Original Article

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Liposome encapsulated of temozolomide for the treatment of glioma tumor: preparation, characterization and evaluation

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Summary **Temozolomide plays a critical role in curing glioma at present. The purpose of this work was to develop a suitable drug delivery system which could prolong the half-life, improve the brain targeting, and reduce the systemic effect of the drug. Temozolomide-liposomes were formulated by the method of proliposomes. They were found to be relatively uniform in size of 156.70 ± 11.40 nm with a narrow polydispersity index (PI) of 0.29 ± 0.04. The average drug entrapment efficiency and loading capacity were 35.45 ± 1.48% and 2.81 ± 0.20%, respectively. The pH of temozolomide-liposomes was 6.46. *In vitro* release studies were conducted by a dynamic dialysis. The results showed that temozolomide released slowly from liposomes compared with the solution group. The release behavior of temozolomide-liposomes was in line with First-order kinetics and Weibull equation. The pharmacokinetics study was evaluated by pharmacokinetics parameters. The $t_{1/2\beta}$ and MRT of temozolomide-liposomes were 3.57 times and 1.27 times greater than that of temozolomide solution. The C_{max} and AUC values of temozolomide-liposomes were 1.10 times and 1.55 times greater than that of temozolomide solution. The results of pharmacokinetics study showed temozolomide-liposomes prolonged the *in vivo* circulation time and increased AUC. Furthermore, the biodistribution in mice showed that temozolomide-liposomes preferentially decreased the accumulation of temozolomide in heart and lung and increased the drug concentration in brain after *i.v.* injection, which implied that temozolomide-liposomes improved the therapeutic effect in the brain and reduced the toxicity in lung and heart.**

Keywords: Temozolomide, liposomes, gliomas, pharmacokinetics, biodistribution

1. Introduction

Glioma with an incidence of 5 per 100,000 persons is one of the most devitalizing malignant diseases (*1*). They are very difficult to resect entirely because of the infiltrative nature of the tumors and these tumors almost invariably recur, rapidly leading to death (*2*). Surgical resection alone is inadequate for cure, so enough effective chemotherapy drugs are desiderated.

Temozolomide is a new type of imidazole tetrazines drugs with demonstrated efficacy for patients with recurrent gliomas. During adjuvant temozolomide, the median survival of the patient with gliomas was about 16 months, and the survival rates of 1-year and 2-year were 58% and 31%, respectively (*3*). Generally, temozolomide is regarded as tolerable, effective, and well absorbed after oral administration. However, because of its short half-life of about 1.8 h in plasma, temozolomide must be administered in high systemic doses to achieve therapeutic brain levels (*4*). Furthermore, prolonged systemic administration is associated with systemic effect including nausea and vomiting, headache, and fatigue (*5*). Temozolomide injection is more suitable for post-operative patients. However, the marketable temozolomide lyophilized powder for injection used Tween 80 due to the poor solubility of the drug, which can cause hemolysis, allergies and other adverse reactions (*6,7*). Therefore, the development of new anti-glioma nano drug delivery systems became the research focuses to prolong the half-life, improve the brain targeting, and reduce the systemic effect of temozolomide. Huang *et al*. (*8*) prepared the solid lipid nanoparticles of

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temozolomide, which exhibited sustained release and brain targeting.

Liposomes as the first closed bilayer phospholipid systems were described in 1965 and they were proposed as drug delivery systems soon (*9*). Both hydrophilic drugs and hydrophobic drug can be entrapped. The significant advance was the ability to keep the integrity of the liposome structure when entrapping drugs in it. This property has been demonstrated to significantly affect the stability and pharmacokinetics of dugs (*10*). With this property liposomes can protect temozolomide from depredating in the plasma, which can improve the stability of the temozolomide and prolong considerably the temozolomide half-life in the circulation. In addition, liposome could be consumed by monocytes, neutrophils, *etc*., which can pass through blood brain barrier in the body circulation process selectively and get to the brain (*11*). Besides, it can get to the brain tissue through the blood brain barrier by pinocytosis of endothelial cells (*12,13*). So the drug concentration in the brain could increase significantly, when the temozolomide was entrapped in liposome.

The aim of this present work was to develop temozolomide-liposomes to prolong the half-life, reduce the systemic effect, and improve the brain targeting, the therapeutic efficacy and the patient compliance. In this study, temozolomide-liposomes was developed by the method of proliposomes method with the materials of soybean lecithin and cholesterol and characterized by the particle size and its distribution, pH value, morphology, drug loading, drug encapsulation efficiency, and drug release behavior *in vitro*. The *in vivo* pharmacokinetics and biodistribution of temozolomide-liposomes were evaluated to elucidate their feasibility as intravenous delivery systems.

2. Materials and Methods

2.1. *Materials*

Temozolomide was purchased from Hangzhou Hesu Chemical Technology Co., Ltd. (Zhejiang, China). Soybean lecithin (injection grade, phosphatidylcholine accounts for 95% pH 5.0-7.0) was provided by Shanghai Taiwei Pharmaceutical Co., Ltd. (Shanghai, China). Cholesterol, Sorbitol, and Mannitol were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Lactin was provided by Tianjin Guangcheng Chemical Agent Co., Ltd. (Tianjin, China). Glacial acetic acid was purchased from Tianjin Fuyu Chemical Co., Ltd. (Tianjin, China). N-caprylic alcohol, hydrochloric acid, and other reagents used were of analytical grade.

2.2. *Formulation of temozolomide-liposomes*

Temozolomide-liposome was prepared by a proliposomes method (*14,15*). Briefly, temozolomide (20 mg), soybean phospholipid (240 mg), and cholesterol (30 mg) were dissolved in methanol. Then sorbitol (1.5 g) was added with the help of ultrasonic cleaner (Kun Shan Ultrasonic Instruments Co., Ltd., Jiangsu, China). The organic solvent was removed using a RE52-98 rotary evaporator (Shanghai Yarong Instrument Co., Shanghai, China) under vacuum (0.02 MPa) with the rotate speed of 80 rpm for 30 min at 40°C. The lipid was thoroughly dried to remove residual organic solvent by placing in a vacuum oven for 5 h and then filtered through the screen (20 mesh). All products were sealed immediately in vials and stored in refrigerator at 4°C. The dried lipid was rehydrated with 15 mL of the solution of temozolomide (1 mg/mL) and obtained suspensions with ultrasound for 5 min when use.

2.3. *Measurement of particle size, and pH value*

The particle size and polydispersity index (PI) of temozolomide-liposome were determined by Delsa™ Nano C Particle Analyzer (Beckman Coulter A53878, Otsuka Blectronics Co. Ltd., USA). The pH value of temozolomide-liposome was determined with a digital pH meter (FE20, Mettler Toledo, Switzerland). Each measurement was made at least in triplicate

2.4. *Transmission election microscope (TEM) examination*

TEM observations of temozolomide-liposomes were performed by transmission electron microscope (H-7000, Hitachi, Japan). Briefly, the liposome samples were dropped onto carbon-coated grids and drawn off excess solution with a piece of filter paper. Then, the grids were immersed in 2.0% phosphomolybdic acid aqueous solution for 1 min. Finally, the grids were dried and imaged using a transmission electron microscope.

2.5. *Determination of entrapment efficiency (EE) and drug-loading rate*

The EE of liposomes was determined using the ultrafiltration technique (molecular weight cutoff 10 kDa) for separating the non-entrapped drug from liposomes (*16*). In short, 50 μL of temozolomide-liposome was mixed with 1 mL of acetonitrile, and diluted it to 10 mL with 5% acetic acid (v/v). The samples were filtered using 0.22 μm membrane filter and then subjected to high-performance liquid chromatography (HPLC). The content of total temozolomide was determined.

Briefly, 100 μL of temozolomide liposome was added in an ultrafiltration centrifuge tube and mixed with 700 μL of distilled water. The free temozolomide was separated from liposome by ultrafiltration at 3,500 rpm for 30 min. After completed the centrifugation, 200 μL of distilled water was added and shake, then centrifuge again by ultracentrifugation at 3,500 rpm

for 20 min. Suck up 50 μL of filtrate and dilute to 10 mL with 5% acetic acid (v/v). The samples were filtered using 0.22 μm membrane filter and injected into the HPLC. The content of free temozolomide was determined.

$$EE\% = \frac{W_{total} - W_{free}}{W_{total}} \times 100\% \quad (1)$$

$$DL\% = \frac{W_{total} - W_{free}}{W_{lipid}} \times 100\% \quad (2)$$

Where W_{free} was the analyzed weight of free drug, W_{total} was the analyzed weight of drug in liposomes, W_{lipid} was the analyzed weight of liposomes.

2.6. *HPLC analysis*

The HPLC equipment was a Shimadzu LC-10A system (Shimadzu, Kyoto, Japan) consisting of a LC-10AT HPLC pump and a SPD-10A UV-VIS detector. Data processing was performed with LC Solution software. Drug analysis was conducted on a Diamonsil-ODS column (150 × 4.6 mm, Dikma Technologues, China). The mobile phase consisted of methanol, deionized water, and glacial acetic acid (10:89.5:0.5, v/v) at a flow rate of 1.0 mL/min and an injection volume was 20 mL. The detection wavelength was set at 329 nm

2.7. *In vitro release study*

In vitro release rate behaviors of temozolomide from liposome vesicles were performed by the dialysis method. Briefly, 3 mL of liposome suspension (the concentration is 1 mg/mL) was loaded in dialysis bag (molecular weight cutoff 8 to 14 kDa) and dialyzed against the release media of PBS with pH 5.0 at 37 ± 0.5°C in an water bath shaker at 100 rpm. At scheduled time (0.083, 0.17, 0.33, 0.5, 0.75, 1, 2, 4, and 6 h) intervals, 0.2 mL of the release media was collected and then the same volume of fresh release media were added. Dilute the solution to 1 mL with 5% acetic acid (v/v). The release amount of temozolomide was determined by HPLC as described earlier. The mean calculated values were obtained from 3 replicates. The drug release profile of temozolomide was examined as a control. The accumulative release percentage of temozolomide (R%) was calculated according to the following equation:

$$R = \frac{c_n v_0 + \sum_{i=0}^{n-1} c_i v_i}{W} \times 100\ \%$$

where R is the release rate, c_n is the drug concentration in the release medium of each time interval, v_0 is the total volume of the release medium, v_i is the volume of the withdrawn medium, c_i is the drug concentration in the release medium at time, and W is the total drug content of the release sample.

2.8. *Pharmacokinetics studies in rabbits*

The *in vivo* pharmacokinetics experiment was carried out with adult white New Zealand rabbits (female and male) weighing 2.0-2.5 kg supplied by the Medical Animal Test Center of Shandong University. The animal experiment protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Shandong University. The rabbits used for this study were housed individually under normal conditions, and fasted overnight before experiment with free access to water. Animals were randomly assigned into two groups with six rabbits each. Rabbits in one group were administered with temozolomide liposomes (equal to 10 mg/kg temozolomide) by injection in one of the ear marginal vein, while control group was administered with 10 mg/kg temozolomide solution (5 mg/mL, dissolved in physiological saline solution). Blood was collected at specified time intervals (0.083, 0.167, 0.333, 0.5, 1, 2, 3, 5, 8, 10, 12 h) by intracardiac puncture and was put into centrifuge tube washed with EDTA-2Na (15 mg/mL) along with 10 μL of a 10% phosphoric acid solution. Plasma samples were harvested by centrifuging at 12,000 rpm for 10 min. Subsequently, 300 μL of the resulting rabbit plasma was added to 300 μL protein precipitation (10% trichloroacetic acid:acetonitrile = 3:2) and the mixture was vortexed for 30 sec. The sample was centrifuged for 10 min at 12,000 rpm. After centrifugation, the supernatant layer was filtered through a 0.22 μm filter, and then 20 μL of the filtered solution was directly injected into HPLC for analysis.

The plasma samples were determined by the HPLC system. The HPLC condition was the same as described above except for the mobile phase consisted of methanol, deionized water, and glacial acetic acid (8:91.5.5:0.5, v/v).

Method validation: weigh 61.2 mg of temozolomide, then dissolve and dilute to 50 mL with water. Put the temozolomide solution as stock solution. Pipe appropriate amount stock solution to prepare a series of concentration of temozolomide (1.5, 3, 30, 150, 306, 612, 1,224 μg/mL). Pipe 10 μL of the series of concentration of temozolomide solution respectively, then add 0.28 mL of plasma and 10 μL of 10% phosphoric acid solution (w/v), mix well. Then a series of concentration of temozolomide (0.051, 0.1, 1.02, 5.1, 10.2, 20.4, 40.8 μg/mL) were obtained. Dispose the plasma with temozolomide with the method as above. Injected 20 μL sample and record the peak area (A). Draw the standard curve and calculate the CV% and recovery.

2.9. *Drug distribution studies in mice*

Kunming strain mice (weighed between 18 and 22 g, female or male were provided by the Medical Animal Test Center of Shandong University) were used for the biodistribution studies. The animals were fasted 12 h

before drug administration. The animal experiment protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Shandong University.

Two groups of 15 mice each were used for the *in vivo* distribution studies. temozolomide-liposomes and temozolomide solution were injected through the tail veins of mice at a dosage of 20 mg/kg. Following drug administration, at each predetermined time point (0.083, 0.25, 0.5, 1, 2 h), three mice in each group were then sacrificed by cervical dislocation, and the spleen, kidneys, liver, brain, heart, and lungs were surgically removed. Each organ sample was washed with physiological saline solution, and the redundant liquid was blotted using absorbent paper. Accurately weighted tissue specimen was homogenized with 1 mL physiological saline solution (except for liver, 2 mL) by homogenizer. Tissue homogenates were processed as the plasma sample. After centrifugation, the supernatant was passed through a 0.22 μm filter. About 20 μL of the filtered tissue homogenate was injected into HPLC for analysis. The HPLC condition was the same as described in Pharmacokinetics studies in rabbits.

3. Results and Discussions

3.1. *Physicochemical characterization of the liposome*

The photographs of three batches of the formulation were shown in Figure 1. The appearance of liposomes after rehydration is semitransparent with opalescence.

The particle size, polydispersity index, entrapment efficiency, drug loading, and pH of three batches of the formulation were reported in Table 1. The mean particle size of the formulation was 156.7 ± 11.4 nm. Furthermore, the PI of temozolomide-liposomes was 0.290 ± 0.041, which revealed that the formulation had a quite narrow size distribution and uniform size. The mean pH values of temozolomide-liposomes was 6.46 ± 0.08, which was within the safe pH scope of 4-9 for intravenous injection. The mean drug entrapment efficiency and drug loading was 35.45 ± 1.48% and 2.81 ± 0.20%, respectively. The size distribution of temozolomide-liposomes shown in Figure 2. The results indicated that singe-peak and narrow distribution were obtained.

To obtain more information about the morphology of temozolomide-liposomes, TEM analysis was performed and the result of TEM image was showed in Figure 3. The image displayed that temozolomide-liposomes had relatively spherical shapes and had obvious hydrophilic layer, which may be due to sorbitol covering in the surface of the liposome. The particle

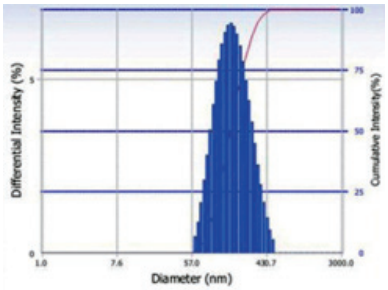

Figure 2. The size distribution of temozolomide liposomes. The results indicated that singe-peak and narrow distribution were obtained.

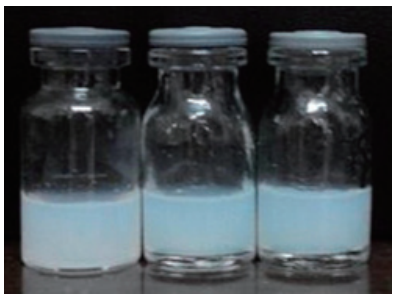

Figure 1. Appearance of liposomes after rehydration. The photographs of three batches of the formulation were shown. The appearance of liposomes after rehydration is semitransparent with opalescence.

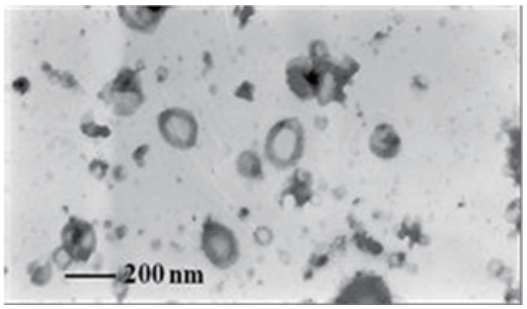

Figure 3. Transmission electron microscopy micrographs of temozolomide. The image displayed that temozolomide-liposomes had relatively spherical shapes and had obvious hydrophilic layer, which may be due to sorbitol covering in the surface of the liposome.

Table 1. The particle size, polydispersity index, entrapment efficiency, drug loading and pH of three batches of the formulation

Batches	Particle size (nm)	PI	EE (%)	DL (%)	pH
1	159.4	0.334	36.40	2.70	6.49
2	144.2	0.282	33.75	2.68	6.53
3	166.5	0.254	36.21	3.04	6.37
$\bar{\chi}$ ± S.D.	156.7 ± 11.4	0.290 ± 0.041	35.45 ± 1.48	2.81 ± 0.20	6.46 ± 0.08

PI, polydispersity index; EE, entrapment efficiency; DL, drug loading.

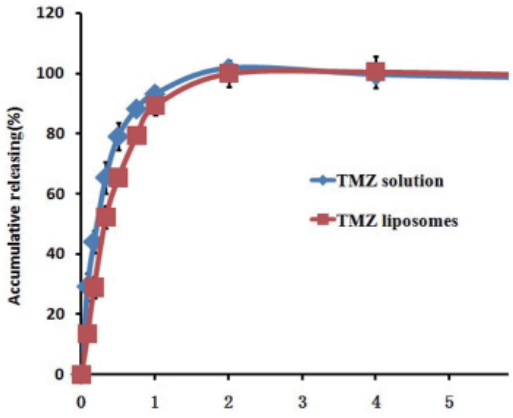

Figure 4. *In vitro* release profile of temozolomide from temozolomide solution and temozolomide-liposomes in phosphate-buffered saline (pH 5.0) at 37 ± 0.5°C (*n* = 3). The release behavior of temozolomide- liposomes was in accordance with the Weibull equation and First-order kinetics.

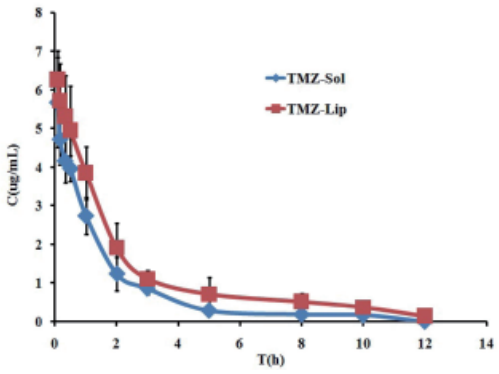

Figure 5. The temozolomide concentration-time curve after intravenous administration of temozolomide solution and temozolomide- liposomes in rabbits (*n* = 6). The plasma temozolomide concentration of temozolomide-liposomes group was much higher than that of the temozolomide solution group at each sampling time.

size determined by TEM agreed well with the results from Delsa™ Nano CParticle Analyzer.

3.2. *In vitro release behaviors of temozolomide-liposomes*

The release behavior profile of temozolomide-liposomes and temozolomide solution *in vitro* was shown in Figure 4. The release behavior of temozolomide-liposomes was in accordance with the Weibull equation and First-order kinetics. It can be expressed using the following equation: $\ln\ln(1/(1 - Q/100)) = 1.0805\ln t + 0.8133$, $r = 0.9988$ and $\ln(100 - Q) = -2.2329t + 4.6426$, $r = 0.9988$. The release behavior of temozolomide solution was in accordance with the Weibull equation model and can be expressed using the following equation: $\ln\ln(1/(1 - Q/100)) = 0.8419\ln t + 1.0014$, $r = 0.9992$. The results of *in vitro* release profile of temozolomide from temozolomide solution and temozolomide-liposomes were shown that temozolomide was released slower from temozolomide-liposomes than temozolomide solution at first. The slow release characteristic at this phase could be attributed to the fact that temozolomide was held by the lipid and therefore temozolomide was released gradually from the lipid matrices mainly through dissolution and diffusion (*17*). However, after 2 h, the release rate of the drug from the liposomes and solution were both observed around 100% and their release behaviors have no obvious difference. This phenomenon may be explained by the existing of the free drugs in the preparations because of the low encapsulation efficiency.

3.3. *Pharmacokinetics study in rabbits*

At the range of 0.051-40.8 μg/mL, the standard curve was $A = 32.423C - 2.6433$ and the standard showed a good linearity with a correlation coefficient of 0.9999. The CV% of is no more than 6% and the recovery is no less than 93%. The mean drug concentration in plasma versus time profile after the intravenous administration of liposomes and suspension was shown in Figure 5, and the pharmacokinetic parameters calculated by DAS 2.0 software were summarized in Table 2. After *i.v.* administration, the plasma temozolomide concentration of temozolomide-liposomes group was much higher than that of the temozolomide solution group at each sampling time. Furthermore, temozolomide-liposomes were eliminated more slowly than temozolomide solution. Besides, following injection of temozolomide-liposomes, temozolomide concentration was still measured to be 0.37 mg/L after 10 h, while temozolomide concentration of temozolomide solution was detected to be 0.29 mg/L after 5 h. Compared with temozolomide solution, the pharmacokinetic parameters of liposome group have been changed: $t_{1/2\beta}$ and MRT were 13.312 h and 3.402 h, which were 3.57 times and 1.27 times of temozolomide solution; C_{max} and AUC values of temozolomide-liposomes were 6.26 mg/L and 16.158 mg/L·h, which were 1.10 times and 1.55 times of temozolomide solution. The results of pharmacokinetics study showed that prolonged *in vivo* circulation time and increased AUC were in favor of temozolomide treatment for gliomas. These results could account for the rapid distribution and metabolism

Table 2. The pharmacokinetic parameters of temozolomide after *i.v.* injection of temozolomide solution and temozolomide-liposomes in rabbits (*n* = 6)

Parameters	Temozolomide -Solution	Temozolomide -Liposomes
t1/2α (h)	0.745	1.050
t1/2β (h)	3.731	13.312
V1 (L/kg)	1.741	1.531
CL (L/h/kg)	0.956	0.619
AUC0-t (mg/L*h)	10.457	16.158
MRT	2.686	3.402
Tmax (h)	0.083	0.083
Cmax (mg/L)	5.680	6.260

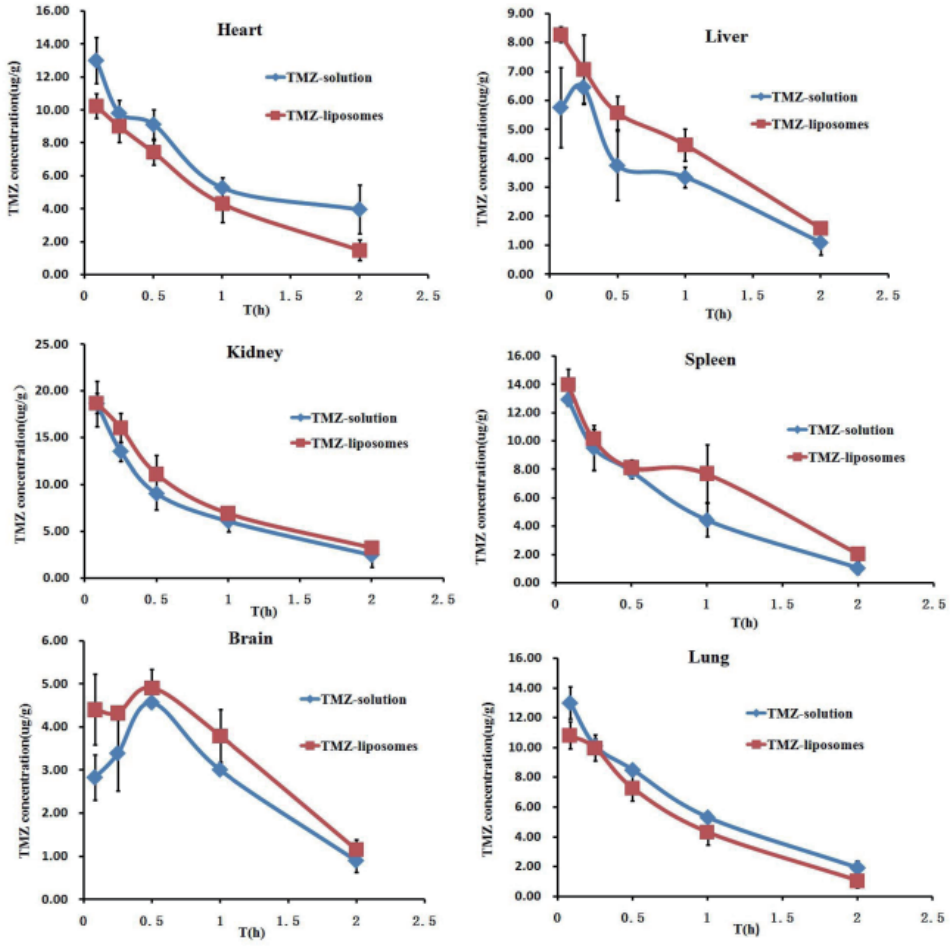

Figure 6. Temozolomide concentration-time curve in tissue at different time points after intravenous (*i.v.*) administration of temozolomide-solution and temozolomide-liposomes ($n = 3$). The biodistribution profiles of temozolomide in mice after intravenous administration of temozolomide solution and temozolomide-liposomes were shown. Briefly, the drug concentrations in brain were elevated after the administration of temozolomide-liposomes compared with the temozolomide solution. Meanwhile, the drug concentration in liver, kidney and spleen increased but decreased in heart and lung after administration of the liposomal formulation compared with temozolomide solution.

of the free temozolomide, compared to the distribution of temozolomide entrapped in liposomes. This might be explained by the protection of the lipid bilayer membranes and slow drug release from liposomes (*18*), which was also shown in the *in vitro* release experiment. At the same time a water shield on the surface of the liposomes reduced adsorption of opsonins, thereby reducing the intake of mononuclear phagocytes. That was one of the reasons why temozolomide-liposomes could remain in the circulation for a longer period of time than temozolomide solution.

3.4. *Drug distribution studies in mice*

The biodistribution profiles of temozolomide in mice after intravenous administration of temozolomide solution and temozolomide-liposomes were shown in Figure 6. Some changes of distribution can be observed between temozolomide solution and liposomes after the intravenous administration. Briefly, the drug concentrations in brain were elevated after the administration of temozolomide-liposomes compared with the temozolomide solution which could improve the efficiency of temozolomide treatment for gliomas. Meanwhile, the drug concentration in liver, kidney and spleen increased but decreased in heart and lung after administration of the liposomal formulation compared with temozolomide solution, which may be expected to reduce the potential side effects in heart and lung (*19*). The increasing concentration of temozolomide in liver and spleen may be explained by the fact that liposomes < 200 nm tend to be taken up by macrophages in liver and spleen more easily. The reason resulted to the increasing concentration of temozolomide in kidney was liposomes tend to be into the kidney easily due to the hydrophilic layer of sorbitol on the surface of liposomes (*20*). Furthermore, temozolomide-liposomes resulted in a higher drug accumulation in brain compared with temozolomide solution, which may be explained by the fact that the RES removal of the nanoparticles can be prevented by surface coating with hydrophilic polymers to increase their availability at brain (*21*). Liposome could be consumed by monocytes, neutrophils, *etc.*, which can pass through blood brain barrier in the body

Table 3. Tissue targeting parameters of temozolomide solution and temozolomide liposomes after *i.v.* administration in mice (n = 6)

Tissues	AUC		Re
	Temozolomide-Sol	Temozolomide-Lip	
Heart	13.58	10.366	0.763
Liver	6.812	9.106	1.337
Spleen	10.975	14.328	1.306
Lung	12.444	10.426	0.838
Kidney	15.178	17.494	1.153
Brain	5.656	6.9	1.220

Re = AUCliposomes/AUCsolution.

circulation process selectively and get to the brain. Besides, it can get to the brain tissue through the blood brain barrier by pinocytosis of endothelial cells. So the drug concentration in the brain could increase significantly. At the same time a water shield on the surface of the liposomes reduce adsorption of opsonins, thereby reducing the passive targeting to lung.

To represent targeting efficiency, AUC and the targeting parameters such as the intake rate (Re), were calculated and are listed in Table 3. temozolomide-liposomes exhibited a larger value of AUC for brain compared with temozolomide solution. The results showed that compared to temozolomide solution, Re value of temozolomide-liposomes in the brain was 1.22, Re values in the liver and spleen were 1.337 and 1.306, Re values in the kidney was 1.153, and Re values in the heart, lungs were 0.763 and 0.838, which also implied that temozolomide-liposomes increased the concentration of the drug in the brain, improved the therapeutic effect and reduced the toxicity in heart and lung.

In this study, temozolomide-liposomes were prepared for intravenous injection with the method of proliposomes successfully. The preparation process was simple with good reproducibility, and temozolomide-liposomes possess displayed excellent performance featured by small and uniform size. The pH was within the safe pH scope of 4-9 for intravenous injection. The results of *in vitro* drug release behavior showed that temozolomide-liposomes released slowly compared with temozolomide solution. The results of pharmacokinetics and tissue distribution study showed that the liposomes had a slow-release effect and brain targeting. Furthermore, temozolomide-liposomes could reduce the C_{max} in some organs such as heart, lung potentially decreasing the side effects. temozolomide-liposomes can change biodistribution, increase the concentration of drug at the treatment site, and reduce side effects in non-target tissues. Furhermore temozolomide- liposomes avoided the use of Tween 80 which was contained in the listed freeze-dried powder for injection which might be helpful to decrease the side effects. The drug delivery system can prolong the half-life, improve the targeting and reduce the systemic effect of the drug, which were promising for developing the new formulation. The following work would be focused on optimizing prescription and process to improve the encapsulation efficiency.

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References

1. Ries L, Eisner M, Kosary C. SEER Cancer Statistics, 1973-1998. Bethesda, MD, National Cancer Institute. 2001; 2:1375-1382.
2. Galanis E, Buckner J. Chemotherapy for high-grade gliomas. Brit J Cancer. 2000; 82:1371.
3. Stupp R, Dietrich P-Y, Kraljevic SO, Pica A, Maillard I, Maeder P, Meuli R, Janzer R, Pizzolato G, Miralbell R. Promising survival for patients with newly diagnosed glioblastoma multiforme treated with concomitant radiation plus temozolomide followed by adjuvant temozolomide. J Clin Oncol. 2002; 20:1375-1382.
4. Baker SD, Wirth M, Statkevich P, Reidenberg P, Alton K, Sartorius SE, Dugan M, Cutler D, Batra V, Grochow LB. Absorption, metabolism, and excretion of 14C-temozolomide following oral administration to patients with advanced cancer. Clin Cancer Res. 1999; 5:309-317.
5. Kopecký J, Priester P, Slováček L, Petera J, Kopecký O, Macingova Z. Aplastic anemia as a cause of death in a patient with glioblastoma multiforme treated with temozolomide. Strahlenther Onkol. 2010; 186:452-457.
6. Deng Y, Zhao J, Dong X, Shi L, Lu Y, Ni D, Zhao H. Composite emulsifier, an emulsion prepared from it and the preparation method thereof. U.S. Patent Application 12/670,820[P]. 2008-7-25.
7. Constantinides PP, Tustian A, Kessler DR. Tocol emulsions for drug solubilization and parenteral delivery. Adv Drug Deliver Rev. 2004; 56:1243-1255.
8. Huang GH, Zhang Z, Bi XL, Dou MJ. Solid lipid nanoparticles of temozolomide: Potential reduction of cardial and nephric toxicity. Int J Pharm. 2008; 355:314-320.
9. Allen TM, Cullis PR. Liposomal drug delivery systems: From concept to clinical applications. Adv Drug Deliver Rev. 2013; 65:36-48.
10. Chonn A, Cullis PR. Recent advances in liposome technologies and their applications for systemic gene delivery. Adv Drug Deliver Rev. 1998; 30:73-83.
11. Afergan E, Epstein H, Dahan R, Koroukhov N, Rohekar K, Danenberg HD, Golomb G. Delivery of serotonin to the brain by monocytes following phagocytosis of liposomes. J Control Release. 2008; 132:84-90.
12. Garcia-Garcia E, Andrieux K, Gil S, Couvreur P. Colloidal carriers and blood-brain barrier (BBB) translocation: A way to deliver drugs to the brain? Int J Pharm. 2005; 298:274-292.
13. Huwyler J, Cerletti A, Fricker G, Eberle AN, Drewe J. By-passing of P-glycoprotein using immunoliposomes. J Drug Target. 2002; 10:73-79.
14. Hwang SY, Kim HK, Choo J, Seong GH, Hien TBD, Lee E. Effects of operating parameters on the efficiency of liposomal encapsulation of enzymes. Colloid Surface B.

2012; 94:296-303.
15. Liu TS, Cai BC, Deng XK, Li WD, Zhao XH, Huang YZ. Preparation of strychnine solid liposome by sorbitol carrier aggradation and freeze-drying method. Chin Tradit Pat Med. 2005; 27:509-511.
16. Li BQ, Deng YJ, Yang JW. Determination of entrapment efficiency of topotecan liposomes by ultrafiltration-HPLC. Chin New Drug J. 2007; 16:58.
17. Lin QP, Guo RP, Wang QS, Wang DC, Liu CH, Xu XY. Preparation, *in vitro* release and cytotoxic effect of docetaxel liposomes for intravenous injection. J Chin Pharm Univ. 2008; 39:417-421.
18. Ait-Oudhia S, Mager DE, Straubinger RM. Application of pharmacokinetic and pharmacodynamic analysis to the development of liposomal formulations for oncology. Pharmaceutics. 2014; 6:137-174.
19. Maldonado F, Limper AH, Lim KG, Aubrey MC. Temozolomide-associated organizing pneumonitis. Mayo Clin Proc. 2007; 86:771-773.
20. Suzuki R, Takizawa T, Kuwata Y, Mutoh M, Ishiguro N, Utoguchi N, Shinohara A, Eriguchi M, Yanagie H, Maruyama K. Effective anti-tumor activity of oxaliplatin encapsulated in transferrin-PEG-liposome. Int J Pharm. 2008; 346:143-150.
21. Kaur IP, Bhandari R, Bhandari S, Kakkar V. Potential of solid lipid nanoparticles in brain targeting. J Control Release. 2008; 127:97-109.

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