Reduced intracellular mobility underlies manganese relaxivity in mouse brain in vivo: MRI at 2.35 and 9.4 T

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Target Audience Anyone who is interested in manganese-enhanced MRI [1], MRI contrast agents, or MRI of manganese poisoning.

Purpose (i) To perform T₁-weighted MRI analyses at two different field strengths and to explore if such measurements provide additional insights into the cellular localization and chemical environment of Mn²⁺ in mouse brain in vivo, (ii) to estimate the enhancement factor ε and relaxivity r₁ for Mn²⁺ in striatum in vivo, and (iii) to assess the access of Mn²⁺ and Gd-DTPA to extra- and intracellular compartments after disruption of cellular membranes.

Methods T₁ measurements. (i) 9 mice received a single intraventricular injection of Gd-DTPA (5 µL, 100 mM). 3 hours later, the T₁ of 5 of these animals was measured at 2.35 T, while the other 4 were measured at 9.4 T. In addition, these 4 mice underwent high-resolution T₁-weighted MRI (see below) as well as post mortem. (ii) Another 6 mice received a single s.c. injection of MnCl₂ (0.5 mmol/kg b.w.). One (n=4), 3 (n=4), and/or 7 (n=3) days later, T₁ was measured at 9.4 T followed by 2.35 T. (iii) Another 4 mice received multiple s.c. injections of MnCl₂ (0.25 mmol/kg b.w. on days 1, 4, and 7). On days 8, 10, and 14, T₁ was measured at 9.4 T and 2.35 T. T₁ was determined with multiple TR spin-echo MRI (in-plane resolution 117 µm, slice thickness 234 µm). Values for cerebral (prelimbic) cortex, striatum, thalamus, and cerebellar cortex were obtained with TE 16 ms (2.35 T) and 10 ms (9.4 T) and 7 TR (0.2–10 s). Relativities r₁ are defined as ΔR₁/Δ[concentration] with ΔR₁ the relaxation rate increase and Δ[concentration] the concentration increase of a contrast agent at a certain point of time after injection. Because it may be assumed that Δ[concentration] is identical for the same animals undergoing MRI at the same time after receiving the same dose of the agent through the same route, the corresponding relaxivity ratios RR at the two field strengths may simply be calculated by 100R₁/400R₁ = 100R₁/400R₁. (iv) Aquous relaxivities r₁ (in s⁻¹·M⁻¹) were determined by measuring T₁ for 5 different concentrations of Mn²⁺ (0.05–0.5 mM) and Gd-DTPA (0.1–1.0 mM) at both 20°C and 37°C.

Enhancement factors for Mn²⁺ in the striatum. Additional 4 mice received the multiple s.c. injections and underwent the T₁ measurements. Results and Discussion Table below summarizes the T₁ relaxation data (T₁ in seconds, mean ± SD. RR = 100R₁/400R₁ = 100R₁/400R₁) as observed for mouse brain in vivo before and after injections of Mn²⁺ or Gd-DTPA at 2.35 T and 9.4 T. Both contrast agents shorten the T₁ in all brain tissues and at both field strengths. However, while 100R₁ and 400R₁ for Gd-DTPA are very similar at 2.35 T and 9.4 T, the values for manganese are up to 4-fold smaller (P<0.05) at the 4-fold higher field. In aqueous solution, the relaxivities 100R₁ and 400R₁ for Gd-DTPA are 4.8 and 4.4 at 20°C as well as 3.8 and 3.7 at 37°C, while for Mn²⁺ the results are 5.4 and 5.0 at 20°C as well as 3.9 and 3.9 at 37°C, respectively. Thus, all relaxivity ratios 100R₁/400R₁ range between 1.0 and 1.1 except for Mn²⁺ in vivo, where respective values range from 2.4 for the striatum to 4.4 for the cerebellar cortex. In the striatum at one day after 3 injections, the relaxivities 100R₁ and 400R₁ are 2.2 and 1.1, respectively, because aqueous Mn²⁺ values of 100R₁ and 400R₁ of 3.94 and 3.86 mM⁻¹·s⁻¹ at 37°C correspond to 4.1 and 4.0 kg mmol⁻¹·s⁻¹, respectively. These results suggest Mn²⁺ ions in vivo are in a viscous fluid and/or bound to macromolecules, because a reduced mobility increases the longitudinal relaxation rate by eliminating the contribution of the rotational correlation time in the frequency range 10–100 MHz [3].

High-field data show that Mn²⁺ is most concentrated in brain one day after repeated injections. 400R₁ is considered to be a more reliable measure of Mn²⁺ concentration due to a lower relaxivity enhancement than 100R₁. For a single injection, Mn²⁺ concentration is highest 3 days after injection. One day after the single injection 100R₁/400R₁ = 2.8 for brain Mn²⁺ is significantly (p<0.005) higher than 100R₁/400R₁ = 1.7 after 3 injections despite a lower brain concentration indicated by a significantly lower 400R₁. At later stages (400R₁ ≤ 2.5, while the assumed brain concentrations (i.e., 400R₁) remain high). These discrepancies indicate that the accumulation of Mn²⁺ in the brain is not a single process which proportionally reduces the mobility. Instead, it suggests that effective immobilization during early exposure to excessive Mn²⁺ is accompanied by a separate Mn²⁺ “storage” with less immobilization in order to minimize the disturbance of cellular function by isolating Mn²⁺ from physiologically relevant macromolecules. While acute manganese poisoning in human may be related to the immediate dysfunction of macromolecules, neurodegenerative processes associated with chronic manganese poisoning may involve an isolation or confinement mechanism.

The Mn²⁺-induced contrast for cell layers in vivo persists post mortem substantially longer than that induced by Gd-DTPA. Figure (right) shows T₁-weighted MRI (9.4 T) of the hippocampus in horizontal sections: (A) before and (B) 3 days after Mn²⁺ injection in vivo. (C) 90–102 min post mortem, (D) 120–132 min post mortem. E) 220–232 min after Gd-DTPA injection in vivo as well as (F) 0–12 min, (G) 30–42 min, and (H) 90–102 min post mortem. Mn²⁺ accumulates predominantly in the pyramidal cell layers (A, B). After death, Mn²⁺ remains for at least 2 hours (C, D). The Gd-DTPA contrast persists for several hours in vivo after injection (E). During the first 12 min after death, Gd-DTPA mostly remains in extracellular spaces (F), but within 42 min Gd-DTPA enters the CA3 pyramidal cells (G; white arrowhead) and within 102 min all cell layers are affected (H). These observations are in line with a binding of Mn²⁺ to intracellular compounds and/or a sequestered intracellular storage, because otherwise smaller Mn²⁺ would rapidly move through the impaired outer plasma membrane according to the concentration gradient.

Conclusion The combined application of low-field and high-field MRI identified a reduced mobility of Mn²⁺ as a mechanism underlying the enhanced relaxivity in vivo. Pertinent studies may be exploited for a characterization of novel contrast agents in vivo as well as for studying neuropathologic alterations as e.g. seen in manganese poisoning.