molecular pharmaceutics

ARTICLE

pubs.acs.org/molecularpharmaceutics

A Perspective for Biowaivers of Human Bioequivalence Studies on the Basis of the Combination of the Ratio of AUC to the Dose and the Biopharmaceutics Classification System

Shinji Sakuma,[*,†] Hidehisa Tachiki,[‡] Hitoshi Uchiyama,[‡] Yasunobu Fukui,[§] Naohiro Takeuchi,[§] Kazuo Kumamoto,[∥] Tomonori Satoh,[∥] Yoshinobu Yamamoto,[⊥] Emi Ishii,[⊥] Yoshiyuki Sakai,[#] Susumu Takeuchi,[○] Masaru Sugita,[○] and Shinji Yamashita[†]

[†]Faculty of Pharmaceutical Sciences, Setsunan University, 45-1, Nagaokatoge-cho, Hirakata, Osaka 573-0101, Japan

[‡]Research & Development Division, Towa Pharmaceutical Co., Ltd., 32-8, Kuwazai-shinmachi, Kadoma, Osaka 571-0043, Japan

[§]Research Center, Kyowa Pharmaceutical Industry Co., Ltd., 6-7-2, Yurinokidai, Sanda, Hyogo 669-1324, Japan

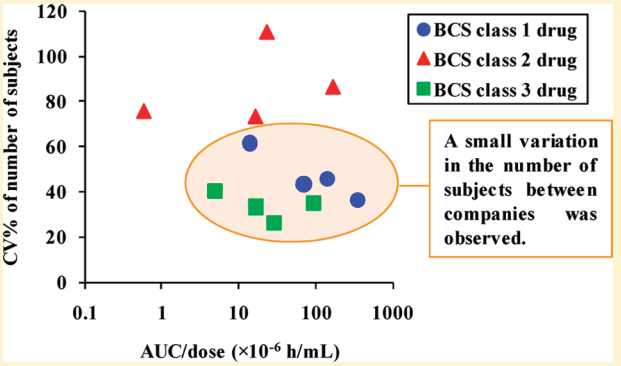
[∥]Pharmaceutical Development Center, Nichi-Iko Pharmaceutical Co., Ltd., 205-1 Shimo-umezawa, Namerikawa, Toyama 936-0857, Japan

[⊥]Drug Development Laboratory, Ohara Pharmaceutical Co., Ltd., 121-15, Toriino, Koka-cho, Koka, Shiga 520-3403, Japan

[#]Research & Development Division, Taiyo Pharmaceutical Industry Co., Ltd., 1-24-11, Taiko, Nakamura-ku, Nagoya, Aichi 453-0801, Japan

[○]Biological Research Department, Sawai Pharmaceutical Co., Ltd., 2-30, Miyahara 5-chome, Yodogawa-ku, Osaka 532-0003, Japan

ABSTRACT: The ratio of AUC to the dose (AUC/dose) was previously found as a parameter that predicts a risk of bioinequivalence of oral drug products. On the basis of the combination of this parameter and the biopharmaceutics classification system (BCS), a perspective for biowaivers of human bioequivalence studies is discussed. Databases of bioequivalence studies using immediate-release solid oral dosage forms were disclosed by 6 Japanese generic pharmaceutical companies, and the number of subjects required for demonstrating bioequivalence between generic and reference products was plotted as a function of AUC/dose for each BCS category. A small variation in the number of subjects was constantly observed in bioequivalence studies using dosage forms containing an identical BCS class 1 or class 3 drug, even though formulations of the generic product differ between companies. The variation was extremely enlarged when the drugs were substituted with BCS class 2 drugs. Rate-determining steps in oral absorption of highly water-soluble BCS class 1 and class 3 drugs are independent of formulations when there is no significant difference in the *in vitro* dissolution profiles between formulations. The small variation observed for both BCS categories indicates that the number of subjects converges into one value for each drug. Our analysis indicates the appropriateness of biowaiver of bioequivalence studies for immediate-release solid oral dosage forms containing not only BCS class 1 drugs but also class 3 drugs.



KEYWORDS: biopharmaceutics classification system (BCS), bioequivalence, biowaiver, immediate-release solid oral dosage forms, formulations

INTRODUCTION

Formulations of oral dosage forms often influence the pharmacokinetics of active pharmaceutical ingredients that are included in the dosage forms. Therefore, human bioequivalence (BE) studies are performed when pharmaceutical industries alter the formulations of their original products or develop generic products.[1] Pharmaceutical scientists tend to persist in the consistency of *in vitro* dissolution profiles between test products (new formulations) and reference products containing an identical drug (original formulations). However, the essence of bioequivalence is that *in vivo* drug dissolution from test products is consistent with the dissolution from reference products. This is because drugs are absorbed from the gastrointestinal (GI) tract according to their intrinsic absorbability after they are released from the dosage forms.[2] However, since direct assessment of *in vivo* dissolution profiles of test and reference products in the human GI tract is impossible,[3] plasma concentration−time profiles of drugs after oral administration of the products are observed in BE studies.

Received: December 8, 2010
Accepted: June 1, 2011
Revised: May 11, 2011
Published: June 01, 2011

Pharmacokinetic parameters such as the area under the plasma concentration−time curve (AUC), maximum drug concentration (C_{max}), and time to reach the maximum drug concentration (T_{max}) are used as a surrogate for bioequivalence from a regulatory point of view.[1,2] The test product is regarded as bioequivalent to the reference product when there is no significant difference in oral bioavailability between products in terms of the extent and rate, which correspond to AUC and C_{max}, respectively.[1,4,5] Recognizing that water solubility and intestinal membrane permeability of drugs are the fundamental parameters controlling the extent and rate of drug absorption,[6] Amidon and co-workers established the biopharmaceutics classification system (BCS).[7,8] Drugs are classified into 4 classes on the basis of their physicochemical properties. For instance, drugs with high solubility and high permeability, which are expected to be highly absorbed from the intestine, are classified into the class 1 of the BCS.

The World Health Organization (WHO) considers that 18−24 subjects are generally required to prove bioequivalence between test and reference products in standard BE studies.[9] However, due to high variation in AUC and C_{max}, pharmaceutical industries should sometimes conduct BE studies with more subjects than the WHO consideration. They face a high risk of finding bioinequivalence between products unless a required entry for the BE study is attained.[10] However, this process reduces cost performance. Impractical BE studies should be also avoided.

On the basis of the intrinsic properties of drugs, Yamashita and Tachiki analyzed risk factors that incur bioinequivalence of immediate-release solid oral dosage forms in BE studies.[2] Forty-four generic products were selected at random from databases of BE studies performed by Towa Pharmaceutical Co., Ltd. (Osaka, Japan) during the past 10 years; the sampled products were almost equally classified into 4 classes of the BCS. The number of subjects required for demonstrating bioequivalence between generic products and the corresponding reference products (brand) was predicted from the results of BE studies. Neither solubility nor permeability correlated well with the number of subjects, indicating that variation in drug absorption was dominated by other factors. They found that there was a well-regulated correlation between the number of subjects and the ratio of AUC to the dose (AUC/dose) when drugs were classified into either BCS class 1 (high solubility and high permeability) or class 3 (high solubility and low permeability). AUC/dose gave a clear criterion to distinguish drugs with a high risk of bioinequivalence. More than 24 subjects, which was defined as a practical upper limit of the number of subjects in standard BE studies on the basis of the WHO consideration, were required when AUC/dose was less than 18.0×10^{-6} h/mL (a threshold value). However, when drugs were classified into BCS class 2 (low solubility and high permeability) or class 4 (low solubility and low permeability), no regularity was observed, irrespective of AUC/dose.

The above-mentioned finding suggests that the combination of the BCS and AUC/dose enables us to predict difficulties in proving bioequivalence of immediate-release solid oral dosage forms containing BCS class 1 and class 3 drugs. On the basis of this combination, a perspective for biowaivers of BE studies, together with the validation of the appropriateness of AUC/dose as the risk parameter, was discussed in cooperation with 6 Japanese generic pharmaceutical companies.

EXPERIMENTAL SECTION

1. Drug Products. Kyowa Pharmaceutical Industry Co., Ltd. (Hyogo, Japan), Nichi-Iko Co., Ltd. (Toyama, Japan), Ohara Pharmaceutical Co., Ltd. (Shiga, Japan), Sawai Pharmaceutical Co., Ltd. (Osaka, Japan), Taiyo Pharmaceutical Industry Co., Ltd. (Aichi, Japan), and Towa Pharmaceutical Co., Ltd. (Osaka, Japan) have participated in this study. Based on 44 drugs used in the previous study,[2] each of the participants independently listed drugs with disclosure-possible databases. Among them, 11 drugs whose immediate-release solid oral dosage forms are being supplied in the Japanese market by at least half of the participating companies were consequently selected as model drugs. Terbinafine hydrochloride was added to compensate for the shortage of BCS class 1 drug. Since BCS class 4 is a minor category of oral drug products,[11] it was not addressed in this report.

2. Data Analysis. *2. 1. Human Intestinal Permeability.* Effective intestinal membrane permeability of drugs in humans (P_{eff}, $\times 10^{-4}$ cm/s) was calculated on the basis of physicochemical properties such as molecular weight and ClogP.[12] ADMET Predictor (version 3.0, Simulation Plus, Inc., Lancaster, CA) was used as an analytical software program. The P_{eff} value of metoprolol was used as the reference value of human intestinal permeability because 95% of orally administered metoprolol is absorbed from the GI tract.[13] The P_{eff} value of metoprolol (1.34×10^{-4} cm/s) was obtained from the online database provided by TSRL Inc. (Ann Arbor, MI).[14] Drugs whose P_{eff} values were higher than 1.34×10^{-4} cm/s were classified as high-permeability drugs. Residues were classified as low-permeability drugs.

2. 2. Dose Number. Dose number (Do) is defined as (Mo/Vo)/Cs, where Mo is the actual dose in human BE studies (mg), Vo is the volume of water which is concomitant with drug administration (150 mL, based on the Japanese guidelines for BE studies[4]), and Cs is the solubility of drugs in water (mg/mL).[7] Since the solubility of model drugs other than terbinafine hydrochloride has already been measured,[2] these values were used in this report. The solubility of terbinafine hydrochloride was measured by a standard method described in the Japanese Pharmacopoeia,[15] as done for 11 model drugs. The reference value of the dose number was 1. Drugs are completely dissolved in 150 mL of water unless calculated dose numbers exceed 1. The drugs whose dose numbers were less than 1 were classified as high-solubility drugs. Residues were classified as low-solubility drugs.

2. 3. BCS Class. The BCS classification was obtained from the online database provided by TSRL Inc.[14] The online database took precedence over both P_{eff} and dose number-based classifications when inconsistencies were observed.

2. 4. AUC/Dose. AUC from 0 to infinity (AUC_{0-inf}) was calculated by numerical integration using a linear trapezoidal formula and extrapolation to infinity based on a single-exponential equation. Values of AUC/dose ($\times 10^{-6}$ h/mL) were obtained by dividing AUC_{0-inf} values by the corresponding dose values, as previously reported.[2]

2. 5. The Number of Subjects. The number of subjects was predicted from the results of BE studies performed by the pharmaceutical industries. The results obtained from BE studies with a small number of subjects, which were previously performed with the aim of estimating the number of subjects to show bioequivalence, was used. Both AUC-based and C_{max}-based predictions were performed under a 90% confidence interval, and a larger number of subjects were used for the predicted value, as described in the Japanese guidelines for human BE studies.[4] Either BESTS (version 3.0.4 or 4.0.0, CAC Co., Tokyo, Japan) or PSAG-CP (version 4.3, Jihou Co. Ltd., Tokyo, Japan) was used as an analytical software program. Regarding the AUC-based

Table 1. Model Drugs Used in This Study

model drugs							pharmaceutical industries[b]					
active drugs	dosage forms	contents	dose in BE studies	P_{eff}, human ($\times 10^{-4}$ cm/s)	dose no.	BCS	A	B	C	D	E	F
amlodipine besilate	tablet	5 mg	5 mg	0.33	1.1×10^{-1}	3	*	*	*	*		*
cetilizine hydrochloride	tablet	10 mg	10 mg	2.01	1.0×10^{-4}	1	*	*	*	*	*	*
doxazosin mesilate	tablet	2 mg	2 mg	0.33	2.3×10^{-3}	1		*		*		*
epalrestat	tablet	50 mg	50 mg	1.73	2.3	2	*		*	*		*
imidapril hydrochloride	tablet	10 mg	10 mg	0.29	1.4×10^{-3}	3		*	*	*	*	*
levofloxacin	tablet	100 mg	100 mg	1.99	3.4×10^{-2}	3		*	*	*	*	*
pilsicainide hydrochloride hydrate	capsule	50 mg	50 mg	2.77	4.0×10^{-4}	1				*	*	*
pranlukast hydrate	dry syrup	10%	100/200 mg[a]	2.13	$1.2 \times 10^{3}/2.4 \times 10^{3}$	2	*		*	*	*	*
pravastatin	tablet	10 mg	10 mg	0.48	3.0×10^{-4}	3	*			*		*
risperidone	tablet	3 mg	3 mg	4.46	2.0	2			*	*	*	
simvastatin	tablet	5 mg	5 mg	3.34	3.5×10^{1}	2	*		*	*		*
terbinafine hydrochloride	tablet	125 mg	125 mg	3.84	1.1×10^{-1}	1		*		*		*

[a] BE studies were performed at a dose of either 100 mg or 200 mg as a pranlukast free form. [b] The name of the company was blinded. *Databases of BE studies were disclosed.

prediction, AUC from 0 to the final sampling point after oral administration of each generic product (AUC_{0-t}) was substituted for the AUC_{0-inf} used previously,[2] because only AUC_{0-t}-based prediction was performed in one-third of the participating companies. AUC_{0-t} was calculated by numerical integration using a linear trapezoidal formula. The final sampling point was determined on the basis of the Japanese guidelines so that AUC_{0-t} was more than 80% of AUC_{0-inf}.[4]

RESULTS AND DISCUSSION

1. Selected Model Drugs and Their Formulations. Table 1 lists the 12 model drugs used in this study. A total of 49 cases of BE studies performed by participating companies were disclosed; however, information on the individual companies that disclosed each database was blinded. All 49 disclosed formulations were immediate-release solid oral dosage forms, and after oral administration to fasted humans, the plasma concentration of parent drugs was monitored. Bioequivalence of each generic product to its corresponding reference product has already been validated by suppliers according to the Japanese guidelines for human BE studies (data not shown).

Single unit-type dosage forms (tablets or capsules) whose drug contents were equal to doses in BE studies were selected, excluding the dry syrups (powders) containing pranlukast hydrate. When dosage forms with different drug contents were commercially available, the dosage form with the highest content was used, except in the case of simvastatin. The tablet containing 5 mg of simvastatin, which is one-fourth of the highest content, was selected so that BE study databases were disclosed by 4 pharmaceutical industries. BE studies of pranlukast were performed at a dose of either 100 or 200 mg/subject. Doses of simvastatin and pranlukast, which are classified into BCS class 2, were not always the highest content; however, we decided to analyze their BE study databases because high dose numbers were constantly observed, irrespective of dose (Table 1). Four drugs were allocated equally to 3 classes of the BCS.

2. Appropriateness of AUC/Dose as a Parameter That Predicts the Risk of Bioinequivalence of Oral Drug Products. The scientific basis on the combination of the BCS and AUC/dose, which was supported by the results of BE studies performed by one company, is too frail to discuss the combination-based perspective for biowaivers of BE studies. Therefore, prior to the discussion, the appropriateness of AUC/dose as the risk parameter was validated using databases of BE studies disclosed by 6 companies.

We first attempted to collect AUC_{0-inf}, C_{max}, the AUC_{0-inf}-based number of subjects, and the C_{max}-based number of subjects, as done in the previous study.[2] However, we noticed that a couple of participating companies calculated only the AUC_{0-t}-based number of subjects because the Japanese regulatory agency accepts not only the AUC_{0-inf}-based but also the AUC_{0-t}-based predictions.[4] Since both predictions were performed for 34 formulations among 49 formulations in the residual companies, a comparison between them was made (data not shown). No difference in the number of subjects was observed for 23 formulations. As the Japanese regulatory agency accepts both predictions, a difference observed for 11 formulations was small (the maximal difference was 1.5 times). In addition, when the AUC_{0-t}-based number of subjects was less than or more than 24, which indicates the criterion of high/low risk in BE studies that incur bioinequivalence between test and reference products containing BCS class 1 or class 3 drugs,[2] the corresponding AUC_{0-inf}-based number of subjects always remained on the same side. Since the difference between both predictions was insignificant, we decided to use the AUC_{0-t}-based number of subjects throughout this report.

AUC and C_{max} are used as a surrogate for bioequivalence;[1,4,5] therefore, pharmaceutical industries perform both AUC-based and C_{max}-based predictions. The AUC_{0-t}-based number of subjects was next compared with the corresponding C_{max}-based one (data not shown). As reported in the literature,[16,17] the number of subjects showed a tendency to increase when the AUC-based prediction was substituted with the C_{max}-based prediction: the latter prediction gave larger and equivalent numbers of subjects in 35 and 8 formulations, respectively, among 49 formulations. C_{max} is defined by the rate and extent

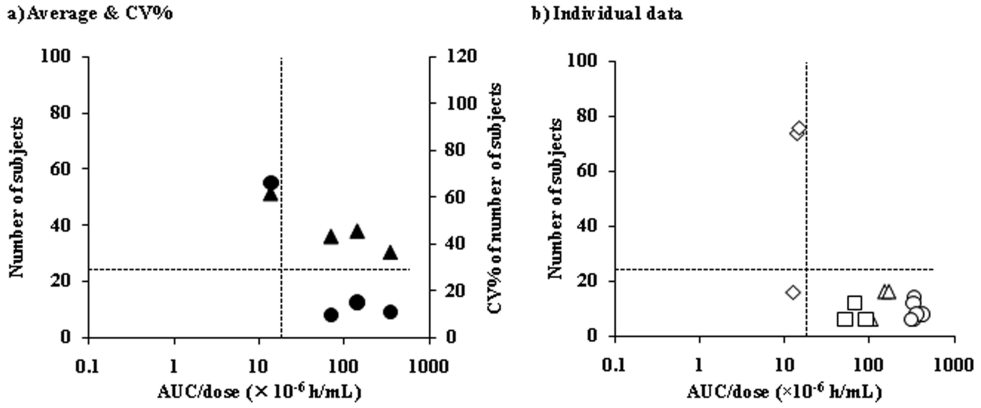


Figure 1. The number of subjects as a function of AUC_{0-inf}/dose for BCS class 1 drugs. Horizontal and vertical dotted lines indicate 24 subjects and 18.0×10^{-6} h/mL, respectively. (a) Average values of number of subjects between different formulations containing an identical drug (●) and coefficients of variation (CV%) of number of subjects (▲). They were plotted as a function of average values of AUC_{0-inf}/dose. (b) Individual data (○, cetilizine; Δ, doxazosin; □, pilsicainide; ◇, terbinafine).

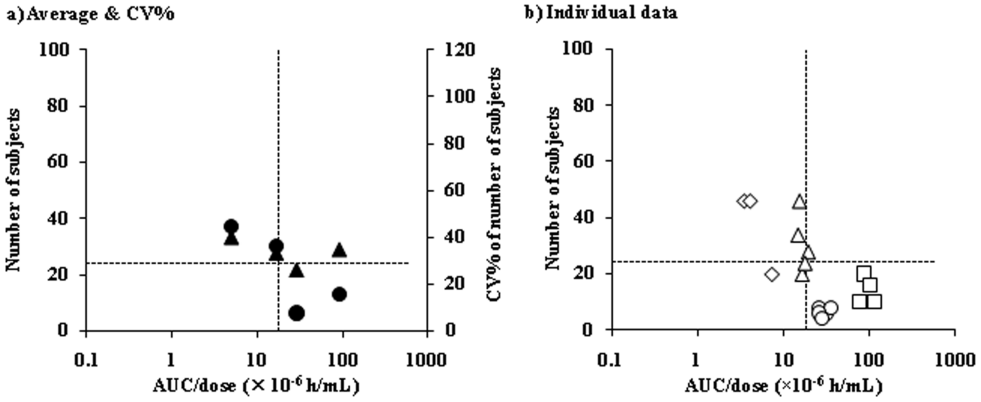


Figure 2. The number of subjects as a function of AUC_{0-inf}/dose for BCS class 3 drugs. Horizontal and vertical dotted lines indicate 24 subjects and 18.0×10^{-6} h/mL, respectively. (a) Average values of number of subjects between different formulations containing an identical drug (●) and CV% of number of subjects (▲). They were plotted as a function of average values of AUC_{0-inf}/dose. (b) Individual data (○, amlodipine; Δ, imidapril; □, levofloxacin; ◇, pravastatin).

of absorption; however, AUC is defined solely by the extent.[18] It was likely that the AUC-based prediction gave a small number of subjects when compared with the corresponding C_{max}-based prediction because AUC, which is total amount of drug absorbed orally, is independent of the absorption rate.

Figure 1 shows the relationship between AUC_{0-inf}/dose and the number of subjects for BCS class 1 drugs. A horizontal dotted line (24 subjects) indicates the WHO consideration-based practical upper limit of the number of subjects in standard BE studies. We have worked on the assumption that there is a high risk of bioinequivalence in BE studies if the predicted number of subjects exceeds 24, as done in the previous study.[2] A vertical dotted line indicates 18.0×10^{-6} h/mL. The previous study revealed that this line indicates a boundary across which the number of subjects changed critically when drugs were classified into either BCS class 1 or class 3 (Yamashita and Tachiki previously reported the boundary as 18.0×10^{-7} h/mL; however, we noticed that there was a calculation mistake in the order of magnitude).[2] In the case of cetirizine, doxazosin, and pilsicainide, whose AUC/dose was more than 18.0×10^{-6} h/mL, the average number of subjects was less than 24. Formulations of these generic products differed between companies; however, a variation in the number of subjects between formulations containing an identical drug was small. On the other hand, a large number of subjects (ca. 55 subjects on average) was required to prove bioequivalence between generic and reference products containing terbinafine hydrochloride whose AUC/dose did not reach 18.0×10^{-6} h/mL. The variation was relatively large when compared with 3 residual BCS class 1 drugs because one outlier was observed (Figure 1b).

As shown in Figure 2, a similar bipolarization whose boundary was formed by both dotted lines was observed for BCS class 3 drugs. When AUC/dose was more than 18.0×10^{-6} h/mL (amlodipine and levofloxacin), the average number of subjects was less than 24. In contrast, the average number of subjects exceeded 24 when AUC/dose fell short of 18.0×10^{-6} h/mL (imidapril and pravastatin). A variation in the number of subjects between different formulations containing an identical BCS class 3 drug was small, irrespective of AUC/dose.

The threshold value of AUC/dose, which distinguishes BCS class 1 and class 3 drugs with a high risk of bioinequivalence, was observed with good reproducibility. Regarding doxazosin and levofloxacin, the online database of the BCS classification was inconsistent with P_{eff}-based classification (Table 1); therefore,

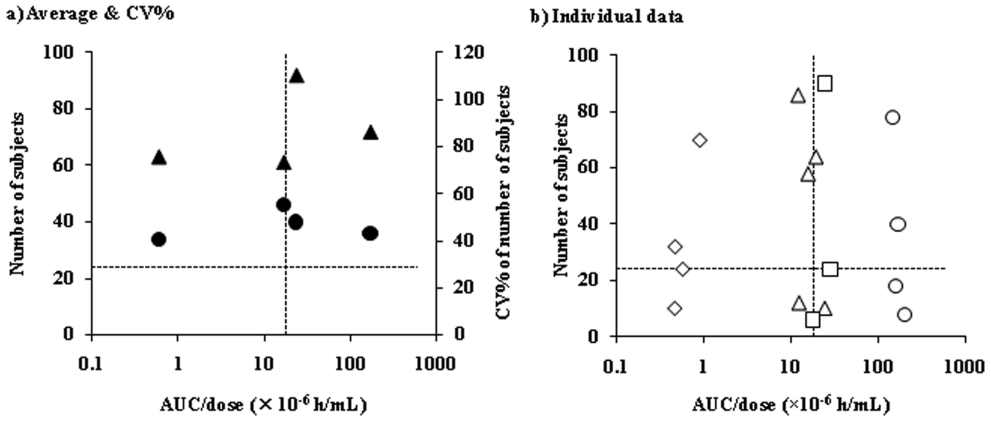


Figure 3. The number of subjects as a function of AUC_{0-inf}/dose for BCS class 2 drugs. Horizontal and vertical dotted lines indicate 24 subjects and 18.0×10^{-6} h/mL, respectively. (a) Average values of number of subjects between different formulations containing an identical drug (●) and CV% of number of subjects (▲). They were plotted as a function of average values of AUC_{0-inf}/dose. (b) Individual data (○, epalrestat; Δ, pranlukast; □, risperidone; ◇, simvastatin).

the former took precedence over the latter, as described earlier. The reason of this inconsistency is unclear; however, our observation is not affected even when data are replaced with each other because both drugs are classified into either BCS class 1 or class 3. Four compounds were selected as model drugs in the respective BCS classes. They were distributed over a wide range of AUC/dose. AUC/dose is equal to F/CL_{tot}, where F is oral bioavailability and CL_{tot} is total body clearance of the drug. As discussed in the previous study,[2] the low AUC/dose of BCS class 1 drugs is attributed to the high first-pass metabolism (thus the low F) and/or high metabolic clearance from the body (thus the high CL_{tot}) because these drugs are absorbed almost completely from the intestine. On the other hand, the metabolic clearance of BCS class 3 drugs is usually low. Therefore, it is considered that their low AUC/dose mainly results from the incomplete oral absorption due to the low membrane permeability in the intestine. Our findings in this study also suggested that the above-mentioned matters were significant factors for bioinequivalence of oral dosage forms containing BCS class 1 and class 3 drugs. We concluded that AUC/dose was appropriate for the parameter that predicts the risk of bioinequivalence of immediate-release solid oral dosage forms containing BCS class 1 or class 3 drugs.

3. A Perspective for Biowaivers of BE Studies on the Basis of the Combination of AUC/Dose and the BCS. *3. 1. The Case of BCS Class 2 Drugs.* The Japanese regulatory agency states that bioinequivalence is a matter of the difference in the quality of formulations and/or manufacturing processes.[19] This statement tends to be observed in BE studies using BCS class 2 drug-containing oral dosage forms although it is independent of the BCS classification. Four model drugs were distributed over a wide range of AUC/dose, as seen in the case of BCS classes 1 and 3. As shown in Figure 3, a large variation in the number of subjects between companies was observed when an identical BCS class 2 drug was formulated. The lowest CV% was 73% for pranlukast; however, the value was even larger than the CV% observed for terbinafine (62%), which was maximal among those of BCS class 1 and class 3 drugs used in this study (Figures 1 and 2). The average number of subjects exceeded 24 constantly, irrespective of AUC/dose. Each of 6 participating companies designed the formulation of the generic product independently and evaluated its bioequivalence to the corresponding brand. Production batches of the brand used in the respective BE studies possibly differed between companies; however, it was considered that the large variation in the number of subjects and the formulation design of generic products was closely linked because the formulation of the brand was identical in each study.

When pharmaceutical industries alter the formulations of their original products or develop generic products, they usually design the new formulation with an *in vitro* dissolution profile similar to that of the original. However, the similarity of *in vitro* dissolution profiles between original and new formulations does not always ensure similarity of *in vivo* dissolution profiles between them. The pharmacopeia-specified test procedures for *in vitro* dissolution are designed with the aim of validating that the quality between production batches is controlled properly. Experimental conditions such as volume and components of dissolution media are not always conditions that completely imitate the human GI tract. Pharmaceutical industries should design formulations containing BCS class 2 drugs with an appreciation for the possibility of the unreliable *in vitro*−*in vivo* correlation caused by poor solubility. The rate-determining step in oral absorption of BCS class 2 drugs is *in vivo* dissolution of the drugs from their dosage forms. Since drug dissolution is a factor typically attributed to formulations, bioequivalence between immediate-release solid oral dosage forms containing poorly water-soluble BCS class 2 drugs is more susceptible to formulation designs. Prior to the discussion on the application of biowaiver of BE studies into BCS class 2 drug-containing oral dosage forms, analytical methods that predict properly *in vivo* drug dissolution from the dosage forms should be established.

3. 2. The Case of BCS Class 1 and Class 3 Drugs. Contrary to the case of BCS class 2 drugs (Figure 3), a small variation in the number of subjects was constantly observed in BE studies using oral dosage forms containing an identical BCS class 1 or class 3 drug, even though formulations of generic products differed between companies (Figures 1 and 2). When these dosage forms are administered orally, most of drugs are released while the dosage forms remain in the stomach because both BCS class 1 and class 3 drugs are highly water-soluble. Since BCS class 1 drugs are highly permeable, their rapid absorption in the small intestine is observed. Therefore, the rate-determining step in their absorption is gastric emptying of them dissolved in the stomach, which is independent of formulations. This is the scientific basis of biowaiver of BE studies for immediate-release

solid oral dosage forms containing BCS class 1 drugs. In many countries, including the USA but excluding Japan, human BE studies are not required if *in vitro* dissolution of BCS class 1 drugs from their oral dosage forms meets the regulation-specified requirements. The expansion of this idea into immediate-release solid oral dosage forms containing BCS class 3 drugs is being advanced.[5,20,21] The absorption of poorly permeable BCS class 3 drugs is rate-limited by membrane permeability of drugs emptied from the stomach after dissolution from the dosage forms. This parameter is also independent of formulations.

Due to the formulation independency of the rate-determining step in oral absorption, it is general that the effect of the quality of formulations and/or manufacturing processes on bioequivalence between test and reference products containing BCS class 1 or class 3 drug is hardly visible in human BE studies when the *in vitro* dissolution meets the regulation-specified requirements for biowaiver.[20−23] The variation in the number of subjects observed in Figures 1 and 2 was relatively small when compared with the case of BCS class 2 drugs (Figure 3). It appears that the number of subjects converges into one value for each drug. This is likely because these BCS class 1 and 3 drugs are absorbed from the GI tract according to their intrinsic absorbability. Our analysis supports the appropriateness of biowaiver of BE studies for immediate-release solid oral dosage forms containing BCS class 1 drugs. A similar view may be also established for BCS class 3 drugs. However, biowaiver of BE studies for immediate-release solid oral dosage forms of them is in the height of discussion. Additional analysis with a large number of BCS class 3 drugs will be required. Databases of BE studies that ended in failure may be also helpful in obtaining a conclusive statement for this issue. Including BCS class 1 drugs, an increase in the number of drugs will be prerequisite to predict the threshold value of AUC/dose accurately although we expect that the current prediction (18.0×10^{-6} h/mL) is not so far from the true value.

CONCLUSIONS

Databases of BE studies using immediate-release solid oral dosage forms were disclosed by 6 Japanese generic pharmaceutical industries. As seen in the previous study performed in cooperation with 1 company, AUC/dose gave a clear criterion to distinguish BCS class 1 and class 3 drugs with a high risk of bioinequivalence. This good reproducibility validated that AUC/dose was appropriate for the risk predictor of highly water-soluble drug-containing oral dosage forms. A small variation in the number of subjects between different formulations containing an identical BCS class 1 or class 3 drug was constantly observed. This indicates that the number of subjects converges into one value for each drug. Our analysis on the basis of the combination of AUC/dose and the BCS indicates that BE studies of the oral dosage forms containing not only BCS class 1 drugs but also class 3 drugs can be biowaived. In contrast, a large variation in the number of subjects was observed when the drugs were substituted with BCS class 2 drugs. This finding indicates that bioequivalence between oral dosage forms containing poorly water-soluble BCS class 2 drugs is more susceptible to formulation designs because *in vivo* drug dissolution, which is a rate-determining step in their oral absorption, is a factor typically attributed to formulations. Currently, the application of biowaiver of BE studies into BCS class 2 drug-containing oral dosage forms is a premature discussion.

AUTHOR INFORMATION

Corresponding Author

*Setsunan University, Faculty of Pharmaceutical Sciences, 45-1, Nagaotoge-cho, Hirakata, Osaka 573-0101, Japan. Tel: +81-72-866-3124. Fax: +81-72-866-3126. E-mail: sakuma@pharm.setsunan.ac.jp.

REFERENCES

(1) Bioavailability and Bioequivalence Studies for Orally Administered Drug Products—General Considerations; Guidance for Industry; Food and Drug Administration: Washington, DC, 2003. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070124.pdf (accessed March 2011).

(2) Yamashita, S.; Tachiki, H. Analysis of Risk Factors in Human Bioequivalence Study That Incur Bioinequivalence of Oral Drug Products. *Mol. Pharmaceutics* **2009**, *6*, 48–59.

(3) Sakuma, S.; Ogura, R.; Masaoka, Y.; Kataoka, M.; Tanno, F. K.; Kokubo, H.; Yamashita, S. Correlation between *In Vitro* Dissolution Profiles from Enteric-coated Dosage Forms and *In Vivo* Absorption in Rats for High-solubility and High-permeability Model Drugs. *J. Pharm. Sci.* **2009**, *98*, 4141–4152.

(4) Guideline for Bioequivalence Studies of Generic Products; National Institute of Health Sciences: Tokyo, Japan, 2006. http://www.nihs.go.jp/drug/be-guide(e)/be2006e.pdf (accessed March 2011).

(5) Guidance on the Investigation of Bioequivalence; European Medicines Agency: London, U.K., 2010. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2010/01/WC500070039.pdf (accessed March 2011).

(6) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. Experimental and Computational Approaches to Estimate Solubility and Permeability in Drug Delivery and Development Setting. *Adv. Drug Deliery. Rev.* **2001**, *46*, 3–26.

(7) Amidon, G. L.; Lennernäs, H.; Shah, V. P.; Crison, J. R. A Theoretical Basis for a Biopharmaceutic Drug Classification: The Correlation of *In Vitro* Drug Product Dissolution and *In Vivo* Bioavailability. *Pharm. Res.* **1995**, *12*, 413–420.

(8) Löbenberg, R.; Amidon, G. L. Modern Bioavailability, Bioequivalence and Biopharmaceutics Classification System: New Scientific Approaches to International Regulatory Standards. *Eur. J. Pharm. Biopharm.* **2000**, *50*, 3–12.

(9) WHO Expert Committee on Specifications for Pharmaceutical Preparations—WHO Technical Report Series, No. 863—Thirty-four Report; World Health Organization: Geneva, Swiss Confederation, 1996. http://apps.who.int/medicinedocs/en/d/Js5516e/19.html (accessed March 2011).

(10) Haidar, S. H.; Davit, B.; Chen, M. L.; Conner, D.; Lee, L.; Li, Q. H.; Lionberger, R.; Makhlouf, F.; Patel, D.; Schuirmann, D. J.; Yu, L. X. Bioequivalence Approaches for Highly Variable Drugs and Drug Products. *Pharm. Res.* **2008**, *25*, 237–241.

(11) Takagi, T.; Ramachandran, C.; Bermejo, M.; Yamashita, S.; Yu, L. X.; Amidon, G. L. A Provisional Biopharmaceutics Classification of the Top 200 Oral Drug Products in the United States, Great Britain, Spain, and Japan. *Mol. Pharmaceutics* **2006**, *3*, 631–643.

(12) Lennernäs, H. Human Intestinal Permeability. *J. Pharm. Sci.* **1998**, *87*, 403–410.

(13) Regårdh, C. G.; Borg, K. O.; Johansson, R.; Johnsson, G.; Palmer, L. Pharmacokinetic Studies on the Selective Beta1-receptor Antagonist Metoprolol in Man. *J. Pharmacokinet. Biopharm.* **1974**, *2*, 347–364.

(14) *Biopharmaceutics Classification System (BCS)*; TSRL Inc.: Ann Arbor, MI, 2011. http://69.20.123.154/services/bcs/search.cfm (accessed March 2011).

(15) *The Japanese Pharmacopeia*, 5th ed.; The Society of Japanese Pharmacopeia: Tokyo, Japan, 2006. http://jpdb.nihs.go.jp/jp15e/ (accessed March 2011).

(16) Endrenyi, L.; Yan, W. Variation of C_{max} and C_{max}/AUC in Investigations of Bioequivalence. *Int. J. Clin. Pharmacol., Ther. Toxicol.* **1993**, *31*, 184–189.

(17) Hauck, W. W.; Parekh, A.; Lesko, L. J.; Chen, M. L.; Williams, R. L. Limits of 80%-125% for AUC and 70%-143% for C_{max}. What Is the Impact on Bioequivalence Studies? *Int. J. Clin. Pharmacol. Ther.* **2001**, *39*, 350–355.

(18) Balan, G.; Timmins, P.; Greene, D. S.; Marathe, P. H. *In-vitro In-vivo* Correlation Models for Glibenclamide after Administration of Metformin/glibenclamide Tablets to Healthy Human Volunteers. *J. Pharm. Pharmacol.* **2000**, *52*, 831–838.

(19) Regulatory Response to BCS in Japan; Harmonization, Globalization and Innovation in Pharmaceutical Science and Technology, September 26, 2005. http://www.nihs.go.jp/drug/section0/Yokohama050926.pdf (accessed March 2011).

(20) 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. L.; Hussain, A. S. Biopharmaceutics Classification System: The Scientific Basis for Biowaiver Extensions. *Pharm. Res.* **2002**, *19*, 921–925.

(21) Tsume, Y.; Amidon, G. L. The Biowaiver Extension for BCS Class III Drugs: The Effect of Dissolution Rate on the Bioequivalence of BCS Class III Immediate-release Drugs Predicted by Computer Simulation. *Mol. Pharmaceutics* **2010**, *7*, 1235–1243.

(22) Cheng, C. L.; Yu, L. X.; Lee, H. L.; Yang, C. Y.; Lue, C. S.; Chou, C. H. Biowaiver Extension Potential to BCS Class III High Solubility-Low Permeability Drugs: Bridging Evidence for Metformin Immediate-release Table. *Eur. J. Pharm. Sci.* **2004**, *22*, 297–304.

(23) Cook, J. A.; Davit, B. M.; Polli, J. E. Impact of Biopharmaceutics Classification System-Based Biowaivers. *Mol. Pharmaceutics* **2010**, *7*, 1539–1544.