Development of Cationic Gd(III) Chelate as Potential Tumor-Selective MRI Contrast Agent

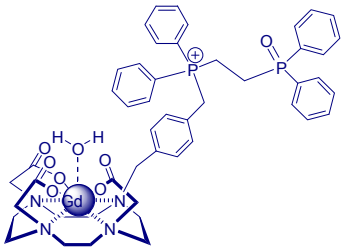
C-T. Yang[1], C-X. Yong[1], C-Y. Tuang[2], Y-T. Chang[2], and K-H. Chuang[1]
[1]Laboratory of Molecular Imaging, Singapore Bioimaging Consortium, Agency for Science,Technology and Research, Singapore, Singapore, Singapore,
[2]Laboratory of Bioimaging Probe Development, Singapore Bioimaging Consortium, Agency for Science,Technology and Research, Singapore, Singapore, Singapore

Introduction

The mitochondrial transmembrane potential in carcinoma cells is significantly higher than that in normal epithelial cells ($\Psi\Delta m$ = 60 mV).[1-2] Cationic molecules can therefore be driven electrophoretically through membranes by the negative mitochondrial transmembrane potential and accumulate inside the energized mitochondria of tumor cells. Cationic PET radiotracers like ^{64}Cu-labeled Triphenylphosphonium have been shown to preferentially accumulate in tumors in athymic nude mice bearing U87MG human glioma xenograft.[3-5] Based on the experiences in PET studies, we developed a new cationic Gd chelate -- GdDO3A-2-(diphenylphosphoryl)ethyldiphenylphosphonium (Gd(DO3A-xy-TPEP)$^+$; Scheme 1) -- as a potential tumor-selective MRI contrast agent.

Materials and methods

Gd(DO3A-xy-TPEP)$^+$ was synthesized and characterized by electrospray ionization mass spectrometry (ESI-MS). The compound was purified by high performance liquid chromatography (HPLC). Three different cell lines, namely HEK 293 (human embryonic kidney cells), HeLa (human epithelial carcinoma ce1ls) and A549 (human lung adenocarcinoma epithelial cells), were used for in vitro cytotoxicity evaluation. All three cell lines were plated ($5x10^3$ cells per well) in 96-well plates in medium. After 24h of incubation at 37˚C, the cells were treated with compound at concentrations ranging from 0 to 1.6mM for another 24h at 37˚C. Each experiment was performed in triplicate. Cell viabilities were presented as the ratio of the number of cells treated to the number of non-treated control cells and determined with Cell Counting Kit-8. IC50 values were obtained from plots of percentage cell viability against Gd concentration (Fig.1). Relaxivity measurements and MRI study were conducted on a Bruker 7T Clinscan MRI system. T_1 was measured by inversion recovery spin echo in aqueous phantoms with concentrations 0.4, 0.2 0.1, 0.05, 0.025, 0.0125 and 0.00625 mM Gd (Fig.2). In vivo study was approved by the local Institutional Animal Care and Use Committee. Gd(DO3A-xy-TPEP)$^+$ was injected (dosage: 0.01 mmol Gd/kg body weight) through tail veins of male C57BL/6 mice (weight 30 ± 2 g) under 1% isoflurane anesthesia. T_1-weighted images were acquired at various time points using T_1-weighted turbo spin-echo sequence (TR/TE=500/7.1 ms, resolution=100 µm, thickness=1 mm). ROI were chosen from several organs and the signals were normalized by the signal of a phantom filled with 0.05 mM [Gd] in saline.



Scheme 1. Structure of Gd(DO3A-xy-TPEP)$^+$

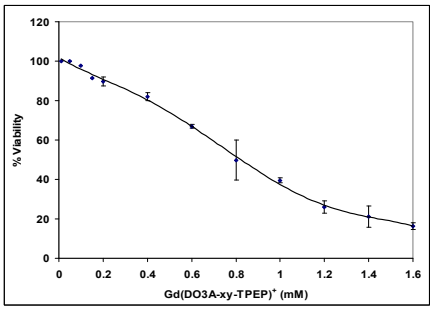


Fig. 1. Cell viability in HeLa Cells

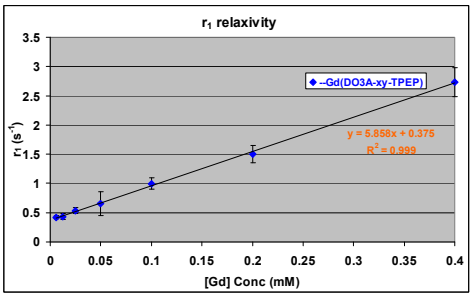


Fig 2. T_1 relaxivity (r_1) of Gd(DO3A-xy-TPEP)$^+$

Results and discussion

In vitro cell viability in three different cell lines showed insignificant cytotoxicity at low [Gd] concentrations up to 0.2 mM for all cell lines. The IC50 values are 0.80 mM, 0.70 mM and 0.82 mM for HEK 293, HeLa, and A549 cell lines, respectively. The T_1 relaxivity r_1 (5.9 $mM^{-1}s^{-1}$) in H_2O at 25 °C is higher than that of the clinically used ones (3.9 and 4.1 $mM^{-1}s^{-1}$ for Gd-DOTA and Gd-DTPA, respectively). In vivo imaging study in mice demonstrated that significant contrast enhancement can be observed in the liver and kidney after tail vein injection (Fig.3). Signal enhancement remained high in kidney and liver even at 5 h post-injection. The long tissue retention of Gd(DO3A-xy-TPEP)$^+$ indicated that cation may involve in potential membrane binding including blood vessel, connective tissues, etc. This Gd(DO3A-xy-TPEP)$^+$ would potentially be advantageous for tumor imaging due to the larger negative transmembrane potential. In vivo imaging study on tumor mouse model is in progress.

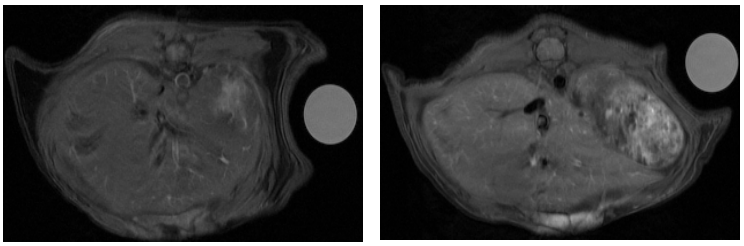


Fig. 3. Representative axial images crossing liver before (left) and 2h after the injection (right)

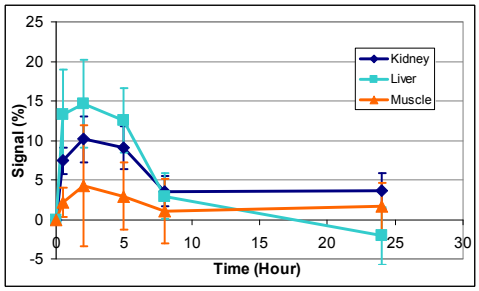


Fig. 4. Time course of MRI signal change in organs.

References:
1. Madar, I.; Anderson, J. H.; Szabo, Z.; Scheffel, U.; Kao, P. F.; Ravert, H. T.; Dannals, R. F. *J. Nucl. Med.* **1999**, 40, 1180.
2. Johnson, L. V.; Walsh, M. L.; Chen, L. B. *Proc. Natl. Acad. Sci. U.S.A.***1980**, 77, 990.
3. Wang, J.; Yang, C.-T.; Sreerama, S. G.; Cao, Q.; Li, Z.-B.; He, Z.; Chen, X.; Liu, S. *J. Med. Chem*. **2007**, 50, 5057.
4. Kim, Y.-S.; Yang, C.-T. Wang, J.; Wang, L.; Li, Z.-B.; Chen, X.; Liu. S. *J. Med.Chem*. **2008**, 51, 2971.
5. Yang, C.-T.; Kim, Y.-S.; Wang, J.; Wang, L.; Shi, J.; Li, Z.-B.; Chen, X.; Fan, M.; Li, J.-J.; Shuang Liu. *Bioconjugated Chem*. **2008**, 2008.