Journal of Pharmaceutical Sciences xxx (2018) 1-8

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Pharmacokinetics, Pharmacodynamics and Drug Transport and Metabolism

Evaluation of Using Dogs to Predict Fraction of Oral Dose Absorbed in Humans for Poorly Water-Soluble Drugs

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ARTICLE INFO

Article history:
Received 22 March 2018
Revised 16 May 2018
Accepted 22 May 2018

Keywords:
solubility
dissolution
permeability
oral absorption
species difference
dog
BCS

ABSTRACT

Dogs have been widely used to study the oral absorption of a drug in drug discovery. However, there has been no quantitative validation of using dogs to predict the fraction of oral dose absorbed (*Fa*) in humans (Fa_h) for poorly water-soluble drugs. Here, we report the results of using dogs for quantitative Fa_h prediction, focusing on poorly water-soluble free acid and neutral drugs. The *Fa* values of 4 acidic and 1 neutral proprietary compounds were measured in humans and dogs. Extensive literature survey was also performed to increase the number of *Fa* data. Fa_h and *Fa* in dogs (Fa_d) were then compared at equivalent body weight−normalized doses. In the case of neutral compounds, Fa_d was found to be similar to Fa_h. In the case of acidic compounds, Fa_d significantly overestimated Fa_h in most cases. A difference in intestinal pH was suggested as the main reason for this discrepancy. In conclusion, the use of dogs would not be appropriate to predict Fa_h for acidic compounds, but more work is required to know about neutral compounds.

Introduction

Recent drug candidates tend to show poor aqueous solubility.[1-4] Poor aqueous solubility can cause incomplete and variable oral absorption in humans. Therefore, the oral absorption of a poorly water-soluble drug candidate should be appropriately evaluated at the preclinical stage for successful drug development.[5] At the preclinical stage, dogs are frequently used to study *in vivo* performances of formulations. However, there are several species differences in the physiology of the gastrointestinal tract between dogs and humans.[6,7] Therefore, the rate and extent of oral drug absorption in dogs could be different from that in humans.

Previously, Chiou et al.[8] investigated the correlation between the fraction of oral dose absorbed (*Fa*) in dogs (Fa_d) and humans (Fa_h) for high solubility drugs. However, for low solubility drugs, the correlation between Fa_d and Fa_h has not been investigated.

The purpose of the present study was to evaluate the use of dogs to predict the *Fa* of poorly water-soluble drug candidates in humans. As the gastric pH in dogs shows interindividual and intraindividual variability,[9,10] to neglect the effect of gastric pH on *Fa*, free acid and neutral drug candidates were selected as test compounds in this study (Table 1). The test compounds were orally administered to humans and dogs. Fa_d and Fa_h were then compared at body weight (BW)-normalized doses. The effects of bile micelles and pH on the solubility of the test compounds were also investigated in this study.

Materials and Methods

Materials

The 5 test compounds were synthesized at Ono Pharmaceutical Co., Ltd. Metoprolol tartrate was purchased from Sigma-Aldrich Japan (Tokyo, Japan). Carbamazepine was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Exjade® 125 mg tablets

Abbreviations used: *Fa*, fraction of oral dose absorbed; Fa_h, *Fa* in humans; Fa_d, *Fa* in dogs; k_{el}, elimination rate constant; *Do*, dose number; *MAD*, maximum absorbable dose; BW, body weight; FaSSIF, fasted state simulated intestinal fluid; $FaSSIF_h$, biorelevant media for humans; $FaSSIF_d$, biorelevant media for dogs; BSA, bovine serum albumin; P_{app}, apparent permeability; UWL, unstirred water layer; BCS, biopharmaceutics classification system; PDE, particle drifting effect.

Conflicts of interest: The authors declare no competing financial interest.

This article contains supplementary material available from the authors by request or via the Internet at https://doi.org/10.1016/j.xphs.2018.05.016.

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https://doi.org/10.1016/j.xphs.2018.05.016
0022-3549/

Table 1
Physicochemical Properties of the Test Compounds

Physicochemical Properties	ONO-A	ONO-B	ONO-C	ONO-D	ONO-E
MW[a]	400	600	500	550	560
Type	Neutral	Acid	Acid	Acid	Acid
p*Ka*[b]	NA	4.72, 4.73	4.24	4.09	4.44
Log*P*[c]	3.70	9.00	5.35	5.21	6.49
Log*D* (pH 6.5)[c]	3.70	5.44	3.09	4.31	5.59
Log*D* (pH 7.4)[d]	3.70	3.65	2.19	1.90	3.53
Melting point (°C)	217	158	214	199	124
Solubility (μg/mL)[e]					
Blank $FaSSIF_h$	0.0706 ± 0.0038	0.00573 ± 0.00339	0.910 ± 0.013	3.87 ± 0.12	3.45 ± 0.10
Blank $FaSSIF_{d,pH\ 7.5}$	0.0478 ± 0.0095	0.635 ± 0.082	15.4 ± 0.2	75.6 ± 3.3	41.5 ± 0.9
$FaSSIF_h$	22.2 ± 0.2	37.4 ± 0.5	5.67 ± 0.04	12.4 ± 0.2	56.3 ± 0.5
$FaSSIF_{d,pH\ 6.5}$	53.1 ± 0.3	64.3 ± 0.5	14.8 ± 0.4	25.2 ± 0.3	105 ± 1
$FaSSIF_{d,pH\ 7.5}$	50.2 ± 0.4	500 ± 4	115 ± 2	168 ± 2	565 ± 7
Permeability[f]					
Caco-2 permeability (10^{-6} cm/s)	102 (27.4)	331 (2.39)	43.8 (19.2)	54.3 (24.6)	256 (12.6)
Unbound fraction in Caco-2 assay	0.269 ± 0.008	0.00721 ± 0.00196	0.438 ± 0.021	0.453 ± 0.011	0.0492 ± 0.0076
Other properties					
Particle diameter (μm)	3.2	2.9	2.1	1.8	2.5
BCS class	2	2	2	2	2

MW, molecular weight; NA, not applicable.
[a] Rounded to the first digit.
[b] Calculated using Advanced Chemistry Development (ACD/Labs) Software, Version 12.0.
[c] Calculated from measured log*D* at pH 7.4.
[d] Measured value.
[e] Mean ± SD, $n = 3$.
[f] The value corrected by unbound fraction of the test compound in the donor solution (the value without corrected by unbound fraction). Mean ± SD, $n = 3$.

were purchased commercially from Japanese market. Deferasirox was extracted from the Exjade tablets and recrystallized from ethanol water. The fasted state simulated intestinal fluid (FaSSIF) powder (previously known as 'SIF Powder') and the dog FaSSIF powder were purchased from Biorelevant.com Ltd. (London, UK). Bovine serum albumin (BSA) was purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals were of standard research grade.

Equilibrium Solubility Measurement

The equilibrium solubility of the test compounds in biorelevant media was determined by a shake-flask method. The composition and pH of the biorelevant media are shown in Table 2.[11,12] The biorelevant media for humans ($FaSSIF_h$) was prepared by diluting the FaSSIF powder for humans with a phosphate buffer (blank $FaSSIF_h$: 106 mM sodium chloride, 28.4 mM monobasic sodium phosphate, adjusted to pH 6.5 by NaOH). The biorelevant media for dogs ($FaSSIF_d$) was prepared in the same manner (blank $FaSSIF_d$: 59.6-mM sodium chloride, 28.7-mM monobasic sodium phosphate, adjusted to pH 6.5 or 7.5 by NaOH) ($FaSSIF_{d,pH\ 6.5}$ and $FaSSIF_{d,pH\ 7.5}$, respectively).

Six milligrams of each test compound was added to 6 mL of a biorelevant medium, and the resulting suspension was stirred for 24 h at 37°C. The suspension was then passed through a Millex GV filter (0.22 μm, polyvinylidene fluoride; Millipore). The first few drops were discarded. The concentration of the test compound in the filtrate was determined using high-performance liquid chromatography or liquid chromatography/tandem mass spectrometry (LC/MS/MS) with electrospray ionization (Tables S1 and S2, Supplemental Information). The experiment was performed in triplicate.

Octanol–Buffer Partition Coefficient

An aliquot (15 μL) of 10 mM stock solution of each test compound in dimethylsulfoxide was added to a test tube containing 0.75 mL of octanol and 0.75 mL of pH 7.4 sodium phosphate buffer (0.07 M). The test tubes were then rotated for 1 h using a bench-top rotator at room temperature. The test tubes were allowed to stand for 1 h to separate the octanol and buffer phases. After serial dilutions, the compound concentration in each phase was determined using LC/MS/MS with electrospray ionization. The experiment was performed in duplicate, and the average value was reported.

Caco-2 Permeation Assay

Caco-2 cells (clone C2BBe1) were obtained from American Type Culture Collection (Manassas, VA). A Caco-2 cell monolayer was grown to confluence on collagen-coated, microporous, polycarbonate membranes in 12-well Costar Transwell plates. The permeability assay buffer for the donor chamber (apical side) was Hank's balanced salt solution containing 10-mM 2-(N-morpholino) ethanesulfonic acid, 15-mM glucose, and 0.01% BSA, at a pH of 6.5. The buffer for the receiving chambers (basal side) was Hank's balanced salt solution containing 10-mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 15-mM glucose, and 4.0% BSA, at a pH of 7.4. The concentration of the test compound in the donor solution was set as 1 μM. The cell monolayer was then incubated at 37°C with 5% CO_2 in a humidified incubator. Samples were taken

Table 2
Composition and pH of Biorelevant Media

Composition	$FaSSIF_h$	$FaSSIF_d$
pH	6.5	6.5 or 7.5
Sodium taurocholate (mM)	3.0	5.0
Sodium taurodeoxycholate (mM)	–	5.0
Lecithin (mM)	0.75	1.25
Lysolecithin (mM)	–	1.25
Sodium oleate (mM)	–	1.25
Sodium chloride (mM)	106	59.6
Monobasic sodium phosphate (mM)	28.4	28.7
Sodium hydroxide (mM)	8.7	21.7