Mn-loaded apoferritin: a high sensitivity, biologically compatible MRI agent.

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Introduction: An innovative approach to the design of MRI Contrast Agents has been pursued through the entrapment of Mn(II) aqua ions inside the inner cavity of Apoferritin. This task has been addressed by partial dissolution of the previously formed β-MnOOH inorganic phase. The dissolution of β-MnOOH occurs via the reduction of Mn(III) to Mn(II) operated with aminopolycarboxylic acids that also act as coordination ligands for sequestering weakly coordinated manganese ions on the outer surface of the protein. The reductive treatment has allowed to generate an apoferritin-based nanocarrier (Mn-Apo) containing up to 300-400 Mn(II) aqua ions encapsulated in the inner cavity. This yielded to the remarkable relaxivity value (per apoferritin) of 4000-7000 $mM^{-1}s^{-1}$. Mn-Apo being formed by endogeneously occuring molecules and ions, displays a high biocompatibility. It is well established that liver is the major organ for ferritin clearance from circulation and a specific receptor for this protein has been described for rat and human hepatocytes. Therefore, this imaging probe can be proposed in the diagnosis of a variety of liver diseases involving an alteration in the hepatic iron storing capabilities (e.g. hepatocellular carcinoma, fibrosis, cirrhosis). In alternative, Mn-Apo can be biotinylated in order to develop a targeting procedure based on the well known biotin/avidin recognition pathway.

Methods: Iron-free horse spleen apoferritin was reconstituted in the presence of $MnCl_2$ solution at pH=9 under air. Reduction/solubilisation of the β-MnOOH phase inside the apoferritin cavity has been carried out with NTA and TETA at room temperature for 1 week. The characterization of the formed Mn-Apo system has been attained by the acquisition of 1/T1 NMRD profile (FFC-NMR relaxometer, Stelar, Mede, Italy) and ESR (Bruker EMX spectrometer, Karlsruhe, DE). MR images were acquired at 7T on a Bruker Avance spectrometer.

Results: The "in vitro" uptake of Mn-Apo is markedly more efficient in healthy hepatocytes with respect to hepatocarcinoma tumour cells (Figure 1)., The cells viability after the incubation with Mn-Apo, evaluated by the trypan blue based assay, was not significantly different from control cells. Competition studies with native ferritin confirmed that the cellular uptake occurs through ferritin receptors in both hepatocytes and hepatoma. Mn-apo was injected in C57BL/6 mice at a dose of 0.01 mmol/Kg of Mn and a very high Signal Intensity (SI) enhancement of the liver in the MRI images was detected 30' and 1h after the administration. 24h after the decreased liver SI indicated the almost complete elimination of the probe. A clear delineation of a hepatocarcinoma lesion in the HBV transgenic mouse model using this Mn based imaging probe has been attained (Figure 2).

Conclusions. Mn-loaded apoferritin is a very efficient probe specific for liver imaging. In particular, it could be useful in the diagnosis of a variety of liver diseases involving an alteration in the hepatic iron storing capabilities.

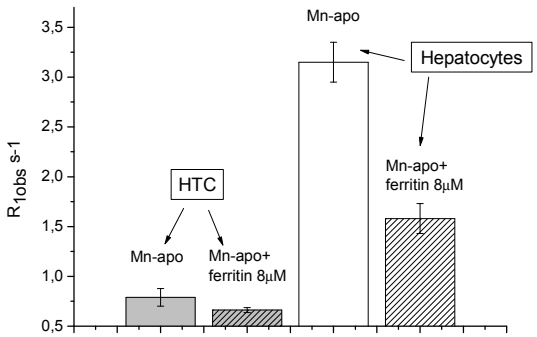

Figure 1

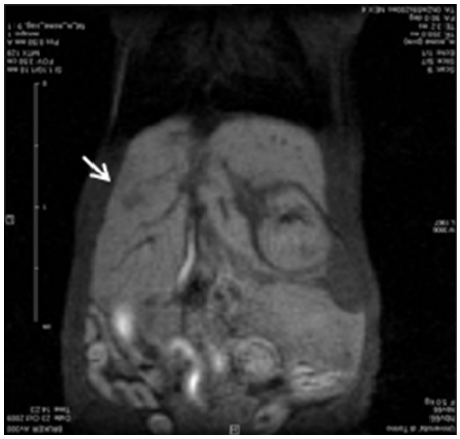

Figure 2