Chromium(VI)-Enhanced MRI of White Matter in Mouse Brain

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Introduction

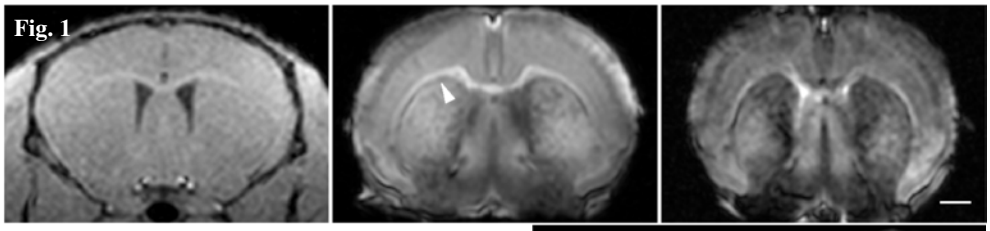
With the growing number of genetically modified mice as models of human brain disorders, MRI is increasingly used for characterizing the mouse central nervous system. In order to improve the visualization of specific structures (or functions), exogeneous contrast agents are becoming more and more popular. Here we adopt a technique developed for histology, where an aqueous solution of potassium dichromate (with chromium in oxidation state Cr(VI)) has long been used as a fixative. A number of histological procedures based on an oxidation by Cr(VI) has since been developed for a retention of lipids in situ. In particular, the site of oxidized lipids in myelinated nerve fibres was visualized by subsequently staining reduced chromium. Although Cr(VI) is diamagnetic, the retention of reduced chromium at the site of the reaction in brain tissue may be of a special value as both Cr(V) and Cr(III) are paramagnetic. Preliminary MRI studies of mouse brain in vivo using a single injection of potassium dichromate [1,2] have shown a potential for highlighting cerebral white matter (WM), but toxicity precluded the application of Cr(VI) concentrations as high as used for a histologic staining of myelinated fibers. The purpose of this in vitro MRI study was to determine whether Cr(VI) selectively highlights WM using both normal and demyelinated mouse brains directly immersed in a solution of potassium dichromate at a histologically relevant concentration.

Methods

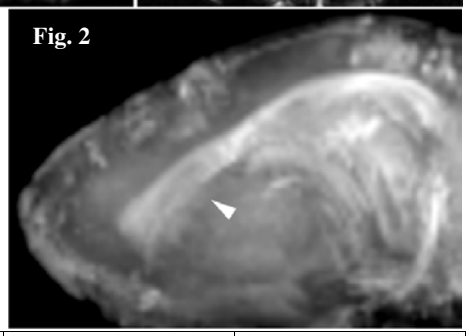
Animals and induction of demyelination. Eight male mice (C57BL/6, 8 weeks old, 24–26 g) were studied. Five mice were fed on a normal diet and served as normal controls. Demyelination of the brain was induced by feeding 3 mice on a powdered diet containing 0.2% (w/w) cuprizone (Sigma, Taufkirchen, Germany) for 6 weeks.
MRI. All MRI measurements were carried out at 2.35 T using a MRBR 4.7/400 mm magnet (Magnex Scientific, Abingdon, UK) and a DBX system (Bruker Biospin MRI GmbH, Ettlingen, Germany) equipped with B-GA20 gradients. Radiofrequency excitation and signal reception was accomplished with use of a Helmholtz coil (inner diameter 100 mm) and an elliptical surface coil (inner diameter 20 × 14 mm), respectively. 3D gradient-echo MRI data sets (rf-spoiled 3D FLASH, TR/TE 17/7.6 ms, α 25°, measuring time 93 min) were acquired at 117 µm isotropic resolution. T_1 of WM and grey matter (GM) were determined using a spin-echo multiple TR saturation recovery method (TR 15, 25, 50, 120, 250, 500, 1200, 2500, 5000 ms, TE 9.5 ms, FOV 76.8 × 25.6 mm, matrix 256 × 128, in-plane resolution 300 × 200 µm, slice thickness 700 µm), while T_2 was determined using a multi-echo spin-echo sequence with the same parameters but with a constant TR and multiple TE (TR 5000 ms, TE 9.5, 19, 28.5, 38, 47.5, 57, 66.5, 76, 85.5, 95, 104.5, 114, 123.5, 133, 142.5, 152 ms). In order to minimize partial volume contributions from neighboring tissue, sections were selected perpendicular to the bundle of subcortical WM near the anterior horn of the right lateral ventricle. MRI signal intensities within subcortical WM or cortical GM was fitted for each TR and TE value with single exponential functions to yield T_1 and T_2 relaxation times.
Immersion of the brain. After in vivo MRI, the brain was excised and immersed in a solution (2 mL, pH 5) of 85 mM potassium dichromate ($K_2Cr_2O_7$, Sigma, Taufkirchen, Germany) dissolved in distilled water for the following 4 days. For postmortem MRI, the brain was taken out of the solution and wrapped up in paraffin wax film. It was then placed in the same position (with respect to the coils) and measured in the same manner as in vivo.

Results

Immersion of the brain in a solution of potassium dichromate resulted in a heterogeneous signal increase in MRI. Figure 1 shows coronal sections of the brain of (left) a normal brain in vivo, (center) the same brain after immersion, and (right) a demyelinated brain after immersion (scale bar 1 mm). Subcortical WM becomes clearly highlighted. In addition, its contrast to GM in cerebral cortex and basal ganglia is also enhanced.



In the demyelinated brain, such a clear and continuous contrast of WM is not seen. Figure 2 shows a lateral maximum intensity projection of the data set shown in the center of Fig.1. It visualizes the 3D structure of subcortical WM (arrowhead). The Table summarizes the SNR values as well as the relaxation times, which are in line with the qualitative findings noted above. In normal brain, WM yielded a SNR of about 66 after immersion, whereas GM yielded only 47. This corresponds to an enhancement of the contrast-to-noise ratio from about 3 in vivo to 20 after immersion. T_1 as well as T_2 relaxation times in normal brain are tremendously shortened after immersion, with more pronounced shortening in GM than in WM. Thus, the above noted SNR increase in WM and GM both results from a reduced T_1 saturation, while the WM-GM contrast reflects the more pronounced shortening of T_2 (and thus of T_2*) in GM. In the demyelinated brain, WM yielded a SNR of only 43 after immersion. This can be explained by the shorter T_2 (about 16 ms) than in normal WM (about 20 ms).



Discussion

Here, the brain was first exposed to diamagnetic 'MRI silent' dichromate ions. As a consequence, the observed MRI signal alterations must be ascribed to a reduction of Cr(VI) to a paramagnetic form due to the oxidation of reactive organic matter. Accordingly, the higher MRI signal intensity with the less pronounced T_2 shortening in normal WM than in normal GM and in demyelinated brain indicates that the presence of normally myelinated nerve fibers inhibits the formation of strongly T_2-dephasing products. In view of a previous histochemical study [3], it is tempting to assume that a stepwise formation of strongly T_2-dephasing products containing Cr(III) is delayed or even stopped at an intermediate stage – which possibly contains Cr(V) – by the presence of normal myelin sheaths.

		Normal Brain		Demyelinated Brain	
		In Vivo	After Cr	In Vivo	After Cr
SNR	WM	27.5 ± 2.3	66.0 ± 8.1	24.1 ± 1.1	42.8 ± 15
	GM	24.3 ± 2.0	46.6 ± 11	24.2 ± 1.1	32.9 ± 10
T_1 (ms)	WM	968 ± 25	86 ± 11	1313 ± 40	86 ± 1.5
	GM	1246 ± 44	74 ± 7	1288 ± 17	62 ± 2.1
T_2 (ms)	WM	60.2 ± 3.6	20.2 ± 3	89.7 ± 4.2	16.2 ± 1.2
	GM	69.4 ± 1.1	14.3 ± 0.7	75.4 ± 2.8	13.3 ± 1.3

References

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