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Docetaxel microemulsion for enhanced oral bioavailability: Preparation and in vitro and in vivo evaluation

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1. Introduction

Docetaxel is an antineoplastic agent belonging to the second generation of the taxoid family. It is about twice as potent as paclitaxel as an inhibitor of microtubule depolymerisation in vitro [1]. Yet, the only licensed indication for docetaxel is in the treatment of locally advanced or metastatic breast cancer with a first-line chemotherapy regimen [2]. Currently, parenteral formulations are available for its clinical use [2] while oral administration is still limited because of its low oral bioavailability (<5% in mice) [3], which is in part due to its practically insoluble property (4.93 µg/mL in water) [4] as well as its high affinity to the multidrug efflux pump P-glycoprotein (P-gp) [5] and hepatic first-pass metabolism [6].

To improve patient compliance and for efficient combination therapy with other antineoplastic agents, an oral formulation of docetaxel would be useful since oral chemotherapy could ease the use of more chronic regimens [3,7]. Studies have shown that cyclosporine A (CsA) or interferon-alpha could increase the oral bioavailability of docetaxel by inhibiting P-gp. When co-administered with 15 mg/kg CsA, the bioavailability of oral docetaxel (5 mg/m²) given as an oral drinking solution of the i.v. formulation increased from 8±6% (monotherapy) to 90±44% [8]. In case of interferon-alpha co-administration in rats, oral bioavailability was improved from 10.4% to 37.2% [9]. However, pre-treatment with a P-gp inhibitor before the oral administration of docetaxel is far from improving patient compliance and may lead to more side effects.

Amongst the various drug delivery systems, the microemulsion system is considered an ideal alternative for the oral delivery of poorly water-soluble compounds [10–12]. Employing the microemulsion formulation to nitrendipine improved its oral bioavailability as indicated in its AUC values which were more than three times larger compared to those of its oil solution [11]. When ibuprofen was orally administered as an o/w microemulsion, its AUC increased about nine times (p<0.001) compared to those of the suspension formulation [12] and enhancing effect of absorption was demonstrated.
Microemulsion is a lipid based delivery system demonstrating absorption enhancement. The major advantages include high solubilization potential, thermodynamic stability, improved dissolution of lipophilic drugs and surfactant-induced permeability enhancement [13,14]. Additionally, several excipients commonly used in these systems including Cremophor, Tween 80, Labrasol and Transcutol could inhibit the function of P-gp [15–17] making the microemulsion system an attractive choice for docetaxel oral delivery. Herein, we report on the preparation of a microemulsion system of docetaxel which could result in an improvement of oral bioavailability by increasing the drug’s solubility and permeability while inhibiting the P-gp efflux system.

2. Materials and methods

2.1. Materials

Docetaxel was purchased from Taihua Co., Xi’an, China. Labrafil WL 2609 BS, Labrafil M 2125 CS, Capryol 90, Labrafil M 1944 CS, Maisin 35–1 were received as gifts from Gattefossé Co. (Saint Priest, Cedex, France), while Pluror Oleique CC 497 was purchased from the same company. Cremophor EL and Cremophor RH40 were purchased from BASF Co. (Ludwigshafen, Germany). Tween 80 and sodium taurocholate (NaTC) were from Tokyo Chemical Industry Co., LTD. (Tokyo, Japan). Transcutol, porcine pancreatin (8 × USP specifications activity) and Trizma maleate were obtained from Sigma-Aldrich (St. Louis, MO, USA). Propylene glycol and PEG 400 were both purchased from Duksan Pure Chemical Co. Ltd. (Ansan, Korea). Egg phosphatidylcholines (PC) was generous gift from Lipoid Company (Ludwigshafen, Germany). 4-Bromophenylboronic acid (4-BPB), 3-(4, 5-dimethyltetrazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Dulbecco’s modified eagle medium (DMEM), penicillin–streptomycin and fetal bovine serum were obtained from Invitrogen (Ontario, Canada). Acetonitrile and methanol were HPLC grade and supplied from Fisher Scientific Korea Ltd. (Seoul, Korea). All other chemicals were of the highest grade possible and obtained from commercial sources.

2.2. Preparation of microemulsion

2.2.1. Solubility study

The solubility of docetaxel in various oils and surfactants was determined by adding excess amount of docetaxel into 1 mL of each vehicle in the centrifugal tube, followed by mixing (100 rpm) in a shaking incubator (Jeio-Tech, Seoul, Korea) at 25 °C for 48 h. The solution was centrifuged at 13,200 rpm for 5 min to remove the excess docetaxel, after which the concentration of docetaxel in the supernatant was measured by HPLC after appropriate dilution with methanol.

2.2.2. Pseudo ternary phase diagrams

Based on the results of solubility study (Table 1), Capryol 90 was selected as an oil phase. The effect of surfactant (i.e., Cremophor EL and Cremophor RH 40) and co-surfactant (i.e., propylene glycol and Transcutol) on the pseudo ternary phase diagram was systematically observed at room temperature. The surfactant and co-surfactant were weighed at different ratios (1:1, 4:3, 3:2 and 2:1, w/w) in each tube, and were vortexed vigorously for 30 s to make the surfactant mixture (Sm). Afterwards, the oil phase (O) and the surfactant mixture (Sm) were mixed, where the ratios of oil to Sm in the mixtures were varied from 9:1 to 1:9 (w/w). Distilled water was added dropwise to each clear oil and Sm mixture with gentle stirring to allow equilibration. Following the addition of aliquot of water phase, the mixture was visually examined for transparency. The points from clear to turbid and turbid to clear were designated as emulsion and microemulsion, respectively.

2.2.3. Preparation of docetaxel-loaded microemulsions and content determination

Based on the results of pseudo ternary phase diagrams, three microemulsions with the following oil:Sm:water (w/w/w) ratio were selected for further experiments: M-1 (30:38:6:31.1), M-2 (28:35:7:36.1), and M-3 (29:4:37:33:3) (Table 2). In order to determine the maximum loading content of docetaxel in microemulsion formulations, excess amount of docetaxel was dissolved into the oil phase by vortexing for 30 s after which the microemulsions were prepared as mentioned above. It was further mixed in a shaking incubator at 100 rpm (Jeio-Tech, Seoul, Korea) for 24 h at 25 °C. Excess docetaxel was removed by centrifugation at 13,200 rpm for 5 min after which the content of docetaxel in the microemulsions was measured by HPLC after appropriate dilution with methanol.

2.3. Characterization of docetaxel-loaded microemulsions

2.3.1. Mean droplet size and distribution

The droplet size and distribution of the microemulsions loaded with docetaxel was measured by an electrophoretic light-scattering spectrophotometer (ELS-8000, OTSUKA Electronics Co. Ltd., Japan). The microemulsions were transferred to a standard quartz cuvette, and the droplet size and polydispersity index of the microemulsions were determined via dynamic He–Ne laser (10 mW) light-scattering at an angle of 90° at 25 °C. Data analysis was conducted using a software package (ELS-8000 software) supplied by the manufacturer.

2.3.2. TEM

The morphologies of the microemulsions were examined by an Energy-Filtering Transmission electron microscopy (TEM) (LIBRA 120, Carl Zeiss, Germany) with a 80 kV accelerating voltage. The microemulsions were negatively stained with 2% phosphotungstic acid

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**Table 1** Solubility of docetaxel in various vehicles at 25 °C saturated for 48 h.

<table>
<thead>
<tr>
<th>Oil Code</th>
<th>Solubility (mg/mL)</th>
<th>Surfactant/co-surfactant</th>
<th>Solubility (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capryol 90</td>
<td>125.86±12.91</td>
<td>Tween 80</td>
<td>35.14±2.47</td>
</tr>
<tr>
<td>Maisine 35-1</td>
<td>20.13±3.76</td>
<td>Cremophor EL</td>
<td>27.61±0.62</td>
</tr>
<tr>
<td>Pluror Oleique CC 497</td>
<td>7.46±3.51</td>
<td>Labrasol</td>
<td>53.08±6.04</td>
</tr>
<tr>
<td>Labrafil WL 2609 BS</td>
<td>7.39±0.51</td>
<td>Transcutol</td>
<td>207.82±4.87</td>
</tr>
<tr>
<td>Labrafil M2125 CS</td>
<td>3.13±0.2</td>
<td>Ethanol</td>
<td>127.32±9.34</td>
</tr>
<tr>
<td>Labrafil M 1944 CS</td>
<td>2.76±0.23</td>
<td>PEG 400</td>
<td>81.03±7.89</td>
</tr>
<tr>
<td>Propylene glycol (PG)</td>
<td>3.12±0.15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each value is the mean±S.D. of three separate determinations.

---

**Table 2** Formulations and droplet size of microemulsions and docetaxel content.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Composition (%)</th>
<th>Droplet size</th>
<th>Docetaxel content (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rx</td>
<td>Oil</td>
<td>Surfactant/co-surfactant (2:1, w/w)</td>
<td>Oil:Sm:water</td>
</tr>
<tr>
<td>M-1</td>
<td>Capryol 90</td>
<td>Cremophor EL/PG</td>
<td>30:39:31</td>
</tr>
<tr>
<td>M-2</td>
<td>Capryol 90</td>
<td>Cremophor RH40/Transcutol</td>
<td>28:36:36</td>
</tr>
<tr>
<td>M-3</td>
<td>Capryol 90</td>
<td>Cremophor EL/Transcutol</td>
<td>29:37:34</td>
</tr>
</tbody>
</table>

Each value is the mean±S.D. of three separate determinations.
(PTA) and placed on carbon-coated 400 mesh copper grids followed by drying at room temperature before measurements.

2.3.3. Changes of docetaxel solubility in microemulsions after dilution

To investigate the solubilization capacity of docetaxel in the diluted microemulsions, each formulation (M-1, M-2, and M-3) containing 20 mg/mL docetaxel was diluted 20 times with normal saline at 37 °C. Commercial parenteral formulation of docetaxel (Taxotere®) in distilled water (10 mg/mL) containing 25% (w/v) of Tween 80 and 9.75% (v/v) of ethanol was prepared and also diluted 20 times with normal saline for comparison. An aliquot from each diluted formulation (50 µL) was taken at predetermined time intervals for 24 h, and was centrifuged at 13,200 rpm for 5 min to remove the precipitated docetaxel, if any. The concentration of docetaxel in the supernatant was measured using HPLC after appropriate dilution with methanol.

2.3.4. In vitro digestion study

An in vitro lipolysis model was used as described in the literature [18]. Docetaxel-loaded microemulsions were dispersed in 18 mL of digestion buffer (50 mM Trizma maleate, 150 mM NaCl, 5 mM CaCl₂·2H₂O, pH 7.5) containing 5 mM NaTC and 1.25 mM PC (to simulate the fasted intestinal conditions). Experiments were initiated by adding 2 mL of pancreatin containing digest buffer (final lipase concentration: 1000 USP units/mL), and conducted for 30 min in a thermostated reaction vessel (37 °C) with continuous stirring. A pH auto-titrator (Titrator T50, Mettler Toledo, Switzerland) was used to maintain the pH at 7.5 with the addition of sodium hydroxide (0.2 M). The content of OH⁻ present in the titrant can be equated with the fatty acids produced by lipolysis. At the end of each experiment, 180 µL of 4-BPB methanol solution (0.5 M) was added to inhibit the lipase activity immediately after which 1 mL of the reaction mixture was taken and centrifuged at 13,200 rpm for 5 min in order to separate the mixture into an aqueous phase and a pellet phase. Samples obtained from the aqueous phase was diluted with methanol and analyzed by HPLC.

2.4. In vitro release of docetaxel

The in vitro release of docetaxel from microemulsions was conducted by both the ultrafiltration and dialysis methods. For the ultrafiltration method, an aliquot of each docetaxel microemulsion (200 µL) was placed in 900 mL of release medium (PBS, pH 7.4) containing 0.1% Tween 80 (w/v) to maintain sink condition. While stirring the release medium using the magnetic stirrer at 100 rpm at 37 ± 0.5 °C, aliquots of dissolution medium (0.5 mL) were withdrawn at predetermined time intervals for 12 h, and were refilled with the equal volume of fresh medium. The samples (0.3 mL) were centrifuged at 7000 ×g for 10 min in an Ultracel YM-3 ultrafiltration tube (MWCO: 3,000, Millipore Corporation, MA, USA). The concentration of docetaxel in the filtrate was determined by HPLC after appropriate dilution with methanol. For the dialysis method, aliquot of each docetaxel microemulsion and Taxotere® (200 µL) was placed in the mini dialysis kits (MWCO 6–8 kDa) (Kfar-Hanagid, Israel), and was immersed in 900 mL of release medium (PBS, pH 7.4) containing 0.1% Tween 80 (w/v). Aliquots of dissolution media (0.5 mL) were withdrawn, and the concentration of docetaxel was determined by HPLC after appropriate dilution with methanol. The percent cumulative amount of docetaxel released from microemulsions was calculated as a function of time.

2.5. Transport of docetaxel through the Caco-2 cell monolayer

For the in vitro transport study of docetaxel microemulsions, Caco-2 cells (passage 20–25, American Type Culture Collection, Rockville, MD) were grown in DMEM containing 10% fetal bovine serum, 1% nonessential amino acid solution, 100 U/mL penicillin, and 0.1 mg/mL streptomycin at 37 °C in an atmosphere of 5% CO₂. Then, they were seeded onto the apical side of collagen-coated Transwell-COL inserts in 12-well Transwell culture plate (Corning Costar Co., Cambridge, MA, USA) at a concentration of 1.5–1.5 × 10⁵ cells/well. Culture medium (DMEM) was added to apical (0.5 mL) and basolateral (1.5 mL) side, and was replaced every other day for the first week and daily thereafter. Cells were incubated for 18–21 days until the transepithelial electrical resistance (TEER), measured by using an EVOM voltohmeter (WPI Inc., FL, USA), increased to between 300 Ω/cm² and 600 Ω/cm².

Before the transport study, the culture medium (DMEM) was replaced with preheated (37 °C) transport medium consisting of Hanks’ balanced salt solution (HBSS) supplemented with 25 mM glucose and 10 mM HEPES (pH 7.4). After the cell monolayer was equilibrated for 30 min at 37 °C, TEER values of monolayers were determined in triplicate. For the apical to basolateral (A to B) transport study, 0.5 mL of each microemulsion or Taxotere® diluted with transport medium (5 µg as docetaxel) was added to apical side, while 0.5 mL of docetaxel (10 µg/mL) in transport medium containing 4% (v/v) DMSO was added as the control. At predetermined time intervals, 0.2 mL of medium in basolateral side was taken for 2 h, and was replaced with the fresh medium. For basolateral to apical (B to A) transport study, blank microemulsions or blank Taxotere® (0.5 mL) in transport medium without docetaxel was added to the apical side to pre-treat the monolayer for 30 min at 37 °C, while the same amount (0.5 mL) of transport medium with 4% (v/v) DMSO was added as the control. After rinsing the monolayer with the transport media, Transwell inserts with 0.5 mL of fresh transport medium at the apical side were placed into the basolateral side containing 1.5 mL docetaxel solution (10 µg/mL) dissolved in transport medium containing 4% (v/v) of DMSO. At predetermined time intervals, 0.2 mL of medium in apical side was taken for 2 h, and was replaced with the fresh medium [19]. The concentration of docetaxel was determined by HPLC analysis, and the cumulative amount of docetaxel permeated was plotted as a function of time. The apparent permeability coefficient (P_app) was determined from the linear slope of the plot using the following equation:

\[
P_{\text{app}} = \frac{dQ}{dt} \frac{1}{A \cdot C_0}
\]

where, \(P_{\text{app}}\) is the apparent permeability coefficient (cm/s), \(dQ/dt\) is the steady state flux, \(A\) is the surface area of membrane (cm²), \(C_0\) is the initial concentration of docetaxel in the apical (for A to B transport) or basolateral (for B to A transport) side.

2.6. Pharmacokinetics studies

Male Sprague–Dawley rats (250–270 g, Dae-Han Biolink, Daejeon, Korea) were used to perform the in vivo pharmacokinetics study. All rats were maintained in a light-controlled room kept at a temperature of 22 ± 2 °C and a relative humidity of 55 ± 5% (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University, Korea). The experimental protocols involving animal study were approved by the Animal Care and Use Committee of the College of Pharmacy, Seoul National University.

Before the experiment, the rats were fasted overnight with access to water ad libitum, and they were randomly divided into five groups. Femoral arteries and veins of the rats were catheterized with polyethylene tubing (PE-50, Clay Adams, Parsippany, NJ, USA) filled with 50 IU/mL of heparin in saline under anesthesia by ketamine at supine position. For the intravenous administration group, Taxotere® (10 mg/mL docetaxel in distilled water containing 25% (w/v) Tween 80 and 9.75% (v/v) ethanol) was diluted with normal saline to 1 mg/mL, and then was bolus administered via the femoral vein at the docetaxel
dose of 8 mg/kg rat. For oral administration groups, docetaxel microemulsions without dilution were directly administrated to the stomach using an oral sonde at the docetaxel dose of 10 mg/kg rat. Blood samples (~0.25 mL) were withdrawn from the femoral artery at predetermined time intervals for 6 h, and 0.2 mL of heparin (50 IU/mL) solution was used to maintain patency of the cannula between sampling. Plasma samples were obtained by immediately centrifuging the blood samples at 7000 xg for 5 min, after which 100 µL of plasma samples were transferred to new glass tubes and stored at −20 °C until analyzed by HPLC. The pharmacokinetic parameters of each formulation were attained using the WinNonlin® program (Version 3.1, Pharsight Co., Mountainview, CA, USA).

2.7. HPLC analysis of docetaxel

HPLC method was used for the analysis of docetaxel concentration in all samples. For the content, the stability, the transport and the in vitro release studies, samples were properly diluted by methanol and directly injected (20 µL) into the HPLC system without further treatment, while plasma samples were extracted twice with diethyl ether before injection, as previously reported with minor modification [20]. Briefly, 100 µL of plasma was spiked with 10 µL of Titrisol buffer (pH 5.0) and 5 µL of paclitaxel (5 µg/mL) as the internal standard. Docetaxel was extracted with 1.0 mL of diethyl ether by vigorous mixing for 5 min. After centrifugation at 13,200 rpm for 5 min, the organic phase was collected. The organic phase was combined after repeating the above extraction procedure, after which was dried under nitrogen gas stream at 40 °C. The residue was then dissolved with 40 µL of methanol and mixed for 5 min. The solution was centrifuged for 5 min at 13,200 rpm, and 20 µL of the supernatant was injected into the HPLC system.

The HPLC system was equipped with a Waters 2487 Dual Absorbance Detector, 717 plus Autosampler and 515 HPLC dual pumps. A CAPCELL PAK C18 MG HPLC packed column (4.6 mm I. D. x 250 mm, 5 µm, Shiseido, Japan) was used at room temperature. The wavelength of the UV detector was set at 230 nm. Mixture of acetonitrile:water (65:35, v/v) was used as the mobile phase at a flow rate of 1.0 mL/min for the in vitro samples, while that for the blood samples consisted of acetonitrile:acetae buffer (pH 5):tetrahydrofuran (45:50:5, v/v) at the same flow rate.

2.8. Statistical analysis

All the experiments in the study were performed at least three times and the data were expressed as the mean ± standard deviation (S.D.). A two-tailed unpaired Student’s t-test was performed at p < 0.05.

3. Results and discussion

3.1. Solubility study

As shown in Table 1, among the six oils tested, docetaxel showed the highest solubility in Capryol 90 (125.86 ± 12.91 mg/mL). Therefore, Capryol 90 was fixed as the oil phase for further studies. Capryol 90 with the chemical name of propylene glycol monocaprylate is commercially available as an oily liquid consisting of 90% of caprylic acid monoester. It has low to medium HLB (4 – 5) and can be used in self-emulsifying lipidic formulations, and has comprehensive regulatory, toxicity and handling dossiers (including Type IV DMF). It is also compatible with gelatin and HPMC capsules [21] and has been used in oral formulations to enhance the bioavailability of poorly soluble drugs [22].

3.2. Pseudo ternary phase diagram study

Commercially available surfactants approved for oral administration [23] including Cremophor EL (HLB = 13.5), Cremophor RH 40 (HLB = 14 – 16), Tween 80 (HLB = 15), and Labrasol (HLB = 14) were selected to compose phase diagrams. Transcutol, propylene glycol (PG), PEG 400, and ethanol were used as co-surfactants. In preliminary studies, a total of 16 phase diagrams prepared by pairing four surfactants and four co-surfactants at the 1:1 (w/w) ratio were examined. After selecting three compositions (Cremophor EL/PG, Cremophor RH 40/Transcutol, and Cremophor EL/Transcutol) which formed a stable and broad microemulsion area, nine more diagrams were constructed by varying the ratios of surfactant and co-surfactant from 1:1 to 4:3, 3:2 and 2:1. The area of microemulsion decreased as the ratio of co-surfactant increased (data not shown), which was consistent with a previous report [24]. Thus, the ratio of surfactant and co-surfactant was fixed at 2:1, and three phase diagrams were finally selected for further investigation (Fig. 1).

As shown in Fig. 1, all three diagrams possessed a broad microemulsion area. When Tween 80 or Labrasol was employed as the surfactant to construct the phase diagram, the microemulsion area was small regardless of what co-surfactant was used (data not shown). When Cremophor EL was used, combination with PG, Transcutol or ethanol could broaden the microemulsion area. However, due to its volatile nature, ethanol was eliminated as a co-surfactant for Cremophor EL. In the case of Cremophor RH 40, introducing PG caused too high a viscosity for the resulting microemulsion, and thus only Transcutol was considered as co-surfactant.

Based on results shown in Fig. 1, M-1, M-2, and M-3 were chosen for further studies (Table 2). These three formulations were those that satisfied an oil:Sm ratio of 4.4:5.6 (w/w) which consisted of the highest oil composition and stability. With these components and proportion of oil and Sm, fine microemulsions could be formed spontaneously by gentle agitation following aqueous dilution and thus the premicroemulsion concentrate of those microemulsion systems could also be used conveniently for clinical oral administration by filling them into capsules.

3.3. Characterization of microemulsions

The average droplet size of microemulsions measured by ELS was around 30 nm (Table 2), and was consistent with the TEM results (Fig. 2). Microemulsions were clear and transparent, and turned sky-blue opalescent as substantially diluted with water. As shown in Table 2, solubility of docetaxel in microemulsions (22.76, 32.34 and 29.25 mg/mL in M-1, M-2 and M-3, respectively) was dramatically improved compared to that in aqueous solution (4.93 µg/mL) [4]. The different co-surfactants used in the formulations could have attributed to the difference in solubility as in the case of M-1 where PG was used while in M-3, Transcutol. Moreover, the relative solubility of the drug in various components would contribute to the drug entrapment in a given microemulsion as previously reported [14]. The solubility of docetaxel was higher in Transcutol as compared to PG (Table 1) which may be the reason that the solubility of docetaxel in M-3 was greater than that in M-1. Among the three microemulsions, M-2 had the highest solubilization capacity towards docetaxel and Cremophor RH40 which was the surfactant used in M-2 could have been the cause for this improved solubility. However, solubility of docetaxel in Cremophor RH40 could not be determined because the surfactant was semi-solid at room temperature. For a more rational comparison among the three microemulsion formulations, a fixed concentration of 20 mg/mL of docetaxel was prepared and used in the following studies.

Microemulsions could be diluted by water in the gastrointestinal tract upon oral administration, which could lead to drug precipitation. Thus, it is important that the solubilization capacity is determined. As
Fig. 1. Pseudo ternary phase diagrams composed of (a) Cremophor EL and propylene glycol (b) Cremophor RH40 and Transcutol, and (c) Cremophor EL and Transcutol as surfactant and co-surfactant (2:1, w/w), respectively, while Capryol 90 was used as an oil phase. The grey area represents the o/w microemulsion existence range and the ‘E’ area means crude emulsion range.

Fig. 2. Transmission electron microphotography (TEM) of docetaxel-loaded microemulsions: (a) M-1, (b) M-2, and (c) M-3. The scale bar for all images represents 50 nm.
shown in Fig. 3, microemulsions of docetaxel exhibited satisfactory solubilization capacity for at least 24 h, while its solubility in Taxotere® decreased dramatically after 2 h despite going through the same dilution process.

Microemulsion formulation which is one of the lipid based delivery systems is subject to enzymatic hydrolysis upon oral administration, resulting in reduced drug solubilization capacity [25]. The in vitro digestion study conducted on M-1, M-2 and M-3 showed that 60–80% of docetaxel remained solubilized in aqueous phase following the 30 min lipolysis process (Table 3). ANNOVA test showed that there was a significant difference between M-1 and M-2, but no significant difference between M-1 and M-3 which mean that the effects of the two surfactants, Cremophor RH40 and Cremophor EL only slightly affected the digestion process. The digestion process seems to affect the oil (Capryol 90) more significantly than the surfactants/co-surfactants.

3.4. In vitro release study

The in vitro release of docetaxel from the microemulsions is shown in Fig. 4. Ultrafiltration study was conducted where no significant difference among the three microemulsions was observed (Fig. 4a). Dialysis studies also showed no significant difference among the three microemulsions: no initial burst release was observed and about 80% of docetaxel was released within 12 h. However, the initial release of docetaxel from Taxotere® was faster than that from the microemulsion within 1 h, after which that of the former slowed down (Fig. 4b) due to the decrease in drug solubility in diluted conditions (Fig. 3). A study on phenytoin has been reported where drug dissolution study showed about 90% release within 10 min in transport medium containing 4% (v/v) DMSO. The A to B transport of docetaxel from each formulation was in the following order: M-3>M-2>M-1>control group (Fig. 5a), while that of B to A transport was in the reverse order: control group>M-1>M-2>M-3 (Fig. 5b). The Papp values of docetaxel from A to B transport and B to A efflux were compared and results are shown in Table 4. It shows that the permeability coefficient of the microemulsions was significantly higher.

3.5. Transport study through Caco-2 cell monolayer

The viability of Caco-2 cells against each blank microemulsion observed by 3-(4, 5-dimethylthiazol-2-ly)-2, 5-diphenyltetrazolium bromide (MTT) assay [27] was higher than 90% for up to 3 h (data not shown). Thus, 2 h-transport studies were conducted. This viability was also observed with docetaxel solutions of higher than 10 µg/mL concentrations in transport medium containing 4% (v/v) DMSO. The A to B transport of docetaxel from each formulation was in the following order: M-3>M-2>M-1>control group (Fig. 5a), while that of B to A transport was in the reverse order: control group>M-1>M-2>M-3 (Fig. 5b). The Papp values of docetaxel from A to B transport and B to A efflux were compared and results are shown in Table 4. It shows that the permeability coefficient of the microemulsions was significantly higher.

Table 3

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Initial content (mg)</th>
<th>Final content (mg)</th>
<th>Final Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-1</td>
<td>2.65 ± 0.17</td>
<td>2.11 ± 0.04</td>
<td>80.1 ± 6.4</td>
</tr>
<tr>
<td>M-2</td>
<td>2.72 ± 0.10</td>
<td>1.69 ± 0.06</td>
<td>62.0 ± 4.5*</td>
</tr>
<tr>
<td>M-3</td>
<td>2.86 ± 0.04</td>
<td>2.00 ± 0.12</td>
<td>70.0 ± 3.7</td>
</tr>
</tbody>
</table>

Each value is the mean ± S.D. of three separate determinations.

*p < 0.05 compared with M-1.

Fig. 3. Change of docetaxel solubility in Taxotere® and microemulsions after 20 times dilution by saline solution at 37 °C. Data are expressed as the mean ± S.D. (n = 3).

Fig. 4. Release profiles of docetaxel from microemulsions and Taxotere® determined by (a) the ultrafiltration method and (b) the dialysis method at 37 °C in PBS (pH = 7.4) containing 0.1% (w/v) Tween 80. Each data point represents the mean ± S.D. of three determinations.
than that of the control or Taxotere® in the A to B transport. In particular, the \( P_{\text{app}} \) value of docetaxel from M-3 was around 43 times higher than that from the control group (7.68 \times 10^{-5} \text{ cm/s} vs. 0.18 \times 10^{-5} \text{ cm/s}, \ p < 0.01).

Fig. 5 shows that in the B to A transport, the permeability coefficient of the microemulsions significantly decreased compared to the control or Taxotere®. For the control and Taxotere®, the B to A permeability coefficient was higher than that of the A to B value (Table 4), indicating that docetaxel is a substrate of P-gp. However, the efflux of docetaxel from M-3 represented by the \( P_{\text{app}} \) value of B to A was significantly lower than that from the control group (7.43 \times 10^{-5} \text{ cm/s} vs. 1.04 \times 10^{-5} \text{ cm/s}, \ p < 0.01). On top of that, no significant difference between the A to B and B to A \( P_{\text{app}} \) values was observed for the M-3 formulation, implying the suppression of P-gp by the microemulsion. Therefore, in addition to the enhancement of drug solubility by the microemulsion formulation, inhibition of the P-gp efflux system could have caused the increase in A to B permeability.

After the transport study, there was about a 20%–30% reduction on the TEER values of Caco-2 monolayers (Table 4). Since a study on Labrasol showed that up to 80% of TEER value reduction could affect the tight junction [17] and results of our MTT assay indicated about 90% cell viability within 3 h, it could be concluded that the microemulsions did not alter the integrity of the Caco-2 cellular tight junctions.

It is well known that docetaxel is a P-gp substrate and the oral absorption is in part affected by P-gp efflux activity [28]. Much work has been done to study the P-gp inhibition effect of Cremophor EL as well as other surfactants [29] and their mechanisms [30]. It was indicated that Cremophor EL may inhibit the function of P-gp by affecting membrane fluidity [15]. And, Cremophor could specifically bind to the hydrophobic domain of the P-gp that may change its secondary and/or tertiary structure and reduce its function [29]. Besides Cremophor EL, the enhanced permeation of docetaxel possibly also comes from other components of microemulsions. As reported in a literature [16], the transport \( P_{\text{app}} \) values of several poorly water-soluble model compounds were much improved by Transcutol and its permeation-enhancing effect on Caco-2 cell monolayer was much stronger than that of same amount of PG. The results of Fig. 5 and Table 4 show that Transcutol contained in formulations M-2 and M-3 showed higher docetaxel absorption enhancing effect than PG contained in formulation M-1. Moreover, although it was reported that both Cremophor EL and Cremophor RH 40 could inhibit P-gp [31], Cremophor EL presented stronger capacity of inhibiting P-gp compared to that of Cremophor RH 40 [32]. That might be the reason that M-3 containing Cremophor EL exhibited higher A to B transport amount of docetaxel than that of M-2 with Cremophor RH 40. The different inhibiting capacity may be

### Table 4

<table>
<thead>
<tr>
<th>Rx</th>
<th>Permeability coefficient ( P_{\text{app}} ) ( \times 10^{-5} \text{ cm/s} )</th>
<th>Change of TEER(^{\text{c}} ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A to B</td>
<td>B to A</td>
</tr>
<tr>
<td>Control(^{\text{b}} )</td>
<td>0.18 ± 0.008</td>
<td>10.44 ± 0.43(*)</td>
</tr>
<tr>
<td>Taxotere®</td>
<td>0.29 ± 0.14</td>
<td>10.34 ± 0.52(**)</td>
</tr>
<tr>
<td>M-1</td>
<td>4.43 ± 0.27(*)</td>
<td>8.73 ± 0.86(**)</td>
</tr>
<tr>
<td>M-2</td>
<td>6.18 ± 0.47(**)</td>
<td>8.30 ± 0.79(**)</td>
</tr>
<tr>
<td>M-3</td>
<td>7.68 ± 0.80(**)</td>
<td>7.43 ± 0.22(**)</td>
</tr>
</tbody>
</table>

Each value is the mean ± S.D. of three separate determinations.

\(^{\text{a}}\) Percent of initial TEER value after 2 h of A to B transport study.

\(^{\text{b}}\) Control was docetaxel (10 µg/mL) in transport medium containing 4% (v/v) DMSO.

\(^{\text{c}}\) Change of TEER between the A to B and B to A transport study.

\(*\) \( p < 0.05 \), \(**\) \( p < 0.01 \) compared with Taxotere® and the control.

\(#\) \( p < 0.05 \), \(**\) \( p < 0.01 \) compared with A to B transport.

\( \text{Rx} \) Permeability coefficient \( P_{\text{app}} \) of docetaxel across Caco-2 monolayer from each formulation, and the change of TEER values after 2 h of A to B transport study.

Fig. 5. (a) apical to basolateral and (b) basolateral to apical transport profiles of docetaxel from Taxotere® or microemulsions across the Caco-2 cell monolayer at 37 °C. The control for the A to B transport study was docetaxel (10 µg/mL) in transport medium containing 4% (v/v) DMSO. For the B to A transport study, the apical side was pretreated with the blank microemulsions or blank Taxotere® in transport medium without docetaxel, while the transport medium with 4% (v/v) DMSO was added as the control. Each data point represents the mean ± S.D. of three determinations.

Fig. 6. Plasma concentration-time profiles of docetaxel after bolus intravenous injection of Taxotere® (8 mg/kg), oral administration of Taxotere® or microemulsions (equivalent to 10 mg/kg as docetaxel) to rats. Each data point represents the mean ± S.D. of five determinations.
resulted from the chemical and physical nature of each excipient [33] but the exactly theoretical principle was still not clear. Thus, it could be concluded that the in vitro absorption of docetaxel could be greatly improved by the components (i.e., surfactants) of the microemulsions through the P-gp inhibition and/or permeability-enhancing effect, and the composition of M-3 could elevate the absorption to the greatest extent among the tested formulations.

3.6. Pharmacokinetic study

Fig. 6 shows the plasma concentration profiles of docetaxel after oral administration of various formulations to rats. The pharmacokinetic parameters are summarized in Table 5. The AUC of docetaxel in M-3 increased 5.2-fold compared with that of docetaxel in the orally administered Taxotere® (389.43 ng·h/mL vs. 74.98 ng·h/mL, p < 0.01). The T_{max} was also enhanced 5.8-fold in M-3 as compared with that of the orally administered Taxotere® (46.39 ng·mL vs. 270.48 ng·mL, p < 0.01). The T_{max} was relatively delayed for about 15 min in the microemulsions compared to Taxotere®. Docetaxel in Taxotere® is readily exposed in the gastrointestinal tract since it is solubilized in Tween 80 and ethanol. However, in case of the microemulsions, the drug needs to be released out from the oil phase thereby resulting in a delayed T_{max}. This result is consistent with that of the in vitro release study (Fig. 4). Additionally, the oral bioavailability of docetaxel from three microemulsions resulted in 1.3, 2.6, and 5.2-fold increase, respectively, with the orally administered Taxotere®. Moreover, M-3 showed a significantly higher absolute oral bioavailability of docetaxel (34.42%), compared to that of orally administered Taxotere® (6.63%).

The improved oral bioavailability of docetaxel in the microemulsions could be explained by the combination of the following effects: (1) significantly improved solubility of docetaxel by microemulsions which could keep the drug as the soluble form during the gastrointestinal dilution and permeation process; (2) the synergistic effect of oil and surfactants as absorption enhancers; and (3) the inhibition of P-gp efflux. Therefore, although the microemulsion formulations could not alter the hepatic first-pass elimination, it could significantly increase the oral bioavailability of docetaxel by the combined effects of enhanced solubility, P-gp inhibition in the gastrointestinal walls and permeability enhancing effects.

4. Conclusions

The o/w microemulsion formulation composed of Capryol 90 (oil), Cremophor (surfactant) and Transcutol (co-surfactant) enhanced the solubility of docetaxel up to 30 mg/mL. Additionally, the in vitro transport study across Caco-2 cell monolayer showed that such formulation could significantly enhance the A to B transport of docetaxel and that the components of the microemulsion exhibited the inhibiting effect against P-gp efflux. Thus, the new microemulsion formulation resulted in a 5.2-fold higher oral bioavailability of docetaxel in rats compared to that of oral Taxotere®. This new microemulsion for oral administration may further be developed into an alternative formulation for docetaxel.

Acknowledgements

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References


Table 5

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Oral Taxotere®</th>
<th>Intravenous Taxotere®</th>
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<tbody>
<tr>
<td></td>
<td>M-1</td>
<td>M-2</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>0.75</td>
<td>0.75</td>
</tr>
<tr>
<td>15</td>
<td>3.23</td>
<td>3.23</td>
</tr>
<tr>
<td>20</td>
<td>5.97</td>
<td>5.97</td>
</tr>
</tbody>
</table>

Each value is the mean ± S.D. of five rats.

*p < 0.05, **p < 0.01 compared with Taxotere® (oral).


