Drug Design, Development and Therapy

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

RETRACTED ARTICLE: Development of a nanoliposomal formulation of erlotinib for lung cancer and in vitro/in vivo antitumoral evaluation

This article was published in the following Dove Press journal:
Drug Design, Development and Therapy

Xiao Zhou
Hui Tao
Kai-Hu Shi

Department of Cardiothoracic Surgery, The Second Hospital of Anhui Medical University, Hefei, People's Republic of China

Abstract: The aim of this study was to develo PEGylation lip o s formulations of erlotinib and evaluate their characteristics, stabi and lease characteristics. The average particle sizes and entrapment efficiency of PEGylati rlotinib l osomes are 102.4±3.1 nm and 85.3%±1.8%, respectively. Transp on electron icro opy images showed that the liposomes dispersed well with a unif m sha and no chan s during the storage. The in vitro drug-release kinetic model of erlotinib release f m the PEGylation liposomes in phosphate-buffered saline fit well with t Higuchi equation. In vitro anticancer activity assay showed that the blank liposomes had low r cellular cyto oxicity and that the cellular cytotoxicity of erlotinib liposomes increased signific tly under the me incubation condition, which should contribute to the increase in intracellular g conc tration by the transportation of liposomes. The two liposomes of erl ni ith and without PEGylation) exhibited similar cellular cytotoxicity with no significant diffe nt centrations. Pharmacokinetic results indicated that erlotinib-loaded P lation l somes can significantly change the pharmacokinetic behavior of drugs and mprove he drug ioavailability by nearly 2 times compared to ordinary liposomes. No s n of dam g such a the appearance of epithelial necrosis or sloughing of epithelial cells was t ted in histological studies.

Keywo : cellular cytotoxicity, drug-release, erlotinib, PEGylation liposomes, pharmacokinetic

Introduction

Erlotinib is a potent and selective tyrosine kinase inhibitor (TKI). It reversibly binds the binding site of ATP in the epidermal growth factor receptor tyrosine kinase domain to inhibit the automatic phosphorylation of tyrosine kinases.[1,2] Inhibition of tyrosine kinase can promote apoptosis, inhibit angiogenesis, and ultimately prevent excessive cell proliferation.[3–5] Erlotinib in treatment of various solid tumors such as non-small-cell lung cancer is available in oral form.[6,7] Oral bioavailability of erlotinib in healthy volunteers and cancer patients was 59% and 76%, respectively.[8,9] There are no data on other forms of such drugs. The new erlotinib delivery system is limited due to its poor solubility. Nevertheless, solid lipid nanoparticles containing erlotinib were produced by Bakhtiary et al[10] recently. Moreover, Dora et al[11] found that cyclodextrin nanosponge containing erlotinib could enhance oral bioavailability with less cytotoxicity. In another study conducted by Barghi et al,[12] erlotinib-loaded functional material nanoparticles were introduced.

Although erlotinib is orally active and has acceptable bioavailability,[13] we hypothesized that drug delivery approaches could improve the efficacy of combination therapies with EGFR inhibitors. Erlotinib accumulates in tumor, skin, kidney, and liver

Correspondence: Kai-Hu Shi
Department of Cardiothoracic Surgery, The Second Hospital of Anhui Medical University, Fu Rong Road, Hefei, Anhui Province 230601, People's Republic of China
Tel/fax +86 551 6386 9531
Email kaihushi@21cn.com

submit your manuscript | www.dovepress.com
Dovepress
http://dx.doi.org/10.2147/DDDT.S146925

at levels significantly higher than in plasma,[14] most likely because of its hydrophobicity. Encapsulation in carriers such as liposomes would reduce concentrations of free drug in the blood and thereby limit deposition in normal tissues such as bone marrow.[15] Accumulation of erlotinib in tumors is just 2 times greater than in skin, and previous studies have shown that liposome encapsulation can result in higher tumor drug concentration compared with that in skin.[16]

Over the past 20 years, liposomal drug delivery systems have held extraordinary potential for the delivery of therapeutics to tumors, and various strategies have been used to improve their targeting specificity and cellular uptake. Earlier studies attempted to load TKI into liposomes at higher percentages, which led to rapid leakage/precipitation during storage or immediately after liposome preparation.[17] Moreover, PEGylation of liposomes aimed to extend the blood half-life of loaded erlotinib served as a factor triggering the leakage of loaded erlotinib.[18] PEGylation has been widely adopted to improve the accumulation of liposomes in tumor tissues through enhanced permeability and retention effects, which is a passive form of targeting.[19]

The primary objective of this work is to develop liposomal formulations of erlotinib and evaluate their characteristics, stability, and release characteristics. The targeti[illegible] and antitumor efficiency in lung cancer were also evaluate[illegible] in vitro and in vivo.

Materials and methods

Materials

Erlotinib was purchased from Sun Ph[illegible]a Ltd., Co. (Mumbai, India). Soybean phosphatidylcho[illegible]e (SP[illegible] DSPE-PEG2000, DSPE, and cholesterol (CH[illegible]L) were obtai[illegible]d from Sinopharm Chemical Reagent (Shanghai, People's Republic of China). A549 cell was p[illegible]rchas[illegible]d from Cobioer Biomedical Ltd., Co. (Nanjin[illegible] People[illegible] Republi[illegible] of China). All other reagents were o[illegible]taine[illegible] from S[illegible]harm Chemical Reagent (Shanghai, [illegible]ople's [illegible]ublic of China). Methanol and acetonitrile (chroma[illegible]graphic grade) were obtained from Sigma Aldrich (St Louis, MO, USA). Water for high-performance liquid chromatography was double distilled, and all other reagents were of analytical grade.

Preparation of erlotinib liposomes

The PEGylation liposomes containing erlotinib were prepared by the thin-film hydration method as described previously.[20] Briefly, erlotinib, SPC, CHOL, and PEG-DSPE were mixed, with the molar ratio of PEG-DSPE:CHOL:SPC being 3:10:22 and the weight ratio of lipid:erlotinib being 17:1. The solvent was then evaporated using an RE52 rotary evaporator (Shanghai Yarong Biochemistry Instrument Company, Shanghai, People's Republic of China) in a round-bottomed flask at 40°C for about 40 minutes to obtain a solid film. This film was then flushed with nitrogen for 30 minutes and stored overnight in a desiccator to remove any traces of chloroform. After that, the thin film was hydrated by sonication in 5% glucose solution and placed in water bath for 10 minutes to produce a suspension of liposomes. And then the liposomes were frozen for 72 hours. The dry powder was rehydrated and sonicated for 3 minutes before a[illegible]lication. For the preparation of erlotinib liposomes [illegible]ithout P[illegible], a similar procedure was carried out except [illegible]at the PEG-[illegible]SPE was replaced by DSPE.

Characterization [illegible]f li[illegible]osomes

The mean partic[illegible] size and [illegible]olydis[illegible]rsity index (PDI) of liposome dis[illegible]ers[illegible]s were det[illegible]ined by dynamic light-scattering method usi[illegible] fiber-optics particle analyzer. The syste[illegible] was set in the au[illegible]-measuring mode. Particle size ana[illegible]sis data were evaluated using volume distribution to dete[illegible] large drop[illegible]s.

Th[illegible]ntrapm[illegible]t efficiency (% EE) was determined by [illegible]easuring the concentration of unentrapped drug in the li[illegible]d d[illegible]ersion. Briefly, erlotinib liposomes were placed [illegible]n a sedimentation centrifuge for 10 minutes at 4°C and [illegible]2,000 rpm, and the amount of erlotinib in supernatant was determined by dissolving the supernatant in an acetone and ethanol (1:2) mixture at 332 nm by high-performance liquid chromatography. The drug encapsulation efficiency was calculated by the following equation:

$$EE\% = \frac{W_{erlotinib}}{W_{liposomes} + W_{erlotinib}} \times 100\%$$

where $W_{erlotinib}$ represents the amount of erlotinib loaded in the liposomes and $W_{liposomes}$ represents the weight of erlotinib liposomes.

The stability of erlotinib liposomes was evaluated by monitoring the changes in mean particle size, PDI, and EE% during storage at 4°C or room temperature. The morphology of erlotinib liposomes by the negative staining transmission electron microscopy (TEM) method was also evaluated. The samples were added to the surface of copper grids and stained with phosphotungstic acid (1%, w/v). The accelerating voltage was 120 kV. The samples were magnified by 100,000 times.

In vitro release study

The release test was performed using a Franz-type vertical diffusion cell.[21] Approximately 10 mg erlotinib liposomes was applied into the donor compartment of each cell, and the receptor chamber was filled with 17 mL of phosphate buffer solution (pH 7.4). Phosphate buffer solution was used to solubilize the leaked erlotinib as a hydrotropic agent. Magnetic stirring speed and temperature were set at 600 rpm and 37°C respectively. Dialysis membrane (molecular weight cutoff 12,000–14,000) was placed between the donor compartment cell and receptor chamber. An aliquot of the sample (2 mL for each time point) was taken from the dissolution medium at different time intervals (0.5, 1, 2, 4, 6, 8, 12, 24, 36, 72, and 96 hours) and analyzed for its drug concentration at 332 nm. The receptor chamber was replaced by an equal volume of phosphate buffer solution.

Ethics in animal experiments

All in vivo experimental protocols were approved by the animal care committee of the Faculty of Medicine, Anhui Medical University animal center, and were carried out in accordance with the guideline of experimental animals of Anhui Medical University. All Sprague–Dawley rats in this study were purchased from Anhui Medical University animal center (Hefei, People's Republic of China).

In vitro anticancer activity assay

In vitro anticancer activity of PEGylation erlotinib liposomes on human lung carcinoma A549 cells was evaluated by MTT assay. Control experiments were carried out using a complete growth culture medium as nontoxic control. A549 cells (1×10^5 cells/well in 1 mL medium) were seeded into 96-well plate. Twenty-four hours later, cells were incubated with varied concentrations of PEGylation erlotinib liposomes, erlotinib liposomes, free drugs, and blank liposomes. After 48 hours of incubation under cell culture conditions, the growth and viability of cells were determined by using MTT assay. The 0.2 mL complete growth culture medium and 60 µL MTT solution (5 mg/mL in phosphate-buffered saline [PBS]) were added to each well and incubated for 4 hours. The absorbance was determined using a microplate reader at 540 nm. The results were expressed as percentages relative to those obtained from the group with a nontoxic control.

Pharmacokinetic studies

Sprague–Dawley rats weighing from 200 to 220 g were used for pharmacokinetic study. Before the experiment, the rats were kept in a state of fasting for 6 hours. In the experiment, the rats were divided into three groups (8 each). One group was given erlotinib injection (free drug, as a control), and the other two were given erlotinib liposomes and PEGylation liposomes (dose =10 mg/kg), respectively. Then all formulations were introduced into rats via intravenous injection. Blood samples (2 mL) were collected from the tail vein into heparinized 5 mL polythene tubes just before administration and 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, and 24 hours after dosing. The plasma obtained was stored at –20°C until analysis.

Histology studies

The histopathological results of erlotinib liposomes and PEGylation liposomes were observed at the end of pharmacokinetic studies. Animals were anesthetized, and their hearts, livers, spleens, lungs, and kidneys were dissected and washed with cold saline. The organs were pressed between filter pads, weighed, and then fixed in 10% neutral formalin using standard techniques and stained with hematoxylin and eosin for histopathological examination. All tissue samples were examined and graded under light microscopy with 1,000× magnification.

Statistical analysis

Statistically significant differences between values obtained under different experimental conditions were determined using two-tailed unpaired Student's *t*-test.

Result and discussion

Characterization of liposomes

Table 1 shows the addition of PEG that did not produce significant influence of erlotinib liposomes in terms of particle size and PDI. The average particle size of erlotinib liposomes and PEGylation erlotinib liposomes was 100.1±2.6 and 102.4±3.1 nm, respectively. The PDI of all samples was <0.8. The EE of erlotinib in liposomes and PEGylation

Table 1 Characterization of PEGylation erlotinib liposomes before and after storage at 4°C (n=3)

Formulations	Time	Particle size (nm)	EE (%)	PDI
PEGylation erlotinib liposomes	0 day	102.4±3.1	85.3±1.8	<0.45
Erlotinib liposomes	0 day	100.1±2.6	84.7±2.9	<0.43
PEGylation erlotinib liposomes	1 month	101.5±2.3	84.8±2.2	<0.47
Erlotinib liposomes	1 month	101.7±1.4	84.3±1.6	<0.48
PEGylation erlotinib liposomes	2 months	101.9±2.6	84.6±2.3	<0.56
Erlotinib liposomes	2 months	102.3±2.4	84.1±2.1	<0.58
PEGylation erlotinib liposomes	3 months	102.3±2.3	83.1±1.3	<0.63
Erlotinib liposomes	3 months	102.9±3.1	83.9±2.8	<0.61

Abbreviations: EE, entrapment efficiency; PDI, polydispersity index.

liposomes were (84.7%±2.9%) and (85.3%±1.8%), respectively. The high EE in the formulation might be related to the strong hydrophobicity of erlotinib. Table 1 also gives the stability data of particle size of erlotinib liposomes stored at 4°C. After 3 months of storage, no dramatic change such as visible aggregation, drug content changes, and precipitation in the appearance of liposomes occurred. TEM images (Figure 1) showed that the liposomes dispersed well with a uniform shape and no changes during storage.

In vitro drug release

The in vitro release of erlotinib from the liposomes was investigated. Figure 2 shows the release profiles of the three groups. Compared with the group of rapid release of free drug, the other two liposome groups exhibited similar and sustained release, and no initial burst release was observed. Slow release occurred due to the fact that with the dissolution and diffusion mechanisms on the lipid matrices, the solubilized or dispersed drug could only be released slowly. As illustrated in Table 2, the in vitro drug-release kinetic model of erlotinib release from the PEGylation liposomes in PBS fits well with the Higuchi equation: $Q = 8.928t_{1/2} - 1.237$ (r=0.993). Thus, it was speculated that the sustained-release property of erlotinib liposomes could enhance cycle in vivo. The in vitro release results indicated that the release of erlotinib from PEGylation liposomes was similar to that from ordinary liposomes, demonstrating that the PEG modification did not affect erlotinib release. The addition of modified materials did not lead to significantly reduced release speed. A possible reason could be that the quantity of added material did not greatly increase the steric hindrance of liposomes.

In vitro anticancer activity assay

The A549 lung cancer cell as model cell was applied to assay the cellular cytotoxicity of erlotinib liposomes. MTT assay method was used to evaluate the viability of cells.

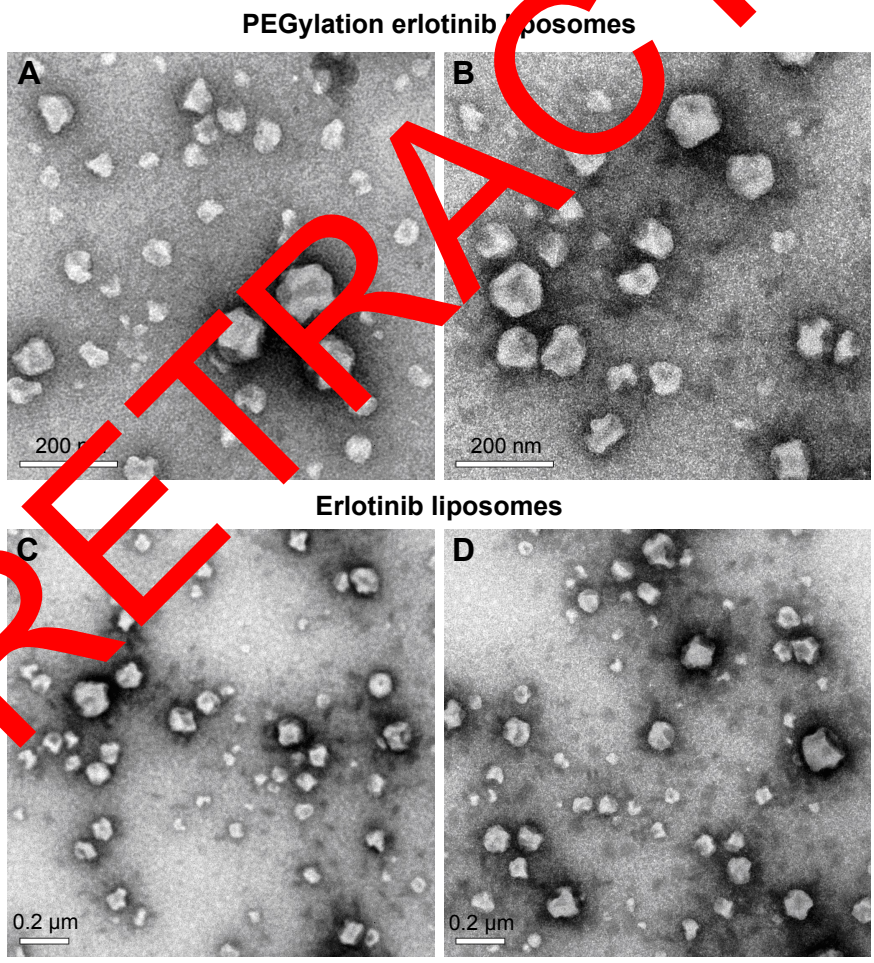

Figure 1 TEM images of PEGylation erlotinib liposomes and erlotinib liposomes before and after storage at 4°C (n=3).
Notes: (**A**) 0 day; (**B**) 3 months; (**C**) 0 day; (**D**) 3 months. Magnification ×10,000.
Abbreviation: TEM, transmission electron microscopy.

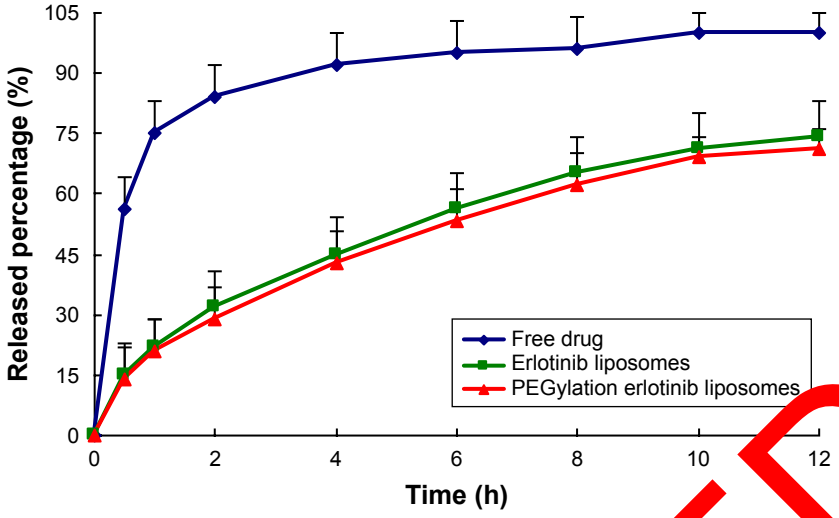

Figure 2 The in vitro drug-release profiles of erlotinib-loaded liposomes of two types and of free drug.
Note: Data are expressed as mean ± SD (n=6).
Abbreviation: SD, standard deviation.

The cellular viability of free drug, blank liposome, and erlotinib liposomes during the different incubation times is shown in Figure 3. Even after the cells were incubated for 48 hours with 0.3 mg/mL blank liposomes, the cellular viability was still >80%. This result indicates that blank liposomes had lower cellular cytotoxicity. By contrast, with the same concentration (free drug), the cellular cytotoxicity of erlotinib liposomes increased significantly under the same incubation condition, which should contribute to the increase in intracellular drug concentration by the transportation of liposomes. In addition, the two liposomes of erlotinib (with and without PEGylation) exhibited a similar trend of cellular cytotoxicity at the same concentration. With the extension of incubation time, liposomes of erlotinib (with PEGylation) showed a larger inhibitory effect on cells. PEG-modified liposomes exhibited high efficiency in the present study, which is expected to be introduced into the development of other drugs. Its value is reflected not only in the changes of erlotinib but also in the possibility of helping establish a platform to transfer the toxicity active substance to become an ideal candidate drug.[22]

Pharmacokinetic studies

The pharmacokinetic profile of erlotinib following administration in the three formulations was evaluated initially in tumor-bearing rats, with results presented in Figure 4. A dose of 10 mg/kg free drug was chosen and employed throughout all studies based on previous studies performed by our group (data not shown). As expected, both the clearance and the volume of distribution of the drug were significantly lower when administered in liposomes as free drug ($p<0.05$). Compared to ordinary liposomes, those composed of PEGylation showed relatively long terminal half-lives (96.8±11.2 hours versus 92.7±10.1 hours), lower clearance rates (0.07±0.03 mL/kg versus 0.09±0.05 mL/kg), and higher area under the curve 0–∞ values (898.5±82.4 mmol/L*h versus 625.5±72.7 mmol/L*h). They were also significantly different from free drug (Table 3). The results also showed that erlotinib-loaded PEGylation liposomes could significantly change the pharmacokinetic behavior of drugs and improve drug bioavailability nearly 2 times compared to ordinary liposomes, which was due to the prolonged in vivo circulation of drug. Furthermore, when the drug is prepared

Table 2 Release kinetic of erlotinib-loaded liposomes (n=6)

Parameters	Erlotinib liposomes		PEGylation erlotinib liposomes	
	Equation	Correlation coefficient (*r*)	Equation	Correlation coefficient (*r*)
Zero-order	Q =2.372t −1.209	0.892	Q =3.281t −1.112	0.929
First-order	Ln(1−Q) =2.093t +0.192	0.933	Ln(1−Q) =2.371t +0.293	0.934
Weibull	lnln(1/(1−Q)) =1.298 lnt +0.381	0.938	lnln(1/(1−Q)) =3.271 lnt +0.234	0.926
Higuchi	$Q = 6.867t_{1/2} - 0.878$	0.989	$Q = 8.928t_{1/2} - 1.237$	0.993

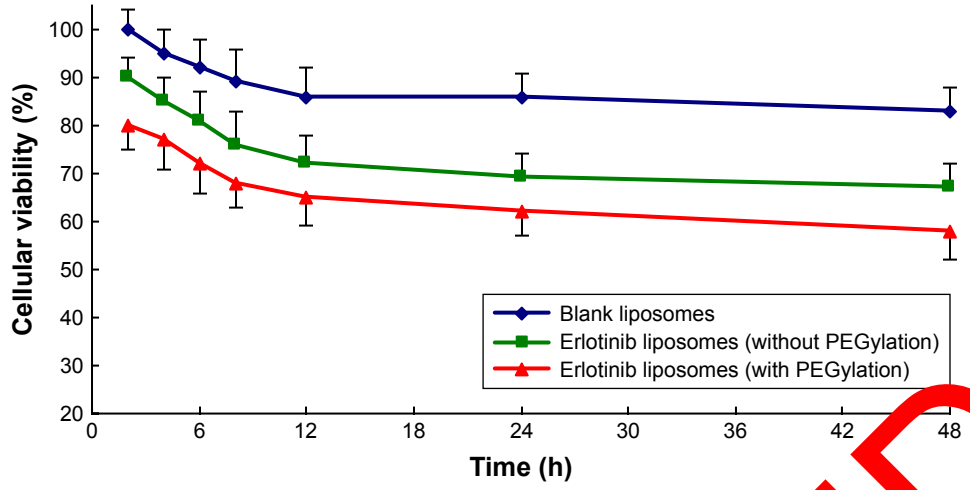

Figure 3 The cellular viability of A549 cells cultured with blank liposomes and erlotinib-loaded liposomes during different incubation time in comparison with that of erlotinib liposomes (without PEGylation) at the same dose (n=6).
Note: Data are expressed as mean ± SD.
Abbreviation: SD, standard deviation.

into PEGylation liposome formulation, its pharmacokinetics is mainly determined by the characteristics of PEGylation liposome rather than the physical and chemical properties of the drug molecule.

Histology studies

The histopathological examinations of the heart, liver, spleen, lung, and kidney were carried out to identify any damage done to the tissue. The microphotographs of the heart, liver, spleen, lung, and kidney were taken following their incubation with erlotinib formulations (Figure 5). No sign of damage, such as the appearance of epithelial necrosis or sloughing of epithelial cells, was detected.

Conclusion

In this study, PEGylation liposomes formulations of erlotinib were developed with an average particle size of 102.4±3.1 nm. TEM images showed that the liposomes dispersed well with a uniform of shapes and with no changes during the storage. The in vitro drug-release kinetic model of erlotinib release from the PEGylation liposomes in PBS fits well with the Higuchi equation. In vitro anticancer activity assay

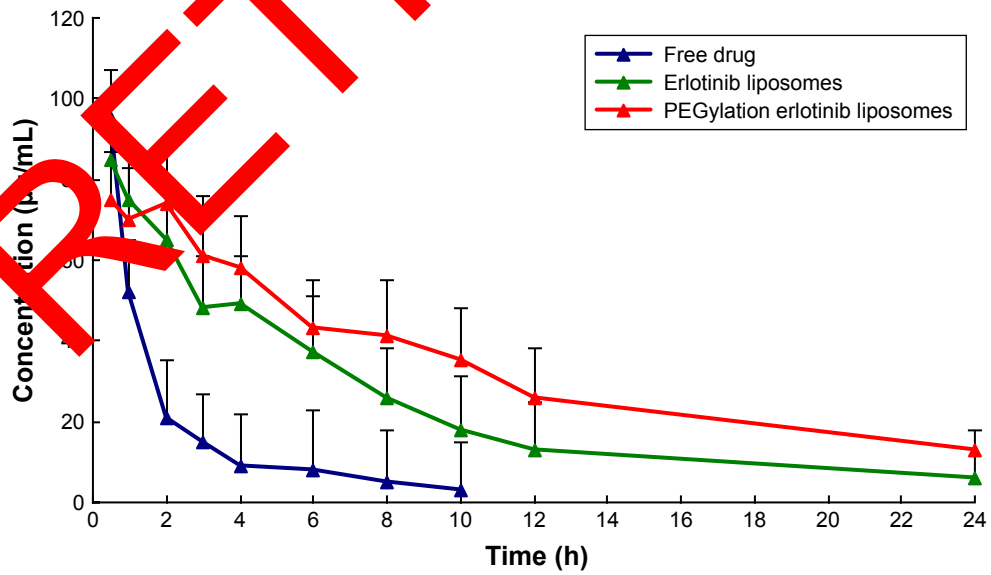

Figure 4 Concentration–time curve of erlotinib in different formulations.
Notes: Free drug (blue line), liposomes (green line), and PEGylation liposomes (red line) (n=6). Data are expressed as mean ± SD.
Abbreviation: SD, standard deviation.

Table 3 Pharmacokinetic parameters of erlotinib after intravenous administration of free drug, liposomes, and PEGylation liposomes to rats (n=6)

Parameter	Intravenous administration		
	Free drug	Liposomes	PEGylation liposomes
$t_{1/2}$(min)	28.9±8.3	92.7±10.1*	96.8±11.2*
AUC_{0-t} (μg·min/mL)	168.8±18.3	574.3±65.3*	792.5±78.6*
$AUC_{0-\infty}$ (μg·min/mL)	186.7±19.6	625.5±72.7*	898.5±82.4*
MRT (min)	29.2±8.7	89.5±9.6*	92.7±10.1*
CL (L/kg/min)	0.21±0.11	0.09±0.05*	0.07±0.03*

Notes: *$p<0.05$ versus free drug.
Abbreviations: AUC_{0-t}, area under the curve calculated to the last measured concentration; $AUC_{0-\infty}$, area under the curve from time 0 extrapolated to infinite time; CL, clearance; MRT, mean residence time; $t_{1/2}$, half-life.

showed that the blank liposomes had lower cellular cytotoxicity and that the cellular cytotoxicity of erlotinib liposomes increased significantly at the same incubation condition, which should contribute to the increased intracellular drug concentration by the transportation of liposomes. The two liposomes of erlotinib (with or without PEGylation) exhibited a similar cellular cytotoxicity at the same concentration with no significant difference. Pharmacokinetic results indicated that erlotinib-loaded PEGylation liposomes can significantly change the pharmacokinetic behavior of drugs and improve the drug bioavailability nearly 2 [illegible] compared to the ordinary liposomes.

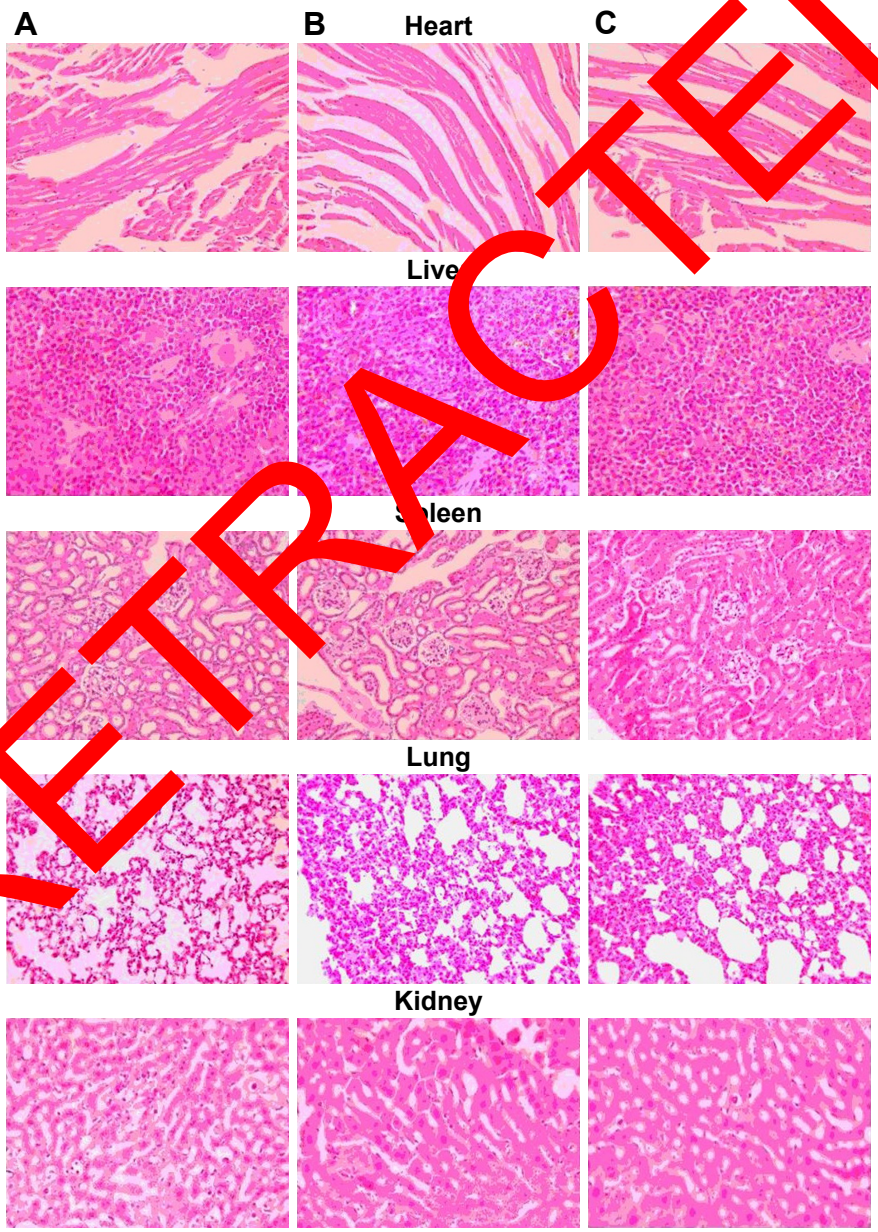

Figure 5 Histopathological studies of the heart, liver, spleen, lung, and kidney.
Notes: (**A**) Free drug; (**B**) erlotinib liposomes; (**C**) PEGylation erlotinib liposomes. Magnification ×5,000.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Aydiner A, Yildiz I, Seyidova A. Clinical outcomes and prognostic factors associated with the response to erlotinib in non-small-cell lung cancer patients with unknown EGFR mutational status. *Asian Pac J Cancer Prev*. 2013;14:3255–3261.
2. Tan PS, Bilger M, de Lima Lopes G, Acharyya S, Haaland B. Meta-analysis of first-line therapies with maintenance regimens for advanced non-small-cell lung cancer (NSCLC) in molecularly and clinically selected populations. *Cancer Med*. 2017;6(8):1847–1860.
3. Lee JG, Wu R. Combination erlotinib-cisplatin and Atg3-mediated autophagy in erlotinib resistant lung cancer. *PLoS One*. 2012;7:e48532.
4. Kirane A, Toombs JE, Ostapoff K, et al. Apricoxib, a novel inhibitor of COX-2, markedly improves standard therapy response in molecularly defined models of pancreatic cancer. *Clin Cancer Res*. 2012;18:5031–5042.
5. Makrilia N, Lappa T, Xyla V, Nikolaidis I, Syrigos K. The role of angiogenesis in solid tumours: an overview. *Eur J Intern Med*. 2009;20:663–671.
6. Sutiman N, Zhang Z, Tan EH, et al. Phase I study of oral vinorelbine in combination with erlotinib in advanced non-small cell lung cancer (NSCLC) using two different schedules. *PLoS One*. 2016;11:e0154316.
7. Kumar R, Lu SK, Minchom A, et al. A phase 1b trial of the combination of an all-oral regimen of capecitabine and erlotinib in advanced non-small cell lung cancer in Caucasian patients. *Cancer Chemother Pharmacol*. 2016;77:375–383.
8. Ranson M, Reck M, Anthoney A. Erlotinib in combination with pemetrexed for patients with advanced non-small-cell lung cancer (NSCLC): a phase I dose-finding study. *Ann Oncol*. 2010;21:2233–2239.
9. Ranson M, Shaw H, Wolf J, et al. A phase I dose-escalation and bioavailability study of oral and intravenous formulations of erlotinib (Tarceva, OSI-774) in patients with advanced solid tumors of epithelial origin. *Cancer Chemother Pharmacol*. 2010;66:53–58.
10. Bakhtiary Z, Barar J, Aghanejad A, et al. Microparticles containing erlotinib-loaded solid lipid nanoparticles for treatment of non-small cell lung cancer. *Drug Dev Ind Pharm*. 2017;43:1244–1253.
11. Dora CP, Trotta F, Kushwah V, et al. Potential of erlotinib cyclodextrin nanosponge complex to enhance solubility, dissolution rate, in vitro cytotoxicity and oral bioavailability. *Carbohydr Polym*. 2016;137:339–349.
12. Barghi L, Asgari D, Barar J, Nakhlband A, Valizadeh H. Synthesis, characterization and in vitro anti-tumoral evaluation of Erlotinib-PCEC nanoparticles. *Asian Pac J Cancer Prev*. 2014;15:10281–10287.
13. Swaisland HC, Smith RP, Laight A, et al. Single-dose clinical pharmacokinetic studies of gefitinib. *Clin Pharmacokinet*. 2005;44:1165–1177.
14. Polli JW, Humphreys JE, Harmon KA, et al. The role of efflux and uptake transporters in [N-{3-chloro-4-[(3-fluorobenzyl)oxy]phenyl}-6-[5-({[2-(methylsulfonyl)ethyl]amino}methyl)-2-furyl]-4-quinazolinamine (GW572016, lapatinib) disposition and drug interactions. *Drug Metab Dispos*. 2008;36:695–701.
15. Cui J, Li C, Guo W, et al. Direct comparison of two pegylated liposomal doxorubicin formulations: is AUC predictive for toxicity and efficacy? *J Control Release*. 2007;118:204–215.
16. Hichert V, Scholl C, Steffens M, et al. Predictive blood plasma biomarkers for EGFR inhibitor induced skin rash. *Oncotarget*. 2017;8:35193–35204.
17. Trummer BJ, Iyer V, Bhatt Iyer S, O'Connor R, Straubinger RM. Physicochemical properties of epidermal growth factor receptor inhibitors and development of a nanoliposomal formulation of gefitinib. *J Pharm Sci*. 2012;101:2763–2777.
18. Koudelka Š, Turanek J, Knotigova P, Mašek J, et al. Liposomes with high encapsulation capacity for paclitaxel: preparation, characterisation and in vitro anticancer effect. *J Pharm Sci*. 2010;99:2309–2319.
19. Maeda H. Macromolecular therapeutics in cancer treatment: the EPR effect and beyond. *J Control Release*. 2012;164:138–144.
20. Zeihamed M, Dorkoosh F, Dashzadeh S, Haeri A, Akhlaghi MF. Potential of lipid nanomes for enhancement of oral drug absorption. *Curr Drug Deliv*. 2016;13:888–903.
21. Kang SN, Hong SS, Lee MK, Lim SJ. Dual function of tributyrin emulsion in solubilization and enhancement of anticancer effect of celecoxib. *Int J Pharm*. 2012;428:76–81.
22. McKillop D, Partridge EA, Kemp JV, et al. Tumor penetration of gefitinib (Iressa), an epidermal growth factor receptor tyrosine kinase inhibitor. *Mol Cancer Ther*. 2005;4:641–649.