Acceptability and characteristics of 124 human bioequivalence studies with active substances classified according to the Biopharmaceutic Classification System

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- In USA and Europe the classical way to guarantee equivalence between different formulations is the bioequivalence (BE) study based on *in vivo* bioavailability.
- The Biopharmaceutic Classification System (BCS) classifies active substances into four different groups according to their aqueous solubility and intestinal permeability.
- Recently the European Medicines Agency (EMA) has released a new bioequivalence guideline for immediate release solid oral dosage forms that includes recommendations on BCS-based biowaivers.

WHAT THIS STUDY ADDS

- As far as we know this is the first time that the probability of proving BE of more than one hundred BE studies with 80 active substances categorized according to BCS was evaluated.
- Despite the usually employed test dissolution methodology proposed as quality control, pharmaceutical products with active substances from the four classes of BCS showed non-BE studies.

AIM

The aim of this study was to evaluate the acceptability of 124 bioequivalence (BE) studies with 80 active substances categorized according to the Biopharmaceutics Classification System (BCS) in order to establish if there were different probabilities of proving BE between the different BCS classes.

METHODS

We evaluated the differences between pharmaceutical products with active substances from different BCS classes in terms of acceptability, number of subjects in the study (*n*), the point estimates, and intra- and inter-subject coefficients of variation data from BE studies with generic products.

RESULTS

Out of 124 BE studies 89 (71.77%) were performed with pharmaceutical products containing active substances classified by the BCS. In all BCS classes there were non-bioequivalent pharmaceutical products: 4 out of 26 (15.38%) in class 1, 14 out of 28 (50%) in class 2, 3 out of 22 (13.63%) in class 3 and 1 out of 13 (7.69%) in class 4. When we removed those pharmaceutical products in which intra-subject variability was higher than predicted (2 in class 1 active substances, 9 in class 2 and 2 in class 3) there were still non-BE pharmaceutical products in classes 1, 2 and 3.

CONCLUSIONS

Comparisons between pharmaceutical products with active substances from the four BCS classes have not allowed us to define differential characteristics of each class in terms of *n*, inter and intra-subject variability for C_{max} or AUC. Despite the usually employed test dissolution methodology proposed as quality control, pharmaceutical products with active substances from the four classes of BCS showed non-BE studies.

Introduction

In many developed countries, the main purpose of regulation related to the manufacture and distribution of medicinal products for human use is the safeguard of public health. In many countries the marketing authorization process for generic drugs is clearly established and relies on the demonstration of bioequivalence (BE). BE is defined as the absence of a significant difference in the availability of the active ingredient at the site of drug action [1]. In Europe, the European Medicines Agency (EMA) has stated that an 'essentially similar medicinal product' shall refer to a medicinal product which has the same qualitative and quantitative composition in terms of active substances and the same pharmaceutical form as the reference medicinal product, and whose bioequivalence with the reference medicinal product has been demonstrated by appropriate bioavailability studies [2].

In the USA [3] and Europe [4] the classical way to guarantee equivalence between different formulations is the so-called *in vivo* equivalence (bioequivalence) based on comparative *in vivo* bioavailability using pharmacokinetic endpoints.

Other countries have incorporated equivalence regulation, with various levels of requirements according to their specific national context and depending on many factors, such as the characteristics of the active drug substance, the health risk and the availability of resources to carry out a specific type of study [5]. In many places there is an interest in developing definitions and guidelines on when *in vivo* equivalence studies are necessary and when *in vitro* studies are acceptable. In this context, some countries are trying to develop regulatory systems to establish equivalence between different formulations supported by *in vitro* testing and Biopharmaceutics Classification System (BCS) considerations.

The BCS, introduced by Amidon *et al.* in 1995 [6], classifies active substances into four different groups according to their aqueous solubility at the highest dose in a volume of 250 ml and intestinal permeability. The idea is that when a drug formulated as an immediate release solid oral dosage form has an *in vivo* high solubility into the gastrointestinal tract and has a high permeability (class 1 drugs) the rate and extent of drug absorption is expected to be equivalent to the *in vitro* dissolution test, being 85% dissolved in less than 30 min with three different buffers, making *in vivo* bioequivalence demonstration unnecessary. This approach has been accepted by the FDA [7] and EMA [4] but is still rarely used, at least in Europe.

Nevertheless the EMA has gone one step further, and has released new bioequivalence guidelines that include recommendations on BCS-based [8] biowaivers. Biowaivers would be applicable to products containing BCS class 1 drugs which exhibit high solubility, complete absorption and rapid *in vitro* dissolution and also to pharmaceutical products containing substances exhibiting high solubility,

limited absorption (BCS class 3) and very rapid *in vitro* dissolution, once the product has been previously justified for the biowaiver and has demonstrated other specific requirements concerning active substances and excipients [9–12]. Also the new EMA guideline said that 'excipients that might affect bioavailability are qualitatively and quantitatively the same. In general, the use of the same excipients in similar amounts is preferred'. More than that, the WHO [13] had relaxed the solubility ratio and permeability criterion for class 1 and class 3 allowing too some bioexceptions in pharmaceutical products containing BCS class 2 drugs that are weak acids with dose solubility ratio of 250 ml or lower at 37°C over a pH range of 1.2–6.8. Such waivers would increase the speed and decrease the cost of bringing orally administered multisource therapeutics to market.

The aim of this study was to evaluate the characteristics in terms of acceptability, sample size, the point estimates, and intra- and inter-subject coefficients of variation of 80 active substances categorized according to the BCS tested in 124 BE studies with generic pharmaceutical products in order to establish if there were different probabilities of proving BE between the different BCS classes.

Methods

Studies

One hundred and twenty-four BE randomized crossover studies with 80 active substances were conducted on approximately 4500 healthy, young volunteers. The studies were all designed according to the requirements of EMA for BE studies for immediate release dosage forms [4] and developed in the Department of Pharmacology of the School of Medicine. The design and the reference medicinal product was always the same for each active substance but the number of subjects was different depending on previous data. All subjects gave written informed consent for participation in the BE studies, which were conducted according to the Helsinki declaration and the Spanish laws and were approved by the Ethics Committee of La Paz University Hospital. In every case, the BE study was developed after Spanish Medicines Agency approval of the protocol and the dissolution *in vitro* tests. In compliance with BE European requisites, in each study, the *in vitro* test dissolution methodology was in accordance with European pharmacopoeial requirements and EMA regulations. Some data from 108 of 124 studies presented here have previously been published [14].

Drug classification

The BCS classification of drugs was obtained from the Therapeutic System Research Laboratories website (<http://www.tsrlinc.com/services/bcs/search.cfm>) directed by Amidon *et al.* [15] (Table 1). Active substances in bold (C_{max}) or underlined (AUC) were non-BE formulations). In order to

Table 1

Biopharmaceutics Classification System (BCS) of active substances (number of studies)

High solubility		Low solubility		Non-classified
High permeability		Class 2		
Class 1		Class 2		
Amlodipine (2)	Pravastatin (1)	Acetoclofenac (1)	Irbesartan (1)	Amisulpride (1)
Bisoprolol (1)	Quinapril (2)	Acetoclofenac (1)	Lamotrigine (1)	Azythromycin (2)
Cetirizine (2) *	Ramipril (1)	Carbamazepine (1)	Lorazepam (1)	Azythromycin (2)
Citalopram/escitalopram (2)	Sertraline (1)	Carvedilol (1)	Lovastatin (1+2)	Bromazepam (1)
Donepezil (1)	Sildenafil (1)	Clopidogrel (3)	Mycophenolate mofetil (1)	Bromazepam (1)
Doxazosin (1)	Sildenafil (1)	Ebastine (1)	Quetiapine (1)	Clarithromycin (1)
Enalapril (2)	Terbinafin (2)	Ibuprofen (1+3)	Risperidone (1)	Clarithromycin (1)
Loratadine (1)	Tramadol (1)	Ibuprofen (4)	Risperidone (1)	Clavulanate (2)
Mirtazapine (1)	Venlafaxine (1)	Irbesartan (1)	Simvastatin (1)	Clavulanate (1)
Ondansetron (1)	Zolpidem (1)			Deflazacort (1)
Low permeability		Class 4		Deflazacort (1)
Class 3		Class 4		Dypirone (4)
Alendronic acid (3)†	Levetiracetam (1)*	Acetaminophen (1)		Flutamide (1)
Anastrozole (1)	Levofloxacin (1)	Amoxicillin (5)		Leflunomide (1)
Cefaclor (2)	<u>Lisinopril (1)</u>	Cefixime (1)		Lercanidipine (1)
Codeine (1)	Losartan (1)	Cefuroxime axetil (1)		Loperamide (1)
Codeine (1)	Pyrazinamide (1)	Famotidine (2)		Lormetazepam (2)
Fluconazole (1)	Ranitidine (1)	Hydrochlorothiazide (2)		Naproxen (1)
Gabapentin (1)	Risedronic acid (1)	Oxcarbazepine (1)		Norfloraxine (1)
Isoniazid (1)	Terazosin (1)			Pentoxifylline (1)
Lamivudine (1)	Topiramate (1)			Piroxicam (1)
Letrozole (1)				Pramipexole (1)
				Prednisone (1)
				Repaglinide (2)
				Rifampicin (1)
				Ropirinol (1)
				Torsemide (1)

Classification of drugs according to ClogP permeability of Biopharmaceutics Classification System (BCS) of Therapeutic System Research Laboratories web site (<http://www.tsrlinc.com/services/bcs/search.cfm>). Drugs in bold for C_{max} and underlined for AUC correspond to non-bioequivalent parameters. (*) Therapeutic Equivalence Evaluation AA Code of FDA (Rest of codes were AB or none classified). †The primary results were maximum observed excretion rate ($U_{rate_{max}}$) and accumulated elimination ($Ae(0,\infty)$).

classify drugs in BCS categories, we selected the ClogP-based permeability list because it classifies more drugs. A sensitivity analysis was performed excluding all drugs with discordant classification in ClogP vs. log P permeability measurements of Amidon vs. Spanish data from Takagi *et al.* [16]. The drugs excluded are listed in Table 2.

Pharmacokinetics

The pharmacokinetic (PK) analysis was performed using the WinNonlin 2.0 program (Pharsight Corporation, Cary, USA) from individual plasma or urine (three studies) concentration values on a non-compartmental extravascular input model. PK was expressed in terms of the rate and extent of absorption as characterized by the maximum observed plasma concentration (C_{max}) and area under the concentration–time curve ($AUC(0,\infty)$), or the truncated AUC as appropriate. In three studies (three alendronic acid formulations) the primary results were the maximum observed excretion rate ($U_{rate_{max}}$) and the accumulated elimination ($Ae(0,\infty)$). Only one analyte per study was included in the analysis, usually the parent drug. In seven BE studies the pharmaceutical products had two active ingredients and in one study three (isoniazid, pyrazina-

Table 2

Drugs excluded in the sensitivity analysis

Drug	Permeability classification		Spanish data from Takagi <i>et al.</i> [16]	
	Data of Amidon <i>et al.</i> [15] ClogP-based	LogP-based	ClogP	LogP
Amlodipine	1	3	1	3
Amoxicillin	4	4	3	3
Loratidine	1	1	2	2
Lorazepam	2	2	1	1
Losartan	3	3	1	NC
Ondansetron	1	3	1	3
Pravastatin	1	3	1	3

Discordances found in permeability classification between ClogP-based vs. logP-based on Amidon *et al.* [15] vs. Spanish Data from Takagi *et al.* [16] of Biopharmaceutics Classification System (BCS).

midate and rifampicin). In these cases all active substance were analyzed independently for BE purposes.

Statistical analysis

Standard analysis of bioequivalence Methodology and statistical notations of BE studies are well described in the

EMA guidelines [4]. These calculations were made with the WinNonlin 2.0 program. From the WinNonlin statistical analysis, we calculated the power *a posteriori*, T : R mean ratios, the 90% CI and the mean square error (MSE) of ANOVA on the log scale,

The products were considered bioequivalent if the 90% confidence intervals (CI) of the log means of ratios of $C_{\max}$ and AUC (Urate_{max} and Ae) fell within 80% and 125%.

Calculating the point estimate, inter and intra-subject variability We showed the T : R mean ratios as a subtraction from 100, resulting in a point estimate. Inter-subject variability ($CV_{\text{Inter-subject}}$) was obtained exclusively from the reference formulation. Intrasubject variability ($CV_{\text{Intrasubject}}$) was calculated from the MSE of the corresponding analysis of variance (ANOVA) using the following formula ($CV_{\text{Intra-subject}} = \text{SQRT}(\text{EXP}(\sigma^2) - 1)$, where σ^2 denotes the residual (intra-subject) variance of the logarithmically transformed characteristics data [17].

Recalculation of the sample size In the case of data from non-BE studies we recalculated the number of subjects (n_r) using the following formula ($n_r = ((Z_{1-\beta} + Z_{1-\alpha})^2 \cdot 2 \cdot \sigma^2) / (\ln\theta - \ln(1.25))^2$ [18].

where σ^2 is the within-subject variability on the log scale and n_r is the retrospective number of subjects calculation. Let $\theta = \mu_T / \mu_R$ where μ_T and μ_R denote the mean parameters for test and reference, and let $\ln$ denote the natural logarithm. This equation provides reasonable approximations of the sample size for $\mu_T / \mu_R \neq 1$, as the power of a study is very sensitive to the assumption about the point estimate. n_r was calculated for a type I error of 5% and $\theta = 0.05$.

Analysis of BCS groups To analyze possible differences between formulations containing active substances from different BCS classes we evaluated the study number of subjects (n), point estimate and $CV_{\text{Intrasubject}}$ and the $CV_{\text{Inter-subject}}$ with respect to $C_{\max}$ and AUC in BE studies by means of a Student's *t*-test for two samples with unequal variances or a Mann-Whitney U-test for non-Gaussian distribution, respectively, using SPSS 15.0 Statistical Analysis Software (SPSS, Chicago, IL, USA).

Results

Out of 124 BE studies 89 (71.77%) were performed with active substances classified by the BCS (Table 1) and 77 (62.1%) were classified in the sensitivity analysis described in the methodology. Table 3 summarizes the number of products that fulfilled BE (for $C_{\max}$ and AUC) vs. those not fulfilling BE, and it also shows the distribution (mean $\pm$ SD) of the number of subjects in the study (n), the point estimate, the $CV_{\text{Intra-subject}}$ and the $CV_{\text{Inter-subject}}$ for $C_{\max}$ and AUC of studies with active substances grouped by BCS.

In all BCS classes there were non-bioequivalent pharmaceutical products, reaching 50% in class 2. Thirty-five out of 124 pharmaceutical products did not fulfil BE criteria because $C_{\max}$ exceeded limits, and 15 did not comply with the AUC. Comparisons between all classes have showed some statistical differences (Table 3).

Table 4 shows the results from non-BE pharmaceutical products for $C_{\max}$ and AUC classified by active substance according to the four categories of BCS. We also have grouped these non-BE products according to differences <20% of point estimate (Group A) or >20% (Group B) (meaning differences between formulations), and by the power *a posteriori* >0.8 (Group 1) or <0.8 (Group 2) (assuming the validity of the results).

In a sensitivity analysis (Table 5) the removal of five studies because of discordances in the classification of active substances of the BCS of three BE and two non-BE active substances of class 1 changed the ratio BE : Total number of studies of products from 15.38% to 9.52% and the removal of another five BE active substances from class 4 improved the ratio from 12.5% to 5.69%. For class 2 and 3 only one active substance were excluded, respectively. Nevertheless sample size, point estimates, $CV_{\text{Intra-subject}}$ and $CV_{\text{Inter-subject}}$ between classes continued to show the same significant differences, reinforcing our results. Excluding the three formulations of class 3 active substances where the primary results were urine analysis, because of extra biological variability, differences remained for the point estimate with respect to class 4 drugs for $C_{\max}$ and we found no other significant differences in both analyses.

Moreover, when we removed those studies in which the intra-subject variability of formulations was higher than predicted (Table 4, Group A2, defined as those active substances from studies with insufficient power *a posteriori* and a point estimate <20%) (two with class 1, nine with class 2 and two with class 3 active substances), there were non-BE formulations with active substances from classes 1, 2 and 3 (Table 4).

Discussion

$C_{\max}$ is the cornerstone for the demonstration of bioequivalence because almost always it is more variable than AUC. In fact, in our data from 124 formulations 35 did not comply with BE acceptance criteria because of $C_{\max}$ and 15 because AUC exceeded the limits, and between these 14 were also out of limits for $C_{\max}$. For class 1, non-BE was always due to $C_{\max}$, not AUC.

Comparison between all classes showed some statistical differences in terms of study n , the point estimate, $CV_{\text{Intra-subject}}$ and the $CV_{\text{Inter-subject}}$ for $C_{\max}$ and AUC, but in our opinion these differences did not define differential characteristics between classes.

Although it is believed that class 1 active substances may not require *in vivo* BEQ demonstration, 4 of 26

Table 3

BCS classification (ClogP-based) of active substances tested in 124 BE studies with generic pharmaceutical products for maximum observed plasma concentration (C_{max}) and area under the concentration–time curve (AUC) or maximum observed excretion rate ($U_{rate_{max}}$) and accumulated elimination ($Ae(0,\infty)$) as appropriate

	BE Non BE	% Non BE	n mean (SD)	Point estimate mean (SD)	Intra-subject CV mean (SD)	Inter-subject CV mean (SD)
Ln C_{max} (124)	89 35	28.23				
Class 1 (26)	22 4	15.38	34 (6.80)	4.99 (3.97)	22.84 (9.57)	37.03 (14.23)
Class 2 (28)	14 14	50	33 (12.28)	13.05 (14.40)	29.65 (16.77)	51.53 (48.67)
Class 3 (22)†	19 3	13.63	36 (19.56)	9.23 (13.75)	26.91 (14.53)	42.22 (24.93)
Class 4 (13)	12 1	7.69	25 (3.59) 24 (24–36)*	2.80 (2.63) 1.09 (0–7.16)*	24.58 (7.78) 22.59 (14.99–43.16)*	31.04 (6.41) 32.25 (23.36–44.23)*
NC (35)	22 13	37.14	30 (9.87)	10.26 (11.97)	29.09 (17.78)	37.72 (14.01)
Ln AUC (124)	109 15	12.10				
Class 1 (26)	26 0	0	34 (6.80)	3.44 (2.49)	16.29 (5.14)	42.89 (21.78)
Class 2 (28)	21 7	25	33 (12.28)	7.90 (6.38)	21.01 (13.87)	55.02 (62.63)
Class 3 (22)†	20 2	9.1	36 (19.56)	4.72 (5.47)	17.17 (14.95)	37.84 (24.04)
Class 4 (13)	13 0	0	25 (3.59) 24 (24–36)*	4.16 (2.70)	17.10 (6.13) 12.55 (5.43–45.71)*	24.39 (6.11) 23.43 (18.03–40.78)*
NC (35)	29 6	17.14	30 (9.87)	7.07 (7.31)	23.71 (17.55)	38.73 (12.90)

Non-classified (NC), Study number of subjects (n), the subtractions from 100% of mean ratios (point estimate), intra-subject coefficient of variability ($CV_{intra-subject}$), intersubject coefficient of variability ($CV_{inter-subject}$). $CV_{intra-subject}$, the coefficient of variation in the multiplicative model, is the estimate from the MSE of the corresponding ANOVA, $ICV = \sqrt{\exp(\sigma^2) - 1}$, where σ^2 denotes the residual (within-subject) variance of the logarithmically transformed characteristics. (p) is the value of Student's *t*-test for two samples with unequal variances or the value of a Mann Whitney test as appropriate, between one class (start dotted/dashed line) with respect other class (end of dotted/dashed line). $P < 0.05$ $P \leq 0.01$ ——— $P \leq 0.005$ ———. †In three studies the primary results were analysis of $U_{rate_{max}}$ and $Ae(0,\infty)$. *Median (range).

pharmaceutical products containing class 1 active substances (two in the sensitivity analysis) did not comply with EMA requirements, particularly because C_{max} exceeded the limits. In two cases (sildenafil and loratadine) the $CV_{intra-subject}$ was higher than predicted (Group A2 Table 4). In another two cases (pravastatin and zolpidem) the differences in point estimates of C_{max} were higher than expected, falling out of the 90% CI limits of 80–125%, with an intra-subject variability as predicted, meaning that the disposition between formulations was truly different (Group A1 Table 4). With our data, the positive predictive value (PPV) of the usually employed *in vitro* dissolution test for quality control purposes was 92% (95% CI 73, 99%) (after excluding two un-powered studies (group A2)) for formulations containing class 1 BCS drugs.

In our study, more than 50% of pharmaceutical products containing class 2 active substances failed the BE demonstration of C_{max} (50% or 51.85% in the sensitivity analysis) or AUC (25% or 25.93% in the sensitivity analysis). Ibuprofen is a class 2 weak acid proposed for biowaiver by WHO [13]. We conducted BE studies with eight formulations of ibuprofen of which four resulted in non-BE, three

of them because of dramatic differences between formulations. These formulations may have presented more problems in determining bioequivalence due to the influence of bioavailable excipients and the highly variable behaviour of these compounds or maybe because the inability of usually employed *in vitro* dissolution tests to predict *in vivo* behaviour. The PPV of conventional usually employed *in vitro* dissolution tests for formulations containing class 2 BCS drugs (excluding two un-powered studies (group A2)) was 61% (95% CI 39, 80%).

Characteristics of class 3 active substances are high solubility and low permeability. Permeation through the intestinal membrane is the rate limiting process for drug absorption. In our results, 13.63% of studies were non-BE, which is similar to class 1 (15.38%). This type of drug seems to be, in terms of differences between point estimates and intra-subject variability, between class 1 and 2. In one case (ranitidine) the intra-subject variability was higher than predicted (Group A2). In another two cases (codeine and isoniazid) the differences in point estimates of C_{max} exceeded 20% (<80% or >125%). In these cases, the probability of acceptance is zero because of dramatic differ-

Table 4

Results of non-BE formulations (study number of subjects, retrospective sample size calculation) classified by BCS active substances for maximum observed plasma concentration (C_{max}) and area under the concentration-time curve (AUC)

Point estimate Group A (<20%)		Group B (>20%)	
Power a posteriori			
Group 1 (>0.8)			
<u>Class 1</u> C _{max} : Pravastatin (36,34), Zolpidem (36, 27)	<u>Class 2</u> C _{max} : Clopidogrel-2 (82,84), Ibuprofen-2 (36, 9), Irbesartan (35, 23) AUC: Clopidogrel-1 (36, 54), Clopidogrel-2 (82, 51), Risperidone (34, 24)	<u>Class 1</u>	<u>Class 2</u> C _{max} : Ibuprofen-1 (24, 15) Ibuprofen-3 (35, 10), Ibuprofen-4 (24, 18), Lovastatin-1 (36,37), Risperidone (34, 27), Simvastatin (36, 43) AUC: Ibuprofen-3 (35, 5), Simvastatin (36, 30)
<u>Class 3</u>	<u>Class 4</u>	<u>Class 3</u> C _{max} : Codeine (35, 34), Isoniazid (24, 25)	<u>Class 4</u>
<u>NC</u> C _{max} : Bromazepam (33, 43), Deflazacort (35, 34), Flutamide (24, 35), Lormetazepam-2 (24, 25), Rifampicin (24, 15) AUC: Bromazepam (33, 26), Clavulanate-2 (24, 18)		<u>NC</u> C _{max} : Azythromycin-2 (35, 33), Clarithromycin (36, 32) AUC: Pentoxifiline (12, 12)	
Group 2 (< 0.8)			
<u>Class 1</u> C _{max} : Sildenafil (35, 66), Loratadine (35, 69)	<u>Class 2</u> C _{max} : Aceclofenac (23, 42), Clopidogrel-1 (36, 137), Clopidogrel-3 (54, 235), Lovastatin-2 (36, 65), Lovastatin-3 (36, 53) AUC: Clopidogrel-3 (54, 88), Lovastatin-1 (36, 63)	<u>Class 1</u>	<u>Class 2</u>
<u>Class 3</u> C _{max} : Ranitidine (23, 50) AUC: Lisinopril (24, 52)	<u>Class 4</u> C _{max} : Oxcarbazepine (24, 69)	<u>Class 3</u> AUC: Codeine (35, 47)	<u>Class 4</u>
<u>NC</u> C _{max} : Azythromycin-1 (35, 70), Lercanidipine (35, 97) AUC: Flutamide (24, 35)		<u>NC</u> C _{max} : Clavulanate-1 (24, 233), Clavulanate-2 (24, 235), Lormetazepam-1 (24, 41), Pentoxifiline (12, 30) AUC: Clarithromycin (24, 32), Clavulanate-1 (24, 170),	

Formulation-Number of study with the same drug. Behind formulation appears between parenthesis, two numbers (n, nr), showing the study number of subjects (n) and retrospective sample size calculation (nr).

ences between formulations (Group B1 Table 4). The PPV of usually employed *in vitro* dissolution tests is 86% (95% CI 70, 99%) (excluding two unpowered studies (Group A2)) for formulations containing class 3 BCS drugs. Wu & Benet [19] developed a Biopharmaceutics Drug Disposition Classification System (BDDCS) based on the extent of metabolism, instead of permeability or extent of absorption. They think it inappropriate to have a biowaiver for this class until more is known about the effect of formulation components on intestinal transport and until there is a validated methodology to predict these effects except, perhaps, in the case of identical excipients [9].

Active substances from class 4 are of low solubility and low permeability and show significant problems for effective oral delivery. The number of pharmaceutical products that fall into this class depends on the precise limit used for the definition of permeability and solubility. Surprisingly, class 4 has the lowest percentage of non-bioequivalent

formulations (7.69%, 0% in the sensitivity analysis). In terms of point estimates, class 4 showed the lowest value, a value significantly lower than class 2 and 3, although this fact should be considered cautiously because of the limited number of studies in our series. We note that these compounds showed the lowest inter-subject variability in AUC of all four BCS classes, significantly lower than class 1 (Table 3), which may be due to the absence of saturation of intestinal transport and enzymes affected by the inter-subject differences in their function. The PPV of conventional *in vitro* dissolution tests is 100% for formulations containing class 4 BCS drugs.

The BCS developed by Amidon *et al.* [6] with the objective to predict from a more specific *in vitro* dissolution test the bioavailability of a drug has produced an important impact based on the possibility to waive *in vivo* bioavailability and bioequivalence testing for immediate release solid dosage forms for class 1 (FDA, EMA and WHO) [7, 8,

Table 5

Sensitivity analysis, BCS classification (ClogP-based) of active substances tested in 124 BE studies for maximum observed plasma concentration (C_{max}) and area under the concentration–time curve (AUC) or or maximum observed excretion rate ($U_{rate\ max}$) and accumulated elimination ($Ae(0,\infty)$), as appropriate, excluding discordances

	BE Non BE	% Non BE	n Mean (SD)	Point estimate Mean (SD)	CV _{Intra-subject} Mean (SD)	CV _{Inter-subject} Mean (SD)
Ln C_{max} (124)	89 35	28.23				
Class 1 (21)	19 2	9.52	33 (7.51)	4.89 (3.87)	22.47 (9.19)	36.01 (14.14)
Class 2 (27)	13 14	51.85	34 (12.37)	13.38 (14.57)	30.27 (16.76)	52.80 (49.11)
Class 3 (21)†	18 3	14.29	36 (20.04)	9.62 (13.97)	26.42 (14.70)	42.14 (25.54)
Class 4 (8)	7 1	12.50	25 (2.12) 24 (24–30)*	2.31 (2.37) 1.05 (0–5.58)*	27.35 (8.46) 24.73 (18.19–43.16)*	32.43 (7.26) 33.14 (23.91–44.23)*
NC (47)	32 15	31.92	30 (8.45)	7.90 (9.95)	25.88 (15.58)	35.61 (14.39)
Ln AUC (124)	109 15	12.10				
Class 1 (21)	21 0	0	33 (7.51)	3.20 (2.29)	15.83 (4.78)	41.58 (22.26)
Class 2 (27)	20 7	25.93	34 (12.37)	8.07 (6.44)	21.46 (13.93)	55.88 (63.89)
Class 3 (21)†	19 2	9.52	34 (20.04)	4.91 (5.53)	17.38 (15.28)	37.60 (24.61)
Class 4 (8)	8 0	0	25 (2.12) 24 (24–30)*	3.75 (2.75) 3.48 (0.53–8)*	17.59 (5.87) 16.53 (10.31–30.53)*	26.59 (6.40) 24.48 (20.49–40.78)*
NC (47)	41 6	12.77	30 (9.87)	5.85 (6.10)	20.37 (14.72)	37.46 (17.70)

Non-classified (NC), Study number of subjects (n), the subtractions from 100% of mean ratios (point estimate), intra-subject coefficient of variability (CV_{Intra-subject}), intersubject coefficient of variability (CV_{Inter-subject}). CV_{Intra-subject}, the coefficient of variation in the multiplicative model, is the estimate from the MSE of the corresponding ANOVA, $ICV = \sqrt{\exp(\sigma^2_2) - 1}$, where σ^2_2 denotes the residual (within-subject) variance of the logarithmically transformed characteristics.

(P) is the value of Student's *t*-test for two samples with unequal variances or the value of a Mann Whitney test as appropriate, between one class (start dotted/dashed line) with respect other class (end of dotted/dashed line). $P < 0.05$ $P \leq 0.01$ ——— $P \leq 0.05$ ———. †In three studies the primary results were $U_{rate\ max}$ and $Ae(0,\infty)$ parameters analysis. *Median (range).

13], for class 3 (EMA and WHO) [8, 13] and for a specific group of class 2 (WHO) [13]. This means there is the possibility of decreasing the cost of bringing multisource orally administered drugs to the market. Nevertheless, more than a decade after the pioneering work of Amidon *et al.*, 35 (28%) of the formulations of our series are still un-classified and in another 10% more there are classification discordances. To this point we must add that FDA guidelines and EMA and WHO proposals to biowaive BE requirements also show discordances in definitions of high solubility (the decrease in pH from 7.5 in the FDA guidance to 6.8 in WHO and EMA proposals) and high permeability (the permeability criterion was relaxed from 90% in the FDA guidance to 85% in the WHO and EMA proposals).

The primary concern from the regulatory point of view is the protection of the patient against approval of products that are not bioequivalent. The current practice in the *in vivo* studies of carrying out two one-sided tests at the 0.05 level of significance ensures that there is no more than a 5% chance that a generic product that is not truly equivalent to the reference will be approved [20]. However, with

our data, with the usually employed *in vitro* dissolution tests, except for class 4, there were more than 5% of false positives.

In fact, our data show that some products containing class 1, class 2 and class 3 active substances do not comply with the EMA regulation requisites for bioequivalence although these formulations satisfied the test dissolution methodology proposed as quality control. Therefore we suggest the possibility that drug manufacturers may not develop these *in vitro* dissolution tests properly, or perhaps these usually employed *in vitro* tests may be insufficient to allow a waiver of *in vivo* bioequivalence.

However we must also point out some limitations in the study design that limit the comparison between BCS classes such as the limited number of studies with class 4 drugs and the absence of a calculation of sample size to ensure the power of comparisons between classes.

In our opinion, the demonstration of bioequivalence is a major concern for the approval and the use of generic products, and the possibility of a biowaiver for *in vivo* BE studies needs to be approached with greater caution and

careful supervision in order to guarantee the efficacy and safety of these medicines.

In conclusion, comparisons between pharmaceutical products with active substances from the four BCS classes have not allowed us to define differential characteristics of each class in terms of n , $CV_{\text{Intra-subject}}$ and $CV_{\text{Inter-subject}}$ for C_{max} or AUC. Despite the usually employed test dissolution methodology proposed as quality control, pharmaceutical products with active substances from the four classes of BCS showed non-BE studies. Pharmaceutical products in classes 1 and 3 were similar but failed to comply with BE requirements in nearly 15% of the cases. Products containing class 2 drugs were the less compliant with BE requirements, failing in at least 50% of cases. Moreover, once those formulations with a $CV_{\text{Intra-subject}}$ higher than predicted were excluded, there were non-BE products containing drugs from classes 1, 2 and 3.

Competing interests

All authors have been working in the Phase I Clinical Trial Unit of the Clinical Pharmacology Service at La Paz University Hospital and the School of Medicine, Autonomous University of Madrid, Spain.

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Author's contributions: Elena Ramirez conceived the study, participated in the design of the study, conducted the review of Phase I Clinical Trial Unit studies and the extraction of data, the performance of the statistical analysis, the interpretation of the results and the writing of the paper. Olga Laosa participated in the design of the study, in the review and interpretation of the results and the writing of the paper. Pedro Guerra participated in the review and interpretation of the results and the writing of the paper. Blanca Duque participated in the review of Phase I Clinical Trial Unit studies, the extraction of data, in the review of the results and the writing of the paper. Beatriz Mosquera participated in the review of the Phase I Clinical Trial Unit studies, the extraction of data, in the review of the results and the writing of the paper. Alberto M. Borobia participated in the performance of the statistical analysis and in the review of the results. Suhua H. Lei participated in the in the performance of the statistical analysis and in the review of the results. Antonio J. Carcas participated in the interpretation of the results and the writing of the paper. Jesús Frias participated in the design of the study, in the review and interpretation of the results and the writing of the paper.

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