Molecular MR Imagining of the Estrogen Receptor using a Novel Contrast Agent

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Purpose:

To develop a non invasive, *in-vivo* method for measuring estrogen receptor (ER) concentration and distribution in breast cancer.

Introduction:

A large fraction of breast cancers are stimulated in the presence of estrogen through the interaction with the ER (1). The induction of growth is due to the activity of the estrogen receptors as potent transcription factors for a variety of genes (2,3). Consequently, hormonal therapy designed to reduce serum estrogen levels or block the effects of estrogens on the cancer cells by means of selective estrogen modulators, are used clinically to improve survival (1,2). The current clinical methods that measure ERα are based on two strategies. The first one involves the competitive binding of radio-labeled ligand and the second relies on recognition of the receptor by immunohistochemical methods (4). In addition to experimental problems resulting from uneven and non specific staining, the analysis is subjective and semi-quantitative (5). Defects in specimen preservation that lead to protein degradation may also distort the final results (6). We propose to determine the level and spatial distribution of ER by developing a non invasive, *in-vivo* MRI method to answer the above demands. We have synthesized a novel contrast agent composed of an estrogenic part tagged to a new gadolinium chelate termed ER-ligand-Gd .The biological activity and MRI delectability of this ER ligand was investigated in ER+ human breast cancer cells *in-vitro* and orthotopic implanted tumors *in-vivo.*

Methods:

Cells: ER-positive human breast cancer cells and ER-negative MDA-MB-231 human breast cancer cells were cultured routinely as previously described (7). The estrogenic activity of ER-ligand-Gd and Gd free ER-ligand were examined in cells that were cultured in estrogen free and phenol red free DMEM adding the different compounds at a concentration indicated in Fig. 2, as well as the vehicle (control). Cell proliferation was determined spectrophotometrically by the MTT method.

Tumors: Human breast cancer cells were implanted into the mammary gland of CD1-NU immunodeficient female mice, as previously described (8). During the MRI experiments, mice were anesthetized by inhalation of 1% Isoflurane in an O_2:N_2O (3:7) mixture, applied through a nose cone. Animal procedures were approved by IACUC.

MRI: MR images were recorded with a 4.7T/30cm bore Biospec spectrometer (Bruker, Germany). The relaxivity of the new ER-Ligand-Gd in a physiological solution was obtained from T1 measurements using varying repetition time - TR (11 TRs, from 50 to 15,000 msec) and fixed echo time of 23 msec. The distribution of ER-ligand-Gd in the blood, inner organs and the tumors was monitored over a period of 24h after a bolus injection of ER-ligand-Gd into the tail vein at a dose ranging from 0.024 to 0.4 mmol/kg. The distribution of the ER-ligand-Gd was compared to that of GdDTPA at a bolus injection of 0.4 mmol/kg. The contrast agent distribution was monitored for 24 h, alternating between two pulse sequences : 1.T_1-weighted, 3D gradient echo with TE/TR of 4.3/18.3 ms; a 30° flip angle, 2. Saturation recovery sequence with varying TI (n=20) ranging from 10 to 10000 msec, and gradient echo acquisition with TE/TR of 3.5/15 msec, 10° flip angel. The latter sequence enabled us to map the T1 relaxation time. The spatial resolution was the same for both sequences and during the whole experiment time of 0.2x0.4x1.2 mm^3. At the end of the experiment tumors were removed and coronal slices were studied after staining with hematoxylin-eosin and immunohistochemical staining with a monoclonal antibody of ERα.

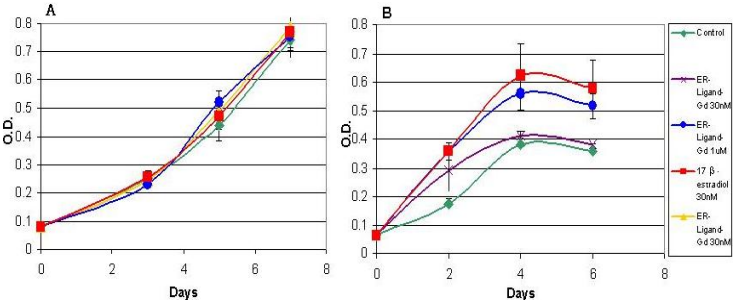

Fig 1: Proliferation of ER-negative MDA-MB-231 (A) and ER-positive MCF-7 (B) human breast cancer in estrogen free medium (control) and in media supplemented with 17β-estrodiol and ER-Ligand-Gd.

Results & Discussion:

Both ER-Ligand and ER-Ligand-Gd induced cell proliferation as 17β-estradiol, however, at different doses as indicated in Fig.1. Neither 17β-estradiol nor the new compounds exerted an effect on the ER negative cells. The dose of ER-Ligand needed for induction of cell proliferation, ~ 50 nM, was of the same order of magnitude as that of 17β estradiol, indicating high affinity of this molecule to ERα. A ~30 fold higher concentration of ER-Ligand–Gd was necessary to induce a proliferation rate as estrogen. However, the optimal dose was still in the pharmacological range of micro-molar.

The capacity of the novel ER-Ligand-Gd to serve as a relaxation contrast agent was determined by measuring its relaxivity. The measured relaxivity of the ER-Ligand-Gd compound in PBS was 6.2 $s^{-1}mM^{-1}$, higher than the relaxivity of GdDTP in the same field. The ER-Ligand-Gd was found to be non toxic at the range of 0.024-0.4 mmol/kg weight. The clearance of the ER-ligand-Gd through the kidneys into the urine was several folds slower than that of GdDTPA and full clearance occurred only 24 hours after the administration of the new paramagnetic ER-ligand. Signal enhancement in the tumors due to ER-ligand-Gd was persistent throughout the experimental time. The slow entrance and clearance of ER-Ligand-Gd was attributed to its entrance to the cells and binding to the ER. To detect the binding of ER-Ligand-Gd to ER we performed measurements 24 hours after its administration, when all the tissues appeared to be cleared from ER-ligand-Gd. Using a dose of 0.024 mmol/kg we found that the ER positive breast tumors still exhibited small but persistent increase in T1 relaxation rate (n=3), while in the muscle this rate returned to the pre administration value (Figure 2). This increase suggested binding of the ER-ligand-Gd to the high ER present in the nuclei of the cells as was confirmed by immunostaining of the receptor.

Conclusions:

We have obtained a novel contrast agent that can selectively bind to the ER and enhance the MRI signal in its vicinity. This prototype probe is non toxic and is potent both as a ligand of ER that induces proliferation and as a sensitive MRI contrast agent for mapping the receptor level. Translation of the use of such a probe in humans opens the way for molecular imaging of the breast for improved diagnostic and prognostic purposes.

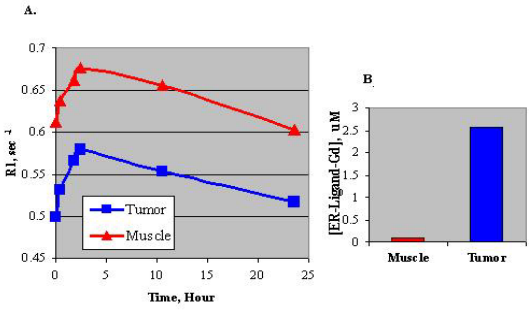

Figure 2: Time course of the R1 (1/T1) relaxation rate in orthotopic MCF7 breast tumor and muscle of female immunodeficient mouse after a bolus administration of ER-Ligand -Gd (0.024 mmol/kg)(A) and the residual contrast agent in these organs after 24h (B).

References: 1. CK Osborne *et al, Scientific American/ Science&Medicine* Jan/Feb: 32-41, 1996**. 2**. JI Macgregor *et. al. Pharmacological Reviews* 50: 151-96, 1998. **3.** M Muramatsu *et. al. Biochem. Biophy.s Res. Commu.n,* 270: 1-10, 2000. **4.** JM Harvey *et. al. J. Clin. Oncol.* 17: 1474-1481, 1999. **5.** DM Barnes *et. al. European journal of cancer* 34: 1677-1682, 1998.**6.** JA Katzenellenbogen, *et.al. Clin. Cancer Res.* 1: 921-932, 1995. **7.** E Furman *et. al. J. Steroid Biochem Mol. Biol.* 43:189-95, 1992 **8.** Y Paran *et. al. NMR Biomed.* 17:170-80, 2004**.**

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