Does MEMRI detect apoptosis in the rat neonatal hypoxic-ischemic stroke model?

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Introduction

Hypoxic-Ischemic insult to the premature brain may result in neurological disorders and death. Animal experiments have shown that in the acute phase neuronal death is largely due to necrosis. However, in the newborn brain ongoing apoptotic cell death in the hours and days after the insult contribute to the extent of injury for as long as 6 weeks[1,2]. Massive cell-influx of Ca^{2+} is one of the final steps in apoptosis, and Mn^{2+}, which mimics Ca^{2+}, has therefore been proposed as a contrast agent to detect apoptotic cells on MRI[3]. The aim of this study was to explore manganese-enhanced MRI (MEMRI) as a method for longitudinal tracing of secondary neuronal loss due to apoptosis and to evaluate the MRI findings in relation to histology.

Materials and methods

*Animal model:*The Rice-Vannucci model for hypoxic-ischemic (HI) brain injury was used. P7 Wistar pups of both genders (n=10) underwent ligation of the right carotid artery under isoflurane anaesthesia (4% induction, 2% maintenance). 2 hrs after surgery the pups were exposed to 8% O_2 (with 92% N_2) at 36ºC for 75minutes. 5 pups were sham operated and not exposed to hypoxia. 8 hrs after the hypoxia and 24hrs before the 6 weeks scan $MnCl_2$ (100mM, 40mg/kg) was injected i.p. to 8 of the HI pups and the 5 shams. 2 HI pups received NaCl instead of $MnCl_2$.

MR imaging and data analysis: MRI was performed at 7T (Bruker 70/20 AS, Bruker Biospsin GmbH, Germany) with a 72mm volume coil for transmission and actively decoupled head receive-only surface coils. During scanning the anaesthetized (2% isoflurane) pups lay prone in designated water heated beds. Key MRI parameters were: 2D T2-mapping (MSME): FOV=18x18mm; Acq.matrix=128x96 zero-filled to 128x128; pixel size=141µm isotropic; slice thk=1mm; TE=7.6ms; TR=2500ms; 40 echoes; 4 averages; TAT 16min. 3D T1-w FLASH: FOV=20x20x17,5mm; Acq.matrix=128x96x84 zero-filled to 128x128x112, voxel size=156µm isotropic; TE=3ms; TR=12ms; FA=30º; 16 averages; TAT=25min. At 6 weeks the MRI scan geometry was adjusted slightly due to the growth of the animals. The 3D data sets were used to reconstruct coronal slices corresponding to histological slices -3,25mm from bregma.

Histology: was performed with ED-1 staining for activated microglia and Fluro-Jade B staining for neuronal injury.

Results and discussion

In HI pups, SI in the two hemispheres were similar at 24hrs, with only minor morphological changes (Fig. 1). Up to 6 weeks after HI, reduced T1-weighted image intensity was seen in a gradually larger area of the cortex with corresponding reduction in T2. This indicates ongoing cell death and cyst formation in cortical tissue from 24hrs to 6 weeks after the stroke. By visual inspection, no significant difference between non-Mn^{2+} and Mn^{2+} enhanced pups was found in this aspect.

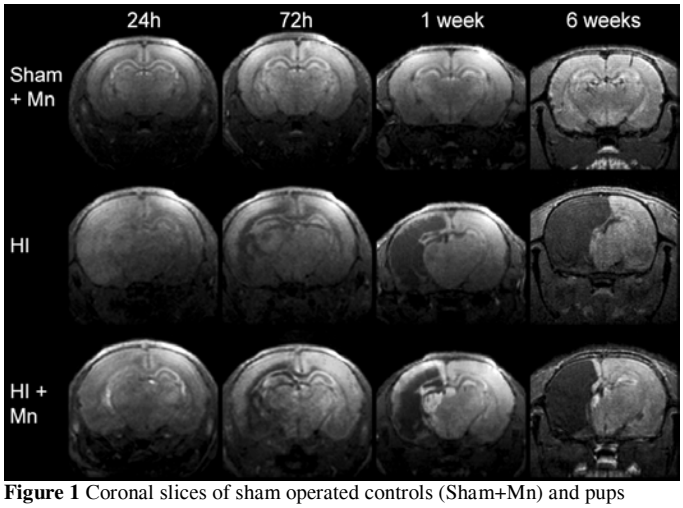

Figure 1 Coronal slices of sham operated controls (Sham+Mn) and pups with hypoxic-ischemic stroke with (HI+Mn) and without (HI) $MnCl_2$-injection at different time points after onset of HI.

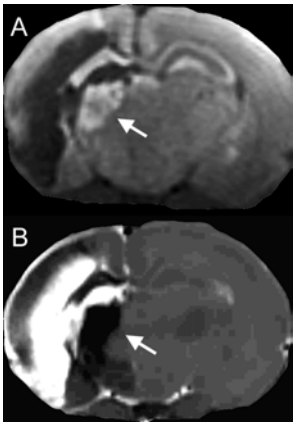

Figure 2 T1-w image (A) and T2-map (B) of stroke at 1 week after HI and $MnCl_2$-injection. Arrows show corresponding areas in thalamus with Mn-enhancement (A) and extreme reduction of T2 (B).

In HI pups injected with $MnCl_2$, a distinct area in the lateral part of the thalamus showed increased SI compared with surrounding tissue at 1 week (Fig. 2A). In HI animals without Mn^{2+} this enhancement was not seen, suggesting uptake of Mn^{2+} in these structures. T2-maps showed extreme reduction in T2 in large parts of the thalamus at 1 week in both Mn^{2+} and non-Mn^{2+} pups (Fig. 2B). The areas with highly reduced T2 corresponded well to the Mn^{2+}-enhanced areas on T1-w images, taking into account a difference in slice thickness between T1-w images and T2-maps. Scans at 6 weeks showed that the Mn^{2+}-enhanced areas at 1 week had largely disappeared and turned cystic, but that a small area in thalamus still was enhanced by Mn^{2+} in some animals.

Preliminary histological analysis and immunohistochemistry showed dying neurons and activated microglia in the areas of Mn^{2+}-enhancement. The extreme reductions in T2 suggests increased iron deposits (iron staining is pending), whereas the Mn^{2+}-enhancement might reflect Mn^{2+}-uptake in both activated microglia and apoptotic neurons. However, it is known that the apoptotic process peaks between 24 and 48hrs after HI stroke. Our results showed no specific Mn^{2+}-enhancement in this time window lending support to the hypothesis that Mn^{2+}-enhancement seen at 1 and 6 weeks is due to Mn^{2+}-uptake in activated microglia.

Conclusion

The current results of MEMRI in the neonatal rat HI stroke model indicate that manganese-enhancement is due to Mn^{2+}-uptake in activated microglia rather than in apoptotic neurons. The extreme T2 shortening seen in the same area may suggest iron deposits.

References: 1 Northington FJ et al: Neurobiol Dis. 200;8:207-19. 2 Geddes R et al: Dev Neurosci. 2001;23:180-5. 3 Silva AC et al: NMR Biomed. 2004;17:532-543