. To verify this cell independent mechanism, particles were injected into the lateral ventricles at different time points post infusion of ET1/saline-control, and scanned immediately. Immediately post injection of particles, dark contrast was observed at the rim of both ET1/saline-control infused sites (Figure 2B). Immunohistochemistry revealed fluorescent-MPIOs within the injured site in association with IBA1-positive microglia (Figure 2C). These results suggested cell independent leakage of particles at the site of injury. Indeed, leakage of Gd-DTPA was observed at both 1 and 14 days post ET1 infusion (n=4), demonstrating impaired BBB (Figure 2D, E).

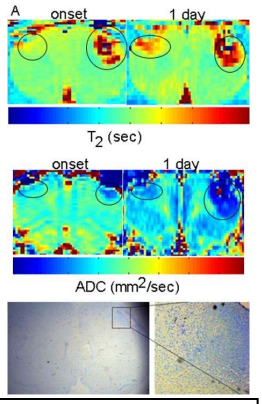

Figure 1: T_2 and ADC maps 3 hours after ET1 infusion and one day post infusion. Nissl staining shows cell death in stroke.

To determine whether cell independent delivery of the particles could be prevented by pre-labeling the NPC population prior to insult event, MPIOs were injected 2 days prior to stroke onset. MRI revealed normal RMS migration at 1 day and 2 days post intracerebroventricular injection of MPIOs as well as clear and homogenous signal intensity at the cortex. T_2* weighted MRI acquired immediately after infusion of ET1 and saline-control revealed dark contrast around the injured sites as well as in the CC (Figure 3). Yet, pre-labeling reduced the extent of immediate leakage of particles at the stroke site. These results were further confirmed by fluorescence microscopy revealing green particles co-localized with cells at the site of injury (Figure 3).

It is our conclusion that while MRI tracking of endogenous NPC via intracerebroventricular injection of MPIOs is a powerful and appropriate method for studying cell migration in naïve rodent brains, when the method is used in the ET1 stroke model data, interpretation is confounded by immediate, cell independent MPIO accumulation at the site of injury. This method should be carefully re-evaluated for use in any disease model that involves breakdown of BBB.

NIH grant: DP2 OD00436 **Refs**: **1)** Granot, et al, Neuroimage 2011; **2)** Windle, et al, Exp Neurology 2006

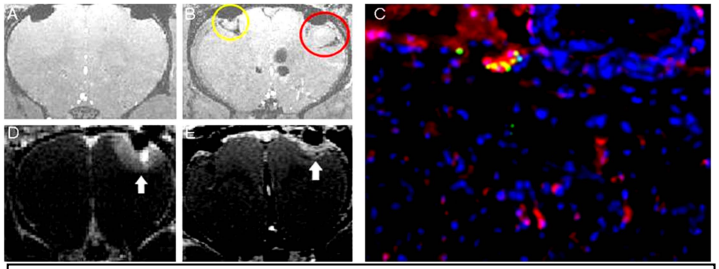

Figure 2: (A) T_2* weighted MRI 10 days post ET1 infusion, prior to MPIOs injection and (B) immediately post MPIOs injection. Red circles, ET1 infused cortex; yellow circles, saline-control infused cortex. C) IHC of injury site immediately post MPIO injection. Green, MPIOs; Red, IBA1+ cells; Blue, Dapi. (D) Gd-DTPA enhanced T_1 weighted MRI 1 (E) and 14 days post ET1 infusion. White arrows point to bright contrast due to leakage of Gd-DTPA.

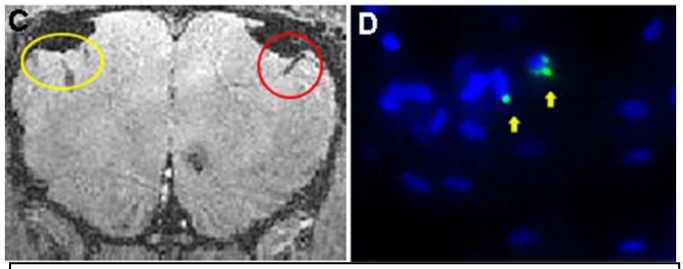

Figure 3: MPIOs were injected 2 days prior to stroke induction. T_2* weighted 3D gradient echo image acquired immediately post ET1 and saline-control infusions. Red circles, ET1 infused cortex; yellow circles, saline-control infused cortex. D) Immunohistochemistry immediately post ET1 infusion, 2 days post MPIO injection. Green, MPIOs; Blue, Dapi. Yellow arrows point to particles in association with cells.