Characterization of HE-24.8: a new contrast agent for experimental Magnetic Resonance angiography

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Synopsis: Micro MR angiographies of small vessels require contrast agents with long intravascular persistence [1]. Also for assessing vessel permeability by means of DCE MRI the use of macromolecular contrast agents has shown potential to better characterize tissue and assess specific treatment effects on tumors. The purpose of this study was to synthesize and biologically and physically characterize a new water-soluble polymeric contrast agent based on a complex of N-(2-hydroxypropyl)methacrylamide copolymer with gadolinium (HE-24.8). Furthermore, its potential for experimental micro-MR-angiography was assessed.

Methods: HE-24.8: A comonomer Ma-Acap-Asp-[(Asp-(OMe)2]2 and two copolymers with HPMA were synthesized (figure 1). The copolymers after deprotection of carboxylic groups formed very stable complexes with gadolinium exhibiting an excellent stability in aqueous solutions. Copolymer HE-24.8 contained HPMA and Ma-Acap-Asp-[(Asp-(OH)2]2-Gd units. The copolymer HE-kin contained small amount of tyrosinamide in its structure enabling radiolabeling and this copolymer was used for pharmacokinetic studies. In both polymer complexes, Gd was embedded in side chains of the hydrophilic polymer formed by three aspartic acid residues extending four carboxylic groups for complexation with Gd. The gadolinium content was determined by atomic mass spectroscopy. Gd-DTPA-human serum albumin (Gd-DTPA-HSA) was synthesized by covalently binding gadolinium-DTPA to albumin as described previously [2].

In vitro determination of cell toxicity: Human fibroblasts (MSU) and human hepatoma cells (HEP-G2) were incubated with HE-24.8 (0.05 and 0.5mmol Gd/ml) for 24 and 48 hours. Control cells were incubated with Gd-DTPA-BMA (Omniscan, Amersham Health, USA) using equal gadolinium concentrations or with growth medium without any contents. Viability was determined by Trypan blue (GIBCO, Gland Island, NY) exclusion.

Kinetics, biodistribution and in vivo toxicity: ^{131}Iodine labeled HE-kin was injected into the tail vein of 4 healthy Copenhagen rats. Blood samples and scintigraphic images were taken during a two week follow up period and the animals were monitored for signs of toxicity. Two weeks after intraveneous injection, the animals were sacrificed and their organs were removed. Residual activity was counted and was corrected for radioactive decay.

MR Imaging: Tumor micro-angiographies were performed in 5 Copenhagen rats with subcutaneous Dunning AT1 prostate tumors, which were grown for 3 weeks. For Magnetic resonance imaging (MRI) a clinical 1.5 Tesla whole-body MR-system (Siemens Symphony, Erlangen, Germany) and a custom-made radio-frequency-(rf) coil for rf excitation and signal reception were used [2]. Determination of the relaxivity of HE-24.8, Gd-DTPA HSA and Gd-DTPA-BMA: The contrast media were diluted in 0.9% NaCl (0.0, 0.01, 0.05, 0.1, 0.5, 1.0mmol Gd/L) and transferred to phantom vials. T1-times were determined with a saturation recovery turbo FLASH-sequence (TR/TE=10/4, flip-angle: 12°, Trec=53-9000ms). The relaxation rates were plotted against concentrations. By linear regression relaxivity was calculated. Micro-MR angiography: Four rats were examined with HE-24.8 (0.05mmol Gd/kg), one after administration of Gd-DTPA-HSA. In 3 rats being examined with HE-24.8 and the animal with Gd-DTPA HSA the tumors were investigated using a 3D gradient echo pulse sequence (TR/TE=28.0/8.6ms, flip-angle=70º, FOV=12.8x30x50mm^3, matrix=128x317x512, 2 acquisitions, total scan time=24min). Subsequently the chest was imaged using a 3D gradient echo pulse sequence (TR/TE=10.2/3.8ms, flip-angle=70º, FOV= 30x55x110mm^3, matrix=60x256x512, 4 acquisition, total scan time=5.18min). In 1 animal first the brain then the tumor were investigated after administration of HE-24.8.

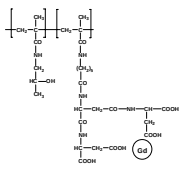
Results: In vitro-toxicity: No increased death rates were observed in MSU and HEP-G2 cells after incubation with HE-24.8 or Gd-DTPA-BMA in dosages of 0.05 and 0.5 mmol Gd/mL growth medium for 24 and 48 hours, respectively.

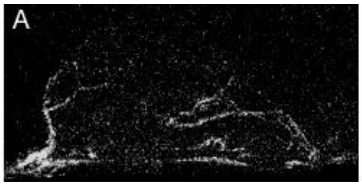
Kinetics, biodistribution and in vivo toxicity: After 15 minutes < 50% of the injected dose of HE-kin was found in the blood. Four hours later the blood concentration had decreased to 16.9% of the injected dose. 24, 168 and 336 hours after injection, 4.67 %, 0.36% and 0.15% of the injected dose could be attributed to the vascular compartment. At the whole animal level 37.6%, 20.0% and 8.0% of the injected dose were still detected at 4, 24 and 336 hours post injection. HPMA copolymer-Gd did not accumulate in any organ specifically. Relatively, the largest fraction accumulated in the spleen, with 0.25% ID per gram tissue. In most other tissues retention rates were comparable, with approximately 0.05% of the initial dose per gram tissue. No decreased overall activity, no reduction in food intake, and no substantial signs of weight loss of the animals were observed during the experiments.

MR-relaxivity: HE-24.8 showed higher T1 relaxivity (21.6±0.7mM-1s-1) than Gd-DTPA HSA (12.4±0.2mM-1s-1) and Gd-DTPA-BMA (4.3±0.1mM-1s-1).

MR-angiography: Gd-DTPA-HSA and HE-24.8 were both capable to visualize small vessels in the subcutaneous tumors (Fig. 2). The contrast of vessels was higher for HE-24.8, however, the number of vessels displayed was not increased, which was most probably reasoned by the limited resolution and signal to noise ratio reached with the 1.5T MR-scanner. In the brain HE-24.8 proved its potential as angiographic contrast agent by visualizing the vascular network with the carotic artery, the jugular vein, the basal cerebral arteries of the circulus arteriosus cerebri and the middle cerebral artery and some of its branches (Fig. 3).

Discussion: In summary, a new polymeric contrast agent with high T1 relaxivity and good tolerability is presented, which is suited for experimental micro-MR-angiography. Its chemical properties allow to flexibly modify the molecular size, which recommends its use also for DCE MRI studies on vessel permeability.





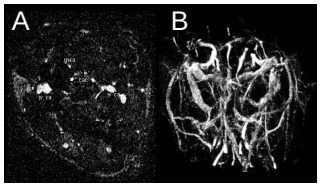


Figure 1 (left): Scheme of HE-24.8; Figure 2 (middle): 3D-MIP of subcutaneous Dunning prostate cancers after administration of HE24.8 (A) and a Gd-DTPA HSA (B); Figure 3 (right): MRA-image (A) and 3D-MIP (B) of a HE-24.8 enhanced scan of the rat brain.

References: [1] Kobayashi H et al. Magn Reson Med 2001, 46: 579-585 [2] Kiessling et al. Invest Radiol 2002, 37:193-198