Abbreviations

RBG	Resibufogenin
PLGA	Polylactic-*co*-glycolic acid
TPGS	D-α-tocopheryl polyethylene glycol 1000 succinate
PLGA-TPGS	Poly(lactic-*co*-glycolic acid)-d-α-tocopheryl polyethylene glycol 1000 succinate
RPTN	RBG-loaded PLGA-TPGS nanoparticles
RCPTN	RBG/coumarin-6-loaded PLGA-TPGS nanoparticles
RPN	RBG-loaded PLGA nanoparticles
DL	Drug loading
EE	Encapsulation efficiency
TCMs	Traditional chinese medicines
MDR	Tumor multidrug resistance
C6	Coumarin-6
EPTN	Empty PLGA-TPGS nanoparticles
TI	Targeting index
SI	Selectivity index
Re	Relative targeting efficiency
Te	Targeting efficiency
PDI	Polydispersity index
FS	Commercial fluorouracil for injection solutions
RS	RBG solutions
AUC	Area under concentration-time curve
MRT	Mean retention time
IR	Inhibition rate
SD	Standard deviation

2. Materials & Methods

2.3.3. Drug loading and encapsulation efficiency and in vitro drug release

The DL of RPTN, RCPTN and RPN was analyzed by RP-HPLC (1200 Series, Agilent Technologies, Santa Clara, CA, USA) using Hypersil C18 column (250×4.6 mm, pore size 5 μm, Thermo, West Palm Beach, FL, USA). Briefly, a predetermined quantity of nanoparticles was dissolved in 2 ml ethyl acetate. After a nitrogen steam was introduced to evaporate ethyl acetate, RBG was extracted

and dissolved in quantum by mobile phase (75/25 (v/v) methanol /0.05% acetic acid aqueous solution) in 10 ml volumetric measuring flask. Then, a clear solution of 20 μl was gained for HPLC analysis (flow rate: 0.8 ml/min, temperature: 30 °C). A UV/VIS detector was used at 298 nm to detected column effluent and the retention time was 6.1 min [1, 2]. DL was defined as ratio between the quantity of RBG loaded in nanoparticles and the quantity of nanoparticles, and EE was determined by the ratio between the quantity of RBG loaded in nanoparticles and total RBG quantity added in the preparation procedure, which was performed in sextuplicate.

RPTN, RCPTN and RPN were evaluated for their *in vitro* release of RBG by dialysis method [3]. In brief, a known quantity of RBG, RPTN, RCPTN and RPN with the same RBG concentration, all of which had already been dispersed in 5 ml phosphate buffer solution (PBS, pH 7.4, containing 0.5% w/v SDS, which was used to increase the solubility of RBG in PBS), were placed into a regenerated cellulose dialysis membrane bag of standard grade (MWCO=1000, Houston, TX, USA), respectively. The sealed sample was then immersed into 15 ml release medium (PBS as mentioned above) in an appropriate beaker, which was kept in an orbital water bath maintained at 37.0 °C shaking at 120 r/min, respectively. At definite intervals, ten milliliters of receiver release solution was removed for HPLC analysis and changed by fresh release medium simultaneously. After RBG was extracted from the collected samples using 10 ml ethyl acetate for three times (the recovery rate was 99.7%, n=6), a nitrogen stream was introduced to evaporate ethyl acetate. The rudimental RBG was dissolved in quantum by mobile phase (75/25 (v/v) methanol/0.05% acetic acid aqueous solution) in 10 ml votilumetric measuring flask. The analysis process was similar as described in the measurement of DL, which was also performed in sextuplicate.

2.4. Cellular uptake of nanoparticles

For the quantitative research，all of HepG2 cells and HCa-F cells in each well were collected and dispersed into purified water after being observed by CLSM, and then sonicated at 100 W for 2 min in the ice bath, respectively. Then the samples were supplemented with 1.0 μg/ml internal standard emodin solution, and a nitrogen steam was used to evaporate purified water. The residues were dissolved in 2 ml ethyl acetate and vortex admixed for 1 min. After ethyl acetate was evaporated by a nitrogen stream, RBG was extracted and dissolved in quantum by appropriate quantity of mobile phase, and a clear solution of 20 μl was gained for HPLC analysis (as the chromatographic conditions described in *Drug loading and encapsulation efficiency* above). The standard curves and linear regressions of RBG were mapped with ratio (A) between peak area of RBG and internal standard emodin as ordinate against the concentration of RBG (C) as abscissa. The cellular uptake efficiency of RBG was determined by the ratio between the quantity of RBG measured after cellular

uptake and total RBG quantity added before cellular uptake, which was performed in sextuplicate.

2.5. In vitro cytotoxicity assays

The *in vitro* cytotoxicity of the nanoparticles was assessed using WST-1 assays. Briefly, HepG2 cells were seeded into 96-well plates at a density of 1×10^4 cells per well in 100 μl of culture medium. After incubation in 5% CO_2 at 37 °C for 24 h, the cells were incubated with RPTN suspensions, RPN suspensions or RBG solutions (RS, 0.1% v/v DMSO culture medium was used as the solvent) at 1.2, 2.5, 5, 10 and 20 μg/ml equivalent RBG concentrations, with fluorouracil solutions (FS, commercial fluorouracil for injection) at 1.2, 2.5, 5, 10 and 20 μg/ml equivalent fluorouracil concentrations, and with EPTN suspensions at the same nanoparticle concentrations (5.5, 11, 22, 44 and 88 μg/ml) for 24, 48 and 72 h. NPs suspensions were formed using RPTN, RPN and EPTN that were dispersed into culture medium and sonicated at 100 W for 1 min in an ice bath. At the determined timepoints, the cells were washed with PBS twice after the medium was discarded. Ten microliters of WST-1 solution was then added to each well, and the plates were incubated in 5% CO_2 at 37 °C for 4 h. The amount of soluble formazan that was transformed from WST-1 in the mitochondria of metabolically active cells was evaluated using a microplate reader (354 Type, Thermo, West Palm Beach, FL, USA) at a wavelength of 450 nm. Six separate experiments were performed and measured. Cell viability was determined as the ratio between the fluorescence absorbance of the cells that were incubated with different experimental combinations and analyzed at different timepoints to the fluorescence absorbance of the cells that were incubated with only the culture medium (the control group, non-treated cells) for the same period of time. IC_{50} values, defined as the drug concentration at which 50% of cancer cell growth was inhibited, were determined by curve fitting the cell viability data to the drug concentration data.

2.6. In vivo drug concentration and freezing slices of RCPTN for evaluating liver targeting property in mice

2.6.1. Pretreatment of biological samples

After being injected drug or not, the mice were given ether by inhalation, and then blood samples were obtained from cardiac apical, supplemented with 109 mM sodium citrate according to the ratio of 1/9 (v/v, sodium citrate/blood samples) for the use of anticoagulation. The supernatant plasma was collected after the blood samples were centrifuged at 5 000 r/min for 10 min. After the mice were euthanized, the organs (liver, heart, spleen, lung and kidney) were immediately isolated and homogenized in 4 volumes (w/v) of normal saline, and the plasma and homogenates of the organs (liver, heart, spleen, lung and kidney) were measured precisely of 400 μl respectively, then supplemented with 1.0 μg/ml internal standard emodin standard solution, respectively. After vortex

admixing for 1 min and extracted three times by appropriate quantity of ethyl acetate, the samples were vortex admixed for another three times and centrifuged at 10 000 r/min for 10 min. Organic phase was collected and ethyl acetate was evaporated by a nitrogen stream (the absolute recovery rates in different samples were all bigger than 90.0%, *n*=9). The residues were dissolved in quantum by 100 μl methanol, and a clear solution of 20 μl was gained for HPLC analysis (as the chromatographic column described above). The standard curves and linear regressions of RBG in plasma and different organs were mapped with ratio (A) between peak area of RBG and internal standard emodin as ordinate against the concentration of RBG (C) as abscissa. Blank plasma and homogenates of different organs without any RBG and internal standard emodin were also studied in the same way.

2.6.2. LD_{50} determination

Six-week-old Kunming male mice with the weight of about 20-22 g were purchased from the Dalian Medical University Laboratory Animal Center (Dalian, China). This study had been permitted by the Institutional Animal Care and Use Committee (IACUC) (Dalian Medical University, Dalian, China). The mice were all fed standard laboratory diet and water before sacrifice, and kept in a room with constant temperature (22±1°C) and a dark-light cycle (12 h/12 h). LD_{50} (median lethal dose) values of RS (RBG solutions, 20% v/v PEG400-normal saline solution) group and RPTN (RPTN suspensions, 20% v/v PEG400-normal saline solution) group were determined in the mice through intravenous injection via tail vein according to Litchfield and Wilcoxon method. The mice in RS group were administered at a dosage of 10.0, 13.2, 17.3, 22.8, 30.0 mg/kg equivalent to RBG concentration while the mice in RPTN group were administered at a dosage of 30.0, 33.8, 38.3, 43.6, 50.0 mg/kg equivalent to RBG concentration. Ten mice were taken in each dose level of the two groups and the mortality was recorded up to 24 h of observation.

2.8. Pharmacokinetics study on rats

2.8.1. Chromatographic conditions

Shimadzu LC20AD HPLC system consisted of a quaternary delivery system, a degasser, an auto-sampler and UV detector. The chromatograph was equipped with an Elite SinoChrom ODS-BP (150 × 2.1 mm, 5 μm, Elite, Dalian, China) analytical column. The mobile phase was consisted of acetonitrile-0.1% formic acid aqueous solution (35:65, v/v) at a flow rate of 0.5 ml/min. The run time was 4.5 min for each injection, and the column temperature was controlled at room temperature.

An AB Sciex QTRAP 4500 (MS/MS) equipped with an electrospray ionization (ESI) source was used, and the system was operated in positive mode. The optimized ionspray voltage and

temperature were set at 4,500 V and 500 °C, respectively. The curtain gas (CUR) was set at 10 psi; gas 1 and gas 2 (nitrogen) were set at 30 and 40 psi, respectively, and the dwell times were 200 ms. The quantification assay was performed using multiple reaction monitoring (MRM). The selected m/z transitions were 385.200→253.200 for Resibufogenin, 544.000→397.000 for adriacin (internal standard). Analyst 1.6.2 software (Applied Biosystems) was used to control the equipment and for data acquisition and analysis. The optimized MRM parameters including declustering potential, entrance potential, collision energy, cell exit potential was 80, 8, 28, 7 for RBG and 80, 12, 16, 11 for adriacin, respectively.

2.8.2. Pretreatment of biological samples

One hundred microliters supernatant plasma was collected after blood samples were centrifuged at 3 000 r/min for 10 min, then supplemented with 50 μl adriacin (580 ng/ml) and vortex admixed for 30 s. The samples were extracted twice by appropriate quantity of ethyl acetate and centrifuged at 10 000 r/min for 10 min. Organic phase was collected and ethyl acetate was evaporated by a nitrogen stream. The residues were dissolved in quantum by 100 μl methanol, and a clear solution of 10 μl was gained for LC-MS analysis.

2.8.3. Drug administration

Male SD rats with the weight of approximate 189.5±9.3 g were provided by the Dalian Medical University Laboratory Animal Center (Dalian, China). This study had been permitted by the Institutional Animal Care and Use Committee (IACUC) (Dalian Medical University, Dalian, China). Eighteen rats were randomly divided into three groups: RBG group, RPN group and RPTN group, all of which received administration via jugular vein. In the RPTN group and RPN group, six rats were administered with RPTN and RPN suspensions respectively at a dosage of 1 mg/kg equivalent to RBG concentration. In the RS group, six rats were administered with RS at the same dosage like above. The method of the drug treatment was performed as mentioned in LD_{50} determination assays. Blood samples (300 μl) were obtained from each rat in the three different groups at an interval of 5, 10, 15, 30, 45, 60, 120, 240, 480, 720 and 1440 min after administration. Treatment and analysis process of blood samples at different intervals were performed as mentioned above. Pharmacokinetic parameters were calculated with 3P97 software (Chinese Society of Mathematical Pharmacology).

3. Results

3.1. Characterization of drug-loaded nanoparticles

3.1.2. Size, size distribution, and surface charge

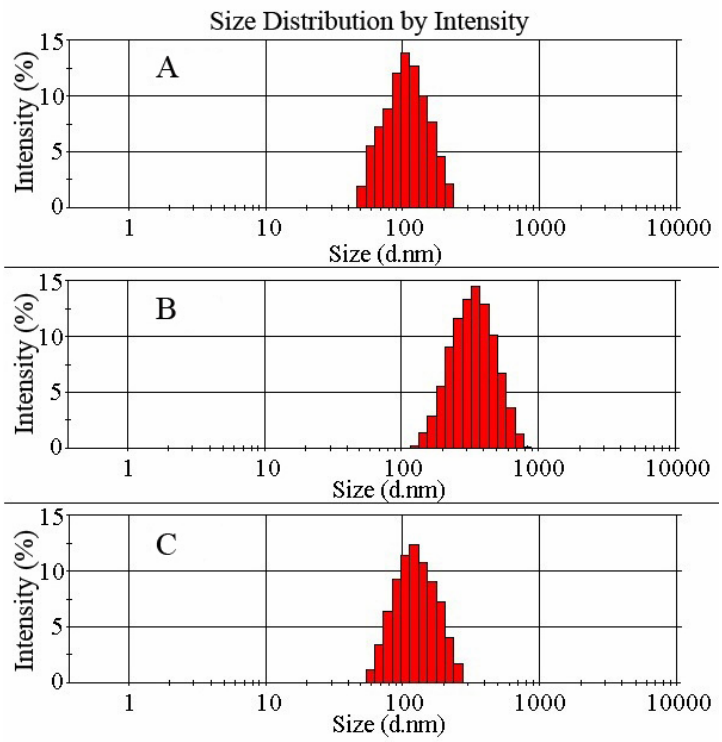

Figure S1. A, B and C are size distribution by intensity images of the RPTN (PDI=0.082), RPN (PDI=0.179) and RCPTN (PDI=0.076), respectively.

3.2. Cellular uptake of nanoparticles by HHSEC

To testify weather RPTN can be uptaken by normal cells in liver or not, we have also chosen human hepatic sinusoidal endothelial cells (HHSEC) as studying on cellular uptake of RCPTN (the concentration of C6 and RBG in RCPTN was quantified at 200 μg/ml and 183.2 μg/ml respectively) the same as HepG2 and HCa-F cells. The results of *Figure S2* showed that the green fluorescence of RCPTN was closely located around the nucleus of HHSEC (blue fluorescence, stained with DAPI), however, of which green fluorescence intensity was much weaker than that of RCPTN internalized into HepG2 and HCa-F cells at 12 h.

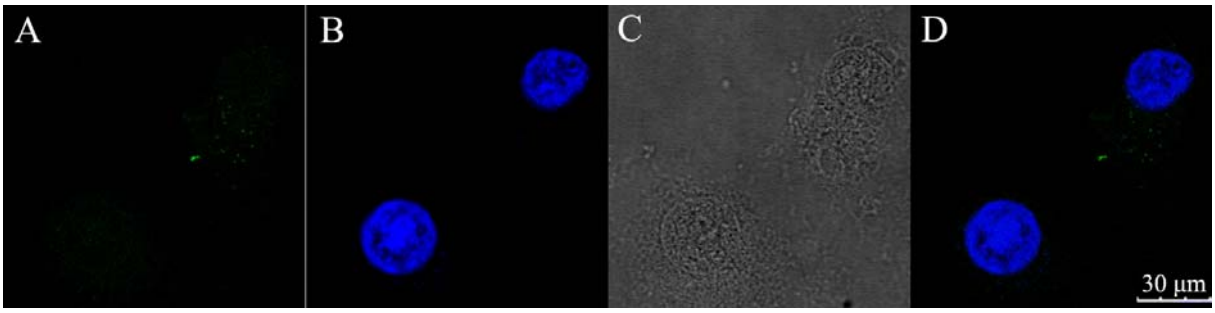

Figure S2. Confocal laser scanning microscopy (CLSM) images of HHSEC after incubation with RCPTN for 12 h at 37 °C. RCPTN were green while nuclei of the cells were blue stained using DAPI. A is the green fluorescence of RCPTN uptaken by HHSEC; B is the blue fluorescence of HHSEC nuclei dyed by DAPI; C is the bright field image of HHSEC; D is the combined image of A and B, respectively.

At the same time, the content of drugs in HHSEC was measured using the same method. The

content of C6 and RBG was 7.90±0.21 μg/ml and 7.08±0.27 μg/ml (n=6), and the cellular uptake efficiency of C6 and RBG was 3.95% and 3.86%. Both of them were lower than HepG2 and HCa-F cells, which illustrated that RPTN had a better targeting ability to liver cancer cells and weaker uptaken property to hepatic sinusoidal endothelial cells at the same time of 4 h and even longer time of 12 h.

3.5. In vitro cytotoxicity assays

The *in vitro* cytotoxicity of RPTN, RPN, RS, FS and EPTN in HepG2 cells was evaluated using WST-1 assays. Commercial fluorouracil injections were used as the reference group. *Figure S3* describes the *in vitro* cell viability of HepG2 cells that were cultured with RPTN, RPN and RS at the same RBG concentrations (1.2, 2.5, 5, 10 and 20 μg/mL) or FS at 1.2, 2.5, 5, 10 and 20 μg/ml equivalent fluorouracil concentrations or EPTN at the same nanoparticle concentrations for 24 h, 48 h and 72 h. The following general results are described in *Figure S3*. (1) EPTN showed little cytotoxicity in HepG2 cells at different nanoparticle concentrations; hence, the synthesized PLGA-TPGS copolymer was considered to be biocompatible and nontoxic to cells. (2) Cell viability in HepG2 cells exposed to RPTN, RPN, RS and FS significantly decreased as the drug dose and the incubation times increased, and all of these samples exhibited dose-dependent and time-dependent effects indicating *in vitro* cytotoxicity, especially those exposed to RPTN and RPN. (3) RPTN and RPN exhibited equivalent or higher cytotoxic effects against cancer cells than FS and RS. Furthermore, RPTN showed better cytotoxic effects than RPN. As shown in *Figure S3*, at different incubation times, RPTN exhibited significantly higher cytotoxic effects than FS. For example, HepG2 cell viability after 24 h of incubation at a concentration of 10 μg/ml decreased from 79.39% for FS to 67.81% (i.e., a 11.58% increase in the inhibition rate, p>0.05) for RPN and to 61.32% (i.e., a 18.07% increase in the inhibition rate, p<0.05) for RPTN (*Figure S3*). Moreover, compared to the commercial FS, the cell viability in HepG2 cells was significantly reduced to 29.21% (p<0.05) and 15.98% (p<0.05) for RPN and to 37.03% (p<0.01) and 25.62% (p<0.01) for RPTN after 48 and 72 h incubation, respectively, at the 10 μg/ml drug concentration.

As listed in *Table S1*, the IC_{50} values of RPTN and RPN were both significantly lower than that of FS at 48 h and 72 h. For example, the IC_{50} values of RPTN, RPN and FS after 48 h of incubation were 4.05, 9.32 and 30.01 μg/ml, respectively, suggesting that the RPTN and RPN formulations may be 7.41- and 3.22-fold more effective than the FS formulation. Furthermore, the IC_{50} values of RPTN and RPN were both significantly lower than that of RS after different periods of incubation. For example, the IC_{50} values of RPTN, RPN and RS after 24 h of incubation were 16.94, 24.53 and 59.57 μg/ml, respectively, indicating that the RPTN and RPN formulations may be 3.52- and

2.43-fold more effective than the RS formulation.

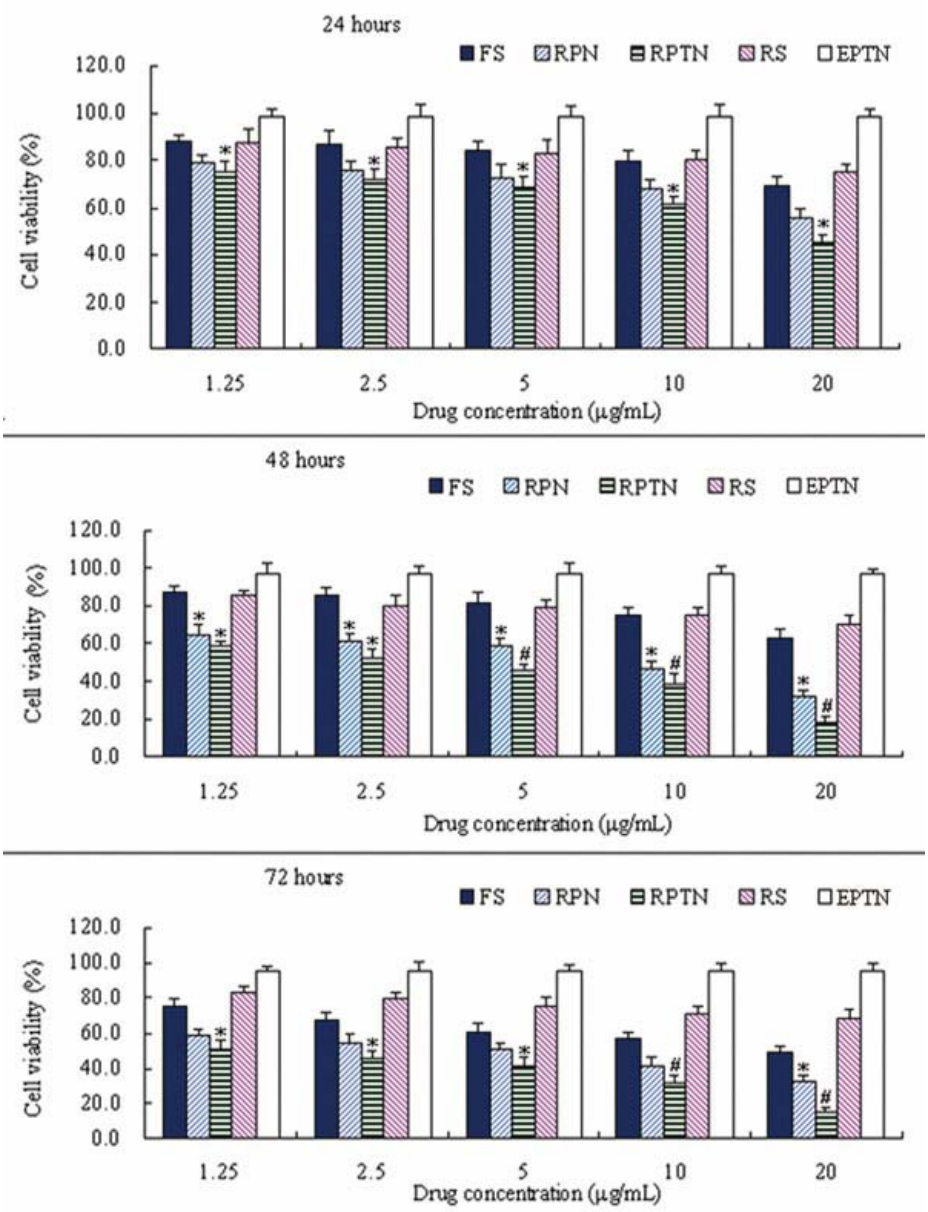

Figure S3. Viability of HepG2 cells cultured *in vitro* with the EPTN, RPTN, RPN, RS and FS group at different drug concentrations after 24, 48, and 72 h of incubation. All data represent the mean ± SD of sextuplicate experiments. * $p<0.05$, # $p<0.01$ versus corresponding the FS group.

Table S1. IC_{50} values of RPTN, RPN, FS and RS against HepG2 cells after 24, 48, and 72 h of incubation. (n=6, mean ± SD)

Incubation time (h)	IC_{50} (μg/ml)			
	RPTN	RPN	FS	RS
24	16.94 ± 0.13*	24.53 ± 0.22*	38.10 ± 0.27	59.57 ± 0.11
48	4.05 ± 0.15*#	9.32 ± 0.19*#	30.01 ± 0.29	46.04 ± 0.14
72	0.66 ± 0.17*#	5.92 ± 0.21*#	17.44 ± 0.17	44.31 ± 0.18

*$p<0.01$ versus corresponding RS group, #$p<0.05$ versus corresponding FS group.

3.5. Fluorescence imaging was used to study frozen sections to evaluate the liver-targeting properties of RCPTN in mice

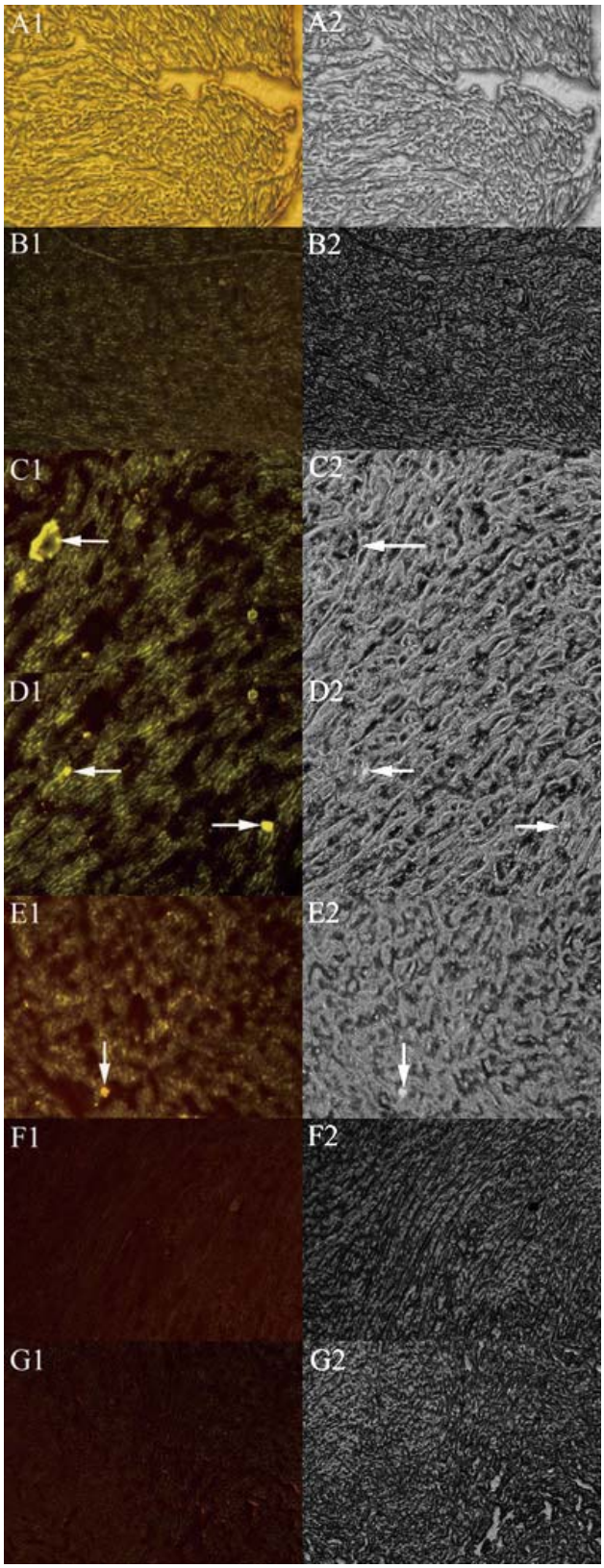

Figure S4. Fluorescence microscopy images (200×) of the heart freezing slices for blank group and experiment groups of the mice injected with RCPTN after 0.5, 1, 2, 4, 8, and 12 hours.

Notes: (A) Images are heart freezing slices of blank group, and (B-G) images are heart freezing slices of experiment group which were picked at 0.5, 1, 2, 4, 8, and 12 hours, respectively. The left images (1) are green fluorescence of QCPTN with C6 (pointed to by the arrows); the right images (2) are observed from white light channel.

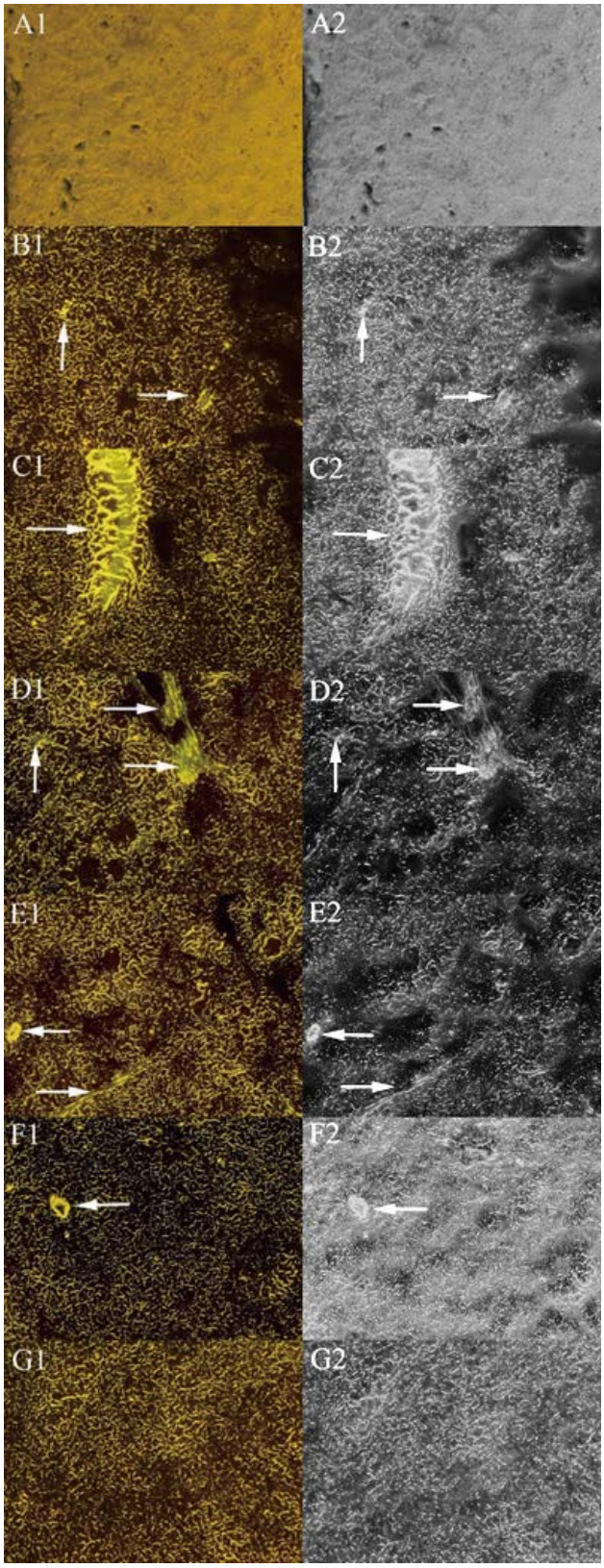

Figure S5. Fluorescence microscopy images (200×) of the spleen freezing slices for blank group and experiment groups of the mice injected with RCPTN after 0.5, 1, 2, 4, 8, and 12 hours.

Notes: (A) Images are spleen freezing slices of blank group, and (B-G) images are spleen freezing slices of experiment group which were picked at 0.5, 1, 2, 4, 8, and 12 hours, respectively. The left images (1) are green fluorescence of QCPTN with C6 (pointed to by the arrows); the right images (2) are observed from white light channel.

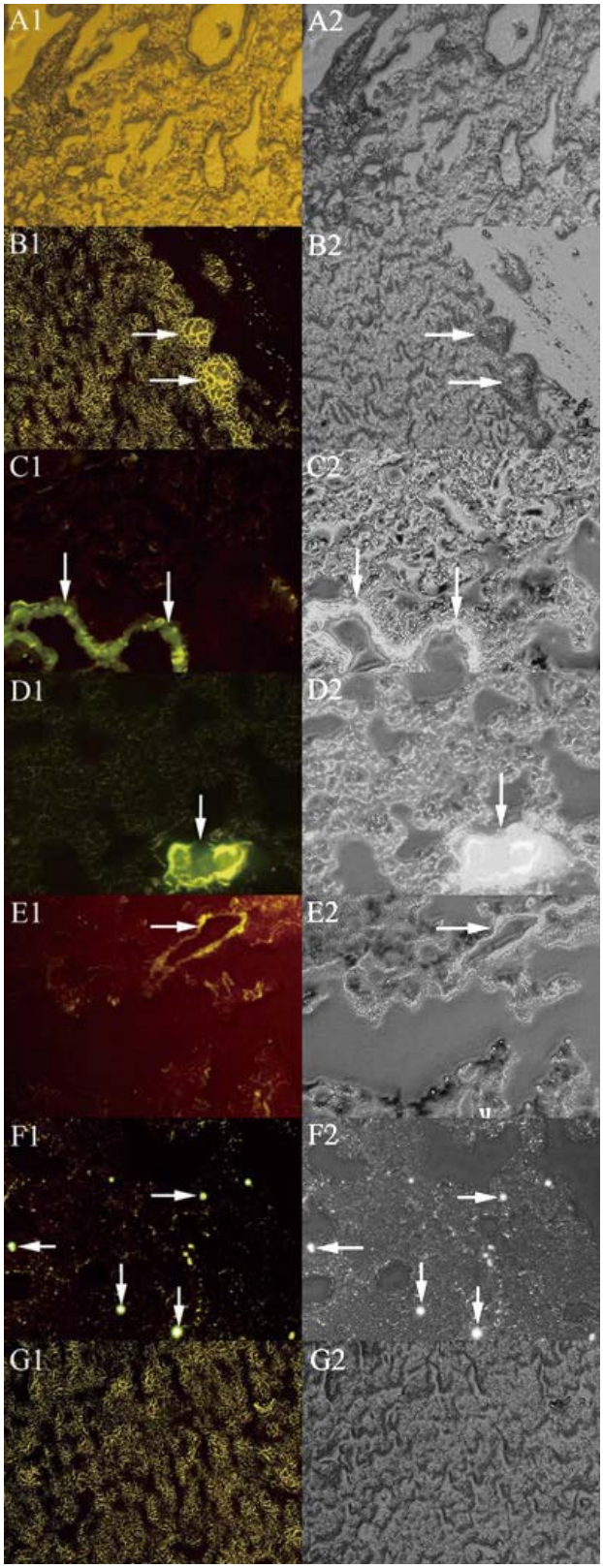

Figure S6. Fluorescence microscopy images (200×) of the lung freezing slices for blank group and experiment groups of the mice injected with RCPTN after 0.5, 1, 2, 4, 8, and 12 hours.

Notes: (A) Images are lung freezing slices of blank group, and (B-G) images are lung freezing slices of experiment group which were picked at 0.5, 1, 2, 4, 8, and 12 hours, respectively. The left images (1) are green fluorescence of QCPTN with C6 (pointed to by the arrows); the right images (2) are observed from white light channel.

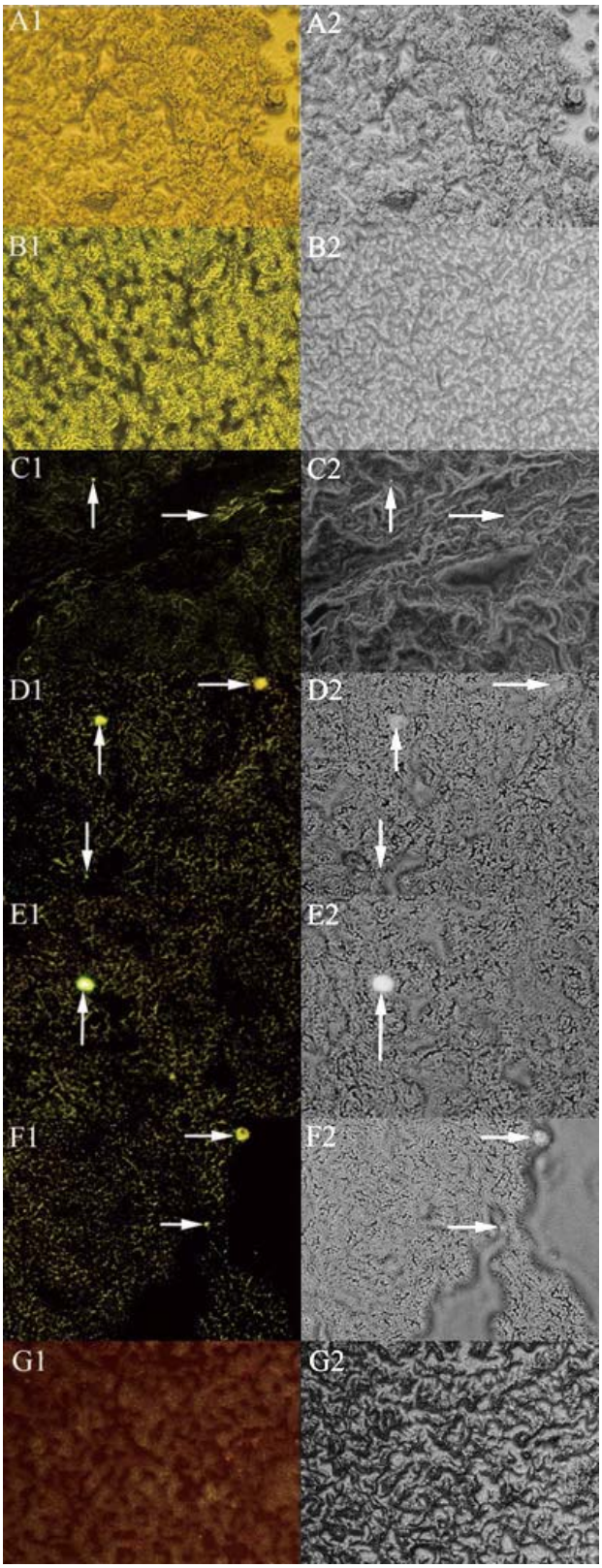

Figure S7. Fluorescence microscopy images (200×) of the kidney freezing slices for blank group and experiment groups of the mice injected with RCPTN after 0.5, 1, 2, 4, 8, and 12 hours.

Notes: (A) Images are kidney freezing slices of blank group, and (B-G) images are kidney freezing slices of experiment group which were picked at 0.5, 1, 2, 4, 8, and 12 hours, respectively. The left images (1) are green fluorescence of QCPTN with C6 (pointed to by the arrows); the right images (2) are observed from white light channel.

3.8. Pharmacokinetics study on rats

To evaluate the results related to the coefficients of correlative equations according to the Akaike information criterion (AIC) being the minimum, the concentration–time curves of RPTN, RPN and RS in rat plasma fitted a two compartment model, the weight coefficients was 1/C. The main pharmacokinetic parameters are listed in *Table S2*.

Table S2. Pharmacokinetic parameters of RPTN, RPN and RS on rats. (n=6, mean ± SD)

Parameter	Unit	RPTN	RPN	RS
A	μg/ml	2.443±0.013	2.469±0.011	2.798±0.009
α	min^{-1}	0.091±0.008	0.112±0.009	0.129±0.007
B	μg/ml	0.024±0.011$^{\#}$	0.096±0.024*	0.280±0.015
β	min^{-1}	0.021±0.012	0.032±0.016	0.035±0.018
V_C	L/kg	0.354±0.029	0.364±0.023	0.373±0.026
$t_{1/2(\alpha)}$	min	16.159±0.025	27.643±0.027*	5.370±0.021
$t_{1/2(\beta)}$	min	72.54±0.12$^{\#}$	51.31±0.15*	19.96±0.24
K_{21}	min^{-1}	0.022±0.017	0.035±0.018	0.044±0.012
K_{10}	min^{-1}	0.085±0.011	0.101±0.013	0.112±0.014
K_{12}	min^{-1}	0.0032±0.0016	0.0036±0.0012	0.0184±0.0013
AUC	(μg/ml)*min	23.013±0.028	25.196±0.026	29.898±0.029
CL	L/(kg·min)	0.032±0.015	0.087±0.014	0.173±0.016
MRT	min	129.583±0.021$^{\#}$	80.364±0.029*	42.479±0.027

*p<0.05, $^{\#}$$p$<0.01 versus corresponding RS group, respectively.

4. Discussion

As shown in *Figure S3*, the synthesized PLGA-TPGS copolymers appeared to be both biocompatible and nontoxic to cells because no significant cytotoxicity to HepG2 cells was observed at different nanoparticle concentrations. This laid the foundation for further *in vivo* studies. As the length of the incubation increased, RPTN and RPN exhibited increasingly stronger cytotoxicity against HepG2 cells than FS and RS, which was mainly attributed to the higher cellular uptake and sustained drug release patterns of RPTN and RPN, as shown in *Figure S3.* These data indicated that the RBG-loaded nanoparticles had better therapeutic effects against HepG2 cells than FS and RS. Moreover, it has been reported that TPGS plays an important role in reducing P-glycoprotein (P-gp)-mediated MDR in cancer cells and in helping drugs to permeate across cell membranes. Hence, the nanoparticles may show higher cytotoxicity. Compared to RPN, the cytotoxicity effects of RPTN were also improved against HepG2 cells, revealing that the PLGA-TPGS copolymers demonstrated a performance that was superior to that of the PLGA

copolymers in these drug-delivery systems. We also studied *in vitro* cytotoxicity using HCa-F cells, and the results were similar to those found in this study. Moreover, determining the *in vitro* cytotoxic effects of RPTN and RPN on other liver cancer cells will require further study.

LD_{50} is the key index to evaluate drug safety, which can estimate the extent of drug toxicity. Previous studies have suggested that RBG has toxicological activities such as cardiotoxic. LD_{50} value of RPTN was found to be 2.02-fold higher than that of RS, which revealed that nanoparticles reduced the toxicity of the drug. This was mainly attributing to the highly dispersed RBG in the copolymer PLGA-TPGS; RBG with the entrapment effect of the polymer matrix was slowly released from RPTN, which obviously reduced the rate of absorption and the toxic effects *in vivo*. Meanwhile, because of the liver targeting effect of RPTN, drug distribution in the non-targeted organs was decreased, thus toxicological activities to heart and kidney were avoided.

For the first time, emodin has been used as the internal standard for determining concentration of RBG in biological samples. To determine concentration of RBG, RP-HPLC method was set up and RBG was well separated from emodin under the described chromatographic condition.

The pharmacokinetics parameters were explained that the pharmacokinetics process of RPTN and RPN suspensions had great different compared with RS. RPTN group and RPN group both got longer $t_{1/2(\beta)}$ and mean retention time (MRT) than RS group, showing determinate sustained drug release, which was accordant with the results of *in vitro* drug release owing to the lower remain of NPs in the blood circulation, which was also coincident with the trend of AUC determined in the *in vivo* drug concentration for evaluating liver targeting property in mice (AUC in mice plasma of RCPTN was 0.8-fold less than that of RS). It was due to that the nanoparticles were mainly uptaken by reticuloendothelial system (RES) after injection. The nanoparticles, of which the particle size was in the range of 100 to 200 nm, got an immediate clearance from plasma by the macrophages of RES and finally gathered in lysosomes of Kupffer cells in liver [4]. Thus, the nanoparticles could deliver the drug to liver which got numerous Kupffer cells and the position was fixed, and then the entrapped drug was slowly released into liver to increase its concentration, which led to a lower drug concentration in blood circulation. Meanwhile, RPTN showed better liver targeting and consequently got less AUC compared with RPN. RPTN was faster and more completely released *in vitro* than RPN, so that RPTN had the longer retention time and the greater $t_{1/2(\beta)}$, but the less AUC in the blood circulation compared with RPN.

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