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Biowaiver Extension Potential and IVIVC for BCS Class II Drugs by Formulation Design: Case Study for Cyclosporine Self-microemulsifying Formulation

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The objective of this work was to suggest the biowaiver potential of biopharmaceutical classification system (BCS) Class II drugs in self-microemulsifying drug delivery systems (SMEDDS) which are known to increase the solubility, dissolution and oral absorption of water-insoluble drugs. Cyclosporine was selected as a representative BCS Class II drug. New generic candidate of cyclosporine SMEDDS (test) was applied for the study with brand SMEDDS (reference I) and cyclosporine self-emulsifying drug delivery systems (SEDDS, reference II). Solubility and dissolution of cyclosporine from SMEDDS were critically enhanced, which were the similar behaviors with BCS class I drug. The test showed the identical dissolution rate and the equivalent bioavailability (0.34, 0.42 and 0.68 of p values for $AUC_{0\rightarrow 24h}$, C_{max} and T_{max}, respectively) with the reference I. Based on the results, level A *in vitro-in vivo* correlation (IVIVC) was established from these two SMEDDS formulations. This study serves as a good example for speculating the biowaiver extension potential of BCS Class II drugs specifically in solubilizing formulation such as SMEDDS.

Key words: Biopharmaceutics classification system (BCS), Biowaiver, *In vitro-in vivo* correlation (IVIVC), Cyclosporine, Self-microemulsifying drug delivery systems (SMEDDS)

INTRODUCTION

Water insolubility of drug is the most challenging problem in pharmaceutics. A great number of drug candidates have been discarded in pre-clinical stage due to their improper solubility (Porter et al., 2007). Water insolubility of a drug also hinders an imperative reformulation of marketed drugs. Various technologies have been studied to solve this problem for the past decades. One of the most promising answers for this problem is the self-microemulsifying drug delivery system (SMEDDS) (Gershanik and Benita, 2000; Gursoy and Benita, 2004). SMEDDS is a pre-concentrate form of microemulsions composed of oils, surfactant and cosurfactant which spontaneously form microemulsions in the external water phase (Lawrence and Rees, 2000; Pouton, 2000; Liu et al., 2010). The potential of SMEDDS has been proven against oil-soluble and water-insoluble drugs such as cyclosporine (Trull et al., 1994), tacrolimus (Borhade et al., 2009), atorvastatin (Shen et al., 2005), silymarin (Wu et al., 2006), itraconazole (Woo et al., 2008), carvedilol (Wei et al., 2005), fenofibrate (Patel and Vavia, 2007), paclitaxel (Yang et al., 2004), ibuprofen (Araya et al., 2005) and simvastatin (Kang et al., 2004). Solubility, dissolution rate and oral bioavailability of these drugs were remarkably improved with the help of SMEDDS (Spernath and Aserin, 2006). This conspicuous improvement of solubility and bioavailability comes from the typical characteristics of microemulsion which is defined by the small droplet size (less than 100 nm) and low surface tension. Interestingly, most drugs which exhibit such an excellent feasibility for incorporation with SMEDDS are classified as BCS class II drugs with low solubility and high permeability. High permeability, which means

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high lipid solubility, seems to make these BCS class II drugs more likely candidate for SMEDDS.

US FDA allows biowaiver of *in vivo* bioavailability and bioequivalence studies for BCS class I drugs in immediate-release (IR) solid oral dosage forms. Currently extending biowaiver to other classes has been suggested extensively and is still a contentious issue (Yu et al., 2002; Cheng et al., 2004; Rinaki et al., 2004; Jantratid et al., 2006). Some of these arguments have been aroused from the emerging pharmaceutical technologies which can highly enhance drug solubility and permeability (Vicosa et al., 2009; Koga et al., 2010). SMEDDS is one of the most prominent technologies for enhancing solubility. So, it is worth of contemplating if SMEDDS could extend the biowaiver potential for BCS class II drugs. Cyclosporine is one of those typical drugs, categorized in BCS class II and formulated in SMEDDS. Sandimmune Neoral® is the first marked cyclosporine SMEDDS. And FDA recently approved five cyclosporine generics (Masri, 2003). The use of generic cyclosporine offers a reduced cost alternative with equal therapeutic efficacy and outcomes. The generic cyclosporine reduced the total cost of the immunosuppressive therapy almost by 40% (Masri, 2003). Although technical difficulties lie on development of cyclosporine SMEDDS, new generic cyclosporine is still on demand from the market. From technical point of view, currently available cyclosporine generics are formulated based on the SMEDDS technology. So, cyclosporine SMEDDS is a good example to answer the current questions for biowaiver extension potential through formulation design.

In this study, a new generic candidate of cyclosporine SMEDDS was introduced to solubility, dissolution and permeability studies to identify the pharmaceutical properties of cyclosporine could be altered by formulation design. Subsequently, the relative bioavailability of cyclosporine SMEDDS was estimated with reference SMEDDS against dogs to verify if the altered pharmaceutical properties (e.g., solubility, dissolution and permeability) also precisely reflect *in vivo* bioavailability. Additionally, *in vivo* and *in vitro* correlation (IVIVC) was estimated based on the US FDA guidance (Emami, 2006).

MATERIALS AND METHODS

Materials

New cyclosporine SMEDDS was employed for the test drug, based on the reported formula (Yang et al., 2006). The test SMEDDS, containing 100 mg of cyclosporine, was filled into empty soft gelatin capsules and heat-sealed. Sandimmune Neoral 100 mg soft gelatin capsule (Novartis Co.) was used for reference SMEDDS (reference I) and purchased in the market. Cyclosporine self-emulsifying formulation (self-emulsifying drug delivery system: SEDDS), which forms macro-emulsions with mean droplet size of more than 2 μm, was selected as another reference (reference II) and fabricated following reported composition (Yang, 2006). ^{3}H-cyclosporine (specific activity; 8 Ci/mmol) was obtained from Amersham Pharmacia Biotech for the cell permeability study.

Solubility of cyclosporine in microemulsions

Solubility of cyclosporine was analyzed. Cyclosporine formulations (test, reference I and reference II), containing 100 mg of cyclosporine, were dropped into 100 mL of pH 1.2, 4.8 and 6.8 buffers at 37°C, vigorously shaken for 24 h. The medium was centrifuged at 1,500 rpm for 10 min. And the upper layer was taken out and analyzed by HPLC (Alliance 2610, Waters) using a capcellpak C_8 column (UG120, Shiseido) at 70°C. The mobile phase, consisted of 65% (v/v) acetonitrile, 15% (v/v) methanol and 20% (v/v) phosphate buffer, was delivered at rate of 1 mL/min and monitored at 210 nm.

Dissolution test

The dissolution test was carried out on cyclosporine formulations in soft capsules, following US FDA BCS guidance (Lobenberg and Amidon, 2000). Test was conducted using the dissolution apparatus II of the USP 24 at 50 rpm under 37°C in 900 mL of each of the following dissolution media: simulated gastric fluid USP without enzymes (pH 1.2), pH 4.5 phosphate buffer and simulated intestinal fluid USP without enzymes (pH 6.8). 5 mL of aliquot was withdrawn for analysis from the gastric juice at scheduled time points, ultra-filtered (0.45 μm) at 2500 rpm and analyzed by HPLC. The same volume of fresh medium was added to the juice after the sampling.

Permeability of cyclosporine on MDCK cell

The absorptive permeability of cyclosporine was estimated by using Madin-Darby canine kidney (MDCK) cell monolayer. Cyclosporine SMEDDS (test and reference I), cyclosporine SEDDS (reference II) and cyclosporine were subjected to the estimation. MDCK cells were seeded on porous (0.4 μm) polycarbonate bottom-layered cup (Transwell #3412, Corning Inc.) at a density of 2.5×10^5 cells/cm^2 and incubated over 3 days. Before the experiment, cell monolayer was washed with pH 7.4 Hank's balanced salts solution (containing 15 mM D(+)glucose, 10 mM HEPES), and then equilibrated for 30 min at 37°C in 95% humidity. Transepi-

thelial electrical resistance (TEER $\Omega \cdot cm^2$) of each monolayer was measured at 37°C using an epitherial votohammeter (World Precision). MDCK monolayer with TEER values <90 $\Omega \cdot cm^2$ were not used for the study (Irvine et al., 1999). Permeability assay was conducted using 0.5 mL of apical (AP) donor solution and 1.5 mL of basolateral (BL) acceptor solution. Donor solution was prepared as followings. Cyclosporine formulations were mixed with ^{3}H-labeled cyclosporine and dispersed in transport medium and then shaken for 30 min to get cyclosporine microemulsions. The resulting solution at the concentration of 10 μM of cyclosporine with 2 μCi of ^{3}H-labeled cyclosporine was placed on the apical side of the transwell plate under incubation at room temperature. Aliquots (200 μL) of medium in the acceptor compartment were taken at 0.5, 1.0, 1.5, and 2.0 h. The same amount of fresh medium was added to the acceptor compartment after sampling. Radioactivity of the samples was determined by a liquid scintillation counter (LSC, System 1400, Wallac). Permeability (P_{app}) of cyclosporine was calculated by dividing the permeation velocity of cyclosporine by the initial concentration of cyclosporine in the donor compartment.

Oral bioavailability study

The oral bioavailability of cyclosporine formulations (test, reference I and reference II) was estimated against dogs. Animal study was performed in accordance with of the Ethical Standards of the International Association for the Study of Pain and based on the animal protocols.

Six male dogs, weighing 12.0~15.0 kg, were subjected for the each formulations. Dogs were fasted for 12 h before dosing and additional 4 h after dosing. 100 mg of cyclosporine in formulation was orally administered via the pharynx. 30 mL of water was administered immediately using a syringe. The whole blood samples (3 mL) were obtained from the cephalic vein into plastic tubes containing EDTA before dosing and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8 and 24 h after dosing. Samples were frozen under −20°C until analysis. The whole blood concentrations of cyclosporine were measured by radioimmunoassay using Cyclo-Trac SP® RIA kit (Diasorin).

Data analysis

Whole blood concentration-time data of cyclosporine were analyzed by conventional non-compartmental method using WinNonlin program (Pharsight Corp.). The estimated pharmacokinetic parameters were statistically compared using the unpaired student's t-test. *P* values less than 0.05 were interpreted as significant. All parameters are expressed as mean ± S.D.

RESULTS AND DISCUSSION

Solubility of cyclosporine in microemulsions

Microemulsions have been regarded as a transparent "one-phase solution" rather than a two-phase mixture because of complete miscibility of oil and water (Kahlweit, 1988). The solubility of cyclosporine in microemulsions was observed based on the same assumption.

Cyclosporine is relatively insoluble with 6 μg/mL of aqueous solubility at 37°C (Ismailos et al., 1991). In this study cyclosporine was solubilized up to 0.91 ± 0.08 and 0.93 ± 0.13 mg/mL in microemulsion, which was acquired from test and reference I, respectively. No statistical difference was observed in solubility at different pH. We could not determine the solubility of cyclosporine from the reference II because it formed a heterogeneous emulsion which caused a large variation of the data.

In the BCS system, the drug substance is considered "highly soluble" when the highest strength of drug is soluble in 250 mL or less of aqueous media over the pH range of 1.0 to 7.5. Our solubility data suggests cyclosporine may be considered as a "highly soluble drug" in microemulsion system.

Absorptive permeability of cyclosporine SMEDDS in MDCK cells

TEER values of the MDCK monolayer did not change significantly after application of these formulations, suggesting that the integrity of the monolayer was maintained during the test. The absorptive P_{app} values (nm /sec) of cyclosporine were 3.84 ± 0.36, 3.68 ± 0.72 and 2.83 ± 0.78 for test, reference I and II, respectively. No significant difference ($p > 0.05$) was detected between the test and reference I. However, the P_{app} (nm/sec) of cyclosporine in these formulations decreased compared to that of cyclosporine by itself (7.3 ± 1.3). P_{app} value of 3.84 is similar to the reported P_{app} value of furosemide which is typically low permeable drug (Irvine et al., 1999; Taub et al., 2002). The absorptive permeability of cyclosporine in the MDCK cell monolayer did not reflect the enhanced solubility. Similar results have been reported in previous works showing a decreased P_{app} of drugs when the drugs were dissolved in solubilizing systems such as cyclodextrin (Taub et al., 2002), microemulsion (Spernath et al., 2007) or micelles (Chiu et al., 2003). Taub et al. reported P_{app} for mefenamic acid decreased after formulating with hydroxypropyl-bbb-cyclodextrin, although the solubility of mefenamic acid was increased by five fold in the formulation (Taub

et al., 2002). Spernath et al. also reported a decreased P_{app} for diclofenac in microemulsions (Spernath et al., 2007). Chiu et al. proved that the P_{app} of cyclosporine in caco-2 cell lines decreased in micelle formulation (Chiu et al., 2003). They explained that micellar solubilization of cyclosporine decreased the fraction of "free" unbound cyclosporine that would be available for transport across the monolayer causing the decrease in P_{app}. On the other hand, they proved that cyclosporine has much higher intrinsic human jejunal permeability. The P_{app} of cyclosporine in the caco-2 cell monolayer might not reflect the exact situation in the gastro-intestinal tract.

Many other factors should be considered to precisely explain the decreased P_{app} of cyclosporine in microemulsions. Cyclosporine is a typical substrate of p-glycoprotein (Chiu et al., 2003). Some ingredients, especially surfactants, interfere with the function of p-glycoprotein and the integrity of the cell monolayer (Kabanov et al., 2003). But these apprehensions are possibly dismissed for the following reasons in this study. One is that both formulations (test and reference I) showed the equivalent P_{app} in MDCK monolayer. This equivalent P_{app} suggests both formulations will probably show the equivalent permeability in the gastro-intestinal membrane as well. Secondly, the previous studies showed that an oral absorption of cyclosporine increased by 2 fold with the help of microemulsions (Trull et al., 1994). This enhanced absorption also suggests that other factors such as the dissolution rate are much more reflective in explaining oral absorption of cyclosporine rather than the cellular permeability (Chiu et al., 2003). US FDA also recommends to observe the rate of mass transfer across human intestinal membrane to get more reasonable P_{app} (Chiu et al., 2003).

Dissolution of cyclosporine from SMEDDS

According to BCS guidance a drug product is considered to be rapidly dissolving when 85% or more of the labeled amount of the drug substance dissolves within 30 min using an USP apparatus I at 100 rpm (or apparatus II at 50 rpm) with a volume of 900 mL or less in each of the following media: (a) 0.1 N HCl or simulated gastric fluid USP without enzymes; (b) a pH 4.5 buffer; and (c) a pH 6.8 buffer or simulated intestinal fluid USP without enzymes.

But the current dissolution methods that are described in the BCS guidelines are irrelevant to estimate the real dissolution of class II drugs in the gastro-intestinal tract (Yu et al., 2002). A more relevant *in vitro* dissolution method is required to precisely predict the oral absorption of class II drugs. US FDA allows the addition of surfactants or solvents to the dissolution medium to mimic the *in vivo* situation for water-insoluble drugs like class II drugs (Yu et al., 2002). However, this method is still under question about its reflective power on *in vivo* dissolution. Likewise, the proper dissolution test for SMEDDS has not been established yet. In this study a centrifugal ultrafiltration method was applied to estimate the dissolved amount of cyclosporine (Chen et al., 2008).

The cumulative percentage of the dissolved cyclosporine is plotted as a function of time in Fig. 1. pH of the medium did not affect the dissolution of cyclosporine from SMEDDS (Fig. 1A). Typically dissolution of cyclosporine from SMEDDS was reproducible (< 10% variability at all time points measured) and rapidly exceeded 85% of dissolution rate within 30 min. The dissolution profiles between the test and reference I were nearly identical when analyzed according to a simple model independent approach (Moore and Flanner 1996), i.e. the difference factor, f_1, was 7.3~12.6 except that at 10 min (18.1) and the other similarity factor, f_2 was 53.4, thus satisfying the criteria for identical dissolution characteristics (i.e. f_1 <15 and f_2 > 50, respectively). The dissolution of cyclosporine from SEDDS was much lower than those from SMEDDS, which showed only 30% of dissolved cyclosporine at 60 min (Fig. 1B).

Relative oral bioavailability of cyclosporine SMEDDS

Fig. 2 shows the whole blood concentration-time profiles of cyclosporine after single oral administration of cyclosporine formulations. The whole blood cyclosporine concentration-time profiles of the test and the reference I are almost identical each other. The mean pharmacokinetic parameters after the oral administration of the test were: $AUC_{0\rightarrow 24h}$ = 7203.1 ± 1297.0 ng·h/mL, C_{max} = 1189.0 ± 132.9 ng/mL, T_{max} = 1.3 ± 0.3 and $T_{1/2}$ = 5.2 ± 1.2 h, as shown in Table I. The relative bioavailability ($AUC_{0\rightarrow 24h}$) and C_{max} of the test were 91.9% and 95.8%, respectively. No significant differences were detected in pharmacokinetic parameters between two formulations ($p > 0.05$). The oral absorption of the reference II (cyclosporine SEDDS) was approximately 40% of that from SMEDDS with a delayed T_{max} (2.3 h).

In vitro and *in vivo* correlation

Recently practical application of IVIVC for optimization of drug formulation has been suggested in the pharmaceutical field (Emami, 2006). IVIVC also serves as another criterion of biowaiver extension. In this study, we estimated IVIVC based on the correlation between the fraction of cyclosporine absorbed and the

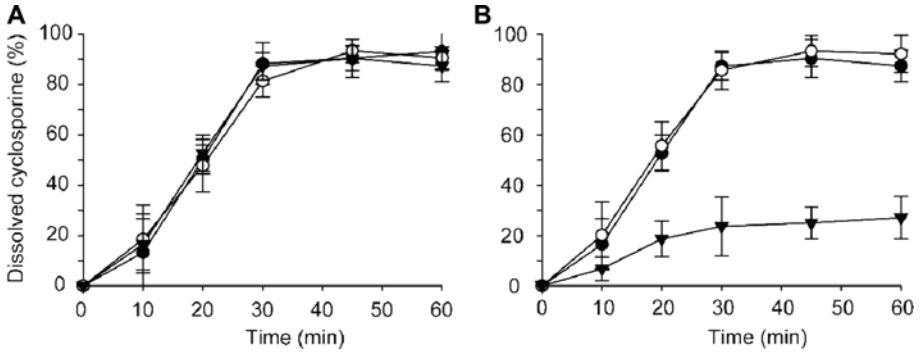

Fig. 1. (**A**) Dissolution of cyclosporine from test SMEDDS formula at pH 1.2 (●), pH 4.5 (○) and pH 6.8 (▼) dissolution medium, (**B**) dissolution of cyclosporine from the test (●), the reference I (○) and the reference II (▼) at pH 6.8 dissolution medium.

Table I. Pharmacokinetic parameters of cyclosporine after single oral administration of test, reference I and reference II against dogs (n = 6)

	SMEDDS		SEDDS	*P* value[a]
	Test	Reference I	Reference II	
$AUC_{0\to24h}$ (ng·h/mL)	7203.1 ± 1297.0	7833.6 ± 874.5	3123.6 ± 366.3	0.34
$AUC_{0\to\infty}$ (ng·h/mL)	7901.6 ± 1525.6	8683.4 ± 1045.7	3345.4 ± 407.3	0.32
C_{max} (ng/mL)	1189.0 ± 132.9	1241.1 ± 69.4	661.7 ± 140.1	0.42
T_{max} (h)	1.3 ± 0.3	1.3 ± 0.4	2.3 ± 1.0	0.68
$T_{1/2}$ (h)	5.2 ± 1.2	7.2 ± 0.7	7.6 ± 0.6	0.35

[a]*P* value was acquired from unpaired t-test between test and reference I.

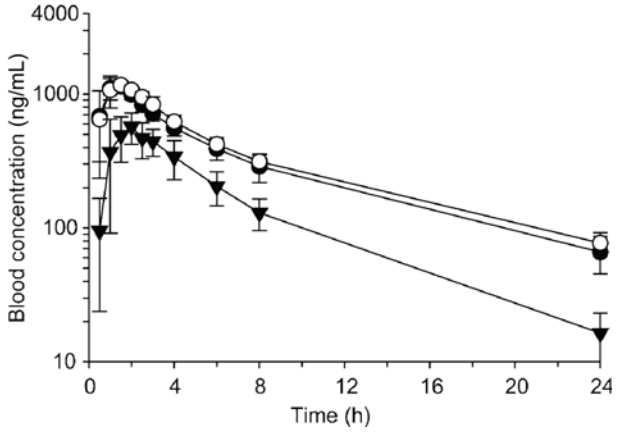

Fig. 2. Whole blood concentration-time profiles of cyclosporine after single oral administration of the test (●), the reference I (○) and the reference II (▼) against dogs at a dose of 100 mg (n=6).

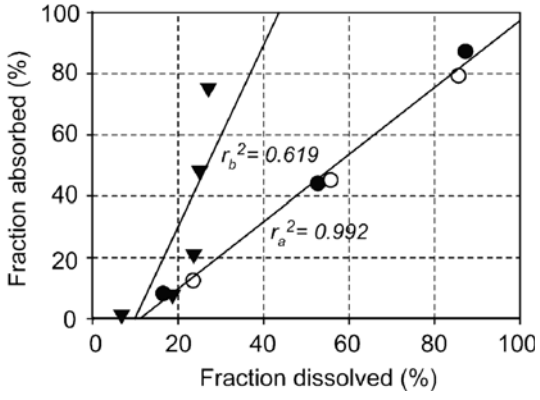

Fig. 3. Relationship between *in vitro* the fraction dissolved and *in vivo* the fraction absorbed; r_a^2 (correlation factor) was acquired from the pooled data of the test (●) and the reference I (○), and r_b^2 was acquired from the data of the reference II (▼) alone.

fraction of cyclosporine dissolved. The fraction of drug dissolved was determined from the dissolution data. The fraction of drug absorbed was determined using the Wagner-Nelson method from the cyclosporine concentration *vs* time data (Emami, 2006) and approximated again by the Hill equation using the non-linear least square regression program (WinNonlin, Pharsight Corp.). As shown in Fig. 3, level A correlation was observed between the fractions of drug dissolved and the fraction of drug absorbed for the pooled data of the test and reference I ($Y = 1.094 \times X - 12.473$; $r_a^2 = 0.992$). Level A correlation indicates *in vitro* dissolution of SMEDDS highly reflects *in vivo* absorption. This also suggests the possibility of biowaiver extension potential for this drug formulation. However, for the reference II (cyclosporine SEDDS), correlation fac-

tor (r_b^2) was 0.619 and too low to tell if a good relationship existed. This finding suggests that formulation based biowaiver extension potential for BCS class II drug is realistic and SMEDDS is the sufficient formulation for supporting this extension potential.

Biowaiver extension potential of class II drugs in SMEDDS

US FDA allows the claim for biowaiver if drug substances satisfy the following conditions: 1) a drug with high solubility; 2) a drug with high permeability; and 3) a drug substance in IR solid oral dosage forms that exhibit rapid *in vitro* dissolution (no less than 85% of dissolution within 30 min) (Emami, 2006).

The data shown in this study support that SMEDDS alters the pharmaceutical properties of cyclosporine which is pretty similar to those of BCS Class I drug. The test formulation, which exhibited an equivalent dissolution with the reference I, showed the identical oral absorption compared to the reference I. Additionally, highly reliable correlation (level A) between *in vitro* dissolution and *in vivo* absorption was detected in both cyclosporine SMEDDS formulations. These results highly support the rationale for biowaiver extension potential to cyclosporine SMEDDS.

US FDA sets another following conditions for biowaivers: 1) the drug must be stable in the gastrointestinal tract; 2) excipients used in the IR solid oral-dosage forms have no significant effect on the rate and extent of oral drug absorption; 3) the drug must not have a narrow therapeutic index; and 4) the product is designed not to be absorbed in the oral cavity (Emami 2006).

Until now, biowaiver could be justified only for solid oral-dosage forms which are eligible for the traditional dissolution test. Unfortunately, SMEDDS is liquid based dosage form in soft gelatin capsules. However recent several studies reported a significant linear relationship (level A) in IVIVC for the drugs in soft gelatin capsules such as ritonavir (Rossi et al., 2007), lopinavir (Donato et al., 2008) and arundic acid (Nishimura et al., 2007). These drugs also belong to BCS class II. These studies indicated liquid based soft gelatin capsules are also eligible for the biowaiver extension with level A correlation in IVIVC.

US FDA recommends not to use excipients that affect the rate or extent of absorption of drugs for the biowaiver of class I drugs. But BCS class II drugs require specific ingredients, usually surfactants, in their formulation to guarantee the reliable and reproducible dissolution and bioavailability. For the formulation of cyclosporine SMEDDS, surfactants also play key roles in achieving the steady absorption of cyclosporine. Microemulsions dissolve the whole dose of cyclosporine without additional endogenous surfactants, such as bile acids in the gastrointestinal tract. The gastrointestinal absorption of cyclosporine from SMEDDS is unaffected by bile juice secretion and food intake in this reason (Trull et al., 1994; Yang et al., 2006). Surfactants act as an absorption enhancer in this formulation. The inevitable incorporation of surfactants in SMEDDS needs to be reviewed in these respects.

Narrow therapeutic index (NTI) is another limitation for cyclosporine SMEDDS to claim biowaiver. But several studies already showed biowaiver potential of drugs with NTI. Carbamazepine, which belongs to BCS class II and NTI drugs, was reported to have a considerable biowaiver potential with level A correlation in IVIVC studies (Lake et al., 1999; Kovacevic et al., 2009). Kovacevic et al. mentioned "A risk associated with a therapy failure and occurrence of dose-dependent adverse drug reactions in target patients should be evaluated additionally" for biowaiver extension of drugs with NTI (Kovacevic et al., 2009). So, biowaiver potential for cyclosporine SMEDDS may be possibly considered in the same way.

Nowadays, many other drugs, especially BCS class II drugs such as silymarin, ibuprofen, saquinavir and ritonavir etc, are on the market in SMEDDS (Gursoy and Benita, 2004; Woo et al., 2007) and the number of SMEDDS products is increasing. Many factors still remain as a limitation for marketing authorization of these drugs with only *in vitro* studies. However our study showed a rational for biowaiver potential of BCS class II drugs in SMEDDS formulation and this issue could be explored more extensively in the future.

CONCLUSION

In this study, cyclosporine SMEDDS showed an enhanced solubility, an increased dissolution rate and an identical oral absorption with prior-marketed SMEDDS product. Cyclosporine SMEDDS satisfied biowaiver conditions and level A IVIVC. These results provide insight for the biowaiver extension potential of BCS class II drugs by SMEDDS formulation.

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REFERENCES

Araya, H., Tomita, M., and Hayashi, M., The novel formulation design of O/W microemulsion for improving the gastrointestinal absorption of poorly water soluble compounds. *Int. J. Pharm*., 305, 61-74 (2005).

Borhade, V. B., Nair, H. A., and Hegde, D. D., Development and characterization of self-microemulsifying drug delivery system of tacrolimus for intravenous administration. *Drug Dev. Ind. Pharm*., 35, 619-630 (2009).

Chen, Y., Li, G., Wu, X., Chen, Z., Hang, J., Qin, B., Chen, S., and Wang, R., Self-microemulsifying drug delivery system (SMEDDS) of vinpocetine: formulation development and in vivo assessment. *Biol. Pharm. Bull.*, 31, 118-125 (2008).

Cheng, C. L., Yu, L. X., Lee, H. L., Yang, C. Y., Lue, C. S., and Chou, C. H., Biowaiver extension potential to BCS Class III high solubility-low permeability drugs: bridging evidence for metformin immediate-release tablet. *Eur. J. Pharm. Sci*., 22, 297-304 (2004).

Chiu, Y. Y., Higaki, K., Neudeck, B. L., Barnett, J. L., Welage, L. S., and Amidon, G. L., Human jejunal permeability of cyclosporin A: influence of surfactants on P-glycoprotein efflux in Caco-2 cells. *Pharm. Res*., 20, 749-756 (2003).

Donato, E. M., Martins, L. A., Froehlich, P. E., and Bergold, A. M., Development and validation of dissolution test for lopinavir, a poorly water-soluble drug, in soft gel capsules, based on in vivo data. *J. Pharm. Biomed. Anal.*, 47, 547-552 (2008).

Emami, J., In vitro - in vivo correlation: from theory to applications. *J. Pharm. Pharm. Sci*., 9, 169-189 (2006).

Gershanik, T. and Benita, S., Self-dispersing lipid formulations for improving oral absorption of lipophilic drugs. *Eur. J. Pharm. Biopharm*., 50, 179-188 (2000).

Gursoy, R. N. and Benita, S., Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs. *Biomed. Pharmacother.*, 58, 173-182 (2004).

Irvine, J. D., Takahashi, L., Lockhart, K., Cheong, J., Tolan, J. W., Selick, H. E., and Grove, J. R., MDCK (Madin-Darby canine kidney) cells: A tool for membrane permeability screening. *J. Pharm. Sci.*, 88, 28-33 (1999).

Ismailos, G., Reppas, C., Dressman, J. B., and Macheras, P., Unusual solubility behaviour of cyclosporin A in aqueous media. *J. Pharm. Pharmacol*., 43, 287-289 (1991).

Jantratid, E., Prakongpan, S., Amidon, G. L., and Dressman, J. B., Feasibility of biowaiver extension to biopharmaceutics classification system class III drug products: cimetidine. *Clin. Pharmacokinet*., 45, 385-399 (2006).

Kabanov, A. V., Batrakova, E. V., and Miller, D. W., Pluronic block copolymers as modulators of drug efflux transporter activity in the blood-brain barrier. *Adv. Drug Deliv. Rev*., 55, 151-164 (2003).

Kahlweit, M., Microemulsions. *Science*, 240, 617-621 (1988).

Kang, B. K., Lee, J. S., Chon, S. K., Jeong, S. Y., Yuk, S. H., Khang, G., Lee, H. B., and Cho, S. H., Development of self-microemulsifying drug delivery systems (SMEDDS) for oral bioavailability enhancement of simvastatin in beagle dogs. *Int. J. Pharm*., 274, 65-73 (2004).

Koga, K., Takarada, N., and Takada, K., Nano-sized water-in-oil-in-water emulsion enhances intestinal absorption of calcein, a high solubility and low permeability compound. *Eur. J. Pharm. Biopharm*., 74, 223-232 (2010).

Kovacevic, I., Parojcic, J., Homsek, I., Tubic-Grozdanis, M., and Langguth, P., Justification of biowaiver for carbamazepine, a low soluble high permeable compound, in solid dosage forms based on IVIVC and gastrointestinal simulation. *Mol. Pharm*., 6, 40-47 (2009).

Lake, O. A., Olling, M., and Barends, D. M., In vitro/in vivo correlations of dissolution data of carbamazepine immediate release tablets with pharmacokinetic data obtained in healthy volunteers. *Eur. J. Pharm. Biopharm*., 48, 13-19 (1999).

Lawrence, M. J. and Rees, G. D., Microemulsion-based media as novel drug delivery systems. *Adv. Drug Deliv. Rev*., 45, 89-121 (2000).

Liu, Y., Chen, Z. Q., Zhang, X., Feng, N. P., Zhao, J. H., Wu, S., and Tan, R., An improved formulation screening and optimization method applied to the development of a self-microemulsifying drug delivery system. *Chem. Pharm. Bull.*, 58, 16-22 (2010).

Lobenberg, R. and Amidon, G. L., Modern bioavailability, bioequivalence and biopharmaceutics classifcation system. New scientific approaches to international regulatory standards. *Eur. J. Pharm. Biopharm*., 50, 3-12 (2000).

Masri, M., The generics in transplantation and the rules on their use. *Exp. Clin. Transplant*., 1, 65-68 (2003).

Moore, J. W. and Flanner, H. H., Mathematical comparison of dissolution profiles. *Pharm. Technol.*, 20, 64-74 (1996).

Nishimura, H., Hayashi, C., Aiba, T., Okamoto, I., Miyamoto, Y., Nakade, S., Takeda, K., and Kurosaki, Y., Application of the correlation of in vitro dissolution behavior and in vivo plasma concentration profile (IVIVC) for soft-gel capsules-a pointless pursuit? *Biol. Pharm. Bull.*, 30, 2221-2225 (2007).

Patel, A. R. and Vavia, P. R., Preparation and in vivo evaluation of SMEDDS (self-microemulsifying drug delivery system) containing fenofibrate. *AAPS J.*, 9, E344-E352 (2007).

Porter, C. J., Trevaskis, N. L., and Charman, W. N., Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs. *Nat. Rev. Drug Discov*., 6, 231-248 (2007).

Pouton, C. W., Lipid formulations for oral administration of drugs: non-emulsifying, self-emulsifying and 'self-microemulsifying' drug delivery systems. *Eur. J. Pharm. Sci*., 11 Suppl 2, S93-S98 (2000).

Rinaki, E., Dokoumetzidis, A., Valsami, G., and Macheras, P., Identification of biowaivers among Class II drugs: theoretical justification and practical examples. *Pharm. Res*., 21, 1567-1572 (2004).

Rossi, R. C., Dias, C. L., Donato, E. M., Martins, L. A., Bergold, A. M., and Froehlich, P. E., Development and validation of

dissolution test for ritonavir soft gelatin capsules based on in vivo data. *Int. J. Pharm*., 338, 119-124 (2007).

Shen, H. R., Li, Z. D., and Zhong, M. K., [Preparation and evaluation of self-microemulsifying drug delivery systems containing atorvastatin]. *Yao Xue Xue Bao*, 40, 982-987 (2005).

Spernath, A. and Aserin, A., Microemulsions as carriers for drugs and nutraceuticals. *Adv. Colloid Interface Sci.*, 128-130, 47-64 (2006).

Spernath, A., Aserin, A., Ziserman, L., Danino, D., and Garti, N., Phosphatidylcholine embedded microemulsions: physical properties and improved Caco-2 cell permeability. *J. Control. Release.*, 119, 279-290 (2007).

Taub, M. E., Kristensen, L., and Frokjaer, S., Optimized conditions for MDCK permeability and turbidimetric solubility studies using compounds representative of BCS classes I-IV. *Eur. J. Pharm. Sci.*, 15, 331-340 (2002).

Trull, A. K., Tan, K. K., Tan, L., Alexander, G. J., and Jamieson, N. V., Enhanced absorption of new oral cyclosporin microemulsion formulation, Neoral, in liver transplant recipients with external biliary diversion. *Transplant. Proc.*, 26, 2977-2978 (1994).

Vicosa, A. L., Chatah, E. N., Santos, T. C., Jones, L. F., Jr., Dantas, C. B., Dornelas, C. B., Rodrigues, C. R., Castro, H. C., Sousa, V. P., Dias, L. R., and Cabral, L. M., Bioequivalence studies and sugar-based excipients effects on the properties of new generic ketoconazole tablets formulations and stability evaluation by using direct compression method. *Pharm. Dev. Technol.*, 14, 530-539 (2009).

Wei, L., Sun, P., Nie, S., and Pan, W., Preparation and evaluation of SEDDS and SMEDDS containing carvedilol. *Drug Dev. Ind. Pharm.*, 31, 785-794 (2005).

Woo, J. S., Kim, T. S., Park, J. H., and Chi, S. C., Formulation and biopharmaceutical evaluation of silymarin using SMEDDS. *Arch. Pharm. Res.*, 30, 82-89 (2007).

Woo, J. S., Song, Y. K., Hong, J. Y., Lim, S. J., and Kim, C. K., Reduced food-effect and enhanced bioavailability of a self-microemulsifying formulation of itraconazole in healthy volunteers. *Eur. J. Pharm. Sci.*, 33, 159-165 (2008).

Wu, W., Wang, Y., and Que, L., Enhanced bioavailability of silymarin by self-microemulsifying drug delivery system. *Eur. J. Pharm. Biopharm*., 63, 288-294 (2006).

Yang, S., Gursoy, R. N., Lambert, G., and Benita, S., Enhanced oral absorption of paclitaxel in a novel self-microemulsifying drug delivery system with or without concomitant use of P-glycoprotein inhibitors. *Pharm. Res*., 21, 261-270 (2004).

Yang, S. G., Novel Nano Delivery System of Cyclosporin A with Enhanced Stability. Seoul National University, Seoul, (2006).

Yang, S. G., Kim, D. D., Chung, S. J., and Shim, C. K., Stable bioavailability of cyclosporin A, regardless of food intake, from soft gelatin capsules containing a new self-nanoemulsifying formulation. *Int. J. Clin. Pharmacol. Ther*., 44, 233-239 (2006).

Yu, L. X., Amidon, G. L., Polli, J. E., Zhao, H., Mehta, M. U., Conner, D. P., Shah, V. P., Lesko, L. J., Chen, M. L., Lee, V. H., and Hussain, A. S., Biopharmaceutics classification system: the scientific basis for biowaiver extensions. *Pharm. Res.*, 19, 921-925 (2002).