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유중수 (w/o)형 마이크로에멀젼을 이용한 독소루비신의 경구 전달

Water-in-oil (w/o) Microemulsion for Oral Delivery of Doxorubicin

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제약학과 약제과학 전공

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ABSTRACT

Water-in-oil (w/o) Microemulsion for Oral Delivery of Doxorubicin

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Purpose: Although doxorubicin (DOX) is a potent anticancer drug, development of its oral formulation has been hindered by its limited intestinal absorption and low oral bioavailability. The limited intestinal absorption via the paracellular pathway may be the primary cause of the low oral bioavailability of DOX. In this study, medium chain glycerides-based colloidal nanosystems were formulated to enhance the intestinal paracellular absorption of DOX and reduce its cardiotoxicity.

Methods: The DOX formulations prepared by the construction of pseudo-ternary phase diagram were characterized in terms of their droplet size distribution, viscosity, drug loading, and drug release. Further evaluation was conducted by an

in vitro Caco-2 transport study as well as *in situ/in vivo* intestinal absorption, bioavailability and toxicity studies.

Results: The water-in-oil (w/o) microemulsion systems consisting of Captex 355 (oil), Span 80/ Tween 80 or Capmul MCM/Labrasol (surfactant mixture) and water were developed for oral delivery of DOX. Compared with DOX solution, these formulations enhanced the absorptive transport of DOX across Caco-2 cell monolayers at least partly due to the paracellular-enhancing effects of their lipidic components. Moreover, the *in situ* intestinal absorption and *in vivo* oral bioavailability of DOX in rats were markedly enhanced. In addition, no discernible damage was observed in the rat jejunum after oral administration of these DOX formulations while the cardiac toxicity was significantly reduced when compared with intravenous DOX solution.

Conclusions: Taken together, the medium chain glycerides-based colloidal nanosystems prepared in this study represents a potentially effective oral delivery system for DOX

Keywords: doxorubicin, oral delivery, Intestinal paracellular absorption, medium chain glyceride, microemulsion.

Abbreviations : DOX, doxorubicin; AUC, total area under the plasma concentration-time curve from time zero to time infinity; C_{max}, peak plasma concentration; T_{max}, time to reach a C_{max}; F_{rel}, extent of relative oral bioavailability.

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1. Introduction

Doxorubicin (DOX) is an anthracycline glycoside antibiotic with a mechanism of impairing DNA synthesis during tumor cell division (1). It is eliminated via the urinary and fecal (intestinal and biliary) excretion as well as metabolism (2). The major metabolites of doxorubicin are doxorubicinol and the aglycones, doxorubicinone and 7-deoxydoxorubicinone (3, 4). The biliary excretion of doxorubicin is almost entirely mediated by P-glycoprotein (P-gp), as shown in a mdr1a knockout mice study which resulted in an approximately 82.0 % reduction of doxorubicin concentration in the bile (5). It is one of the most widely used anticancer drugs for the treatment of lymphoma, osteosarcoma and other sarcomas, carcinomas, and melanoma (6). The most common dosing mode of DOX is a single intravenous injection, but this may lead to an undesired systemic exposure profile with an excessively high (toxic) level in the initial and subsequent fast decay below the minimum therapeutic level (7). It has been generally believed that long-term exposure to drug at modest concentrations would be more beneficial than a pulsed supply of drug at higher concentrations (8). Thus, much effort has been devoted for achieving prolonged systemic exposure to DOX, and the most successful case has been DOXIL®, a pegylated liposomal DOX.

However, oral chemotherapy would be more advantageous over the current regimens via the intravenous route (9, 10). Oral delivery could provide a relatively prolonged systemic exposure profile with less fluctuation leading to lower toxicity

and improved efficacy (11). Moreover, the oral mode of cancer treatment is non-invasive, cost (time and labor)-saving, and available for outpatient, resulting in a better patient compliance and improved quality of life, particularly for the elderly and for patients with advanced or relapsed cancer (12, 13). Thus, oral chemotherapy may be a potential alternative to the current DOX regimen.

Despite many recent studies on the oral delivery of DOX (6, 8, 14-16), its development still remains challenging due to the limited intestinal absorption and low oral bioavailability. In these previous studies, it has been often assumed that the P-gp-mediated efflux and cytochrome P450 (CYP) 3A-mediated first-pass metabolism in the intestine and liver are the main barriers to the oral absorption of DOX. However, a recent work has revealed that the limited and paracellular intestinal absorption of DOX (corresponding to BCS class III) may be the major factor that is responsible for the low oral bioavailability in contrary to what had been earlier reported (17). The absorptive transport of doxorubicin across the Caco-2 cell monolayers is not significantly changed in the presence of a P-gp inhibitor (18). Doxorubicin, the hydrophilic cation, is prone to paracellular transport across Caco-2 cell monolayers, and thus active transport is not attenuated by P-gp-mediated efflux activity (18, 19). Moreover, it has been revealed that doxorubicin crosses the intestinal epithelium primarily via paracellular route (accounting for 85.6 % of the overall absorptive transport) probably due to its physicochemical properties (hydrophilic cation; pKa = 9.63, log P = –0.5, polar surface area = 206.1, aqueous solubility = 50 mM, apparent Caco-2 permeability = 0.102×10^{-6} cm/s). Clearly, only drugs absorbed via transcellular, but not

paracellular, pathway are subject to the intestinal P-gp-mediated efflux (20). Thus, it can be suggested that P-gp-mediated efflux does not play a significant role in limiting the intestinal absorption of doxorubicin (attenuating the absorptive transport by only 5.56 – 13.2 %). Therefore, this requires a new formulation strategy for developing an effective oral delivery system of DOX, i.e., enhancing the intestinal absorption of paracellularly-transported BCS class III drugs.

Over the past decades, lipid-based colloidal systems including microemulsion have been used mainly for the oral delivery of poorly water-soluble drugs (BCS class II and IV) (21-23). Microemulsions are thermodynamically stable, isotropic, optically clear mixtures of oil, water and surfactant, frequently in combination with a cosurfactant (24). Microemulsions have high solubilizing capacity, thermodynamically stability allows self-emulsification with little energy input and long shelf life compared to other emulsions (25). However, recent studies tend to focus on microemulsion as a drug delivery system for enhancing the oral absorption of BCS class III drugs which include fexofenadine, famotidine, calcein, hydroxysafflor yellow A, and earthworm fibrinolytic enzyme (26-30). Microemulsions may enhance the oral absorption of paracellularly-transported BCS class III drugs because they contain oils and surfactants, some of which have been well recognized as paracellular permeation enhancers. Microemulsion could thus be applied in developing oral delivery systems for DOX.

Microemulsions are prepared by the spontaneous emulsification method (titration method) (25). The tendency toward water-in-oil (w/o) or oil-in-water (o/w) microemulsion is dependent on the properties of the oil and surfactant (24). In this

study, in order to form stable w/o microemulsion, the low HLB surfactant (such as Capmul MCM and span80) was combined with high HLB surfactant (tween 80 and Labrasol).

Medium-chain glycerides (MCG), such as medium-chain (C6-C12) fatty acids, mono-, di- and tri-glycerides were used in mixed micelle and emulsion formulations as absorption enhancers of a number of different drugs by these neutral lipids (24).

The objective of this study was to design medium chain glycerides-based water-in-oil (w/o) microemulsion systems which consist of oil, a blend of a low and high hydrophilic-lipophilic balance (HLB) surfactant and an aqueous phase for the oral delivery of DOX, with the expectation to enhance the intestinal permeation of DOX via the paracellular pathway. To date, very few attempts have been made to develop w/o microemulsions for the oral delivery of paracellularly-transported BCS class III drugs including DOX. Therefore, this study could provide new findings regarding the application of microemulsion in these oral drug delivery systems.

2. Materials and methods

2.1. Materials

A human colonic epithelial cell line, Caco-2 cells, was obtained from the American Type Culture Collection (Rockville, MD). DOX (hydrochloride salt) was purchased from Boryung Pharmaceutical Co. (Gunpo, South Korea). [^{14}C] Mannitol (51 mCi/mmoL) was purchased from Amersham Pharmacia Biotech (Buckinghamshire, UK). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum, penicillin-streptomycin, and trypsin-EDTA were purchased from Gibco Laboratories (Grand Island, NY, USA). Captex 355 (C8/C10 triglyceride) and Capmul MCM (C8/C10 mono-/di-glyceride) were kindly donated by Abitec Co. (Columbus, OH, USA). PEG-8 caprylic/capric glycerides (Labrasol) were kindly donated by Gattefossé Co. (Saint Priest, Cedex, France). Span 80 and Tween 80 were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Propranolol hydrochloride (an internal standard for the high-performance liquid chromatographic (HPLC) analysis of DOX), non-essential amino acid solution, Hank's balanced salt solution (HBSS), N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), D-glucose, and DMSO were purchased from Sigma-Aldrich Co. (St. Louis, MO). Other chemicals were of reagent grade or HPLC grade.

2.2. Construction of Pseudo-Ternary Phase Diagrams

The pseudo-ternary phase diagram was constructed to determine the components and contents for the formation of w/o microemulsions (31). The surfactant mixtures (S_{mix}) were prepared by blending Span 80 and Tween 80 (F1) or Capmul MCM and Labrasol (F2) in a fixed weight ratio of 2:1. Then, the oil phase (Captex 355) and S_{mix} were mixed, where the ratios of oil to S_{mix} were varied from 9:1 to 1:9 (w/w). Each clear mixture was titrated with distilled water (DW), while stirring the mixture at room temperature to allow equilibrium. 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. Based on the results of the pseudo ternary phase diagrams, two microemulsion formulations (F1 and F2) were selected for further experiments: 50% Captex 355, 40% Span 80/Tween 80 mixture, and 10% aqueous phase for F1; 55% Captex 355, 35% Capmul MCM/Labrasol mixture, and 10% aqueous phase for F2.

2.3. Determination of maximum loading content and preparation of DOX formulations

In order to determine the maximum loading content of DOX in microemulsion formulations, excess amount of DOX was first dissolved into water followed by mixing in a shaking incubator (Jeio-Tech, Seoul, Korea) at 100 rpm for 48 h at 25℃. Then, excess DOX was removed by centrifugation at 16000 g for 5 min at 25℃. The supernatant (saturated DOX aqueous solution) was taken as an aqueous phase

to prepare the microemulsions following the above mentioned compositions. They were further mixed in a shaking incubator at 100 rpm for 48 h at 25℃. Excess DOX, if any, was removed by centrifugation at 16000 g for 5 min at 25℃. The content of DOX in the formulation was measured by HPLC assay after an appropriate dilution with methanol. Based on these results of the maximum loading content of DOX in microemulsions, 20 mg/mL of DOX aqueous solution was used to prepare F1 and F2 formulations containing 2 mg/mL of DOX for further studies.

2.4. Characterization of DOX Formulations

2.4.1. Mean Droplet Size and Distribution

The droplet size and distribution of DOX formulations (F1 and F2) was measured by an electrophoretic light-scattering spectrophotometer (ELS-8000, OTSUKA Electronics Co. Ltd., Japan). The DOX formulations were transferred to a standard quartz cuvette, and their droplet size and polydispersity index were determined via dynamic He–Ne laser (10 mW) light-scattering at an angle of 90 ° at 25 ℃. Data analysis was conducted using a software package (ELS-8000 software) supplied by the manufacturer.

2.4.2. Viscosity

The viscosity of F1 and F2 was measured by DV-E Viscometer (BROOKFIELD, USA) using a #16 spindle at a speed of 100 rpm at room temperature.

2.4.3. Transmission Electron Microscopy

The morphology of F1 and F2 was examined by an Energy-Filtering Transmission electron microscopy (TEM) (LIBRA120, Carl Zeiss, Germany) with a 80 kV accelerating voltage. The DOX formulations were negatively stained by 2 % sodium phosphotungstate (pH 7) and placed on carbon-coated 400 mesh copper grids followed by drying at room temperature before measurements.

2.4.4. Changes of DOX Formulations after Dilution

To evaluate the changes in the droplet size of DOX formulations after dilution, each formulation (F1 and F2) containing 2 mg/mL DOX was 10-fold diluted with normal saline at 37 ℃, and then, the droplet size of the diluted formulations was measured as mentioned above. To evaluate the changes in the formulations' colloidal structure after dilution, the leakage of DOX from each formulation (F1 and F2) after 500-fold dilution with normal saline at 37 ^{o}C was measured (26, 27). After centrifugation at 16000 g for 10 min at 25 ^{o}C, aliquots from the upper coarse emulsion and lower aqueous phase were collected. The content of DOX in the samples was measured by HPLC assay after an appropriate dilution with methanol.

2.5. *In vitro* Release Study

An aliquot of each DOX solution in DW, F1 and F2 (200 μL) was placed in the mini dialysis kits (MWCO 6–8 kDa) (Kfar-Hanagid, Israel), and was immersed in 100 mL of release medium (PBS, pH 7.4) in a shaking incubator at 100 rpm at 37 ℃.

Then, aliquots of dissolution media (0.5 mL) were withdrawn, and the concentration of DOX was determined by HPLC analysis after an appropriate dilution with methanol. The percent cumulative amount of DOX released from formulations was calculated as a function of time.

2.6. Caco-2 Cell Culture

Caco-2 cells were routinely cultured in DMEM containing 10 % FBS, 1 % non-essential amino acids, 100 U/mL penicillin, and 0.1 mg/mL streptomycin at 37 ℃ in an atmosphere of 5 % CO_2 and 90 % relative humidity. For the transport studies, Caco-2 cells from passage numbers 40 to 55 were seeded on permeable polycarbonate filter inserts (1.12-cm^2 surface area, 0.4-μm pore size; Corning Costar Corp., Cambridge, MA) in 12-Transwell plates at a density of 1–1.5×10^5 cells/insert and were cultured for 21 days. The integrity of cell monolayers was evaluated prior to the transport studies by measuring transepithelial electrical resistance (TEER) and [^{14}C] mannitol permeability across the monolayers. Cell monolayers were considered intact and suitable for use in transport studies when TEER values were 300–600 Ω·cm^2 (32). Mannitol transport in the Caco-2 cell monolayers was < 0.35 % of the dose/h.

2.7. *In vitro* Cytotoxicity Test in Caco-2 Cells

Cytotoxicity of DOX solution, F1 and F2 on Caco-2 cells was evaluated by MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide) assay. Caco-2

cells were seeded onto 96-well plates at a seeding density of 1×10^4 cells/well and used for cytotoxicity test after 24-h incubation. MTT assay was performed using a commercially available MTT assay kit (TOX-1, Sigma-Aldrich, St. Louis, MO). The culture medium was replaced with 200 μL of DOX solution in PBS (20 μg/mL) and DOX formulations 500-fold diluted with PBS (final concentration: 4 μg/mL). Blank PBS (pH 7.4) was employed as a negative control. After 2-h incubation, 20 μL of MTT assay reagent in PBS was added to each well and the plate was incubated for another 4 h. The absorbance of the mixture in the 96-well plate was then measured with an Emax® microplate spectrophotometer at 560 nm (Molecular Devices, Sunnyvale, CA, USA). The percent viability of the cells was determined from the absorbance values considering that of the negative control as 100 %.

2.8. *In vitro* Transport Study in Caco-2 Cell Monolayers

The absorptive transport of DOX solution in transport medium (20 μg/mL) and DOX formulations 500-fold diluted with transport medium (final concentration: 4 μg/mL) in Caco-2 cell monolayer was evaluated. The transport experiments were conducted in Transwell plates that were placed on an orbital shaker; the plates were shaken at 60 rpm during the transport experiments to minimize the influence of the aqueous boundary layer on transport. Prior to transport experiments, cell monolayers were washed three times with transport medium (pH 7.4, HBSS containing 25 mM HEPES and 25 mM glucose). After each wash, the plates were incubated in the transport medium for 30 min at 37 ℃, and then TEER was

measured. For the measurement of absorptive (i.e., apical to basolateral) drug transport, 0.5 mL of DOX solution in transport medium (20 μg/mL) or DOX formulations 500-fold diluted with transport medium containing 1 % DMSO (final concentration: 4 μg/mL) was added to the apical side of the cell monolayer, and 1.5 mL of transport medium containing 1 % DMSO was added to the basolateral side. The inserts were moved to wells containing fresh transport medium containing 1 % DMSO (1.5 mL) every 30 min for 2 hr. At each time point, a 0.2-mL aliquot of the transport medium was removed from the basolateral side, and the concentrations of DOX in each sample were determined by HPLC assay. TEER values were measured at 0, 1, 2, 4, 6, 8, and 24 h after the start of transport experiments. The apparent permeability coefficient (P_{app}; cm/s) was calculated by dividing the unidirectional fluxes by the initial drug concentration with the following equation (26):

$$P_{app} = \frac{dQ / dt}{A \cdot C_0}$$

where the dQ / dt, A, and C_0 are the rate of appearance of drug in the basolateral side, surface area of cell monolayer (1.12 cm^2), and initial drug concentration in the apical side, respectively.

2.9. Animals

Protocols for the animal studies were approved by the Institutional Animal Care and Use Committee of Seoul National University, Seoul, South Korea. Male ICR mice (5–6 weeks old and weighing 20–25 g) and Sprague–Dawley rats (7–9 weeks old and weighing 200–250 g) were purchased from Orient Bio, Inc. (Seongnam, South

Korea). They were maintained in a clean-room (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University) at a temperature of 20 to 23 ℃ with 12-h light (07:00–19:00) and dark (19:00–07:00) cycles, and a relative humidity of 50 ± 5 %. The mice and rats were housed in metabolic cages (Tecniplast, Varese, Italy) under filtered, pathogen-free air, with food (Agribrands Purina Korea, Pyeongtaek, South Korea) and water available *ad libitum*.

2.10.*In situ* Closed Loop Study in Rats

The absorption of DOX solution in 0.9 % NaCl-injectable solution, F1 and F2 in various rat intestinal segments was evaluated by *in situ* closed loop study (17). After minimal abdominal incision under light ether anesthetization and sufficient washing of the contents within the gastrointestinal (GI) tract, a 5-cm long jejunum and colon loops were closed by ligation made at approximately 2-cm distal to both ends of each intestinal section. Special care was exercised to avoid damaging blood vessels and to include as much of a complete mesenteric blood vessel arch as possible for each loop. After injection of 0.5-mL DOX solution, F1 and F2 (2 mg/mL) into each loop by means of an l-mL syringe with a 31-gauge needle, the whole GI tract was carefully replaced into the abdominal cavity, and the incision was closed using clamps and kept moist by covering with gauze pads presoaked with normal saline. The rat was warmed by a lamp. At 120 min after drug injection, each loop was removed, transferred into a beaker containing 50 mL of methanol, and the gastrointestinal tract was cut into small pieces using scissors to facilitate the

extraction of DOX. After manual shaking and stirring with a glass rod for 1 min, a 50-μL aliquot of the supernatant was collected from each beaker and stored in a -80 ^{o}C freezer until the HPLC analysis of DOX.

2.11.*In vivo* Pharmacokinetic Study in Rats

The femoral artery was cannulated with a polyethylene tube (PE-50; Clay Adams, Parsippany, NJ, USA) under light ketamine anesthetization (50 mg/kg, intramuscular injection) as reported previously (33, 34). DOX solution in 0.9 % NaCl-injectable solution and DOX formulations (F1 and F2) at a dose of 10 mg/kg was orally administered (dosing volume: 5 mL/kg) to rats using a feeding tube after overnight fasting with free access to water. An approximately 300-μL aliquot of blood sample was collected via the femoral artery at 0, 15, 30, 45, 60, 75, 90, 120, 180, and 240 min after oral administration of DOX solution and formulations. An approximately 300-μL aliquot of heparinized 0.9 % NaCl-injectable solution (20 U/mL) was used to flush the cannula immediately after each blood sampling to prevent blood clotting. After centrifugation of blood sample, a 150-μL aliquot of plasma sample was stored in a -80 ^{o}C freezer (Model DF8517; Ilshin Laboratory Company, Seoul, South Korea) until the HPLC analysis of DOX.

2.12.*In vivo* Toxicity Test in Mice and Rats

To evaluate the intestinal toxicity of DOX formulations, the jejunum (approximately 5 cm) was carved out at 24 h after oral administration of DW, F1 or F2 to rats. The

segment was then washed with PBS and fixed in 4 % paraformaldehyde for 24 h. A vertical section was prepared, stained with hematoxylin-eosin (H&E), and observed under light microscopy (Magnification: ×200; Model: Nikon Microscope ECLIPSE 80i, Nikon Corp., Tokyo, Japan).

To evaluate the cardiac toxicity of DOX formulations, saline and DOX solution in 0.9 % NaCl-injectable solution (5 mg/kg) were administered intravenously, and DOX formulations (F1 and F2, 10 mg/kg) were administered orally to mice and rats on day 1 and on day 15. The animals were sacrificed on day 28, and cardiac toxicity was evaluated by the activity of superoxide dismutase (SOD) in rat heart homogenate. SOD activity was determined using assay kits (Sigma–Aldrich Co., St. Louis, MO). The heart of mice was carved out and processed for H&E staining in the same manner as the rat intestinal toxicity study.

2.13.HPLC Analysis of DOX

The concentrations of DOX in the samples of the *in vitro, in situ* and *in vivo* experiments were determined by a reported HPLC method (17). A 150-μL aliquot of plasma sample (a 100-μL aliquot of the other samples) was deproteinized with a 300-μL aliquot of acetonitrile, and a 100-μL aliquot of methanol that contained 5 μg/mL of propranolol (an internal standard) was added. After vortex-mixing and centrifugation at 16,000 g for 10 min, a 400-μL aliquot of the supernatant was transferred to another clean eppendorf tube, and then evaporated under a gentle stream of nitrogen gas at room temperature. The residue was reconstituted in 60-μL

mobile phase for plasma sample (100-µL mobile phase for the other samples). After vortex mixing and centrifugation, a 25-µL aliquot was injected onto a reversed phase (C18) HPLC column. The mobile phases, 10 mM KH_2PO_4 (pH 4.0): acetonitrile: methanol (70:25:5, v/v/v) with 0.1 % TEA (v/v) for *in vivo* studies, and 10 mM KH_2PO_4 (pH 4.0): acetonitrile (70:30, v/v) for *in vitro* and *in situ* studies, were run at a flow-rate of 1 mL/min. The column effluent was monitored by a fluorescence detector set at excitation/emission wavelengths of 470 nm/575 nm for DOX and 230 nm/320 nm for propranolol. The retention times of DOX and propranolol were approximately 3.5 and 6 min, respectively. The quantitation limits of DOX in rat plasma, urine and GI samples were 0.01, 0.1, and 0.1 µg/mL, respectively. The inter- and intra-day coefficients of variation were below 12.1 %.

2.14.Pharmacokinetic Analysis

The total area under the plasma concentration–time curve from time zero to time infinity (AUC) was calculated using standard software (WinNonlin®; Pharsight Corporation, Mountain View, CA, USA). The peak plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were directly read from the experimental data. The extent of relative oral bioavailability (F_{rel}) was calculated by dividing the AUC after oral administration of DOX formulations (F1 and F2) by the AUC after oral administration of DOX solution.

2.15.Statistical Analysis

A p-value of less than 0.05 was considered to be statistically significant using a t-test between the two means for the unpaired data or a Duncan's multiple range test of Statistical Package for the Social Sciences (SPSS) posteriori analysis of variance (ANOVA) among the more than three means for the unpaired data. All data were expressed as mean ± standard deviation (SD) except median (ranges) for T_{max}.

3. Results

3.1. Preparation of DOX Formulations

Fig. 1 shows the pseudo-ternary phase diagrams of systems containing water, Captex 355, and surfactant mixture (S_{mix}). The S_{mix} was the blend of a low HLB (Span 80, Fig. 1a, HLB = 4.3; Capmul MCM, Fig. 1b, HLB = 5) and high HLB (Tween 80, Fig. 1a, HLB = 15; Labrasol, Fig. 1b, HLB = 14) non-ionic surfactant at a fixed ratio of 2:1 w/w. A clear and transparent microemulsion existed in the w/o area. Based on the principle of high drug-loading efficiency and low proportion of surfactants, the DOX formulations was selected from this area, and composed of 50 % Captex 355, 40 % Span 80/Tween 80 mixture, and 10 % aqueous phase for F1 and 55 % Captex 355, 35 % Capmul MCM/Labrasol mixture, and 10 % aqueous phase for F2.

3.2. Characterization of DOX Formulations

Table 1 summarizes the droplet size, polydispersity, viscosity, and maximal drug loading of the DOX formulations (F1 and F2). Both F1 and F2 had droplet sizes of less than 200 nm and viscosities ranging from 75 to 110 cP. The TEM images of DOX formulations are shown in Fig. 2. A number of spherical droplets were observed, and their sizes seemed to be approximately 100–200 nm. The DOX formulations were changed to the coarse (turbid) emulsion with 10-fold and 500-fold dilution with PBS. After a 10-fold dilution, the mean droplet sizes of F1

and F2 increased by 8.96 and 8.66 fold (1470 ± 336 nm for F1 and 1230 ± 251 nm for F2), respectively (Table 1). After a 500-fold dilution and subsequent centrifugation, only minor portion (less than 10 %) of the loaded DOX was detected in the lower aqueous phase (% leakage), and most of the DOX was still detected in the upper coarse emulsion (Table 1). However, as shown in Table 1, no significant differences between F1 and F2 were observed in terms of the droplet size, polydispersity, viscosity, maximal drug loading, and characteristics after dilution. All the results indicated a successful formation of w/o microemulsion containing oil, surfactant and aqueous phase.

3.3. *In vitro* Release Study

The time profiles of in vitro release of DOX from solution, F1 and F2 are shown in Fig. 3. The release of DOX from the solution reached the plateau at 4 h, and up to 69.6 % cumulative release of DOX was observed within 12 h. However, the cumulative release of DOX from F1 and F2 were significantly lower than that from the DOX solution at all the time points studied. The cumulative release of DOX from F1 was not significantly different from that from F2.

3.4. *In vitro* Transport Study in Caco-2 Cell Monolayers

The viability of Caco-2 cells after 2-h exposure to the DOX solution, F1 and F2 is shown in Fig. 4. The Caco-2 cell viability against DOX solution (20 μg/mL) was not significantly different from that against the control (PBS). Moreover, no

significant reduction in cell viability was observed in the presence of F1 and F2. The time profiles of in vitro absorptive transport of DOX from the solution, F1 and F2 across Caco-2 cell monolayers are shown in Fig. 5. The apparent permeability coefficients (P_{app}; cm/s) of DOX from the solution, F1, and F2 were $0.159 \times 10^{-6} \pm 0.0229$, $0.968 \times 10^{-6} \pm 0.183$, and $1.71 \times 10^{-6} \pm 0.443$, respectively. The P_{app} values of DOX from F1 and F2 were significantly higher than those from the solution, and the P_{app} values of DOX from F2 were significantly higher than those from F1. The time profiles of relative TEER values with respect to the initial TEER value during the 2-h transport study of the DOX solution, F1 and F2 across Caco-2 cell monolayers and subsequent incubation in fresh media up to 24 h are shown in Fig. 6. There was no significant change in TEER values throughout the transport study of the DOX solution. However, TEER values were reduced to 70.7 % and 62.6 % of their initial values in the presence of F1 and F2, respectively. After wash-out of the formulations and change to fresh media, the TEER values of Caco-2 cell monolayers increased constantly and reached control level at 24 h.

3.5. *In situ* Closed Loop Study in Rats

The remaining fractions of DOX at 2 h after the injection of the DOX solution, F1 and F2 into the rat jejunum and colon loops are listed in Table 2. In both the jejunal and colonic loops, the remaining fractions of DOX after injection of the F1 and F2 were significantly lower than that of the solution, indicating the enhanced intestinal absorption of DOX in F1 and F2 compared with the DOX solution. Moreover, in the

jejunal loops, the remaining fractions of DOX after injection of F2 were significantly lower than those of F1, indicating the enhanced intestinal absorption of DOX in F2 compared with F1.

3.6. *In vivo* Pharmacokinetic Study in Rats

Fig. 7 shows the time profiles of arterial plasma concentrations of DOX after oral administration of the DOX solution, F1 and F2 at a dose of 10 mg/kg to rats. Relevant pharmacokinetic parameters of DOX are listed in Table 3. The AUC, C_{max} and F_{rel} values of the DOX solution, F1 and F2 were significantly different from each other, in the following order: F2 > F1 > solution. The T_{max} values were comparable among the DOX solution, F1 and F2, while the detection periods of DOX in rat plasma were 75, 180 and 240 min for the solution, F1 and F2, respectively.

3.7. *In vivo* Toxicity Study in Rats

Fig. 8 shows representative histological sections of jejunal segments at 24 hr after oral administration of DW, F1 and F2 to rats. In those three groups of rats, any evidence of damage to the intestinal wall, such as villi fusion, occasional epithelial cell shedding, and congestion of mucosal capillary with blood and focal trauma, was not found in parts of the jejunum. There was no discernible difference among the control (DW), F1 and F2, indicating that oral DOX formulations are not toxic to the rat intestinal mucosa (Figs. 8a–c). Fig. 9 shows SOD activity in the heart tissue

after intravenous injection of saline and the DOX solution and after oral administration of F1 and F2 to rats. Compared to the control (treated with intravenous saline) group, SOD activities in heart homogenate decreased significantly in rats treated with intravenous DOX solution, indicating the significant cardiac toxicity of intravenous DOX therapy. However, after oral administration of F1 and F2, SOD levels were comparable with the control group, indicating the reduced cardiotoxicity of oral F1 and F2 compared with intravenous DOX solution. Fig. 10 shows representative histological sections of heart segments after intravenous injection of saline and DOX solution and after oral administration of F1 and F2 to mice. As shown in Fig. 10b, a marked disorganization of cardiac muscles and structural changes in heart tissues were observed after intravenous injection of DOX solution to mice. However, no significant difference in the heart histopathology was observed between the control (saline) and the oral DOX formulations (Figs. 10a, c, d).

4. Discussion

Medium chain glycerides-based colloidal system (w/o microemulsion) containing Captex 355, water and S_{mix} was formulated by the construction of pseudo ternary phase diagram (Fig. 1). The pseudo ternary phase diagram has been widely used to determine the concentration range of components for the existence of microemulsions (23, 31). The mixture of a low and high HLB non-ionic surfactant was used as the S_{mix} of DOX formulation, based on several previous studies reporting a successful preparation of a stable w/o microemulsion using various combinations of low and high HLB non-ionic surfactants (26, 28, 31, 35, 36). The nano-sized round droplet and relatively low viscosity were observed in the prepared DOX formulations (Table 1), which are consistent with previously reported oral w/o microemulsions (100-600 cp) (26, 28, 30).

Colloidal systems including microemulsion are diluted by the gastrointestinal fluid upon oral administration, and thus the characteristics of F1 and F2 after dilution were evaluated. Since oral dilution factor in humans has not been clarified yet, it was assumed to be 500 fold in this study, based on relevant previous studies using the oral dilution factors of approximately 100–2200 fold (14, 23, 26, 37). In the *in vitro* dilution study, the leakage of only minor portions (less than 10 %) of DOX into the outer bulk aqueous phase was observed after the 500-fold dilution of DOX formulation (Table 1). This result suggests that there was no burst release of DOX

when the DOX formulations are diluted, and that the phase conversion may occur primarily into w/o/w rather than o/w emulsion (26).

Drug release characteristics of DOX formulations were evaluated using the dialysis method (Fig. 3). Compared with the DOX solution, slower and sustained release of DOX was observed in the DOX formulations. Since the different appearance profiles of DOX in the receptor side were observed, it is obvious that the penetration process of DOX through the dialysis membrane is not the rate-limiting step in the overall release process of DOX from the inner phase of microemulsion to the receptor side. Moreover, since the molecular cut off of dialysis membrane used is 6–8 kDa, it is unlikely that the droplet of microemulsion itself may penetrate the dialysis membrane. Thus, this result may be attributed to the slow diffusion of DOX through the oil phase of microemulsions being the rate-limiting step in the overall release process of DOX from microemulsions. In w/o microemulsion, water-soluble drugs were solubilized and incorporated mainly in the water-phase core and therefore released rather slowly, which indicates that the microstructure of microemulsion is important for the rate of drug release (26). Taken together, those pseudo-ternary phase diagram and characterization data in terms of droplet size, morphology, viscosity, drug loading, and drug release suggest that DOX-loaded w/o microemulsion formulations have been successfully prepared (Figs. 1–3, Table 1).

The Caco-2 cell monolayer is widely used as an *in vitro* model of the human small intestinal mucosa to predict the intestinal absorption of a drug. The correlation between the *in vitro* apparent permeability coefficient across Caco-2 monolayers and the *in vivo* fraction of oral dose absorbed is well established (38, 39). Moreover,

Caco-2 cell assay is widely known as a useful *in vitro* model for determining the absorptive characteristics of a drug and elucidating its transport mechanism (40). Thus, the intestinal permeation mechanism of DOX solution and formulations was investigated using Caco-2 cell model.

No significant cellular toxicity of DOX solution in Caco-2 cells was observed for 2 h (Fig. 4), which is consistent with a previous study reporting no significant effect of 0.1-mM DOX on Caco-2 cell viability up to 6-h exposure (41). Our recent study has revealed that the absorptive transport of DOX across Caco-2 cell monolayers occurs primarily via the paracellular pathway (17). In this study, medium chain tri-glyceride (Captex 355) was incorporated as the oil phase of both F1 and F2. Medium chain glycerides have been reported to markedly enhance the intestinal permeability of paracellular marker compounds (26). However, evidence on the paracellular enhancing activity of Tween 80 or Span 80 (the surfactant mixture of F1) has not yet been reported (42). Thus, Captex 355 seems to be responsible for the significantly higher P_{app} of F1 and F2 compared with the DOX solution (Fig. 5). The F2 contains Capmul MCM (medium chain mono- and di-glyceride) and Labrasol as a surfactant mixture. It has been reported that medium chain mono- and di-glycerides are more active than tri-glyceride as a membrane permeation enhancer (42). Moreover, Labrasol has been reported to enhance the intestinal permeation of a paracellular marker compound, mannitol, and a poorly-absorbed antibiotic, gentamicin (43, 44). Thus, the difference in surfactant mixture between F1 and F2 seems to be mainly responsible for the significantly higher P_{app} of F2 compared with F1 (Fig. 5). The change in TEER values has been generally accepted as an indicator

of tight junction integrity, which is the major determinant of paracellular permeation (40,45). The reduction of TEER value by F1 and F2 during the 2-h transport study suggests that DOX formulations may enhance the absorptive transport of DOX at least partly via the paracellular pathway. Moreover, the restoration of TEER value during 22 h after wash-out of the formulations and change to fresh media suggests that the paracellular permeation enhancing effects of F1 and F2 are reversible.

The *in situ* intestinal absorption and *in vivo* oral bioavailability of DOX formulations were evaluated using the rat model, based on a good correlation between the fraction of oral dose absorbed in rats and that in humans (34, 46). The results of the *in situ* closed loop study in rats suggest that the intestinal absorption of DOX may be in the following order: F2 > F1 > DOX solution (Table 2), which is consistent with the results of the *in vitro* transport study in Caco-2 cells (Fig. 5). In the *in vivo* rat pharmacokinetic study, 10-mg/kg oral dose of DOX solution were selected. Oral doses of 10–20 mg/kg were used in several previous studies for the oral delivery of DOX (6, 8, 14-16). Moreover, our recent study has revealed that DOX solution exhibits linear pharmacokinetics at the oral dose range of 20–100 mg/kg (17). After oral administration of DOX solution and formulations, systemic DOX exposures were in the following order: F2 > F1 > DOX solution (Table 3), which is consistent with the *in vitro* Caco-2 cell and *in situ* close loop studies (Fig. 5 and Table 2). As discussed earlier, the lipidic components of DOX formulations such as Captex 355, Capmul MCM and Labrasol may be responsible for the differences in oral bioavailability of DOX between the DOX solution and the oral formulations F1 and F2.

The intestinal toxicity of DOX formulations in rats was evaluated by histological H&E staining. As shown in Fig. 8, no significant intestinal toxicity of DOX solution, F1 and F2 in rats was observed, which is consistent with the results of the Caco-2 cell study where the restoration of TEER values (Fig. 4c) as well as no significant cellular toxicity (Fig. 4a) were observed. In addition, because cardiac toxicity is the major complication of the DOX therapy, the activity of SOD, a cardiac toxicity marker, and histological test using H&E staining of the DOX solution, F1 and F2 in mice and rats were evaluated (15). Significant SOD reduction (Fig. 9) and marked disruption of fine structure in heart tissues (Fib. 10b) indicate the significant cardiac toxicity of intravenous DOX therapy (8, 15, 16). However, markedly reduced cardiotoxicity in terms of SOD level (Fig. 9) and heart histopathology (Figs. 10a, c, d) was observed in oral F1 and F2. Thus, these results in intestinal and cardiac toxicity studies suggest that oral DOX formulations prepared in this study are less toxic than conventional intravenous DOX formulations, indicating a potentially safe oral delivery system for DOX.

In summary, this study is the first report on the oral delivery of DOX through the enhancement of intestinal permeation via the paracellular pathway. Moreover, very few attempts have been made to develop w/o microemulsions for the oral delivery of paracellularly-transported BCS class III drugs including DOX. Therefore, this study provides new insights for the application of microemulsion in oral DOX delivery via the paracellular pathway.

5. Conclusion

Medium chain glycerides-based colloidal systems containing Captex 355, Capmul MCM, and/or Labrasol were developed for the oral delivery of DOX. The DOX formulations were successfully prepared by the construction of the pseudo-ternary phase diagram, and their droplet size distribution, viscosity, drug loading, and drug release were characterized. Compared with the DOX solution, the new DOX formulations enhanced the absorptive transport of DOX across the Caco-2 cell monolayers at least partly due to the paracellular-enhancing effects of their lipidic components. Moreover, the *in situ* intestinal absorption and *in vivo* oral bioavailability of DOX in rats were markedly enhanced in these new DOX formulations. In addition, no discernible damage was observed in the rat jejunum after oral administration of the DOX formulations, and the cardiac toxicity was significantly reduced when compared with the intravenous DOX solution. Taken together, the medium chain glycerides-based colloidal system prepared in this study represents a potentially effective oral delivery system for DOX.

6. References

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Table 1 Physicochemical Properties of DOX Formulations (n = 3).

Physicochemical property	F1	F2
Droplet size (nm)		
Before dilution	164 ± 30.5	142 ± 38.5
After 10-fold dilution	1470 ± 336	1230 ± 251
Polydispersity index	0.184 ± 0.0340	0.170 ± 0.0462
Viscosity (cP)	99.3 ± 12.2	84.3 ± 8.08
Leakage (%) after 500-fold dilution	8.63 ± 1.03	9.21 ± 1.20
Maximum drug loading (mg/mL)	3.02 ± 0.129	2.92 ± 0.378

Table 2 Remaining Fractions of DOX at 2 h after Injection of the DOX Solution, F1 and F2 into the Rat Jejunum and Colon Loops (n = 3–4).

Intestinal segment	DOX remaining (%)		
	Solution	F1	F2
Jejunum	87.6 ± 19.8*	64.4 ± 9.52*	40.5 ± 10.4*
Colon	90.8 ± 12.8*	57.8 ± 8.39	46.7 ± 11.0

* Significantly different from the other groups ($p < 0.05$).

Table 3 Pharmacokinetic Parameters of DOX after Oral Administration of the DOX Solution, F1 and F2 at a Dose of 10 mg/kg to Rats (n = 4).

Parameter	Solution	F1	F2
AUC (ng·min/mL)	2740 ± 1030*	5310 ± 1310*	9040 ± 1730*
C_{max} (ng/mL)	17.5 ± 2.76*	29.4 ± 6.42*	57.7 ± 9.28*
T_{max} (min)	45 (30–45)	45 (30–45)	60 (30–60)
F_{rel} (%)	100	214	364

* Significantly different from the other groups ($p < 0.05$).

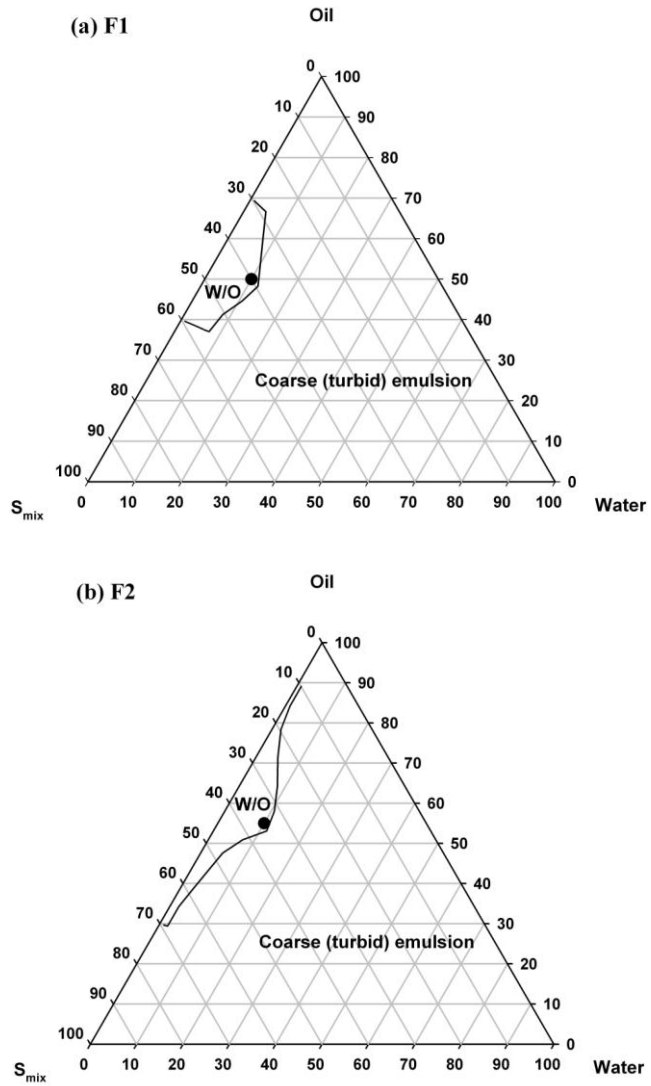
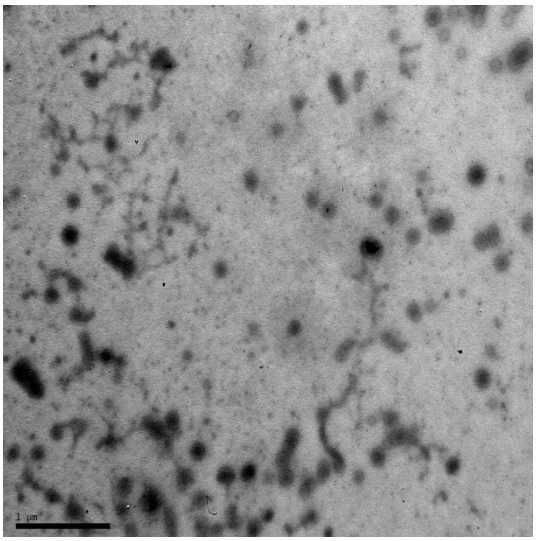


Figure 1 Pseudo-ternary phase diagrams of systems containing water, Captex 355 (Oil), and surfactant mixture (S_{mix}). The S_{mix} was the blend of Span 80 and Tween 80 (a) or Capmul MCM and Labrasol (b) at 2 : 1 w/w. Clear and transparent microemulsions were formed in the w/o area, and other area represents coarse (turbid) emulsion. The closed circles (●) represent the DOX formulations.

(a) F1



(b) F2

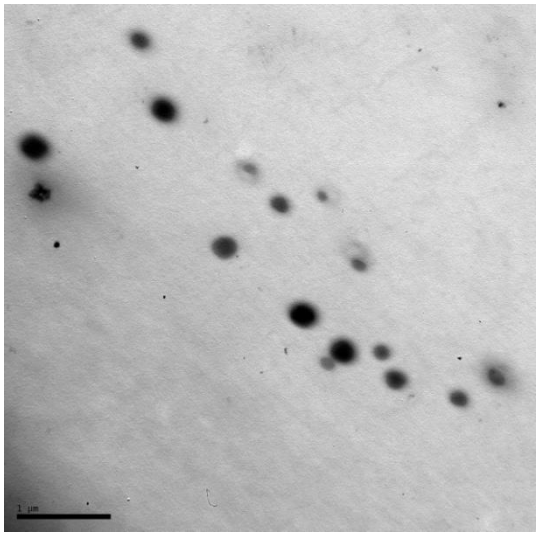


Figure 2 TEM images of DOX formulations. The scale bars represent 1 μm.

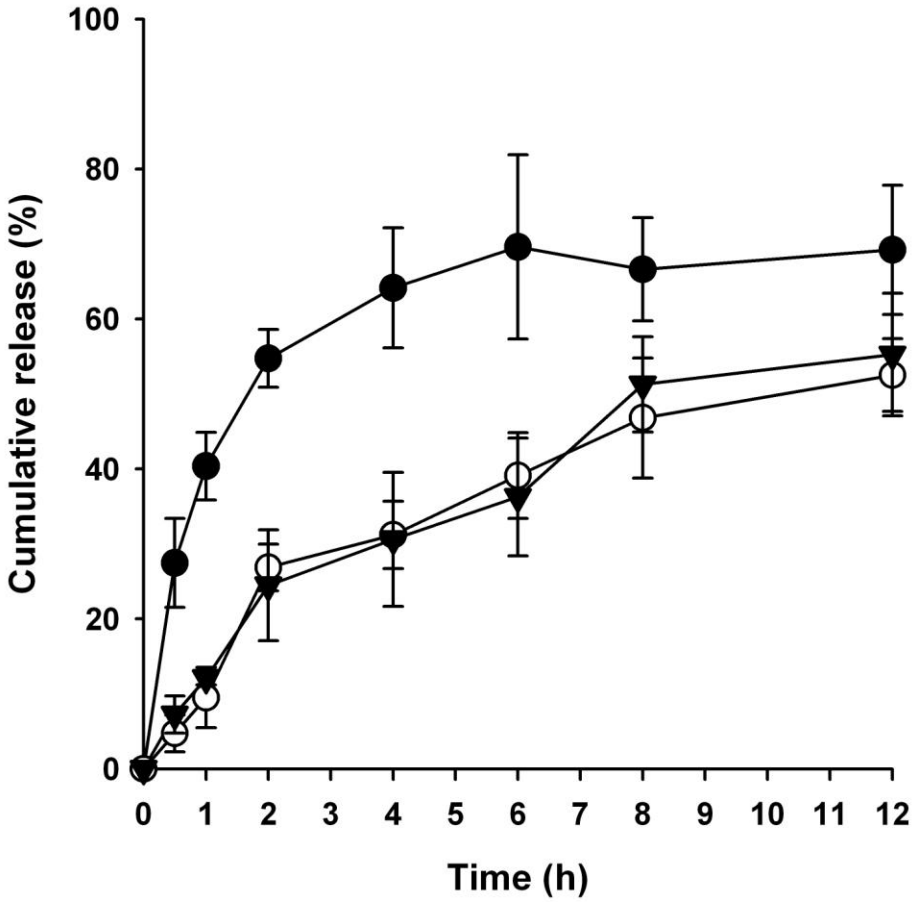


Figure 3 Time profiles of *in vitro* release of DOX from solution (●), F1 (○) and F2 (▼) at 37 °C in PBS (pH 7.4). Vertical bars represent standard deviation (n = 3).

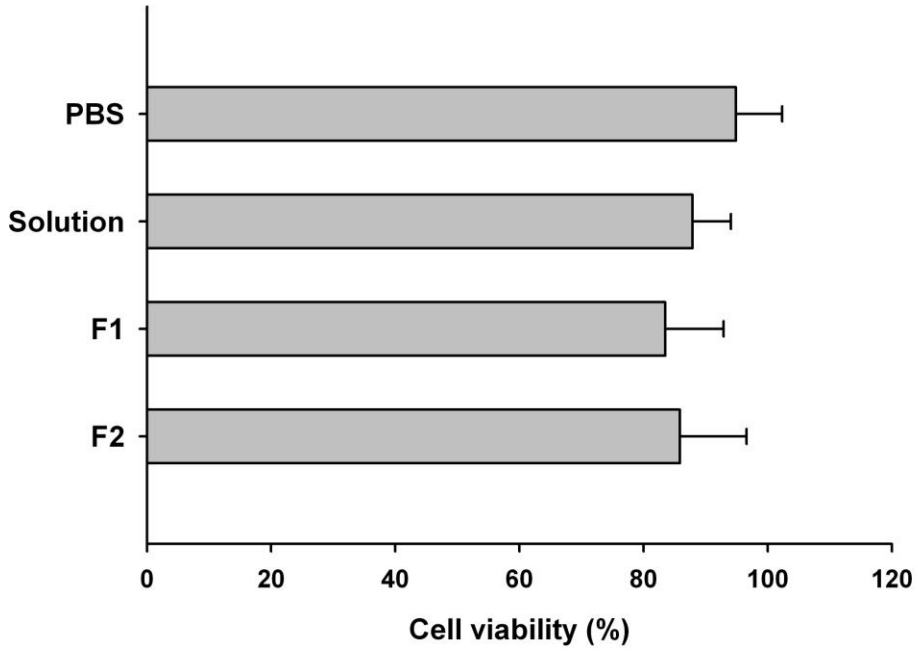


Figure 4 Viability of Caco-2 cells after 2-h exposure to DOX solution, F1 and F2 (n = 5). Horizontal bars represent standard deviation.

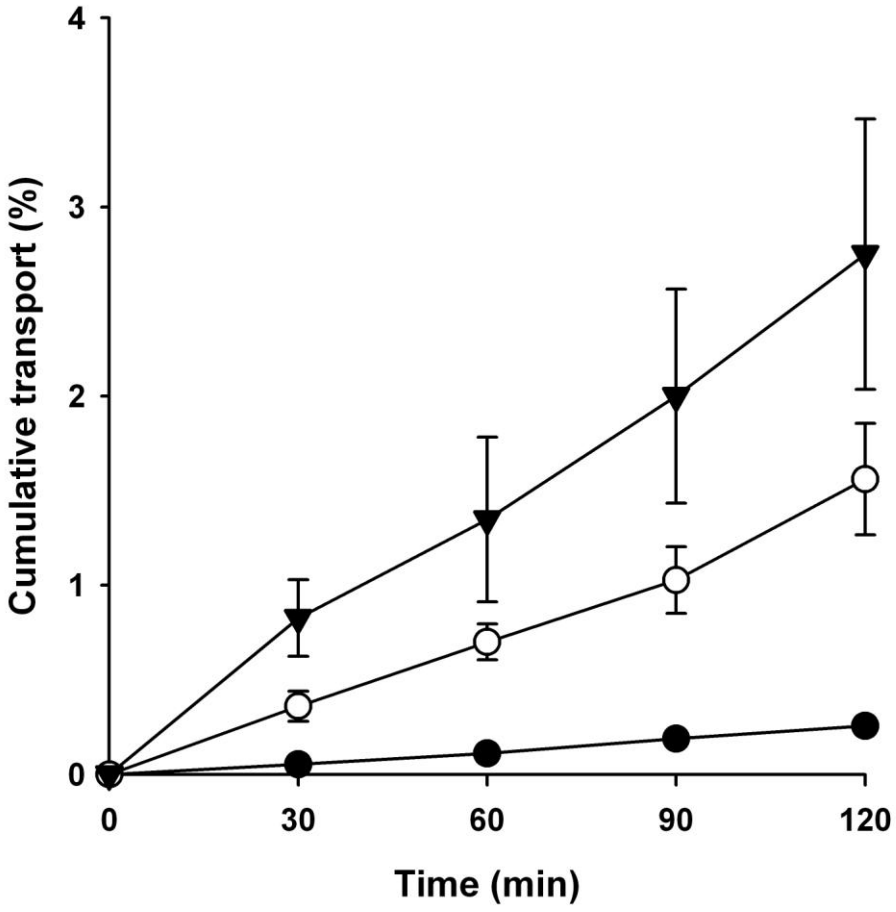


Figure 5 Time profiles of *in vitro* absorptive transport of DOX from solution (●), F1 (○) and F2 (▼) across Caco-2 cell monolayers at 37 °C (n = 4). Vertical bars represent standard deviation.

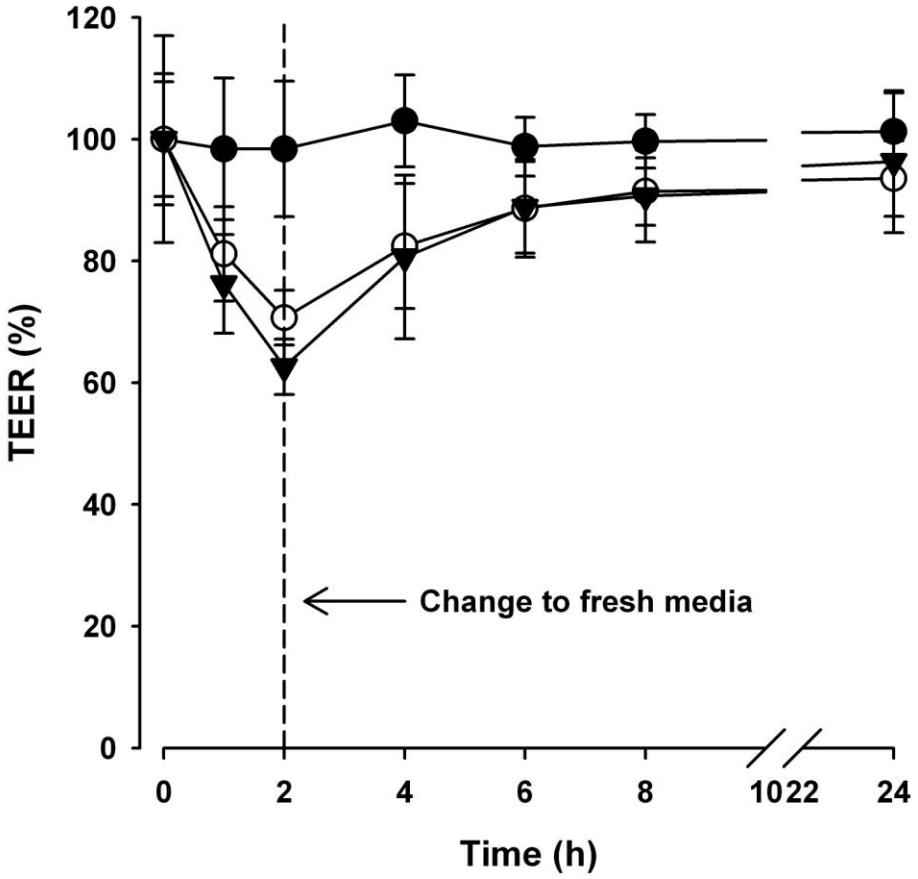


Figure 6 Time profiles of TEER values during the 2-h transport study of DOX solution (●), F1 (○) and F2 (▼) across Caco-2 cell monolayers and subsequent incubation in fresh media up to 24 h (n = 4). Vertical bars represent standard deviation.

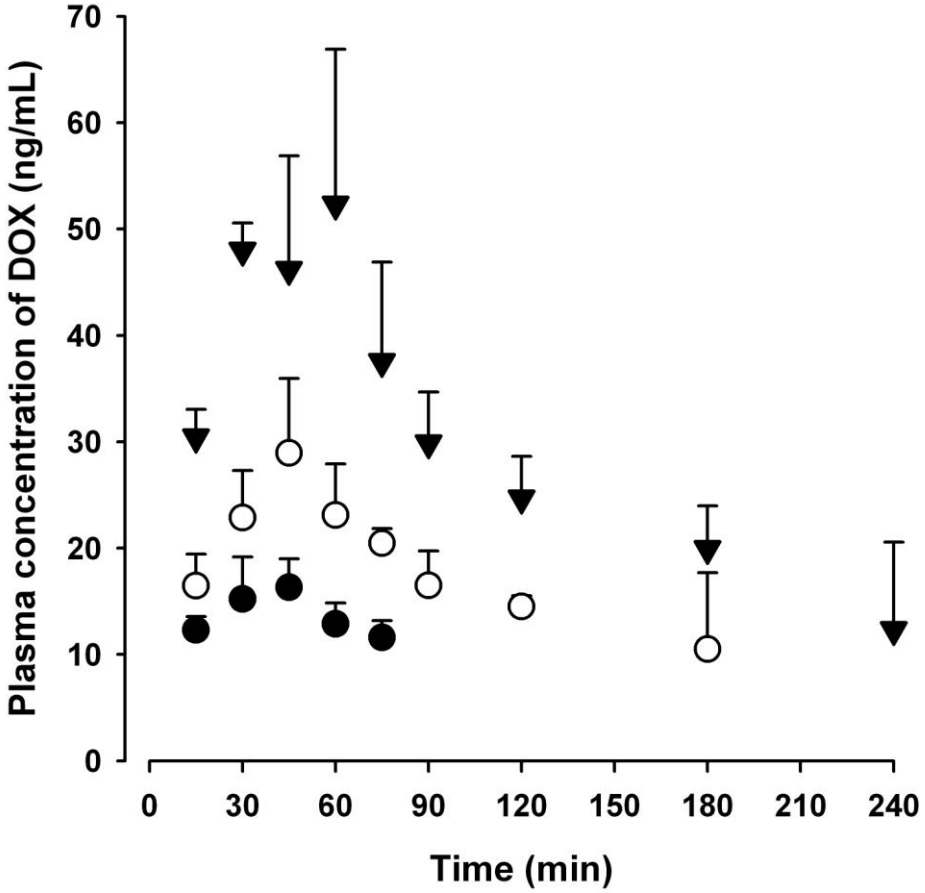
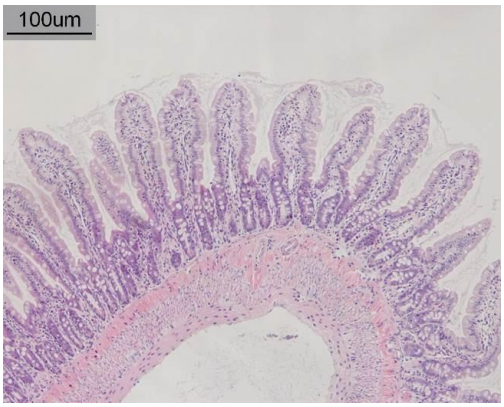


Figure 7 Time profiles of arterial plasma concentrations of DOX after oral administration of DOX solution (●), F1 (○) and F2 (▼) at a dose of 10 mg/kg to rats (n = 4). Vertical bars represent standard deviation.

(a) DW



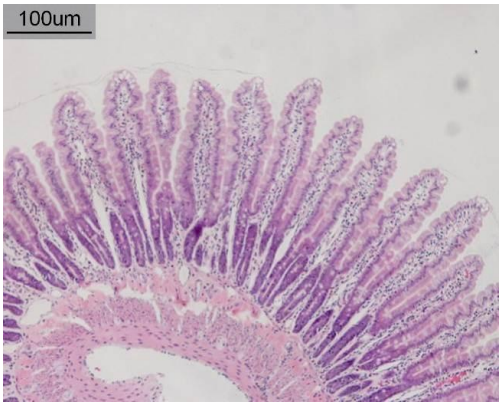
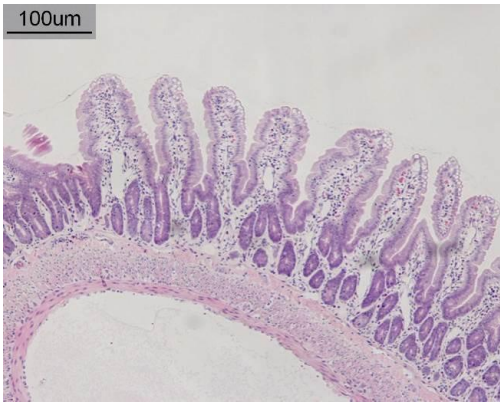
(b) F1	(c) F2
	

Figure 8 Representative histological sections of jejunal segments at 24 hr after oral administration of DW (a), F1 (b) and F2 (c) to rats. The scale bars represent 100 μm.

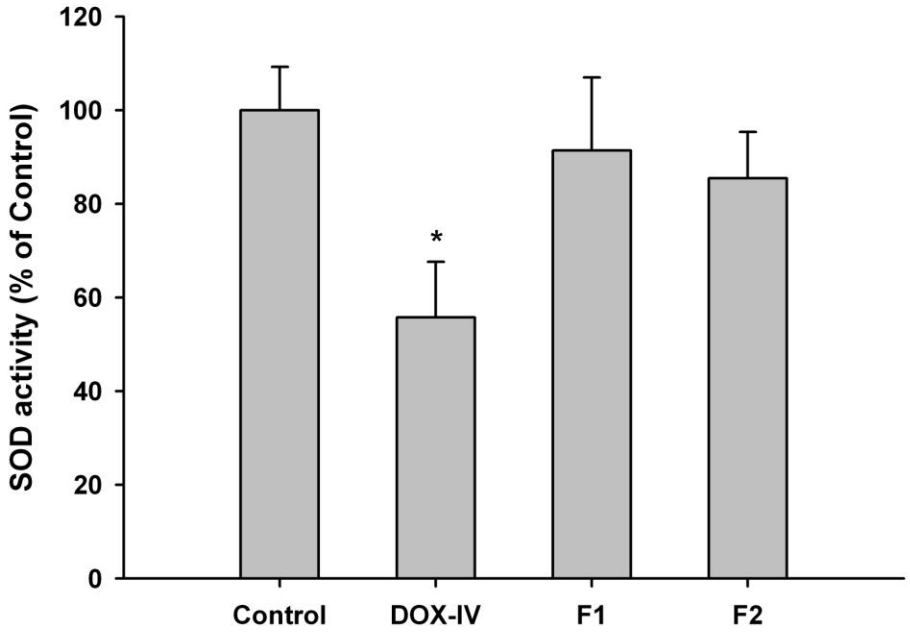


Figure 9 SOD activity in heart tissue after intravenous injection of saline (Control) and DOX solution (DOX-IV) and after oral administration of F1 and F2 to rats (n = 3). *; Significantly different from the other groups ($p < 0.05$).

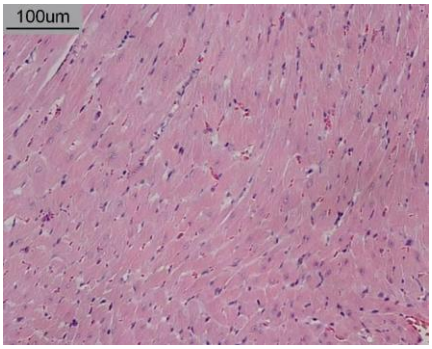
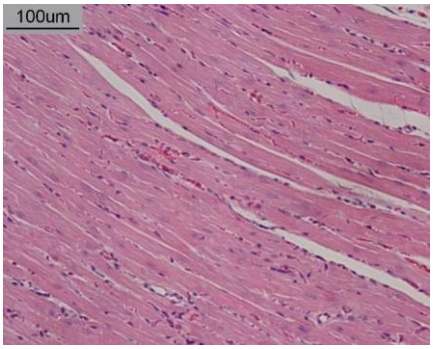
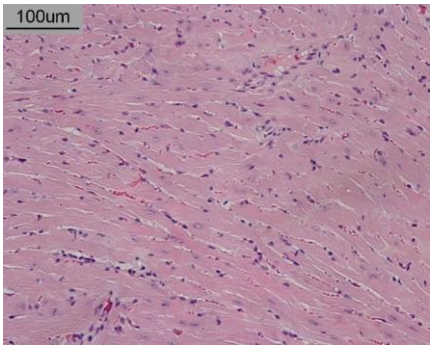
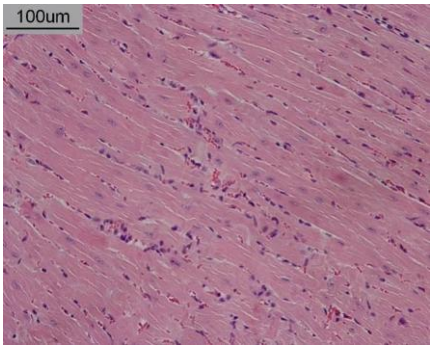
(a) Saline	(b) DOX solution
	
(c) F1	(d) F2
	

Figure10 Representative histological sections of heart segments after intravenous injection of saline (a) and DOX solution (b) and after oral administration of F1 (c) and F2 (d) to mice. The scale bars represent 100 μm.

국문초록

유중수 (w/o)형 마이크로에멀젼을 이용한 독소루비신의 경구 전달

서론

독소루비신 (Doxorubicin)은 BCS Class III 에 속하는 안트라사이클린 (Anthracycline) 계 항암제로서 림프종, 골육종, 육종, 상피성암, 흑색종 등에 널리 쓰인다. 독소루비신의 작용기전은 DNA 염기사이에 삽입되어 DNA 구조에 손상을 줘서 세포분열을 차단하는 것으로 주로 정맥주사로 투여되며 주요 부작용은 심장독성이다. 경구 투여 시 독소루비신의 생체이용률 (bioavailability)은 매우 낮고 주로 간 대사 및 담즙 배설에 의해 소실된다. 독소루비신의 낮은 경구 생체이용률의 주 원인은 장에서의 P-gp-mediated efflux 나 간에서의 cytochrome P450 (CYP) 3A-mediated first-pass metabolism 보다는 세포곁 통로 (paracellular pathway)를 통해 일어나는 제한된 소장흡수 때문임이 기존에 이미 규명되어있으므로, 독소루비신의 경구투여를 향상시키기 위해서는 세포곁 통로를 통한 흡수를 증가시킬 수 있는 제형이 필요할 것으로 생각된다.

마이크로에멀젼은 열역학적으로 안정한 성질을 가지며, 기름(Oil)과 계면활성제(surfactant)와 같은 paracellular permeation enhancer 를 포함하고 있어 세포곁을 통해 전달되는 (paracellularly-transported) BCS Class III 약물의 경구 흡수를 증가시킬 것으로 예상되므로 독소루비신의 경구 전달 시스템 개발에 적용할 수 있을 것으로 기대된다.

본 시험에서는 소장에서 세포곁 통로 (paracellular pathway)를 통한 독소루비신의 흡수를 증가시킴으로써 독소루비신의 경구 전달은 향상시키고 심장독성은 낮춘 중쇄 글리세리드 (medium chain glyceride)를 기반으로 한 유중수 (w/o)형 마이크로에멀젼 (microemulsion) 시스템을 개발하고자 하였다.

방법

중쇄 글리세리드를 기반으로 한 독소루비신의 콜로이드 나노시스템 제형은 거짓 삼원 상태도 (pseudo-ternary phase diagram)를 이용하여 선정하였고, 선정된 제형의 특성은 액적 크기 (droplet size) 와 분포, 점도, 약물 부하 (drug loading), *in-vitro* 약물방출을 평가하여 확인하였고, 추가로 Caco-2 cell 에서의 *in-vitro* 세포독성 (cytotoxicity) 시험, Caco-2 cell monolayer 에서의 *in-vitro* transport 시험, 래트 (rat)에서의 *in-situ* closed loop 시험, 래트에서의 *in-vivo* 약물동태 (pharmacokinetic) 시험과 *in-vivo* 독성시험을 평가하였다.

결과 및 논의

그림 1 과 같이 작성된 pseudo-ternary phase diagram 에 의해 독소루비신의 두 가지 마이크로에멀젼 제형을 선정하였다. Surfactant mixture (S_{mix})는 low HLB non-ionic surfactant (Span80, Capmul MCM)와 high HLB non-ionic surfactant (Tween80, Labrasol)를 2:1 무게비로 섞어 사용하였다. High drug loading efficiency 와 low proportion of surfactant 의 성질을 갖기 때문에 w/o 층에 존재하는 맑고 투명한 마이크로에멀젼을 선정하였으며, 선정된 두 가지 제형의 조성은 다음과 같다. 제형 1 (F1)의 조성은 50 % Captex 355, 40 % Span 80/Tween 80 mixture (2:1 w/w), 10 % aqueous phase 였고, 제형 2 (F2)의 조성은 55 % Captex 355, 35 % Capmul MCM/Labrasol mixture (2:1 w/w), 10 % aqueous phase 였다.

독소루비신의 콜로이드 나노시스템 제형의 특성은 표 1 에 정리하였다. 두 가지 제형 (F1, F2) 모두 200 nm 미만의 액적 크기를 가졌고 점도는 기존의 경구용 w/o 마이크로에멀젼과 유사하게 낮은 75-110 Cp 범위에 있었으며 두 제형 간에 큰 차이는 없었다. TEM images 는 그림 2 에 나타내었고 약 100–200 nm 크기를 가진 구형의 액적 (spherical droplet)이 관찰되었다.

3 가지 제형 (용액, F1, F2)에서의 투석법 (dialysis method)를 이용한 시간에 따른 독소루비신의 *in-vitro* 방출 패턴은 그림 3 에 나타내었다. 용액제형에서는 4 시간만에 plateau 에 도달하였고, F1 과 F2 에서는

용액제형에 비해 느리고 천천히 방출되었다. F1 과 F2 에서의 독소루비신 누적용출량은 용액제형보다 현저히 낮았으며 F1 과 F2 간에는 큰 차이가 없었다. 독소루비신이 w/o 마이크로에멀젼의 유상 (oil phase)을 통해 천천히 확산되기 때문에 이 단계가 전체 방출 과정에서의 율속단계가 된다.

Caco-2 cell monolayer 에서의 *in-vitro* transport 시험 결과는 그림 4 에 나타내었다. Control (PBS)과 독소루비신 용액 (20 μg/mL), F1, F2 간에 Caco-2 cell viability 는 차이가 없었다 (그림 4). 독소루비신의 겉보기 투과계수 (P_{app}; cm/s)는 각각 0.159×10^{-6} ± 0.0229 (DOX solution), 0.968×10^{-6} ± 0.183 (F1), 1.71×10^{-6} ± 0.443 (F2)로 F2 > F1 > 용액제형의 순으로 P_{app} values 가 높았다 (그림 5). F1 과 F2 제형에 들어있는 유상의 medium chain tri-glyceride 인 Captex 355 가 paracellular permeability 를 증가시키는 역할을 하여 독소루비신 용액보다 intestinal permeability 를 증가시킨 것으로 보여지며, F2 제형은 medium chain tri-glyceride 보다 membrane permeation enhancer 효과가 더 우수하다고 알려진 medium chain mono- and di-glyceride 인 Capmul MCM 와 역시 intestinal permeation of paracellular marker 로 알려진 Labrasol 을 추가로 함유하여 membrane permeation enhancer 효과가 F1 보다 더 크게 나타난 것으로 추정된다. F1 의 조성인 Tween 80 과 Span 80 의 paracellular enhancing activity 는 아직 보고되지 않았다. 독소루비신 용액, F1, F2 의 Caco-2 cell monolayers 을 통과하는

transport study 를 2 시간동안 진행한 뒤 media 를 교체하여 24 시간동안 배양했을 때의 상대 TEER values 를 측정한 결과, 용액은 TEER 값의 변화가 거의 없었으나 F1 과 F2 에서는 Initial TEER value 대비 각각 70.7 %와 62.6 %까지 감소하였다가 신선한 media 로 교체한 뒤에는 서서히 증가하여 24 시간에 control level 에 도달하였다 (그림 6). TEER 값의 변화는 paracellular permeation 의 주요 결정인자인 tight junction integrity 의 척도이다. 따라서 TEER 값의 감소는 paracellular pathway 를 통한 독소루비신의 absorptive transport 가 어느 정도 증가했다는 것을 의미하며, TEER 값이 복원된 것은 F1 과 F2 의 paracellular permeation 증진 효과가 가역적임을 의미한다.

독소루비신 용액, F1, F2 를 래트의 공장 (jejunum) 과 colon loops 내에 주입한 후 2 시간 경과했을 때의 독소루비신 잔량을 측정한 결과는 표 2 와 같다. F1 과 F2 의 잔량이 용액제형보다 현저히 낮았고 이는 w/o 마이크로에멀젼이 독소루비신의 소장 흡수를 증가시켰음을 나타낸다. 또한 F2 가 F1 보다 잔량이 더 적었으므로 F2 가 소장 흡수를 더욱 증가시킴을 알 수 있었다.

그림 7 는 독소루비신 3 가지 제형 (DOX 용액, F1, F2)을 각각 10 mg/kg 농도로 래트에게 경구 투여했을 때의 시간에 따른 동맥 혈중 농도를 나타낸 것이다. 독소루비신의 relevant pharmacokinetic parameters 는 표 3 에

정리하였다. DOX 용액, F1, F2 의 AUC, C_{max}, F_{rel} values 는 F2 > F1 > solution 의 순서로 증가했다.

그림 8 은 정제수 (control)와 독소루비신의 2 가지 마이크로에멀젼 제형 (F1, F2)을 각각 래트에게 경구투여하고 24 시간이 지난 후 공장의 대표적인 조직을 잘라낸 것으로 세 그룹 모두 소장벽에 융모결합이나 점막 모세혈관의 충혈과 같은 손상이 보이지 않았고 control 과 제형 간에 식별할만한 차이는 보이지 않았다. 이는 독소루비신의 경구 제형 (F1, F2)이 래트의 소장 점막에 독성을 나타내지 않음을 의미한다.

그림 9 은 래트에게 생리식염수 (control)와 독소루비신 용액은 정맥주사로, F1 과 F2 는 경구투여한 후 심장 조직에서의 superoxide dismutase (SOD) 활성을 측정한 것이다. Control 군과 비교하여 독소루비신 용액을 정맥투여한 군에서는 SOD 활성이 현저히 낮아졌으나, F1 과 F2 를 경구투여한 군에서는 활성에 변화가 거의 없었다. 이는 free radical 제거효소인 SOD 활성도가 감소하여 심장독성이 크게 나타날 수 있는 용액제형에 비해 F1 과 F2 가 독소루비신에 의한 심장독성을 완화시킬 수 있음을 보여준다.

그림 10 은 mice 에게 생리식염수 (control)와 독소루비신 용액은 정맥주사로, F1 과 F2 는 경구투여한 후 심장 조직을 잘라낸 것으로 용액제형에서는 심각한 심근 해체와 심장조직의 구조적 변성이

일어났으나 독소루비신의 경구 제형 (F1, F2)에서는 control 과 별다른 차이가 없었다.

결론

본 연구에서는 pseudo-ternary phase diagram 를 이용하여 Captex 355, Capmul MCM, and/or Labrasol 를 함유하는 w/o 마이크로에멀젼을 만들었고 그 특성을 평가하였다. 그 결과 본 연구에서 개발된 중쇄 글리세리드를 기반으로 한 콜로이드 나노시스템 (medium chain glycerides-based colloidal system)은 독소루비신의 경구전달시스템으로 매우 효과적이었다.

주요어: 독소루비신 (Doxorubicin), 경구 투여(oral delivery), 장관 세포곁 흡수 (intestinal paracellular absorption), 중쇄 글리세리드 (medium chain glyceride), 마이크로에멀젼 (microemulsion).

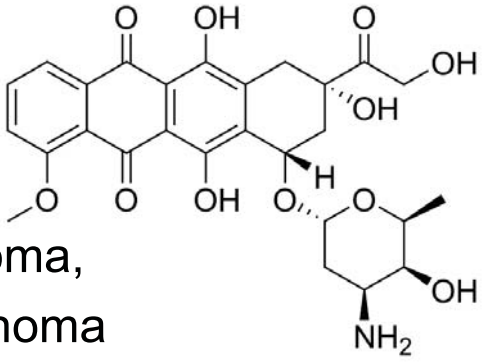
학번: 2006-30446

Appendix

Water-in-oil (w/o) Microemulsion for Oral Delivery of Doxorubicin

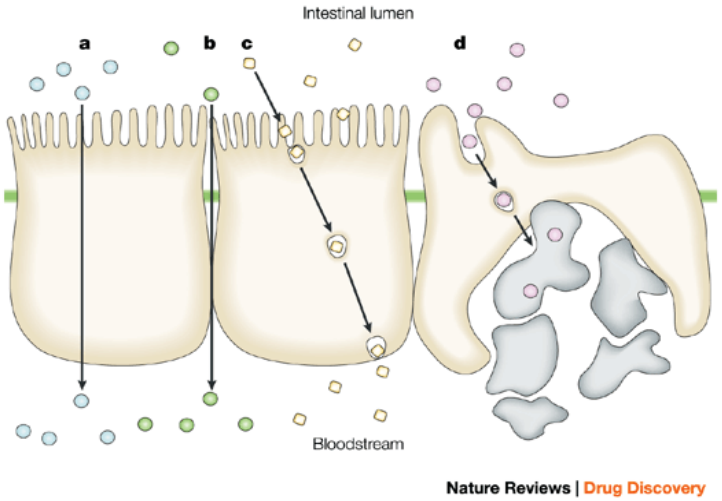
2013.11.26
서울대학교 약학대학
약제과학 박사과정
김지언

Doxorubicin (DOX)



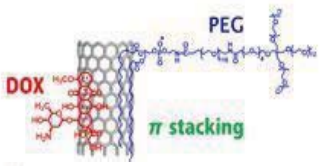
- BCS class III drug
  : High Solubility, Low Permeability
- Anthracycline antibiotics
  : treatment of lymphoma, osteosarcoma, other sarcomas, carcinomas, melanoma
- Mechanism of action
  : Impairing DNA synthesis during tumor cell division
- Poor oral bioavailability (1%)
- P-gp and CYP substrate
- Exhibits high first pass metabolism in liver
- Route of administration: Intravenous injection
- Adverse effect: Cardiotoxicity

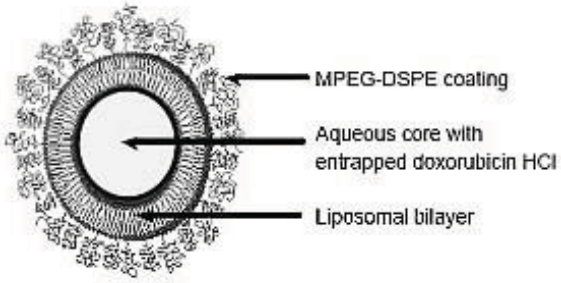
Doxorubicin (DOX)



Nature Reviews | Drug Discovery

Doxorubicin attached carbon nanotubes





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RESEARCH ARTICLE

The limited intestinal absorption via paracellular pathway is responsible for the low oral bioavailability of doxorubicin

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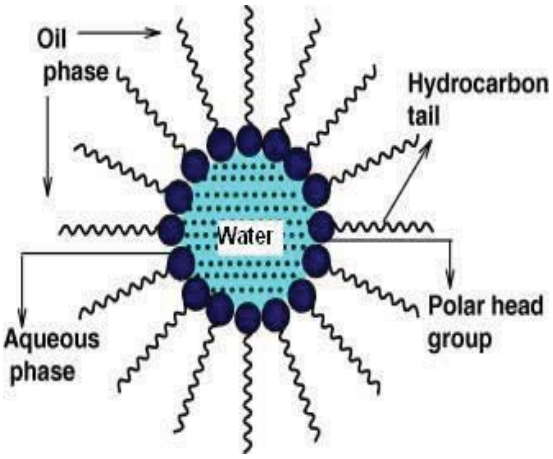
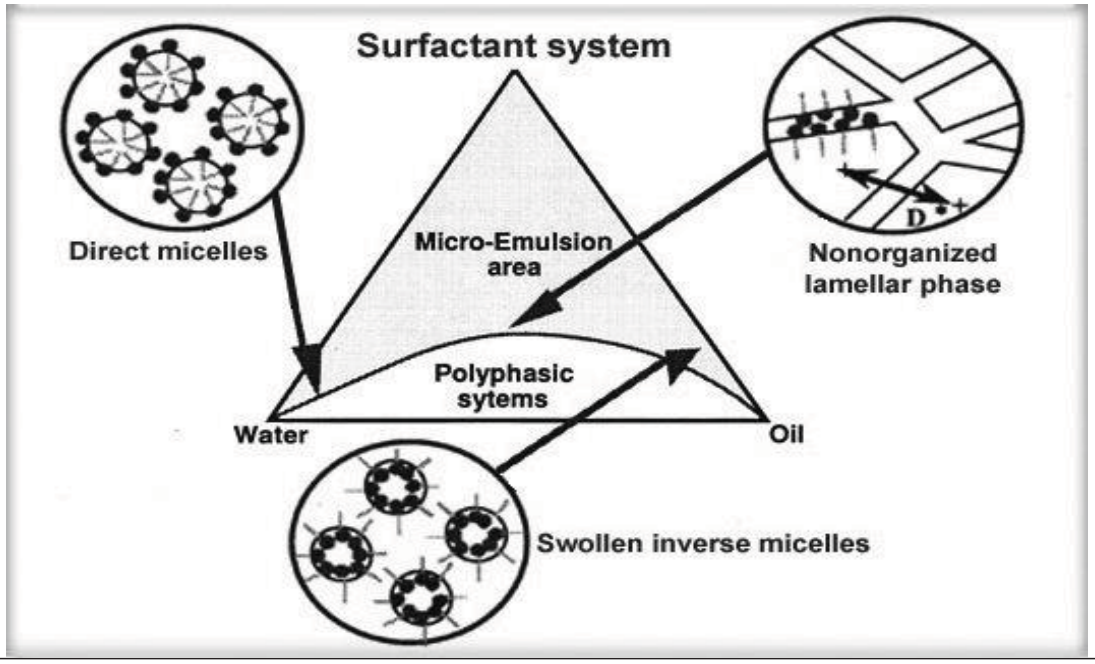
Characteristics of Microemulsions

- Droplet size: 10-200 nm
- Isotropic, optically clear
- High solubilizing capabilities
  → Increase drug solubility
- Low surface tension
- Thermodynamically stable
  → Easy to prepare (self-emulsification system)
- Reversible temperature behaviour
  → Formation of microemulsion is reversible
- Low viscosity compared to other emulsions



Systems of Microemulsions

- Ternary system : water, oil, surfactant
- Quaternary system
  : water, oil, surfactant, and co-surfactant

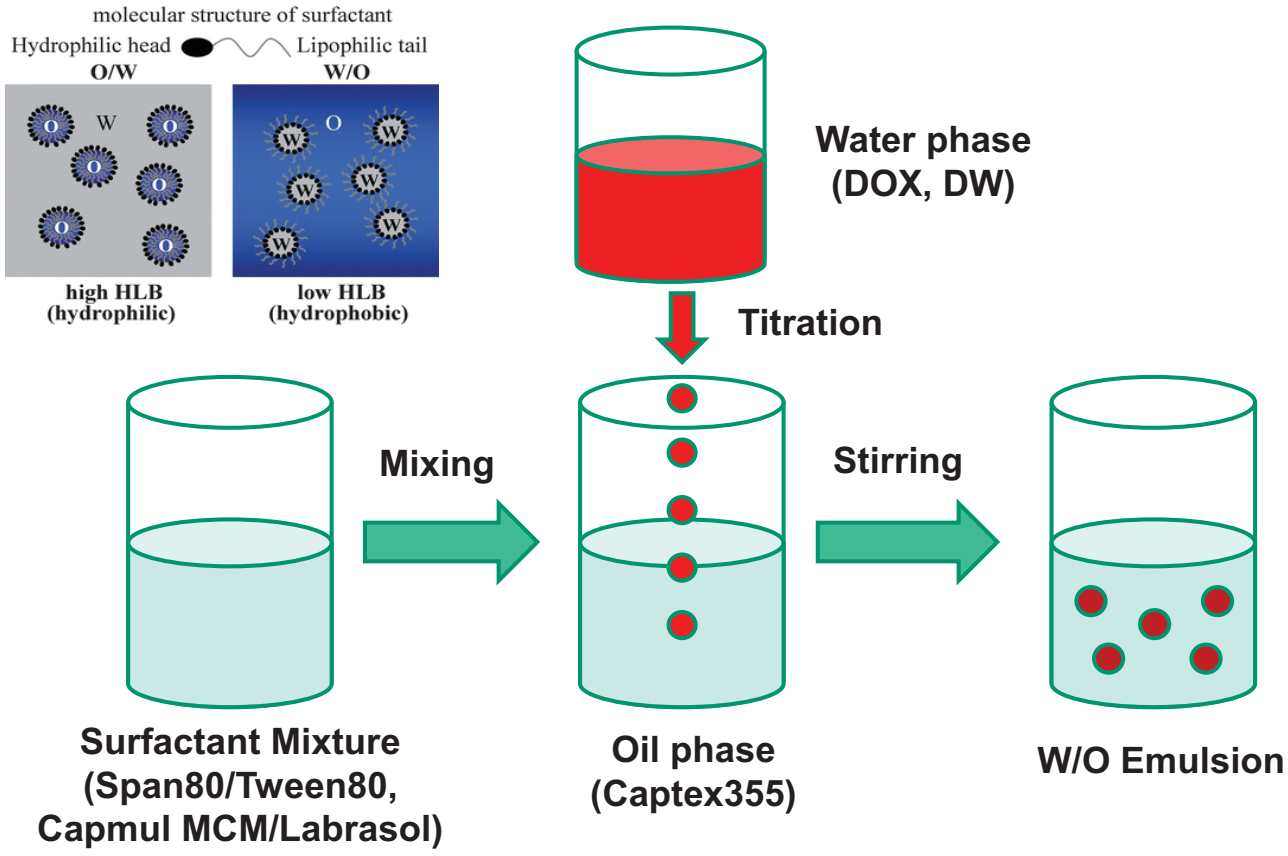


Objects

- Microemulsions contain oils and surfactants as paracellular permeation enhancers ⇒ enhance the oral absorption of paracellular-transported BCS class III drug.

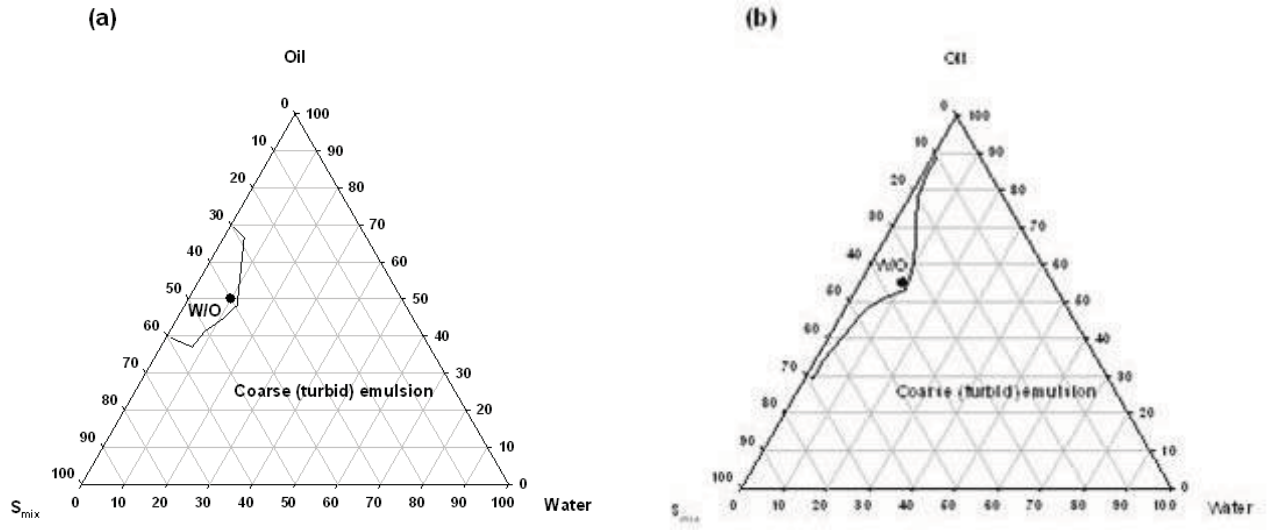
- In this study, we have formulated medium chain glycerides (MCG)-based colloidal systems to enhance the intestinal paracellular absorption of doxorubicin (DOX) thereby improving its oral delivery.

Preparation of DOX Formulations



Composion of DOX Formulations

Pseudo-ternary phase diagram

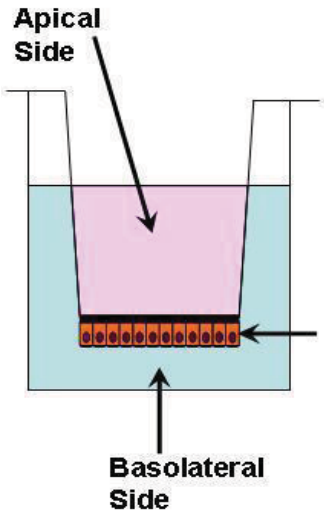


Formulation Code	Oil phase (Captex 355)	S_{mix} (2:1 w/w)	Aqueous phase	Surfactant
F1	50%	40%	10%	Span 80/Tween 80
F2	55%	35%	10%	Capmul MCM/Labrasol

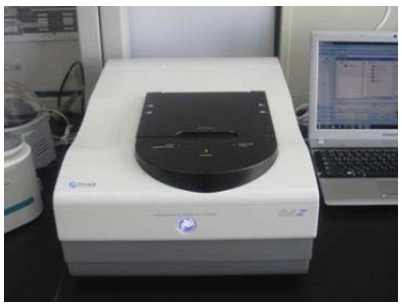
Medium-chain Glyceride

	Captex355	CAPMUL MCM	Labrasol
Physical form	Clear Liquid	Liquid/semi-solid	Liquid
Category	MCTs (Medium Chain **Tri**glyceride)	Medium Chain Glyceride (MCG), Medium Chain **Mono & Di-**Glyceride	**PEG-8** Caprylic/Capric Glycerides
EP Name	Glyceryl/Caprate tricaprylate	Glyceryl Caprylate/ Caprate	Caprylocaproyl macrogol-8 glycerides
HLB	-	5-6 (친유성)	14 (친수성)
Use	Actives or polar lipids solubilizing agent Saponification score of 325-340	Partially water soluble emulsifier. Increases the absorption and delivery of Paracellular marker compound .	Solubilizer and wetting agent. Bioavailability enhancer (known as P-gP inhibition).

Experimental Method

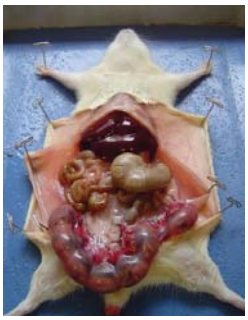


In vitro study



ELS

TEM

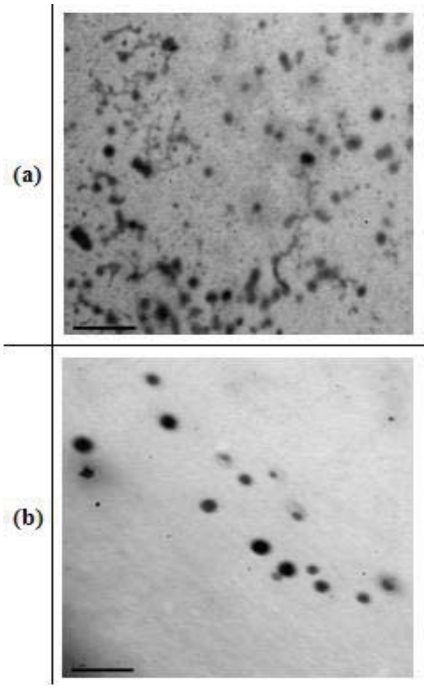


In situ study

HPLC

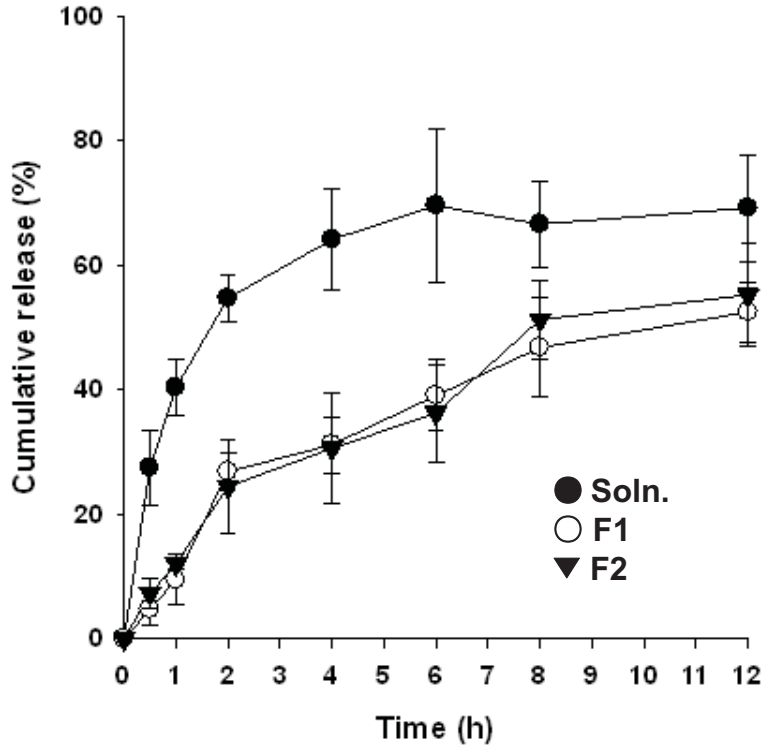
Characterization of DOX Formulations

Physicochemical property	F1	F2
Droplet size (nm)		
Before dilution	164±30.5	142±38.5
After x10dil. PBS	1470±336	1230±251
Polydispersity index	0.184±0.034	0.170±0.046
Viscosity (cP)	99.3±12.2	84.3±8.08
Leakage (%) after x500dil. PBS	8.63±1.03	9.21±1.20
Max. drug loading (mg/mL)	3.02±0.129	2.92±0.378



100-200nm size
Spherical droplet

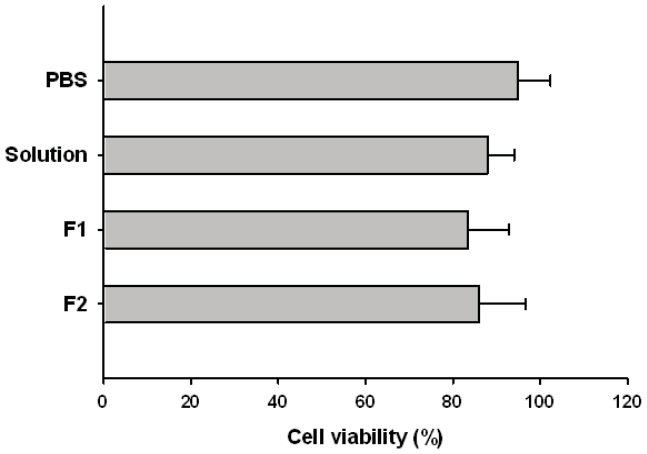
In Vitro Release Study



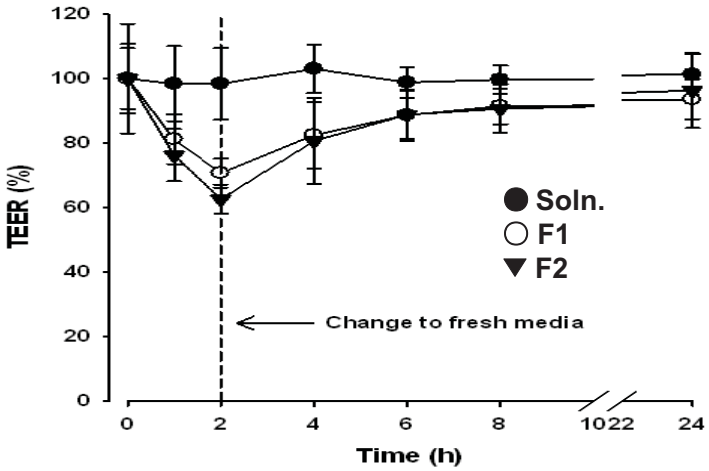
Time profiles of *in vitro* release of DOX from solution (●), F1 (○), and F2 (▼) at 100rpm, 37°C in PBS (pH 7.4).

- using dialysis method (n=3): MWCO 6-8 kDa
- Release of DOX: DOX Soln.>F1≈F2
- Slow diffusion of DOX through oil phase of w/o emulsion ⇒ Rate-limiting step

In Vitro Transport Study in Caco-2 cell



Viability of Caco-2 cells after 2-h exposure to 0.1mM DOX solution, F1 and F2 (*n* = 5)

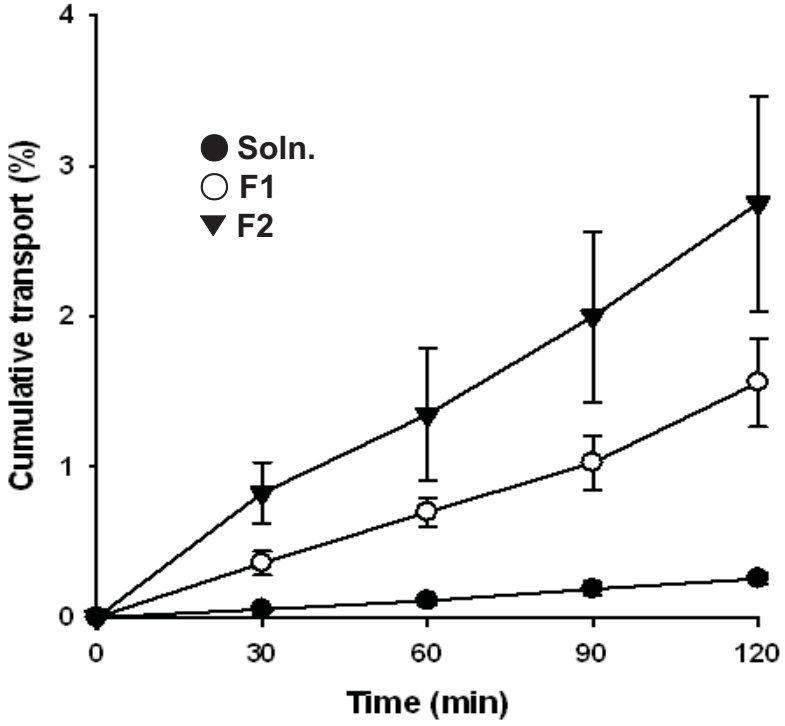


Time profiles of TEER values during the 2-h transport study of 0.1mM DOX solution (●), F1 (O), and F2 (▼) across Caco-2 cell monolayer and subsequent incubation in fresh media up to 24 h (*n* = 4).

➢Not significant difference between Control(PBS) and DOX soln.,F1,F2
→ No cellular toxicity

➢TEER value reduced during 2 h & restored after change media in F1,F2
➢ Disruption of tight junction → Paracellular permeability ↑

In Vitro Transport Study in Caco-2 cell



Time profiles of *in vitro* absorptive transport of DOX from solution (●), F1 (O), and F2 (▼) across Caco-2 cell monolayers at 37°C (*n* = 4).

➢ P_{app} values
: F2>F1> DOX soln.

➢ due to Paracellular-enhancing effects of lipidic components (e.g. MCT,MCG)

➢ Labrasol also acts as membrane permeability enhancer

In Situ Closed Loop Study

Remaining Fractions of DOX at 2 h after Injection of DOX Soln., F1 and F2 (2mg/mL) into the Rat Jejunum and Colon Loops (*n*=3–4)

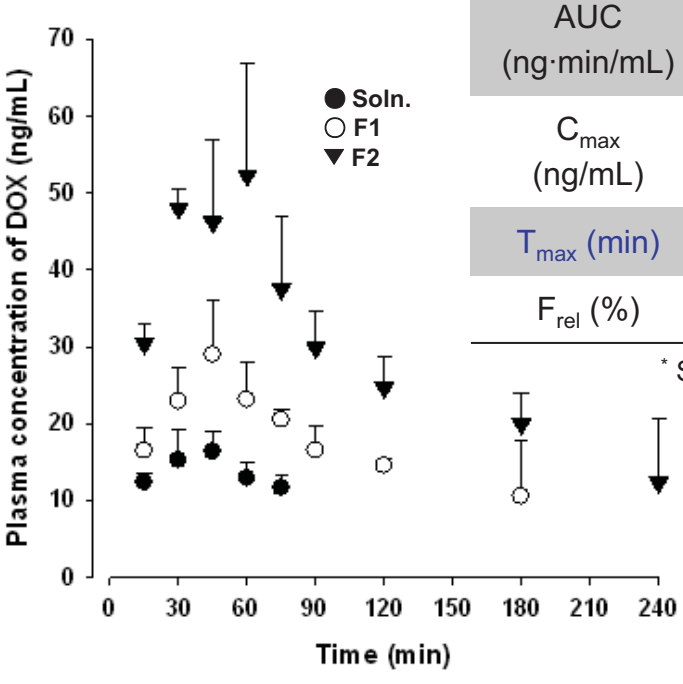
Intestinal Segment	DOX remaining (%)		
	Solution	F1	F2
Jejunum	87.6±19.8*	64.4±9.52*	40.5±10.4*
Colon	90.8±12.8*	57.8±8.39	46.7±11.0

* Significantly different from the other groups ($p < 0.05$).

- Remaining Fractions of DOX
  : DOX Solution > F1 > F2

⇒ Intestinal absorption of DOX (may be)
: F2 > F1 > DOX solution

In Vivo Pharmacokinetic Study



Time profiles of arterial plasma concentrations of DOX after oral administration of DOX solution (●), F1(○) and F2 (▼) at a dose of 10 mg/kg to rats (*n* = 4).

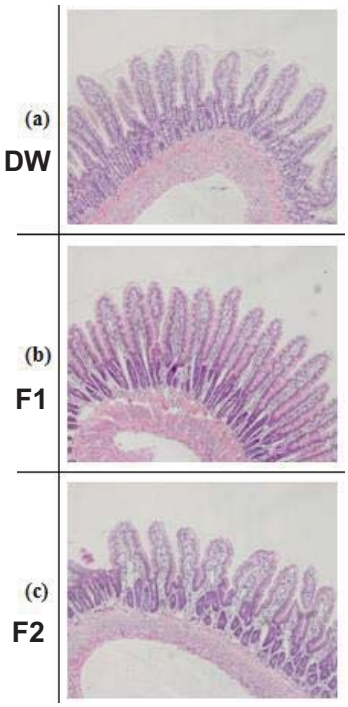
Parameter	Solution	F1	F2
AUC (ng·min/mL)	2740±1030*	5310±1310*	9040±1730*
C_{max} (ng/mL)	17.5±2.76*	29.4±6.42*	57.7±9.28*
T_{max} (min)	45 (30–45)	45 (30–45)	60 (30–60)
F_{rel} (%)	100	214	364

* Significantly different from the other groups ($p < 0.05$).

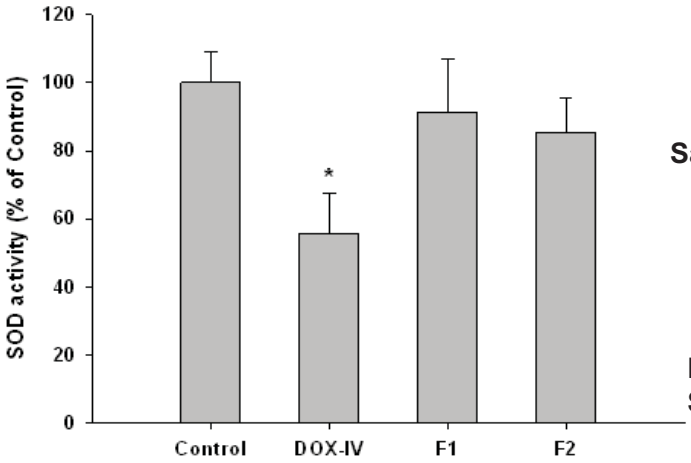
- PK parameter of DOX
  : F2 > F1 > DOX soln.
- Prolonged exposure of drug at modest conc. would be beneficial

In Vivo Toxicity Study

SD Rat



No discernible damage

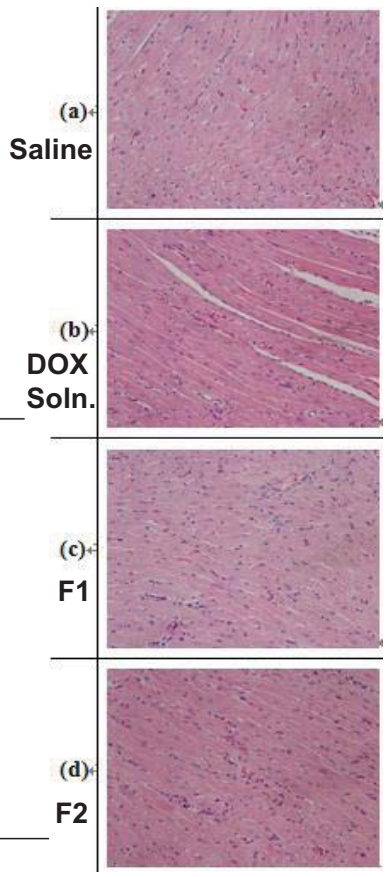


* Significantly different from the other groups ($p < 0.05$).

SOD activity in heart tissue after I.V injection of saline (Control) and DOX solution (DOX-IV) and after oral administration of F1 and F2 to rats (n = 3).

- No difference between Control(Saline) & F1,F2 in SOD activity
  ⇒ Cardiac toxicity ↓

ICR Mice



Conclusions

- MCG-based colloidal nanosystems based on w/o microemulsion containing Captex355, Capmul MCM, and/or Labrasol were developed for the oral delivery of DOX
- New formulations enhanced the absorptive transport of DOX due to the paracellular-enhancing effects of their lipidic components and reduced cardiac toxicity after oral administration.
- MCG-based colloidal system prepared in this study represents a potentially effective oral delivery system for DOX.

Thank you!

Appendix

[SCI 게재 논문]

- Ji-Eon Kim, Hyun-Jong Cho, Jung Sun Kim, Chang-Koo Shim, Suk-Jae Chung, Min-Ho Oak, In-Soo Yoon*, Dae-Duk Kim*, The limited intestinal absorption via paracellular pathway is responsible for the low oral bioavailability of doxorubicin. Xenobiotica, 43(7), 579-591, 2013
- Ji-Eon Kim, Hyun-Jong Cho* and Dae-Duk Kim*, Budesonide/cyclodextrin complex-loaded lyophilized microparticles for intranasal application. Drug Development and Industrial Pharmacy, Posted online on April 3, 2013.
- Hyun-Jong Cho, Ji-Eon Kim, Dae-Duk Kim, In-Soo Yoon*, *In vitro–in vivo* extrapolation (IVIVE) for predicting human intestinal absorption and first-pass elimination of drugs: principles and applications, Drug Development and Industrial Pharmacy, Posted online on August 28, 2013. (Review Article)

[현재 SCI Review 중인 논문]

- Ji-Eon Kim, Min-Hyo Ki, Hyun-Jong Cho, In-Soo Yoon, Ree-Sun Kim, Geun-Tae Kim*, Dae-Duk Kim*, Pharmacokinetics and Bioequivalence of Two Formulations of Valsartan 160-mg Tablets in Healthy Korean Male Volunteers: A Randomized, Single-Dose, 2-Period Crossover Study, Clinical Therapeutics
- Ji-Eon Kim†, In-Soo Yoon†, Hyun-Jong Cho, Dong-Hwan Kim, Young-Hee Choi, Dae-Duk Kim*, Emulsion-based colloidal nanosystems for oral delivery of doxorubicin: Improved intestinal paracellular absorption and alleviated cardiotoxicity, International journal of pharmaceutics

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Xenobiotica

Xenobiotica, 2013; 43(7): 579–591

DOI: 10.3109/00498254.2012.751140

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RESEARCH ARTICLE

The limited intestinal absorption via paracellular pathway is responsible for the low oral bioavailability of doxorubicin

Ji-Eon Kim[1], Hyun-Jong Cho[2], Jung Sun Kim[3], Chang-Koo Shim[1], Suk-Jae Chung[1], Min-Ho Oak[4], In-Soo Yoon[4]*, and Dae-Duk Kim[1]*

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Abstract

1. Doxorubicin exhibited dose-independent pharmacokinetics after intravenous (5–20 mg/kg) and oral (20–100 mg/kg) administration to rats. Nearly all (82.1–99.7%) of the orally administered doxorubicin remained unabsorbed, and the hepatic first-pass extraction ratio and oral bioavailability of doxorubicin were approximately 0.5% and 1%, respectively. Based on these results, it is likely that the primary factor responsible for the low oral bioavailability of doxorubicin is the limited intestinal absorption, rather than the CYP3A4-mediated first-pass metabolism.
2. Moreover, the *in vitro* transport and cellular uptake studies using Caco-2 cell monolayers have revealed that doxorubicin crosses the intestinal epithelium primarily via the paracellular pathway (accounting for 85.6% of the overall absorptive transport) probably due to its physicochemical properties (hydrophilic cation; $pK_a = 9.67$, $\log P = -0.5$). These results suggest that P-glycoprotein (P-gp)-mediated efflux activity does not play a significant role in limiting the intestinal absorption of doxorubicin, attenuating the absorptive transport by only 5.56–13.2%.
3. Taken together, the present study demonstrated that the limited and paracellular intestinal absorption of doxorubicin was a major factor responsible for its low oral bioavailability, restricting the role of CYP3A4-mediated first-pass metabolism and P-gp-mediated efflux.

Keywords

Bioavailability, Caco-2 cells, doxorubicin, intestinal absorption, paracellular transport, rats

History

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Introduction

Doxorubicin (Figure 1) is an anthracycline glycoside antibiotic known to impair DNA synthesis during tumor cell division (Schwarzbach et al., 2002). When bonded to cellular DNA and anionic lipids, doxorubicin is extensively distributed in tissues (Gustafson & Thamm, 2010). It is eliminated via the urinary and fecal (intestinal and biliary) excretion as well as metabolism (Lee et al., 1995). The major metabolites of doxorubicin are doxorubicinol and the aglycones, doxorubicinone and 7-deoxydoxorubicinone (Gustafson & Thamm, 2010; Kivistö et al., 1995). Metabolism to doxorubicinol occurs by aldoketoreductases in the cytoplasm, while the aglycones are formed by the microsomal CYPs (Kivistö et al., 1995). The biliary excretion of doxorubicin is almost entirely mediated by P-glycoprotein (P-gp), as shown in a mdr1a knockout mice study which resulted in an approximately 82.0% reduction of doxorubicin concentration in the bile (van Asperen et al., 2000).

Although doxorubicin is one of the most widely used anticancer drugs for the treatment of lymphoma, osteosarcoma and other sarcomas, carcinomas, and melanoma (Son & Choi, 2009), its therapeutic effects are limited due to its dose-dependent cardiotoxicity and myelosuppression. Epirubicin and idarubicin are second generation analogs of doxorubicin, which have made it to clinical development and approval among the nearly 2000 analogs synthesized to overcome the toxicity problems. Yet, despite their reduced cardiotoxicity, they revealed lower efficacy compared to doxorubicin. Hence, doxorubicin is still indispensable in the area of cancer chemotherapy (Kalaria et al., 2009; Ryberg et al., 1998).

Single intravenous (IV) injection, which is the most common dosing mode of doxorubicin, may result in an undesired systemic exposure profile with an excessively high level in the initial and subsequent fast decay below the minimum therapeutic level (ONS Clinical Practice Committee, 1999). Moreover, it is generally believed that

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RESEARCH ARTICLE

Budesonide/cyclodextrin complex-loaded lyophilized microparticles for intranasal application

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Abstract

Objective: Lyophilized microparticles composed of budesonide (BDS), hydroxypropyl-β-cyclodextrin (HP-β-CD), and hydroxypropylmethylcellulose (HPMC) or sodium carboxymethylcellulose (CMC-Na) were developed for intranasal delivery and their characteristics were evaluated.
Materials and methods: The particle size and morphology were assessed by mean diameter measurement and scanning electron microscopy (SEM) image, respectively. The solid-state of products was tested by X-ray powder diffraction (XRPD), Fourier transform infrared spectroscopy (FT-IR) and differential scanning calorimetry (DSC). *In vitro* drug release and cytotoxicity to the primary human nasal epithelial (HNE) cells were also evaluated.
Results and discussion: Lyophilized microparticles exhibited vanishment of crystallinity of drug in XRPD analysis, the enfeeblement of carbonyl (C=O) stretching bands of carboxyl group in BDS in FT-IR spectra and the disappearance of endothermic peak of drug in the results of DSC study. Based on the results of solid-state studies, BDS was existed as an amorphous form in the lyophilized microparticles. CD complexation enhanced drug solubility and release rate, and HPMC or CMC-Na also improved drug dissolution rates. Cytotoxicity of developed microparticles to the HNE cells was measured and their safety to HNE cell was identified.
Conclusion: Developed microparticles can efficiently deliver insoluble drug, such as BDS, to the nasal epithelium and thus it may improve therapeutic efficacy in the respiratory tract.

Keywords

Budesonide, hydroxypropyl-β-cyclodextrin, intranasal delivery, lyophilized microparticle, solid-state study

History

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Introduction

Intranasal corticosteroids (INS) are considered as important agents for the treatment of allergic rhinitis, nonallergic rhinitis, chronic rhinosinusitis and nasal polyposis[1–6]. Although systemic administration of corticosteroids has been tried, it might induce several side effects such as growth suppression, suppression of hypothalamic-pituitary-adrenal (HPA) axis function, alteration in bone metabolism and skin thinning[7,8]. Thus, direct delivery to the respiratory epithelium has been investigated to reduce those side effects and maximize local therapeutic effects. Budesonide ([RS]-1β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione cyclic 16,17-acetal with butyraldehyde; BDS) is one of the frequently used inhaled glucocorticosteroids and it was approved for allergic rhinitis and asthma. Compared to other inhaled steroids, BDS has a high-relative affinity for the glucocorticoid receptor; it has about a 200-fold higher affinity for the glucocorticoid receptor and a 1000-fold higher topical anti-inflammatory potency than cortisol[9]. It is also reported that its half-life ($t_{1/2}$) after intranasal administration (2.9 h) is similar with that after intravenous injection (2.3 h)[10].

Nevertheless, BDS (log *P*: 3.2) is practically insoluble in water (0.02 mg/mL), which restricts its therapeutic application[11]. For the delivery of BDS, many approaches have been tried to enhance its solubility by the formulation development and the addition of solubilizer: liposome[12,13], microparticle[14,15], nanoparticle[16,17], nanosuspension[18,19], nanoemulsion[20], solid dispersion[21] and cyclodextrin complex[11,22]. Among various trials, several types of cyclodextrin have been widely used for the enhancement of drug solubility and dissolution[23,24]. Cyclodextrin (CD) is a natural cyclic oligosaccharide that is formed through an enzymatic degradation of starch. Three most common CDs, α-CD, β-CD and γ-CD, are composed of six, seven and eight (1,4)-linked-D-glucopyranose units, respectively, with a hydrophilic outer surface and a somewhat lipophilic central cavity[25]. Drug absorption-enhancing effects of CDs across mucosal membranes were already reported[26–28]. In addition, it is known that drug-CD complex can control drug release[29] and it may influence on the drug permeation across mucosal membrane[30].

Though a few investigations about BDS–CD complex were already reported[11,22], its intranasal delivery system has not been thoroughly studied. In this investigation, hydrophilic polymers (HPMC and CMC-Na) were added to develop microparticles based on a ternary phase system. HPMC exhibits bioadhesiveness[31,32] and it is a generally regarded as safe (GRAS) excipient[33].

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REVIEW ARTICLE

In vitro–in vivo extrapolation (IVIVE) for predicting human intestinal absorption and first-pass elimination of drugs: principles and applications

Hyun-Jong Cho[1], Ji-Eon Kim[2], Dae-Duk Kim[2], and In-Soo Yoon[3]

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Abstract

Oral administration remains the preferred dosing method in clinical practice and drug development. Oral bioavailability (F) is a function of the fraction absorbed (F_{abs}), gastrointestinal or gut wall availability (F_G), and hepatic availability (F_H). Therefore, predicting intestinal absorption (F_{abs}) and first-pass elimination (F_G and F_H) from *in vitro* data may facilitate the selection of more orally bioavailable drug candidates in earlier stages of drug discovery and development. This review provides an overview of the determinants of intestinal absorption and first-pass elimination of drugs and focuses on the principles and applications of conventional *in vitro–in vivo* extrapolation (IVIVE) methods to predict F_{abs}, F_G, and F_H in humans.

Keywords

First-pass elimination, intestinal absorption, *in vitro–in vivo* extrapolation (IVIVE), oral bioavailability, pharmacokinetics

History

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Introduction

Oral administration remains the preferred dosing method in clinical practice and drug development[1–5]. However, the development of oral dosage forms and regimens has often been hindered by low oral bioavailability (F)[6–10]. It has also been reported that low F is associated with high variability in systemic exposure of 143 drugs[11]. Potential benefits of high F include smaller dosage forms, lower costs, and improved patient compliance[6,12]. Therefore, it is crucial to find more orally bioavailable drug candidates in drug discovery and development[13,14].

F can be determined by comparing the dose-normalized area under plasma concentration-time curve (AUC) after oral administration to that after intravenous administration. Orally administered drugs pass sequentially from the gastrointestinal (GI or gut) lumen, through the GI wall, liver and lung, and then reach the systemic circulation as shown in the following equation:

$$F_S = F_{abs} \times F_G \times F_H \times F_L \quad (1)$$

where F_S is the fraction of the dose that reaches the systemic circulation as unchanged drug after oral administration; F_{abs} (fraction absorbed) is the fraction of the dose absorbed into the GI wall from the GI lumen (primarily in the intestine); F_G, F_H, and F_L are the fractions of the dose that escapes the first-pass elimination in the GI wall (primarily in intestinal epithelium), liver, and lung, respectively. However, when determining F referred to the systemic exposure (represented by AUC) after intravenous administration, F_L is canceled out because intravenously administered drug also has to first-pass through the lung before reaching the systemic circulation[15]. Therefore, F is only a function of the F_{abs}, F_G, and F_H as shown in the following equation:

$$F = F_{abs} \times F_G \times F_H = F_{abs} \times (1 - E_G) \times (1 - E_H) \quad (2)$$

where E_G and E_H are the GI and hepatic first-pass extraction ratio, respectively. The concept of the F based on Equation (2) is depicted in Figure 1 [16].

Therefore, predicting intestinal absorption (F_{abs}) and first-pass elimination (F_G and F_H) from *in vitro* data may facilitate the selection of more orally bioavailable drug candidates in earlier stages of drug discovery and development[6,17,18]. Here, we review the principles and applications of conventional *in vitro–in vivo* extrapolation (IVIVE) methods to predict human intestinal absorption and first-pass elimination of drugs.

IVIVE for predicting Fabs in human

Determinants of F_{abs}

The major determinants of F_{abs} are drug stability in GI fluids, time available for absorption, solubility/dissolution in GI fluids as well as permeability/permeation through the GI epithelial membrane[6,19,20]. Stability can readily be determined by assessing the degradation of compounds in the human gastric fluids, simulated gastric fluids (SGF; 0.2% NaCl, 1% pepsin, 0.7% HCl with pH 1.2), and/or simulated intestinal fluids (SIF; 0.685% KH_2PO_4, 1% pancreatin, 1% KOH with pH 7.4)[21–23]. The time available for

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Section/Category: Pharmacology, Pharmacokinetics, Pharmacodynamics, Biosimilars, Bioequivalence

Keywords: valsartan; pharmacokinetic; bioequivalence; wet granulation; Korean male volunteer

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Abstract: Purpose: The aim of this study is to assess the quality of Valsa V® tablet (valsartan 160 mg, test formulation, wet granulation) by comparing its pharmacokinetic parameters with Diovan® tablet (valsartan 160 mg, reference formulation, dry granulation) in healthy Korean male volunteers.Method: This study was performed under fasted condition with a randomized, single-dose, 2-period crossover design. Sixty healthy Korean male volunteers were randomly divided into two groups. Each subject received a single dose of the test and reference formulations orally under fasted condition with washout period of 7 days between the administrations. Blood samples were collected up to 24 hours after dosing, and pharmacokinetic parameters were determined after analyzing plasma concentration of valsartan by using UPLC-MS-MS. The dissolution studies of both formulations were conducted using USP apparatus 2 at 50 rpm with 1000 mL of phosphate buffer solution (pH 6.8) at 37±0.5°C. Finding: Age 21-31 years old (mean±SD: 23.6±2.4), height 161-190 cm (173.7±6.6), weight 54-85 kg (68.0±8.7) of sixty healthy Korean male volunteers were enrolled in this study. The mean AUC0-∞ of test tablet and reference tablet was 31784±13844 ng·h/mL and 32714±14512 ng·h/mL, respectively; the mean Cmax was 5094±2061 and 5064±1864 ng/mL, respectively; the mean Tmax was 2.92±1.04 and 3.08±1.01 hours, respectively. The 90% confidence intervals (CIs) for geometric mean ratios of test to reference formulation of AUC0-t and Cmax were 0.9295-1.0546 and 0.9190-1.0848, respectively and were met the KFDA regulatory criteria for bioequivalence (0.8 to 1.25). Implications: The results of this study in healthy Korean male volunteers showed that the test and reference formulations of 160-mg valsartan met the KFDA regulatory criteria for bioequivalence despite its difference in formulation (wet granulation vs. drug granulation). Both formulations were well tolerated, with no serious adverse events reported.

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Abstract: We have previously reported that the limited intestinal absorption via the paracellular pathway may be the primary cause of the low oral bioavailability of doxorubicin (DOX). In this study, we have formulated medium chain glycerides-based colloidal nanosystems to enhance the intestinal paracellular absorption of DOX and reduce its cardiotoxicity. The DOX formulations prepared by the construction of pseudo-ternary phase diagram were characterized in terms of their droplet size distribution, viscosity, drug loading, and drug release. Further evaluation was conducted by an in vitro Caco-2 transport study as well as in situ/in vivo intestinal absorption, bioavailability and toxicity studies. Compared with DOX solution, these formulations enhanced the absorptive transport of DOX across Caco-2 cell monolayers at least partly due to the paracellular-enhancing effects of their lipidic components. Moreover, the in situ intestinal absorption and in vivo oral bioavailability of DOX in rats were markedly enhanced. In addition, no discernible damage was observed in the rat jejunum after oral administration of these DOX formulations while the cardiac toxicity was significantly reduced when compared with intravenous DOX solution. Taken together, the medium chain glycerides-based colloidal nanosystems prepared in this study represents a potentially effective oral delivery system for DOX.