Supporting information

MRI-guided tumor chem-photodynamic therapy with Gd/Pt bifunctionalized porphyrin

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Experimental

Materials

All chemicals were of reagent grade quality or better, obtained from commercial suppliers and used without further purification. N, N-dimethyl formamide (DMF) and dimethylsulfoxide (DMSO) were all purchased from Shanghai Chemical Co. Ltd. All solvents used in this study were analytical grade. C6 and Hela cell line was purchased from the China Center for Type Culture Collection (Wuhan University) and cultured in RPMI 1640 medium (Beijing Dingguo changsheng Biotechnology Co. Ltd.), supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (100 U/mL penicillin and 100 mg/mL strepto-mycin) at 37 °C in a humidified atmosphere containing 5% CO_2. Because of light sensitivity of platinum complexes, it is

important to note that synthesis of porphyrin-platinum conjugates was performed in the dark condition.

Synthesis of [cis-PtCl(NH_3)$_2$] 4-5,10,15,20-tetra-(4-pyridyl)-porphyrin nitrate (Pt-P1)

4 mL of DMF was added to a 25 mL dry round-bottomed flask containing 60.0 mg cisplatin (0.195 mmol) and 33 mg silver nitrate (0.195 mmol). The mixture was stirred at room temperature for 24 h. Then the resulted turbid solution was filtered to remove white silver chloride precipitate. The solution of 5, 10, 15, 20-Tetra (4-pyridyl) porphyrin (0.49 mmol, 30 mg) in 3 mL DMF was added to the light yellow colored filtrate and stirred at 80 °C for 48 h. After the mixture was cooled down to room temperature, the suspension was precipitated with 0 °C diethyl ether. The solid was filtered and further washed with methanol, dichloromethane and diethyl ether. The solid was further dried in vacuum and 72 mg of the product were obtained in 78 % yield.

Synthesis of Gd/Pt-P1

Gd/Pt-P1 was prepared according to the reported procedure [Gadolinium-Chlorin]. Briefly, 10 mL DMF solution of metal-free Pt-P1 (50 mg, 0.03 mmol) and gadolinium acetate hydrate (35.2 mg, 0.1 mmol) was stirred at 50 °C for 24 h. The solvent was removed and then purified by alumina column chromatography (toluene-acetone-DMSO as eluants, the fractions of DMSO:H_2O = 1/0–8/2).

Characterizations of Gd/Pt-P1

UV-Vis spectrophotometer (Lambda Bio40, Perkin-Elmer), fourier transform infrared spectroscopy (FTIR) (Perkin Elmer Spectrum, USA), and RF-530/PC spectrofluorophotometer (Shimadzu) were used to characterize the compound. The content of Gd(III) in Gd/Pt-P1 was measured by inductively coupled plasma mass spectrometry (ICP-AES; IRIS Intrepid II XSP, Thermo Elemental [Waltham, MA,

USA]) after the nitric acid digestion, and calculated with standard series comparison quota.

Measurement of ROS generation of Gd/Pt-P1

The Gd/Pt-P1 aqueous solutions with different concentration were incubated with 40 mM DCFH for 5 min and irradiated using a 630 nm laser, 200 mW/cm^2 for 30 s. Subsequently, the fluorescence intensity of DCF was detected by fluorescence spectrophotometer, which was proportional to the amount of ROS produced.

Photocytotoxicity

The vitro photocytotoxicity of Gd/Pt-P1 was performed on C6 cells using MTT assay. Briefly, C6 cells were seeded on 96-well plate (Costar, IL, USA) at a density of 5.0 × 10^3 cells/well in 100 μL 1640 medium containing 10% FBS. The cells were cultured for 12 h at 37 °C in 5% CO_2 atmosphere. Afterwards, the cells were treatment with different concentrations of cisplatin, Pt-P1 and Gd/Pt-P1 for another 6 h, and the medium was then replaced by fresh medium. The plates were irradiated with 630 nm (0.2 W/cm^2) LED illuminant for 10 minutes respectively. As a control experiment, the other group was treated without irradiation. After further incubation for 44 h, MTT stock solution (5 mg/mL in PBS, 20 μL) was added to each well and incubated for another 4 h. The media were completely removed and 150 μL of DMSO was added to each well to dissolve the formazan blue crystal. The absorbance of the solution was measured using a microplate reader at 570 nm. Cell viability was expressed as follows and compared with the cells treated without irradiation:

$$\text{Cell viability (\%)} = A_{sample}/A_{control} \times 100\%.$$

Where A_{sample} and $A_{control}$ are the absorbance values for the treated cells and the untreated control cells, respectively. The Asample and Acontrol values were obtained after subtracting the absorbance of DMSO. Data are presented as average ± SD (n = 4).

In vitro cellular uptake

Confocal laser scanning microscope (CLSM, Nikon EZ-C1, Japan) was used to examine the intracellular distribution of Gd/Pt-P1. C6 cells were seeded on plate at a density of 5.0×10^4 cells per well in 1 mL of 1640 medium containing 10% FBS. The cells were then incubated with cisplatin, Pt-P1, Gd/Pt-P1 at a concentration of 2 μg mL^{-1}. At predetermined intervals, the cells were washed with PBS and fixed with 4% (w/v) paraformaldehyde aqueous solution for 10 min. The slides were then stained with Hoechst 33258 (5 mg mL^{-1} in PBS) at 37 °C for 10 min. The fixed cell monolayer was finally observed by using CLSM.

T_1-weighted images and T_1 maps of Gd/Pt-P1

The T_1-weighted images and T_1 maps of Gd/Pt-P1 were measured at 37 °C using a Siemens Prisma 3T MRI scanner. The Gd/Pt-P1 was dispersed in water at the concentrations in the range of 0.00025-4.95 mM. For MRI measurements, 0.2 mL solutions were filled into each of the test tubes to achieve T_1-weighted images and T_1 maps. The T_1 -weighted image parameters were depicted as follows: repetition time 700 ms; echo time 12 ms; field of view 120×120 mm; matrix size 400×400; slice thickness 2.0 mm; number of acquisition 14. The T1 map parameters were depicted as follows: repetition time 15 ms; echo time 2.7 ms; field of view 159×159 mm; matrix size 768×768; slice thickness 2.0 mm; number of acquisition 14. The measurement of T_1 maps at each manganese concentration was done in triplicate, and the average value of T_1 was taken. The longitudinal relaxivity (r_1) was determined by a linear fit of the inverse longitudinal relaxation time ($1/T_1$) as a function of the manganese concentrations.

In vivo T1 -Weighted MRI

To evaluate the enhancement of Gd/Pt-P1 in MRI imaging, a tumor-bering mouse is scanned by a Siemens Prima 3.0T MRI scanner. Before and after i.v injection of

Gd/Pt-P1, the transversal cross-section images including T1 images and its pseudocolor images of the nude mouse was obtained. During the whole scan process, the mouse was anaesthetized by isoflurane (5 %) and maintained the normal temperature. The T_1-weighted image parameters were depicted as follows: repetition time 700 ms; echo time 12 ms; field of view 120 ×120 mm; matrix size 400×400; slice thickness 2.0 mm; number of acquisition 14. The T1 map parameters were depicted as follows: repetition time 15 ms; echo time 2.7 ms; field of view 159×159 mm; matrix size 768×768; slice thickness 2.0 mm; number of acquisition 14. Injected dimeglumine gadopentetate (a commercial MRI contrast agent) and PBS were used as controls, respectively.

Animals and therapeutic studies *in vivo*

All animals received care in compliance with the guidelines outlined in the Guide for the Care and Use of Laboratory Animals and the procedures were approved by the Wuhan University of China Animal Care and Use Committee. Female BALB/c nude mice were obtained from Beijing HFK Bioscience Co. Ltd. with body weights of 18-20 g and housed under specific pathogen-free conditions (60 % relative humidity, 20 °C room temperature) with a 12 h light/12 h dark cycle. Before treatment, the mice were kept in quarantine for 5 days. Then the mice were subcutaneously inoculated C6 cells (2×10^6 per mice) at backside to establish the subcutaneous transplanted glioma model. All procedures used in this experiment were compliant with the local animal ethics committee. When tumor grew to a volume of 50 mm^3 (about 5 days after inoculation), 30 mice were numbered and randomized into 5 groups. After that, 200 μL of formulated drug were injected through the tail vein, which was designated day 0. Tumor volume size (V) was monitored every 2 days for up to 11 days. Tumor volume was estimated by the following equation: $V = a \times b^2/2$, where a and b was the longest and shortest diameters, respectively. The tumor inhibitory rate (TIR) was calculated as $TIR=[1-(V_{tf}-V_{ti})/(V_{pf}-V_{pi})]\times100\%$. V_{tf} and V_{ti} represent the final and initial tumor volume of the treatment group, while the V_{pf} and V_{pi} represent the final

and initial tumor volume of the PBS group, respectively. Statistical analyses were performed using Pasw Statistics (version 17.0) software.

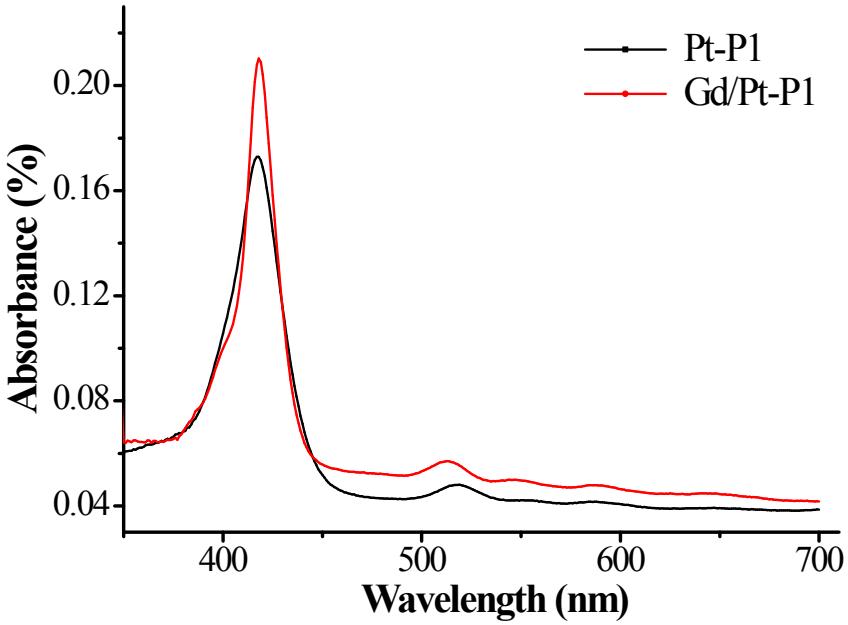

Fig. S1 UV-vis absorbance spectra of Pt-P1and Gd/Pt-P1.

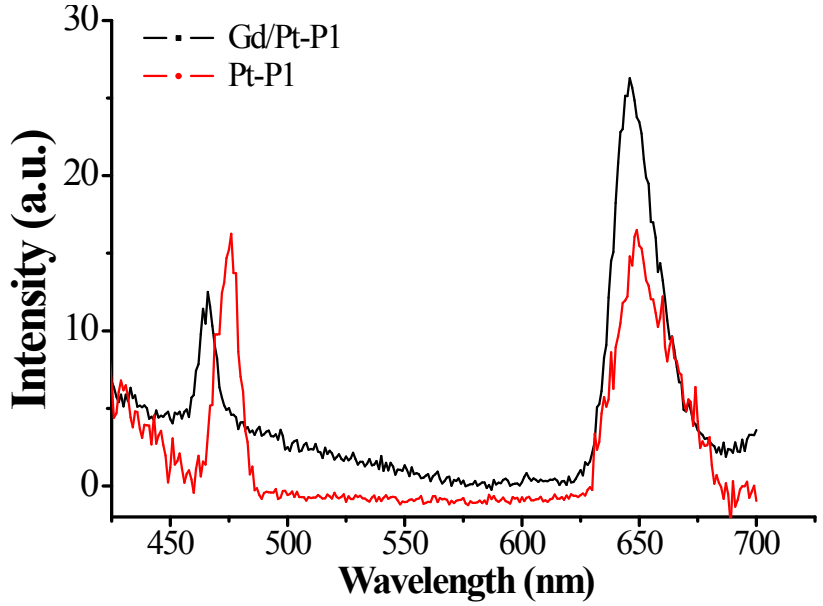

Fig. S2 Emission spectra of Pt-P1and Gd/Pt-P1.

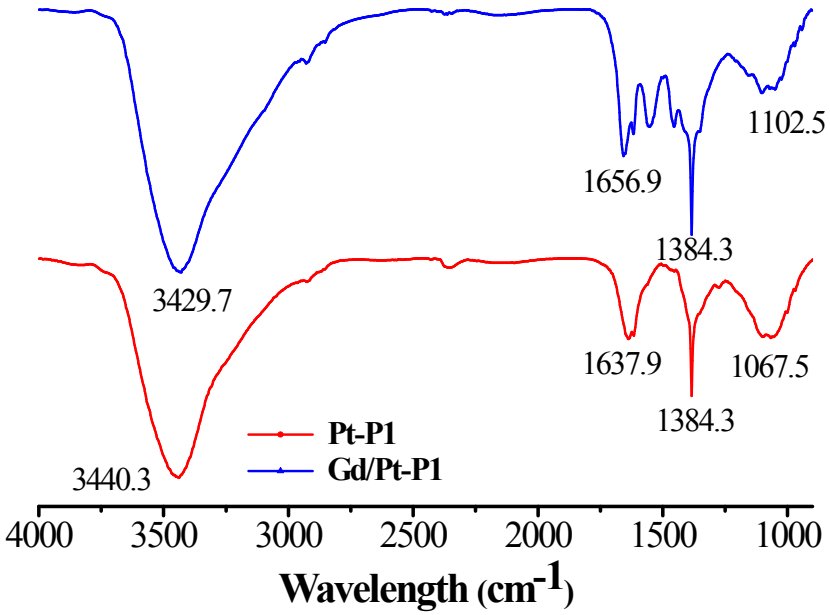

Fig. S3 FTIR spectra of Gd/Pt-P1 and Pt-P1.

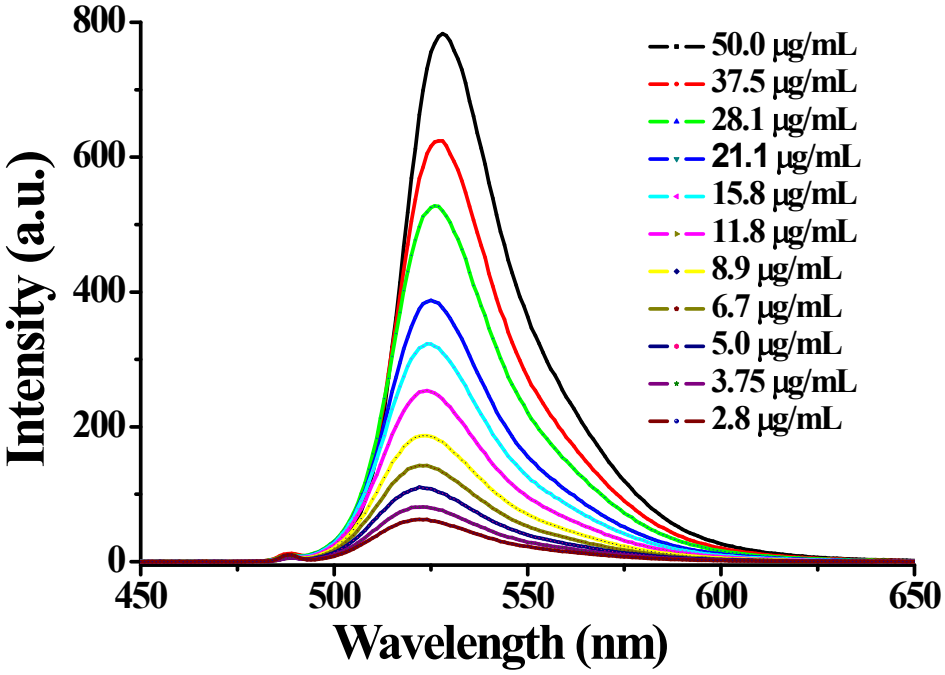

Fig. S4 Fluorescence spectrum of DCFH activated (40 mM) with different concentration of Gd/Pt-P1 (Ex: 488 nm, Em: 550 nm)

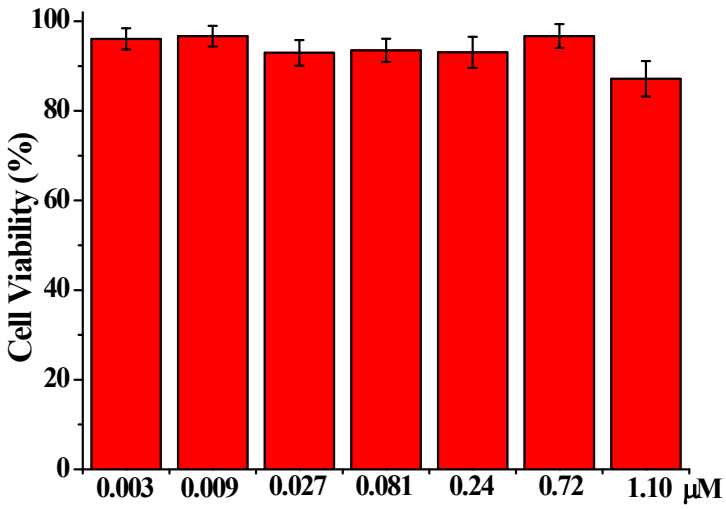

Fig. S5 Relative viability of COS-7 cells incubated with various concentrations of Gd/Pt-P1 with or without 630 nm laser (0.2 W/cm^2, 10 min).

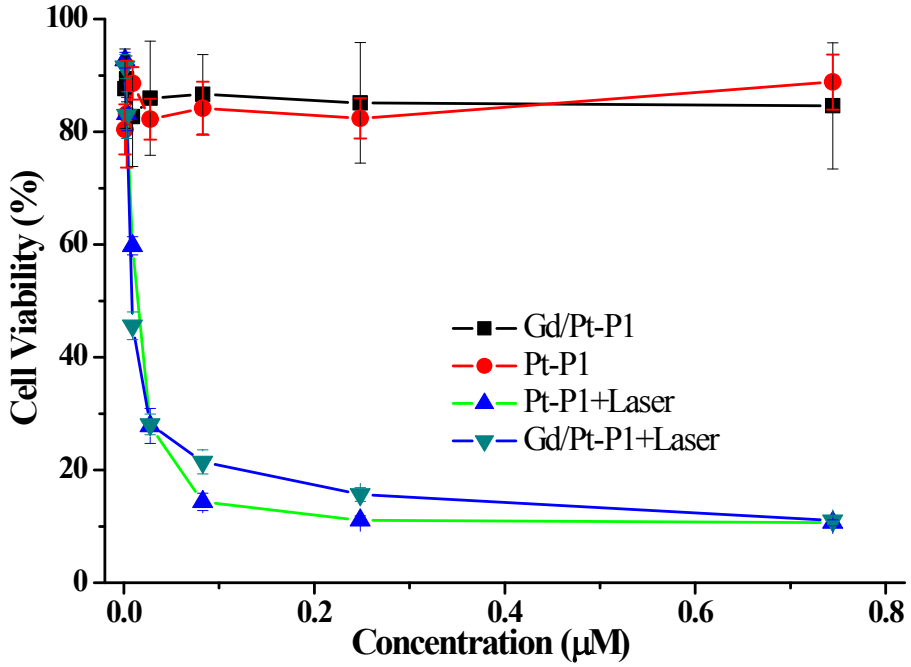

Fig. S6 Relative viability of Hela cells incubated with various concentrations of porphyrins with or without 630 nm laser (0.2 W/cm^2, 10 min).

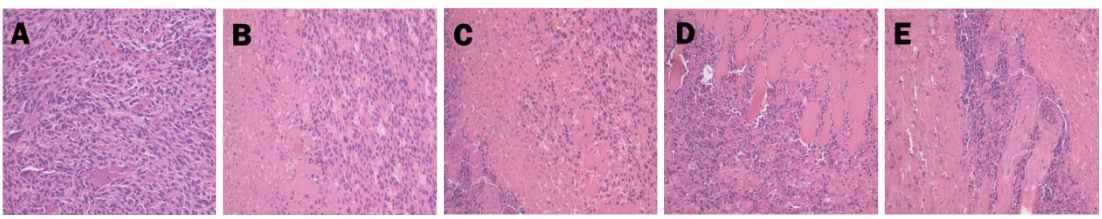

Fig. S7 H&E staining of major organs from the mice treated with: (A) PBS, (B) P1+Laser, (C) Gd/Pt-P1, (D) Pt/P1 + laser, (E) Gd/Pt-P1+ laser.