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RESEARCH ARTICLE

Targeted delivery of etoposide to cancer cells by folate-modified nanostructured lipid drug delivery system

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Abstract

Context: Cardiotoxicity and myelosuppression of etoposide (ETP) limited its clinical application. Targeted drug delivery system could deliver anticancer agents to the target cancerous cells, thus reducing their toxicity.

Objective: In this study, folate (FA) was applied for the construction of nanostructured lipid carriers (NLCs), and used for targeted delivery of ETP to tumors overexpresses the FA receptors.

Methods: FA-poly (ethylene glycol)-distearoylphosphatidylethanolamine was synthesized. FA decorated and ETP-loaded NLCs (FA-ETP-NLCs) were prepared and the formulation was optimized by Box–Behnken design. Their particle size (PS), zeta potential and drug encapsulation efficiency (EE) was evaluated. *In vitro* cytotoxicity studies of FA-ETP-NLCs were tested in CT26, SGC7901, NCI-H209 cell lines. *In vivo* antitumor efficacies of the carriers were evaluated on mice bearing CT26 cells xenografts.

Results: The optimum FA-ETP-NLCs formulations had a PS of 120.86 nm. The growth of CT26, SGC790 or NCI-H209 cells *in vitro* was obviously inhibited. FA-ETP-NLCs also displayed the best antitumor activity than other formulations *in vivo*.

Conclusion: The results demonstrated that FA-ETP-NLCs were efficient in selective delivery to CT26, SGC790 or NCI-H209 cells overexpressing the FA receptors. Also, FA-ETP-NLCs can sufficiently transfer ETP to the cancer cells, enhance the antitumor capacity. Thus, FA-ETP-NLCs could prove to be a superior nanomedicine to achieve tumor therapeutic efficacy.

Keywords

Box–Behnken design, etoposide, folic acid, nanostructured lipid carriers, tumor target

History

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Introduction

Etoposide (ETP), a semi-synthetic derivative of podophyllotoxin, is the inhibitor of deoxyribonucleic acid topoisomerase II that has a significant activity against malignant lymphoma, small cell lung cancer, stomach cancer and ovarian cancer (Yordanov et al., 2013). However, because of its low solubility, the short biological half-life (1.5 h), poor bioavailability and severe side effects, it is urgent to overcome these drawbacks and improve the clinical therapy effect (Wang et al., 2014).

Drug targeting and transport to solid tumors have been the area of extensive focus in the field of drug delivery to reduce off-target effects of drugs and increases their efficacies (Taghdisi et al., 2013; Yan et al., 2015). Recently, the entrapment of anticancer drugs in lipid-based nanoparticles can be beneficial for cancer chemotherapy because they combine the advantages of fat emulsions, polymeric nanoparticles and liposomes while simultaneously overcoming their drawbacks (Grinberg et al., 2014). Recent attempts to develop lipid-based nanoparticles for ETP include liposomes, solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) (Zhang et al., 2011; Kuo & Wang, 2014; Wang et al., 2014). In our previous study, we designed ETP-NLCs and evaluated their *in vitro* and *in vivo* antitumor effect against human gastric cancer cells (SGC7901 cells). Results demonstrated that the ETP-loaded NLCs might be a promising nanomedicine for the treatment of gastric carcinoma (Jiang et al., 2015). In order to evaluate antitumor effect against various cancers of ETP-NLCs, we further constructed long circulation and targeted NLCs for ETP.

NLCs, the second generation of SLNs, were developed in the midlines of the 1990s as an alternative carrier system to the existing traditional carries (Singh et al., 2015). Poly (ethylene glycol)-distearoylphosphatidylethanolamine (PEG-DSPE) block copolymers have been widely applied in the preparation of lipid-based nanoparticles. PEG-DSPE for the formation of nanostructures could prolong the body circulation time and release drugs at a sustained rate (Wang et al., 2012). Moreover, the terminal groups of PEG can be linked to various targeting ligands on the surface of nanocarriers.

Folic acid (FA) is a stable, inexpensive and non-immunogenic chemical with a high affinity for folate receptor which is frequently overexpressed on the surface of human cancer cells such as lung, colon cancer and glioblastoma multiforme (Kuo & Lee, 2015; Wang et al., 2015). It has been found that

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the half maximal inhibitory concentration (IC_{50}) of ETP using FA targeted dextran stearate polymeric micelles was 0.49 μg/ml for CT-26 cells while 9.41 μg/ml for the pure drug (Varshosaz et al., 2014). In a study on the antitumor activity, the uptake of FA-modified polymeric nanoparticles loaded with ETP by Hela cells and L929 cells was much higher than that of normal fibroblast cells (Kılıçay et al., 2011). Thus, FA decorated NLCs can be potential carriers for tumor-targeted drug delivery.

The experimental design has been frequently applied for nanoparticle optimization. Box–Behnken design is one of the most efficient designs for response surface methodologies that could be a suitable approach for understanding the effects of formulation variables and the interactions between factors on the responses (Ji et al., 2015). Therefore, in this present study, FA-PEG-DSPE was synthesized. Based on the previously optimized formulation of ETP-NLCs (Jiang et al., 2015), we optimized the FA decorated ETP-NLCs formulation containing lipid/drug ratio, the amount of surfactant and FA-PEG-DSPE by Box–Behnken design. We investigated the *in vitro* cytotoxicity studies of FA-ETP-NLCs in three cell lines (CT26, SGC7901 and NCI-H209 cell lines). *In vivo* antitumor efficacies of the carriers were evaluated on mice bearing CT26 cells xenografts.

Materials and methods

Materials

ETP was provided by Qilu Pharmaceutical Co. Ltd. (Jinan, China). FA, glycerol monostearate (GM), soybean phosphatidylcholine (SPC), oleic acid and 3-[4,5-dimehyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide (MTT), were purchased from Sigma-Aldrich (St Louis, MO). PEGDSPE was purchased from Corden Pharma International (Plankstadt, Germany). Labrafac PG (propylene glycol dicaprylocaprate) was a kind offer from Gattefossé (Gennevilliers, France). 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) were obtained from Avanti Polar Lipids (Alabaster, AL). All other chemicals were of analytical grade or high-performance liquid chromatography grade.

Cells and animals

CT26, SGC7901 and NCI-H209 cells were obtained from the American type culture collection (Manassas, VA). Cells were cultured in Dulbecco's modified Eagle's medium (Sigma) supplemented with 10% fetal bovine serum (FBS) (Fisher Chemicals, Fairlawn, NJ) in a 5% CO_2 fully humidified atmosphere. BALB/c nude mice (4–6 weeks old, 18–22 g weight) were purchased from Medical Animal Test Center of Shandong Province (Jinan, China), and were maintained under specific pathogen-free conditions.

Synthesis of FA-PEG-DSPE

FA-PEG-DSPE was synthesized by conjugating FA to DSPE-PEG-NH_2 through the amide bond (Zhang & Yao, 2012; Zhang et al., 2015a). Briefly, 5 mg of FA, 5 mg of DCC and 10 mg of NHS were dissolved in dimethyl sulfoxide (DMSO) for 18 h in a dark place. Then, DSPE-PEG-NH_2 was added to the resulting solution and the mixture was stirred for 24 h under nitrogen. Then, the reactants were isolation in a gel column (Sephadex G-50) eluted by 0.1 M $NaHCO_3$ and 0.05 M acetic acid solution, respectively, to remove DMSO and unreacted FA. The resulting solution was then lyophilized and kept at 4 °C until use. The production rate of the FA-PEG-DSPE was 73.5%.

Construction of FA-ETP-NLCs

FA-ETP-NLCs were constructed by preparing the solvent injection technique (Jiang et al., 2015). Briefly, 10 mg of ETP, 50 mg of GM, 50 mg of SPC and 5 ml of oleic acid were dissolved in isopropyl alcohol with heating at 80 °C. The resulting solution was rapidly injected into the aqueous phase containing 20 mg of FA-PEG-DSPE and 1 mg of DOTAP. The resulting mixture was continuously stirred at 600 rpm for 30 min. Thereafter, the dispersion was centrifuged at 10 000 rpm for 10 min and aggregates were resuspended in double-distilled water. ETP-loaded NLCs do not contain FA (ETP-NLCs) were prepared using the same method without adding the FA-PEG-DSPE. Blank NLCs do not contain ETP (NLCs) were prepared using the same method without adding the FA-PEG-DSPE and ETP. The FA-ETP-NLCs, FA-ETP-NLCs and NLCs were stored in 2–8 °C for further research.

Characterization of NLCs

The surface morphology of the FA-ETP-NLCs was examined by transmission electronic microscopy (TEM). The particle size (PS), size distribution and zeta potential of the prepared NLCs was measured by photon correlation spectrometer (Zetasizer 3000HS, Malvern Instruments Co. Ltd., Malvern, UK) (Taratula et al., 2013).

The amount of ETP encapsulated in the FA-ETP-NLCs and ETP-NLCs was determined by UV-Vis method (Wang et al., 2014). Samples were dissolved by adding a specific amount of ethanol and the concentration of the ETP was determined with a UV-Vis spectrophotometer. The selected wavelength for ETP measurement was 285 nm. The concentration of the ETP was calculated according to an already-obtained calibrating curve. The drug encapsulation efficiency (EE) and drug loading content (DL) was calculated as follows:

$$\text{EE}\ (\%) = (\text{Weight of ETP in NLCs})/(\text{Weight of ETP totally added}) \times 100.$$

$$\text{DL}\ (\%) = (\text{Weight of ETP in NLCs})/(\text{Weight of total carriers}) \times 100.$$

Box–Behnken experimental design

A 17-run, 3-factor, 3-level Box–Behnken design was used for exploring quadratic response surfaces and constructing second-order polynomial models with Design Expert software version 8.0.5b (Stat-Ease Inc., Minneapolis, MN) (Table 1). The independent variables were lipid/drug ratio (5–15), surfactant concentration (1–3%) and FA-PEG-DSPE concentration (0.2–0.6%), whereas the dependent variables constituted PS, polydispersity index (PDI) and drug EE.

Table 1. The Box–Behnken design of FA-ETP-NLCs and evaluated response parameters.

Formulation codes	Lipid/drug ratio (X_1)	Surfactant (X_2) (%w/v)	FA-PEG-DSPE (X_3) (%w/v)	Particle size (nm)	PDI	EE (%)
1	15	3	0.4	126.87	0.172	77.28
2	10	2	0.4	126.23	0.143	82.47
3	10	3	0.2	142.01	0.201	75.26
4	10	2	0.4	124.25	0.138	82.61
5	15	2	0.2	118.16	0.183	78.95
6	15	1	0.4	202.73	0.329	75.33
7	5	1	0.4	119.37	0.188	76.24
8	10	1	0.2	125.57	0.19	80.11
9	5	3	0.4	258.19	0.4	68.32
10	10	2	0.4	123.45	0.141	82.76
11	10	2	0.4	125.32	0.136	82.64
12	5	2	0.6	309.21	0.247	76.27
13	5	2	0.2	137.78	0.203	77.34
14	15	2	0.6	273.15	0.19	82.31
15	10	3	0.6	312.46	0.248	78
16	10	2	0.4	122.77	0.146	82.81
17	10	1	0.6	274.38	0.206	78.38

The optimized FA-ETP-NLCs formula was selected on the basis of smaller PS, smaller PDI and higher EE.

Serum stability

Serum stability of FA-ETP-NLCs was evaluated by incubation of the NLCs with 50% (v/v) FBS at 37 °C for 24 h (Beg et al., 2015). At scheduled times (0, 4, 8, 16 and 24 h), 100 µl volumes were withdrawn, diluted in Milli-Q water and the size and PDI and EE were measured. At each time point, 1 ml of each sample was diluted with 2 ml THF and the mixture was bath sonicated for 5 min, followed by centrifugation at 10 000 rpm for 5 min. The variation trends of the EE were measured by UV-Vis method the same as the above section.

In vitro drug release

In vitro ETP release from FA-ETP-NLCs and ETP-NLCs was assessed by the dialysis method (Song et al., 2015). FA-ETP-NLCs and ETP-NLCs were positioned in the dialysis bag separately. Then, the bag was incubated with 50 ml of release medium at 37 °C (0.1% Tween-80 in PBS, pH 7.4). One milliliter of the medium was harvested at predetermined time points and replaced with 50 ml of fresh medium. The concentrations of released ETP were determined by the UV-Vis method mentioned above.

Cell viability study

The CT26, SGC7901 and NCI-H209 cells were treated with FA-ETP-NLCs, ETP-NLCs, ETP solution and NLCs, respectively (Mishra et al., 2015). The cells were seeded in a 96-well plate and allowed to adhere for 24 h prior to the assay. The cells were subsequently treated with 0.2 ml serum-free medium containing various concentrations of NLCs. After 72 h of incubation, a total of 20 µl (5 mg/ml) of MTT dye solution was added to each well before the cells were incubated for 4 h at 37 °C under a light-blocking condition. The medium was then removed and 150 µl of DMSO was added into each well. Relative viability was obtained from the absorbance at 590 nm of the treated cells divided by the absorbance at 590 nm of the untreated cells.

Tissues distribution assay

Tissues distribution assay was investigated in gastric tumor-bearing BALB/c nude mice models (Zhang et al., 2015b). BALB/c mice were inoculating subcutaneously (s.c.) in the left armpit with 100 µl SGC7901 cells suspended in PBS. When tumor volume reached about 100 mm^3, FA-ETP-NLCs, ETP-NLCs and ETP solution were given into the mice by tail vein injection, separately. At predetermined time intervals, mice were sacrificed and the tumor, heart, liver, spleen, lung and kidney of mice were collected and stored. Tissues were initially weighed and homogenized with physiological saline to determine the amount of ETP in each tissue. The concentrations of released ETP were determined by the UV-Vis method mentioned above.

Antitumor effects *in vivo*

The antitumor effects of NLCs were investigated in gastric tumor-bearing BALB/c nude mice models (Tian et al., 2014). BALB/c mice were inoculating s.c. in the left armpit with SGC7901 cells suspended in PBS. When tumor volume reached about 100 mm^3, FA-ETP-NLCs, ETP-NLCs, ETP solution, NLCs and 0.9% saline were injected through the tail vein of the mice, once every 3 d. Twenty-one days later, all the mice were sacrificed by cervical dislocation and the tumor tissue samples were taken out. Tumor volume of each mouse was measured with a digital caliper every 3 d, and was calculated according to the below equation:

$$\text{Tumor volume } \left(mm^3\right) = (\text{longest diameter}) \times (\text{shortest diameter})\ 2/2$$

The tumor inhibition efficiency was calculated according to the tumor volume results:

Tumor inhibition efficiency (%) = (volume of control − volume of treated)/volume of control × 100.

The behaviors and the body weight loss of the mice during the *in vivo* study were also observed.

Statistical analysis

All the results expressed as means ± standard deviation were representative of three independent experiments. Statistical data analysis was performed using the Student's *t*-test with $p < 0.05$ as the minimum level of significance.

Results and discussion

Box–Behnken experimental design

A 3-factor, 3-level Box–Behnken design was applied to precisely understand the effect of lipid/drug ratio (X_1), amount of surfactant (X_2) and amount of FA-PEG-DSPE (X_3) on PS, PDI and EE of FA-ETP-NLCs. The results of the experimental run were shown in Table 1.

Quadratic equations establishing main effects and interaction factors were determined. Statistical validation of quadratic equations was confirmed by ANOVA. The Model F-value of PS, PDI and EE were 2300.11, 662.44 and 512.71, respectively, which indicate the model was significant. Values of ''Prob > F'' less than 0.05 demonstrated model terms such as X_1, X_2, X_3, X_1X_2, X_1X_3, X_2X_3, etc., were significant model terms. Non-significant lack of fit for PS, PDI and EE imply that models were well fitted.

The effect could be explained by the following quadratic equations:

$$Y_1(\text{PS}) = 124.40 - 12.95X_1 + 14.69X_2 + 80.71X_3 - 53.67X_1X_2 - 4.11X_1X_3 + 5.41X_2X_3 + 24.18X_1^2 + 28.21X_2^2 + 60.99X_3^2$$

$$Y_2(\text{PDI}) = 0.14 - 0.020X_1 + 0.014X_2 + 0.014X_3 - 0.092X_1X_2 - 0.0093X_1X_3 + 0.0078X_2X_3 + 0.063X_1^2 + 0.068X_2^2 + 0.002X_3^2$$

$$Y_3(\text{EE}) = 82.66 + 1.96X_1 - 1.40X_2 + 0.41X_3 + 2.47X_1X_2 + 1.11X_1X_3 + 1.12X_2X_3 - 3.79X_1^2 - 4.57X_2^2 - 0.15X_3^2.$$

Response surface analyses plotted in three-dimensional model graphs for depicting the effects of the predetermined factors on the PS, PDI or EE were shown in Figure 1.

Optimization and validation

The optimum FA-ETP-NLCs formulation was based on the set criteria of smaller PS, minimum PDI and maximum EE. The composition of optimized formulation was as follows: lipid/drug ratio was 10.86:1, the amount of surfactant was 2.03% and the amount of FA-PEG-DSPE was 0.39%. A new batch of NLCs with the predicted levels was prepared. PS, PDI and EE of the optimized formulation were 120.86 nm, 0.142 and 82.67%, respectively, which were in good agreement with the predicted values. The DL of optimized formulation was 1.65%. The zeta potential of FA-ETP-NLCs was +21.3 mV. Surface charge is an important indication for the structure and stability of nanoparticle system. The positive charged particle surfaces could have a higher binding affinity toward the negatively charged cell surfaces (Mishra et al., 2015).

TEM images clearly delineated that the FA-ETP-NLCs had spheroidal shapes with the lighter shell on the darker core (Figure 2). We consider the darker core as ETP-loaded NLCs, the lighter shell is the FA-PEG-DSPE coating. The size of FA-ETP-NLCs was about 120 nm. PS is a key influence factor

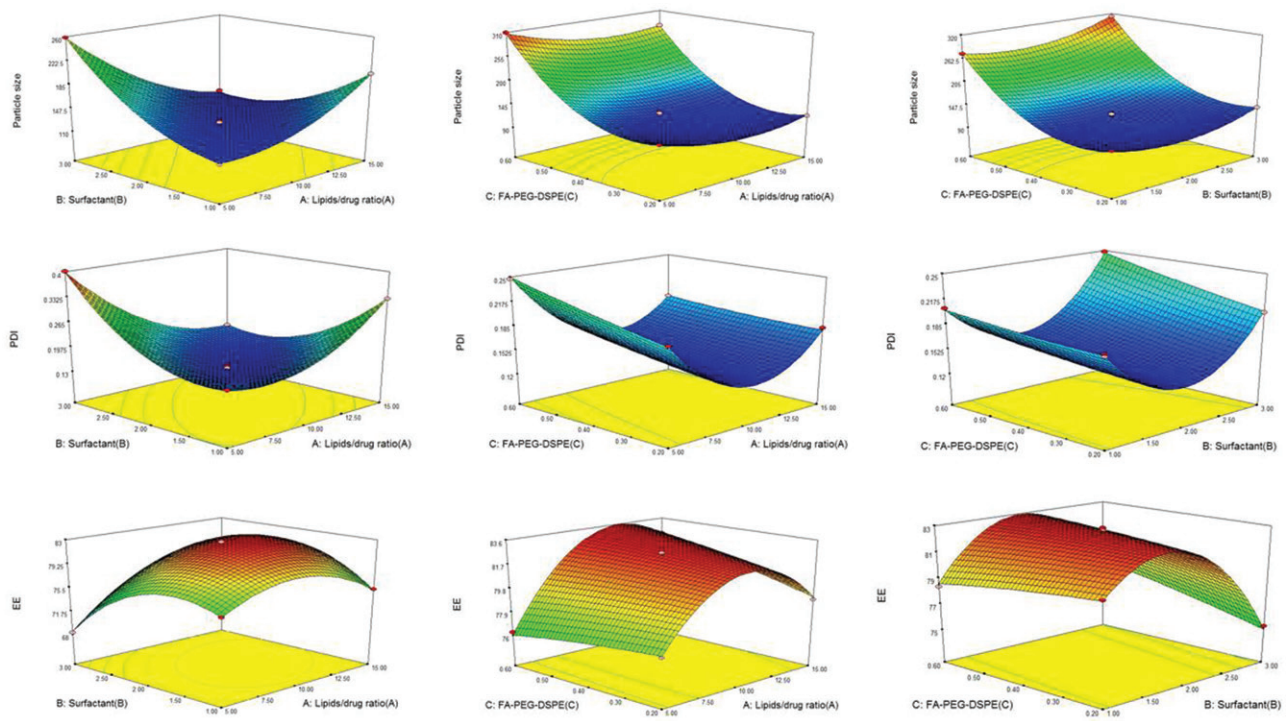


Figure 1. Response surface analyses plotted in three-dimensional model graphs for depicting the effects of the predetermined factors on the PS, PDI or EE.

of the carriers: The size of carriers has a great impact on the *in vitro* cell toxicity and *in vivo* delivery efficiency of the NPs, including decreased uptake by the liver, and prolonged the blood circulation time (Liu et al., 2015). The small PS of FA-ETP-NLCs is expected to enhance the adhesion with biological cells, increase the cellular uptake of delivery systems and improved bioavailability of ETP. The EE is another fundamental effect of drug therapeutic effect. The high EE achieved by the FA-ETP-NLCs could prove that the solvent injection technique and the excipients used are appropriate for the loading of ETP into the NLCs.

Serum stability

Stability of FA-ETP-NLCs and other NLCs in serum was evaluated and described in Table 2. Blank and drug-loaded NLCs were stable up to 24 h without any significant size, PDI or EE changes. The mean diameters of all kinds of NLCs in serum remained at around 100 nm making them suitable for an efficient tumor targeting following intravenous administration (Yoon et al., 2015). The size distribution after 24 h of incubation was similar to the initial measurement. A narrow size distribution may also contribute to accurate drug delivery. It can therefore be surmised that the NLCs were considered stable in the presence of FBS, the particles will not aggregate and the drug loaded in the NLCs will not leak out. The results illustrated that the FA-ETP-NLCs was stable in serum and suggests that they will exhibit high stability *in vivo* following intravenous administration.

In vitro ETP release from ETP-NLCs

The *in vitro* release profiles of FA-ETP-NLCs, ETP-NLCs, as well as ETP solution were depicted in Figure 3. The drug release of ETP solution was fast, over 50% of ETP release was observed at 2 h and more than 80% of the drug was released after 4 h. FA-ETP-NLCs, ETP-NLCs showed the sustained-release behavior, and the release of FA-ETP-NLCs was slower than ETP-NLCs. The sustained-release mechanism could belong to drug diffusion, lipid matrix swelling and the carrier erosion or degradation. Also, the coating of FA-PEG-DSPE on the surface layer of the NLCs may lead to the more sustained release. The sustained release can be beneficial for drugs with irritation effects at high concentrations (Feng et al., 2014). This behavior may assist with the antitumor progress bringing about the continuous antitumor effect.

Cell viability study

The ability of FA-ETP-NLCs, ETP-NLCs, ETP solution was evaluated on CT26, SGC7901 and NCI-H209 cells using the MTT assay. The IC_{50} values of various formulations in different cells were summarized in Table 3. As we can tell from the results in all the three kinds of tested cells, the IC_{50} values of ETP-NLCs were 4–5-fold dose advantage over ETP

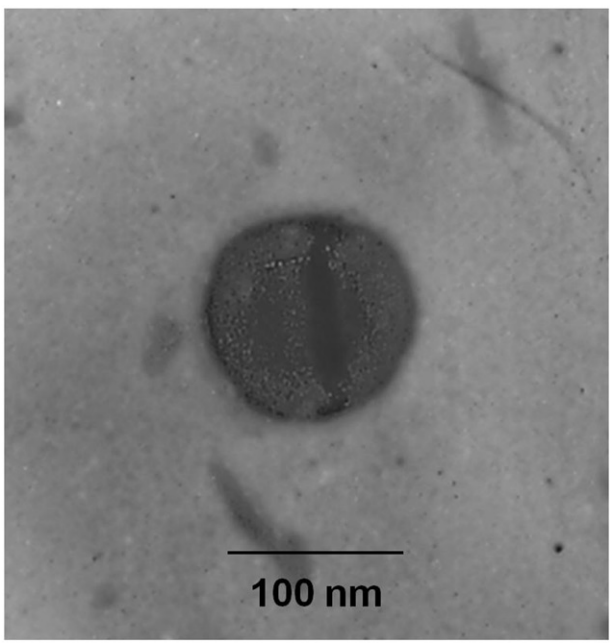


Figure 2. TEM image of FA-ETP-NLCs.

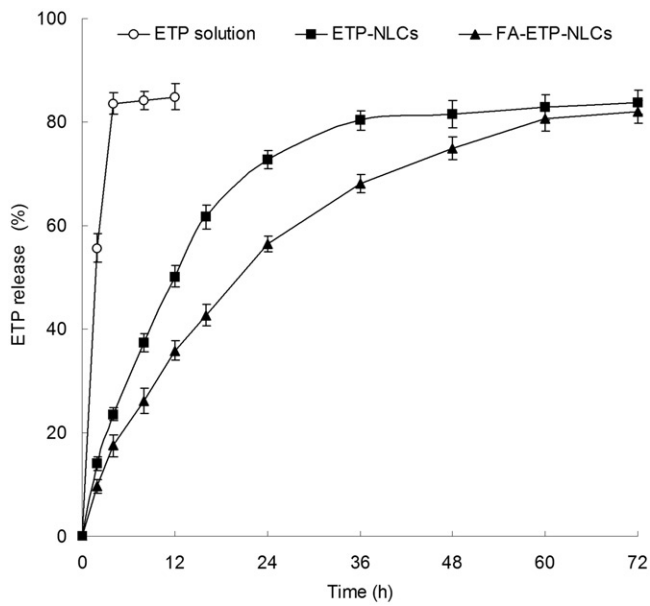


Figure 3. The *in vitro* release profiles of FA-ETP-NLCs, ETP-NLCs and ETP solution.

Table 2. Serum stability of NLCs.

	Particle size (nm)		Size distribution (PDI)		EE (%)	
Samples	Milli-Q water	50% FBS	Milli-Q water	50% FBS	Milli-Q water	50% FBS
Blank NLCs	81.74 ± 2.31	82.35 ± 3.86	0.091 ± 0.009	0.112 ± 0.019	/	/
ETP-NLCs	82.16 ± 2.87	83.94 ± 3.17	0.116 ± 0.013	0.136 ± 0.016	84.71 ± 2.14	83.59 ± 3.76
FA-ETP-NLCs	120.91 ± 3.59	122.13 ± 4.41	0.139 ± 0.026	0.181 ± 0.033	83.86 ± 3.21	82.92 ± 4.18

solution; and the IC_{50} values of FA-ETP-NLCs were 3–4-fold dose advantage over ETP-NLCs. FA-ETP-NLCs showed significantly higher inhibition rates and obviously higher suppression efficiency than ETP-NLCs and ETP solution. It could be explained that effective property of NLCs formula could facilitate greater cellular uptake than free drugs. The mechanism of action of ETP NLCs was proposed to be a cell uptake followed by a sustained drug release from the NLCs in combination with an intracellular P-gp inhibition ensuring a higher anticancer drug concentration inside the cancer cells (Varshosaz et al., 2014). Moreover, with the adding of the FA ligands, better cancer cell delivery effect of NLCs was observed than their non-modified ETP-NLCs counterparts.

In vivo tissues distribution and antitumor efficacy

The *in vivo* tissues distribution and antitumor efficacy of NLCs were investigated in SGC7901 gastric tumor-bearing BALB/c nude mice models. Tissue distribution results of FA-ETP-NLCs, ETP-NLCs and ETP solution were showed in Figures 4–6. Figures 4 and 5 illustrated that the distribution of ETP in FA-ETP-NLCs and ETP-NLCs was higher in the tumor tissue compared with the other tissues, which was expected to reduce the side effects. However, the drug solution mainly distributes in heart and kidney, this could lead to systemic toxicity (Figure 6). The higher concentration in the tumor tissue remained relatively stable at all time points until 24 h after injection, indicate the sustained-release behavior of the FA-ETP-NLCs.

Table 3. IC_{50} values (μg/ml) of various formulations in different cells.

Cells	ETP solution	ETP-NLCs	FA-ETP-NLCs
CT26	11.81 ± 0.86	2.13 ± 0.34	0.58 ± 0.11
SGC7901	57.2 ± 3.15	10.46 ± 0.98	3.87 ± 0.59
NCI-H209	41.75 ± 4.37	12.37 ± 1.08	4.42 ± 0.85

Figure 7 illustrated *in vivo* antitumor efficacy of different formulations in gastric tumor-bearing mice. The increase of tumor volume was substantially inhibited by ETP-loaded NLCs groups than ETP solution group ($p < 0.05$). The blank NLCs and saline control groups did not have any impact on tumor inhibition ($p > 0.05$). Tumor volume of the FA-ETP-NLCs group was about 219 mm^3 after 21 d of treatment, obviously smaller than ETP-NLCs group (416 mm^3) and ETP solution group (1130 mm^3). Tumor volume of blank NLCs and saline control group reached 1806 and 1815 mm^3, respectively. The tumor inhibition efficiency of FA-ETP-NLCs, ETP-NLCs and was 88%, 77% and 38%, respectively. Better tumor inhibition efficiency indicates that FA-ETP-NLCs showed better antitumor activity than ETP-NLCs and ETP solution for the treatment of gastric cancer *in vivo* (Dai et al., 2015).

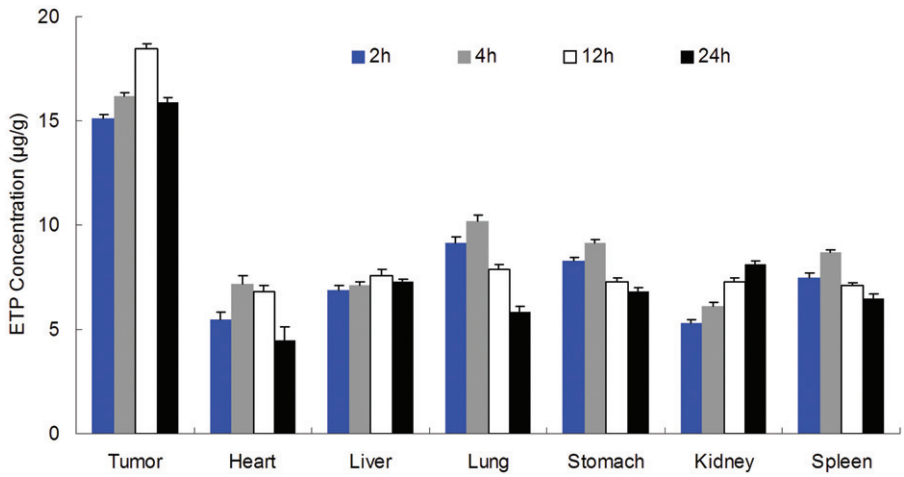


Figure 4. Tissue distribution results of FA-ETP-NLCs.

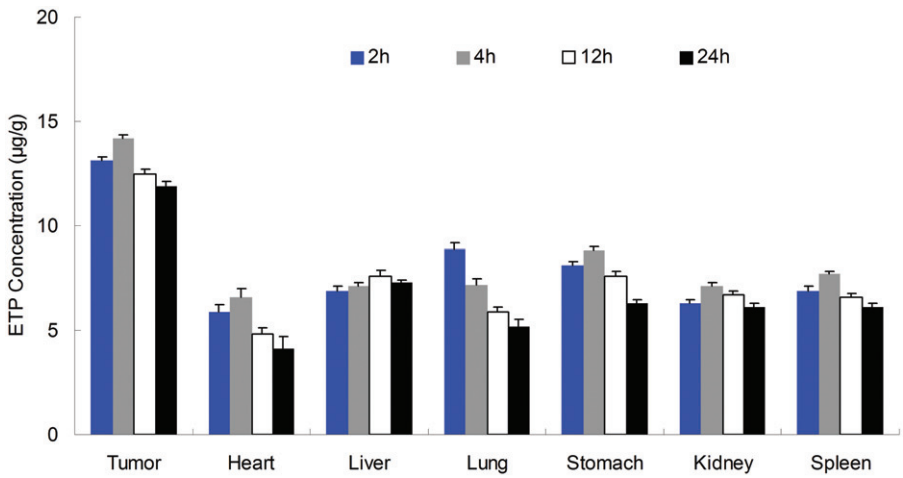
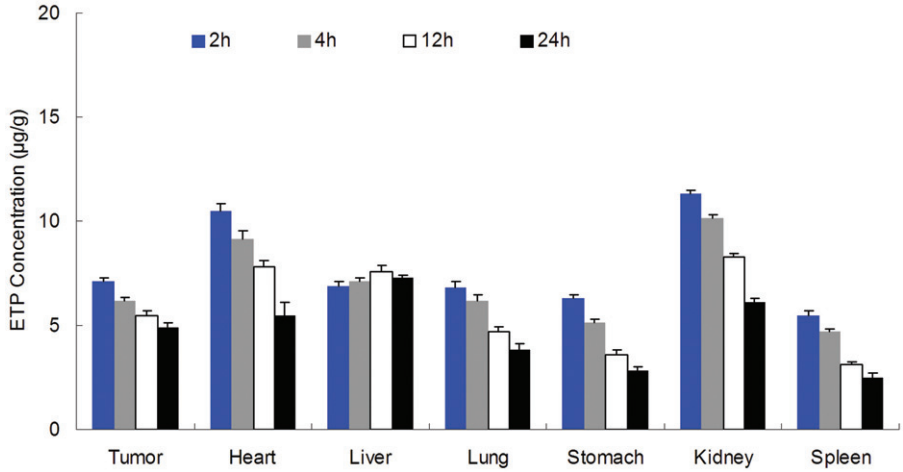


Figure 5. Tissue distribution results of ETP-NLCs.

Figure 6. Tissue distribution results of ETP solution.



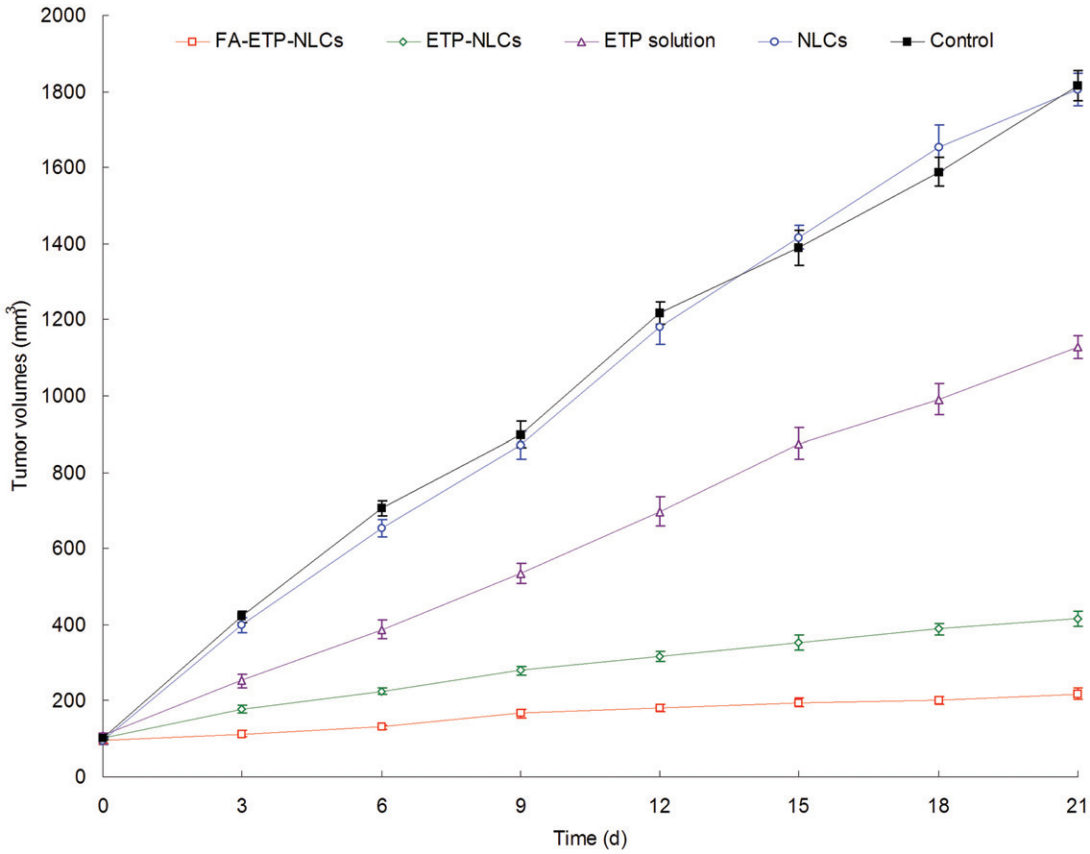


Figure 7. *In vivo* antitumor efficacy of different formulations in gastric tumor-bearing mice.

Obviously, decreased body weights, reduced foods intake and inactive moving behavior were found in ETP solution, blank NLCs and saline control groups. In contrast, stable body weights, normal food intake and movement were found in FA-ETP-NLCs and ETP-NLCs groups. These results may suggest the less systemic toxic side effect of the NLCs formulations for the treatment of gastric cancer *in vivo*.

Conclusion

FA-modified, ETP-loaded NLCs-FA-ETP-NLCs were developed in this study. The optimized FA-ETP-NLCs have small PS, narrow size distribution and high drug EE. FA-ETP-NLCs showed the highest cytotoxicity in three kinds of tumor cells *in vitro*. The *in vivo* study illustrated that FA-ETP-NLCs had the best biodistribution in tumor tissue and the highest antitumor activity on gastric cancer animal model compared to ETP-NLCs and ETP solution. It could be concluded that FA-ETP-NLCs could be used as a promising system for the treatment of cancer.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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