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Original Article

Gadolinium-porphyrins: new potential magnetic resonance imaging contrast agents for melanoma detection

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Abstract

BACKGROUND: Two new porphyrin-based magnetic resonance imaging (MRI) contrast agents, Gd-hematoporphyrin (Gd-H) and Gd-tetra-carboranylmethoxyphenyl-porphyrin (Gd-TCP) were synthesized and tested in nude mice with human melanoma (MM-138) xenografts as new melanoma contrast agents.

METHODS: Subcutaneous xenografts of human melanoma cells (MM-138) were studied in 30 (five groups of six) nude mice. The effect of different contrast agents (Gd-TCP, Gd-H, $GdCl_3$ and Gd-DTPA) on proton relaxation times was measured in tumors and other organs. T_1 values, signal enhancement and the Gd concentration for different contrast agent solutions were also investigated.

RESULTS: The porphyrin agents showed higher relaxivity compared to the clincal agent, Gd-DTPA. A significant 16% and 21% modification in T_1 relaxation time of the water in human melanoma tumors grafted in the nude mice was revealed 24 hours after injection of Gd-TCP and Gd-H, respectively. The percentage of injected Gd localized to the tumor measured by inductively coupled plasma atomic emission spectrometry (ICP-AES) was approximately 21% for Gd-TCP and 28% for Gd-H which were higher than that of Gd-DTPA (10%).

CONCLUSIONS: The high concentration of Gd in the tumor is indicative of a selective retention of the compounds and indicates that Gd-TCP and Gd-H are promising MR imaging contrast agents for melanoma detection. Gd-porphyrins have considerable promise for further diagnostic applications in magnetic resonance imaging.

KEY WORDS: MRI, porphyrin-based contrast agent, hematoporphyrin, melanoma.

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The use of contrast agents to shorten relaxation times following enhanced signal intensity may extend the potential of magnetic resonance imaging (MRI) for diagnosis of tumors in the early stages. Porphyrin-based agents have been synthesized and investigated [1-4] and showed selective affinity for a variety of tumors [5]. Studies have shown that these materials have high selective uptake and retention in tumors [1, 2, 4].

Porphyrin-based agents are in the investigation stage [2-8]. A related class of organic molecules called texaphyrin, which is a modified porphyrin, has recently received considerable interest for its high tumor-selective uptake [2]. The gadolinium complex of texaphyrin is a tumor-selective radiation sensitizer that is detectable by MRI [2]. The porphyrins chosen for this study are natural porphyrins, hematoporphyrin [8, 13-bis (hydroxyethyl) -3, 7, 12, 17-tetramethyl 21H, 23H-porphine-2, 18-dipropionic acid] and a synthetic boronated porphyrin, 1, 6, 11, 16-tetra (3-*o*-carboranyl- methoxy) phenyl-porphyrin (TCP). TCP is synthesized by modification of method of Miura et al [6]. The beauty of porphyrins is that these materials offer not only a stable chelate for the transportation of paramagnetic metals into the tumors, but also the potential attachment of molecules that could destroy the cancer cell. The synthetic porphyrin TCP is an example of this concept where boron atoms have been attached chemically to the porphyrin, thus offering the potential for boron neutron capture

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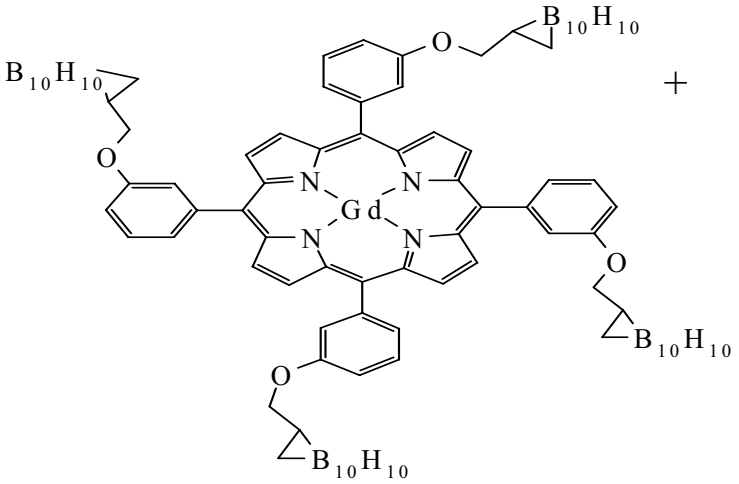
therapy (BNCT). This may facilitate tumor detection and treatment planning and allows radiologists and radiation oncologists to better diagnose and define the radiation treatment field in BNCT.

The two porphyrin-based agents, TCP and hematoporphyrin, can be used simultaneously as MRI contrast agents by choosing gadolinium as the metal for incorporation. The synthesis of these compounds has been described previously [9,10]. In this study, Gd-H and Gd-TCP are injected into the nude mice with a human melanoma (MM-138) xenograft. The biodistribution, the T_1 relaxation times, and the signal enhancement of the contrast agents are presented and the results are compared for the first time.

Methods

The porphyrin-based MRI contrast agents were prepared as described previously [9,10]. Briefly, hematoporphyrin powder was suspended in distilled water and was added to the gadolinium solution and refluxed until the solution became homogeneous to yield Gd-H. The synthesis of Gd-TCP involved the formation of an aldehyde containing the *o*-carborane group. This aldehyde reacted with pyrrole to form the porphyrin, TCP-H_2. The gadolinium ion was inserted into TCP-H_2 by adaptation of Muira's method [6] for the nickel complex. The chemical structure of Gd-TCP is shown in figure 1.



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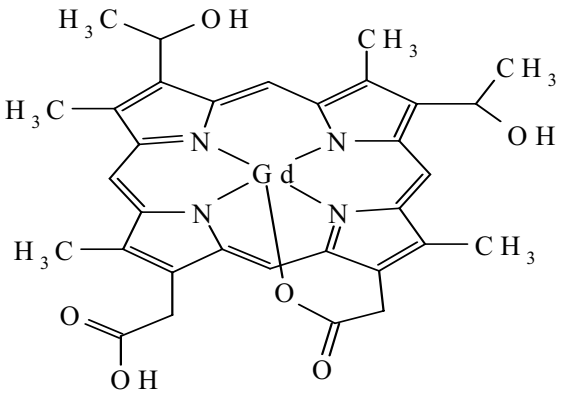

(II)

Figure 1. The proposed structure of I) Gd-tetra-carboranylmethoxyphenyl-porphyrin (Gd-TCP) and II) Gd-hematoporphyrin (Gd-H).

Solutions of Gd-H and two discrete agents ($GdCl_3$, Gd-DTPA) were prepared by accurately dissolving the required amount in 0.9% saline solution. Gd-TCP (15 mg, 0.010 mmol) was dissolved in 1 ml of cremophor EL (CRM) and 2 ml of 1,2-propanediol. This solution was transferred into a 10 ml volumetric flask, and a 0.9% saline solution was added to the mark. This gave a final concentration of 1.0 mM.

The animal studies were performed with nude mice (nun, Blab/c) 6-8 weeks old with a mean weight of 20 g. The animals were randomly divided into five groups of six. Each group was housed in cages in humidity and temperature controlled, isolated animal house.

The human melanoma cells, MM-138, originally derived from human malignant melanoma and grown in tissue culture, were injected (2.5×10^6 cells) subcutaneously into both flanks of nude mice. Three to four weeks after cancer cell inoculation, when the tumor diameter was 3-5 mm, the mice were injected with the different contrast agent conjugates. All contrast agents were diluted in physiological saline to a final concentration as injected in bolus doses (0.1 mmol /kg of body weight). Two groups of six mice received an intraperitoneal (IP) injection of Gd-H or Gd-TCP. One group received Gd-DTPA and the fourth group (i.e. the control group) received $GdCl_3$. The injected volume was 200 µL. The animals were sacrificed by an over-dose of pentobarbital sodium 24 hours after IP injection, followed by removal of tumor, kidney, liver, and spleen. These were minced for MRI and ICP-AES experiments.

All MRI measurements were obtained on a 300 MHz, 7.0 Tesla, Varian UNITY Plus (Varian Associated, Inc., CA) with a vertical Oxford Instruments magnet of bore size 89-mm using the 15 mm saddle coil (DOTY Scientific Instruments) resonator. The effect of contrast agents on proton relaxation times was measured in tumors and other harvested organs using an inversion recovery (IR) pulse sequence technique.

The T_1 relaxation time was measured using an imaging probe based on IR sequence (180° rf-τ_i-90° rf-collect FID). The repetition time chosen was 2.5 times the estimated T_1. Ten inversion delays (T_I) were used with each increasing by a factor of two. The minimum inversion time used was approximately one-tenth of the estimated T_1 value. The T_1 values and Gd concentration data for different contrast agent solutions were used to generate relaxivity rate constants, r_1 in reciprocal millimolar seconds. This was accomplished via linear regression analyses of $1/T_1$ versus Gd concentration which r_1 calculated as the slope of the fitted lines for data collected at different concentrations.

All images were obtained using the T_1-weighted imaging method using IR pulse sequence technique, with T_E = 20 msec, T_R = 400 msec, T_I = 200 msec, 3 mm slice thickness, 5×5 cm^2 field of view, and matrix size of 256 × 256. The enhancement effect of these agents on MRI signal was measured by averaging the individual SI of five randomly selected voxels. The values of echo time and repetition time were optimized for the tumor of the control mice and were used for all other tissue samples in the series of experiments.

All samples were frozen until used for ICP-AES measurements. The gadolinium content was measured based on an acid digestion procedure using ICP-AES (Applied Research Laboratory, UK) instrument according to the method of Tamat et al [11]. The 342.249 nm atomic emission line of Gd was chosen for the ICP-AES analysis. The tissue uptake of the Gd was calculated as a percentage of the initial injected dose of contrast agent (% ID).

Results

Relaxivity values of the porphyrin-based contrast agents are shown in table 1. As can be seen, relaxivity values of these agents are higher than that of Gd-DTPA.

The gadolinium uptake by the tumor was 7% for $GdCl_3$ and 10% for Gd-DTPA. For Gd-TCP and Gd-H, tumor uptakes of 21% and 28% of injected gadolinium were recorded. As indicated in figure 2, liver retained the highest

amount of gadolinium for both Gd-H and Gd-TCP (34% and 32%, respectively).

Table 2 shows the T_1 measurements of organs using different contrast agents and untreated mice (control). As this table illustrates, for $GdCl_3$, the T_1 relaxation time was reduced by approximately 10% in the tumor compared to the control. For Gd-DTPA, a modification of about 14% was observed in T_1 values of the tumor. This standard clinical contrast agent showed a similar reduction of T_1 relaxation time for both spleen and kidney. Both porphyrin-based contrast agents, Gd-TCP and Gd-H, showed a 16% and 21% decrease in the T_1 value for the tumor, respectively.

The graphs of MRI signal intensity for removed organs and different contrast agents are shown in figure 3. In the control group, the MRI signal intensity for tumors was lower than that recorded for the normal tissues under study. This may result from the T_1 relaxation time for the tumor being longer than that in the normal tissues, which decreases MRI signal intensity and is consistent with T_1 values measured in this work (table 2).

The highest MRI signal intensity (120%) was observed for the tumor upon injection of Gd-H compared to the control. After Gd-H, the other porphyrin-based agent, Gd-TCP, showed good signal enhancement (70%) relative to the control. The results showed the enhancement of Gd-DTPA and $GdCl_3$ was also significant, but lower than those of porphyrin-based contrast agents.

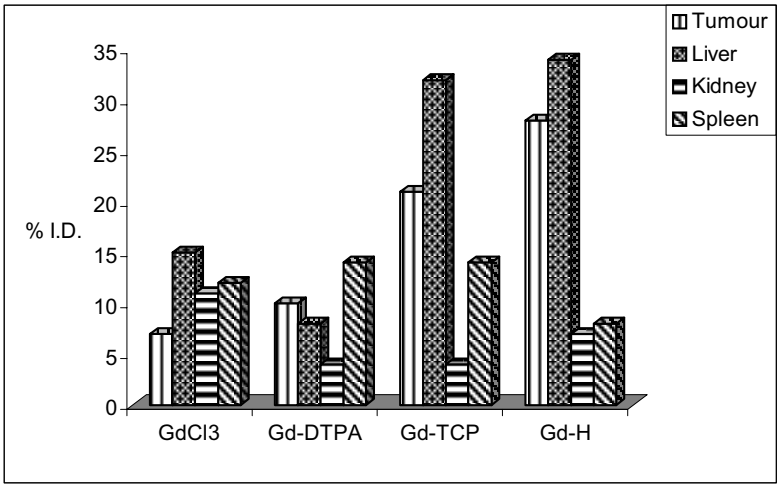

Figure 2. Comparison of biodistribution of the gadolinium uptake in melanoma xenografted in nude mice for different compounds of MRI contrast agents.

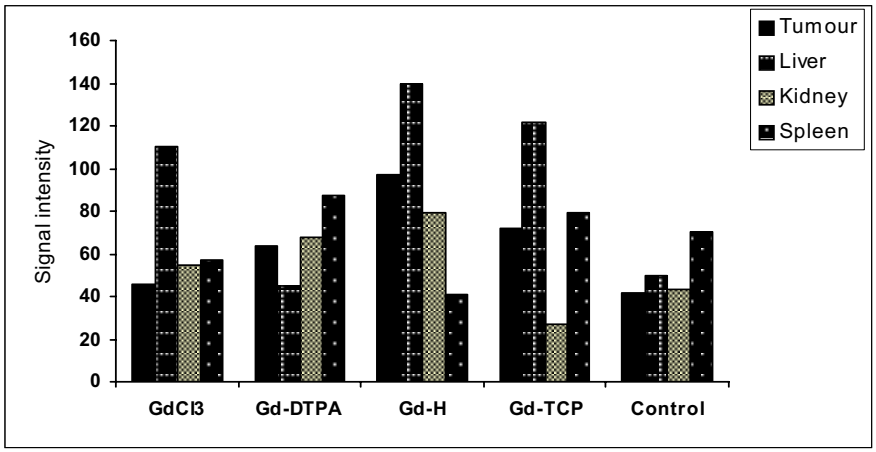

Figure 3. MRI signal intensity imaging at 24 hr after injection of different gadolinium compounds.

Table 1. Relaxivity values of gadolinium concentrations in aqueous solutions of MRI contrast agents at room temperature (23 °C).

Contrast agents	r_1 ($mM^{-1} s^{-1}$)	r_2 ($mM^{-1} s^{-1}$)
$GdCl_3$	12.3 ± 0.7	15.8 ± 1.1
Gd-DTPA	3.7 ± 0.1	4.8 ± 0.2
Gd-H	16.3 ± 1.4	20 ± 1.6
Gd-TCP	31.7 ± 0.3	38.2 ± 0.6

Note: Measurements were made at 7.0 T magnetic field strength.

Table 2. T_1 relaxation times(s) of different organs (mean ± SEM of values obtained from an average of five mice). Tissues were removed 24 hr post-injection of contrast agents.

Compound / Sample	Tumor	Liver	Kidney	Spleen
$GdCl_3$	1.43 ± 0.03	0.74 ± 0.02	0.85 ± 0.01	0.84 ± 0.03
Gd-DTPA	1.37 ± 0.02	0.77 ± 0.01	0.87 ± 0.02	0.88 ± 0.02
Gd-TCP	1.33 ± 0.02	0.78 ± 0.02	0.94 ± 0.02	0.83 ± 0.02
Gd-H	1.26 ± 0.01	0.74 ± 0.02	0.98 ± 0.03	0.71 ± 0.03
Control	1.59 ± 0.03	0.96 ± 0.01	0.99 ± 0.04	0.96 ± 0.02

Note: Data are mean ± SEM of values obtained from an average of five mice.

Discussion

Specific targeting of MRI contrast agents depends on the selected receptors on the cancer cell membrane, stability of the ligand and uptake and clearance kinetics. Relaxivity measurements were performed to specify investigation of the tissue-specific contrast agents. The results indicated that relaxivity values of porphyrin-based agents were greater than those for the Gd-DTPA and $GdCl_3$ agents, and this is due to its greater potential to coordinate water molecules (table 1). These results are consistent with reported relaxivity of porphyrin-based contrast agents [2] which were also significantly greater than those for Gd-DTPA and $GdCl_3$ in other studies [9, 12]. The field strength dependence of the relaxivity may be another reason for the higher values of studied agents compared to clinically relevant field strengths (1.5 and 3.0 T).

Tumor uptake of the two porphyrin agents was more likely higher than that of $GdCl_3$ and Gd-DTPA. This identified the potential of the porphyrin-based compounds as tumor-specific detection agents.

The Gd-porphyrin agents gave at least 2 times greater Gd concentration in the liver than Gd-chelates. Some of the gadolinium found in the liver might represent gadolinium dissociated from the DTPA. It is known that free gadolinium accumulates in the liver and this may explain some of the high uptake in this reticuloendothelial organ. This was consistent with results of gadolinium content observations in the literature [13]. Significant gadolinium accumulated in the liver, spleen and kidney probably reflects clearance and metabolism of the gadolinium complexes [13].

As these results indicate, the lowest uptake of gadolinium was observed in the kidney 24 hours after injection. This is due to the properties of gadolinium-based contrast agents, which are hydrophilic and accumulated in the extracellular water of tissues and have rapid renal excretion. The results showed that T_1 relaxation times of tumor were significantly greater than those in normal tissue. The general theory that T_1 values are longer in tumors was also confirmed by another animal study [14]. This difference has been reported to arise from

an increase in water content and the large extracellular volumes of the cancerous tissues [13].

The decreases in the T_1 values of the contrast agents were in line with the concentrations of gadolinium absorbed by the tumors [15]. These reductions in the T_1 relaxation times of the tumor upon administration of contrast agent are highly significant. The signal enhancement of the porphyrin-based complexes in this study are in good agreement with that reported previously by conjugation of Gd-DTPA with porphyrins under in-vivo conditions in mice [9]. The liver showed the greatest enhancement for both porphyrin-based agents and revealed the accumulation of gadolinium complexes. In spite of good accumulation of porphyrin-based agents into the tumor, the accumulation of these agents in the liver is problematic. Further developments in MRI contrast agents, in combination with improved imaging techniques, may lead to novel applications in diagnostic MRI. In this study, using Gd-porphyrins and melanoma tumor xenograft model, quantitative studies of paramagnetic contrast agent uptake supported the possible application of porphyrin-based contrast agents for the detection of melanoma. The findings also indicated that with satisfactory Gd uptake by the tumor and low levels in kidney and spleen, these agents could be useful for melanoma detection. The promising potential of porphyrin-based contrast agents may therefore provide a new application for further diagnostic applications in MRI.

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