International Journal of Nanomedicine

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Intravenous microemulsion of docetaxel containing an anti-tumor synergistic ingredient (Brucea javanica oil): formulation and pharmacokinetics

This article was published in the following Dove Press journal:
International Journal of Nanomedicine
24 October 2013
Number of times this article has been viewed

Shilin Ma[1]
Fen Chen[1]
Xiaohui Ye[2]
Yingjie Dong[2]
Yingna Xue[1]
Heming Xu[1]
Wenji Zhang[1]
Shuangshuang Song[1]
Li Ai[2]
Naixian Zhang[2]
Weisan Pan[1]

[1]School of Pharmacy, Shenyang Pharmaceutical University, Shenyang, [2]Liaoning Institute of Pharmaceutical Industry, Liaoning, The People's Republic of China

Abstract: The purpose of this study was to develop a docetaxel microemulsion containing an anti-tumor synergistic ingredient (Brucea javanica oil) and to investigate the characteristics of the microemulsion. Brucea javanica oil contains oleic acid and linoleic acids that have been shown by animal and human studies to inhibit tumor formation. The microemulsion containing Brucea javanica oil, medium-chain triglyceride, soybean lecithin, Solutol®HS 15, PEG 400, and water was developed for docetaxel intravenous administration. A formulation with higher drug content, lower viscosity, and smaller particle size was developed. The droplet size distribution of the dispersed phase of the optimized microemulsion was 13.5 nm, determined using a dynamic light scattering technique. The small droplet size enabled the microemulsion droplets to escape from uptake and phagocytosis by the reticuloendothelial system and increased the circulation time of the drug. The zeta potential was –41.3 mV. The optimized microemulsion was pale yellow, transparent, and non-opalescent in appearance. The value of the combination index was 0.58, showing that there was a synergistic effect when docetaxel was combined with Brucea javanica oil. After a single intravenous infusion dose (10 mg/kg) in male Sprague Dawley rats, the area under the curve of the microemulsion was higher and the half-time was longer compared with that of docetaxel solution alone, and showed superior pharmacokinetic characteristics. These results indicate that this preparation of docetaxel in emulsion is likely to provide an excellent prospect for clinical tumor treatment.

Keywords: microemulsion, docetaxel, synergistic ingredient, formulation, pharmacokinetic

Introduction

Cancer is a serious threat to health and one of the main causes of death worldwide. Based on GLOBOCAN 2008 estimates, about 12.7 million cancer cases and 7.6 million cancer deaths are estimated to have occurred in 2008; of these, 56% of the cases and 64% of the deaths occurred in the economically developing world.[1] Chemotherapy, the primary means of treating cancer, has undergone extensive improvement in recent years. However, despite the development of new anticancer agents, taxanes remain the cornerstone of adjuvant and metastatic chemotherapy against solid tumors.[2] Docetaxel (DOC), a second-generation semi-synthetic taxane derived from the needles of the European yew tree *Taxus baccata*, is an anti-mitotic chemotherapeutic agent.[3] DOC is considered to be one of the most important anticancer drugs for clinical use. It acts by inhibiting the microtubular network that is essential for mitotic and interphase cellular functions. It also accelerates the assembly of tubulin into stable microtubules

Correspondence: Weisan Pan
School of Pharmacy,
Shenyang Pharmaceutical University,
Wenhua Road 103, Shenyang 110015,
The People's Republic of China
Tel +86 24 2398 6313
Fax +86 24 2398 6313
Email pwstfzy@163.com

and hinders their disassembly, thereby inhibiting cell division and causing eventual cell death.[4,5]

DOC is registered as first line therapy for metastatic breast, non-small cell lung, prostate, gastric, and head and neck cancer.[6] Although the solubility of DOC is increased compared with that of paclitaxel, it is still low. The nonionic surfactant Tween® 80 and ethanol used in clinical dosage Taxotere® causes some side effects, such as neurotoxicity, fluid retention and musculoskeletal toxicity.[7,8] Multidrug resistance is another problem inherent to DOC. To overcome these disadvantages and to improve solubility of DOC, alternative methods of drug delivery have been suggested, including liposomes,[9,10] nanoparticles,[11,12] micelles,[13] prodrugs,[14] macromolecular conjugates,[15] and cyclodextrins.[16]

Microemulsions (MEs) are transparent, thermodynamically stable isotropic mixtures of oil, water, and surfactant, and are frequently combined with a cosurfactant.[17,18] MEs have more favorable solubilization capacities for lipophilic drugs than do micellar systems because of the extra locus for solubilization provided by the internal droplet oil phase.[17] Currently, tumor blood vessels are characterized by abnormalities such as a high proportion of proliferating endothelial cells, pericyte deficiency, and aberrant basement membrane formation leading to an enhanced vascular permeability. Particles such as nanocarriers (in the size range 20–200 nm) can extravasate and accumulate inside the interstitial space.[19]

Brucea javanica oil (BJO) (ya-dan-zi oil) is an extract of the ripe fruit of the simaroubaceae plant *Brucea javanica* (L.) Merr., which was first recorded in the Supplement to Compendium of Materia Medica.[20] BJO contains oleic acid, linoleic acid, stearic acid, palmitic acid, arachidonic acid, and other unsaturated fatty acids. A number of clinical studies have suggested that BJO emulsions can be used alone as a conventional treatment for various cancers;[21–23] however, the present study can show only that BJO emulsions have synergistic effects when combined with certain anticancer drugs or radiotherapy. Our research does not confirm that BJO emulsions result in satisfactory treatment for cancer when used alone. In this study, BJO was used as the carrier of another anticancer drug and also as an antitumor synergistic ingredient. It possesses emulsifying and embolism properties, and allows the drug to remain for a prolonged time in the tumor area and to be released slowly.

Materials and methods

Materials and instruments

Docetaxel and paclitaxel were purchased from Zhongxi Sunve Pharmaceutical Co., Ltd. (Shanghai, People's Republic of China). BJO was obtained from Yaoda Pharmaceutical Co., Ltd., Shenyang, Liaoning, People's Republic of China. Soybean lecithin (S75) for injection was purchased from Shanghai Taiwei Pharmaceutical Co., Ltd., People's Republic of China. Solutol® HS 15 (PEG 660-12-hydroxystearate, BASF, Ludwigshafen, Germany) and PEG 400 were gifts from the Beijing Fengli Jingqiu Commerce and Trade Co., Ltd. (Beijing, People's Republic of China). Tween-80 was supplied by Xi'an Haotian Bio-engineering Technology Co., Ltd. (Xi'an, Shaanxi, People's Republic of China). Medium-chain triglyceride (C8, MCT) was purchased from Tieling Beiya Medical Oil Co., Ltd. (Tieling, Liaoning, People's Republic of China). Methanol, ethanol, and acetonitrile were chromatography grade.

Male Sprague Dawley (SD) rats were obtained from the Central Animal Laboratory of Shenyang Pharmaceutical University.

Methods

Preparation of MEs

Selecting appropriate components is an important prerequisite for successful preparation of an ME. We studied the safety of oil phases of injectable grade and nonionic surfactants as components of the ME.

As DOC is a poorly soluble drug, it is important to select an oil in which it dissolves well. In our preliminary test, the solubility of DOC was determined in several types of oil that could be used for an injection. An MCT that has good solubility and high safety was chosen as the oil phase for the formulation. The surfactant and cosurfactant were selected based on their efficacy in formulating MEs for intravenous products as determined by our previous extensive research in related fields.[24–26]

In our preliminary test, the best solubilization, microemulsifying effect, and resistance to infinite dilution were found for the MCT/S75/HS 15/PEG 400 combinations. The appropriate components and their ratio of surfactant phase (S75, HS 15), oil phase, and aqueous phase (5% PEG 400 aqueous solution) were determined by aqueous phase titration. In this study, the ratio of oil phase and surfactant has an optimal range that allows a high concentration of DOC and BJO in the ME (DOC:BJO, 1:1), and ensures a low viscosity of the ME. The two surfactants were mixed at a weight ratio of 1:1, 2:3, 3:2, or 1:2 to obtain the surfactant mix (Smix). The oil phase and Smix were then mixed at various weight ratios (3:7, 4:6, 5:5, and 6:4), and each mixture was titrated with the aqueous phase under magnetic stirring at 55°C. The equilibrated samples were assessed visually

and determined to be either clear and transparent MEs or crude emulsions.

Preparation of MEs containing DOC

The MEs containing DOC were prepared by dissolving an appropriate amount of DOC in the oil phase and surfactant. The above-mentioned ingredients were weighed into glass vials and stirred; it was ascertained that DOC and S75 were completely solubilized in the mixed solution. An aqueous solution of cosurfactant was then added and shaken to form the ME.

Characterization of the ME

Particle size and size distribution of DOC ME

The droplet size distribution (D90) of the dispersed phase and zeta potential of the microemulsion were determined using dynamic light scattering with the ZetaSizer Nano ZS90 (Malvern Instrument Ltd, Malvern, Worcestershire, UK). The D90 and zeta potential were calculated using manufacturer's Dispersion Technology Software. MEs were diluted 1:100 with water before measurements. The samples were carefully poured into the sample cell to avoid air bubbles.

Determination of drug encapsulation efficiency of DOC

The encapsulation efficiency of the ME was determined using an ultrafiltration-centrifugation technique. Three millilitres of the ME was transferred to the upper chamber of the centrifuge tubes fitted with an ultrafilter (MWCO 3 KD, Millipore, USA). Then, the tubes were centrifuged at 15,000 rpm for 10 minutes. The supernatant was determined by high-performance liquid chromatography (HPLC).

Hemolysis test

An in vitro test was used to assess the hemolytic potential of the DOC ME. Blood was obtained from the ear vein of the rabbits, and fibrin was removed by stirring with glass beads for several minutes. Then, the fibrin-free rabbit blood was centrifuged at 4,000 rpm for 5 minutes to remove the upper layer of plasma. Red blood cells in the pellet were washed four times with isotonic saline solution (centrifugation followed by dispersion). A suitable amount of isotonic saline solution was added to the red blood cells to prepare a 2% erythrocyte dispersion. Different amounts of ME (0.1, 0.2, 0.3, 0.4, and 0.5 mL) were added to the tubes along with 2.5 mL volumes of the erythrocyte dispersion. Normal saline was added to the tubes to obtain a final volume of 5 mL. A positive control was prepared by addition of 2.5 mL distilled water to 2.5 mL of the erythrocyte dispersion; and for the negative control, 2.5 mL normal saline was added to 2.5 mL of the erythrocyte dispersion. After vortexing, the tubes were incubated at 37°C and observed for 4 hours.[27]

In vitro release study

The in vitro release test was performed using a paddle method. The DOC ME and DOC solution were respectively put into pretreated dialysis tubing (molecular weight cut-off 8,000–14,000 Da, Viskase Sale Co., Chicago, IL, USA) previously moistened with phosphate-buffered saline (PBS); both ends were clamped tightly and without enclosing bubbles. The bag was placed in a 1,000 mL cup with a solvent medium (0.5% w/v Tween-80 in PBS, pH 7.4). The rotation speed of the paddle was 60 rpm in a water bath maintained at 37°C ± 0.5°C. At fixed time intervals, 5 mL of the medium was removed and 5 mL of fresh PBS was added. The samples were analyzed by HPLC (LC-2010A; Shimadzu Corporation, Kyoto, Japan) to determine the drug concentration.

The concentration of DOC in the ME

First, a 0.5 mL portion of the ME was diluted to a 25 mL transparent solution using ethanol. The samples were then filtered using microporous membranes and analyzed by HPLC to determine the drug concentration.

HPLC assay for DOC in vitro

DOC concentration was determined using an HPLC apparatus. The chromatographic conditions were as follows. The column was the Diamonsil® reversed-phase C18 column (200 mm × 4.6 mm, 5 μm); the mobile phase, 60% acetonitrile in water, was pumped at a flow rate of 1 mL/min at 25°C. The UV-detection wavelength for DOC was 228 nm.

In vitro cytotoxicity studies

A549 cells were used for an in vitro cytotoxicity study by 3-[4,5-dimethylthiazol-2-yl]-3,5-diphenyltetrazolium bromide dye (MTT) assays. Cells were plated in a 96-well plate at a density of 1×10^4 cells per well and incubated at 37°C in a humidified atmosphere with 5% CO_2. After the cells were attached to flat-bottomed plates, the medium was replaced with the samples in the medium solution. A series of sample solutions at different concentrations of DOC and BJO was tested: the ratio of DOC and BJO in the combined test samples was consistent with their proportion in the preparation for study on the synergistic effect. The cells were incubated with the solutions for a duration of 72 hours;

5.0 mg/mL MTT solution was added, and the plates were incubated for another 4 hours at 37°C. The purple formazan crystals were dissolved in 100 µL dimethylsulfoxide (DMSO) and the plates were read on an ELISA reader at 492 nm. The half inhibit concentration (IC50) values were calculated using the Statistical Product and Service Solutions software (SPSS 20.0; IBM Corporation, Armonk, NY, USA). The combination effect given by Equation 1 was compared with the combination index (CI).[28] Synergy is indicated if CI <1, additivity if CI =1, antagonism if CI >1:

$$CI = \frac{D_A}{D_{x,A}} + \frac{D_B}{D_{x,B}} + \alpha \frac{D_A D_B}{D_{x,A} D_{x,B}} \quad (1)$$

where D_A and D_B are the concentrations of drug A and drug B used in combination to achieve x% drug effect. $D_{x,A}$ and $D_{x,B}$ are the concentrations for single agents to achieve the same effect.

Pharmacokinetics of solutions of DOC and ME in rats

Experimental design

The pharmacokinetics of the ME were compared to that of a concentrated solution of DOC, 2 mg/mL, in rats. The SD rats (230–250 g, male) were randomly assigned into two groups of five rats each. All rats were fasted for 12 hours before the experiment but allowed free access to drinking water. The ME and concentrated solution of DOC were administered to SD rats by tail vein injection at 10 mg/kg. Blood samples were drawn into heparinized tubes at sampling times of 1, 4, 7, 15, 30, 60, 120, and 240 minutes after the drugs had been administered to the rats.

Bioanalysis of the DOC

Blood samples were centrifuged at 10,000 rpm for 5 minutes, and plasma was collected for analysis of DOC by HPLC.

An aliquot of 100 µL blood was placed into a centrifuge tube and 10 µL solution of paclitaxel (Internal Standard) (Zhongxi Sunve Pharmaceutical Co., Ltd., Shanghai, People's Republic of China) was added, then vortexed for 1 minute. After addition of 300 µL acetonitrile to the tube, the sample was vortexed for 3 minutes. Then, the mixture was centrifuged at 4,000 rpm for 10 minutes. The supernatant was placed into a fresh tube and stored until analyzed by HPLC. The HPLC conditions were as follows: the mobile phase was composed of acetonitrile:water (55:45, v/v); the flow rate was 1.0 mL/minute; and the detection wavelength was 228 nm.

Pharmacokinetic parameters were calculated using DAS 2.0 software (Anhui Provincial Center for Drug Clinical Evaluation, Wuhu, Anhui Province, People's Republic of China).

Results and discussion

ME formulation

The excipients, including the oil phase, surfactant, and cosurfactant, that are available for injectable ME must have very low toxicity, good biocompatibility, and the surfactant/cosurfactant must be in the appropriate concentration range.

The oil phase must not only dissolve the hydrophobic drug, but must also facilitate drug delivery to the body; eg, it must enhance absorption and improve bioavailability. In general, the smaller the molecular volume of the oil phase, the greater is its ability to dissolve the drug. In most cases, single-chain surfactants alone are unable to reduce the oil/water interfacial tension sufficiently to enable the formation of an ME.[29–31] The presence of cosurfactants provides the interfacial film sufficient flexibility to assume the different curvatures required to form MEs over a wide range of compositions.[32–34] In our preliminary test, the best solubilization and microemulsifying effect and resistance to infinite dilution were found for the MCT/BJO/S75/HS 15/PEG 400 combination, the ratio of which was confirmed by aqueous phase titration.

The purpose of the formulation of the ME was to combine less surfactant content with an optimal solubilization of the lipophilic drug. In this study, the ratio of the oil phase was fixed at 1:1. A formulation with the highest oil content, lesser amount of surfactant, small size, and low viscosity was chosen as criteria to determine the composition of the ME. The proportion of the different ingredients and ME/emulsion particle diameter is shown in Table 1. Based on the above screening criteria, the ratio of the oil phase and Smix (S75:HS, 1:2) was 6:4, and 5% PEG 400 aqueous solution was confirmed as the optimal formulation for the ME.

Characteristics of the ME

Droplet size, zeta potential, and encapsulation efficiency are the properties that largely influence the biopharmaceutical characteristics and preparation stability of an ME. The smaller droplets can more easily evade the reticuloendothelial system and prolong the time in circulation. As seen from Figure 1A, the D90 of the DOC MEs was 13.5 nm in diameter. The polydispersity index was 0.26.

The zeta potential is an important tool for understanding the state of the nanoparticle surface and predicting

Table 1 The proportion of the different ingredients and microemulsion/emulsion particle diameter

S75:HS 15	Oil phase: Smix	V (mL)	Particle size (nm)
1:1	4:6	3.23	36.3
	5:5	2.83	19.1
	6:4	2.68	44.9
	7:3	–	110
2:3	4:6	1.80	16.4
	5:5	2.16	26.2
	6:4	1.84	33.1
	7:3	2.90	61.0
3:2	4:6	–	306
	5:5	3.30	36.2
	6:4	–	421
	7:3	–	610
1:2	4:6	1.29	16.7
	5:5	1.56	26.1
	6:4	1.60	7.85
	7:3	–	260

Note: BJO: MCT =1:1; V, volume of the aqueous phase when a phase transition occurs; "–", no phase transition was observed obviously.
Abbreviations: S75, Soybean lecithin; HS 15, PEG660-12-hydroxystearate; Smix, surfactant mixed; BJO, Brucea javanica oil; MCT, Medium-chain triglyceride.

the long-term stability of the nanoparticle. Nanoparticles with zeta potential values higher than +25 mV or lower than –25 mV typically have a high degree of stability. Dispersions with a low zeta potential value will eventually aggregate as a result of van der Waals interparticle attractions. The low zeta potential (–41.3 mV) of our ME formulation showed that it was stable (Figure 1).

The entrapment efficiency of DOC in the ME was 99.98%±. This high entrapment efficiency probably resulted from the good solubilization of the oil phase and surfactants for the drug. DOC is a poorly soluble drug: its solubility is very low in water, which makes it suitable for dissolution in the oil phase, thereby producing high encapsulation efficiency. The drug loading was 2 mg/mL. This confirms that the ME can solubilize the DOC.

The calibration curve for DOC was y =23464x – 373 over the range of 0.05–100 μg/mL with a coefficient of correlation r =1. The result of the in vitro release study is shown in Figure 2. The DOC was released from the ME more slowly than it was from the DOC solution. Because of the high encapsulation efficiency, no burst release was observed.

Many surfactants have been used to prepare an ME, and surfactants are known to cause hemolysis of red blood cells. To examine the safety of our formulation for injection, hemolysis of the ME was assessed using the rabbit red blood cells. Complete hemolysis was observed in the positive control tube: the upper solution was a transparent red; erythrocytes were at the bottom of the other six tubes; and the upper solution was colorless and transparent. In the negative control and ME tubes, the erythrocytes were

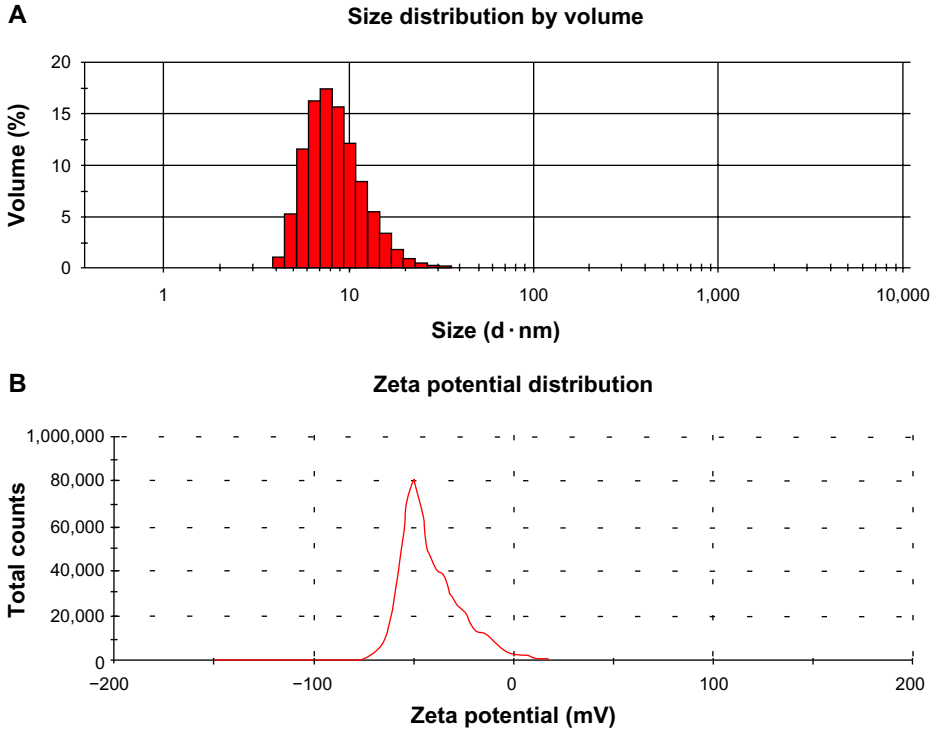

Figure 1 (**A**) Particle size and distribution of the DOC microemulsion. (**B**) Zeta potential of the DOC microemulsion.
Abbreviation: DOC, docetaxel.

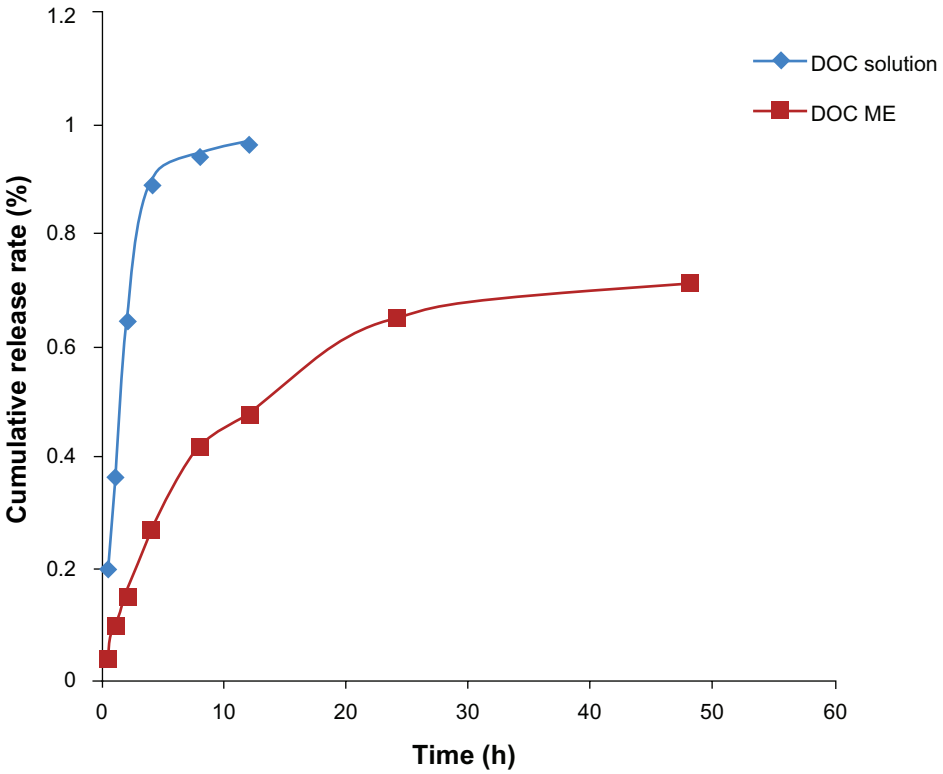

Figure 2 Cumulative release of DOC from microemulsion and solution.
Abbreviations: DOC, docetaxel; DOC ME, docetaxel microemulsion.

precipitated at the bottom of the tubes. The supernatant was colorless and transparent, and no erythrocyte agglutination was observed. These results demonstrated that the DOC ME did not cause hemolysis.

In vitro cytotoxicity studies

Because a surfactant is an amphiphilic molecule, it can act on cell membranes and will affect cell growth. In addition, studies have shown that HS 15 also has certain effects on tumor cells.[35–37] An ME contains a large amount of surfactant, which has some level of cytotoxicity and will affect the validity of the experiment when the ME comes into contact with cells. To avoid the interference of the surfactant and to investigate the synergistic antitumor effect of the BJO, an in vitro cytotoxicity study was carried out without excipient. When used in combination, the IC50 of DOC and BJO values were 5.69 μmol (D_A) and 13.77 mg/L (D_B); when used separately, the IC50 of DOC and BJO were 23.88 μmol ($D_{A,50}$) and 40.36 mg/L ($D_{B,50}$). After calculation, the value of the CI was 0.58, and because it was less than 1, it showed a synergistic effect. The results of that calculation provide an adequate theoretical foundation for this study. An in vivo antitumor effect study will be carried out with this ME in the near future.

Pharmacokinetics

MEs are beneficial for the parenteral delivery of insoluble drugs. They provide a means of obtaining relatively high concentration of the drug in the administering formulation. MEs are more stable in plasma than liposomes or other vesicles, and the internal oil phase is more resistant against drug leakage.[38]

The pharmacokinetic parameters of the DOC ME were investigated and compared with DOC solution after a single intravenous administration (10 mg/kg) to SD rats. Because the standard clinical dose of DOC for humans is 75 mg/m^2, the dose for rats is 10 mg/kg, after a skin surface area conversion.[39] The main pharmacokinetic parameters were calculated based on a non-compartment analysis method and are summarized in Table 2. Compared with the DOC solution, the $t_{1/2}$ was significantly longer, which means that DOC in the microemulsion circulated longer in the body. In addition, the area under the curve (AUC) of the microemulsion was higher than that of DOC solution. These data suggest that with the same dosage, the ME changed the pharmacokinetic parameters of DOC and prolonged its circulation time, which suggests an improved antitumor efficacy of the ME compared with the DOC solution in rats. These observations were possibly a result

Table 2 The main pharmacokinetic parameters of DOC after intravenous administration of the DOC solution or microemulsion at 10 mg/kg DOC in SD rats

Parameter	Units	DOC solution	DOC microemulsion
AUC (0-t)	mg/L*min	199.583 ± 16.622	284.265 ± 71.959
AUC (0-∞)	mg/L*min	223.297 ± 10.516	316.743 ± 76.926
AUMC (0-t)		8,075.472 ± 449.714	9,688.277 ± 3,014.139
AUMC (0-∞)		17,687.71 ± 3,454.528	23,708.703 ± 4,793.893
MRT (0-t)	min	40.529 ± 1.26	33.789 ± 1.923
MRT (0-∞)	min	79.724 ± 18.983	75.413 ± 5.903
VRT (0-t)	min^2	3,657.084 ± 281.291	3,010.26 ± 107.587
VRT (0-∞)	min^2	18,541.597 ± 8,786.664	20,857.89 ± 3,711.747
$t_{1/2z}$	min	103.867 ± 29.392	127.363 ± 13.149
CLz	L/min/kg	0.045 ± 0.002	0.033 ± 0.007
Cmax	mg/L	23.599 ± 1.143	27.193 ± 1.772

Abbreviations: AUC, area under the curve; AUMC, area under the moment curve; MRT, mean residence time; VRT, variance of the residence time; $t_{1/2z}$, elimination half-time; CLz, plasma clearance; Cmax, maximum plasma concentration; DOC, docetaxel; SD, Sprague Dawley.

of the small size of the ME droplets (D90 =13.5 nm) and the hydrophilic surface of the ME droplets, allowing them to escape from uptake and phagocytosis by the reticuloendothelial system.

Conclusion

By considering the lower toxicity and irritation, the enhanced stability and safety of intravenous injection, formulations for the intravenous DOC ME consisted of oil phase (MCT:BJO =1:1):Smix (S75:HS 15 = 1:2) = 3:2 with 5% PEG 400 aqueous solution and DOC at 2 mg/mL. The D90 was 13.5 nm in diameter and the zeta potential value was −41.3 mV. The ME also changed the pharmacokinetic parameters of DOC compared with DOC in solution, which suggested that a DOC microemulsion might have a better antitumor effect. Further research will be conducted to explore that possibility.

Acknowledgments

This research was supported by the Hebei National Science Foundation (C2011319007). The authors thank Professor Xiuyun Hou of Boston University for the advice and useful suggestions on this paper.

Disclosure

The authors declare that there are no conflicts of interest in this work.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA: Cancer J Clin*. 2011;61(2):69–90.
2. Moes JJ, Koolen SL, Huitema AD, Schellens JHM, Beijnen JH, Nuijen B. Pharmaceutical development and preliminary clinical testing of an oral solid dispersion formulation of docetaxel (ModraDoc001). *Int J Pharm*. 2011;420(2):244–250.
3. Herbst RS, Khuri FR, Lu C, et al. The novel and effective nonplatinum, nontaxane combination of gemcitabine and vinorelbine in advanced nonsmall cell lung carcinoma. *Cancer*. 2002;95(2):340–353.
4. Guéritte-Voegelein F, Guénard D, Lavelle F, Le Goff MT, Mangatal L, Poitier P. Relationships between the structure of taxol analogues and their antimitotic activity. *J Med Chem*. 1991;34(3):992–998.
5. Ringel I, Horwitz SB. Studies with RP 56976 (Taxotere): a semisynthetic analog of taxol. *J Natl Cancer Inst*. 1991;83(4):288–291.
6. Hong WK. The current status of docetaxel in solid tumors. An MD Anderson Cancer Center Review. *Oncology (Williston Park)*. 2002;16(Suppl 6):9–15.
7. Van Zuylen L, Verweij J, Sparreboom A. Role of formulation vehicles in taxane pharmacology. *Invest New Drugs*. 2001;19(2):125–141.
8. Gelderblom H, Verweij J, Nooter K, Sparreboom A. Cremophor EL: the drawbacks and advantages of vehicle selection for drug formulation. *Eur J Cancer*. 2001;37(13):1590–1598.
9. Alexopoulos A, Karamouzis MV, Stavrinides H, et al. Phase II study of pegylated liposomal doxorubicin (Caelyx) and docetaxel as first-line treatment in metastatic breast cancer. *Ann Oncol*. 2004;15(6):891–895.
10. Immordino ML, Brusa P, Arpicco S, Stella B, Dosio F, Cattel L. Preparation, characterization, cytotoxicity and pharmacokinetics of liposomes containing docetaxel. *J Control Release*. 2003;91(3): 417–429.
11. Chan JM, Zhang L, Yuet KP, et al. PLGA lecithin-PEG core-shell nanoparticles for controlled drug delivery. *Biomaterials*. 2009;30(8):1627–1634.
12. Hwang HY, Kim IS, Kwon IC, Kim YH. Tumor targetability and antitumor effect of docetaxel-loaded hydrophobically modified glycol chitosan nanoparticles. *J Control Release*. 2008;128(1):23–31.
13. Liu B, Yang M, Li R, et al. The antitumor effect of novel docetaxel-loaded thermosensitive micelle. *Eur J Pharm Biopharm*. 2008;69(2):527–534.
14. Lee E, Kim H, Lee IH, Jon SY. In vivo antitumor effects of chitosan-conjugated docetaxel after oral administration. *J Control Release*. 2009;140(2):79–85.
15. Esmaeili F, Dinarvand R, Ghahremani MH, et al. Docetaxel-albumin conjugates: preparation, in vitro evaluation and biodistribution studies. *J Pharm Sci*. 2009;98(8):2718–2730.
16. Grosse PY, Bressolle F, Pinguet F. In vitro modulation of doxorubicin and docetaxel antitumoral activity by methyl-β-cyclodextrin. *Eur J Cancer*. 1998;34(1):168–174.
17. Lawrence MJ, Rees GD. Microemulsion-based media as novel drug delivery systems. *Adv Drug Deliv Rev*. 2000;45(1):89–121.
18. Eccleston GM. Emulsions and microemulsions. In: Swarbrick J. Encyclopedia of pharmaceutical technology. New York: Informa Healthcare USA. 2007:1561–1565.
19. Park JH, Lee S, Kim JH, Park K, Kim K, Kwon IC. Polymeric nanomedicine for cancer therapy. *Prog Polym Sci*. 2008;33(1):113–137.
20. Su BN, Chang LC, Park EJ, et al. Bioactive constituents of the seed of Brucea javanica. *Planta med*. 2002;68(8):730–733.
21. Nie YL, Liu KX, Mao XY, Li YL, Li J, Zhang MM. Effect of injection of brucea javanica oil emulsion plus chemoradiotherapy for lung cancer: a review of clinical evidence. *J Evid Based Med*. 2012;5(4):216–225.
22. Liu Y, Wang H, FU Q. An experimental study of Brucea javanica oil emulsion on bladder carcinoma. *Chin J Urol*. 2001:336–338.
23. Lou GG, Yao HP, Xie LP. Brucea javanica oil induces apoptosis in T24 bladder cancer cells via upregulation of caspase-3, caspase-9, and inhibition of NF-kappaB and COX-2 expressions. *Am J Chin Med*. 2010;38(3):613–624.
24. Cai WH, Deng WD, Yang HH, Chen XP, Jin F. A propofol microemulsion with low free propofol in the aqueous phase: formulation, physicochemical characterization, stability and pharmacokinetics. *Int J Pharm*. 2012;436(1–2):536–544.

25. Zhao X, Chen D, Gao P, Ding P, Li K. Synthesis of ibuprofen eugenol ester and its microemulsion formulation for parenteral delivery. *Chem Pharm Bull (Tokyo)*. 2005;53(10):1246–1250.
26. Zhuang CY, Li N, Wang M, et al. Preparation and characterization of vinpocetine loaded nanostructured lipid carriers (NLC) for improved oral bioavailability. *Int J Pharm*. 2010;394(1–2):179–185.
27. Zheng X, Kan B, Gou M, et al. Preparation of MPEG–PLA nanoparticle for honokiol delivery in vitro. *Int J Pharm*. 2010;386(1):262–267.
28. Zhao L, Wientjes MG, Au JL. Evaluation of combination chemotherapy: integration of nonlinear regression, curve shift, isobologram, and combination index analyses. *Clin Cancer Res*. 2004;10(23):7994–8004.
29. Bhargava HN, Narurkar A, Lieb LM. Using microemulsions for drug delivery. *Pharm Tech*. 1987;11(3):46–52.
30. Lawrence MJ. Surfactant systems: microemulsions and vesicles as vehicles for drug delivery. *Eur J Drug Metab Pharmacokinet*. 1994;19(3):257–269.
31. Tenjarla S. Microemulsions: an overview and pharmaceutical applications. *Crit Rev Ther Drug Carrier Syst*. 1999;16(5):461–521.
32. Ghosh PK, Murthy RSR. Microemulsions: a potential drug delivery system. *Curr Drug Deliv*. 2006;3(2):167–180.
33. Aboofazeli R, Lawrence CB, Wicks SR, Lawrence MJ. Investigations into the formation and characterization of phospholipid microemulsions. III. Pseudo-ternary phase diagrams of systems containing water-lecithin-isopropyl myristate and either an alkanoic acid, amine, alkanediol, polyethylene glycol alkyl ether or alcohol as cosurfactant. *Int J Pharm*. 1994;111(1):63–72.
34. Stilbs P, Rapacki K, Lindman B. Effect of alcohol cosurfactant length on microemulsion structure. *J Colloid Interface Sci*. 1983;95(2):583–585.
35. Coon JS, Knudson W, Clodfelter K, Lu B, Weinstein RS. Solutol HS 15, nontoxic polyoxyethylene esters of 12-hydroxystearic acid, reverses multidrug resistance. *Cancer Res*. 1991;51(3):897–902.
36. Woodcock DM, Linsenmeyer ME, Chojnowski G, et al. Reversal of multidrug resistance by surfactants. *Br J Cancer*. 1992;66(1):62–68.
37. Buckingham LE, Balasubramanian M, Emanuele RM, Clodfelter KE, Coon JS. Comparison of solutol HS 15, Cremophor EL and novel ethoxylated fatty acid surfactants as multidrug resistance modification agents. *Int J Cancer*. 1995;62(4):436–442.
38. Talegaonkar S, Azeem A, Ahmad FJ, Khar RK, Pathan SA, Khan ZI. Microemulsions: a novel approach to enhanced drug delivery. *Recent Pat Drug Deliv Formul*. 2008;2(3):238–257.
39. Zhao M, Su M, Lin X, et al. Evaluation of docetaxel-loaded intravenous lipid emulsion: pharmacokinetics, tissue distribution, antitumor activity, safety and toxicity. *Pharm Res*. 2010;27(8):1687–1702.