European Journal of Pharmaceutics and Biopharmaceutics 80 (2012) 402–409

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

In vitro vs. canine data for assessing early exposure of doxazosin base and its mesylate salt

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ARTICLE INFO

Article history:
Received 31 May 2011
Accepted in revised form 5 October 2011
Available online 14 October 2011

Keywords:
Doxazosin base
Doxazosin salts
Dissolution
Early exposure
Humans
Dogs

ABSTRACT

In this study, we evaluated the usefulness of biorelevant *in vitro* data and of canine data in forecasting early exposure after the administration of two phases of a BCS Class II compound, i.e., doxazosin base (DB) and its mesylate salt (DM). DB, DM, and doxazosin hydrochloride (DH) were prepared and characterized. *In vitro* data were collected in various media, including human aspirates. Solubilities of DB and DM in human gastric fluid were forecasted by data in fasted state simulating gastric fluid containing physiological components (FaSSGF-V2) but not by data in $HCl_{pH\ 1.8}$. Unlike data in FaSSGF-V2, dissolution of DB and DM tablets in $HCl_{pH\ 1.6}$ is rapid. Dissolution of DB tablet in FaSSGF-V2 is incomplete and conversion to DH seems to occur. Differences between DB and DM in dissolution in the small intestine are overestimated in the absence of physiological solubilizers. Using the *in vitro* data and previously described modeling procedures, the cumulative doxazosin profile in plasma was simulated and the 0–2 h profile was used for evaluating early exposure. Individual cumulative doxazosin profiles in plasma, after single DM tablet administrations to 24 adults, were constructed from corresponding actual plasma profiles. Compared with *in vitro* DM data in pure aqueous buffers, DM data in biorelevant media led to better prediction of early exposure. Based on intersubject variability in early exposure after DM administration and simulated profiles, the administered phase, DB or DM, does not have a significant impact on early exposure. Partial AUCs were used for evaluating early exposure after DB and DM administration in 4 dogs. Early exposure was significantly higher after administration of DM to dogs. Dogs are not appropriate for evaluating differences in early exposure after DB and DM administrations.

1. Introduction

Drug design based on high throughput screening has in general led to more lipophilic compounds exhibiting low aqueous solubility [1]. Due to their ionization properties, lipophilic bases dissolve easier in the acidic gastric environment than in the almost neutral pH of the upper small intestine but complete dissolution of the dose prior to reaching the small intestine, may not be possible, due to limited gastric residence times in the fasted state [2]. Incomplete dissolution of the dose of a lipophilic base in the fasted stomach is likely in cases where the subject is hypochlorhydric, the dosage form disintegrates slowly, and/or the compound is highly dosed.

Salt formation is a well-known and frequently used technique to modify and optimize the physicochemical properties of ionizable compounds, including solubility and dissolution rates, hygroscopicity, stability, impurity profiles, and crystal habit [3]. For most practical purposes, identification and selection of salt forms of new chemical entities still remain a trial and error process that takes place early during drug development and is based on *in vitro* and/or animal (usually, canine) data.

Improvement of dissolution of the dose during gastric residence with the use of salt of a base is typically decided on the basis of dissolution data and/or equilibrium solubility data in hydrochloric acid solutions [4]. However, equilibrium solubility of weak bases is not exclusively dependent on hydrochloric acid concentration [5]. If dissolution is more complete *in vitro* than in stomach, its importance on plasma levels may (depending on disposition characteristics) be underestimated and vice versa [6]. Furthermore, if the dose is partly dissolved during gastric residence, problematic

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doi:10.1016/j.ejpb.2011.10.004

dissolution in the small intestine can lead to low and/or variable oral bioavailability, especially in cases where intra-lumenal concentrations control the overall absorption process. The extent at which the environment in the small intestine needs to be simulated for evaluating differences in dissolution between a free base and its salts has not been addressed in the literature.

On the other hand, dogs are one of the most frequently used animal models for the assessment of oral drug absorption, especially early during the drug development process when the appropriate phase for human studies is to be chosen. However, dogs have faster gastric emptying and often less acidic fasting intragastric pH compared with humans [7]. Also, they may have lower osmolarity in the stomach and higher bile salts concentrations in the small intestine [8]. Such differences from the human lumenal environment may limit their usefulness in the comparison of salt(s) with the free form of a base.

The objective of the present investigation was to evaluate the usefulness of biorelevant *in vitro* data and of canine data in forecasting early exposure, after administration of doxazosin base (DB) and doxazosin mesylate (DM) (Fig. 1). DM is a highly permeable compound [9]. DB has a p*K*a of 6.93 at 25 °C (Product monograph Cardura®, Pfizer), and a log*P* between 2.1 and 2.8 [http://www.drugbank.ca/drugs/DB00590; http://www.69.20.123.154/services/bcs/results.cfm (accessed December 27, 2010)]. Since bases and/or non-HCl salts can be converted to their HCl salts in stomach [4], apart from DB and DM, the hydrochloric salt of doxazosin (DH, Fig. 1) was also prepared, characterized, and studied *in vitro*.

2. Materials and methods

2.1. Chemicals and materials

Sodium taurocholate, min 95% pure (lot no. 033K5306) and 99.5% pure (lot no. 2003040161) were purchased from Sigma–Aldrich Chemie GmbH (Steinheim, Germany) and Prodotti Chimici e Alimentary S.p.a. (Basaluzzo, Italy), respectively. Egg phosphatidylcholine min. 98 % pure (lot no. 105019-1/129 and 105026-1/62) was donated by Lipoid GmbH, (Ludwigshafen, Germany). Potassium dihydrogen phosphate, sodium hydroxide, sodium chloride, hydrochloric acid, and trichloromethane, all analytical grade, were purchased from Panreac Quimica SA (Barcelona, Spain). Maleic anhydride was purchased from Sigma–Aldrich (Steinheim, Germany). Acetic acid (glacial) of analytical grade and acetonitrile and methanol, both HPLC grade were purchased from E. Merck (Darmstadt, Germany).

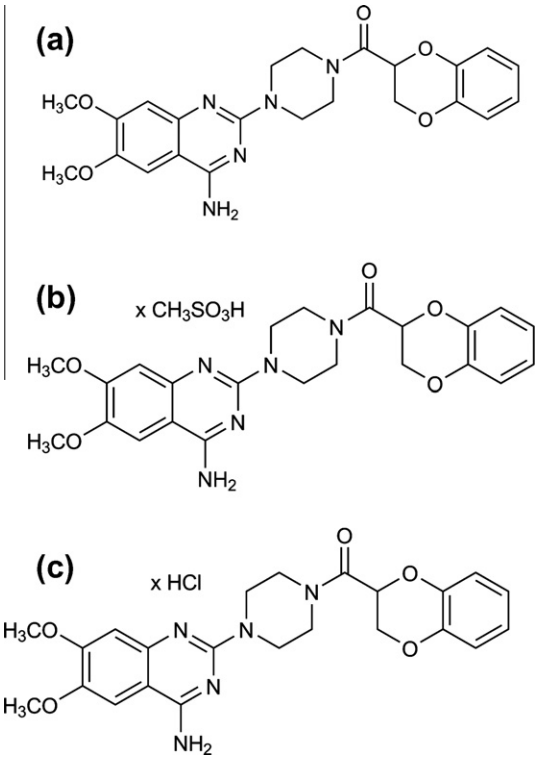

Fig. 1. Structure of doxazosin base (DB, MW: 451.5) (a), doxazosin mesylate (DM, MW: 547.6) (b), and doxazosin hydrochloride (DH, MW: 487.9) (c).

2.2. Preparation and characterization of DB, DM, and DH

Doxazosin base (DB), doxazosin mesylate (DM), and doxazosin hydrochloride (DH) powders were prepared at PLIVA (Zagreb, Croatia). Briefly, DB was prepared by the reaction of 2-chloro-6,7-dimethoxyquinazolin-4-amine and (2,3-dihydrobenzo[b]-[1,4]dioxin-2-yl)(piperazin-1-yl)methanone, according to established procedures [10,11]. DM was prepared by the reaction of doxazosin base with methanesulphonic acid. DH was prepared by the reaction of doxazosin base with HCl. The three forms were assayed for purity by a validated HPLC method (described in Section 2.3), and the results were 97.2% for DB, 101.2% for DM, and 98.0% for DH.

DB, DM, and DH were characterized with nuclear magnetic resonance (^{1}H NMR), dynamic vapor sorption (DVS), X-ray powder diffraction (XRPD), differential scanning calorimetry (DSC), and scanning electron microscopy (SEM). ^{1}H NMR spectra were recorded on Varian Gemini 300 spectrometer in DMSO at room temperature using tetramethylsilane as internal standard. DVS data were recorded on DVS1 instrument (Surface Measurement Systems, UK) in 10 steps (dm/dt 0.003) in relative humidity range 0–90%, at 25 °C. The XRPD patterns were recorded using a Phillips X'pert Pro powder diffractometer at 40 mA, 45 kV and with monochromatized Cu Kα radiation (λ = 1.54056 Å). The samples were scanned at room temperature in continuous scan mode over the range 3–40° with step size of 0.01671 2θ. Data were analyzed using software package Xpert Plus, version 1.3e. DSC thermograms of DB, DM, and DH were recorded on Perkin Elmer Pyris 1 (Perkin Elmer, UK). The instrument was calibrated with indium and zinc prior to analyzing the samples. Accurately weighed samples (2–5 mg) were placed in sealed aluminum pans and scanned at the heating rate of 10 °C min^{-1} over the temperature range 30–300 °C under dry nitrogen (35 ml/min). SEM analysis was carried out using JSM 5800 (JEOL, Tokyo, Japan) microscope. The samples were previously gold sputtered using Edwards S150 sputter coater under argon atmosphere to render them electrically conductive. Images were analyzed using software package Link ISIS, Series 300, Version 3.35.

2.3. Solubility measurements of DB, DM, and DH

Equilibrium solubilities were measured in triplicate with the shake-flask method in human gastric fluid (HGF), in fasted state simulating gastric fluid (FaSSGF-V2), in USP pH 1.2 buffer solution [12], and in pH 1.8, 2.6, and 3.0 HCl solutions. To make it more physiologically relevant (in terms of sodium chloride concentration [13] and osmolality [14]), FaSSGF-V2 (pH 1.6) contains 68 mM NaCl [5] instead of 34 mM NaCl (FaSSGF, [15]). USP pH 1.2 buffer solution contains 50 m M KCl [12].

Human gastric fluids were collected from the stomach of fasted healthy adults, after receiving appropriate approvals from the Scientific and the Executive Committee of the Red Cross Hospital of Athens (AP 8203) as described previously [14]. Individual human gastric fluids were kept frozen at −70 °C until used. Assuming that during a bioavailability study, the particles of an immediate release dosage form will empty from the stomach together with the

co-administered water, and in order to aspirate fluids that reflect the average gastric composition, fluids were aspirated between 20 and 40 min post-administration of 250 ml of water. On the day of solubility measurement, individual fluids from 5 subjects were brought to room temperature and pooled so that from each individual, a total of two samples were obtained (one aspirated at 20 min and one at 40 min) and each sample had a volume of approximately 3 ml. Following to centrifugation at a low speed to remove possible solid particles, the pooled sample (~30 ml, human gastric fluid, HGF) was used immediately for the solubility measurements. The pH of HGF was 1.8.

The solubility medium (5 ml) and the drug phase in excess (150 mg) were transferred into Erlenmeyer flasks (ca. 25 ml). Flasks were covered with parafilm and put in a shaking water bath (37 °C). Based on the equilibration time measured in FaSSGF-V2, 6 h was considered adequate for the determination of equilibrium solubility of DB, DM, and DH in all media. At equilibrium, the pH in each flask was measured and samples were filtered through regenerated cellulose 0.45-μm filters (Titan®, Scientific resources INC, Eatown, NJ/USA), discarding the first 1 ml. Drug content of the filtrate (appropriately diluted with FaSSGF-V2 or HCl solution) was measured by HPLC. Assays were performed using a validated HPLC-UV method. Specifically, the analytical column was a Merck Superspher® 100 RP-18 endcapped (250 × 4 mm, 5 μm), the mobile phase consisted of methanol, acetonitrile, and 50 mM phosphate buffer pH 3.0 (40:15:45, v/v/v), and the flow rate was 1 ml/min. The injection volume was 20 μl, and the retention time was about 7 min. The detection wavelength was 246 nm.

Differences between solubility data in two different media were evaluated with unpaired *t* test at the 0.05 level.

2.4. Preparation of DB, DM, and DH tablets

Uncoated immediate release tablets of DB (2 mg DB per tablet) were prepared by using a combination of commonly used excipients (lactose monohydrate, croscarmellose sodium, sodium lauryl sulfate, colloidal anhydrous silica, starch, and magnesium stearate) and a direct compression method.

Composition and methodology of the preparation of immediate release tablets of DM and DH (equivalent of 2 mg DB per tablet) were identical to that of DB tablets.

2.5. Dissolution studies of DB, DM, and DH tablets

Dissolution experiments were run in triplicate at 37.0 ± 0.5 °C using the USP II Apparatus (Distek® dissolution tester, model 2100B, North Brunswick, NJ, USA and Varian; VanKel, model 7010, Cary, NC) with the paddle rotating at 100 rpm. Experiments were performed in 500 ml of pH 1.6 HCl solution, FaSSGF-V2, pH 6.5 phosphate buffer (29 mM potassium dihydrogen phosphate), pH 6.5 maleate buffer (25 mM maleic anhydride), FaSSIF [16], and FaSSIF containing maleates (FaSSIF$_m$, [17]). Dissolution media were prepared freshly on the day of each experiment.

Samples were withdrawn using a 5 ml Fortuna Optima® syringe (Fischer Labortechnik, Frankfurt/Main, Germany) fitted with stainless tubing to facilitate representative sampling with sample replacement. Samples were filtered through Titan® membrane filters (regenerated cellulose, 0.45 μm, Scientific Resources INC, Eatown, NJ/USA), discarding the first 1 ml. Absorption of the active substance on the filters was found to be negligible. The analytical method applied for quantifying the dissolution data was identical to that used in solubility measurements.

Apart from cases where more than 85% dissolved was observed in less than 15 min, the difference factor, $f_{1,area}$, was used for testing the difference in cumulative % dissolved vs. time profiles according to a previously proposed methodology [18]. Since the coefficient of variation of data points was in almost all cases less than 20%, comparison involved the use of mean data sets. The reference profile varied with the comparison: DB was the reference phase, whereas FaSSGF-V2 or FaSSIF was the reference media. The limit for identifying differences between the test and reference set of data was set to $f_{1,area} > 0.15$ [18].

2.6. Simulated data after administration of DB and DM tablets to adults

Simulated cumulative doxazosin in plasma vs. time profiles after single-dose administrations of DB and DM tablets were constructed (Stella® 9.0.2 software, isee systems, Inc., USA) by using the *in vitro* dissolution data and previously described procedures [20,21]; after taking into account the *in vitro* solubility data, the average bioavailability after oral administration of doxazosin [19], and the enterohepatic circulation of doxazosin (Cardura®, Summary of product characteristics). Briefly, dissolution of the appropriate phase in stomach was assumed to occur until gastric contents are saturated or the entire dose is dissolved, based on *in vitro* dissolution data. If *in vitro* dissolution under gastric conditions was rapid, then a 250 ml solution with concentration equal to the maximum concentration observed in *in vitro* experiments was assumed to have been administered. Absorption by the gastric mucosa was assumed to be negligible, gastric emptying of solids and liquid (250 ml at administration time) occurred with the same rate constant, 2.8 h^{-1}, according to population values [15,20], and absorption from the small intestine occurred without any limitation. In all cases, dissolution was assumed to occur according to a dissolution rate constant that had been estimated from the *in vitro* data based on the Noyes-Whitney theory for dissolution [15,20]. Characterization of the enterohepatic circulation of doxazosin was possible by fitting a previously described model [21] to intravenous doxazosin data [22] and assuming that the gallbladder emptied into the duodenum at 4, 10, and 24 h post-dosing (according to the feeding schedule of the volunteers in the actual *in vivo* study). The cumulative doxazosin profile in plasma until 2 h after administration was used for evaluating early exposure.

2.7. Data after administration of DM tablets to adults

After receiving all required approvals and following ethical principles from Declaration of Helsinki, actual plasma data following to single administration of DM tablets to 24 healthy adults were collected. Each volunteer was administered one DM tablet with 240 ml of water after a 10-h fast. Blood samples were drawn prior to dosing and at 0.5, 1, 1.5, 2, 2.33, 2.67, 3, 3.5, 4, 5, 6, 8, 10, 12, 16, 24, 36, 48, and 72 h post-dosing. Plasma samples were assayed for their doxazosin content using a validated HPLC method that involved liquid–liquid extraction and fluorescence detection (unpublished, developed in house at PLIVA). A two-compartment model with first-order absorption was fitted to each individual concentration vs. time profile (WinNonlin® 5.2, Pharsight Corporation, Mountain View, CA, USA). The correlation coefficient for the linear correlation between an observed individual data set and the calculated by the best fitted line data set ranged from 0.93 to 0.997. Individual cumulative doxazosin in plasma vs. time plots were then constructed by using the estimated apparent absorption rate constant and the average bioavailability coefficient of doxazosin in the literature (0.65, [19]). These data were used for validating the simulated cumulative doxazosin in plasma vs. time plot after DM administration and evaluating the importance of using biorelevant *in vitro* data in such simulation. Profiles in plasma until 2 h after administration were used for the evaluation of early exposure.

2.8. Data after administration of DB and DM tablets to dogs

Canine studies were performed in four healthy female mongrel dogs (4 years old, 28–32 kg) that were accommodated in an animal facility operating according to the European Union regulations for the maintenance and experimentation on animals, and it has been approved by the Veterinary Directorate of the Municipality of Athens (EL 25 BIO 08). After 16 h fasting from food but not water, each dog was administered one DB tablet or one DM tablet with 250 ml of water via an orogastric tube. Blood samples were collected by means of an indwelling catheter positioned in a suitable foreleg vein. Five to eight hours after drug administration, each dog consumed a standard meal (150 g pellets and 250 ml tap water). Twelve hours after dosing, the catheter was removed and the dog returned to her cage, where she was allowed to eat and drink *ad libitum*. Samples after 12 h were collected by individual venipuncture. Blood samples were centrifuged, and plasma was stored at −20 °C in brown glass vials. The LC–MS analytical method that was used for the determination of doxazosin in plasma has been published recently [23].

After each administration, total exposure was estimated by the total area under the plasma profile, AUC_{0-24h}. Since canine plasma vs. time data after intravenous administration are not available in literature and modeling of enterohepatic circulation in dogs is problematic due to inconsistent emptying of canine gallbladder [8], cumulative doxazosin in plasma profiles could not be constructed and early exposure was estimated by the partial area under the plasma profiles, $AUC_{0-Tmax,DB}$, i.e., from $t = 0$ up to time, t, at which the first peak on plasma profile, after the administration of DB to the specific dog, was observed. Areas were estimated with the trapezoidal rule, and statistically significant differences between DB and DM administrations were evaluated with the paired t test at the 0.05 level.

3. Results and discussion

3.1. Characterization of DB, DM, and DH

^{1}H NMR spectrum of DB shows signals corresponding to both quinazoline and piperazine-benzodioxane moiety of molecule. In ^{1}H NMR spectrum of DM, two additional signals appear, corresponding to the protonated quinazoline cation and methanesulphonyl counteranion. ^{1}H NMR spectrum of doxazosin hydrochloride showed signal corresponding to the protonated quinazoline amine, designating the substance as a salt. DVS data show that all three phases, DB, DM, and DH, are non-hygroscopic with similar adsorption and desorption cycles. The X-ray diffractograms are shown in Fig. 2. X-ray diffractogram of DB and DH could not be found in literature. DM exists in a number of different crystalline forms [24]. The X-ray diffractogram of DM is identical to crystal form III standard reference pattern [25] (Fig. 2). DSC thermograms show a single sharp endothermic peak corresponding to the melting of DB [255.8 °C ($\Delta H = 121.3$ Jg^{-1})], DM [278.4 °C ($\Delta H = 113.2$ Jg^{-1})], and DH [288.1 °C ($\Delta H = 201.2$ Jg^{-1})].

SEM pictures show that particles of DB and DM have plate like regular shape, and size less than 10 μm, with DB particles being smaller than DM (Fig. 3). DH particles have also plate like regular shape but their size is about 50 μm (Fig. 3).

3.2. DB vs. DM in the gastric environment

Solubility of DB in HGF is significantly lower than solubility in pH 1.8 HCl solution, despite the fact that both initial and equilibrium pH, pH_{eq}, are similar (Table 1), due to the much higher ionic strength of HGF [5,13]. This may further be confirmed by the fact

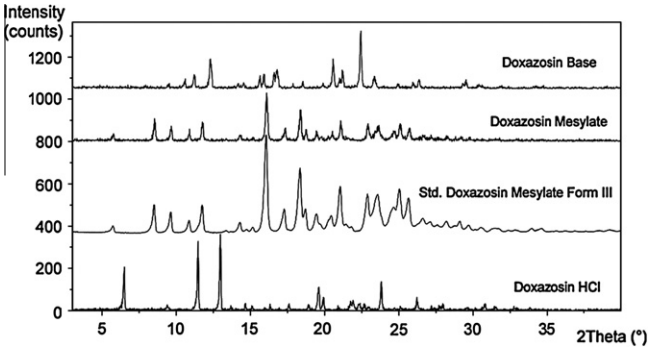

Fig. 2. X-ray powder diffraction pattern of DB, DM, standard DM, and DH.

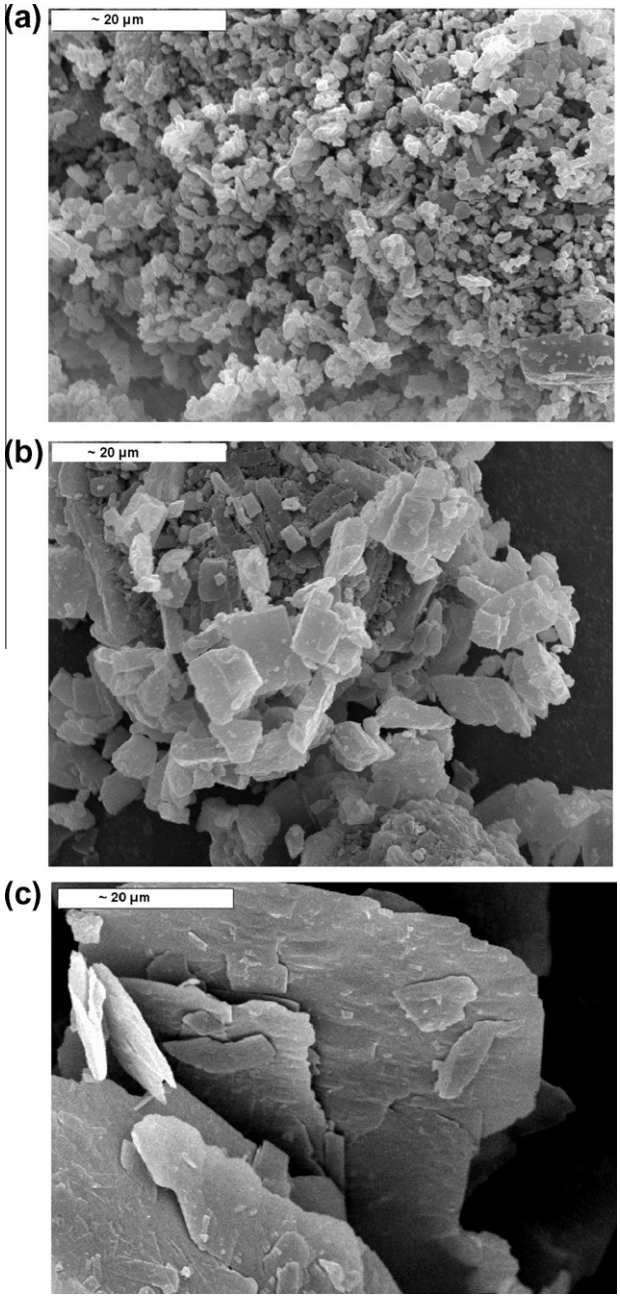

Fig. 3. SEM micrographs of DB powder (a), DM powder (b), and DH powder (c) used in the present study.

that solubility data of DB in FaSSGF-V2 are similar with solubility data in HGF at approximately similar pH_{eq} values (Table 1). DB solubility in pH 1.2 USP buffer is significantly higher than the solubility of DH (Table 1). This difference should be attributable to common ion effects (e.g., [26]), especially if conversion of DB to DH occurs (please see below).

The amount of DM in excess did not affect pH_{eq} values [4], as in all media, pH_{eq} was similar with the initial pH (Table 1). Solubility of DM is higher than DH in HCl solutions, due to its lower melting point and the lack of common ion effect. Due to differences in ionic strength between pH 1.8 HCl and FaSSGF-V2, solubility of DM in FaSSGF-V2 is much closer to solubility in HGF than in pH 1.8 HCl (Table 1).

Dissolution of DM, DH, and, to a lesser extent, DB tablets in pH 1.6 HCl is rapid (Fig. 4). However, in contrast to what equilibrium solubility data suggest (Table 1), dissolution of the dose is slightly less than complete (Fig. 4). As previously observed with other ionized weak bases [27–29], the cationic form of doxazosin could exchange with sodium ions associated with insoluble croscarmellose (the disintegrant in the tablets). For all three phases, dissolution of the dose in FaSSGF-V2 was slower than in pH 1.6 HCl, in accordance with the lower solubilities of the phases in FaSSGF-V2. In addition, dissolution was less complete than in pH 1.6 HCl. Since the increased ionic strength does not affect the interaction of protonated compounds with croscarmellose sodium [27–29], an interaction between protonated doxazosin and taurocholate with subsequent formation of insoluble salt(s) can be postulated. Such interaction indeed would have a significant impact on % dissolution profile, due to the low dose of doxazosin.

The difference between the dissolution profiles of DB and DM tablets is significant ($f_{1,area} = 0.22$), mostly due to the more complete dissolution of DM tablets. For DB and DH tablets, dissolution in FaSSGF-V2 is incomplete, pH remains unaltered until the completion of the process, and dissolution profiles do not differ significantly ($f_{1,area} = 0.11$). Based on solubility data of DB in pH 1.2 USP buffer and in FaSSGF-V2 (please note that pH_{eq} of FaSSGF-V2 is significantly increased), solubility of DB in FaSSGF-V2 (pH 1.6) is expected to be about ten times higher than solubility of DH in FaSSGF-V2 (Table 1). The similar dissolution profiles of DB and DH in FaSSGF-V2, therefore, suggest conversion of DB to DH during dissolution of the dose. The slightly slower dissolution of DH tablets at early time points is in agreement with its bigger particle size (Fig. 3).

3.3. DB vs. DM in the environment of the upper small intestine

Dissolution of DB tablets in media simulating the environment in the upper small intestine was not complete (Fig. 5a). Based on the plateau levels (last experimental point in relevant curve in Fig. 5a), solubility of DB in phosphate and maleate buffer is 1.0 and 0.9 μg/ml, respectively, whereas in FaSSIF and $FaSSIF_m$ is 1.6 and 1.9 μg/ml, respectively. These data indicate that, unlike with other weakly alkaline compounds with similar p*K*as and in similar media prepared with crude materials [17], the effect of the anion of the buffer system on doxazosin solubility is minimal.

Dissolution of DM tablets was more complete and unaffected by the presence of solubilizing agents (Fig. 5b). As a result, the difference between DM and DB in FaSSIF ($f_{1,area} = 0.42$) and in $FaSSIF_m$ ($f_{1,area} = 0.36$) is smaller than the difference between DM and DB in phosphate buffer ($f_{1,area} = 1.44$) and in maleates ($f_{1,area} = 1.10$), i.e., accurate simulation of the environment in the small intestine increases solubility with subsequently smaller differences in dissolution profiles between DB and DM.

Based on the plateau level in pH 6.5 buffer (Fig. 5a), DB is a BCS low solubility compound (the dose to solubility ratio is 2036 ml). Similarly, DM is also a low solubility compound (based on the plateau level in pH 6.5 buffer (Fig. 5b) dose to solubility ratio is 612 ml), as indicated in literature [9].

Table 1
Mean ± SD ($n = 3$) solubility (μg/ml) of doxazosin base (DB), doxazosin mesylate (DM) and doxazosin hydrochloride (DH) in human gastric fluid (HGF) and in various simulated gastric fluids.[a]

	DB	DM	DH
HGF	0.256 ± 0.049	0.200 ± 0.014	N/A
pH 1.8	(pH_{eq} 2.7)	(pH_{eq} 1.8)	
FaSSGF-V2	0.235 ± 0.016	0.141 ± 0.013	0.021 ± 0.002
pH 1.6	(pH_{eq} 2.3)	(pH_{eq} 1.6)	(pH_{eq} 1.6)
USP	0.535 ± 0.013	0.254 ± 0.008	0.040 ± 0.008
pH 1.2	(pH_{eq} 1.2)	(pH_{eq} 1.2)	(pH_{eq} 1.2)
HCl	2.763 ± 0.599	1.511 ± 0.419	0.099 ± 0.006
pH 1.8	(pH_{eq} 2.6)	(pH_{eq} 1.8)	(pH_{eq} 1.8)
HCl	0.751 ± 0.014	3.364 ± 0.187	0.501 ± 0.018
pH 2.6	(pH_{eq} 3.7)	(pH_{eq} 2.7)	(pH_{eq} 2.6)
HCl	0.521 ± 0.054	N/A	N/A
pH 3.0	(pH_{eq} 4.1)		

[a] pH_{eq} is pH at equilibrium; N/A means not available.

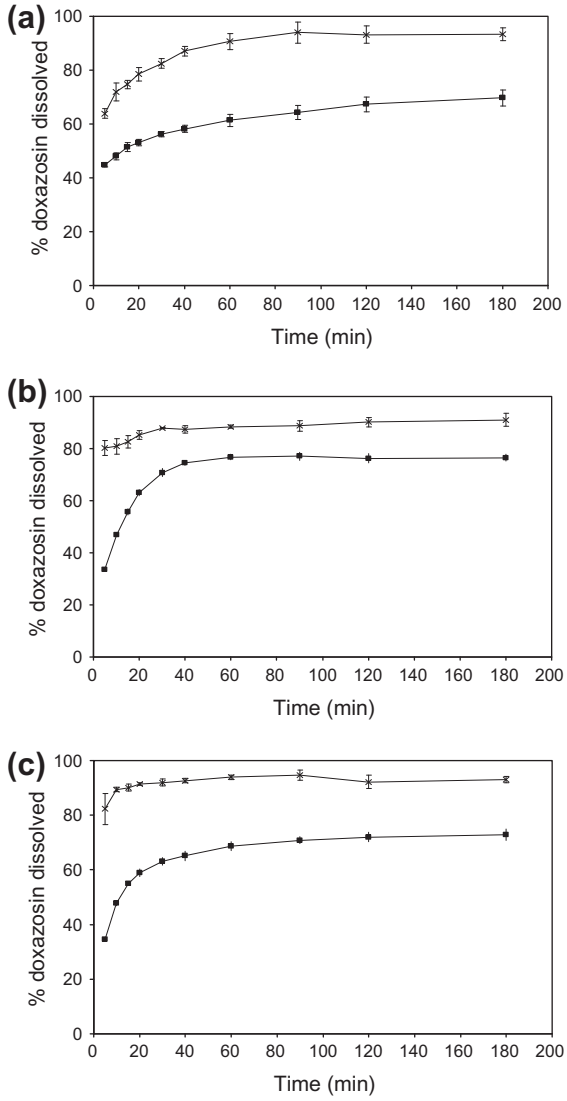

Fig. 4. Mean ± SD ($n = 3$) % dissolved DB tablets (a), DM tablets (b), and DH tablets (c), in pH 1.6 HCl solution (×) and in FaSSGF-V2 (■).

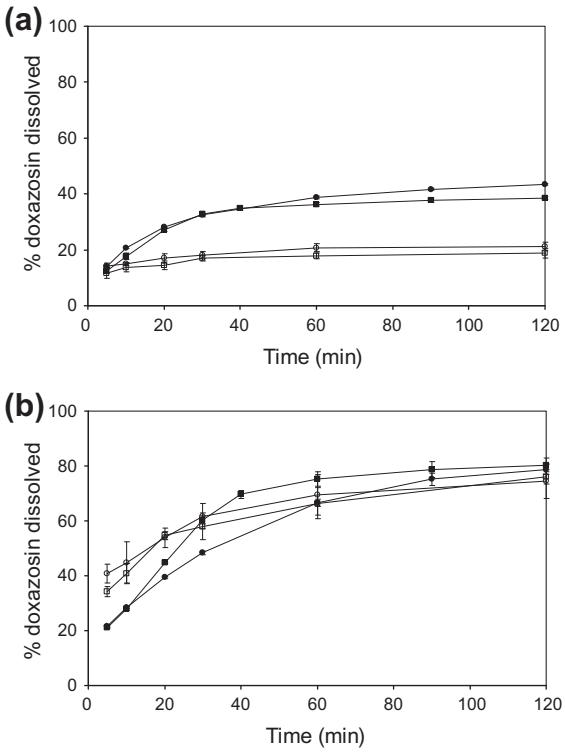

Fig. 5. Mean ± SD (n = 3) % dissolved DB tablets (a) and DM tablets (b) in FaSSIF (■), FaSSIF$_m$ (●), pH 6.5 phosphate buffer (□), and pH 6.5 maleate buffer (○).

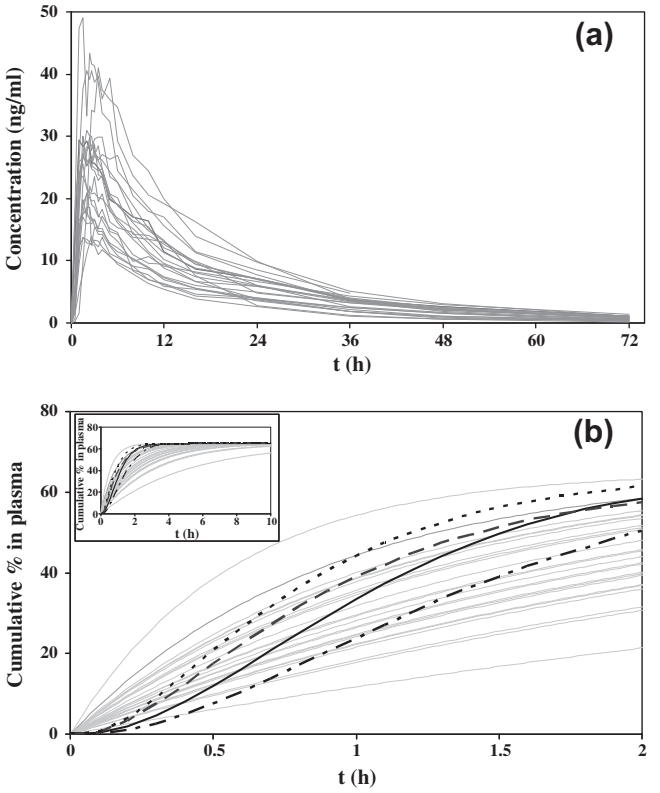

Fig. 6. (a) Individual doxazosin concentration in plasma vs. time profiles (n = 24), after single-dose administrations of DM tablets to healthy adults (b) Partial (and complete, in insert) individual cumulative % doxazosin in plasma vs. time profiles (n = 24, gray lines), simulated cumulative % doxazosin in plasma vs. time profiles after administration of one DB tablet that was constructed by using dissolution data in $HCl_{pH\ 1.6}$ and in phosphate buffer pH 6.8 (– –) and by using dissolution data in FaSSGF-V2 and FaSSIF (–·–), and simulated cumulative % doxazosin in plasma vs. time profiles after administration of one DM tablet that was constructed by using dissolution data in $HCl_{pH\ 1.6}$ and in phosphate buffer pH 6.8 (·····) and by using dissolution data in FaSSGF-V2 and FaSSIF (—).

3.4. Assessment of early exposure after the administration of DB and DM tablets by using in vitro data

Fig. 6a shows the actual individual plasma concentration vs. time profiles, after single administrations of DM tablets to 24 healthy adults. In all profiles, secondary peaks were observed, in agreement with the enterohepatic circulation of doxazosin (Cardura®, Summary of product characteristics). As with plasma concentration profiles, individual cumulative doxazosin profiles in plasma after single administrations of DM tablets were highly variable (Fig. 6b).

Regardless of the type of *in vitro* data used for generating the simulated cumulative input profile of DM, the early phase, 0–0.5 h post-dosing of DM tablets, is underestimated by the simulated profiles (Fig. 6b). This is related to the methodology applied for estimating the kinetics of excretion into the bile (data not shown). Simulation of enterohepatic circulation involved the use of mean intravenous plasma concentration vs. time data that were estimated from a limited number of individual profiles [22]. Also, simulated profiles of DM at late times after administration (times >3 h) seem to overpredict the average actual profile (insert of Fig. 6b). This can be related to the variability of gallbladder emptying patterns.

Compared with *in vitro* data collected in simple aqueous buffers, biorelevant *in vitro* data led to better evaluation of the average input profile of DM, during the 0.5–2 h post-dosing (Fig. 6b).

Simulated cumulative % in plasma vs. time profiles during the first 2 h after administration constructed by using *in vitro* data in simple aqueous buffers or in biorelevant media suggest that the administered phase, DB or DM, does not have a significant impact on early exposure (Fig. 6b).

3.5. Assessment of early exposure after administration of DB and DM tablets by using canine data

Individual canine plasma profiles are shown in Fig. 7. In every profile, a second peak was observed at about 6 h post-dosing suggesting that doxazosin is enterohepatically circulated in dogs as it has been observed in humans. Total exposure after DB administration was not statistically different from that measured after DM administration (p = 0.171). The first peak of doxazosin concentration in plasma after DB administration ranged 5.1–7.7 ng ml^{-1} and was observed 2–4 h post-dosing (Fig. 7). There are no human data in literature, after administration of DB. The first peak of doxazosin concentration in plasma after DM administration ranged 7.3–13.2 ng ml^{-1} and was observed 2–4 h post-dosing (Fig. 7), i.e., not much different from human data collected in this (Fig. 6a) and in previous studies (∼9 ng ml^{-1}, 2–3 h post-dosing [22]). Oral bioavailability of DM has been reported to be similar in dogs and humans [30]. However, this study shows that early exposure after DM administration to dogs is higher than after DB. Specifically, $AUC_{0-Tmax,DB}$ ranged from 10.2 to 16.4 ng ml^{-1} h^{-1} after DB and from 15.4 to 29.2 ng ml^{-1} h^{-1} after DM and difference is significant (p = 0.048). The apparent discrepancy compared with the minimal (if any) difference in early exposure after DB and DM administration in humans (Fig. 6b) could be attributed to species-related differences. Dissolution data in gastric pH of the dogs used in this study and solubility data in their gastric aspirates (data not shown) suggest that dissolution in the canine stomach occurs similarly to that in the human stomach. Therefore, reasons for the inappropriateness of canine data may relate to the faster gastric emptying rates of dogs [31,32] and different dissolution characteristics of DB and DM in the upper small intestine of dogs; individual

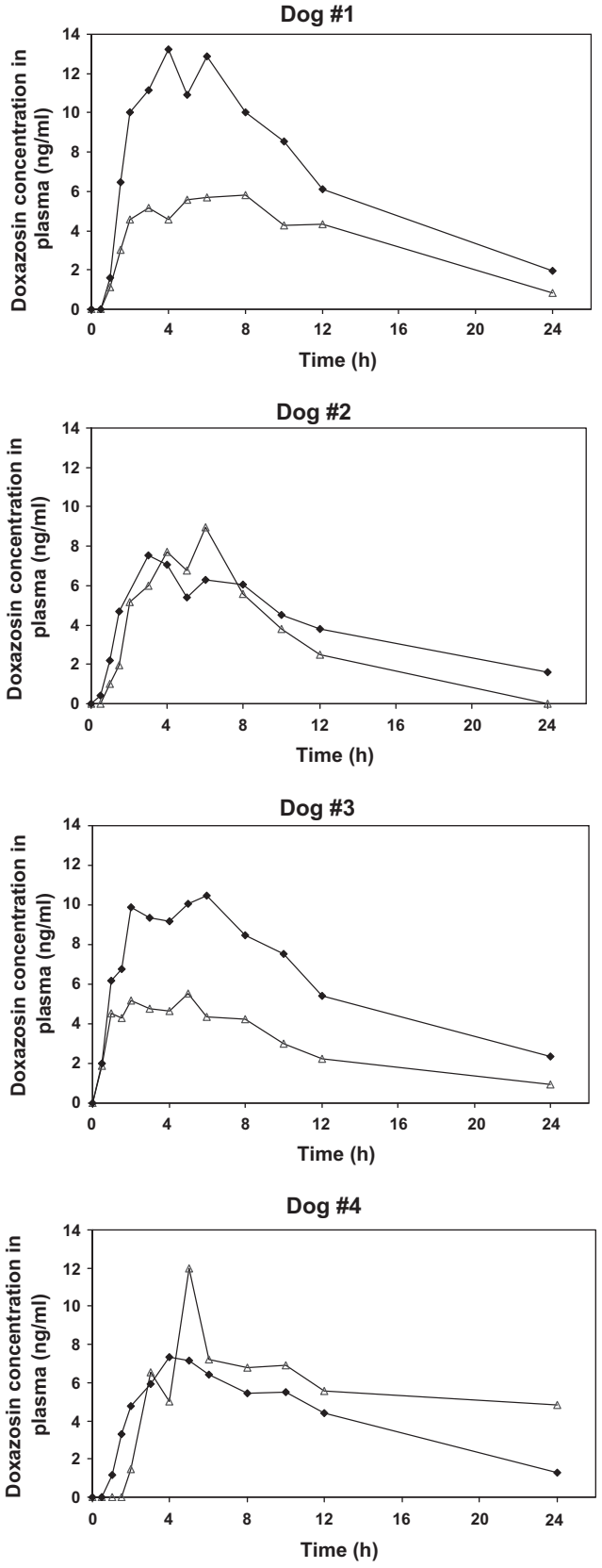

Fig. 7. Individual doxazosin plasma profiles after single administration of one DB tablet (△) and one DM tablet (▲) to four dogs in the fasting state.

bile acids identity and concentrations in the contents of the canine upper small intestine are different between dogs and humans [8].

4. Conclusions

For salts of weak acids, there are human data published half a century ago indicating their potential superiority over the free acid in regard to the absorption characteristics [33–35]. In contrast, although bases are most frequently administered as salts and, as of the end of 2006, 37.9% of APIs approved in the USA after 1981 for oral administration were salts formed from basic molecules [3], there are no published human data for the superiority of their salts in regard to the absorption characteristics [35]. The present study showed that differences in dissolution characteristics between DB and DM tablets are small to have an impact on early exposure. Canine data overestimated differences in early exposure between DB and DM tablets and should be used cautiously in other relevant studies.

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