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Gadolinium-Chlorin is Potentially a New Tumor Specific MRI Contrast Agent

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In this study, a newly-synthesized metalloporphyrin, Gd-chlorin (PB Chlorin), was investigated by using a simple tissue phantom to test its efficacy as an MRI contrast agent. This study demonstrated the potential activity of Gd-chlorin as not only a MRI contrast agent, but also as a PDT photosensitizer by using a simple tissue phantom and conducting a very brief MRI experiment.

Key words: Metalloporphyrin, Gadolinium, MRI contrast agent, PDT photosensitizer

INTRODUCTION

Photodynamic therapy (PDT) is an evolving modality for the treatment of certain malignant tumors (Almeida *et al.*, 2004). The treatment is based on the retention of photosensitizers by cancerous tissues in combination with oxygen and light irradiation. PDT involves the (hopefully) selective uptake and retention of a photosensitizer in a tumor; this is followed by irradiation with light of a particular wavelength and so tumor necrosis is then initiated, presumably through the formation of singlet oxygen. PDT is the only thing that can kill cancerous tissue and precancerous tissues without killing the normal tissue (Chwilkowska *et al.*, 2003; Damoiseau *et al.*, 2001;, Sharman *et al.*, 1999; Decreau *et al.*, 1999). Porphyrins and their analogues are most frequently used as photosensitizers (Lim *et al.*, 2003; Sternberg *et al.*, 1998). In particular, many kinds of malignant tumors in humans have been effectively treated by Photofrin®. However, Photofrin® only weakly absorbs at the therapeutic wavelength of 630 nm, which is a wavelength where the penetration of light in tissue is not optimal. An ideal photosensitizer must absorb at a longer wavelength because low energy light travels further through the tissue than does high energy light. Another important disadvantage of Photofrin® is that it is readily taken up and retained by cutaneous tissue for up to ten weeks post-injection (Ichikawa *et al.*, 2004; Lim *et al.*, 2004; Lee *et al.*, 2004). Contrast agents for shortening the relaxation times, which results in enhanced signal intensity, may extend the potency of MRI for use in tumor diagnosis at an early disease stage, and particularly when the contrast agent is selectively taken up by the tumor tissue. We have developed a new synthetic photosensitizer, a porphyrin-Gd complex, which was created from chlorine derivatives. Several synthetic porphyrin-based agents are currently being investigated and they have shown selective affinity for a variety of tumors.

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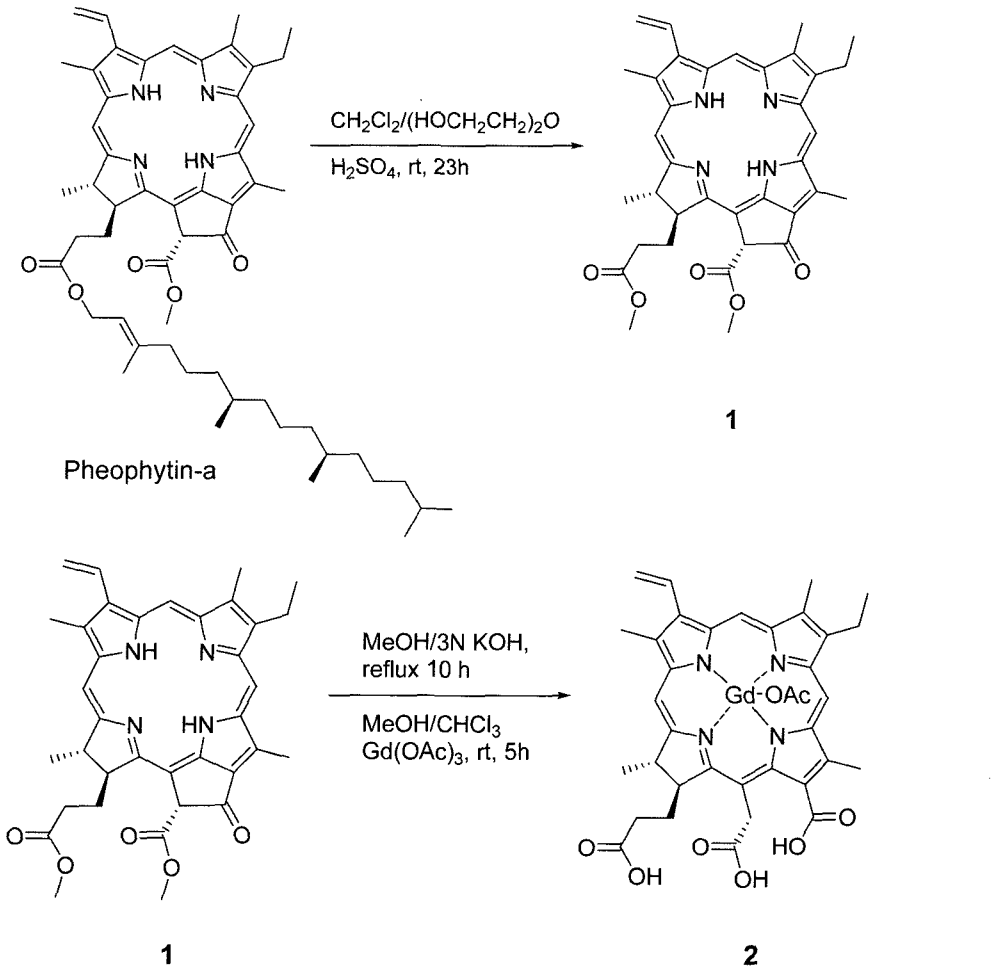
MATERIALS AND METHODS

All the reactions were carried out in an inert atmosphere (N_2) and at room temperature unless otherwise noted. The solvents and reagents were obtained commercially and they were used without further purification. All the reported yields were of the isolated products and they were not optimized. The reactions were routinely monitored by thin-layer chromatography (TLC) on silica gel (pre-coated F_{254} Merck plates). The infrared spectra (IR) were measured on a Jasco FT-IR instrument. The ^{1}H-NMR and ^{13}C-NMR data were determined in a $CDCl_3$ and D_2O solution with using a verian. Gemini 200 spectrometer. Peaks positions are given in parts per million (δ) downfield from tetramethylsilane as the internal standard, with multiplicities reported in the usual manner, and the *J*



Scheme 1. Synthesis of Gd-chlorin complex

values are given in hertz. Flash chromatograpy was performed using Merck 60-200 mesh silica gel. Mass spectrometry was performed by the Korea University Mass Spectroscopy center.

Pheophytin-a was obtained from *Spirulina maxima* alga according to the method of K. M. Smith (Smith *et al.*, 1985).

Methyl pheophorbide a methyl ester (1)

Pheophytin a (60 mg, 0.069 mmol) was dissolved in dichloromethane (3 mL) and diethyleneglycol (20 mL). To this reaction mixture was added H_2SO_4 (1 mL), and the resulting mixture was stirred at room temperature for 23 h. The reaction mixture was poured into a saturated $NaHCO_3$ solution and the entire mixture was extracted with chloroform. The organic solution was concentrated at reduced pressure to yield the crude product. The crude product was purified by column chromatography to obtain compound **1** (39 mg, 93% pure); ^{1}H-NMR (500 MHz, $CDCl_3$) δ 9.51 (s, 1H, *meso*-H), 9.37 (s, 1H, *meso*-H), 8.56 (s, 1H, *meso*-H), 7.99 (dd, 1H), 6.29 (d, 1H), 6.28 (s, 1H), 6.18 (d, 1H), 4.48-4.41 (m, 1H), 4.24-4.22 (m, 1H), 4.19-4.04 (m, 2H), 3.87 (s, 3H), 3.71-3.66 (m, 2H), 3.68 (s, 3H), 3.64-3.42 (m, 6H), 3.40 (s, 3H), 3.22 (s, 3H), 2.68-2.15 (m, 4H), 1.82 (d, 3H), 1.69 (t, 3H), 0.56 (br s, 1H), -1.61 (br s, 1H).

Gadolinium-chlorin (2)

A methyl ester **1** (50 mg, 0.082 mmol) dissolved in methanol (20 mL) was added with 3 N KOH (5 mL) to the solution of methyl pheophorbideThe reaction mixture was refluxed for 10 h, then cooled to room temperature and next dried over $MgSO_4$. The resulting reaction mixture was filtered and the organic solution was concentrated at a reduced pressure. After adding chloroform (10 mL), methanol (10 mL) and gadolinium (III) acetate hydrate (40 mg), the reaction mixture was stirred at room temperature for 5 h. The solvent was removed and then purified by column chromatography to obtain compound **2**; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 9.74 (s, 1H, *meso*-H), 9.54 (s, 1H, *meso*-H), 9.06 (s, 1H, *meso*-H), 8.01 (dd, 1H), 6.24 (d,

1H), 5.98 (d, 1H), 5.24 (m, 1H), 4.48 (m, 1H), 3.70 (m, 2H), 3.69 (s, 3H), 3.65-3.43 (m, 6H), 3.42 (s, 3H), 3.24 (s, 3H), 2.70-2.24 (m, 4H), 1.85 (d, 3H), 1. 65 (t, 3H).

Tissue phantom

Three sets of 2% agar gel were constructed in 15 mL glass tubes in which 0.0, 0.05, and 0.1 mM Gd-Radachlorin were added to each agar solution, respectively, at 100°C.

MRI Imaging

The Spin-Echo (SE) T1-weighted images of the three tissue phantom were obtained with using an echo time (TE) of 15 ms and a repetition time (TR) of 450 ms with a 1.5 T GE clinical MRI scanner. Six coronal plane images (each 5 mm thick) of each phantom were recorded simultaneously and their image intensities were measured.

RESULTS AND DISCUSSION

Table I shows the relative 20%-25% enhancement in the intensity of the tissue plane containing Gd-Radachlorin, as compared with the control plane without Gd-Radachlorin.

This trend was also observed as the concentration of Gd-radachlorin increased, as compared in two phantoms having different concentrations of Gd-radachlorin. In addition, the Gd-Radachlorin was stable even at 100 since it was shown to still be active with its chelated structure on the porphyrin ring. Essentially, it was not washed out as a free Gd ion.

Radachlorin, a derivative of the well-known water soluble green pigment chlorophyll a, has been recently shown to be a promising PDT photosensitizer. It has a maximal tumor uptake 3-5 h post injection, with a high tumor-to-normal tissue ratio, almost 32, and a clearance period of about 24-48 h.

The signal enhancement of the porphyrin-based agent in this study was also in good agreement with the results of a study on the conjugation of Gd-DTPA with porphyrin under *in vivo* conditions in mice (JMRI 2001, 14: 169-74).

Further *in vivo* studies with using an animal model are necessary to investigate the pharmacokinetics and stability of metal chelation on a porphyrin ring. So, this study has demonstrated the potential activity of Gd-Radachlorin as not only a MRI contrast agent, but also as a PDT photosensitizer with using a simple tissue phantom and a very brief MRI experiment.

Table I. Intensity measurements for the three different phantoms

	control (no Gd-Radachlorin)	500 uM Gd-Radachlorin	0.1 mM Gd-Radachlorin
plane 1	168.75±17.69	197.53± 8.53	206.70±20.90
plane 2	161.44±17.51	199.75± 9.55	208.68±21.49
plane 3	174.36±17.44	205.80±10.82	212.51±21.39
plane 4	171.69±18.82	210.66±10.35	219.17±23.05
plane 5	188.74±19.16	218.85±10.43	226.10±23.47
plane 6	188.12±19.68	224.00± 8.92	232.74±24.46
average	175.52	209.43	217.65

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