High-resolution imaging of non-small cell lung cancer in a mouse model of brain metastasis

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INTRODUCTION

Humanized anti-human epidermal growth factor receptor (EGFR) monoclonal antibody is currently and in clinical use as a component of combination therapy for treating head and neck, brain, pancreatic, colorectal and non-small cell lung cancers. Due to high affinity to EGFR they can be potentially developed into MR imaging probes by conjugating nanoparticles or paramagnetic chelates. However, the use of antibodies in imaging is limited due to insufficient delivery of signal enhancer and slow clearance from the blood stream. We previously reported several paramagnetic chelates that undergo rapid oxidation and oligermerization in the presence of peroxidase or myeloperoxidase resulting in a strong signal enhancement in vitro as well as in vivo [1-3]. In our study a combination of a pre-targeting system of antibody-enzyme conjugates and a paramagnetic substrate has been used for targeted EGFR MR imaging in model of lung cancer metastasis to the brain. Furthermore, the specificity of antibody conjugate for delivery to the tumor site and the sensitivity to EGFR expression in vivo were examined for ultimate goal of clinical application.

METHODS

Synthesis Monoclonal antibody (EMD72000) was conjugated to horseradish peroxidase (HRP) and glucose oxidase (GO) by using bisaromatic hydrazone bonds (mAb-HRP, mAb-GO) [2]. Bis-phenolic substrate bis-tyramide of gadolinium (III) DTPA (di-Tyr-DTPA(Gd) was used as HRP substrate [3].

Non-small cell lung cancer (NSCLC) NSCLC cell line (PC14PE) was cultured in RPMI 1640 containing 10% fetal bovine serum. The cells were trypsinized to prior to implantation to mice.

Animal model A mouse model of brain metastasis was prepared by intraarterial injection of PC14PE6 (20,000 cells) through carotid artery using a micro dissectoscope. The imaging was performed after the formation of 1 mm tumors was proven by using T1w imaging enhanced by GdDTPA (0.2 mmol/kg).

MRI MR images were obtained by using a 7T Bruker BioSpec MRI equipped with a phase array coil using T2w and T1w TSE and GRE pulse sequences (TR/TE=200/5, Flip angle=60). The animals were divided into experimental and control groups. One μg of mAb-HRP and two μg of mAb-GO were administered to the experimental mice by intravenous injection at 4 hours before injection of HRP-substrate of di-Tyr-DTPA-Gd (0.2 mmol/kg). The control group of mice received di-Tyr-DTPA-Gd only.

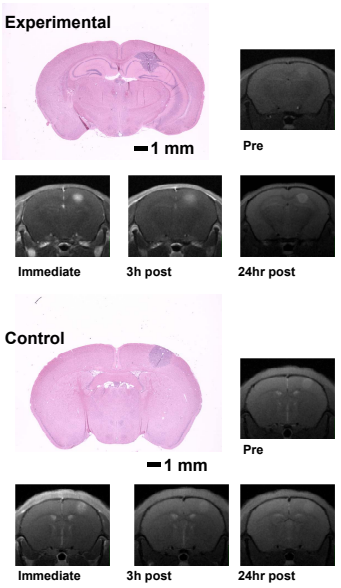

Fig 1. H&E histology correlation of 7.0T imaging of PC14PE6 metastasis in the brain. The corresponding MR slices showing pre and post-contrast of di-Tyr-DTPA-Gd. T1w FSE (200/5, 8NEX).

RESULTS AND DISCUSSION

The paramagnetic substrate showed a very rapid oxidation in the presence of HRP, glucose and glucose oxidase in vitro. The apparent pseudo-first order kinetic constant was $k_1=5.0\cdot10^{-3}$ s^{-1}. The oxidation lead to a 2.7 fold increase of molar relaxivity of Gd (r1= 11.8 $[mMs]^{-1}$). Tumor formation and growth were monitored by T2w and T1w GdDTPA enhanced MRI. Five weeks after the intracarotid tumor cell injection we observed the formation of small tumors that were randomly distributed in the brain. The tumors showed heterogeneous growth patterns. Di-Tyr-DTPA-Gd showed improved enzyme-mediated enhancement of tumors after the preinjection of conjugates. Fig. 1 shows the macro-image of H&R histology correlate that confirms tumor location in hippocampus/dentate gyrus region on MRI slices. The experimental mouse was injected of antibody conjugates (pre-targeting) followed by the injection of the HRP substrate, di-Tyr-DTPA-Gd resulted in a strong enhancement of the tumor which was apparent at 10 min post injection. The enhancement of the tumor was still apparent at 1-3h post injection and the higher signal around the tumor periphery was retained for up to 24 h. After the injection of di-Tyr-DTPA-Gd the control mice showed rapid MR signal increase followed by a gradual wash-out of the paramagnetic substrate. The ROI measurements of normalized enhancement ratios performed using tumor and contralateral brain ROIs proved that average ratios were higher in experimental group when compared with control group. The signal increase in the experimental group was larger and the peak of enhancement was reached earlier than in the control group (Fig. 2).

The current study suggests that receptor targeting using paramagnetic "MR stains" allow tracking metastastic caner cells by detecting the receptor-specific imaging signatures using MRI. The use of therapeutic antibodies in imaging enabled the identification of tumor metastasis. This could be an advantage during the development and testing of anti-EGFR therapies and as a tool for monitoring of treatment efficacy. It is also conceivable that a combination of antibody-enzyme conjugate pretargeting system and a substrate can be used in antibody directed enzyme-prodrug therapy (ADEPT).

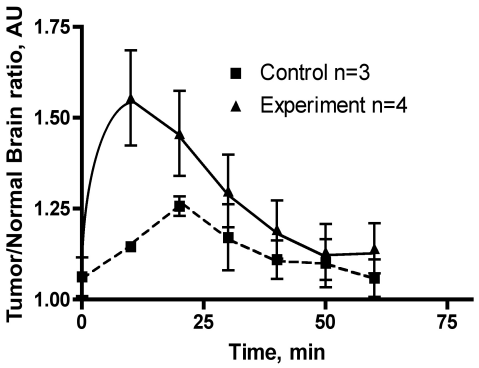

Fig. 2. Quantification of normalized MR SI in the intraarterial model of PC14PE6 brain metastasis using 7.0 T MRI. Shown is tumor/normal brain ROI analysis with contrast/noise correction in a group of control animals (no conjugates, only contrast agent injected) and experimental group (both conjugates and contrast agent injected). Results are presented as mean±SEM.

REFERENCES

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3. Querol M, et al. Org. Lett, 7: 1719 (2005).