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# Analysis of Risk Factors in Human Bioequivalence Study That Incur Bioinequivalence of Oral Drug Products

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**Abstract:** In the study of human bioequivalence (BE), newly developed oral products sometimes fail to prove BE with a reference product due to the high variability in pharmacokinetic (PK) parameters after oral absorption. In this study, risk factors that incur bioinequivalence in BE study were analyzed by applying the Biopharmaceutics Classification System (BCS). Forty-four generic products were selected from a database of BE studies in the past 10 years at Towa Pharmaceutical Co., Ltd. (Osaka, Japan), and 90% confidence interval (CI) of AUC and $C_{max}$ in human BE study for all products were converted into coefficient of variation ($CV_{90}$). Then, the required number of subjects to confirm BE was estimated from the 90% CI in human BE study of new products. It was found that both the permeability of drugs to human intestinal membrane ($P_{eff}$) and the dose number calculated from their water solubility did not correlate well to $CV_{90}$ and the estimated subject number in human BE study, suggesting the contribution of other factors to cause the variability in oral drug absorption. As the PK parameter of drugs, the value of AUC/dose was calculated and plotted against $CV_{90}$ and the estimated subject number by classifying drugs into 4 BCS classes. For drugs in classes 1 and 3, AUC/dose gave a clear criterion to distinguish the drugs with a high risk of bioinequivalence, where drugs with low AUC/dose showed high $CV_{90}$ and large number of subjects. It was suggested that the high metabolic clearance (for class 1 drug) and low oral absorption (for class 3 drug) could be significant factors to incur bioinequivalence in human BE study, although for drugs in classes 2 and 4, clear factors were not defined. Consequently, for drugs in BCS classes 1 and 3, risks in human BE study to incur bioinequivalence could be predicted by calculating the AUC/dose. In the case of generic drugs, since the parameter of AUC/dose is available before initiating human BE study, this finding is expected to promote an efficient and cost-saving strategy for the development of oral drug products.

**Keywords:** Human BE study; bioinequivalence; BCS; human intestinal permeability; dose number; AUC/dose

## Introduction

For oral drug products, bioequivalence (BE) study is performed to confirm that a test product has the same quality as a reference product in terms of oral drug absorption. Bioequivalent products must show the same bioavailability of active drugs in both amount and rate after oral administration. Since, after being dissolved, drug is absorbed from the gastrointestinal (GI) tract according to its intrinsic absorbability, if the test product shows the same pattern of drug dissolution in the GI tract *in vivo* as the reference one, that product must be bioequivalent unless other ingredients do not modulate the absorption of active drug. In this meaning,

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physicochemical properties of drugs such as water solubility and membrane permeability do not affect the bioequivalency of oral product.

Since the U.S. Food and Drug Administration (FDA) published the guidance for waiver of *in vivo* bioavailability and bioequivalence studies based on the Biopharmaceutics Classification System (BCS) in 2000,[1] in Japan, advantages and disadvantages of BCS in drug development have been discussed eagerly in both pharmaceutical industries and the government.[2] However, the BCS concept has not yet been employed in Japan for BE study because of the opinion of Japanese regulatory agency that the permeability and solubility of drugs are not the direct causes of bioinequivalence in oral drug products. They stated that bioinequivalence is a matter of the differences in the quality of formulation and/or manufacturing processes.

In the human BE study, the blood−concentration pattern of the active drug is observed instead of its dissolution pattern in the GI tract, because *in vivo* drug dissolution cannot be assessed directly. Although BE is a matter of drug dissolution from the formulation, parameters such as area under the blood concentration time curve (AUC), maximum drug concentration ($C_{max}$) and time to reach the maximum drug concentration ($T_{max}$) or mean residence time (MRT) are used as surrogates for BE. Those pharmacokinetic (PK) parameters in human BE study are affected by solubility, permeability and metabolism of active drugs. In the case of immediate release products of BCS class 1 drugs, the oral absorption is rate limited by gastric emptying due to the high absorbability of drugs after they are dissolved in the stomach.[3] This is the scientific basis of biowaiver of human BE study for class 1 drugs. On the other hand, human BE study sometimes fails to prove the BE of new products due to the inter- and/or intravariability in PK parameters but not to the differences in drug dissolution. In such cases, a pharmaceutical company must increase the number of subjects in the BE study until BE is proved statistically. This process needs unnecessary human volunteers and raises the cost of drug development.

High variability in oral drug absorption is caused by several factors. Deviations in physiological conditions in the GI tract of volunteers would affect dissolution and permeation of drugs even in the same individual. For example, bile acid secretion into the small intestine promotes the dissolution of poorly soluble drugs to enhance the bioavailability.[4] On the other hand, it was reported that food intake often reduced the oral absorption of BCS class 3 drugs.[5] These facts indicate that low solubility and low permeability of drugs may cause not only the incomplete oral absorption but also the high variability in it.

Metabolism in the intestine and liver affects the oral BA as the first-pass effects after absorption. Also, deviations of the metabolic activity cause variability in PK parameters due to the change in total body clearance of drugs. High clearance of drugs, therefore, might be one of the risk factors for high variability in human BE study.[6]

In this study, data of human BE study performed in Japan by Towa Pharmaceutical Co., Ltd. were analyzed to determine the risk factors in BE studies that incur bioinequivalence of oral drug products. Forty-four drugs that were developed as generic products in Towa company were selected and, at first, were classified into 4 BCS classes according to the criteria of FDA guidance. Then, 90% confidence interval (CI) of AUC and $C_{max}$ in human BE study for their generic products were converted into coefficient of variation ($CV_{90}$) and plotted against the permeability or solubility (dose number) of drugs. In addition, as the PK parameter of those drugs, the value of AUC/dose was selected and used to consider the risk factors in human BE study.

## Materials and Methods

**Drug Products.** Forty-four drugs were selected from a database of BE studies in the past 10 years at Towa Pharmaceutical Co., Ltd. (Osaka, Japan). The drug products were immediate-release solid oral dosage forms, and confirmed bioequivalence to reference products according to the Japanese guideline for BE studies.[7] As the highest strength product of each drug, actual dose values (mg) for the BE studies were employed in this study. The actual dose is usually equal to one dose unit of drug product, and not usually equal to maximum dose which is not always one dose unit but a multiple dose unit. The reason for employing the actual dose instead of the maximum dose was that the purpose of this study was to analyze the risk factors in BE study but not to classify drugs.

**Human Intestinal Permeability.** The values of effective intestinal membrane permeability in human, $P_{eff}$ ( $\times 10^{-4}$ cm/s), of drugs were calculated from their physicochemical

(1) Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System; FDA Guidance for Industry; FDA: Washington, DC, 2000.http://www.fda.gov/CDER/GUIDANCE/3618fnl.pdf (accessed August 2008).

(2) Aoyagi, N. Regulatory Response to BCS in Japan *Harmonization, Globalization and Innovation in Pharmaceutical Science and Technology, September 26, 2005* (Japanese). http://www.nihs.go.jp/drug/section0/Yokohama050926.pdf (accessed August 2008).

(3) 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.

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***Table 1.*** Physicochemical Properties of the 44 Drugs

| no. | compound | dosage form | dose (mg) | $P_{eff}$ (human, $\times 10^{-4}$ cm/s) | dose no. | BCS[a] class |
|---|---|---|---|---|---|---|
| 1 | acetaminophen | dry syrup | 200.0 | 0.91 | 0.0839 | 4 |
| 2 | amlodipine besilate | tablet | 6.9 | 0.33 | 0.1100 | 3 |
| 3 | ampiroxicam | capsule | 27.0 | 1.05 | 25.1046 | uc[b] |
| 4 | azelastine hydrochloride | tablet | 0.5 | 3.76 | 0.0002 | 1 |
| 5 | azithromycin hydrate | tablet | 524.0 | 3.15 | 38.8148 | uc |
| 6 | benazepril hydrochloride | tablet | 5.0 | 0.54 | 0.0002 | 1 |
| 7 | betaxolol hydrochloride | tablet | 10.0 | 1.09 | 0.0001 | uc |
| 8 | bicalutamide | tablet | 80.0 | 2.46 | 235.9882 | 2 |
| 9 | carvedilol | tablet | 20.0 | 0.50 | 18.6667 | 2 |
| 10 | cefcapene pivoxil hydrochloride hydrate | fine granule | 135.0 | 1.34 | 4.5000 | 1 |
| 11 | cefdinir | capsule | 100.0 | 0.77 | 1.8519 | 4 |
| 12 | cefditoren pivoxil | tablet | 125.0 | 0.33 | 22.5225 | 2 |
| 13 | cefixime | capsule | 100.0 | 0.48 | 2.1716 | 4 |
| 14 | cefpodoxime proxetil | tablet | 135.0 | 0.27 | 3.6043 | 4 |
| 15 | cefteram pivoxil | fine granule | 125.0 | 0.28 | 3.3333 | uc |
| 16 | cetirizine hydrochloride | tablet | 10.0 | 2.01 | 0.0001 | 1 |
| 17 | cibenzoline succinate | tablet | 100.0 | 4.68 | 0.0262 | uc |
| 18 | cilazapril | tablet | 1.0 | 0.41 | 0.0013 | 3 |
| 19 | ciprofloxacin hydrochloride | tablet | 232.9 | 1.54 | 0.0419 | 3 |
| 20 | clarithromycin | tablet | 200.0 | 0.17 | 25.6410 | uc |
| 21 | doxazosin mesilate | tablet | 2.4 | 0.33 | 0.0023 | 1 |
| 22 | ebastine | tablet | 10.0 | 12.00 | 50.8518 | 2 |
| 23 | epalrestat | tablet | 50.0 | 1.73 | 2.3474 | 2 |
| 24 | etodolac | tablet | 200.0 | 1.17 | 14.7813 | uc |
| 25 | famotidine | OD tablet | 20.0 | 0.58 | 0.1667 | 4 |
| 26 | glimepiride | tablet | 3.0 | 0.31 | 5.1282 | 2 |
| 27 | imidapril hydrochloride | tablet | 10.0 | 0.29 | 0.0014 | 3 |
| 28 | itopride hydrochloride | tablet | 50.0 | 0.97 | 0.0004 | uc |
| 29 | L-carbocisteine | tablet | 500.0 | 1.00 | 1.9380 | 4 |
| 30 | levofloxacin | tablet | 100.0 | 1.99 | 0.0340 | 3 |
| 31 | meloxicam | tablet | 10.0 | 4.56 | 13.0463 | 4 |
| 32 | metformin hydrochloride | tablet | 250.0 | 0.25 | 0.0052 | 3 |
| 33 | milnacipran hydrochloride | tablet | 25.0 | 1.91 | 0.0002 | uc |
| 34 | nilvadipine | tablet | 4.0 | 0.43 | 0.2667 | 2 |
| 35 | pilsicainide hydrochloride | capsule | 50.0 | 2.77 | 0.0004 | 1 |
| 36 | pranlukast hydrate | dry syrup | 100.0 | 2.13 | 1190.4762 | 2 |
| 37 | pravastatin sodium | tablet | 10.0 | 0.48 | 0.0003 | 3 |
| 38 | quazepam | tablet | 20.0 | 12.00 | 166.6667 | uc |
| 39 | risperidone | tablet | 2.0 | 4.46 | 0.1333 | 2 |
| 40 | sarpogrelate hydrochloride | tablet | 100.0 | 1.19 | 0.0428 | 1 |
| 41 | simvastatin | tablet | 5.0 | 3.34 | 34.7222 | 2 |
| 42 | tandospirone citrate | tablet | 10.0 | 1.84 | 0.0053 | uc |
| 43 | temocapril hydrochloride | tablet | 4.0 | 1.66 | 0.1212 | 1 |
| 44 | zaltoprofen | tablet | 80.0 | 3.98 | 41.5136 | 4 |

[a] The BCS classification based on LogP value was obtained from the online database provided by TSRL Inc.[10] [b] uc: unclassified.

properties, such as molecular weight and ClogP, using a computer software named ADMET Predictor version 3.0 (Simulation Plus, Inc., Lancaster, CA). The $P_{eff}$ value of metoprolol was employed as the reference value of human permeability since 95% of this drug is known to be absorbed from the gastrointestinal tract.[8] The $P_{eff}$ value of metoprolol, $1.34 \times 10^{-4}$ cm/s, was obtained from the online database provided by TSRL Inc. The drugs with higher and lower $P_{eff}$ than $1.34 \times 10^{-4}$ cm/s were classified as high and low permeability drug, respectively.

**Dose Number.** Values of the dose number (Do) were calculated using the following equation:[3]

$$\mathrm{Do} = \frac{M_o/V_o}{C_s}$$

where $M_o$ is the actual dose value for the BE study (mg), $C_s$ is solubility in water (mg/mL), and $V_o$ is 150 mL according to the Japanese guideline for BE studies. Values of solubility

(7) *Guidelines for Bioequivalence Studies of Generic Products* (Japanese); 2006. http://www.nihs.go.jp/drug/be-guide/GL061124_BE.pdf (accessed August 2008).

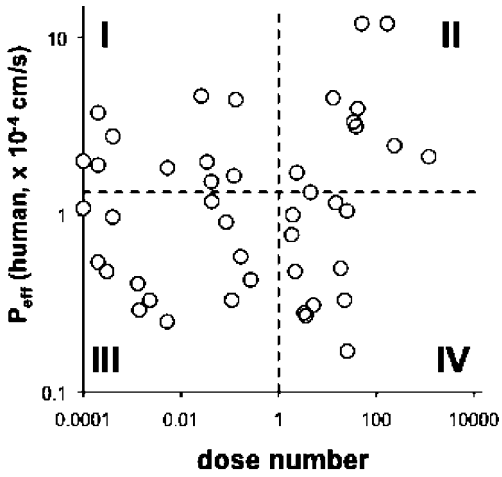


***Figure 1.*** BCS classification of 44 drugs according to solubility in water (dose number) and human permeability ($P_{eff}$). The vertical dotted line marks the reference value of dose number (dose number = 1). The horizontal dotted line marks the reference value of $P_{eff}$ ($P_{eff} = 1.34 \times 10^{-4}$ cm/s). Roman numerals I, II, III and IV each indicate corresponding BCS class. All data are taken from Table 1.

in water were measured by a previously reported method.[9] The value of one was employed as the reference value of dose number for classification of drugs by the solubility. If the dose number is in excess of one, the administered dose of the drug cannot be dissolved in 150 mL of water. Therefore, the drugs with lower and higher dose number than one were classified as high and low solubility drug, respectively.

**BCS Class.** The BCS classification based on the experimental LogP values was obtained from the online database provided by TSRL Inc. (Ann Arbor, MI).[10]

**Bioequivalence Study.** BE studies were carried out according to the Japanese guideline for BE studies. The 90% CI were not suitable to display on the two-dimensional plot because the range was not able to display as a single point on a graph. Therefore, the 90% CI of AUC and $C_{max}$ ratios were converted into coefficient of variation ($CV_{90}$, %) using the following equation:[11]

$$CV_{90}(\%) = \sqrt{\exp(\sigma r^2) - 1}$$

where $\sigma r^2$ is population variance. The Japanese guideline for BE studies has shown literature described for this equation.[12] Refer to the literature for details of this equation.

**AUC/Dose.** Values of AUC/dose ($\times 10^{-7}$ h/mL) were calculated from the AUC value (ng $\times$ h/mL) of test drug products and the dose values (mg).

**Number of Subjects.** We estimated required numbers of subjects to confirm BE between a test product and a reference product based on the result of each BE study. In the analysis of variance according to the Japanese guideline for BE studies, the statistical power (Power($\Delta_0$)) is calculated by the following equation:[13]

$$\mathrm{Power}(\Delta_0) = P\left\{\frac{\ln 0.8 - \Delta_0}{\sqrt{\hat{\sigma}^2/n}} + t_{2n-2}(0.05) \le \frac{\hat{\Delta} - \Delta_0}{\sqrt{\hat{\sigma}^2/n}} \le \frac{\ln 1.25 - \Delta_0}{\sqrt{\hat{\sigma}^2/n}} - t_{2n-2}(0.05)\right\}$$

where $P\{*\}$ is probability, $n$ is number of subjects, $\sigma$ is estimated number of residual variance of logarithmically transformed parameters, and $\Delta$ is estimated number of difference of the arithmetic means for logarithmically transformed parameters. Power($\Delta_0$) means probability that BE can be confirmed with a number of subjects ($n$) based on 90% CI. Refer to the literature for details of this equation.[12] In this study, we did not employ traditional power analysis[14] for estimating the number of subjects in BE study, because the traditional power analysis has calculated the number of subjects based on only power value but not based on 90% CI. Especially, if the mean difference in AUC or $C_{max}$ is large between test and reference products, the traditional power analysis may give a smaller number of subjects than actually required number for concluding BE based on 90% CI. The estimated numbers of subjects were calculated using this equation as mentioned below. The lowest number of $n$ which made Power($\Delta_0$) 0.8 and over was calculated for AUC and $C_{max}$. The number of Power($\Delta_0$) 0.8 means that BE can be confirmed with number of subjects ($n$) based on 90% CI with 80% probability. We have used power 0.8 according to the Japanese guideline for BE studies. Also, FDA guidance concerning statistical approaches to Establishing Bioequivalence[15] has commented as "The study should have 80 or 90% power to conclude BE between these two formulations." According to this description, we have considered that the power 0.8 is adequate to calculate required number of subjects for BE study. Then, the higher value of calculated numbers for AUC and $C_{max}$ was employed as the estimated number of subjects for the drug products. When the number was larger than 100, the estimated number

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(9) *The Japanese Pharmacopoeia Technical Information 2006* (Japanese); The Society of Japanese Pharmacopoeia: Tokyo, Japan, 2006; pp 31−33.

(10) The database provided by TSRL Inc., http://www.tsrlinc.com/services/bcs/search.cfm# (accessed August2008).

(11) *Q&A of Guidelines for Bioequivalence Studies of Generic Products* (Japanese); 2006. http://www.nihs.go.jp/drug/be-guide/QA061124_BE.pdf (accessed August 2008).

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***Table 2.*** Parameters in Bioequivalence Studies for 44 Drugs

| | | 90% CI ($CV_{90}$, %) | | | | |
|---|---|---|---|---|---|---|
| no. | compound | AUC | $C_{max}$ | AUC/dose ($\times 10^{-7}$ h/mL) | no. of subjects | dissolution rate |
| 1 | acetaminophen | 3.6 | 13.5 | 58.2 | 18 | rapid |
| 2 | amlodipine besilate | 6.9 | 6.0 | 20.0 | 6 | slow |
| 3 | ampiroxicam | 2.1 | 6.0 | 7293.2 | 6 | slow |
| 4 | azelastine hydrochloride | 9.4 | 6.9 | 5836.8 | 22 | rapid |
| 5 | azithromycin hydrate | 15.1 | 25.4 | 7.2 | 50 | slow |
| 6 | benazepril hydrochloride | 11.5 | 18.9 | 15.6 | 32 | rapid |
| 7 | betaxolol hydrochloride | 3.5 | 2.7 | 57.1 | 4 | rapid |
| 8 | bicalutamide | 9.1 | 6.6 | 3756.8 | 10 | slow |
| 9 | carvedilol | 8.8 | 15.5 | 10.9 | 20 | slow |
| 10 | cefcapene pivoxil hydrochloride hydrate | 4.2 | 7.3 | 41.2 | 14 | slow |
| 11 | cefdinir | 8.6 | 7.1 | 61.2 | 18 | slow |
| 12 | cefditoren pivoxil | 13.7 | 15.3 | 37.2 | 26 | slow |
| 13 | cefixime | 11.8 | 11.2 | 107.0 | 32 | slow |
| 14 | cefpodoxime proxetil | 6.6 | 8.1 | 75.2 | 8 | slow |
| 15 | cefteram pivoxil | 16.8 | 13.9 | 33.0 | 34 | slow |
| 16 | cetirizine hydrochloride | 4.3 | 6.6 | 310.3 | 6 | rapid |
| 17 | cibenzoline succinate | 2.9 | 5.8 | 14.1 | 6 | rapid |
| 18 | cilazapril | 8.6 | 10.5 | 64.1 | 16 | rapid |
| 19 | ciprofloxacin hydrochloride | 6.4 | 13.2 | 26.3 | 18 | slow |
| 20 | clarithromycin | 17.3 | 21.1 | 28.9 | 36 | slow |
| 21 | doxazosin mesilate | 7.1 | 7.7 | 140.7 | 8 | slow |
| 22 | ebastine | 10.5 | 13.2 | 303.8 | 10 | slow |
| 23 | epalrestat | 13.0 | 22.3 | 166.3 | 82 | slow |
| 24 | etodolac | 7.5 | 14.3 | 468.4 | 36 | slow |
| 25 | famotidine | 8.9 | 8.4 | 27.1 | 8 | rapid |
| 26 | glimepiride | 5.0 | 8.5 | 455.9 | 24 | slow |
| 27 | imidapril hydrochloride | 18.5 | 22.6 | 16.1 | 50 | na[a] |
| 28 | itopride hydrochloride | 6.8 | 20.5 | 16.0 | 42 | rapid |
| 29 | L-carbocisteine | 9.7 | 13.8 | 33.5 | 66 | slow |
| 30 | levofloxacin | 3.3 | 11.2 | 102.3 | 12 | slow |
| 31 | meloxicam | 2.9 | 8.2 | 3408.7 | 8 | slow |
| 32 | metformin hydrochloride | 6.3 | 10.6 | 20.9 | 12 | rapid |
| 33 | milnacipran hydrochloride | 3.6 | 4.9 | 24.3 | 6 | rapid |
| 34 | nilvadipine | 13.7 | 21.2 | 4.4 | ≥100 | na |
| 35 | pilsicainide hydrochloride | 5.3 | 6.2 | 67.1 | 6 | na |
| 36 | pranlukast hydrate | 10.4 | 13.2 | 24.5 | 22 | slow |
| 37 | pravastatin sodium | 21.5 | 30.1 | 4.0 | 92 | rapid |
| 38 | quazepam | 15.2 | 17.5 | 16.9 | 48 | slow |
| 39 | risperidone | 14.1 | 18.4 | 33.8 | 28 | rapid |
| 40 | sarpogrelate hydrochloride | 17.3 | 37.6 | 6.4 | ≥100 | rapid |
| 41 | simvastatin | 13.3 | 13.3 | 0.4 | 18 | slow |
| 42 | tandospirone citrate | 17.1 | 26.2 | 0.2 | 66 | rapid |
| 43 | temocapril hydrochloride | 6.4 | 7.4 | 274.0 | 10 | rapid |
| 44 | zaltoprofen | 6.6 | 24.4 | 191.8 | 56 | slow |

[a] na: not available.

of subjects was shown as 100, because a subject number greater than 100 poses severe constraints on the conduct of a BE study and is not suitable to display on the two-dimensional plots.

**Dissolution Test.** Dissolution tests of drug products were carried out according to the Japanese guideline for BE studies. Based on the dissolution rate, each drug product was classified as "rapid" or "slow" dissolution. The classification was defined according to the FDA guidance.[1] A drug product was classified "rapid" dissolving when not less than 85% of

(15) Statistical Approaches to Establishing Bioequivalence; FDA Guidance for Industry; FDA: Washington, DC, 2001.http://www.fda.gov/Cder/guidance/3616fnl.pdf (accessed November 2008).

(16) *The Japanese Pharmacopoeia*, 15th ed. (English); 2006.http://jpdb.nihs.go.jp/jp15e/ (accessed August 2008).

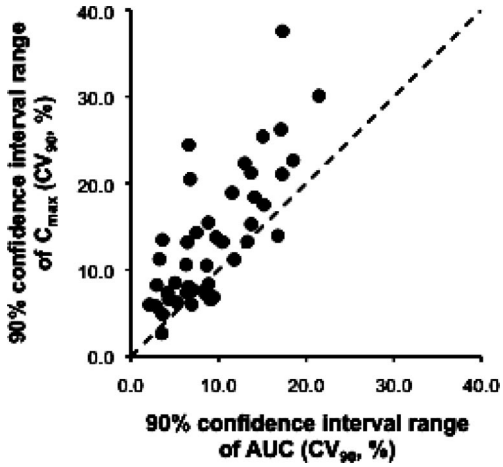


***Figure 2.*** Correlation of 90% confidence interval ($CV_{90}$, %) between AUC and $C_{max}$ in BE studies for 44 drugs. The diagonal dotted line has a slope of unity. All data are taken from Table 2.

the label amount of the drug substance dissolves within 30 min using the Japanese Pharmacopoeia[16] paddle apparatus in each of the following media, the first fluid of the Japanese Pharmacopoeia (as acidic solution of pH 1.2), second fluid of the Japanese Pharmacopoeia (as neutral solution of pH6.8), and diluted McIlvaine buffer (0.05 M disodium hydrogenphosphate/0.025 M citric acid, as solution of pH 3−5). In addition, the criterion of rapid and slow dissolution should be confirmed for both test product and reference products.

**Statistical Analysis Software.** The analytical software for BE studies, BESTS version 4.0.0.2 (Arm Systex Co., Ltd., Osaka, Japan) and Microsoft Office Excel 2002 (Microsoft Corp., Redmond, WA) were used for the statistical analyses.

## Results

Forty-four drug products used for the analysis in this study are shown in Table 1. All formulations were immediate-release solid oral dosage forms. The numbers of drugs whose dosage forms were tablet, capsule, dry syrup, fine granule, and orally disintegrating (OD) tablet were 35, 4, 2, 2, and 1, respectively. The dose strengths of the products were in a usual range from 0.5 to 524.0 mg. Two drugs, ebastine and quazepam (No. 22 and 38 in Table 1), indicated higher $P_{eff}$ values, $12.00 \times 10^{-4}$ cm/s, than others. The others indicated $P_{eff}$ values in the range of 0.17 to $4.56 \times 10^{-4}$ cm/s. Three drugs, bicalutamide, pranlukast hydrate, and quazepam (No. 8, 36, and 38 in Table 1), indicated extremely large values of dose number, 236, 1190, and 167, respectively. The others indicated values of dose number in the range of 0.0001 to 50.9. Thirty-three among 44 drugs were found in the online BCS database provided by TSRL Inc. Numbers of drugs classified into BCS classes 1, 2, 3, and 4 were 8, 10, 7, and 8, respectively.

BCS classification of 44 drugs with solubility in water (measured in Towa Pharmaceutical Co., Ltd) and the estimated human permeability is demonstrated in Figure 1. The vertical dotted line marks the reference value of dose number (dose number = 1). The drugs with higher dose number than the reference value were classified as low solubility. The horizontal dotted line marks the reference value of $P_{eff}$ ($P_{eff} = 1.34 \times 10^{-4}$ cm/s). The drugs with higher $P_{eff}$ than the reference value were classified as high permeability. Forty-four drugs were classified into 4 classes by two dotted lines in Figure 1. The numbers of drugs classified into 1, 2, 3, and 4 were 10, 9, 13 and 12, respectively.

The data of human BE study for 44 drug products are shown in Table 2. The 90% CI ranges of AUC and $C_{max}$ were converted into CV ($CV_{90}$, %). The $CV_{90}$ values of AUC and $C_{max}$ were in the range of 2.1 to 21.5% and 2.7 to 37.6%, respectively. The values of AUC/dose were in the range of $0.2 \times 10^{-7}$ to $7293.2 \times 10^{-7}$ h/mL. Required numbers of subjects were estimated based on the 90% confidence interval ranges of AUC and $C_{max}$. Although the estimated numbers of subjects for most drug products were in the range of 4 to 92, only two products, No. 34 and 40, showed numbers larger than 100. This number is considered to indicate the impossibility of proving bioequivalence in human BE study. Numbers of drug products classified as rapid and slow dissolution rate were 16 and 25, respectively. Three drug products have insufficient data of dissolution tests for classification, and were shown as "na" in Table 2.

The correlation of $CV_{90}$ of AUC and of $C_{max}$ in human BE studies are displayed in Figure 2. In the figure, most of the drug products (36/44) showed higher $CV_{90}$% value of $C_{max}$ than that of AUC.

Figure 3 shows the effect of human intestinal permeability of 44 drugs on the results of BE study. The reference value of $P_{eff}$, $1.34 \times 10^{-4}$ cm/s, for classifying drugs into high and low permeability was demonstrated by the vertical dotted line in Figure 3. The $CV_{90}$ values of AUC and $C_{max}$ showed no relation to $P_{eff}$ (Figure 3A,B). Higher $CV_{90}$ values of $C_{max}$ were found for drugs with $P_{eff}$ value around $1 \times 10^{-4}$ cm/s. In contrast, the estimated subject number showed weak dependency on $P_{eff}$ although significant correlation was not observed. Drugs that require a large number of subjects for BE study were found in the low permeability range in Figure 3C.

Figure 4 shows the effect of dose number of 44 drugs on the results of BE study. The reference value of dose number, one, for classifying the drugs into high and low solubility is demonstrated by the vertical dotted line in Figure 4. The $CV_{90}$ values of AUC and $C_{max}$ showed no relation to dose number, however, large $CV_{90}$ values of $C_{max}$ were found for the drugs with low value of dose number (Figure 4A,B). Also, relatively smaller subject number was required for the drugs with high dose number, although the drugs with high dose number are considered to be poorly soluble in the GI tract (Figure 4C). Results in Figures 3 and 4 suggested that the number of subjects required to confirm BE is possible to be affected by intestinal permeability and solubility in water of drugs. Although the poorly permeable drugs showed a tendency

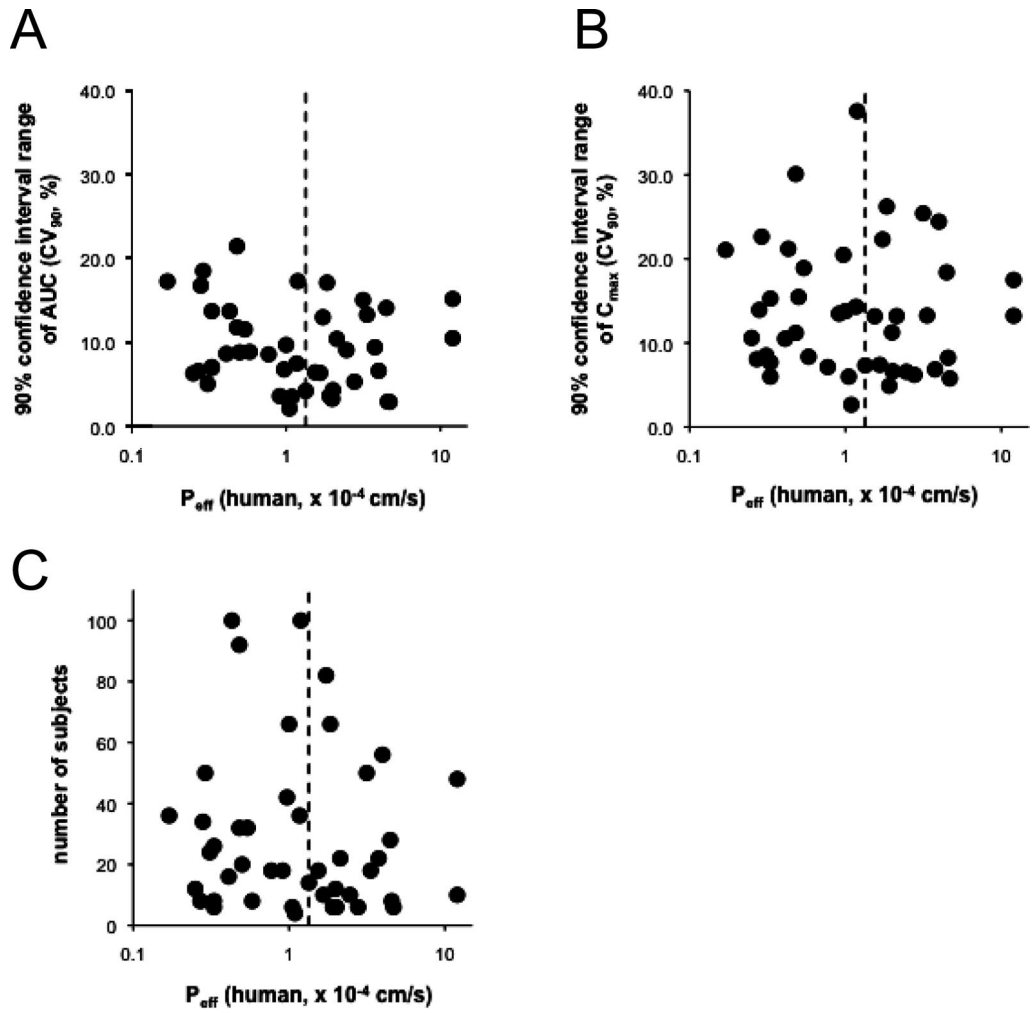


***Figure 3.*** The effects of human permeability ($P_{eff}$) of drugs on the result of BE studies. The vertical dotted line marks the reference value of $P_{eff}$ ($P_{eff} = 1.34 \times 10^{-4}$ cm/s). A: $P_{eff}$ versus 90% CI of AUC ($CV_{90}$). B: $P_{eff}$ versus 90% CI of $C_{max}$ ($CV_{90}$). C: $P_{eff}$ versus required number of subjects. More than 100 number of subjects are indicated as 100. All data are taken from Table 1 or 2.

to require a large number of subjects, in the case of solubility (dose number), a large number of subjects was required for the drugs with low dose number (poor solubility).

Since both $P_{eff}$ and dose number did not give a clear relation to the variability in human BE study, we have picked up next parameter of AUC/dose. In the PK analysis, AUC/dose of oral drug is equal to $F$/CLtot, where $F$ is an oral BA and CLtot is a total body clearance of the drug. Therefore, low oral BA and/or high CLtot led to the low value of AUC/dose. In Figures 5, 6 and 7 the relationship between AUC/dose ( $\times 10^{-7}$ h/mL) and the $CV_{90}$ value of $C_{max}$, the $CV_{90}$ value of AUC and estimated number of subjects are described by dividing drugs into four BCS classes. In these figures, 33 drugs that were found in the online BCS database provided by TSRL Inc. were used for analysis.

In Figure 5, $CV_{90}$ value of $C_{max}$ of BCS class 1 and 3 drugs (Figure 5I,III) showed clear dependency on the value of AUC/dose where drugs with low AUC/dose showed the high variability in $C_{max}$. The distribution of the $CV_{90}$ value of BCS class 1 and 3 drugs provided the threshold value of AUC/dose that can indicate the border of highly variable drugs (AUC/dose $= 18.0 \times 10^{-7}$ h/mL, shown in Figure 5 as the vertical dotted line). Two drugs in class 1 showed a lower value of AUC/dose than threshold. Because class 1 drugs should be absorbed almost completely from the GI tract, low value of AUC/dose could be attributed to the high first-pass metabolism and/or high clearance from the body. In class 3, two drugs also showed a lower value of AUC/dose than threshold. In the case of class 3 drugs, the main cause of low AUC/dose is considered to be incomplete oral absorption due to the low intestinal permeability because metabolic clearance of hydrophilic drugs is usually low. In contrast, $CV_{90}$ values of BCS class 2 and 4 drugs were independent of AUC/dose and the distribution of $CV_{90}$ value in Figure 5II,IV did not provide a clear threshold. As shown in Figure 6, AUC showed almost the same results with those for $C_{max}$.

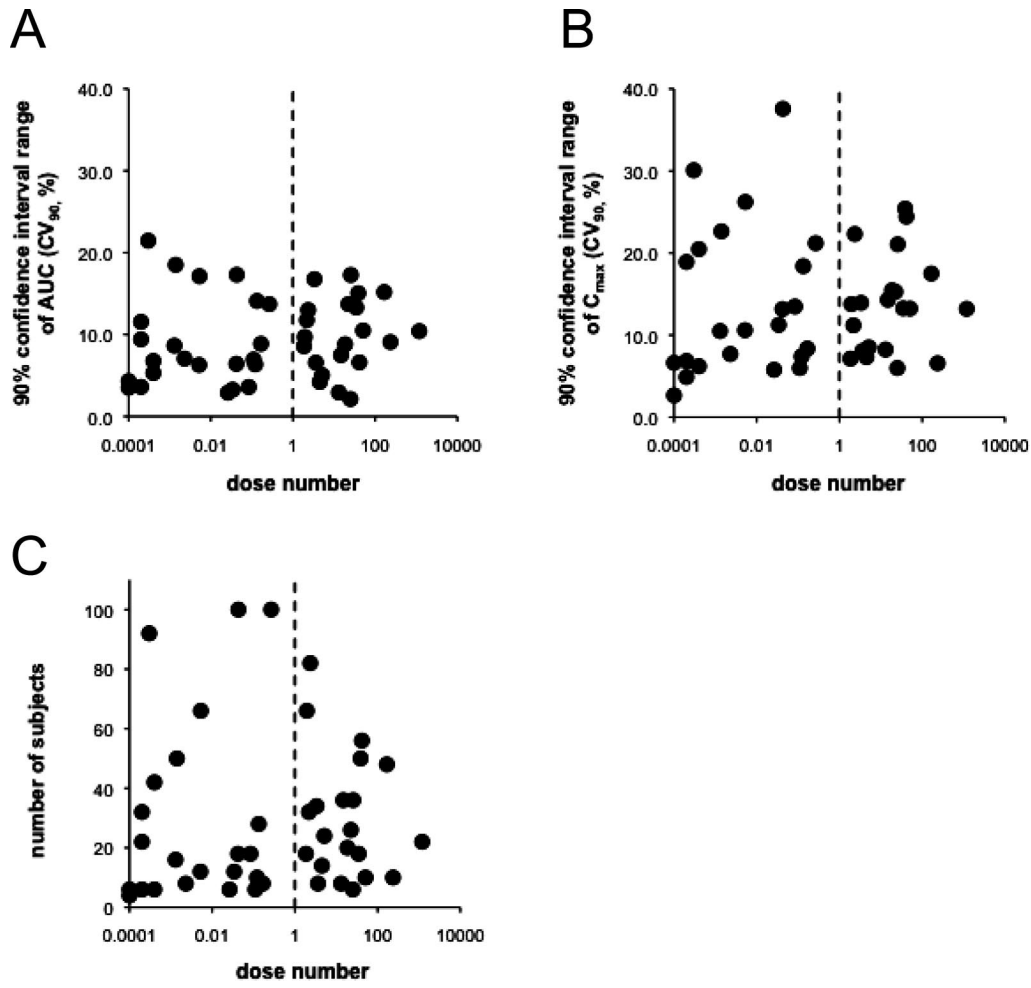


***Figure 4.*** The effects of dose number of drugs on the result of BE studies. The vertical dotted line marks the reference value of dose number (dose number = 1). A: Dose number versus 90% CI of AUC ($CV_{90}$). B: Dose number versus 90% CI of $C_{max}$ ($CV_{90}$). C: dose number versus required number of subjects. More than 100 number of subjects are indicated as 100. All data are taken from Table 1 or 2.

In Figure 7, relationships between the value of AUC/dose and the estimated number of subjects were shown for each class of drugs. For all classes, almost the same results were obtained in Figure 7 with that observed in Figures 5 and 6. Concerning the number of subjects for BE study, WHO technical report has said, "In most studies, 18−24 subjects will be needed".[17] According to this description, we defined the threshold value of AUC/dose which can discriminate the drugs that require more than 24 subjects for BE study. If the estimated number of subjects were more than 24, the products could be considered to have a high risk of bioinequivalence in human BE study. For BCS class 1 and 3 drugs (Figure 7I,III), the distribution of subject number clearly provided the threshold of AUC/dose as $18.0 \times 10^{-7}$ h/mL (the vertical dotted line of Figure 7). This value is the same as that defined in Figures 5 and 6. In both classes, drugs with a higher value of AUC/dose than threshold required a lower number of subjects than 24. Therefore, two drugs in each class 1 and 3 were considered to have a high risk of bioinequivalence due to the high variability in PK parameters (AUC and $C_{max}$) after oral administration. For BCS class 2 and 4 drugs (Figure 7II,IV), the distribution of estimated subject numbers did not provide a clear threshold.

## Discussion

In this study, effects of physicochemical and PK properties of 44 drugs on the result of human BE studies were analyzed in order to determine the risk factors in BE studies that incur bioinequivalence of oral drug products. The drug products used in this study were five types of immediate-release solid oral dosage forms: tablet, capsule, dry syrup, fine granule and OD tablet. Since the dose strength of all products was within the common range of clinical use (Table 1), listed drugs were regarded as not containing uncommon drug products and were suitable for the analysis. In the list, two drugs (ebastine and quazepam) showed higher $P_{eff}$ than

(17) WHO Expert Committee on Specifications for Pharmaceutical Preparations - WHO Technical Report Series, No. 863—Thirty-fourth Report, Annex 9—Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability; 1996. http://www.who.int/medicinedocs/en/d/Js5516e/#Js5516e.19.5.1.2 (accessed August 2008).

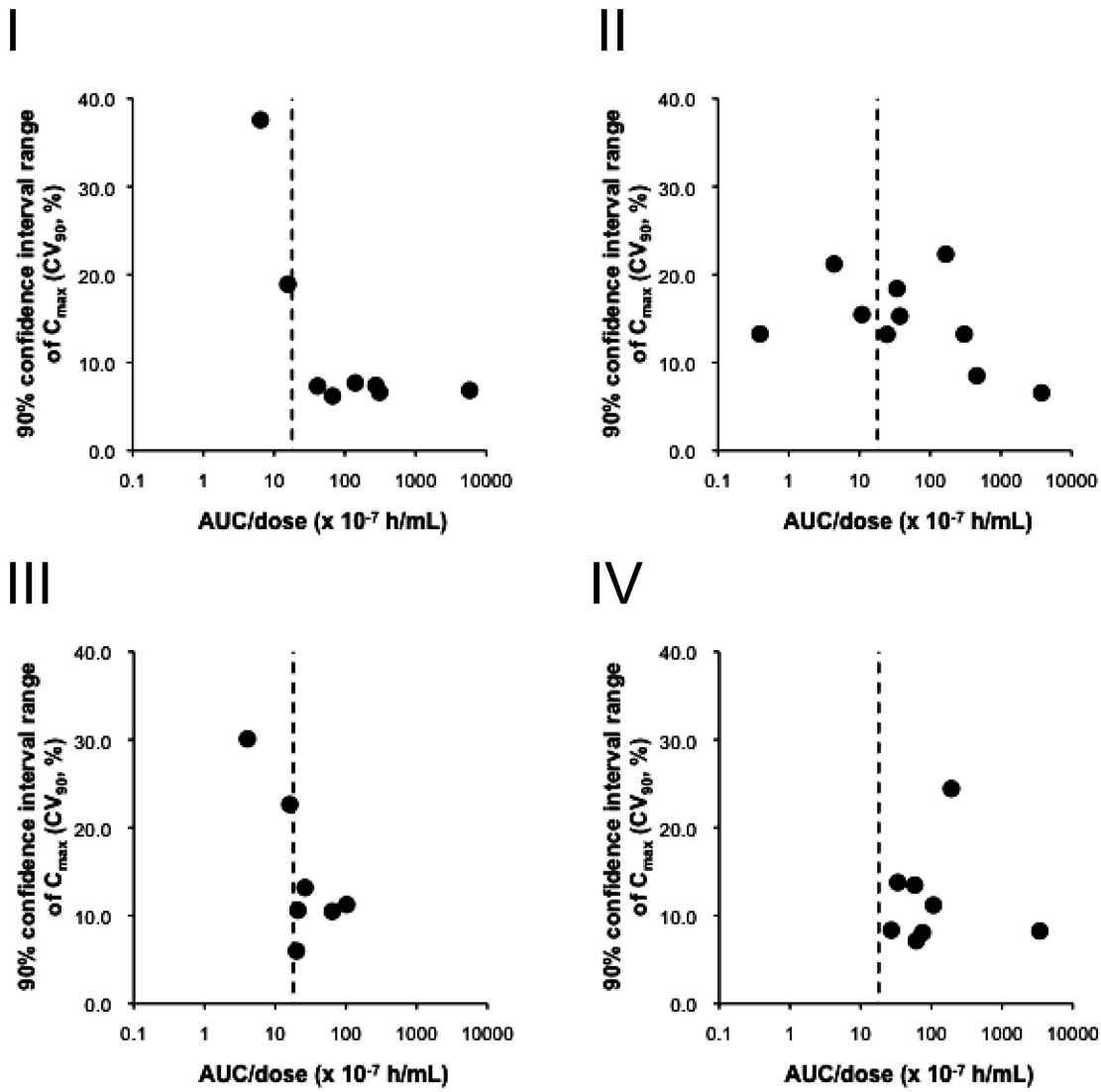


***Figure 5.*** The effects of AUC/dose of drugs on the 90% confidence interval of $C_{max}$ ($CV_{90}$, %) of BE studies. The vertical dotted line marks a threshold of AUC/dose (AUC/dose = $18.0 \times 10^{-7}$ h/mL). I: BCS class 1 drugs (high solubility, high permeability). II: BCS class 2 drugs (low solubility, high permeability). III: BCS class 3 drugs (high solubility, low permeability). IV: BCS class 4 drugs (low solubility, low permeability). All data are taken from Table 2.

others, and three drugs (bicalutamide, pranlukast hydrate, and quazepam) showed extremely large dose number (Table 1).

Forty-four drugs were classified into four BCS classes according to the solubility in water (converted to dose number) and estimated human intestinal permeability ($P_{eff}$). Consequently, the number of drugs in the four BCS classes 1, 2, 3 and 4 were 10, 9, 13 and 12, respectively (Figure 1), showing that the drugs were distributed in four classes almost equally. In Figure 1, the population of high solubility drugs was 50.0% (22/44), and that of high permeability drugs was 43.2% (20/44). In the previous report[18] where the drugs in Japanese Orange Book[19] were classified into 4 BCS classes, population of high solubility drugs was 54.9% (based on dose number using maximum dose strength), and that of high permeability drugs was 69.0% (based on CLogP). Compared with the list in Table 1, population of high solubility drugs was approximately equal (50.0 and 54.9%), whereas this study contained a lower population of high permeability drugs (43.2 and 69.0%). Although the reason for this difference in the percentages of high permeability drugs is not clear, the difference in the method to estimate $P_{eff}$ (CLogP method versus computer simulation) might cause the difference in $P_{eff}$ value for some drugs.

In the human BE study, value of $CV_{90}$ for $C_{max}$ was larger than that for AUC in most of drug products (Table 2 and

(18) Takagi, T.; Ramachandran, C.; Bermejo, M.; Yamashita, S.; Yu, L. X.; Amidon, G. L. A provisional biopharmaceutical classification of the top 200 oral drug products in the United States, Great Britain, Spain, and Japan. *Mol. Pharmaceutics* **2006**, *3*, 631–643.

(19) *Japanese Orange Book 2007* (Japanese); 2007.http://www.jp-orangebook.gr.jp/ (accessed August 2008).

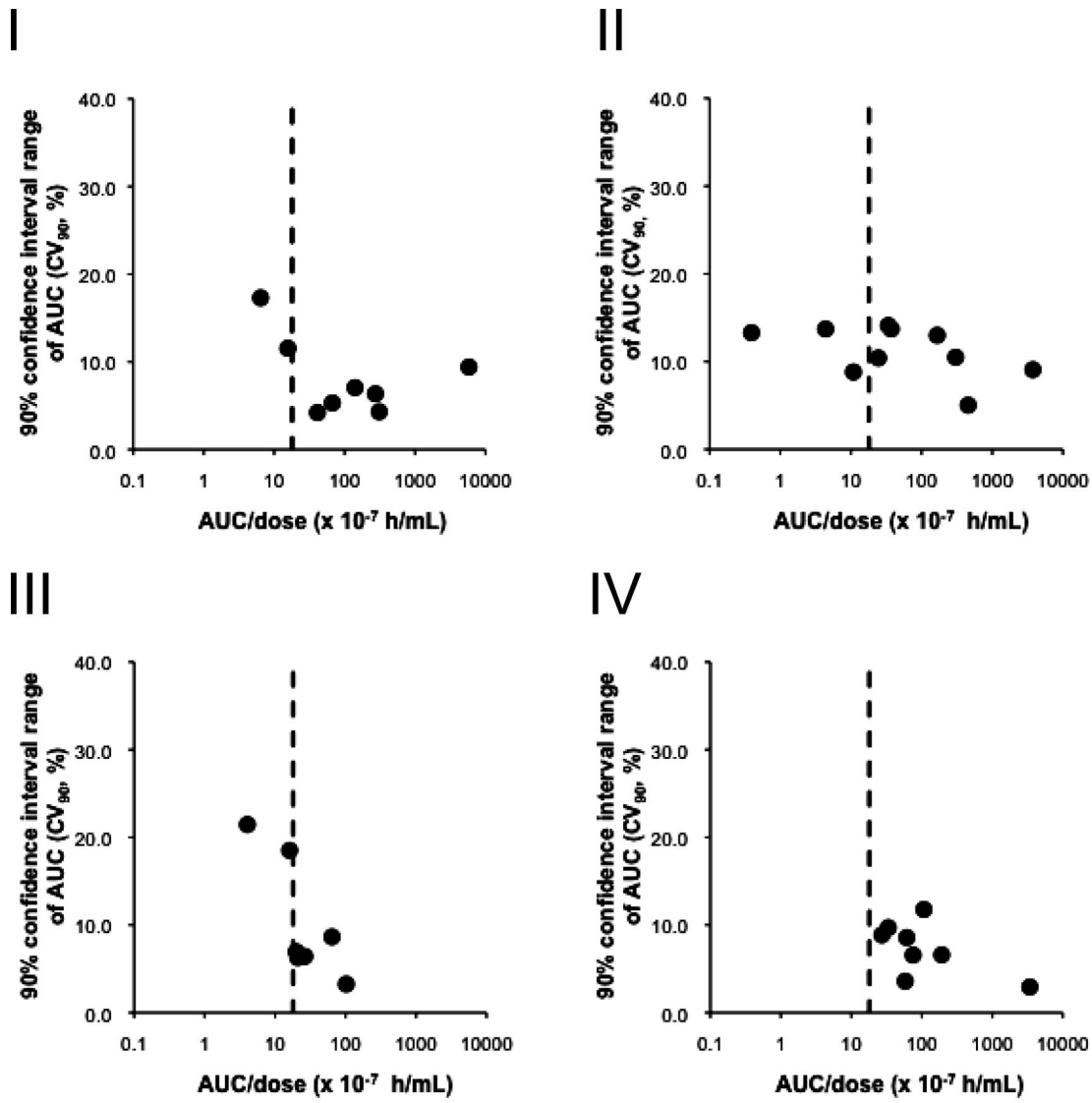


***Figure 6.*** The effects of AUC/dose of drugs on the 90% confidence interval of AUC ($CV_{90}$, %) of BE studies. The vertical dotted line marks a threshold of AUC/dose (AUC/dose = $18.0 \times 10^{-7}$ h/mL). I: BCS class 1 drugs (high solubility, high permeability). II: BCS class 2 drugs (low solubility, high permeability). III: BCS class 3 drugs (high solubility, low permeability). IV: BCS class 4 drugs (low solubility, low permeability). All data are taken from Table 2.

Figure 2). Similar observations have been reported in previous studies,[20,21] in which 90% CI of $C_{max}$ was larger than AUC in the BE studies. These results are considered to be reasonable because AUC of oral drugs is a parameter defined solely by the amount of absorbed drugs while $C_{max}$ is defined by both amount and rate of drug absorption. Total amount of drugs absorbed after oral administration should be insensitive to the variability in drug dissolution rate or gastric emptying rate which profoundly affects the rate of drug absorption. Therefore, in the following analyses, results of AUC were less clear than those of $C_{max}$.

As the first attempt to analyze risk factors, two parameters used for BCS classification, $P_{eff}$ and dose number, were employed and were correlated with the $CV_{90}$ of AUC and $C_{max}$ and the estimated number of subjects. However, as shown in Figures 3 and 4, both parameters failed to show clear relations with the variability in human BE study. In the case of $P_{eff}$, weak tendency was observed which may suggest the necessity of large number of subjects for low permeability drugs. However, there existed many drugs that require only a small number of subjects despite of the low permeability. In this study, $P_{eff}$ of drugs listed in Table 1 were obtained by computer calculation using ADMET predictor without taking into account the contribution of transporters, such as peptide transporter (absorptive direction)

(20) Endrenyi, L.; Yan, W. Variation of Cmax and Cmax/AUC in investigations of bioequivalence. *Int. J. Clin. Pharmacol. Ther. Toxicol.* **1993**, *31*, 184–189.

(21) Hauck, W. W.; Parekh, A.; Lesko, L. J.; Chen, M. L.; Williams, R. L. Limits of 80%-125% for AUC and 70%-143% for Cmax. What is the impact on bioequivalence studies? *Int. J. Clin. Pharmacol. Ther.* **2001**, *39*, 350–355.

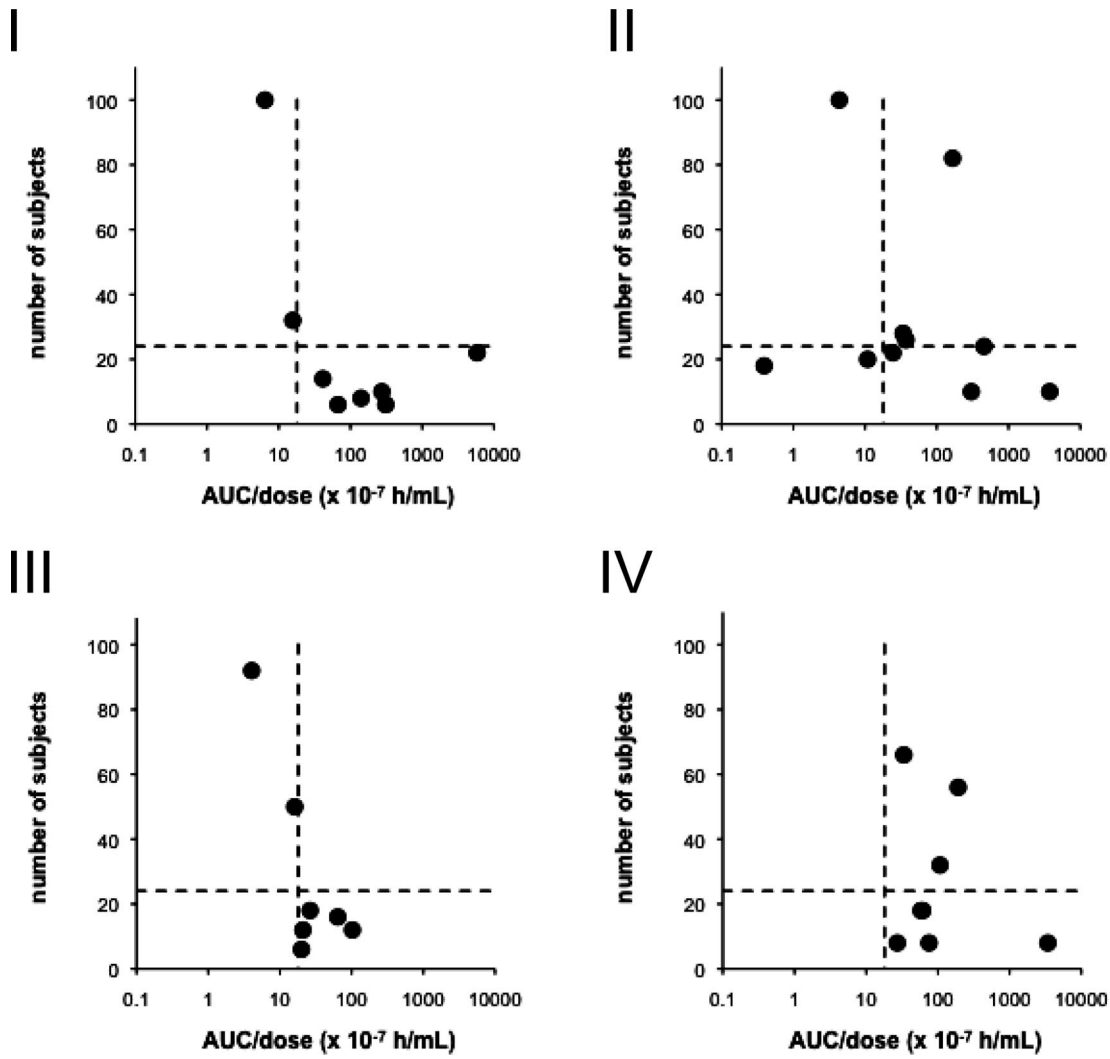


***Figure 7.*** The effects of AUC/dose of drugs on the require number of subjects of BE studies. More than 100 number of subjects are indicated as 100. The vertical dotted line marks a threshold of AUC/dose (AUC/dose = $18.0 \times 10^{-7}$ h/mL). The horizontal dotted line marks a threshold of number of subjects (number of subjects = 24). I: BCS class 1 drugs (high solubility, high permeability). II: BCS class 2 drugs (low solubility, high permeability). III: BCS class 3 drugs (high solubility, low permeability). IV: BCS class 4 drugs (low solubility, low permeability). All data are taken from Table 2.

or P-glycoprotein (secretive direction). This may be one of the reasons for the poor relation between calculated $P_{eff}$ and 90% CI and number of subjects in Figure 3. Measurement of real $P_{eff}$ values for listed drugs should be a next issue to be performed. Furthermore, in Figure 4, high value of the $CV_{90}$ for $C_{max}$ and, thus, the large estimated number of subjects are required for the drugs with low dose number (less than one). Since low permeability and/or low solubility of drugs are considered to cause incomplete and variable oral absorption, parameters of $P_{eff}$ and dose number were expected to give some idea to consider the risk factors in human BE study. Therefore, the result in Figure 4 that suggested the high variability for highly soluble drugs is difficult to understand. One of the plausible explanations is the effect of other factors that cause high variability independently of drug solubility and permeability.

Another factor to affect the bioavailability of oral drugs is first pass metabolism in the intestine and the liver. Metabolism of drugs in the liver also determines total body clearance, thus, affects the PK parameters after absorption. Metabolic clearance of drugs depends on the activity of corresponding enzymes that may vary inter- and intraindividually. Then, AUC/dose of oral drugs that equal to *F*/CLtot was picked up as the next parameter to consider the risk factors in BE study.

In Figures 5 and 6, the relation of AUC/dose to the $CV_{90}$ of $C_{max}$ and AUC was demonstrated with classifying drugs into 4 BCS classes. For drugs in classes 1 and 3, $CV_{90}$ values of drugs clearly depended on AUC/dose where high $CV_{90}$ value was observed only for the drugs with lower value of AUC/dose than $18.0 \times 10^{-7}$ h/mL. This relation was also found between AUC/dose and the estimated number of subjects. In Figure 7, threshold value of AUC/dose, $18.0 \times 10^{-7}$ h/mL, can detect the drug that requires more than 24 subjects to confirm BE. According to the WHO technical report,[17] most of oral drug product needs 18−24 subjects in

***Table 3.*** Risk Factors in Human BE Study for Immediate Release Oral Solid Products Based on BCS and AUC/Dose

| BCS class | AUC/dose | risk factor and the expected subject number in BE study |
|---|---|---|
| 1 | high | no risk factor: BE can be confirmed with a usual number of subjects (24 or less) |
| | low | high metabolic clearance: large number of subjects (more than 24) may be required to confirm BE |
| 2 | | *in vivo* dissolution rate and high metabolic clearance: number of subjects cannot be estimated from the value of AUC/dose |
| 3 | high | no risk factor: BE can be confirmed with a usual number of subjects (24 or less) |
| | low | low oral absorption due to the low membrane permeability: large number of subjects (more than 24) may be required to confirm BE |
| 4 | | undefined |

BE study. Therefore, this number can be considered to indicate the criteria of high/low risks in human BE study to incur bioinequivalence. Since 3 of 4 drug products that gave a lower value of AUC/dose, benazepril hydrochloride, sarpogrelate hydrochloride, and pravastatin sodium, demonstrated a "rapid" dissolution rate (Table 2), it is reasonable to consider that these results were not attributed to the variability in drug dissolution from the formulation, but to the variability in PK of drugs.[21] Actual examples to confirm the results of this study are shown in the interview forms of commercially available drug products provided by Towa Pharmaceutical Co., Ltd.[22] BE studies for high AUC/dose drug products azelastine hydrochloride tablets 1 mg (class 1) and amlodipine besilate tablets 5 mg (class 3) were carried out with 14 and 16 subjects, respectively. On the other hand, low AUC/dose drug products benazepril hydrochloride tablets 5 mg (class 1) and pravastatin sodium tablets 10 mg (class 3) required 48 and 60 subjects, respectively.

Because class 1 drugs should be absorbed almost completely from the GI tract, low value of AUC/dose of drugs in this class could be attributed to the high first-pass metabolism (thus the low value of $F$) and/or high metabolic clearance from the body (thus the high value of CLtot).[23] In the case of class 3 drugs, the main cause of low AUC/dose is considered to be the incomplete oral absorption due to the low intestinal permeability, because most of the drugs in this class are hydrophilic and the metabolic clearances are usually low.[23,24] The results for class 3 drugs in Figures 5, 6 and 7, therefore, suggested that the variability in oral absorption is significant only for the drugs with quite low intestinal permeability. Other than low lipophilicity, carrier mediated secretion by efflux transporters such as P-glycoprotein might be a reason of low permeability of some class 3 drugs. For such drugs, interindividual difference in the expression level of transporters in the GI tract is a possible factor to cause a high variability in the oral absorption.

In contrast, for drugs in BCS classes 2 and 4, both $CV_{90}$ of $C_{max}$ and the estimated number of subjects varied independently on the value of AUC/dose, thus, the results in Figures 5, 6 and 7 did not imply any idea to predict the risk factors in human BE study. In the case of class 2 drugs, dissolution of drugs *in vivo* is the rate controlling step in oral absorption which is affected by the factors attributable to formulation.[3] Therefore, the variability in BE study for class 2 drugs might be caused by both the differences in the quality of formulation and the deviations in PK parameters. For class 4 drugs, the main factor to control oral absorption may vary among drugs and cannot be determined only from the results in this study.

Consequently, for drugs in BCS classes 1 and 3, (1) the risk in human BE study to incur bioinequivalence due to the variability in PK and (2) the number of subjects required to confirm BE could be predicted by calculating the AUC/dose. In the case of generic drugs, for which values of AUC/dose are available before initiating human BE study, this finding can promote an efficient and cost-saving strategy for the development of oral drug products. In addition, results in Figures 5, 6 and 7 can support the idea of a waiver of human BE study not only for class 1 but also for class 3 drugs.

## Conclusion

Risk factors that incur bioinequivalence in human BE study are summarized in Table 3 with an expected number of subjects to confirm BE. BCS classification system and the PK parameter of AUC/dose are quite useful to design an efficient plan of human BE study of oral drug products.

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(22) Interview forms of commercially drug products provided by Towa Pharmaceutical Co., Ltd. (Japanese). http://di.towayakuhin.co.jp/towa5/search (accessed November 2008).

(23) Yang, Y.; Faustino, P. J.; Volpe, D. A.; Ellison, C. D.; Lyon, R. C.; Yu, L. X. Biopharmaceutics classification of selected beta-blockers: solubility and permeability class membership. *Mol. Pharmaceutics.* **2007**, *4*, 608–614.

(24) Lentz, K. A.; Hayashi, J.; Lucisano, L. J.; Polli, J. E. Development of a more rapid, reduced serum culture system for Caco-2 monolayers and application to the biopharmaceutics classification system. *Int. J. Pharm.* **2000**, *200*, 41–51.