Pharmacokinetics and tissue distribution of larotaxel in rats: comparison of larotaxel solution with larotaxel-loaded folate receptor-targeting amphiphilic copolymer-modified liposomes

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Abstract

1. The aim of this study was to compare the pharmacokinetics (PKs) and tissue distribution of larotaxel (LTX) solution with a newly developed formulation called LTX-loaded folate-poly (PEG-cyanoacrylate-co-cholesteryl cyanoacrylate) (FA-PEG-PCHL)-modified liposomes in rats.

2. An ultra-performance liquid chromatography-tandem mass spectrometry method has been developed and validated for the determination of LTX in rat plasma and tissues to investigate the influence of FA-PEG-PCHL-modified lipid carrier on LTX PKs and tissue distribution.

3. The PK study result showed significantly higher area under the concentration-time curve (97.2%, **p < 0.01), slower clearance (49.2%, **p < 0.01) and lower volume of distribution (42.5%, **p < 0.01) in rats following intravenous administration of modified liposomes. The biodistribution results exhibited significantly lower uptake of LTX-loaded modified liposomes in heart (20.4%, **p < 0.01), lung (8.33%, **p < 0.01), muscle (13.4%, *p < 0.05) and spleen (15.0%, **p < 0.01) among all sampled tissues, indicating that the modified lipid carriers may avoid the trapping by the reticuloendothelial system and the modified liposomes may reduce toxicity in cardiovascular system compared to LTX solution. Moreover, markedly higher concentrations of LTX in the kidney (100%, **p < 0.01) were found in LTX-loaded modified liposome treated rats and could be explained by the high folate receptor level in kidney.

4. These results indicated that the FA-PEG-PCHL-modified liposome could be an effective parenteral carrier for the delivery of LTX in cancer treatment.

Keywords
Folate-poly (PEG-cyanoacrylate-co-cholesteryl cyanoacrylate), larotaxel, liposomes, pharmacokinetics, tissue distribution

Introduction

Larotaxel (LTX) is a novel semisynthetic taxoid made from 10-deacetylbaccatin III (Diéras et al., 2008; Sessa et al., 2002). Among the chemotherapeutic options, LTX is one of the most efficacious and safe anticancer agents used for the treatment of a wide range of advanced tumours, including breast, gastric, prostate, head and neck, ovarian cancers and non-small cell lung cancer (Yamamoto et al., 2009). LTX also demonstrated in vitro activities in a range of taxane-resistant tumour models and was active when administered orally or intravenously (Bissery, 2001; Yamamoto et al., 2009). LTX was also characterised by an ability to cross the blood-brain barrier (BBB), with marked antitumour activity, demonstrated in nude mice bearing early stage intracranial glioblastomas (Bissery, 2001; Robert et al., 2010).

LTX is a highly lipophilic agent, sharing common features with other taxanes (e.g. paclitaxel and docetaxel (DTX)). Due to its poor solubility in water, LTX is currently dissolved for clinical use in polysorbate 80 and ethanol (Diéras et al., 2008; Sessa et al., 2002; Yamamoto et al., 2009). However, polysorbate 80 may cause some adverse effects in patients such as haemolysis, hepatotoxicity, peripheral neurotoxicity and severe hypersensitivity reaction, especially when administered intravenously (Alade et al., 1986; Shelley et al., 1995). These adverse effects caused by polysorbate 80 are a limitation to its clinical use. Consequently, some alternative formulation approaches such as liposomes, nanospheres and lipid microspheres need to be taken into account.

Nanotechnology has been extensively exploited to improve conventional cancer therapy or avoid serious adverse effects recently (Cho et al., 2008; Farokhzad & Langer, 2009). The nanocarriers, such as liposomes (mean particle size...
Liposomes are spherical vesicles composed of single or multiple lipid bilayers, and have a number of appealing features, including high biocompatibility, high delivery efficiency and ease of surface modification (Wang et al., 2011; Zhao et al., 2009). The performance of liposome drug delivery systems for tumour therapy could be further improved by using a ligand coupled to the surface of vesicles to achieve an active targeting effect. The selective overexpression of folate receptors in tumour cells and the high affinity of tumours for folic acid provide a unique opportunity for folic acid to be used as a targeting ligand to deliver therapeutic agents to cancer cells via folate receptor-mediated endocytosis (Leamon & Low, 1991). Therefore, the development of drug-loaded intravenous folate-mediated liposomes should be a worthwhile and promising strategy (Lee & Low, 1995).

Several phase I (Robert et al., 2010; Sessa et al., 2002; Yamamoto et al., 2009) and phase II multicenter (Diéras et al., 2008; Zatloukal et al., 2008) studies have been conducted in patients with advanced solid tumours such as non-small lung cancer and metastatic breast cancer. The results showed that LTX has good activity, manageable toxicity and a favourable therapeutic index in patients with advanced solid tumours. Given its potent anticancer activity and acceptable toxicity, it is possible that LTX may eventually progress to human clinical trials. As such, it will be necessary to obtain a suitable formulation for LTX and a better understanding of its pharmacological characteristics, including its pharmacokinetics (PKs) and tissue distribution. In the current study, a new formulation called folate-poly (PEG-cyanoacrylate-co-cholesteryl cyanoacrylate) (FA-PEG-PCHL)-modified liposome (Figure 1) has been developed, and the influence of this lipid carrier on LTX PKs and tissue distribution in rats has been evaluated by comparing with LTX solution.

Materials and methods
Chemicals, reagents and animals
LTX (purity > 99.0%) and DTX (purity > 98.0%) reference standards were purchased from the National Institutes for Food and Drug Control (Beijing, China). LTX raw materials (purity > 98%) used for preparing the formulations were kindly provided by the Department of Pharmaceutics, Shenyang Pharmaceutical University (Shenyang, China). Methanol of HPLC grade was purchased from Fisher Scientific (Fair Lawn, NJ). Distilled water was prepared from demineralised water throughout the study. Other chemicals were of analytical grade.

Male Sprague–Dawley rats (220 ± 20 g) were kindly provided by the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China) and fed with unlimited access to food and water in an air conditioned animal centre at a temperature of 22 ± 2°C and a relative humidity of 50±10%, with a natural light–dark cycle for a week and then fasted with only access to water for 12 h prior to the experiment. The animal study was carried out in accordance with the Guideline for Animal Experimentation of Shenyang Pharmaceutical University, and the protocol was approved by the Animal Ethics Committee of the institution.

Preparation of LTX solution and modified liposomes
The preparation procedure of LTX solution was as follows: the drug was supplied as 94.4 mg of LTX in 2.36 ml of polysorbate 80. The solvent was supplied and composed of ethanol/water (13:87, w/w). Before administration, the drug was diluted in 5% glucose maintaining a concentration of drug of about 1 mg ml⁻¹.

The synthesis procedure of the copolymers of FA-PEG-PCHL (Figure 1) were all detailed in our previous paper (Li et al., 2011). The preparation procedure of LTX-loaded liposomes was as follows: first, a mixture of 0.005 mmol FA-PEG-PCHL, 0.012 mmol LTX and 0.25 mmol soybean lecithin was dissolved in 5 ml tetrahydrofuran. This solution was then injected into purified water at 25°C and the suspension obtained was stirred for 180 min to facilitate the removal of tetrahydrofuran. Second, to obtain a uniform particle size distribution, the suspension was sonicated at a frequency of 25 kHz and power of 500 W for 2.5 min by a probe-type sonicator (JY92-II, Scientz, Ningbo, China) and then sterilised by filtering through a 0.22 μm cellulose acetate filter. After that, appropriate amounts of cryoprotectants (5%, w/v) were dissolved in the liposome dispersion. Finally, the samples were frozen at −80°C for 8 h before being lyophilised using a freeze drier (Eyela FDU-1100, Prkakikai, Tokyo, Japan). The drying time was 36 h. The resulting solid matrix was collected for later experiments after rehydration with deionised water. Particle size distribution of the liposomes was determined by dynamic light scattering using ELS 800 apparatus (Otsuka Electronics, Osaka, Japan) at 25°C after dilution of the dispersion to an appropriate volume with Millipore-filtered water. The prepared liposomes had a...
similar particle size distribution, with a mean diameter of 111.6 ± 9.6 nm. LTX concentration in liposomes preparation was 1 mg ml⁻¹.

**Pharmacokinetic study of LTX solution and modified liposomes**

A plasma PK study was designed to evaluate the LTX-loaded FA-PEG-PCHL-modified liposomes by comparing with LTX solution. A total of 12 rats were randomly divided into two groups, with six in each (Li et al., 2011; Liu et al., 2013). The animal number was calculated based on the power analysis with a power of larger than 80% in the pre-experiment. Group 1 was randomly selected and treated with LTX solution at a dose of 4 mg kg⁻¹ (1 ml of the injection at the concentration 1.0 mg ml⁻¹ of LTX was administered via the tail vein), while a corresponding dose of LTX-loaded liposomes were administered to Group 2 (Kurata et al., 2000; Liu et al., 2013; Robert et al., 2010; Sessa et al., 2002; Yamamoto et al., 2009). Blood samples of about 0.3 ml were collected from the suborbital vein into heparinised centrifuge tubes, at 0.083, 0.167, 0.333, 0.5, 1, 2, 3, 4, 6, 8 and 12 h after dosing. These samples were immediately processed following centrifugation at 4000 × g for 5 min and then stored at −80°C until analysis.

**Biodistribution of LTX solution and modified liposomes**

The tissue distribution study was carried out on 60 rats which were randomly divided into 10 groups, with six in each. The animal number was calculated based on the power analysis with a power of larger than 80% in the pre-experiment. LTX solution/modified liposomes were given intravenously at a dose of 4 mg kg⁻¹ via the caudal vein. After administration, the rats were sacrificed at 0.083, 0.5, 2, 8 and 16 h, and tissues (brain, fat, heart, intestine, kidneys, liver, lungs, muscle, spleen and stomach) were collected at the same time. The tissue samples were rinsed in ice-cold normal saline, blotted dry with filter paper, and then stored at −80°C until analysis.

**LTX assay**

The quantitative analytical method of LTX in rat plasma/tissues is established based on a ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS, Waters Co., Milford, MA) method. Briefly, chromatographic separation was performed using an ACQUITY UPLC® BEH C₁₈, 1.7 μm particle size (50 × 2.1 mm, internal diameter, Waters Co.) column kept at 35°C with a constant flow rate of 0.2 ml min⁻¹. The mobile phase consisted of acetonitrile – 5 mmol l⁻¹ ammonium acetate (Table S1). The MS was performed in the positive ion, multiple reaction monitoring modes following the transition m/z 832.5→551.39 for LTX, and the transition m/z 808.2→527.17 for internal standard (IS, docetaxel). The IS stock solution was prepared at a concentration of 0.1 mg ml⁻¹ and further diluted with acetonitrile to achieve a final concentration of 8 μg ml⁻¹ for plasma samples, and 4 μg ml⁻¹ for tissue samples. All the bio-samples were pre-treated by a liquid–liquid extraction procedure. A volume of 20 μl acetonitrile and 20 μl IS solution were added into 100 μl plasma samples. For the tissue samples, about 0.2 g of the tissue sample was accurately weighed and homogenised with 0.5 ml methanol, while the entire tissue sample was taken and homogenised with 0.5 ml methanol if the total weight of the tissue is less than 0.2 g. A volume of 50 μl acetonitrile and 50 μl IS solution were added into 100 μl of the tissue homogenates. These bio-samples were extracted with a volume of 3 ml methyl tert-butyl ether, vortex-mixed for 5 min and centrifuged at 4000 × g for 10 min. The upper extract was then evaporated to dryness at 35°C under a nitrogen stream. The residue of plasma/tissue samples was reconstituted with 200/500μl aliquot of a mixture of acetonitrile/5 mmol l⁻¹ ammonium acetate (50/50, v/v), and a 5 μl aliquot was injected directly onto the UPLC-MS/MS system.

The retention times for LTX and IS were approximately 1.8 and 1.4 min, respectively. No interference from any endogenous substances was observed. The method was linear over the concentration range of 10.0–10 000 ng ml⁻¹ for rat plasma (10.0–30 000 ng g⁻¹ for rat tissues) (Table S2), with the lower limit of qualification of 10.0 ng ml⁻¹ for rat plasma and 10.0 ng g⁻¹ for rat tissues, respectively. The method showed good intra-assay precision and accuracy with relative standard deviation (% RSD) values from 2.78% to 10.3% and relative error (% MRE) from −8.53% to 7.93%, as well as good inter-assay precision and accuracy with RSD from 2.95% to 11.1%. The recoveries for all the bio-samples were over 85.4% (85.4–93.9%). There was no significant matrix effect for all the ratios within the range 97.6–99.5% for all the bio-samples. The MREs of short-term stability (−8.70–4.47%), freeze-thaw stability (−5.91–6.21%), autosampler stability (−7.07–5.17%) and long-term stability (−10.5–6.53%) of LTX in all the bio-samples were found to be within the range ±15.0%.

**Data analysis**

The PK parameters of LTX were calculated by non-compartmental analysis of plasma concentration versus time data utilising WinNonlin version 5.2 (Pharsight Corporation, Mountain View, CA). All statistical analyses were conducted utilising MATLAB version 8.2.0.701 (MathWorks Corporation, Natick, MA). Normality test of the PK parameters were performed using Lilliefors test. The comparison of PK parameters between administration of the LTX solution and LTX-loaded liposomes was possessed using independent samples t-test (data normally distributed) or the Mann–Whitney U-test (data non-normally distributed). All statistical tests were two-sided with *p < 0.05 as the probability required to declare a difference.

**Results**

**PK study of LTX solution and modified liposomes**

The developed analytical method has been successfully applied to the comparative PK study of LTX in plasma after a single intravenous administration (4 mg kg⁻¹) of LTX solution and LTX-loaded FA-PEG-PCHL-modified liposomes to rats. After visual inspection of the concentration-time profiles (Figure 2), significant higher LTX concentrations and area under the concentration–time curves (AUCs) were
revealed in rats following liposomes administration compared to those after simple solution administration. Changes in LTX PKs in rats following different dosage forms administration are also reflected in altered PK parameters based on the non-compartmental analysis (Table 1). As in the case of AUC, plasma clearance (CL\textsubscript{p}) and volume of distribution (V\textsubscript{d}), rats following liposomes administration were characterised by markedly higher AUCs (97.2%, \(*\text{**p}<0.01\)), slower CL\textsubscript{p} (49.2%, \(*\text{**p}<0.01\)) and lower V\textsubscript{d} (42.5%, \(*\text{**p}<0.01\)). It was in accordance with previous findings, where lipophilic drugs given in lipid carriers showed higher plasma concentration following intravenous injection than solution forms (Shi et al., 2009; Zhao et al., 2010).

Biodistribution of LTX solution and modified liposomes

The tissue LTX concentration-time curves and the significant values of LTX concentrations at different time points in different tissues after intravenous administration of LTX solution and LTX-loaded FA-PEG-PCHL-modified liposomes to rats are presented in Figure 3, while the AUC\textsubscript{0–16h} values in various tissues of the two formulations are listed in Table 2. The comparison of the AUCs between the two groups was conducted based on the estimates assessed using bootstrap analysis, in which subjects were randomly sampled with replacement from the original observations to obtain 1000 datasets that have the same number of subjects as the original dataset. The median and variability (as measured by standard deviation, SD) of the parameter estimates from the 1000 runs were then calculated and compared with the point estimates (median values) obtained with the original dataset (Table 2).

For the modified liposomes, the highest LTX concentrations were found in kidney, followed by liver, lung, fat, stomach, spleen, intestine, heart, muscle and brain, while the AUC\textsubscript{0–16h} of LTX in tissues was in descending order of stomach, spleen, intestine, heart, muscle and brain (Table 2). Significant lower LTX AUCs were detected in muscle (13.4%, \(*\text{p}<0.05\)), especially in heart (20.4%, \(*\text{**p}<0.01\)), lung (8.33%, \(*\text{**p}<0.01\)) and spleen (15.0%, \(*\text{**p}<0.01\)) of rats after modified liposomes administration. While, in contrast, AUCs in kidney increased significantly (100%, \(*\text{**p}<0.01\)) compared with the LTX solution.

Discussion

Microtubule protein has been considered as an attractive target for cancer therapy. LTX, as a microtubule active agent, has shown desirable PK and pharmacodynamic properties (Diéras et al., 2008; Robert et al., 2010; Sessa et al., 2002; Yamamoto et al., 2009; Zatloukal et al., 2008). However, polysorbate 80 used in traditional injections may cause several side effects. As such, some alternative formulation approaches need to be taken into account. In the current study, a novel formulation called LTX-loaded FA-PEG-PCHL-modified liposomes has been developed, and its PKs and biodistribution were evaluated by comparing with simple LTX solution.

The comparative PKs result indicated that the modified liposomes could postpone the elimination and lead to a longer blood circulating effect of LTX in rats. LTX is highly lipophilic substance and almost insoluble in water. When LTX is loaded in a liposome vehicle, it may be embedded in the lipophilic group of the phospholipids molecules to form a tight combination with phospholipids. This structure may delay the drug degradation and slowed down the release of free LTX into blood, which may lead to a prolonged terminal phase of elimination (Zhao et al., 2010).

Another explanation for the differences between LTX solution and modified liposomes may be due to the PEG

Table 1. Plasma pharmacokinetic parameters of LTX after intravenous administration of LTX solution and LTX-loaded FA-PEG-PCHL-modified liposomes in rats (4 mg kg\textsuperscript{-1}) (Median ± SD, n = 6).

| Parameters       | Unit               | LTX solution   | LTX-loaded liposomes | Statistical test |
|------------------|--------------------|----------------|----------------------|-----------------|
| AUC\textsubscript{0–12h} | mg h\textsuperscript{-1} | 0.791 ± 0.108 | 1.56 ± 0.173 | \(*\text{**p}<0.01, U\text{-test}\) |
| AUC\textsubscript{0–infinity} | mg h\textsuperscript{-1} | 0.794 ± 0.109 | 1.56 ± 0.173 | \(*\text{**p}<0.01, U\text{-test}\) |
| MRT              | h                  | 0.198 ± 0.101 | 0.206 ± 0.0970 | p > 0.05, U\text{-test} |
| t\textsubscript{1/2}   | h                  | 0.131 ± 0.0699 | 0.143 ± 0.0626 | p > 0.05, U\text{-test} |
| CL\textsubscript{p}   | l h\textsuperscript{-1} | 1.28 ± 0.155 | 0.650 ± 0.0710 | \(*\text{**p}<0.01, t\text{-test}\) |
| V\textsubscript{d}    | l                  | 0.233 ± 0.103 | 0.134 ± 0.146 | \(*\text{**p}<0.01, t\text{-test}\) |

AUC\textsubscript{0–12h}, area under the concentration-time curve from zero to the last measured time (12 h); AUC\textsubscript{0–infinity}, area under the concentration-time curve from zero to time infinity; CL\textsubscript{p}, plasma clearance; MRT, mean residence time; t\textsubscript{1/2}, elimination half-life; V\textsubscript{d}, volume of distribution. \(*\text{**p}, \text{the null hypothesis at the 1% significance level.}\)
Figure 3. (A) Median concentration-time curves of LTX in rat tissues after a single intravenous administration of LTX solution (circles and black line) and LTX-loaded FA-PEG-PCHL-modified liposomes (squares and grey line) at a dose of 4 mg kg\(^{-1}\) (\(n=6\)). (B) \(p\) Values of LTX concentrations at different time points in different tissues by comparing LTX solution with LTX-loaded FA-PEG-PCHL-modified liposomes.

Table 2. AUC\(_{0-16h}\) values in various tissues of intravenous administration of LTX solution and LTX-loaded FA-PEG-PCHL-modified liposomes.

| Time (h) | Brain | Fat | Heart | Intestine | Kidney | Liver | Lung | Muscle | Spleen | Stomach |
|---------|-------|-----|-------|-----------|--------|-------|------|--------|--------|---------|
| 0.083   | 0.969 | 0.862 | 0.970 ± 0.361 | 0.856 ± 0.241 | p > 0.05, U-test |
| 0.5     | 48.6  | 43.4 | 48.6 ± 7.19 | 43.1 ± 6.12 | p > 0.05, U-test |
| 2       | 10.9  | 8.69 | 10.9 ± 0.890 | 8.68 ± 0.313 | ** \(p < 0.01\), U-test |
| 8       | 26.1  | 24.0 | 26.2 ± 2.47 | 23.8 ± 3.54 | p > 0.05, U-test |
| 16      | 33.9  | 67.8 | 33.9 ± 0.878 | 67.9 ± 0.424 | ** \(p < 0.01\), t-test |
| 34.9    | 32.1  | 34.8 ± 2.43 | 31.9 ± 1.49 | ** \(p < 0.01\), U-test |
| 7.72    | 6.69  | 7.73 ± 0.757 | 6.69 ± 0.769 | \(p < 0.05\), U-test |
| 40.6    | 34.4  | 40.6 ± 0.628 | 34.5 ± 0.397 | ** \(p < 0.01\), U-test |
| 30.5    | 26.8  | 30.6 ± 4.25 | 26.9 ± 3.71 | p > 0.05, U-test |

AUC\(_{0-16h}\), area under the concentration-time curve from zero to the last measured time (16h).

\(p\), the null hypothesis at the 5% significance level; **\(p\), the null hypothesis at the 1% significance level.

Tissues were estimated based on tissue LTX concentrations after a single intravenous administration in rats (4 mg kg\(^{-1}\), \(n=6\)).

Data are presented as median ± SD.

Based on the bootstrap result.
chains of the FA-PEG-PCHL (Figure 1). The lipophilic part cholesterol merged with the core of the lipid carrier, therefore, allows the hydrophilic part of PE-PEG to swing on the surface of the nanoparticles, mask the surface charge and ensure efficient steric stabilisation. The hydrophilicity and steric barrier of PE-PEG can prevent the opsonin–nanoparticle interaction, which is the first step of the recognition by the immune system. The interference with the recognition by opsonin allows the drug-loaded lipid carrier to reduce the phagocytosis by the mononuclear phagocyte system cells, and achieve a prolonged blood circulation of the nanoparticles.

When liposomes were administrated by intravenous injection, they are usually easily taken up by the reticuloendothelial system (RES) such as liver, lung and spleen (Mizushima et al., 1982; Yamaguchi et al., 1984). The mechanism of uptake of the lipid emulsions has been demonstrated to be either by phagocytosis or endocytosis (Yanagikawa, 1982). However, unlike liposomes described above, the AUCs of LTX-loaded FA-PEG-PCHL-modified liposomes in the liver are not increased in comparison with LTX solution. In addition, the AUCs of modified liposomes in lung and spleen were even lower than that of LTX solution (**p < 0.01, Table 2). This phenomenon can be attributed to the particle size and lipid composition (Kawakami et al., 2000). Small emulsion (e.g. <200 nm) could avoid the trapping by RES (Takino et al., 1994), and the composition of the phospholipids used in the liposomes could influence the ability to be transported and metabolised resulting in low RES uptake (Manjunath & Venkateswarlu, 2005; Takino et al., 1994). Moreover, the clearance behaviour and tissue distribution of intravenously injected particulate drug carriers are also influenced by their surface features and opsonisation. Opsonins are adsorbed on the nanoparticle surface and promote particle recognition by the RES (Manjunath & Venkateswarlu, 2005; Moghimi et al., 2001). Lipid emulsions are rapidly taken up by the RES in the liver, lung and spleen after intravenous administration (Jia et al., 2010). However, the presence of a long hydrophilic chain in PEG around the shell of FA-PEG-PCHL-modified liposomes would prevent the recognition by the immune system and lead to a longer retention time in the circulation. As a result, the LTX concentrations in lung and spleen in the FA-PEG-PCHL-modified liposomes treated group were significantly lower than those in the LTX solution-treated group (***p < 0.01).

On the other hand, the LTX crystals might precipitate in the vessel after intravenous administration of LTX solution. The smaller precipitation of LTX may be recognised and trapped by macrophages into RES, and the bigger one may be trapped in the capillary bed of the tissues with abundant blood flow such as lung and heart. So, it is reasonable that the AUCs of LTX solution in lung and heart were significantly higher than that of LTX-loaded liposomes. The lower AUCs of LTX in the heart may reduce the cardiac toxicity, which indicated that LTX-loaded modified liposomes were safer on reducing the side effect to some extent.

Notably, rats treated with LTX-loaded modified liposomes were characterised by higher drug concentrations in kidney than those treated with simple LTX solution, and the higher concentrations of LTX in kidney in LTX-loaded modified liposomes treated rats could be explained by the folate receptor effect – folate receptor level is naturally rich in kidney (Yamaguchi et al., 1984). In addition, the presence of the drug in the brain indicated that both LTX molecules and LTX-loaded liposomes could penetrate the BBB. As there was no significant difference in LTX concentrations for the two formulations in the brain (p > 0.05), the uptake of modified lipid carriers by the brain was not improved because of lack of the folate receptor in the inner side walls of brain capillaries (Kennedy et al., 2003).

LTX did not show a typical bi-exponential distribution in the brain. One possible explanation for the complex wave pattern of the concentration-time profiles is that LTX is a lipophilic compound, which may rapidly cross the BBB, but which then exhibit a high degree of non-specific binding within the brain. It is possible that the bound or trapped LTX molecules are released back to the plasma very slowly. Under such conditions, the binding or trapping of LTX in brain forms the prolonged terminal phase of the concentration-time profiles. However, there is little information available on LTX distribution in the brain. Further studies should be conducted to explain this phenomenon.

Conclusion

A UPLC–MS/MS method was developed, validated and applied to the comparative PKs and tissue distribution study of LTX in rats after intravenous administration of LTX solution and a newly developed LTX-loaded FA-PEG-PCHL-modified liposomes. The results of the current study indicated that the FA-PEG-PCHL-modified liposome could be an effective parenteral carrier for the delivery of LTX in cancer treatment and could be useful in the design of long blood circulating and intravenously injectable biodegradable drug carriers of LTX in the future.

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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