Supporting Information

Potentiating photodynamic therapy of ICG-loaded nanoparticles by depleting GSH with PEITC

Hang Hu[a,b,#], Jitang Chen[a,b,#], Hai Yang[a,b,#], Xuetao Huang[a,b], Honglian Wu[a,b], Yuxin Wu[a,b], Fenfang Li[a,b], Yunfei Yi[a,b], Chen Xiao[a,b], Yihui Li[a,b], Yuxiang Tang[a,b], Zifu Li[a,b,c,d*], Bixiang Zhang[e*], Xiangliang Yang[a,b,c*]

[a]National Engineering Research Center for Nanomedicine, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, 430074, China

[b]Department of Nanomedicine and Biopharmaceutics, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, 430074, China

[c]Hubei Key Laboratory of Bioinorganic Chemistry and Materia Medica, Huazhong University of Science and Technology, Wuhan, 430074, China

[d]Wuhan Institute of Biotechnology, High Tech Road 666, East Lake High Tech Zone, Wuhan, 430040, China

[e]Institute of Hepato-Pancreato-Bililary Surgery, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, 430030, China

Author Contributions:

Dr. Hang Hu, Jitang Chen and Dr. Hai Yang contributed equally to this work.

*Corresponding authors:

Professor Zifu Li, Fax: 86 27 87792234, E-mail: zifuli@hust.edu.cn

Professor Xiangliang Yang, Fax: 86 27 87792234, E-mail: yangxl@hust.edu.cn

Professor Bixiang Zhang, Fax: +86 27 83662851, E-mail: bixiangzhang@163.com

Materials and methods

Materials

HES with average molecular weight (Mw) of 130 kDa and hydroxyethyl molar substitution (MS) of 0.4 was a gift from Wuhan HUST life Sci & Tech Co., Ltd (Wuhan, China). Oleic acid (OA, AR) was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI, 98%), 4-dimethylaminopyridine (DMAP, 99%), indocyanine green (ICG, 75%), 2-phenylethyl isothiocyanate (PEITC, 99%), 1,3-Diphenylisobenzofuran (DPBF, 97 %), and 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA, 97%) were purchased from Aladdin Reagent Co., Ltd (Shanghai, China). Annexin V-FITC/PI Apoptosis Detection Kit and GSH Assay Kit was purchased from Solarbio Science & Technology Co., Ltd (Beijing, China). All other chemicals were of analytical grade and used as received.

Synthesis of HES-OA

Oleic acid (156.9 mg) was dissolved in 10 mL of DMSO. To the solution, EDCI (213.0 mg), DMAP (63.9 mg), and pre-dried (105 ℃, 2 h) HES 130/0.4 were added and the reaction mixture was vigorously stirred at 30 ℃ for 24 h. Then, the reaction mixture was precipitated by pouring into 100 mL of ethanol/ethyl ether mixture (V/V=1:1). The precipitate was isolated by centrifugation (8000 rpm, 10 min) and washed with 20 mL of ethanol/ethyl ether mixture (V/V=1:1) for 3 times. The roughly purified precipitate was then dispersed in deionized water and dialyzed against deionized water for 3 days (MWCO: 3500 Da) and freeze dried. The structure of the synthesized HES-OA was characterized by ^{1}H NMR and FT-IR. The molar substitution of OA was determined by ^{1}H NMR and calculated as

$$MS_{OA} = \frac{(\frac{I_{methyl}}{3})}{(\frac{I_{AGU}}{4 - MS_{OA}})} \times 100\% \quad (1)$$

Where I_{methyl} is the integral for the protons of methyl group of oleic acid, and I_{AGU} is the integral for the protons of hydroxyl and C_1H of AGU in HES between 4.5 and 6.0 ppm. After rearranging Eq. (1), the MS of OA can be calculated as

$$MS_{OA} = \frac{4 * I_{methyl}}{I_{methyl} + 3 * I_{AGU}} \times 100\% \quad (2)$$

Table S1. Feeding ratio for different HES-OA samples.

Product	Feeding amount (mg)				Molar ratio
	OA	EDCI	DMAP	HES 130/0.4	(OA:EDCI:DMAP:AGU[a])
HES-OA1	156.9	213.0	63.9	2000.0	1:2:1:20
HES-OA2	156.9	213.0	63.9	1600.0	1:2:1:16
HES-OA3	156.9	213.0	63.9	1300.0	1:2:1:13
HES-OA4	156.9	213.0	63.9	1000.0	1:2:1:10
HES-OA5	156.9	213.0	63.9	400.0	1:2:1:4

[a] anhydrous glucose unit

Preparation of HES-OA nanoparticles (NPs)

Different HES-OA samples were suspended in deionized water and sonicated for 5 min. HES-OA self-assemble into nanoparticles spontaneously.

Critical aggregation concentration (CAC)

The CAC of HES-OA was determined by using pyrene as the fluorescent probe. Briefly, pyrene acetone solution was added to HES-OA aqueous solution to prepare samples with concentration of HES-OA ranging from 0.0001 mg/mL to 0.5 mg/mL and fixed concentration of pyrene (6×10^{-7} mol/L). The as-prepared samples were stirred at room temperature overnight to allow for equilibrium distribution of pyrene in NPs. Then, the samples were measured by fluorescence spectrometer. The emission was fixed at 373 nm, the excitation was measured at 334 and 337 nm.

Preparation of ICG@HES-OA NPs

Briefly, HES-OA (95 mg) was dispersed in 8 mL of deionized water. To the suspension, 2 mL of ICG DMSO solution (2.5 mg/mL) was dropwise added and the resulting mixture was stirred at room temperature for 2 h. Then, the above mixture was dialyzed against deionized water for 2 days (MWCO: 3500 kDa) to remove DMSO, and the unencapsulated ICG was removed by ultrafiltration (5000 rpm, 10 min, MWCO: 10000 Da). The drug loading content (DLC) of the prepared ICG@HES-OA NPs was determined by UV-vis spectrophotometer. Briefly, a certain amount of freeze dried ICG@HES-OA NPs was dissolved in DMSO, the amount of ICG was determined by UV-vis spectrophotometer at 795 nm. The drug loading content (DLC) was calculated as

$$\text{DLC (\%)} = \frac{\text{Wt (loaded ICG)}}{\text{Wt (ICG - loaded NPs)}} \times 100\%$$

Where Wt (loaded ICG) is the weight of the loaded ICG, and Wt (ICG-loaded NCs) is the weight

of ICG-loaded NPs. The encapsulation efficiency (EE) was calculated as

$$EE\ (\%) = \frac{Wt\ (loaded\ ICG)}{Wt\ (feeding\ ICG)} \times 100\%$$

Where Wt (loaded ICG) is the weight of the loaded ICG, and Wt (feeding ICG) is the weight of the feeding ICG.

Characterization

^{1}HNMR spectra were recorded on a nuclear magnetic resonance spectrometer (AscendTM 600 MHz, Bruker) using tetramethylsilane (TMS) as an internal reference. FT-IR spectra were recorded on a flourier transform infrared spectrometer (Vertex70, Bruker) with an attenuate total reflection (ATR) accessory. UV-vis spectra were recorded on a UV-vis spectrophotometer (TU-1901, Beijing Purkinje General Instrument Co., Ltd). The hydrodynamic diameter was measured by dynamic light scattering (DLS, Nano-ZS90, Malvern). The morphology of the prepared NPs was imaged by transmission electron microscopy (TEM, HT7700, Hitachi) operated at an accelerating voltage of 100 KV. The samples for TEM imaging were prepared by placing a small drop of the prepared NPs suspension onto a carbon-coated copper grid and dried at room temperature followed by negatively stained with phosphotungstic acid at a concentration of 2 % (w/w) for 1 min.

Stability

Free ICG and ICG@HES-OA NPs (5 μg/mL as ICG) were incubated at 37 ℃ in deionized water for 5 days. The absorbance at 780 nm was measured by UV-vis spectrophotometer every day.

In vitro singlet oxygen generation under NIR laser irradiation

Free ICG and ICG@HES-OA NPs (10 μg/mL as ICG) with DPBF (20 μg/mL) were irradiated with 808 nm laser (0.8 W/cm^2) for 1, 2, 3, 4, and 5 min. Then, the absorbance was measured at 405 nm using a microplate reader (318C Microplate Reader).

Cell culture

Human hepatoma cells HepG-2 were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin and 100 μg/mL streptomycin at 37 ℃ in 5% CO_2 atmosphere. Murine hepatoma cells H22 were cultured in RPMI1640 medium supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin and 100 μg/mL streptomycin at 37 ℃ in 5% CO_2 atmosphere.

Cellular uptake

The cellular uptake of free ICG and ICG@HES-OA NPs were investigated by confocal laser scanning microscope (CLSM). Briefly, HepG-2 cells were seeded on cell culture dishes with glass bottom at a cell density of 1×10^5 cells per well. After incubation the cells with free ICG and ICG@HES-OA NPs (20 μg/mL) for 8 h, the medium was removed and the cells were washed with PBS buffer (pH 7.4, 6.7 mmol/L) for 3 times. Then, the cells were fixed with 4 % paraformaldehyde and stained with 4',6-diamidino-2-phenylindole (DAPI) for CLSM observation.

The intracellular ICG amount was also quantified. Briefly, HepG-2 cells were seeded on 12-well plates a cell density of 1×10^5 cells per well. Each group was tested in three wells. After

incubating the cells with free ICG and ICG@HES-OA NPs (20 μg/mL) for 8 h, the medium was removed and the cells were washed with PBS buffer (pH 7.4, 6.7 mmol/L) for 3 times. Then, the cells were tripsinized for cell counting and disrupted under ultrasonication. The intracellular ICG was extracted with methanol and determined by UV-vis spectrophotometer.

Intracellular GSH depletion

HepG-2 cells were seeded on 12-well plates a cell density of 2×10^5 cells per well. Each group was tested in three wells. After incubating the cells with 0.1, 0.2, and 0.4 μg/mL of PEITC for 4 and 8 h, the medium was removed and the cells were washed with PBS buffer (pH 7.4, 6.7 mmol/L) for 3 times. Then, the cells were lysed by incubation with 0.5 mL of Triton X-100 (1%) at 4 ℃ for 2 h and the lysates were centrifuged at 10000 rpm for 10 min. After that, 200 μL of supernatant was mixed with GSH assay reagent and the absorbance was determined at 414 nm by a microplate reader. The intracellular GSH level of H22 cells after incubation with different concentration of PEITC for 8 h was also quantified.

Intracellular ROS quantification

DCFH-DA was used to determine the intracellular ROS level. HepG-2 cells were seeded on 12-well plates at a cell density of 2×10^5 cells per well. Each group was tested in three wells. After treatment with different formulations (20 μg/mL as ICG) for 8 h, the medium was removed and the cells were washed with PBS buffer (pH 7.4, 6.7 mmol/L) for 3 times. Then, the cells were incubated with DCFH-DA (10 μg/mL) for 20 min followed by washed with PBS buffer (pH 7.4, 6.7 mmol/L) for 3 times and irradiated with 808 nm laser (0.8 W/cm^2) for 3min (or no irradiation).

The cells were observed by fluorescence microscope. The fluorescence intensity of each well was also quantified. Briefly, the cells were lysed by incubation with 1 mL of Triton X-100 (1%) at 4 ℃ for 2 h and the lysates were centrifuged at 10000 rpm for 10 min. The supernatant was measured by fluorescence spectrometer (excitation at 485 nm, emission detected at 525 nm). The intracellular ROS level of ICG@HES-OA + irradiation and ICG@HES-OA/PEITC + irradiation (20 μg/mL as ICG, 0.4 μg/mL as PEITC) was also determined 1 h after laser irradiation.

Cell apoptosis assays

HepG-2 cells were seeded on 12-well plates at a cell density of 2×10^5 cells per well. After treatment with different formulations (20 μg/mL as ICG, 0.4 μg/mL as PEITC) for 8 h, the medium was removed and the cells were washed with PBS buffer (pH 7.4, 6.7 mmol/L) for 3 times. The cells were irradiated with 808 nm laser (0.8 W/cm^2) for 3 min (or no irradiation). Then, the cells were incubated for 1 h. After that, the cells were tripsinized, collected by centrifugation and washed with PBS buffer (pH 7.4, 6.7 mmol/L). The cells were suspended in 0.5 ml of 1×binding buffer and stained with annexin V-FITC and PI at room temperature for 10 min before performing flow cytometry.

H22 cells were seeded on 12-well plates at a cell density of 2×10^5 cells per well. After treatment with different formulations (20 μg/mL as ICG, 2.0 μg/mL as PEITC) for 8 h, the cells were collected by centrifugation and washed with PBS buffer (pH 7.4, 6.7 mmol/L) for 3 times. The cells were irradiated with 808 nm laser (0.8 W/cm^2) for 3 min (or no irradiation). Then, the cells were incubated for 16 h. After that, the cells were collected by centrifugation and washed with PBS buffer (pH 7.4, 6.7 mmol/L). The cells were suspended in 0.5 ml of 1×binding buffer

and stained with annexin V-FITC and PI at room temperature for 10 min before performing flow cytometry.

In vitro antitumor activity

The in vitro antitumor activity of different formulations against HepG-2 cells was assessed by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) assays. HepG-2 cells were seeded on 96-well plates at a cell density of 5×10^3 cells per well. Each group was tested in four wells. After incubation with different formulations for 8 h, the medium was removed and the cells were washed with PBS buffer (pH 7.4, 6.7 mmol/L) for 3 times. Then, the cells were washed with PBS buffer (pH 7.4, 6.7 mmol/L) for 3 times and irradiated with 808 nm laser (0.8 W/cm^2) for 3 min (or no irradiation). The cells were further incubated for 16 h. After that, 20 μL of MTT dye (5 mg/mL) was added to each well and incubated with the cells for 4 h. The medium was removed and 150 μL of DMSO was added to dissolve the formed crystals. The absorbance was measured at 492 nm using a microplate reader (318C Microplate Reader). The in vitro antitumor activity of PEITC against H22 cells was assessed by CCK-8 assays after 24 h incubation.

Animal subjects

Male BALB/c mice were purchased from Hubei Research Center of Experimental Animals. All experimental procedures, the animal use and care protocols were carried out under a protocol approved by the Animal Care and Use Committee of Huazhong University of Science and Technology.

Pharmacokinetics

Male BALB/c mice were intravenously injected with free ICG and ICG@HES-OA NPs (6 mg/kg as ICG). At 1 min, 5 min, 15 min, 30 min, 1 h, 6 h, 12 h, and 24 h, eyeballs of mice from each group (n = 3) were removed to collect blood samples (in heparin treated tubes). The blood samples were centrifuged at 3500 rpm for 10 min to obtain the plasma samples. The obtained plasma samples were extracted by methanol and analyzed by fluorescence spectrometer (excitation wavelength: 740 nm, detection wavelength: 812 nm). The pharmacokinetic data were analyzed by Drug and Statistic software version 2.0.

Biodistribution

H22 tumor-bearing mice were randomly divided into 2 groups, with 3 mice in each group. Free ICG and ICG@HES-OA NPs (6 mg/kg as ICG) were intravenously injected to each group. At 12, 24, 48, 72, 96, and 120 h, mice were anesthetised with 2% isoflurane in oxygen at a flow of 2 L/min and the fluorescence images were acquired by using an in vivo imaging system (IVIS lumina XR, Caliper). At the end of the test, mice were sacrificed. The major organs including heart, liver, spleen, lung, kidney, and tumors were harvested for ex vivo fluorescence imaging. The biodistribution of ICG of each group 12 h post injection was also investigated by ex vivo imaging.

In vivo photothermal response and ROS generation

H22 tumor-bearing mice were randomly divided into 3 groups, with 3 mice in each group.

PBS buffer (pH 7.4, 6.7 mmol/L), free ICG and ICG@HES-OA NPs (6 mg/kg as ICG) were intravenously injected to each group. 24 h after injection, the tumors were irradiated with 808 nm laser (0.8 W/cm^2) for 5 min and the infrared images were collected by an infrared imaging camera. After that, mice were sacrificed and the tumors were harvested for dihydroethidium (DHE) staining.

In vivo GSH depletion

H22 tumor-bearing mice were randomly assigned to 5 groups (n=3) and intraperitoneally injected with 10, 15, 20 and 30 mg/kg PEITC. Mice intraperitoneally injected with PBS buffer were used as control. 24 h after administration, mice from each group were sacrificed. The tumors were harvest and homogenized. The tumor homogenate was centrifuged at 10000 rpm for 10 min. After that, 200 μL of supernatant was mixed with GSH assay reagent and the absorbance was determined at 414 nm by a microplate reader.

In vivo apoptosis assays

H22 tumor-bearing mice were randomly assigned to 6 groups (n=3) and injected with each formulation. Free ICG and ICG@HES-OA NPs (6 mg/kg as ICG) were injected to each group by tail vein injection, PEITC (25 mg/kg as PEITC) was injected to each group by intraperitoneal injection subsequently. 24 h after administration, the tumors were irradiated with 808 nm laser (0.8 W/cm^2) for 5 min (or no irradiation). 48 h after administration, mice were sacrificed and the tumors were harvested for TUNEL staining.

In vivo antitumor activity

Male BALB/c mice (19-25 g) were inoculated subcutaneously with H22 tumor cells (each with 1×10^5 H22 tumor cells) on the right lower back. When the H22 tumor volumes reached about 0.2 cm^3, mice were randomly assigned to 6 groups (n=3) and injected with each formulation. Free ICG and ICG@HES-OA NPs (6 mg/kg as ICG) were injected to each group by tail vein injection, PEITC (25 mg/kg as PEITC) was injected to each group by intraperitoneal injection subsequently. The tumors were irradiated with 808 nm laser (0.8 W/cm^2) for 5 min (or no irradiation) 24 h post administration. The second administration was conducted 4 days after the first administration and the tumors were irradiated with 808 nm laser (0.8 W/cm^2) 24 h after the second administration (or no irradiation). Tumor volumes and bodyweight of mice from each group were measured every 2 days. At the end of the test, mice from each group were sacrificed. The blood samples were harvested for serological analysis. The tumors were weighted, imaged, and analyzed by hematoxylin and eosin (H&E) staining. The major organs including heart, liver, spleen, lung, and kidneys were also harvested and analyzed by H&E staining.

Statistical analysis

All data were presented as the mean value ± standard deviation (SD). Statistical analysis was performed by Statistical Product and Service Solutions (SPSS) with independent samples T-test. Statistic significance was established at $p < 0.05$. * $p < 0.05$， ** $p < 0.01$, *** $p < 0.001$.

The molar substitution of OA for different HES-OA samples was determined by ^{1}H NMR

spectra and summarized in Table S2. The DLS characters of the prepared different HES-OA NPs were also summarized in Table S2. The diameter of HES-OA NPs increases with the molar substitution of OA. Due to the optimal size for in vivo application, HES-OA2 NPs were chosen for further study.

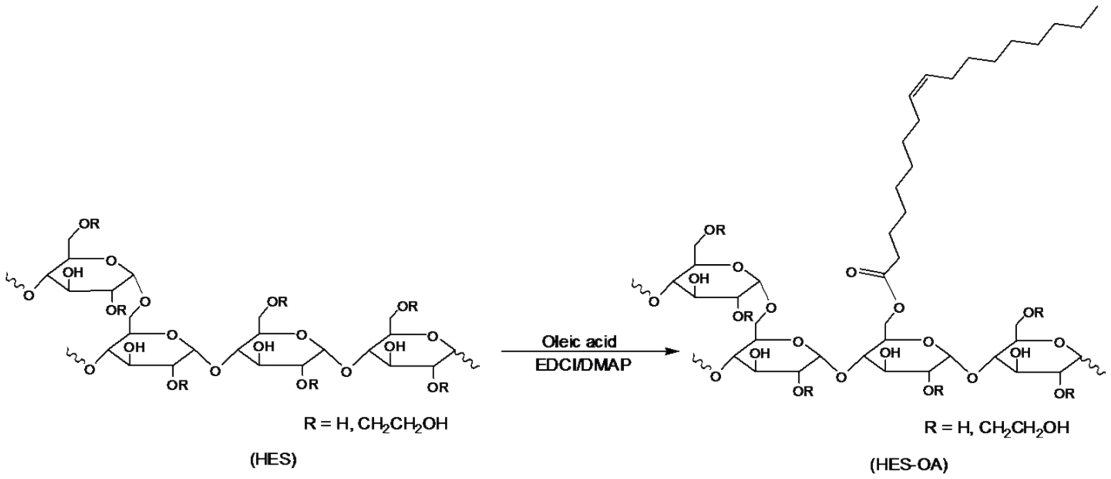

Figure S1. Synthetic scheme of HES-OA.

Table S2. OA molar substitution (MS_{OA}) and DLS characters of different HES-OA samples. Data represent the mean ± SD (n = 3).

Sample	MS_{OA} (%)	D_h (nm)	PDI
HES-OA1	5.1	27.1 ± 0.3	0.26 ± 0.01
HES-OA2	6.0	47.3 ± 0.7	0.23 ± 0.01
HES-OA3	7.0	216.7 ± 4.1	0.19 ± 0.01
HES-OA4	9.5	206.9 ± 4.0	0.12 ± 0.02
HES-OA5	21.9	-[a]	-[a]

[a] not soluble in water. D_h, hydrodynamic diameter. PDI, polydispersity index.

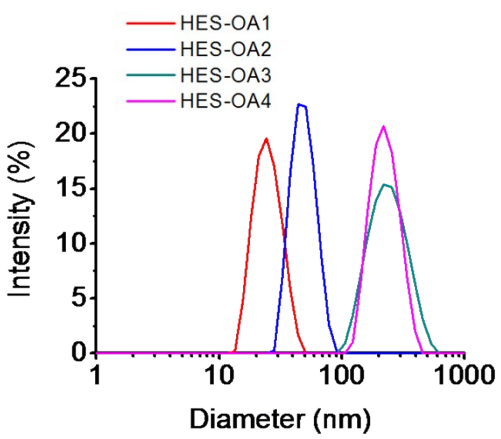

Figure S2. Size distribution of HES-OA1, HES-OA2, HES-OA3, and HES-OA4 NPs measured by DLS.

Table S3. DLS and drug loading characters of the prepared ICG@HES-OA NPs. Data represent the mean ± SD (n = 3).

Sample	D_h (nm)	PDI	DLC (%)	EE (%)
ICG@HES-OA	54.7 ± 5.8	0.26 ± 0.06	4.2	84

D_h, hydrodynamic diameter. PDI, polydispersity index. DLC, drug loading content. EE, encapsulation efficiency.

Table S4. Combination index of in vitro antitumor activity against HepG-2 cells of free ICG and ICG@HES-OA NPs with PEITC under 808 nm laser irradiation (0.8 W/cm^2, 3 min). Data calculated from Figure 3 B, C and E.

Sample	ICG (μg/mL)	PEITC (μg/mL)	CI value
ICG@HES-OA + PEITC	5	0.2	0.454
	5	0.4	0.632
	20	0.2	0.646
	20	0.4	0.692
ICG + PEITC	5	0.2	0.669
	5	0.4	0.684
	20	0.2	0.869
	20	0.4	0.721

CI, combination index. Combination index was calculated by the Chou-Talalay method based on the median-effect equation.

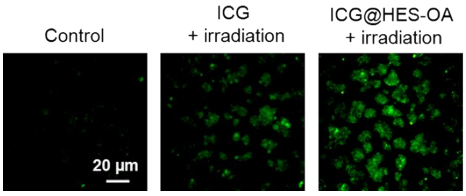

Figure S3. Detection of intracellular ROS of HepG-2 cells treated with different formulations (20 μg/mL as ICG) after 808 nm laser irradiation (0.8 W/cm^2, 3 min) by DCFH-DA.

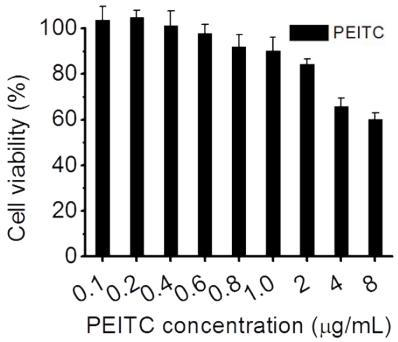

Figure S4. In vitro antitumor activity of PEITC against H22 cells. Data represent the mean ± SD (n = 4).

The GSH depletion effect of PEITC and cell apoptosis were also examined in H22 cells, Figure S4, 5 and 6. PEITC depletes the intracellular GSH of H22 cells in a dose-dependent manner, Figure S5. ICG@HES-OA NPs exhibit enhanced cell apoptosis (17.73 %) as compared to free ICG (10.49 %) under laser irradiation. PEITC (2.0 μg/mL) induces no significant cell apoptosis. Free ICG or ICG@HES-OA NPs combined with PEITC show enhanced cell apoptosis (33.12 % ICG/PEITC, 60.19 % for ICG@HES-OA/PEITC) as compared to free ICG or ICG@HES-OA NPs alone under laser irradiation, Figure S6. The cell apoptosis results obtained in H22 cells are in the same line with those obtained in HepG-2 cells. Thus, the in vivo evaluation was conducted on H22 tumor-bearing mice.

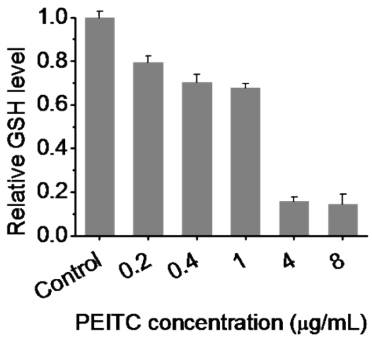

Figure S5. GSH depletion of H22 cells after incubation with different concentration of PEITC for 8 h. Data represent the mean ± SD (n = 3).

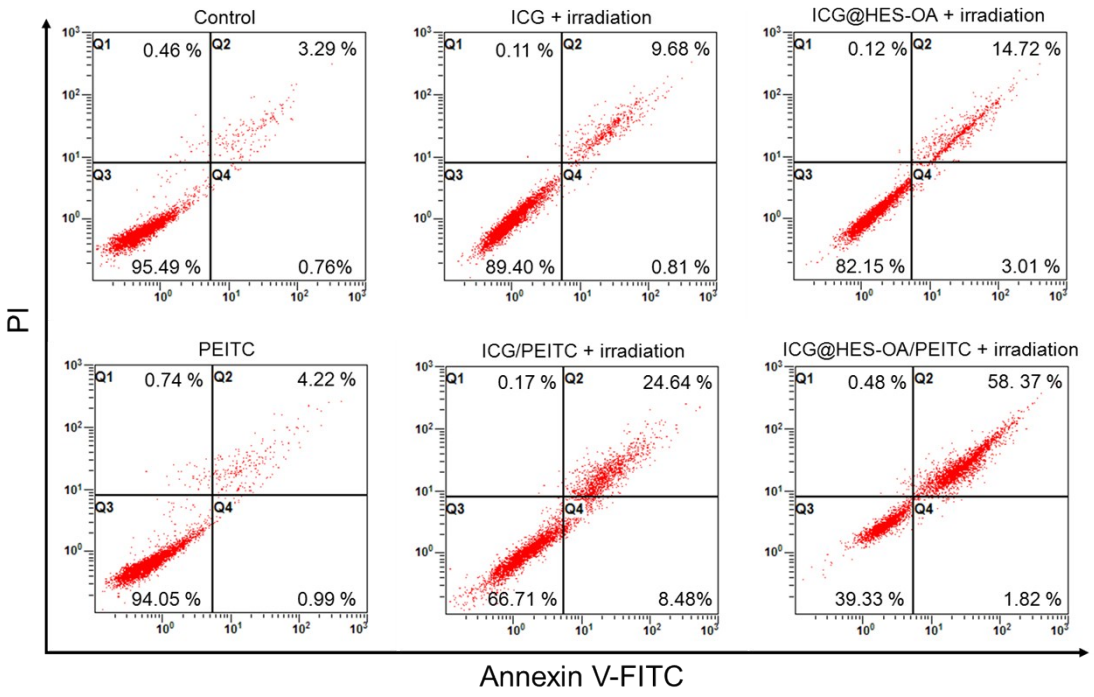

Figure S6. The apoptosis of H22 cells treated with different formulations (20 μg/mL as ICG, 2.0 μg/mL as PEITC) 16 h after 808 nm laser irradiation (0.8 W/cm^2, 3 min).

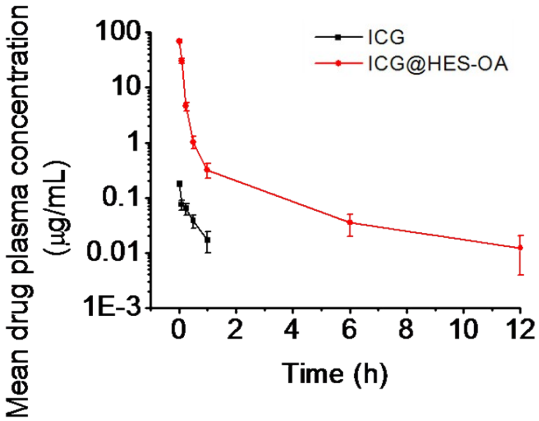

Figure S7. Mean drug plasma concentration-time profiles of free ICG and ICG@HES-OA NPs after intravenous injection (equivalent to 6 mg ICG/kg bodyweight). Data represent the mean ± SD (n = 3).

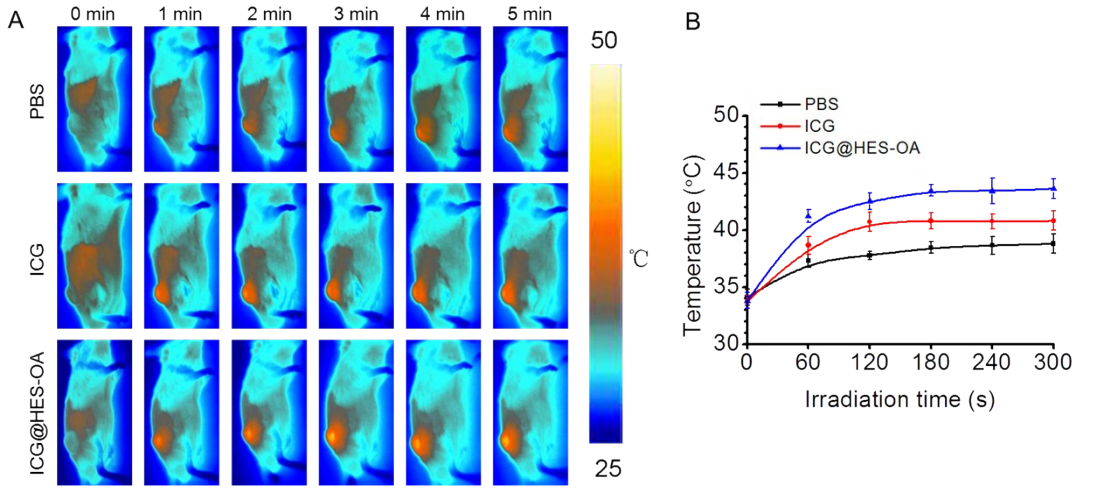

Figure S8. In vivo photothermal response of free ICG and ICG@HES-OA NPs under 808 nm laser irradiation (0.8 W/cm^2, 5 min). (A) Thermal images of mice receiving different formulations and exposed to NIR laser 24 h post intravenous injection (equivalent to 6 mg ICG/kg bodyweight). (B) Tumor temperature profiles of mice receiving different formulations and exposed to NIR laser 24 h post intravenous injection. Data represent the mean ± SD (n = 3).

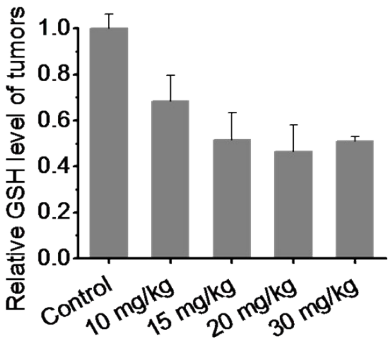

Figure S9. Tumor GSH depletion of mice receiving different doses of PEITC. Data represent the mean ± SD (n = 3).

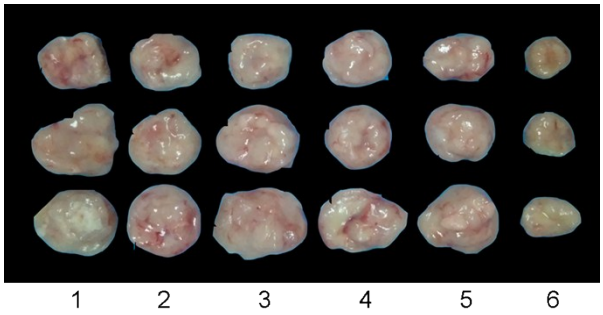

Figure S10. Tumor images of each group at the end of the test. 1, PBS; 2, PEITC; 3, ICG + irradiation; 4, ICG@HES-OA + irradiation; 5, ICG/PEITC + irradiation; 6, ICG@HES-OA/PEITC + irradiation.

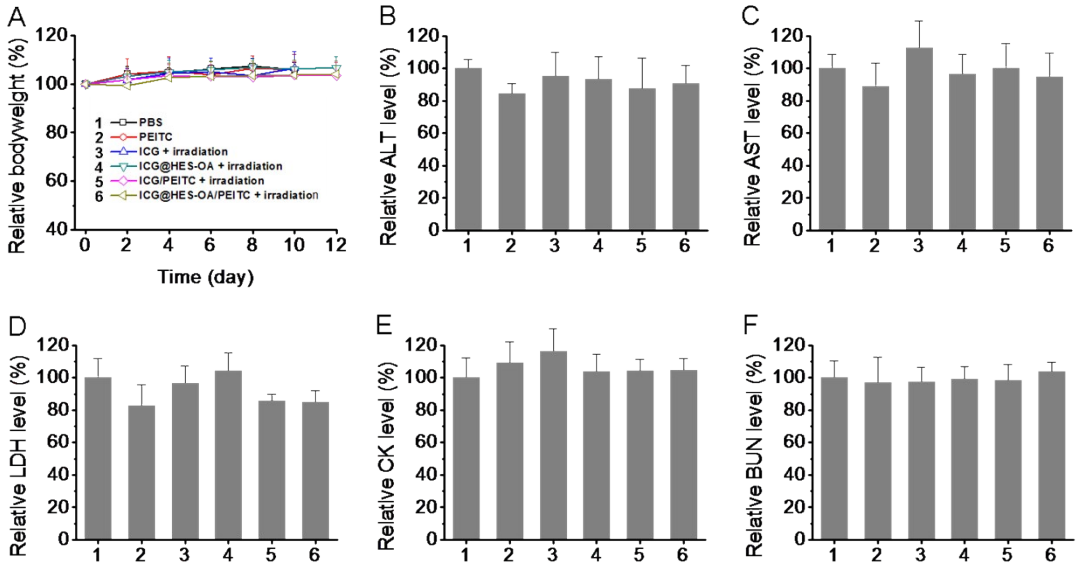

Figure S11. Safety evaluation. (A) Relative bodyweight changes of mice from different groups. (B) Relative ALT level of each group at the end of the test. (C) Relative AST level of each group at the end of the test. (D) Relative LDH level of each group at the end of the test. (E) Relative BUN level of each group at the end of the test. (F) Relative CK level of each group at the end of the test. Data represent the mean ± SD (n = 3).

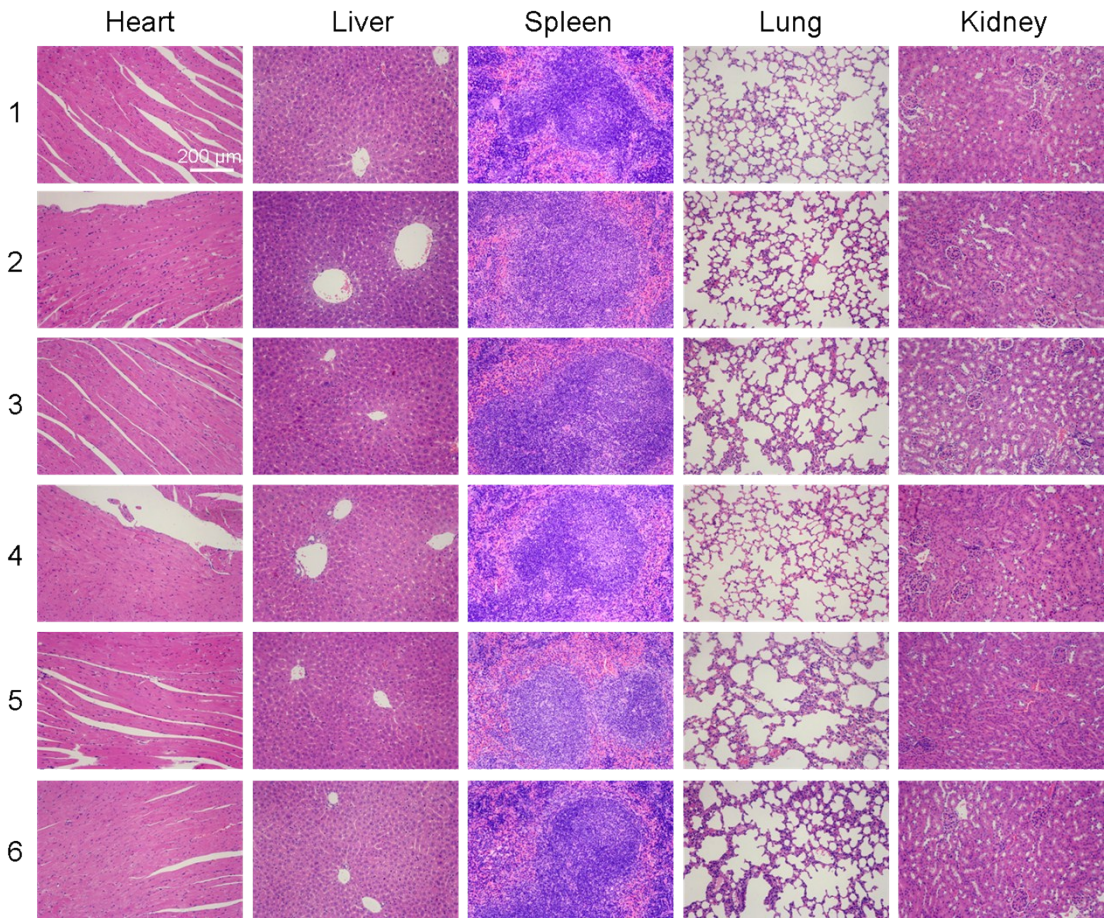

Figure S12. Tissue sections (stained by H&E) of heart, liver, spleen, lung, and kidney of mice from each group at the end of the test. 1, PBS; 2, PEITC; 3, ICG + irradiation; 4, ICG@HES-OA + irradiation; 5, ICG/PEITC + irradiation; 6, ICG@HES-OA/PEITC + irradiation. The scale bar is 200 μm and applied for all images.