MEMRI and Tumors: a method for the evaluation of the contribution of Mn(II) ions in the intra- and extra-cellular compartments

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PURPOSE: Mn^{2+} ions are used as positive contrast agents in Manganese Enhanced MRI (MEMRI) to mimic Ca^{2+} transport in biological systems. Recently, MEMRI has been considered useful for tumor detection as uptake of Mn^{2+} ions may be up-regulated in cancer cells. In the context of tumor detection and staging, it is relevant to assess whether the observed signal enhancement has to be assigned to the intra- or the extra-cellular compartments. The purpose of the work was to set-up a simple method to differentiate the two contributions based on sequestering all the Mn^{2+} ions in the extracellular compartment into a highly stable chelate (Mn-DO2A) characterized by a very low relaxivity ("MRI silent").

METHODS: *In vitro* relaxometric measurements (at 0.47T) were carried out in order to determine the relaxivity of $MnCl_2$ and Mn-DO2A at 25°C and 37°C in PBS and serum, and to estimate the sequestering efficiency of DO2A ligand toward Mn ions. Next, B16F10 melanoma cells were incubated in the presence of $MnCl_2$ and Mn-DO2A and the amount of internalized Mn2+ was assessed by either MRI and ICP-MS. Finally, *in vivo* MRI experiments were carried out at 1T on melanoma mouse models administered with $MnCl_2$ and, after 30 min, with Ca-DO2A. Control experiments were carried out after the single administration of $MnCl_2$ or Mn-DO2A. In vivo MRI analysis was followed by ICP-MS determination of Mn content in tissues.

RESULTS: *In vitro* relaxometric measurements allowed confirming the good sequestering activity of DO2A towards Mn^{2+} ions, as the addition of Ca-DO2A to a solution of $MnCl_2$ causes a drop of relaxivity upon the formation of the highly stable and low-relaxivity (1.3 and 1.5 $mM^{-1}s^{-1}$ in PBS and in serum respectively) Mn-DO2A. It was proved that the sequestering ability of DO2A towards Mn^{2+} ions is fully effective also in the presence of serum albumin. Moreover, it was shown that Mn-DO2A does not enter cell's membranes neither the presence of Ca-DO2A in the extracellular space prompts migration of Mn ions from the intracellular to the extracellular compartment. On this basis the *in vivo* drop in SE% in T1-weighted images is taken as an evidence of the sequestration of extracellular Mn^{2+} ions upon addition of Ca-DO2A. This effect is well detected in the tumor region and, to a less extent, in kidneys, whereas a negligible change in SE% is observed in liver and muscle. It has been found that, 2 hours after the administration of $MnCl_2$ (0.058 mmol/Kg), in the tumor region, 45% and 25% of the observed signal enhancement is due to manganese distributed into the intra- and extra-cellular compartments, respectively.

CONCLUSION: The herein reported method may represent a powerful tool to differentiate vascular perfusion and cellular uptake in a MEMRI experiment by making "silent" the paramagnetic contribution which is not specifically ascribable to the uptake from cancer cells. This approach may further strengthen the MEMRI investigations for early detection and characterization of tumors.

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