Vectorization of Iron Oxide Nanoparticles with a Neuron-penetrating RVG Peptide: An Attempt to Cross the Blood Brain Barrier

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Objectives: to develop a MRI contrast-agent able to cross the blood brain barrier (BBB) and distribute within the brain.

Background: Neurotropic viruses cross the BBB to infect brain cells. Amongst those, rabies virus shows a high degree of neurotropism *in vivo* through very well characterized cellular mechanisms. As recently demonstrated by Kumar P. et al (Nature Vol 448/ July 2007: 39-43), a short 29-amino-acid peptide from rabies virus glycoprotein (RVG) interacts specifically with the nicotinic acetylcholine receptor (AchR) on endothelial and neuronal cells to enable viral entry into the brain. USPIO are ultrasmall superparamagnetic iron oxide nanoparticles presenting a prolonged plasma half-life. In the brain, they are restricted to the vascular space due to their physico-chemical properties that prevent them to cross the BBB. In this study, USPIO were vectorized with the 29-amino-acid peptide from RVG to promote their way through the BBB.

Materials and Methods: 29-amino-acid peptide from RVG (8-amino-3,6-dioxaoctanoyl-Tyr-Thr-Ile-Trp-Met-Pro-Glu-Asn-Pro-Arg-Pro-Gly-Thr-Pro-Cys-Asp-Ile-Phe-Thr-Asn-Ser-Arg-Gly-Lys(TFA)-Arg-Ala-Ser-Asn-Gly) was purchased from NeoMPS (Strasbourg, France). USPIO were functionalized in 2 successive steps with the short RVG peptide first and then with an amino-PEG 750 (Fluka, Bornem, Belgium) to avoid rapid elimination. Imaging of anaesthetized mice brain was performed on a Bruker Avance machine at 4.7T. Images were acquired with the TURBO-RARE sequence (TR = 3090 ms / TE = 42.1 ms / Tacq = 13.1 min.) before and 0.5, 4, and 22h after i.v. administration of either naked USPIO or RVG-USPIO at a single dose of 300 µmol of iron/kg of body weight.

Results: ***1) Synthesis/characterization***: The short 29-amino-acid peptide was successfully grafted on USPIO. The grafting did not alter significantly the relaxivity or stability of the contrast agent. ***2) Imaging***: 30 minutes after the administration of RVG-USPIO, a 37% reduction in signal intensity was reproducibly observed in brain sub-regions. 22h post-RGV-USPIO injection, a signal reduction of 18% was still measured, as compared to a 8% signal reduction with naked USPIO.

Conclusions: In this study, we have successfully developed new vectorized nanoparticles with the RVG peptide known to allow crossing of BBB. Preliminary *in vivo* imaging assessment of mouse brain showed sustained (>22h) reduction of signal intensity, suggesting that a fraction of the injected particles effectively crossed the BBB.

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