Research Article

A Cremophor-Free Self-Microemulsified Delivery System for Intravenous Injection of Teniposide: Evaluation *In Vitro* and *In Vivo*

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Abstract. In order to tackle the problems on low water solubility of teniposide, involvement of toxic surfactant in its injection, and the poor stability during infusion, a Cremophor-free teniposide self-micro-emulsified drug delivery system (TEN-SMEDDS) was prepared for the first time, characterized, and evaluated in comparison with teniposide injection (VUMON) *in vitro* and *in vivo*. The optimized formulation contained N, N-dimethylacetamide, medium-chain triglyceride, lecithin, and dehydrated alcohol besides teniposide. The TEN-SMEDDS could form fine droplets with mean diameter of 282 ± 21 nm and zeta potential of -7.5 ± 1.7 mV after dilution with 5% glucose, which were stable within 4 h. The release of teniposide from TEN-SMEDDS and VUMON was similar. However, the pharmacokinetic behavior of TEN-SMEDDS in rats was different from that of VUMON, evidenced by the lower area under the concentration–time curve and larger volume of distribution in emulsion group. Finally, TEN-SMEDDS was found to distribute more teniposide in most tissues, especially in reticuloendothelial system, after intravenous administration to rats. Importantly, brain drug level in TEN-SMEDDS group was higher than or similar to that in control group, although the emulsion system had a lower plasma drug concentration. In conclusion, the novel SMEDDS prepared here, without toxic surfactant and as an oil solution before use, may be potential for clinical use due to its low toxicity and high store stability. It may be favorable for the treatment of some tumors like cerebroma, since it may achieve the relatively higher drug level in brain but lower blood concentration.

KEY WORDS: characterization; pharmacokinetics; self-microemulsified drug delivery system; teniposide; tissue distribution.

INTRODUCTION

Teniposide 4′-demethylepipodophylotoxin-4-(4,6-O-theny-lidine-β-D-glucopyranoside), which is a semisynthetic derivative of podophyllotoxin resin, is effective for treatment of various malignancies, such as neuroblastoma (1,2), small cell lung cancer, non-Hodgkin's and Hodgkin's lymphoma, and acute leukemia, especially cerebroma (3). However, its application in clinic was limited due to the poor aqueous solubility. In addition, teniposide was unstable in an aqueous solution. Currently, its intravenous injection available in market (VUMON, Bristol-Myers Squibb S.r.l.) contains high concentration of polyethoxylated castor oil (Cremophor EL) as a solubilization agent (4). VUMON can cause several severe side effects such as hypersensitivity, hypertension, hypoeosinophilia, and hematological toxicity (5–10). These are most probably caused by the surfactant Cremophor contained in the vehicle, rather than the drug itself (11). Also, Cremophor may affect red blood cells because the surfactant can penetrate biological membranes to cause an increase in permeability and cell damage. A change in the shape of human white blood cells (WBCs) has also been reported in the studies on the Cremophor (12–14). Hence, monitoring the WBC and platelet count needs to be executed regularly during VUMON administration in clinic (15). In order to avoid these disadvantages, several kinds of nanocarriers, such as liposomes, submicron lipid particles, and lipid emulsions, have been investigated. These explorations partially overcame the problem on the poor solubility of teniposide; however, the stability of these delivery systems was still a big challenge (4,16).

Self-microemulsified drug delivery system (SMEDDS) has recently attracted much attention (17–21). It holds promise for pharmaceutical industry as a safer and an efficacious alternative for drugs with poor solubility (22). With oil and surfactant, SMEDDS was a concentrated solution without water, so it is physically or chemically more stable than emulsion. This system can dissolve many water-insoluble drugs in a proper formulation, while it is less in volume, leading to the easy storage and transportation. Before use, it can form fine oil-in-water microemulsions with gentle agitation following dilution by aqueous phases (23,24), which is favorable for clinical use as intravenous injection. It was reported that such system was superior to micelle or co-solvent system in terms of drug solubilization and stability (25–27). Nevertheless, there is no SMEDDS available for intravenous injection right now,

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Table I. The Effect of the Oil and Emulsifier on the Droplet Size and Stability of TEN-SMEDDS ($n=3$)

Formulation	E-80 (mg)	MCT (mg)	Appearance	Droplet size (nm)	PDI	Stablity (h)
1	2,000	0	Opalescence	222±15	0.273±0.032	<2
2	2,500	0	Opalescence	294±20	0.413±0.026	<4
3	3,000	0	Ivory	441±23	0.509±0.021	>4
4	2,000	250	Ivory	282±21	0.423±0.035	>4
5	2,500	250	Ivory	354±29	0.624±0.035	>6
6	3,000	250	Ivory, great viscosity	432±31	0.797±0.031	>8
7	2,000	500	Ivory	394±36	0.909±0.030	>6
8	2,500	500	Ivory, great viscosity	278±41	0.911±0.028	>8
9	3,000	500	Ivory, great viscosity	390±38	0.885±0.030	>8

possibly due to the difficulty in getting rid of the toxic surfactants, such as Cremophor.

In the present study, we try to develop a teniposide self-microemulsified drug delivery system (TEN-SMEDDS) with a good stability and low toxicity by a simple method. All the excipients used in TEN-SMEDDS are commercially available and proven to be safe for i.v. administration by FDA. The physicochemical characteristics of the TEN-SMEDDS were investigated, and the *in vitro* release, *in vivo* pharmacokinetics, and tissue distribution were assessed using VUMON as a reference. The results proved the possibility of making the water-insoluble teniposide into a Cremophor-free SMEDDS and demonstrated the characteristics of TEN-SMEDDS *in vitro* and *in vivo*, laying a good foundation for its clinical use in the future.

MATERIALS AND METHODS

Materials and Animals

Teniposide was kindly donated by Kelun Pharmaceutical Co., Ltd (Sichuan, China). Lipoid E80 was purchased from Lipoid (German). Medium-chain triglyceride (MCT) was provided by Magna-Kron Co. (USA), and N,N-dimethylacetamide (DMA) was from Acros (Geel, Belgium). Methanol and acetonitrile of HPLC-grade were obtained from Promptar (Elk Grove, USA). All other reagents were of analytical grade.

Preparation of TEN-SMEDDS

TEN-SMEDDS was prepared by a simple process at room temperature. Firstly, 50 mg of teniposide was dissolved in 300 μL DMA. Next, E-80 (2,000–3,000 mg) and MCT (0–500 mg) were added and an appropriate amount of dehydrated alcohol was used to make the formulation up to 5 mL (or 10 mL). Finally, the solution was uniformly mixed and filtered through a 0.22-μm filter.

HPLC Analysis of Teniposide *In Vitro*

A reversed-phase HPLC system composed of an LC-20AT pump (Shimadzu, Kyoto, Japan), a SPD-20A UV detector (Shimadzu, Kyoto, Japan), and an analysis column (octa decyl silane (ODS) column, 5 μm, 200×4.6 mm) was used for the determination of teniposide. The mobile phase consisted of acetonitrile and double-distilled water (45/55, *v/v*). The elution was carried out at a flow rate of 1.0 mL/min at 35°C and the detect wavelength was 240 nm. The HPLC method was validated by the studies on precision, accuracy, and standard curve (data not shown).
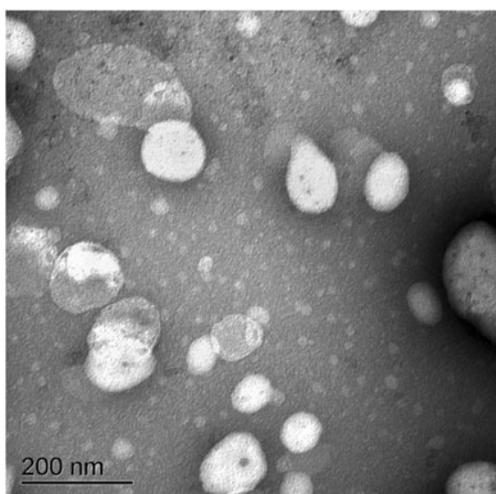
Characterization of TEN-SMEDDS

Transmission Electron Microscopy

The morphology of TEN-SMEDDS after dilution was observed using a transmission electron microscope (TEM)
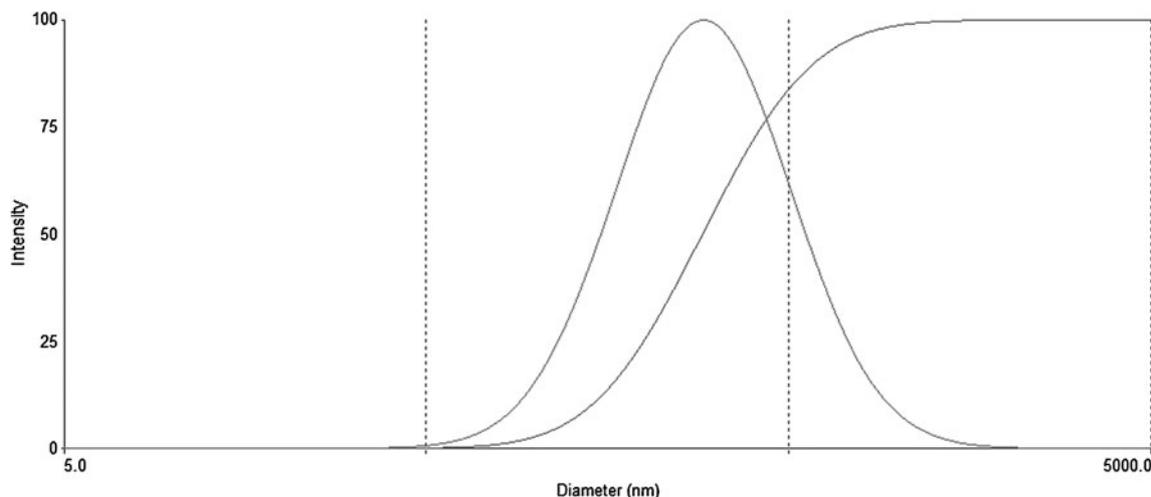
Fig. 1. Droplet size distribution of the optimized TEN-SMEDDS determined by DLS

Fig. 2. TEM image of the TEN-SMEDDS

(JEM-1200, JEOL Co., Ltd., Japan). The samples were diluted with purified water at a ratio of 1:200 by gentle shaking. Then, a drop of sample was deposited on a copper grid. The excess fluid was drawn off with filter paper. Subsequently, the grid was stained with 1% (*w/v*) uranyl acetate and allowed to dry before examination.

Droplet Size and Zeta Potential

A certain volume of TEN-SMEDDS was diluted with 5% glucose injection to a definite volume and shaken gently to mix thoroughly before measurement. The droplet size, polydispersity index (PDI), and zeta potential of so-formed microemulsion were determined by dynamic light scattering (DLS) analysis in a Brookhaven ZetaPlus (Brookhaven, USA) at 25°C. The correlation decay functions were analyzed by the cumulant method to determine the *Z*-average size, and the regularized CONTIN method was used to obtain the droplet size distributions. The results were the mean values of three experiments for the same sample.

Compatibility with Different Medium

Clinically, the commercial injection of teniposide (VUMON) was diluted with 5% glucose or 0.9% sodium chloride injection before intravenous administration. In order to evaluate the stability of the TEN-SMEDDS when diluted with different medium, it was diluted in 5% glucose or 0.9% sodium chloride injection to the final concentrations of 0.1, 0.2, 0.4, 0.8, and 1.0 mg/mL, respectively, and the physicochemical

properties of the so-formed microemulsion, including appearance, droplet size, size distribution, and stability, were then investigated.

In Vitro Release

The release behavior of teniposide from the TEN-SMEDDS or VUMON was determined in phosphate buffer (0.01 M, pH 7.4±0.1) containing 1.0% (*w/v*) Tween 80. Briefly, 1 mL of TEN-SMEDDS or VUMON diluted with 5% glucose (equivalent to 0.2 mg of teniposide) was put into dialysis bags (MW cut off 12,000–14,000 kDa) which were placed in 100 mL release medium stirred at 100 rpm at 37°C. The dialysis bags were soaked in double-distilled water for 24 h before use. An aliquot of 1 mL release medium was withdrawn at intervals of 0.5, 1, 2, 4, 6, 8, and 12 h and replaced by 1 mL of fresh medium. Each sample was passed through a 0.45-μm syringe filter and then determined by HPLC method described above.

In Vivo Pharmacokinetic Study

Animals and Dosing Protocol

Male Sprague–Dawley rats were provided by the Animal Institute of Peking University Health Science Center (Beijing, China). All care and handling of animals were approved by the Ethics Committee of Peking University. Twelve rats weighting 200±10 g were fasted overnight with free access to water. The animals were then divided into two groups, with six rats in each group. After dilution with 5% glucose, TEN-SMEDDS or VUMON was given intravenously to rats *via* the tail vein at a dose of 10 mg/Kg. Blood samples were taken into heparinized tubes at pre-designed time points (5, 15, 30, 60, 120, 240, and 360 min) after administration. The plasma was immediately collected by centrifugation at 5,000 rpm for 10 min and stored at −20°C until analysis.

Plasma Processing and Analysis

Plasma processing and HPLC analysis were performed according to literatures with a little modification (28). An aliquot of 3 mL acetoacetate was added into 200 μL of plasma. The mixture was vortexed for 1 min followed by centrifugation at 10,000 rpm for 10 min at 4°C, and then the organic layer was separated and evaporated under a stream of nitrogen gas. The residue was dissolved with 200 μL methanol. The same HPLC system as mentioned above was used except the equipment of an ODS pre-column (12.5×4.6 mm, 5 μm). The mobile phase consisted of water and acetonitrile (60:40, *v/v*),

Table II. The Effect of Dilution with 5% Glucose on the Stability of TEN-SMEDDS (*n*=3)

Concentration of teniposide (mg/mL)	Appearance	Droplet size (nm)	PDI	Stability (h)
0.1	Translucence	236±22	0.416±0.032	>12
0.2	Opalescence	242±27	0.438±0.029	>12
0.4	Ivory	282±21	0.423±0.035	>4
0.8	Ivory, great viscosity	626±16	0.929±0.024	<4
1.0	Ivory, great viscosity	704±19	0.954±0.032	<4

and 20 μL of sample was injected into the HPLC system for the detection of teniposide.

Pharmacokinetic Parameters

Standard non-compartmental pharmacokinetic parameters were calculated using DAS software (ver. 2.1.1, Mathematical Pharmacology Professional Committee of China). The calculated pharmacokinetic parameters included the maximum peak concentration of the drug in plasma (C_{max}), the area under the concentration–time curve ($AUC_{0-\infty}$), the total elimination rate (CL), and the apparent volume of distribution at a steady state (V_d).

Tissue Distribution

The animals and dosing protocol were the same as in pharmacokinetic study. At predetermined time intervals (5, 30, and 120 min) after i.v. administration, three rats were sacrificed in each group, and the tissues of interest, including heart, liver, spleen, lungs, kidneys, and brain, were harvested. The collected tissues were dried, weighed, and frozen at −20°C. Then, each tissue was homogenized in normal saline before analysis. Sample processing and HPLC analysis were the same as in pharmacokinetic study.

Statistics

Quantitative data were expressed as means ± standard deviation. Statistical significance on the difference of pharmacokinetic parameters between treatment and control group was evaluated by Student's *t* test. A *p* value less than 0.05 was considered to be significant, while less than 0.01 was highly significant.

RESULTS AND DISCUSSION

Effect of the Oil, Emulsifier, and Co-emulsifier on the Droplet Size and Stability

The TEN-SMEDDS must be diluted to form microemulsion prior to infusion. The droplet size and the stability of the formed microemulsion are critical to clinical use. As the components of this formulation, MCT, emulsifier E-80, and co-emulsifier dehydrated alcohol might have effect on the droplet size and stability of the microemulsion. In order to investigate the effect of dehydrated alcohol, the final volume of the system containing 50 mg teniposide was made with 5 and 10 mL of dehydrated alcohol, respectively. However, it was difficult to form a microemulsion in 5 mL specification because the concentrated solution was so viscous when diluted with 5% glucose. Therefore, in the following study, the 10-mL formulation containing 50 mg teniposide was used. And in the characterization of TEN-SMEDDS, the final concentration of

Table III. Apparent Solubility of Teniposide in Double-Distilled Water with Different Percentage of Tween 80 (*n*=3)

% of Tween 80 (*v*/*v*)	0	0.1	1.0
Solubility (μg/mL)	0.54±0.32	8.35±0.15	12.07±0.21
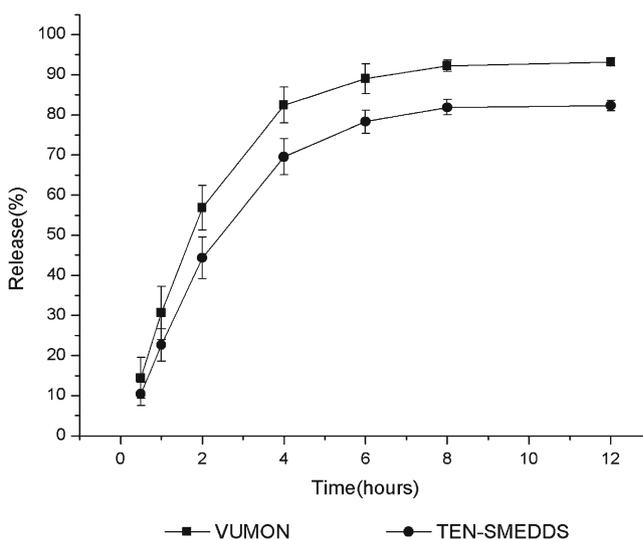
Fig. 3. Release profiles of teniposide from TEN-SMEDDS and VUMON (*n*=3)

teniposide in all the samples was 0.4 mg/mL after dilution with 5% glucose.

Table I shows the effect of E-80 and MCT on the appearance, droplet size, and stability of the TEN-SMEDDS. The stability was evaluated according to the time that teniposide forms crystal in microemulsion under microscope. The stability of TEN-SMEDDS, as well as its droplet size and PDI, was found to increase as the amount of E-80 and MCT increased. The formulation with 2,000 mg E-80 and 50 mg MCT was considered to be suitable here since its droplet size was relatively smaller and uniform, and it was stable within 4 h which might be enough for clinical application. Therefore, based on the screening here by the single factor method, the optimal formulation of TEN-SMEDDS was composed of 50 mg teniposide, 300 μL DMA, 2,000 mg E-80, 250 mg MCT, and a suitable amount of dehydrated alcohol to make a final volume of 10 mL.
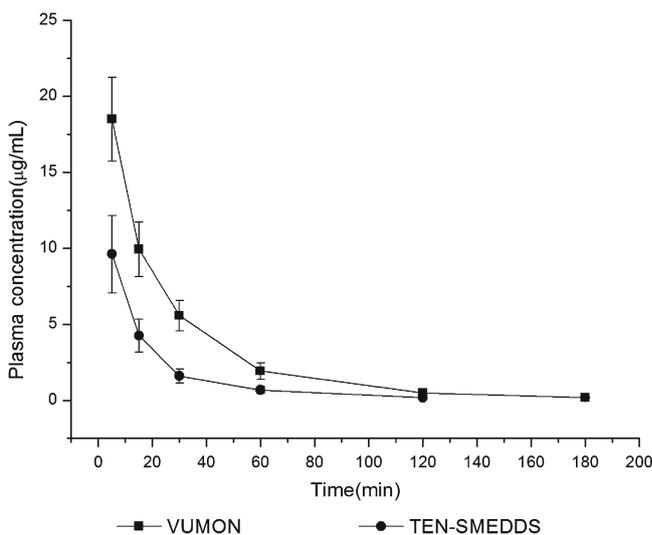
Fig. 4. Plasma concentration–time curves after intravenous administration of TEN-SMEDDS or VUMON at a dose of 10 mg/Kg in rats (*n*=6)

Table IV. Pharmacokinetic Parameters of Teniposide after Intravenous Administration of TEN-SMEDDS or VUMON at a Dose of 10 mg/Kg in Rats ($n=6$)

Formulation	C_{max} (mg/L)	$AUC_{0-\infty}$ (mg/min/L)	CL (L/min)	V_d (L)
TEN-SMEDDS	9.62±2.55**	235.01±54.84**	0.0088±0.0019*	0.27±0.094*
VUMON	18.49±2.75	569.29±71.33	0.0035±0.00054	0.16±0.052

*$p<0.05$ *versus* VUMON group; **$p<0.001$ *versus* VUMON group

Characterization of TEN-SMEDDS

Measurement of Droplet Size and Zeta Potential

As the result of DLS analysis, the mean diameter, PDI, and zeta potential of teniposide microemulsion were 282± 21 nm, 0.423±0.035, and −7.5±1.7 mV, respectively. The droplet size distribution was shown in Fig. 1.

Morphological Investigation

TEM image of teniposide microemulsion is shown in Fig. 2, which confirmed that microemulsion droplets were well dispersed without any aggregation or cluster and were almost spherical in shape. In the TEM image, the droplet size was about 150 nm, smaller than that by DLS. This was understandable because these two methods were based on different mechanisms and the samples were also different: DLS using liquid sample where the microemulsion remains unchanged, while TEM involving a drying step that results in dehydration or shrinkage of microemulsion droplet (29,30).

Compatibility with Different Dilution Medium

In this study, TEN-SMEDDS was diluted with 5% glucose and 0.9% NaCl injection, respectively. After diluted with normal saline to 0.1–1.0 mg/mL (calculated as teniposide), the microemulsion appeared flocculated within 2 h, indicating that teniposide microemulsion was unstable in physiologic saline. The phenomenon may be caused by high ionic strength. The effect of dilution with 5% glucose on the properties of TEN-SMEDDS is presented in Table II. As shown in Table II, as the teniposide concentration increased, the mean diameter and PDI of microemulsion increased, while its stability decreased. When the concentration was more than 0.4 mg/mL,

the droplets of microemulsion enlarged dramatically, and its stability was less than 4 h. In conclusion, the TEN-SMEDDS should be emulsified by 5% glucose to produce a 0.1–0.4-mg/mL microemulsion which should be used within 4 h.

In Vitro Release Study

A dialysis technique was used to determine the *in vitro* release behavior of teniposide from VUMON and SMEDDS. To ensure the sink condition, according to the apparent solubility of teniposide in different media (Table III), 1.0% Tween 80 was added into the release medium. The release profiles of teniposide from VUMON and TEN-SMEDDS are shown in Fig. 3. At 12 h, the cumulative release of teniposide from VUMON and SMEDDS was 92.3% and 83.2%, respectively. Although the rate and extent of teniposide released from SMEDDS were less than those from VUMON, there were no significant differences ($p>0.05$). As we know, VUMON is a colloidal solution containing Cremophor, and in TEN-SMEDDS, teniposide is solubilized in the oil phase of the microemulsion. So, in the sink condition, drug diffusion from these two systems was different but somehow similar (15).

The obtained release data were then fitted into first-order, Higuchi, Hixcon–Crowell, Nibergull, Ritger–Peppas, and Weibull equations. The regression results indicated that the Ritger–Peppas model best fitted the release data (VUMON, $R=0.9491$; SMEDDS, $R=0.9583$).

In Vivo Pharmacokinetic Studies

The plasma drug concentration–time profiles and pharmacokinetic parameters of teniposide after a single intravenous administration of VUMON and TEN-SMEDDS are presented in Fig. 4 and Table IV, respectively. As shown in Table IV, there were significant differences between these two formulations in
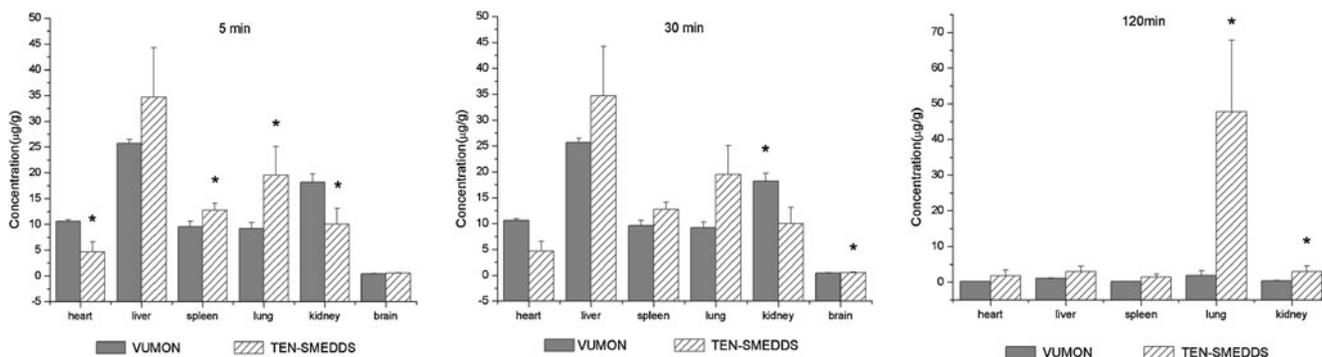
Fig. 5. Concentrations of teniposide in tissues after intravenous administration of TEN-SMEDDS or VUMON at a dose of 10 mg/Kg in rats ($n=3$). *$p<0.05$ *versus* VUMON group

several pharmacokinetic parameters of teniposide, including C_{max}, $AUC_{(0-\infty)}$, CL, and V_d. Typically, the $AUC_{(0-\infty)}$ and C_{max} of TEN-SMEDDS were significantly lower than that of the VUMON ($p<0.01$), respectively, while the CL and V_d in TEN-SMEDDS group were significantly larger than that of commercial injection ($p<0.05$). This seemed to say that teniposide in SMEDDS could be more widely distributed than that in VUMON and eliminated more quickly.

It was previously reported that solubilization with Cremophor could prolong the drug plasma level after i.v. administration compared to an emulsion system (31–34) because Cremophor was supposed to inhibit P-glycoprotein-mediated biliary secretion and caused lipoprotein dissociation that would alter protein binding (35,36). The significantly higher CL and V_d in TEN-SMEDDS group than that in the commercial injection group indicated that Cremophor might prevent the distribution of teniposide into the tissues (37). On the other hand, the faster clearance of TEN-SMEDDS may partially be explained by the fact that lecithin is an endogenous substance, but Cremophor is not (14). Of course, the wide and fast distribution of teniposide in SMEDDS group to peripheral tissues may also cause additional safety concerns, which needs further studies.

From the literatures cited in our manuscript (5–10), we noticed that the main side effects for VUMON were mainly allergic reaction and hematological toxicity. Without Cremophor EL, our formulation may be favorable for reducing the allergic reaction, and our test proved this also (data not shown). On the other hand, the lower C_{max} and AUC values compared to VUMON indicated the lower plasma level of teniposide, likely being advantageous for decreasing the hematological toxicity.

Tissue Distribution

The drug distribution in different tissues after intravenous administration of TEN-SMEDDS and VUMON is shown in Fig. 5. Firstly, the concentrations of teniposide in TEN-SMEDDS group were higher than those in the VUMON group in most tissues tested for 5–120 min and in all tissues at 120 min, confirming the wide distribution of the drug suggested by the larger V_d value in pharmacokinetic study. Secondly, the drug in the TEN-SMEDDS group was found to distribute much more than those in VUMON group in reticuloendothelial system (RES), like liver, spleen, and lung. This reveals that SMEDDS may relatively be easier to accumulate in tissues where phagocytosis cells are rich. Similar observation on rapid uptake of emulsion by the RES after intravenous administration was previously reported (38,39). Several factors, such as droplet size, zeta potential, and opsonization may influence the tissue distribution of intravenously injected TEN-SMEDDS.

Most importantly, it was found here that accumulation of teniposide in the brain was higher in TEN-SMEDDS group than that in its control, although there were no significant differences between these two treatments at 5 and 30 min($p>0.05$), and it could not be detected in the brain at 120 min. It means that the distribution of teniposide in the brain in TEN-SMEDDS group was not less than that in VUMON group though the drug plasma level in the former group was much low. This was significant because teniposide is mostly used for cerebroma in the clinic. So, TEN-SMEDDS may lead to the same drug level in the brain but much low system drug exposure which may result in low system

toxicity. For instance, TEN-SMEDDS may be favorable to decrease the main side effect of teniposide, the hematological toxicity (8–10).

CONCLUSIONS

In this study, Cremophor-free TEN-SMEDDS for i.v. injection was designed and successfully constructed. The novel system solved a series of related problems, such as making a water-insoluble drug into an injection, the involvement of toxic surfactant in teniposide injection, and the poor stability of an emulsion system, guaranteeing a low toxicity and a high stability. Its physicochemical characteristics, *in vitro* release, *in vivo* pharmacokinetics, and tissue distribution were investigated using VUMON as the reference. It was found that these two systems were similar in drug release but different in pharmacokinetic parameters and tissue biodistribution. After i.v. injection to rats, TEN-SMEDDS achieved a high distribution in most tissues but low drug level in plasma. The brain drug level in SMEDDS group was higher than or similar to its control. This interesting finding may be favorable for teniposide since one of its main indications in clinic is cerebroma and it is a drug with hematological toxicity.

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