Curcumin/Gd loaded Apoferritin: a novel "theranostic" agent to prevent hepatocellular damage in models of hepatic injuries

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Introduction. Apoferritin has been exploited to deliver simultaneously therapeutic and imaging agents (loaded into its internal cavity) to hepatocytes as this protein is efficiently taken up from blood by hepatocyte scavenger receptor class A type 5 via the ferritin transporting route. To this purpose the apoferritin has been loaded with the MRI contrast agent GdHPDO3A and Curcumin, a polyphenolic substance endowed with multiple pharmacological actions (namely: antioxidant, anti-inflammatory, antineoplastic). Although clinical trials have demonstrated the safety of curcumin the clinical translation of this promising natural compound is hampered by its poor water solubility, short biological half-life, and low bioavailability in both plasma and tissues. Herein we propose the use of apoferritin as a protecting and specific carrier for curcumin. Curcumin and GdHPDO3A loaded apoferritin (figure 1) has been used for the attenuation of thioacetamide (TA)-induced hepatitis together with the evaluation by MRI of drug delivery efficiency.

Methods. The loading procedure consists of lowering the pH of the apoferritin containing solution followed by the addition of the solutes to be uploaded. The pH is then returned to 7 in order to restore the spherical supramolecular shape of apoferritin, now entrapping the desired solutes in its inner cavity. Apo-CUR-Gd (63 mg/kg) was administered 24h before the treatment with a toxic dose of TA (60 mg/Kg).. MRI images have been recorded at 7T on a Bruker Avance 300 MRI scanner.

Results. Mice were treated intraperitoneal with apoferritin loaded curcumin and Gd-HPDO3A Biodistribution of the imaging probe was followed by MRI measuring liver signal intensity enhancement of T_1-weighted images (figure 2). The high relaxation enhancement efficiency of Gd complexes inside the cavity permits the detection of low concentrations of the imaging probe. By using an average curcumin/Gd ratio of 24 a liver curcumin concentration of about 0.43±0.09 mM has been calculated. This concentration corresponds to ca. 12% of the administered dose (calculated using an average liver volume of 0.91 cm^3) and it is five times higher than the amount found after the i.p. administration of a similar dose of curcumin alone. The extent of hepatic injury has been evaluated by measuring alanine aminotransferase (ALT) activity in plasma and by histology assessment. Clearly, the plasma level of ALT was attenuated to 110 (37% of curcumin untreated animals) and the necro-inflammation score was reduced to 1.3 (52% of curcumin untreated animals) Hepatocytes appeared with a better preserved morphology and trabeculae integrity was maintained. Moreover, the degree of leukocytes infiltration was drastically reduced as well as the presence of apoptotic cells. Furthermore, Apo-CUR has been administered for 8 weeks to hepatocellular carcinoma developing HBV-Tg mice (3 months old) and its ability to prevent hepatocytes neoplastic transformation has been assessed.

Conclusions. In conclusion, apoferritin can be considered as an efficient carrier for therapeutics and probes for imaging guided treatment of liver diseases. The encapsulation of curcumin inside the apoferritin cavity significantly increases its stability and bioavailability while maintaining its anti-oxidant and anti-inflammatory properties.

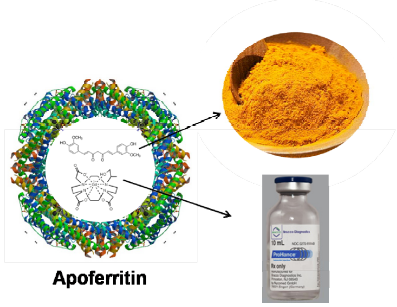

Figure 1

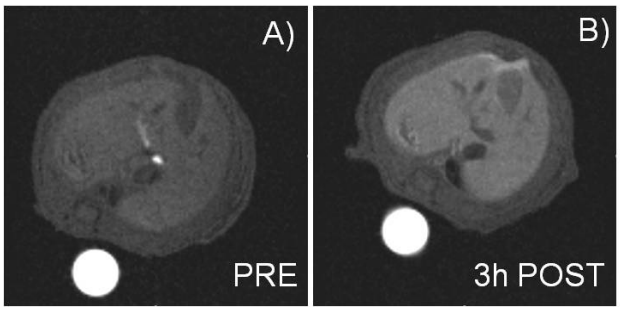

Figure 2