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Original Article

Gadolinium-Hematoporphyrin: new potential MRI contrast agent for detection of breast cancer cell line (MCF-7)

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ABSTRACT

Background: Gadolinium-porphyrins have been synthesized and are currently being investigated as magnetic resonance imaging (MRI) contrast agents. This study aimed to synthesize Gd-hematoporphyrin and applicate it for in vitro detection of breast cancer cell line (MCF-7).

Methods: The naturally occurring porphyrin (hematoporphyrin) was inserted with gadolinium (III) nitrate hexahydrate to yield Gd-H. T_1 relaxation times and signal enhancement of the contrast agents were presented, and the results were compared. UV spectrophotometer measured the attachment of Gd to the cell membrane of MCF-7.

Results: Most of gadolinium chloride ($GdCl_3$) was found in the washing solution, indicate that it didn`t fixed to the breast cell membranes during incubation. Gd-DTPA showed some uptake into the MCF-7 cell membranes with incubation, however, its uptake was significantly lower than Gd-H.

Conclusion: Good cell memberan uptake of Gd-porphyrin is comparable to controls, indicating selective delivery it to the breast cell line and considerable potency in diagnostic MR imaging for detection of breast cancer.

Key Words: Porphyrin, Contrast agent, MRI, Hematoporphyrin, Breast cancer cell (MCF-7)

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Contrast agents for shortening relaxation times, which resulted in enhanced signal intensity, may extend the potency of MRI for tumor diagnosis in the early stages. Paramagnetic chelates using endogenous porphyrin ring as the chelating agents are a promising and interesting family group of potential MRI contrast agents [1].

Synthetic porphyrin-based agents are currently being investigated [2-4], and have shown selective affinity for a variety of tumors[5]. High water solubility and stability under physiological conditions, low propensity for causing phtotoxicity, and intracellular localization in mitochondria for more efficient tumor cell killing, are the reasons why these complexes have been used as tumor-specific contrast agents[1-6]. Many studies have shown that these materials have high selective uptake and retention in tumors [2, 4, 7].

A related class of organic molecules called texaphyrins (a modified porphyrin) has recently received considerable interest for its high tumor selective uptake[2]. The gadolinium complex of texaphyrin is a selective radiation sensitizer that is detectable at MRI and will be commercially available in the near future.

By choosing gadolinium as the metal for incorporation into porphyrin-based agent, hematoporphyrin [18, 13-bis (hydroxyethyl)-3,7,12,17-tetramethyl-21H, 23H porphine-2, 18-dipropionic acid] can be used simultaneously

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as MRI contrast agents[7-9]. This study aimed to synthesize Gd-hematoporphyrin and evaluate in vitro detection of breast cancer cell line (MCF-7) by it.

Materials and Methods

Gadolinium (III) nitrate hexahydrate (0.30 g, 0.66 mmol) was dissolved in 2 ml of distilled water. Hematoporphyrin powder [8,13-bis (1-hydroxyethyl) -3,7, 12, 17-tetramethyl-21 H,23H-porphine-2,18-dipropionic acid] (0.40 g, 0.66 mmol) was suspended in 2 ml of distilled water and added to the gadolinium solution and refluxed until the solution become homogeneous to yield Gd-H [7]. The solution was allowed to cool as room temperature and reduced to 1 ml, under reduced pressure with heating. The resulting white solid was filtered, washed carefully with ice-cold water (2 × 0.5 ml) and dried in the oven at 80 ºC. The yield was 0.11 g (21%).

The solutions of Gd-H, $GdCl_3$ and Gd-DTPA were prepared through accurately dissolving required amount in 0.9% saline solution. 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 human breast cells (MCF7;4×10^6 cells) and different contrast agents were purchased from Pasteur institute. The incubation time for all contrast agents was 12 hours, and more than it, there was no more attachment of agents to the cell membranes. After incubation, all cells were washed twice with PBS/2%FCS, centrifuged, and resuspended in PBS/2%FCS solutions. All solutions were tested (seven samples for each agent) by both UV-Spectrophotometer and NMR.

The UV-spectrophotometer (Spectronic Gene Sys2, Spectronic Instrument) measured the concentration of Gd in the washing solutions, using 342.249 nm of Gd. All sample preparations and UV-Spectrophotometry experiments was done in the Research center, Shahrekord University of Medical Sciences and in the Department of Medical Physics, Isfahan University of Medical Sciences, at 2004.

The T_1 relaxation times and signal intensities of washing solution of samples were measured, using an inversion recovery (IR) pulse sequence technique through 11.0 T Bruker instrument (500 MHz, Tarbiat Modarres University, Iran). The values of echo time and repetition time were optimised for different washing solutions.

Results

Most of $GdCl_3$ was found in the washing solution. This indicated that it remained in solution during incubation and did not fix to the breast cells (MCF-7). Gd-DTPA showed some uptake into the MCF-7 cell membranes with incubation, however, its amount was significantly lower than Gd-H (Figure 1).

Table 1 shows the T_1 values of washing solution of different contrast agents and controls. T_1 relaxation time of $GdCl_3$ was reduced approximately 51% in the washing solutions compared to the control. The standard clinical used contrast agent (Gd-DTPA) showed modification of about 43% in T_1 values of the washing solutions, but the porphyrin-based contrast agents (Gd-H) showed 31% in T_1 values.

Figure 2 shows the signal intensity of MRI for washing solution of different contrast agents; Gd-DPT, $GdCl_3$ and controls had low MRI signal intensity of washing solutions, but the highest MRI signal intensity was observed for the washing solution of Gd-H. The enhancement of Gd-DTPA and $GdCl_3$ was significant, but were lower than porphyrin-based contrast agent.

Table 1. T_1 relaxation times of different contrast agents from an average of seven samples.

Contrast Agent	Mean T_1 ± SD(ms)
Gd-DTPA	1162 ± 138
$GdCl_3$	1018 ± 18.2
Gd-H	1407.5 ± 126.8
Water(pure)	2048.3
PBS(pure)	1013.8

Discussion

Specific targeting of MRI contrast agents demands a detailed knowledge of their properties[10], including the feasibility, injection dose, their uptake by the selected ligands. The results indicated that the relaxivity values of porphyrin-based agents were greater than others, due to its more potential coordination to water molecules. The chemical structure of Gd-H is shown in Figure 1.

These results are consistent with reported relaxivity of porphyrin-based contrast agents, and are significantly 4 to 5 times greater than Gd-DTPA and $GdCl_3$ [2, 7-10].

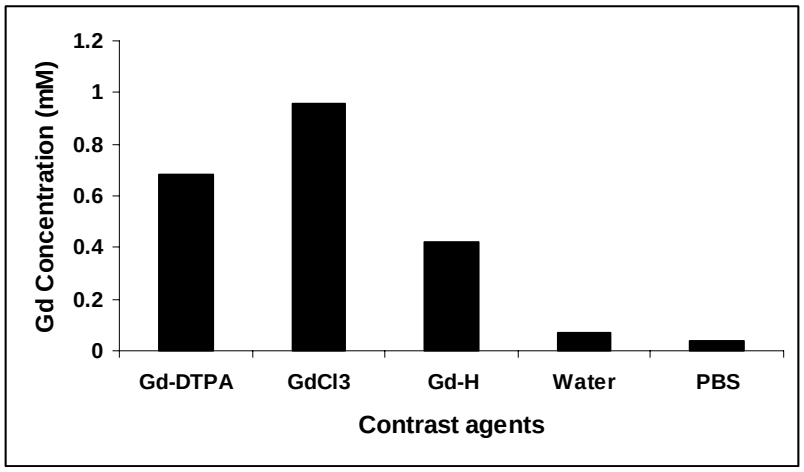

Figure 2. Gadolinium concentrations of different contrast agents in washing solutions.

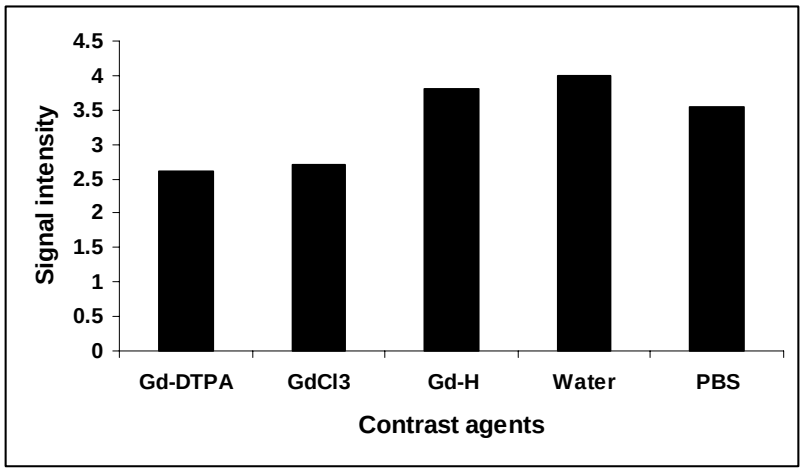

Figure 2. Signal intensity of different contrast agents at 12 hours after incubation.

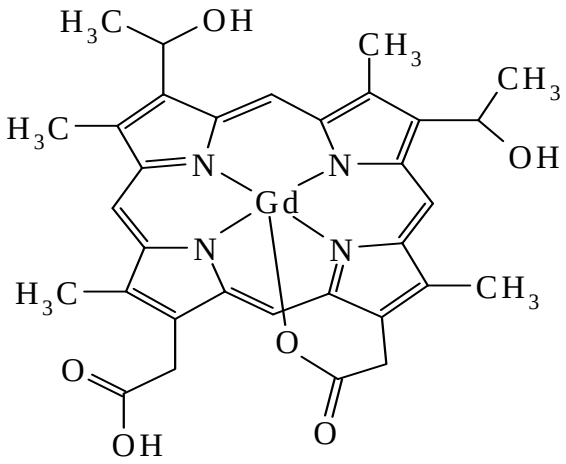

Figure 3. Proposed structure of Gd-hematoporphyrin (Gd-H).

Most of gadolinium was observed in washing solutions of $GdCl_3$ and Gd-DTPA, respectively. The concentration of Gd-H in washing solution of studied cell line was much less than $GdCl_3$ and Gd-DTPA, which indicates better attachment of Gd-H into the cell membranes (MCF-7), and identifies the porphyrin-based compound as a potential specific agent for detection of MCF-7. In the washing solutions of Gd-H, T_1 relaxation time was significantly greater than Gd-DTPA and $GdCl_3$. The increases in the T_1 values of Gd-H are in line with the concentrations of gadolinium attached into the cell membranes, and are in agreement with the previous studies [7, 11].

The signal enhancement of porphyrin-based agent in this study is also in good agreement with the conjugation of Gd-DTPA with porphyrin under in-vivo conditions in mice [6, 7]. In spite of good accumulation of porphyrin-based agent into the cell membranes, its accumulation under in-vivo conditions is problematic, because the pharmacokinetic and stability of studied agents is not investigated. Further in-vivo studies for development of such compounds may lead to novel applications in diagnostic MRI. Our study on Gd-porphyrin and breast cancer cell (MCF-7) makes possible quantitative studies of paramagnetic-based contrast agent. Gd-H also can be used as a dual probe for both MRI contrast agent and a radiation sensitizer for photodynamic therapy[12, 13].

Conclusion

Our findings showed that porphyrin-based contrast agent can detectes tumor, especially breast cancer. Higher concentration of Gd into the cell memberane compares with controls, and indicates selective delivery of Gd-porphyrins to the breast cell line (MCF-7). Overally, with the satisfactory low levels of Gd in the washing solution and good cell membrane uptake, Gd-porphyrins have considerable potency in diagnostic MR imaging for detection of breast cancer.

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