Myelin Mapping in Living Mice Using Magnetization Transfer and Manganese

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Introduction

Magnetization transfer (MT) weighted MRI (for review see [1]) of mouse brain has identified pronounced saturation of mobile protons in myelin-rich structures through interactions with the large pool of bound protons [2]. As the saturation of mobile protons due to MT competes with the longitudinal recovery, the contrast between myelin-rich structures and the "background" may be improved if an exogenous T1-shortening contrast agent can be delivered predominantly to the tissue in the background. Theoretically, ionic contrast agents are expected to become less concentrated in myelin-rich structures unless the agents exceptionally have access to the hydrophobic parts of the myelin, because neural tissue with higher content of the myelin has accordingly lower content of water. The free manganese ion (Mn^{2+}) may be a useful contrast agent for this purpose because this hydrophilic T1-shortening agent is expected to have no access to the hydrophobic parts of the myelin and thus to be delivered more to water-rich tissue in the background. The purpose of this study is to examine whether administration of manganese can be useful for mapping myelin-rich structures in MT-weighted MRI of the brain in living mice.

Methods

Animals. Five female mice (NMRI, 8—12 weeks, 28—38g) were used. Each mouse received manganese chloride (0.5 mmol/kg body weight) dissolved in distilled water via subcutaneous injection. The mice were returned to a chamber with unlimited access to food and water.

MRI. Before, 24, 48, and/or 72 hours after manganese injection, MRI measurements were carried out at 2.35 T using a MRBR 4.7/400 mm magnet (Magnex Scientific, Abingdon, UK). Radiofrequency (RF) excitation and signal reception were accomplished with use of a Helmholtz coil (inner diameter 100 mm) and an elliptical surface coil (inner diameter 20×14 mm), respectively. For MT-weighted MRI, an off-resonance RF irradiation with a frequency offset of 5 kHz and a mean amplitude of 200 Hz (flip angle 1045°) was incorporated into a spin-density weighted gradient-echo MRI (RF-spoiled 3D FLASH, TR/TE 30/7.6 ms, α 5°) at 117×156×156 µm resolution [2]. Magnetization-transfer ratio (MTR) was obtained from acquisitions with and without the off-resonance RF irradiation. In some animals, before and/or after manganese injection, T1 relaxation times of WM and GM were determined using a spin-echo multiple TR saturation recovery method. For evaluation of signal intensities, regions-of-interest were chosen in the white matter (WM; corpus callosum, external capsule, fimbria, cerebellar white matter) as well as in the gray matter (GM; prelimbic cortex, paraventricular thalamic nucleus, rostral hippocampal formation, which present with weak myelin staining in histology).

Results

MT-weighted MRI of the brain of mice before the manganese injection (Figure, left) showed pronounced saturation in WM yielding lower signal intensity, while the background tissue yields higher signal intensity. After manganese injection, this contrast was improved (Figure, right). A quantitative evaluation (Table) agrees with this finding. For example, at 72 hours after injection, signal intensity increased 18% in WM, while in GM it increased 23%. As a result, the contrast-to-noise ratio between WM and GM increased 37%. MTR became lower by 22% and 31% in WM and GM, respectively. The mean T1 values obtained for WM and GM were reduced from 733 ms and 1017 ms before manganese injection to 426 ms and 477 ms at 72 hours after injection (which correspond to 42% and 53% reduction), respectively. These data indicate that the saturation due to MT was reduced by T1 shortening effect of Mn^{2+} ions, of which the effect was less pronounced in WM.

Discussion

This work shows for the first time the use of Mn^{2+} ions for mapping WM in MT-weighted MRI. First of all, administration of manganese increases the signal intensities of the whole brain by reducing the saturation due to MT. In general, signal enhancement can be useful for imaging neural structure of mice. Here, importantly, the signal enhancement due to the reduced saturation does not obscure the MT contrast between WM and GM, but rather improves it. This can be explained by a less pronounced T1 shortening effect of Mn^{2+} ions in WM than in GM. After taken up into the systemic blood circulation, the ions cross the capillary endothelium of the cerebral circulation (mainly of the circumventricular organs) and are distributed throughout the tissue fluid of the whole brain. Here, however, this ionic contrast agent does not diffuse into the hydrophobic regions of the cell membrane (and thus of the myelin), because the non-polar hydrocarbon tails of the lipid bilayer are shielded from any ions in surrounding fluid. Thus, Mn^{2+} ions become less concentrated in WM (lipid content >15%, water content about 70%) than in GM (lipid content <6%, water content >80%). In other words, the ions are less concentrated in those structures where the hydrocarbon chains of the lipids are highly concentrated, whose "semi-solid" protons may play a role in the MT contrast. Furthermore, with respect to a cytoplasmic manganese concentration, the cytoplasm of the myelin is expected to take up Mn^{2+} ions less than the cytoplasm of the other types of cells in the brain, because the oligodendrocytes are rather inactive in healthy adult animals. The ions taken up by active neurons through calcium ion channels become less concentrated in WM, because the intracellular fluid and thus the capacity for manganese accumulation is assumed to be less in the axons than in the cell bodies. These assumptions are in line with earlier observations, where a less pronounced T1-shortening effect of Mn^{2+} ions was seen in WM than in GM [3, 4]. In particular, after manganese application to the retinal ganglion cells [4], the signal enhancement of the axons was weaker than that of the tissue formed by cell bodies. Further, the enhancement of the axons was fading earlier probably due to axonal transport.

In conclusion, administration of Mn^{2+} ions to neural tissue fluid is useful for MT-weighted MRI of the brain in vivo by increasing the signal intensity favorably for mapping myelin-rich structures.

References

[1] Henkelman RM et al. *NMR Biomed* 2001;14:57-64. [2] Natt O et al. *Magn Reson Imaging* 2003;21:1113-1120. [3] Koretsky AP, Silva AC. *NMR Biomed* 2004;17:527-531. [4] Watanabe T et al. *NMR Biomed* 2004;17:554-568.

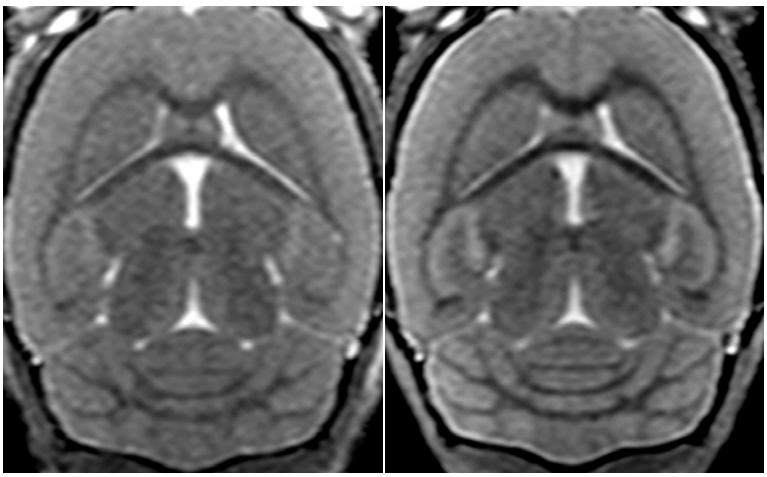

Figure Magnetization-transfer weighted (see Methods) images of the brain of a mouse (left) before and (right) 72 hours after a $MnCl_2$ injection (horizontal section).

Table. Signal Intensities and Magnetization-Transfer Ratios of the White/Gray Matter of Mice Before and 72 Hours After $MnCl_2$ Injection

		Before Mn	After Mn (72 h)
		$n = 4$	$n = 4$
SI	**WM**	87.3 ± 9.1	103 ± 3.9 (+18%)
	GM	116 ± 13	143 ± 9.1 (+23%)
CNR (GM-WM)		4.1 ± 0.5	5.6 ± 0.6 (+37%)
MTR	**WM**	0.51 ± 0.02	0.40 ± 0.02 (-22%)
	GM	0.45 ± 0.02	0.31 ± 0.01 (-31%)

Values are given as mean ± SD; Values given in parentheses: % change from Before Mn; SI: signal intensity (arbitrary unit); CNR: contrast-to-noise ratio.