European Journal of Pharmaceutics and Biopharmaceutics xxx (2009) xxx–xxx

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research paper

Computer simulations using GastroPlus™ to justify a biowaiver for etoricoxib solid oral drug products

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ARTICLE INFO

Article history:
Received 12 May 2008
Accepted in revised form 29 October 2008
Available online xxxx

Keywords:
BCS
Bioequivalence
IVIVC
In-silico
Permeability
Etoricoxib

ABSTRACT

Purpose: The purpose of this study was to compare the dissolution behaviour of etoricoxib in different dissolution media and to establish *in vitro/in vivo* correlation (IVIVC) using computer simulations.
Methods: Drug solubility was measured in different media. The dissolution behaviour of etoricoxib was studied in the USP Apparatus 2 using different dissolution media. A dissolution transfer model was used to investigate if the drug stays in solution when the pH of the medium changes. Drug permeability assessment was performed using the caco-2 cell culture technique. The *in vitro* data were used as input functions in GastroPlus™ to simulate the *in vivo* profiles of the drug.
Results: Solubility of etoricoxib was highest at low pH, and there was no significant difference in the solubility observed between blank buffers and biorelevant media of similar pH. The drug remained solubilised when transferred into simulated intestinal fluids. Using the *in vitro* data as input function in Gastro Plus, an IVIVC was established. Further simulations confirmed that the drug absorption occurs similar to the absorption of an oral solution.
Conclusions: Due to the solubility behaviour within the physiological pH gradient of the gastrointestinal tract, etoricoxib can be classified as an intermediate class 1/2 drug rather than BCS class 2. *In vitro* results combined with *in silico* simulations using GastroPlus support scientifically that a biowaiver for immediate release etoricoxib solid oral dosage forms is justified.

1. Introduction

Etoricoxib, [5-chloro-2-(6-methylpyridin-3-yl)-3-(4-methylsulfonylphenyl) pyridine], (Fig. 1) is a novel orally active agent that selectively inhibits cyclooxygenase-2 (COX-2) [1]. Etoricoxib is used in the treatment of Rheumatoid arthritis, osteoarthritis, acute gout, chronic musculoskeletal pain (including chronic low back pain), postoperative dental pain and primary dysmenorrhoea [2]. The drug is available as oral tablets, and the recommended dosage is between 60 and 120 mg/day. It is a poorly soluble, lipophilic drug with estimated log*P* of 3.14 and pK_a of 4.6. Etoricoxib behaves like a weak base. Its aqueous solubility is low and highly pH-dependent. Pharmacokinetic studies, however, show that when administered orally, etoricoxib is completely and rapidly absorbed, with an oral bioavailability of up to 100% [3].

Dissolution testing is an *in vitro* test used to assess and estimate the *in vivo* behaviour of orally administered solid dosage forms [4]. Dissolution testing is an industry standard and is used both for quality control (QC) purposes and during drug product development (R&D). For R&D purposes, dissolution tests are intended to be an *in vitro* indicator of the *in vivo* performance of the dosage form [5]. Ideally, dissolution data can be used to establish *in vitro/in vivo* correlation with clinically observed plasma-time curves. This can be achieved using computer-based models such as the Advanced Compartmental Absorption and Transit (ACAT) model. The *in vitro* data are used as input function, and the software uses convolution algorithms to estimate the plasma-time curves observed *in vivo*.

The rate and extent to which an orally administered dosage form is absorbed depend on various physiochemical and physiological factors [6–9]. Galia et al. demonstrated the use of biorelevant dissolution media (BDM) in forecasting trends in the *in vivo* performance of BCS class 1 and class 2 immediate release drug products [10]. However, physiological pH changes that occur in the transfer from the stomach to the small intestine should not affect the solubility of class 1 drugs.

In 2002, the FDA implemented a waiver of *in vivo* bioavailability and bioequivalence testing of immediate release solid dosage forms for class 1 highly soluble, highly permeable drugs based on the BCS [11]. In addition, biowaiver for certain class 2 and class 3 drugs is scientifically justified using the BCS approach [12–14].

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doi:10.1016/j.ejpb.2008.10.019

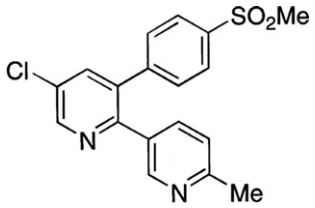

Fig. 1. Etoricoxib chemical structure.

Concerns have been raised that the BCS class boundary may be too strict for acidic drugs that show a high solubility at only high pH values [15]. Tubic-Grozdanis et al. using selected weak acid and weak base BCS class II drugs demonstrated that simulation of oral drug absorption using physicochemical drug properties can aid the justification of biowaiver for some BCS class II compounds [16].

In this study, the dissolution behaviour of etoricoxib (Arcoxia®) was investigated in the USP Apparatus 2 using FaSSIF and SIF. Additionally, a dissolution media transfer model as described by Kostewicz et al. [17] was used to investigate the solubility of the drug when entering the small intestine. GastroPlus™ was used to simulate the drug absorption and to establish an IVIVC.

2. Materials and methods

2.1. Materials

Etoricoxib API powder (Form-V unmilled) and etoricoxib tablets (Arcoxia®-60 mg film-coated talets) were provided by Merck Frosst, Canada. Sodium taurocholate (crude bile salts batch # 015K0585), high purity sodium taurocholate (batch # 115K1109, 95% purity), sodium lauryl sulfate (batch # 084K0187), Lucifer yellow and trifluoroacetic acid were purchased from Sigma–Aldrich (St Louis, MO); soy lecithin (phosphatidycholine Lot # 5568H) was purchased from MP Biomedicals Inc. (Solon, Ohio, USA); egg phosphatidycholine, Lipoid E PC 99.1% pure (HQ), was purchased from Lipoid GmbH (Ludwigshafen, Germany); and potassium phosphate monobasic monohydrate, potassium chloride, sodium phosphate monobasic monohydrate, sodium acetate monohydrate, sodium hydroxide, sodium chloride, hydrochloric acid (ACS grade) and glacial acetic acid were purchased from Fisher Scientific (Fisher scientific Canada Inc.). Dichloromethane, methanol and acetonitrile were all of HPLC grade, 25 mm–0.45 μm Whatman glass microfibre filter and 25 mm, 1 μm Acrodisc glass fibre filter were purchased from Life Sciences (Life Sciences Canada Inc).

Dulbecco's modified eagle's medium (DMEM), L-glutamine, trypsin with 0.25% EDTA, HEPES and minimum essential medium (MEM) containing non-essential amino acids were purchased from GIBCO BRL (Carlsbad, California, USA). Fetal bovine serum (FBS) and Hanks Balanced Salts Solution (HBSS) were purchased from Sigma–Aldrich (St. Louis, Missouri, USA). Phosphate buffer saline (PBS) containing 140 mM NaCl, 8.1 mM $Na_2PO_4.H_2O$, and 1.47 mM $KH_2PO_4.H_2O$, pH 7.2, was prepared using chemicals obtained from Sigma (St. Louis, Missouri, USA). Cell culture flasks (75 cm^2, 25 cm^2 growth surface area) and Transwell® inserts (24 mm, 0.4 μm pore size, and 4.7 cm^2 growth surface areas) were purchased from Corning Costar (Acton, MA, USA).

2.2. Media preparation

Simulated gastric fluid (SGF)- 0.01 M HCl, pH 1.2 (without enzymes), containing 2 g/L NaCl, acetate buffer, pH 4.0, and simulated intestinal fluid (SIF), pH 6.8 (without enzymes), were prepared following the USP 27. Simulated gastric fluid (SGF-SLS), pH 2.0, without enzymes but with 0.25% SLS was prepared as proposed by Dressman et al. [4].

The biorelevant media containing bile salts and lecithin were prepared following the procedure and modification outlined by Marques [18], which was adopted from the composition proposed by Galia et al. [10]. The recommended volume for simulating fasted state conditions (FaSSIF) in the upper small intestine is 500 ml, and that for simulating fed state conditions (FeSSIF) in the upper small intestine [10] is 1000 ml.

2.3. Solubility studies in different media

An excess of the drug powder was added into 10 mL of the different media in glass vials. The vials were sealed and placed into a shaking incubator water bath (Dubnoff Metabolic Shaking Incubator- Precision scientific), and the temperature was maintained at 37 ± 0.5 °C. Samples were taken at 1, 4, 24 and 48 h, filtered using a 0.45 μm Whatman glass microfibre filter (Life Sciences, Canada Inc.) and analysed by HPLC.

2.4. X-ray powder diffraction (XRPD) pattern

To assess the impact of pressure and dwell time on the powder property, about 60–70 mg of the active pharmaceutical ingredient (API) powder was compressed at three different compression pressures and dwell times using a hydraulic lab press (Enerpac P142, Globe Pharma, USA). X-ray diffraction patterns were performed on the compacts using the Scintag XDS-2000 X-ray diffractometer (Scintag Inc. USA). Measurements were taken at a voltage of 45 kV and 40 mA using Si (Li) Peltier-cooled solid state detector. The compression pressure and dwell time which caused the minimum change in powder property were chosen to compress the discs that were used for the intrinsic dissolution tests.

2.5. Intrinsic dissolution test

The intrinsic dissolution tests were performed using the static disc intrinsic dissolution apparatus (Distek Inc., New Brunswick, NJ, USA). Compact powder discs of 0.5 cm^2 surface area were prepared by compressing between 60 and 70 mg of etoricoxib drug powder at 2000 PSI for 2 min using a hydraulic lab press (Enerpac P142, Globe Pharma, USA).

The USP Apparatus 2 (paddle) and a flat-bottomed vessel (Distek, New Brunswick, NJ, USA) were used. The distance between the top of the disc and the bottom of the paddle was adjusted to about 2.5 cm before adding the test media. The water bath temperature was maintained at 37 ± 0.5 °C. The test was performed with a spindle operated at 50 RPM, at specified time intervals 5-ml samples were removed from the vessels and replaced with an equal amount of pre-warmed media. Samples were filtered using Whatman glass microfibre filter (25 mm, 0.45 μm, Life Sciences, Canada Inc.), discarding the first 3 ml. The intrinsic dissolution rate (IDR) was estimated by dividing the initial slope of the plot of concentration versus time by the surface area of the compact exposed to the dissolution media.

2.6. Dissolution tests using the USP Apparatus 2

Dissolution tests in the USP Apparatus 2 (Erweka DT 6, Germany) were performed using SGF and the conventional USP-SIF, pH 6.8, using media volumes of 900 mL. The biorelevant media used were FaSSIF, pH 6.5, at the recommended volumes of 500 mL [10,18], and an additional test was performed with 900 mL FaSSIF medium volume. The paddle speed used in all the tests was 75 RPM. At pre-determined time intervals, 5-mL samples

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were taken, and were replaced with 5 mL of pre-warmed medium. The samples were filtered using Whatman glass microfibre filter (25 mm, 0.45 μm, Life Sciences), the first 3 mL was discarded and the remainder was analysed by HPLC.

2.7. Investigating possible in vivo precipitation under physiological conditions

A transfer model described by Kostewicz et al. [17] was used to investigate a possible *in vivo* precipitation of etoricoxib. An amount of etoricoxib drug powder equivalent to the highest recommended dose (120 mg) was dissolved in 120 ml of SGF to produce a 1.0 mg/ml solution. Using a peristaltic pump (Piper Pump, Dungey Inc. Agincourt, Ontario.), the dissolved drug was pumped into 500 ml of FaSSIF or SIF maintained at 37 ± 0.2 °C in a dissolution vessel (Erweka DT 6, Germany) and stirred at 75 RPM. The pH in the acceptor vessel was monitored and adjusted using 1 N NaOH solution to maintain pH 6.5. Possible drug precipitation was monitored using HPLC.

2.8. Drug permeability assessment using cell culture technique

Drug permeability assessment was performed as previously described by Wei and Loebenberg [19]. Briefly, caco-2 cells (ATTC, Rockville, MD, USA), passages 50–55, were maintained at 37 °C in Dulbecco's modified eagle's medium (DMEM) with 4.5 g/L glucose, 10% fetal bovine serum (FBS), 1% non-essential amino acids, 2 mM L-glutamine and HEPES buffer, in an atmosphere of 5% CO_2 and 95% relative humidity. About 5×10^4 cells were seeded in each apical chamber (medium volume 1.5 mL) of transwell® inserts (4.7 cm^2 area per insert), and 3 mL of transport medium was transferred to the basal (receiver) side.

The integrity of the cell mononlayer was determined by measuring the Trans epithelial electrical resistance (TEER) value using EndOhm volt-ohm meter (World Precision Instruments, Sarasota, FL, USA). The resistance of the bare filter insert was determined and subtracted from the monolayer resistance values, and the results obtained were multiplied by the membrane area of the inserts to obtain a TEER value for each monolayer ($\Omega\ cm^2$). Transport experiments were initiated and performed when the TEER values were about 400 $\Omega\ cm^2$ or higher. Lucifer yellow, 100 μM, which was used as a quality control fluorescence marker to verify the integrity of the tight junctions, was measured at 485 nm excitation and at 530 nm emission using a spectrofluorometer (Model: FLUOROMAX, SPEX industries inc., USA). Its effective permeability should be less than 2×10^{-7} cm/s.

The test compound assay consisted of 100 μM etoricoxib solution prepared in HBSS, and the pH was adjusted to 6.5 for the apical side, while the pH of the receiver side was adjusted to 7.4. The cells were incubated at 37 °C in an atmosphere of 5% CO_2 and 95% relative humidity; the concentration of etoricoxib in both the chambers was analysed by HPLC at pre-determined time intervals. In order to maintain sink condition, the transwell® inserts were moved to pre-incubated wells that contained fresh transport medium. After each experiment, the TEER values were measured in all inserts and the integrity of the cell monolayer was verified.

The apparent permeability coefficient (P_{eff}) was calculated using the following equation:

$$P_{eff} = \frac{V}{A \times C_0} \times \frac{dC}{dt}(cm/s)$$

where dc/dt is the flux across the monolayer (μM/s), and is obtained from the linear slope of the plot of the drug concentration in the acceptor chamber vs. time, A is the area of the transwell inserts used in this experiment (4.7 cm^2), V is the volume of the receiver chamber (cm^3), and C_0 is the initial drug concentration (μM).

2.9. HPLC assay

The analytical column used for the analysis of etoricoxib was Metachem Inertsil-ODS2 10 cm × 3.0 mm, 5 μm (Metachem Technolgies Inc.). The column temperature was maintained at 40 °C using an external column heater (Eppendoff model TC-50), and the mobile phase consisted of double distilled water with 0.1% trifluoroacetic acid (TFA) and acetonitrile with 0.1%TFA in the ratio H_2O:ACN of 77:23. The chromatograms were acquired using Clarity™ (version. 2.4.4.83, Data Apex, Prague, Czech Republic) data acquisition software, using a Shimadzu LC-600 pump, with SIL-9A auto sampler (Shimadzu, Japan) and Dynamax UV detector (Dynamax Corporation, Elkhart, IN). The injection volume was 5 μL and the flow rate was 0.6 mL/min with UV detection at 280 nm. Analyses for the intrinsic dissolution test were performed using Agilent HPLC systems (Agilent 1100, USA) equipped with a UV detector, auto sampler, built-in column heater and Atlas TS™ data acquisition software.

2.10. Computer simulations using Gastroplus™

Results obtained from the *in vitro* tests were used as input functions in Gastroplus™ version. 5.2.0 (Simulations Plus Inc., Lancaster, CA, USA) to simulate the absorption profile of the drug. The three main interfaces (tabs) used for data input are the compound, physiology and pharmacokinetic tabs. In the compound tab, the basic data pertaining to the physicochemical properties of the drug such as bulk density, solubility, pK_a, dose and particle radius were entered. The human effective permeability for etoricoxib used in the simulations was estimated using caco-2 data obtained from a study described in the previous section. The human jejunum effective permeability (P_{eff}) value was also estimated using the ADMET Predictor™ (version 2.0, Simulations Plus Inc, Lancaster, CA, USA), and was compared with the *in vitro* caco-2 value. The log$P$ value and diffusion coefficient were estimated using the ADMET Predictor™ and Gastroplus™.

The *in vitro* dissolution profiles of etoricoxib tablets were used as input functions in Gastroplus™ using the tabulated *in vitro* dissolution data input function together with the controlled release-dispersed dosage form function (CR-dispersed). The drug release profiles were used by the software to calculate the drug concentration in each compartment. The human log$D$ absorption model was used to estimate the changes in permeability as the drug travels along the GI tract. A simulation was performed using the software preset "solution model", and the profiles were compared with that using the dissolution profiles.

The clinical data used in the simulations provided by the manufacturer included those for 60 mg tablets for the oral data and 25 mg for the IV data. Values for the pharmacokinetic inter-compartmental rate constants (k_1k_2, k_2k_1, etc.), volume of distribution (V_d) and clearance were estimated using the clinical data and the PK Plus module in Gastroplus™ and were directly imported into the pharmacokinetic tab to enable the software to calculate the plasma concentration-time curves. In the physiology tab, the default values for the transit times were selected. The outputs obtained include the fraction of oral dose absorbed and the plasma concentration-time profile.

2.11. Statistical analysis

Regression analysis values were automatically generated by Gastroplus™. Values displayed included the regression coefficient (r^2), the sums of square error (SSE), the root mean square error (RMSE), and the mean absolute error (MAE) of prediction . The percent prediction error (PE) was estimated using the equation given below [20]:

$$\%PE = \frac{\text{observed} - \text{predicted}}{\text{observed}} \times 100$$

3. Results

3.1. Solubility studies in different media

The drug equilibrium solubility results (Table 1) indicate that etoricoxib has a high solubility in the gastric media at low pH, and that solubility decreases as pH increases. The presence of bile salts and lecithin in biorelevant media does not appear to impact its solubility. The dose/solubility ratio calculated using solubility value at each pH for the three dosage strengths shows that from pH 5.0 and above, etoricoxib exceeds the critical value of 250 mL for all the three strengths and does not meet the BCS class 1 definition.

3.2. Intrinsic dissolution test

X-ray diffraction patterns obtained by compressing etoricoxib pure drug powder at various compression pressures and dwell times were evaluated to select the appropriate parameter to prepare compressed samples for intrinsic dissolution measurements. The reduction in the sizes of the peaks with increasing pressure and dwell time indicates loss in crystalline structure or conversion to different polymorphic forms [21]. Yu et al. suggested that high compression forces might induce polymorphic changes as well, which might result into an incorrect measurement [22]. The compression pressure of 2000 PSI for 2 min was chosen to prepare the compacts that were used for the intrinsic dissolution test, because it provided a compact whose X-ray diffraction pattern closely matched that of the raw powder (XRDP not shown).

The results (Table 2) show the intrinsic dissolution rate (IDR) data from the studies of etoricoxib in four different media. The IDR is highest in the USP-SGF without enzymes, pH 1.2, followed by SGF with 0.25% SLS, pH 2.0 (5.99 and 3.06 mg/min/cm^2, respectively). The IDRs are 0.026 and 0.023 mg/min/cm^2 in FeSSIF, pH 5.0, and FaSSIF, pH 6.5, respectively, which compared with the low pH media are 130-to 260-fold lower.

3.3. Dissolution tests results

Fig. 2 shows the mean dissolution profile of etoricoxib (60 mg tablets) in the USP Apparatus 2. In all the tests, disintegration was fast and complete in less than 5 min. The dissolution rate is fast in SGF, with complete dissolution in about 5 min, and is slowest in FaSSIF-500 mL compared with FaSSIF-900 mL and SIF-900 mL. At the end of the dissolution test (90 min), the percentages of the drug dissolved in the different media were-SGF-100%, FaSSIF-500 mL, 79.7%; the USP-SIF, 84.7% and FaSSIF-900 mL, 91.6%.

Table 2
Intrinsic dissolution rate (IDR) of etoricoxib in different media and pH values.

Medium	pH	Intrinsic dissolution rate (IDR) (mg/min/cm^2)
USP-SGF (without enzymes)	1.2	5.990
SGF-0.25% SLS	2.0	3.060
FeSSIF	5.0	0.026
FaSSIF	6.5	0.023

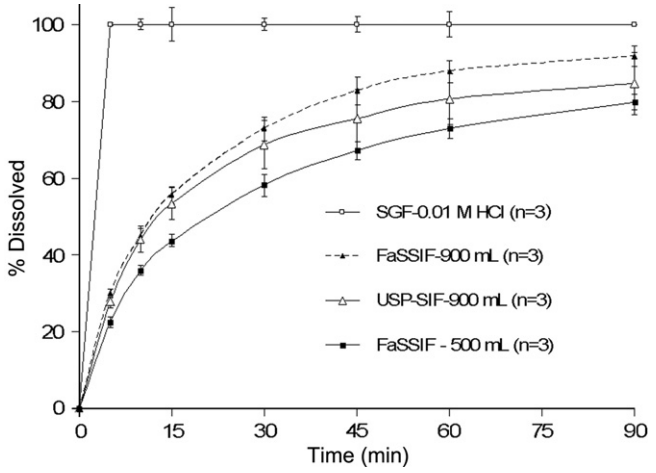

Fig. 2. Comparison of dissolution profiles in the USP Apparatus 2 (n = 3 and all tests were performed at 75 RPM.).

3.4. Investigating a possible in vivo precipitation under physiological conditions

Fig. 3 shows the mean concentration-time profile of etoricoxib when dissolved in simulated gastric fluid and added into 500 mL of SIF and FaSSIF at 2.2 ml/min. Another test was done in which the drug solution in SGF was added into FaSSIF at a higher flow rate of 4.8 ml/min. The final concentration of the drug after transfer into FaSSIF and SIF was higher than the solubility of the drug in FaSSIF or in SIF by itself, with no precipitation observed within two hours. The flow rates were chosen to cover the range of gastric emptying rates under fasting conditions that have been suggested in the literature [23,24].

3.5. Cell culture permeability studies

The caco-2 cell culture permeability results showed that the apical/basolateral (A/B) transport was 5.23×10^{-5} cm/s and that the basolateral/apical (B/A) transport was 5.07×10^{-5} cm/s. The permeability directional ratio (PDR), which is the ratio of BA/AB transport, was estimated to be 0.969. The human jejunum effective

Table 1
Solubility and dose/solubility ratio at 37 °C of the three strengths of etoricoxib in different media.

	pH	Solubility (mg/mL)	Dose (mg)		
			60	90	120
			Dose/solubility ratio		
SGF (Without enzymes)	1.2	13.21 ± 1.39	4.5	6.8	9.1
Acetate Buffer	4.1	0.60 ± 0.12	100.0	150.0	200.0
Blank FeSSIF	5.0	0.22 ± 0.04	272.7	409.1	545.5
FeSSIF (with bile salts and lecithin)	5.0	0.28 ± 0.03	214.3	321.4	428.6
Blank FaSSIF	6.5	0.16 ± 0.04	375.0	562.5	750.0
FaSSIF (with bile salts and lecithin)	6.5	0.14 ± 0.03	428.6	642.9	857.1
SIF pH 6.8	6.8	0.14 ± 0.02	428.6	642.9	857.1

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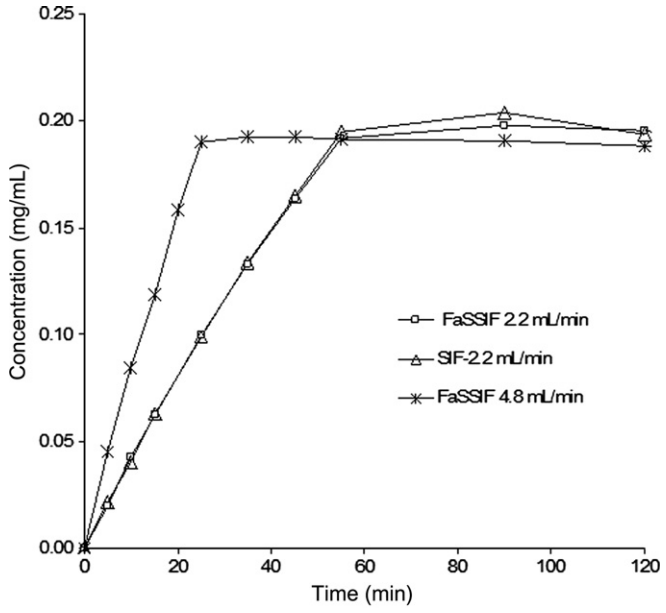

Fig. 3. Measured concentrations when a 1 mg/mL solution of etoricoxib in SGF is transferred into FaSSIF and SIF at 2.2 mL/min and into FaSSIF alone at 4.8 mL/min.

permeability (P_{eff}) was estimated using the permeability converter utility in Gastroplus™, using the *in vitro* caco-2 permeability data. The value obtained was 4.07×10^{-4} cm/s. The human effective permeability value estimated using ADMET Predictor™ version. 2.0 (Simulations Plus Inc, Lancaster, CA, USA) was 3.5×10^{-4} cm/s, and is close to the value estimated using the *in vitro* caco-2 cell culture technique.

3.6. Computer simulations using dissolution data

Fig. 4 shows the observed and simulated plasma profiles using dissolution data from the USP Apparatus 2 in SGF, FaSSIF-900 mL, the USP-SIF and FaSSIF-500 mL. The profile in SGF and FaSSIF-900 mL appears to simulate the *in vivo* profile better compared with that in SIF and FaSSIF-500 mL. The C_{max} (maximum plasma concentration) is lower in profiles simulated using the USP-SIF and FaSSIF-500 mL compared to that in profiles simulated using FaSSIF-900 mL.

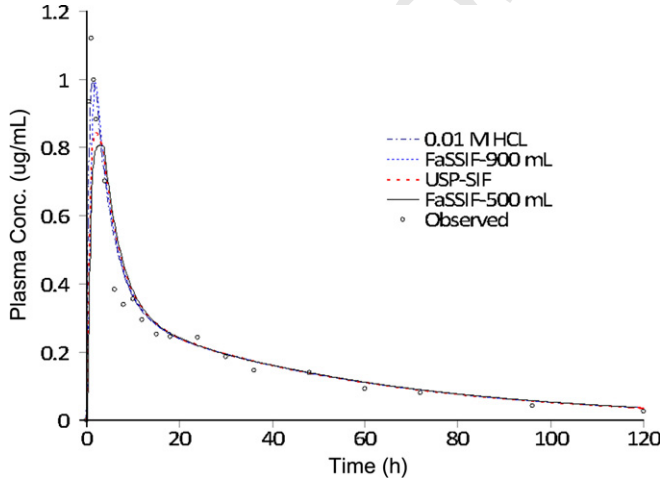

Fig. 4. Etoricoxib: comparison of simulated profiles and observed *in vivo* data (+0 mg tablet) using dissolution data as input function in GastroPlus. The simulated curves of 0.01 M HCl and 900 mL FaSSIF are superimposable and predict the observed data well, however, the simulated curves using SIF or 500 mL FaSSIF as input function show lower C_{max} values.

Table 3
Etoricoxib: regression analysis output.

Medium/method	Power of prediction values			
	r^2	SSE	RMSE	MAE
Solution	0.900	0.193	0.101	0.054
FaSSIF-900 mL	0.899	0.195	0.101	0.058
0.01 M HCl	0.898	0.197	0.102	0.054
USP-SIF	0.676	0.613	0.180	0.093
FaSSIF-500 mL	0.593	0.820	0.208	0.114

Table 4
Etoricoxib: percent prediction error (PE) statistics.

Observed values:- AUC = 1.818×10^4 ng h/mL, C_{max} = 1.12 μg/mL				
Media	AUC (ng h/mL)	C_{max} (ug/mL)	%PE	%PE
			AUC	C_{max}
Solution	1.943×10^4	1.007	−6.88	10.05
0.01 M HCl	1.942×10^4	0.989	−6.82	11.69
FaSSIF-900 mL	1.941×10^4	0.990	−6.77	11.59
USP-SIF	1.939×10^4	0.844	−6.66	24.64
FaSSIF-500 mL	1.937×10^4	0.806	−6.55	28.04

−ve sign means predicted is > mean observed, and no sign means predicted is < observed.

To investigate whether etoricoxib behaves like an oral solution, a set of simulations were performed using the preset software model, where the software assumes that all drug is dissolved and behaves like a solution. The simulated profile was compared with the simulated profile using FaSSIF-900 mL. The simulated profiles were similar and superimposable (data not shown) indicating that there is no difference between the oral absorption of a tablet and of a drug solution.

3.7. Statistical analysis

Results from regression analysis to compare the simulated and observed profiles are shown in Table 3. The results indicate that the best *in vitro*/*in vivo* correlations can be established using the solution model (r^2 = 0.90). Dissolution profiles from SGF and FaSSIF-900 mL as input function provide a similarly good IVIVC (r^2 = 0.899 and 0.898, respectively). Dissolution profiles from SIF and FaSSIF-500 mL provide weak correlations with the *in vivo* profile (r^2 = 0.676 and 0.593, respectively). The percent prediction error (PE) shown in Table 4 indicates that the SGF and the FaSSIF-900 mL dissolution data predicted both the AUC and C_{max} similar to solution, and better than the USP-SIF and FaSSIF-500 mL, respectively.

4. Discussion

4.1. Solubility studies

The drug equilibrium solubility of etoricoxib between pH 5.0 and 6.8 indicates that it does not meet the current FDA criteria for high solubility to be classified as class 1 drug. The values (0.28 and 0.14 mg/mL) are lower than the required solubility of 0.48 mg/mL to dissolve the highest dose strength of 120 mg in 250 mL. These solubility values were obtained using the equilibrium solubility at a constant pH. Increasing the dose volume to 500 mL as suggested by Yu et al. [13] will not change the classification of etoricoxib. However, at pHs 1.2 and 4.1, the solubility of etoricoxib is high enough to completely dissolve the entire high-

est dose of 120 mg in 250 mL of the media without saturation. The solubility does not take into account the dynamic pH changes in the gastrointestinal tract as present *in vivo* [15].

4.2. Intrinsic dissolution studies

The intrinsic dissolution rate (IDR) is a rate phenomenon rather than a measure of equilibrium solubility; therefore it is expected to correlate more closely with the *in vivo* drug dissolution rather than with equilibrium solubility [22]. As such, Yu et al. proposed that an intrinsic dissolution rate of 0.1 mg/min/cm^2 might be used as a class boundary for highly soluble drugs according to the BCS, taking into consideration the dose of the drug [22]. Based on this observation, etoricoxib may be considered as a poorly soluble drug due to the low IDR at high pH values.

The lower intrinsic dissolution rate in the two bile salt solutions (FaSSIF, pH 6.5, and FeSSIF, pH 5.0) is most likely due to the higher pH. The difference in the intrinsic dissolution rate in SGF-SLS compared with that in the USP-SGF could possibly be explained by the presence of micelles in the former. The effective diffusivity of a micelle is reduced compared to that of a drug molecule which is smaller than a micelle, resulting in a reduced diffusion coefficient, and a slower diffusion from the surface of the dissolving compact into the bulk medium [25] This is in accordance with results reported by Crison et al., who showed a decrease in the effective diffusivity of carbamezepine with increasing concentration of sodium lauryl sulfate [26].

4.3. Dissolution studies

The observed incomplete drug dissolution in FaSSIF-500 mL, FaSSIF-900 mL and SIF-900 mL might be due to a lack of sink conditions. Based on the solubility of etoricoxib of ~0.14 mg/mL in these media and pH range, about 1.3 L of dissolution media would be required to provide sink conditions [27]. Complete dissolution in SGF is due to the high solubility of the drug in the medium, and therefore sink conditions existed.

4.4. Investigating possible in vivo precipitation under physiological conditions

Etoricoxib a weak base has shown a high solubility and dissolution rate in the acidic environment of the stomach. Theoretically it is possible that as it moves down the GI tract and the pH rises, its solubility and dissolution rate decrease and it may precipitate out. Therefore, it was investigated if etoricoxib drug powder dissolved in simulated gastric fluid may precipitate when added to FaSSIF or SIF. No precipitation was, however, observed to occur in FaSSIF or SIF within two hours. This suggests that a higher than expected solubility can be achieved in the small intestine, if the drug undergoes complete dissolution in the stomach and is emptied into the duodenum. Since the concentration in solution is the driving force for passive diffusion absorption [17], it appears that the rate of absorption can be even higher than that predicted from aqueous solubility or media simulating the intestinal conditions.

This finding is, however, different from the results reported by Kostewicz et al. [17] where three weakly basic drugs (dipyridamole, BIBU 104 XX and BIMT 17 BS) precipitate in solution under fasted state conditions at concentrations corresponding to their usual doses. The three drugs in the report have aqueous solubilities between 0.002 and 0.008 mg/mL, which compared with that of etoricoxib are 17-to 70-fold lower.

Precipitation of a supersaturated solution depends on nucleation and crystallization. The formation of the initial nuclei depends on the relative supersaturation, which is the difference between the actual concentration of the solute before crystallization and its solubility limit. Von Weimarn recognised that stable nucleation rarely takes place when the supersaturation is less than 3 [28]. With a solubility of 0.14 mg/mL in SIF and a concentration of ~0.2 mg/ml, etoricoxib in SIF has a relative supersaturation of only ~1.42, which is far below 3. This might be the reason why no precipitation did occur. This explains why the concentration of etoricoxib in SIF can exceed its equilibrium solubility. The dynamic solubility observed using the pH gradient allows etoricoxib to be classified as a class 1 drug.

4.5. Cell culture permeability studies

The estimated value for the human effective permeability of 4.07×10^{-4} cm/s suggests that etoricoxib is highly permeable. Amidon et al. demonstrated that the limit for greater than 90% absorption corresponded to a permeability of 2×10^{-4} cm/s [29]. The oral bioavailability for etoricoxib is reported to be 100% [3]. The permeability directional ratio (PDR), which is the ratio of BA/AB transport, was estimated to be 0.969. Yazdanian et al. suggested that drugs with a PDR ratio between 0.7 and 1.3 do not appear to have affinity for cellular efflux pumps [15]. Based on the permeability value and the observed bioavailability, etoricoxib can be considered a BCS class 1 or class 2 drug.

4.6. Computer simulations

A comparison of the simulated profiles generated using data from 0.01 M hydrochloric acid, FaSSIF-900 mL and the software pre-defined solution model indicates that when taken as a tablet, etoricoxib is absorbed similar to a solution. This is due to its rapid dissolution in the gastric compartment. Any drug entering the small intestine is dissolved and stays in solution.

Since the effective permeability of etoricoxib (4.07×10^{-4} cm/s) is considered high, it appears that the dissolved drug is readily absorbed from the upper parts of the GI tract. Its bioavailability therefore seems to be regulated by the gastric emptying rate. This behaviour is similar to that of BCS class 1 drugs, which are highly soluble and highly permeable, and their bioavailability therefore is dependent on the gastric emptying rate [29].

The percent prediction error (PE) statistics shows that all four dissolution profiles over-predicted the AUC to a similar extent (6.8%, 6.7%, 6.6% and 6.5%), indicating that the extent of absorption is not affected by the dissolution rate in the four media. This might also be an indication of borderline behaviour, since Galia et al. had demonstrated that the behaviour of BCS class 1 drugs is not affected by the choice of dissolution media. The FaSSIF-500 mL and the USP-SIF under-predicted C_{max} to an extent greater than the 0.01 M HCl and the FaSSIF-900 mL (24% and 28% compared with 11.7% and 11.6%). This suggests that the USP-SIF may not be the best choice of media, while using FaSSIF at 500 mL may not be the right choice of volume, for *in vitro* testing of etoricoxib, to establish IVIVC. When simulated as an oral solution, the prediction error statistics are similar to the 0.01 M HCl and FaSSIF-900 mL dissolution.

4.7. BCS classification

Following the strict definition of the BCS, etoricoxib may only fit into BCS class 2. Our results indicate that the pH-dependent solubility may not result in *in vivo* precipitation of the drug as it moves down the gastrointestinal tract. The scientific criterion for a drug to be classified as intermediate class 1/2 drug is that the relevant *in vivo* solubility in the small intestine can dissolve the highest dose rather than the solubility between pH 1.2 and 6.8 [13,15,30]. The data from our transfer model support a classification into the intermediate solubility class 1/2 since the entire drug

dose of etoricoxib stays in solution at higher pH values after it is dissolved at a low pH. Furthermore, our computer simulations show that there is no difference in the drug plasma concentrations if the simulation is performed as solution or if the actual dissolution data are used for the simulation. The *in vitro* and *in silico* findings indicate that this drug behaves like a BCS class 1 drug, which is supported by its *in vivo* fast and complete absorption [3].

4.8. Biowaivers

Under certain circumstances Biowaivers can be requested to demonstrate bioequivalence between two drug formulations. Currently, Biowaivers are only granted for BCS class 1 drugs [11], which are formulated as immediate release dosage forms, or for minor formulation changes under SUPAC [31], or if an IVIVC was established for extended release dosage forms [20]. However, as mentioned earlier there are scientific discussions to extend Biowaivers to class 3 and intermediate class 1/2 drugs. The fortieth report of the WHO expert committee on specifications for Pharmaceutical preparations states that Biowaivers could be extended to BCS class 3 drugs if the product dissolves very rapidly (85% <15 min) and to BCS class 2 weak acids if the API has a dose: solubility ratio of 250 ml or less at pH 6.8 and has rapid dissolution (85% <30 min) [32]. Our results show that weak bases such as etoricoxib, which dissolve in the acetic environment of the stomach and stay supersaturated in solution in the small intestine, might also be candidates for Biowaivers.

4.9. Conclusions

The Caco-2 cell culture permeability results indicate that etoricoxib is highly permeable, and pharmacokinetic studies show that 100% bioavailability is obtained when the drug is administered orally as a tablet. Experiments using the *in vitro* transfer model showed that if the entire dose of etoricoxib is dissolved in the gastric media and added to FaSSIF or SIF, a higher concentration and hence higher solubility can be achieved under such simulated intestinal conditions. Computer simulations using a solution model were similar to simulations using tablet dissolution data from 0.01 M HCl and biorelevant media. The simulations show that dissolution behaviour similar to that in FaSSIF is at least needed to be bioequivalent, and any profile with lower release rates might impact on the *in vivo* pharmacokinetic profile.

Q4 Considering the pH gradient in the GI tract, the *in vitro* and *in vivo* behaviours of etoricoxib is similar to those of a BCS class 1 drug. From this perspective, etoricoxib can be considered highly soluble, since its highest dose does not precipitate when transferred from the stomach into the small intestine. The properties of etoricoxib as studied in the media transfer model show that it behaves like a BCS class 1 drug. In view of its therapeutic use, its wide therapeutic index and simple pharmacokinetic properties, a biowaiver for immediate release etoricoxib solid oral drug products can be scientifically justified.

For that *in vitro* bioequivalence test, the WHO requirements can be applied: the release profiles of the test product and its comparator have to show similarity in media having pHs 1.2, 4.5 and 6.8 Q5 using the f2 factor analysis with a critical value of at least 50. Very rapidly dissolving drugs are considered similar. Also, the WHO requirements with respect to the excipients present in the test product need to be taken into account.

Acknowledgments

This work was supported financially by a research grant from NSERC and Merck Frosst Canada Inc. We thank the staff at Merck Frosst Laboratories, Kirkland, Quebec, for their support with X-ray diffraction and intrinsic dissolution studies, and Simulations Plus Inc.

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Please cite this article in press as: A. Okumu et al., Computer simulations using GastroPlus™ to justify a biowaiver for etoricoxib ..., Eur. J. Pharm. Biopharm. (2009), doi:10.1016/j.ejpb.2008.10.019