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Can the USP paddle method be used to represent in-vivo hydrodynamics?

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Abstract

Experiments in-vitro and in dogs were conducted to find in-vitro hydrodynamic conditions that can be used to represent gastrointestinal motility patterns. Specifically, the dissolution performance of micronised and coarse-grade felodipine (a poorly soluble, neutral, lipophilic drug) was studied in a biorelevant medium in the USP paddle apparatus at various paddle speeds. Ratios of percentage dissolved (slower:faster rev min^{-1}) were calculated pairwise. These ratios were then compared with AUC ratios obtained in a corresponding pharmacokinetic study in Labradors, in which the absorption of both the micronised and coarse-grade felodipine had been compared under two gastrointestinal hydrodynamic conditions. Using a paddle speed combination of 75 and 125 rev min^{-1} to represent the motility patterns in response to administration of normal saline and 5% glucose, respectively, the in-vitro ratios (75:125 rev min^{-1} dissolution ratio was 91% for the micronised and 46% for the coarse-grade powder) showed good agreement with the pharmacokinetic data (saline-to-glucose absorption ratio was 98% for the micronised and 46% for the coarse-grade powder). It was concluded that, provided an appropriate composition is chosen for the dissolution test, the USP paddle apparatus can be used to reflect variations in hydrodynamic conditions in the upper gastrointestinal tract.

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Introduction

Dissolution testing is an important tool to forecast the in-vivo performance of drugs and their formulations. In recent years, many efforts have been made to improve the in-vitro conditions to better predict in-vivo behaviour. For example, biorelevant media have been established (Galia et al 1998; Nicolaides et al 1999) to reflect the conditions in the fasted and fed state in the stomach and small intestine. Yet little is known about in-vivo hydrodynamics and how well the in-vitro testing conditions correspond to them.

Recently performed pharmacokinetic studies in fistulated Labradors (Scholz et al 2002), in which the hydrodynamic influence on the bioavailability of the poorly soluble drug felodipine (aqueous solubility: $1\,\mu g\,mL^{-1}$ at 37 °C, log P 4.5) was selectively investigated, revealed a dependency of the hydrodynamic effect on the particle size. A two-fold higher bioavailability after administration of a felodipine suspension under hydrodynamic conditions representative of the fed state compared with the fasted state was observed for coarse-grade felodipine. In contrast, no change in the bioavailability with hydrodynamic conditions was observed for micronised felodipine. Based on these results, the aim of this study was to align hydrodynamic conditions in-vitro using the USP Apparatus II (paddle method) to reflect the in-vivo results. Specifically, we wanted to find a pair of rotational speeds at which differences in the dissolution profiles would be observed for coarse-grade but not for micronised felodipine.

To be able to compare the in-vitro data directly with the in-vivo data, batches and amounts of felodipine used and sampling times were identical to those used in the pharmacokinetic studies; the medium was composed to match the solubility of felodipine in chyme and volumes were chosen to ensure that sink conditions would prevail throughout most of the dissolution test period. In the in-vitro experiments only the hydrodynamic conditions (i.e. the rotational speed) were varied.

Materials and Methods

Materials

Felodipine and the internal standard (H 165/04), a structural analogue of felodipine, were supplied by AstraZeneca (Mölndal, Sweden). Two particle sizes of felodipine were used: micronised powder, lot no. 41688-01, with a median particle size of 8 μm (95% CL 0–24.1 μm) and coarse-grade powder, lot no. 14-01 (sieve fraction 100–200 μm), with a median particle size of 125 μm (95% CL 0–272 μm). Particle sizes were determined using a Coulter LS 130 (Beckmann Coulter Inc., Fullerton, CA) equipped with a fluid micro volume module and the corresponding software. Taurocholic acid sodium salt (NaTC) (>99% purity, lot no. 2000060181) was purchased from Chimici E Alimentari S.p.A. (Basaluzzo, Italy). Egg phosphatidylcholine (99.1% purity, lot 0101-L) was kindly donated by Lipoid GmbH (Ludwigshafen, Germany). All other chemicals were AR grade or equivalent and purchased commercially.

Chyme was collected from three male Labradors, weighing 30–35 kg, fistulated at mid jejunum (Wilsson-Rahmberg & Jonsson 1997), approximately 76 cm distal to the pylorus. Either normal saline (198 mL), which was used to maintain fasted state motility conditions (n = 8 experiments), or isotonic glucose (198 mL), which was used to induce fed state motility conditions (n = 7 experiments), was administered orally 15 min after completion of an infusion of 100 mL of the same fluid directly into the gut over a 3-min interval. Collection in graduated 10-mL vials was then performed continually for 2 h. Both the pH (PHM 93 Reference pH meter; Radiometer, Copenhagen, Denmark) and osmolality (3 MO microosmometer; Advanced Instruments Inc., Norwood, MA) of the freshly collected chyme were measured. The chyme collections were performed in the same dogs as the pharmacokinetic study described in the introduction. The study was approved by the Animal Ethics Committee Gothenburg (ethics approval no.: 2091997).

Solubility studies

In canine chyme

An excess of felodipine was added to 5-mL glass vials containing approximately 3 mL chyme collected individually from the fistulated dogs. After 72 h of gentle shaking in an oven at 37 °C (see Figure 1 for baseline data showing the approach to equilibrium in saline–PEG) the chyme was filtered using a Sartorius (SRP 25, 0.45 μm) filter. The filtrate was diluted using 99% (v/v) ethanol in preparation for analysis of the felodipine concentration by HPLC. Forty-seven determinations were made in chyme collected after administration of normal saline and forty-one in chyme collected after administration of 5% glucose, and each determination was run in duplicate.

In simulated intestinal fluids

An excess of felodipine was added to 20 mL scintillation vials containing simulated intestinal fluids based on the composition of FaSSIF and FeSSIF (Galia et al 1998). In the case of fluids simulating the fasted state, bile salt concentration was varied from 3 to 10 mM, while for fluids simulating the fed state the concentration was varied from 3 to 40 mM. In all media the ratio of bile salt to lecithin (egg phosphatidylcholine) was held constant at 4:1. The samples (n = 3 per medium) were shaken by gentle agitation in an oven at 37 °C. After 4, 24 and 72 h, 5-mL samples were removed and immediately filtered (Rezist 30/0.45 PTFE, 0.45 μm; Schleicher & Schüll). The first 2 mL were discarded and the remaining 3 mL were diluted using 99% (v/v) ethanol for analysis by HPLC.

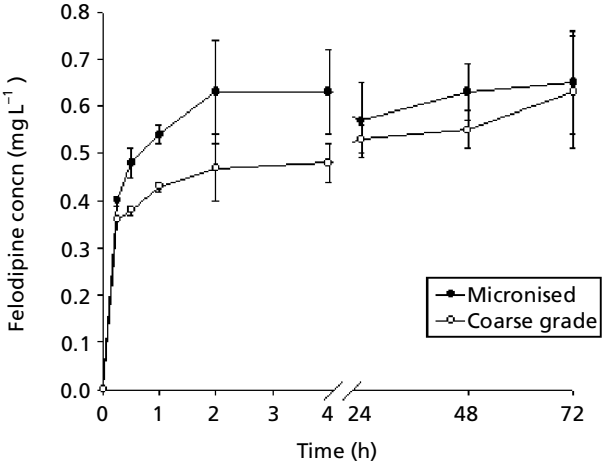

Figure 1 Concentration of micronised and coarse-grade felodipine in 0.9% saline containing 0.8% PEG 4000 at 37 °C: approach to the equilibrium solubility.

In 0.9% NaCl containing 0.8% PEG 4000

An excess of micronised or coarse-grade felodipine was added to 50 mL 0.9% NaCl containing 0.8% PEG 4000 solution in 50-mL stoppered conical flasks. The samples (n = 3 per powder size) were gently shaken in an oven at 37 °C. Samples (5 mL) were removed after 0.25, 0.5, 1, 2, 4, 24, 48 and 72 h and immediately filtered (Sartorius; SRP 25, 0.45 μm). The first 2 mL were discarded and the remaining 3 mL were diluted using 50% (v/v) ethanol for analysis by HPLC.

Dissolution medium

All dissolution studies were performed in the medium in which the solubility of felodipine was nearest to its solubility in chyme samples (Table 1). The osmolality and pH of this medium were also similar to those of recovered chyme (Table 2). It should be noted that because felodipine does not ionise in the physiologically relevant pH range, minor differences in pH between chyme samples and the dissolution medium are unlikely to have any effect on either solubility or dissolution. Furthermore, due to the presence of bile salts and lecithin in the dissolution medium, both the coarse and micronised felodipine particles were immediately wetted by the medium.

Table 1 Composition of medium used to simulate conditions in the canine small intestine, after administration of normal saline or isotonic glucose.

Sodium taurocholate	5 mM
Lecithin	1.25 mM
NaOH	0.27 g
KH_2PO_4	1.95 g
KCl	3.85 g
HCl	qs pH 6.5
distilled water	qs 0.5 L

Dissolution studies

Dissolution experiments were conducted in USP Apparatus II (paddle apparatus, Model DT 6, Erweka, Heusenstamm, Germany). Either micronised or coarse-grade felodipine (10 mg) were sprinkled on 500 mL dissolution medium at 37 °C. Sampling times were identical to the blood sampling times in the pharmacokinetic study: 5, 10, 15, 20, 30, 60, 90, 120, 180, 300, 420 and 1440 min. Samples were removed from the vessel at these intervals using a 5-mL glass syringe (Fortuna Optima) fitted with a stainless-steel cannula. The tip of the cannula was fitted with a 10-μm Protoplast frit to prevent undissolved particles from being withdrawn. The samples were immediately filtered (0.45 μm pore size; Schleicher & Schüll Rezist 30/0.45 PTFE), the first 2 mL were discarded and the remaining 3 mL were diluted with mobile phase (acetonitrile–methanol–phosphate buffer, pH 3, 40:20:40 (v/v/v)) before HPLC analysis. The volume of medium withdrawn from the vessel was replaced with an equal volume of pre-warmed, blank medium. Rotational speeds of 50, 75, 100, 125 and 150 rev min^{-1} were tested to find the right combination to reflect the in-vivo performance of the two felodipine particle sizes. All dissolution experiments were performed in triplicate.

HPLC analysis

In simulated intestinal fluids

Felodipine concentration was determined by injecting (Merck Hitachi autosampler, model L-7200; Merck, Darmstadt, Germany) a 100-μL volume of sample on a LiChrospher 100, RP-18, 125 mm (5 μm) column combined with a LiChrospher 100, RP-18, 4 × 4 mm (5 μm) guard column (Merck, Darmstadt, Germany). The mobile phase consisted of acetonitrile–methanol–phosphate buffer, pH 3, 40:20:40 (v/v/v). The flow rate was set at 1.5 mL min^{-1} (Merck Hitachi pump, model L-7110) and detection was at 362 nm using a Spectroflow 757 absorbance detector (Kratos Analytical, NJ). Felodipine typically eluted at approximately 6.0 min. Peak areas were recorded and analysed by a Chromatopac Shimadzu integrator, model C-R5A and concentrations were calculated with Microsoft Excel from a standard curve covering a concentration range of 0.2–100 mg L^{-1}.

In canine chyme

Before injection on a Nova PAK TM C18, 150 mm (5 μm) (Waters Corporation, Milford, MA) column, chyme samples were extracted with toluene containing the internal standard, evaporated to dryness and re-dissolved in mobile phase. The flow rate was set at 1.0 mL min^{-1} and detection was at 362 nm. Typical retention times were 8.5 and 16.5 min for felodipine and the internal standard, respectively.

Statistics

Results are presented as means and their standard deviations (±s.d.). Student's unpaired *t*-test was used to test for difference between means. A *P*-value less than 0.05 was considered to be significant (SigmaStat 2.0).

Data fitting

The film model of dissolution proposed by Noyes & Whitney (1897) and later modified by Nernst (1904) and Brunner (1904) can be used as a basis for describing dissolution. The film model can be summarized by equation 1.

$$\frac{dX_d}{dt} = \frac{D \times A}{\delta}\left(Cs - \frac{X_t}{V}\right) \quad (1)$$

where X_t is the amount dissolved at time t, V is the volume of dissolution medium, Cs is the solubility in the dissolution medium, A is the surface area, D is the diffusion coefficient and δ is the boundary layer thickness. The thickness of the boundary layer depends on the in-situ hydrodynamic conditions (Levich 1962). Depending on the prevailing conditions during the test, various

Table 2 Comparison of key parameters of chyme obtained after administration of normal saline or 5% glucose and the dissolution medium (see Table 1) used for the in-vitro studies.

	Chyme		Dissolution medium
	0.9% NaCl (fasted)	5% Glucose (fed)	
Solubility of felodipine (mg L^{-1})[a]	77 ± 47	56 ± 34	77.1 ± 1.3
pH	6.35 ± 1.32	4.93 ± 0.97	6.5
Osmolality (mOsm kg^{-1})[a]	277 ± 17	293 ± 15	270 ± 10

[a]No significant difference (unpaired *t*-test, $P > 0.05$) between fasted and fed.

approaches can be used to describe in-vitro dissolution mathematically.

Equation 1 can be integrated to give a first-order equation (Reppas & Nicolaides 2000). This first-order equation assumes that A is constant. Since A obviously will decrease over the course of the experiment, the equation can yield, at best, an empirical fit to the data. Assuming that sink conditions prevail throughout the process ($X_t/V < 0.2\,Cs$) and the surface area is constant, zero-order kinetics can be used to fit the data (Reppas & Nicolaides 2000). Zero-order kinetics can often be applied to calculate the initial dissolution rate. Taking the continuously decreasing surface area under prevailing sink conditions into account, the cube-root equation (Hixon & Crowell 1931) can be applied.

All the approaches listed above are approximations, addressing different periods and aspects of the dissolution process. Their goodness of fit, therefore, depends on which aspects of the dissolution are dominant under the prevailing experimental conditions (Reppas & Nicolaides 2000).

An empirical description of dissolution can be achieved with the Weibull distribution (Weibull 1951; equation 2).

$$X_d = X_{d,max} \times \left(1 - e^{-(t-\gamma/\tau_d)^{\beta}}\right) \quad (2)$$

where $X_{d,max}$ is the maximum amount that can dissolve, X_d is the amount dissolved at time t, γ is the lag time before the onset of dissolution, β is the shape factor and τ_d is the time parameter. Data obtained from dissolution experiments can often be fitted well with this distribution. In addition, parameters describing the process can be calculated and different curves can be compared using the lag time, the final plateau value, the shape factor and the rate of dissolution (Langenbucher 1976). An advantage using this distribution is that parameter calculation is independent of whether or not sink conditions prevail.

Mean data were linearised to the four above-mentioned models and regression analysis was performed with Microsoft Excel 2000.

f_2 calculations

To statistically evaluate differences/similarities between the dissolution profiles, f_2 factors (i.e. similarity factors (Moore & Flanner 1996)) were calculated according to equation 3.

$$f_2 = 50 \log\left\{\left[1 + \frac{1}{n}\sum_{t=1}^{n} w_t (R_t - T_t)^2\right]^{-0.5} \times 100\right\} \quad (3)$$

where R_t is the reference assay at time point t, T_t is the test assay at time point t, n is the number of sampling points and w_t is an optional weighting factor (for the current purpose, $w_t = 1$). The values obtained were in the range 0–100. If the two profiles are identical, f_2 is 100. Values of $f_2 \geqslant 50$ indicate similarity of two profiles under the assumption of a maximum allowable difference of 10%. f_2 data were calculated using mean values of percentage dissolved at the various sampling times.

In-vivo–in-vitro comparisons

In-vivo–in-vitro comparisons were made between the extents of absorption and dissolution.

The ratio of percentage absorbed from the micronised felodipine suspended in normal saline and isoosmotic glucose was calculated according to equation 4.

$$AR = \frac{AUC_{(0-7)}(NS)}{AUC_{(0-7)}(G)} \times 100\% \quad (4)$$

where AR is the absorption ratio, $AUC_{(0-7)}(NS)$ is the truncated AUC up to 7 h after administration in a 0.9% NaCl suspension and $AUC_{(0-7)}(G)$ is the truncated AUC up to 7 h after administration in a 5% glucose suspension.

The ratio of percentage dissolved from the micronised felodipine was based on results for paired rotational speeds (e.g. 50 with 100 rev min^{-1}; equation 5):

$$DR = \frac{\%Diss_{7h}(RS_{lower})}{\%Diss_{7h}(RS_{higher})} \times 100\% \quad (5)$$

where DR is the dissolution ratio, $\%Diss_{7h}(RS_{lower})$ is the percentage dissolved up to 7 h at the lower of the two rotational speeds and $\%Diss_{7h}(RS_{higher})$ is the percentage dissolved up to 7 h at the higher of the two rotational speeds.

The same procedure was followed for the coarse-grade felodipine. The aim was to find a pair of rotational speeds that produced the same ratios for the micronised and coarse-grade felodipine as had been observed in the pharmacokinetic study.

Results

Pharmacokinetic study

The aim of the pharmacokinetic study in fistulated Labradors (Scholz et al 2002) was to investigate the influence of hydrodynamics and drug particle size on the gastrointestinal dissolution and absorption of felodipine. To do this, it was necessary to maintain fasted-state or induce fed-state motility without affecting the solubility of felodipine or producing different fluid volumes in the gastrointestinal tract. This was achieved by the administration of either normal saline (fasted) or 5% glucose (fed). The dosing conditions selected for the fasted and fed states, while not representative of dosing conditions typically used in pharmacokinetic studies, allowed us to selectively study the influence of hydrodynamics on absorption of the poorly soluble felodipine. The relationships between fasted- and fed-state AUC values for micronised and coarse-grade felodipine are summarized in Table 3. The AR was 98% for the micronised powder and 46% for the coarse-grade powder.

Table 3 AUC values (0–7 hours) following the administration of either micronised or coarse-grade felodipine (dose: 10 mg) in either 0.9% saline or 5% glucose obtained in Labradors.

	$AUC_{(0-7)}$ (ng h L^{-1})	
	Micronised felodipine	Coarse-grade felodipine
Normal saline	24 486 (CV: 55%)	1734 (CV: 39%)
Glucose	25 014 (CV: 69%)	3746 (CV: 54%)
***AR (%)**	**97.9**	**46.3**

*see Eq. 3

Solubility

To perform the dissolution studies under the same solubility conditions as existed in the pharmacokinetic studies, felodipine solubility was investigated in biorelevant media containing different bile salt concentrations. A proportional relationship between NaTC–egg phosphatidylcholine (4:1) concentrations and felodipine solubility was observed (Table 4). Values obtained in modified FaSSIF ($77.1 \pm .3$ mg L^{-1}) and FeSSIF (56.6 ± 3.1 mg L^{-1}) media containing 5 mM NaTC and 1.25 mM egg phosphatidylcholine were identical to the values obtained in chyme recovered after administration of normal saline or 5% glucose, respectively. Mean values determined in canine chyme are summarized in Table 2.

Solubility–time profiles for micronised and coarse-grade felodipine in 0.9% saline solution containing 0.8% PEG 4000 suggested the significant influence of particle size on dissolution and time to equilibrium (Figure 1). While the micronised felodipine quickly achieved equilibrium, this was reached only after 72 h for the coarse grade.

Mathematical evaluation of dissolution profiles

Parameters calculated from the Weibull distribution and the first-order kinetics are given in Table 5. Dissolution of both powders was best described by the Weibull distribution, with coefficients of correlation (r^2) obtained from linear regression greater than 0.98 in every case. Slopes of the regression lines representing the shape factor β were slightly less than 1 for both powders (0.5–0.8 for the coarse powder and staying close to 0.6 for the micronised powder). The values obtained for τ_d, the time point at which 63.2% is dissolved, showed a wide range, from approximately 27 200 min at 50 rev min^{-1} to 550 min at 150 rev min^{-1} for the coarse powder, whereas a narrower range of 456 min at 50 rev min^{-1} to 191 min at 150 rev min^{-1} was observed for the micronised powder (Figure 2). For neither powder was an initial lag time observed, nor was the final plateau less than 100%.

Application of first-order kinetics resulted in a fit for both powders at all rotational speeds, with r^2 always greater than 0.95. Application of zero-order kinetics for the coarse powder at rotational speeds of 50 and 75 rev min^{-1} resulted in a similarly good fit to that obtained for the first-order kinetics, with $r^2 \geqslant 0.95$. Fitting to cube-root kinetics resulted in coefficients of correlation lower than those obtained for the Weibull distribution, first-order or zero-order kinetics.

Table 4 Felodipine solubility as a function of different NaTC–egg phosphatidylcholine (4:1) concentrations in simulated intestinal fluids at 37 °C.

NaTC (mM)	Cs (mg L^{-1})	
	Fasted (pH 6.5)[a]	Fed (pH 5.0)[b]
3	48.9 ± 0.9	35 ± 2.4
5	77.1 ± 1.3	56.6 ± 3.1
10	129 ± 2.7	133.4 ± 2.1
15		237.2 ± 19.8
20		332.5 ± 4.2
40		720 ± 6.8

[a]Based on FaSSIF; [b]based on FeSSIF. Values are means ± s.d., n = 3.

Dissolution studies—comparison of results for micronised and coarse felodipine

For the micronised powder, similar dissolution profiles ($f_2 \geqslant 50$) were obtained at rotational speeds of 75 rev min^{-1} and higher (Figure 3B, Table 6); at 50 rev min^{-1} results were lower due to coning. Using the criterion of $C \leqslant 20\%$ Cs of the drug in the corresponding medium to define sink conditions (Gibaldi & Feldman 1967), these prevailed up to 7 h at rotational speeds of 50 and 75 rev min^{-1} and up to 5 h at 100, 125 and 150 rev min^{-1}.

Figure 3A shows the dissolution profiles for the coarse powder tested at 50, 75, 100, 125 and 150 rev min^{-1}.

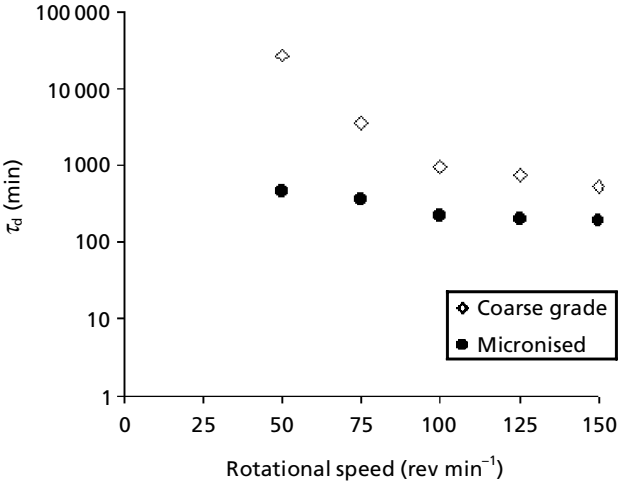

Figure 2 Time parameters τ_d of the Weibull function for the coarse-grade and the micronised powder as a function of rotational speed.

Table 5 Weibull distribution and first-order kinetic parameters for the dissolution of the coarse-grade and the micronised powder.

	Micronised felodipine Rotational speed (rev min^{-1})					**Coarse-grade felodipine Rotational speed (rev min^{-1})**				
	50	75	100	125	150	50	75	100	125	150
Weibull distribution										
β	0.58	0.61	0.66	0.64	0.63	0.52	0.65	0.82	0.77	0.81
τ_d(min)	456	367	225	207	191	27227	3541	985	761	551
k (min^{-1})	0.0022	0.0027	0.0044	0.0048	0.0052	0.00004	0.0003	0.0010	0.0013	0.0018
r^2	0.9956	0.9963	0.9906	0.9937	0.9964	0.9835	0.9904	0.9919	0.9985	0.9984
First-order kinetics										
k (min^{-1})	0.0023	0.0027	0.0036	0.0041	0.0041	0.0003	0.0006	0.0011	0.0016	0.002
r^2	0.976	0.9803	0.982	0.9913	0.9868	0.9654	0.961	0.981	0.96882	0.9961

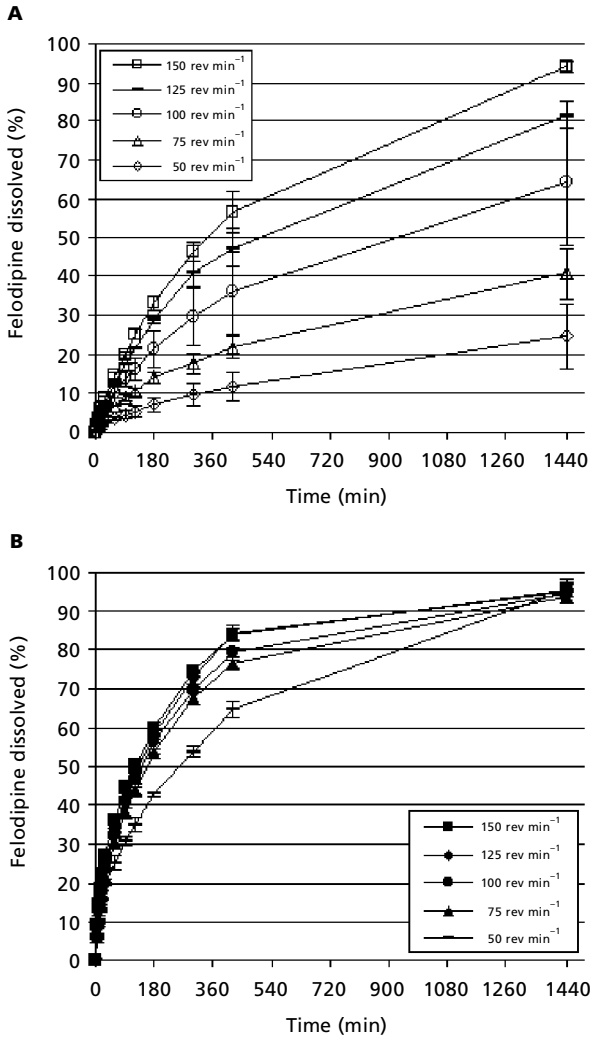

Figure 3 Mean dissolution profiles (n = 3) of felodipine in the paddle apparatus at various rotational speeds up to 24 h for coarse-grade powder (A) and micronised powder (B).

Differences among the dissolution profiles were observed for all rotational speed pairs ($f_2 < 50$), with the exception of the pairs with a difference of 25 rev min^{-1} ($f_2 \geqslant 50$). Sink conditions prevailed throughout the entire experiment at rotational speeds of 50, 75 and 100 rev min^{-1}, and for up to 7 h at rotational speeds of 125 and 150 rev min^{-1}.

In Table 6, the relative percentage dissolved felodipine at the different rotational speeds at 7 h are presented. The results with the rotational speed combination of 75 and 125 rev min^{-1} show an appropriate relationship to the in-vivo data with a DR of 91% for the micronised powder and 46% for the coarse powder.

Discussion

For the corresponding pharmacokinetic study (Scholz et al 2002) a fistulated Labrador model was developed in which the motility pattern, and hence hydrodynamics, could be selectively influenced. Using this model, the interaction between gastrointestinal hydrodynamics and particle size on the absorption of felodipine was investigated. Results of the study indicated that particle size has the greater effect on bioavailability (AUC values were approximately 10-fold higher for the micronised than for the coarse powder). A hydrodynamic influence on absorption was observed for coarse-grade felodipine with a two-fold increase in the AUC values under fed compared with fasted conditions. By contrast, hydrodynamics were unimportant to the absorption of the micronised drug. Appropriate choice of in-vitro hydrodynamic conditions in the paddle apparatus should therefore reflect a two-fold increase in the percentage dissolved felodipine for the coarse grade, but little or no difference for the micronised powder at the higher of the two rotational speeds chosen.

Solubility and dissolution studies

As indicated by the modified Noyes Whitney equation (equation 1), not only hydrodynamic conditions influence the dissolution process. Because of this, other dissolution parameters had to be chosen carefully to enable in-vitro results to be compared with the pharmacokinetic data. The composition of the dissolution medium was especially important in terms of achieving the same solubility driving force for dissolution.

Table 6 Relative percentage of dissolved felodipine for micronised and coarse-grade powder in the paddle apparatus at 7 h and the respective f_2 values for the corresponding dissolution profiles.

Rotational speed (rev min^{-1}/rev min^{-1})	DR felodipine (%) f_2	
	Micronised	Coarse grade
50/75	84.5	53.4
	51	*58*
50/100	81.6	32.5
	47	*40*
50/125	76.9	24.6
	44	*32*
50/150	77.1	20.7
	42	*27*
75/100	96.7	60.6
	55	*51*
75/125	91	45.9
	52	*39*
75/150	91.1	38.5
	50	*33*
100/125	94.3	75.8
	54	*55*
100/150	94.5	63.6
	52	*44*
125/150	100	83.9
	55	*60*

Solubility

The proportional increase of the solubility of felodipine with increasing NaTC–egg phosphatidylcholine (4:1) concentrations indicated the importance of the bile salt concentration in the medium used for the dissolution tests. Concentrations of bile components were therefore adjusted to reproduce the solubility of felodipine observed in the chyme samples. Note that because of the special design conditions needed to selectively study the hydrodynamic influences on absorption, the composition of the in-vitro dissolution medium was slightly different to that of the standard FaSSIF and FeSSIF media. Values of osmolality and pH were adjusted near to those of the canine chyme and also concur with data obtained in human chyme aspirated in the jejunum approximately 60 cm below the pylorus of fasted subjects (Pedersen et al 2000).

Particle size effects on approach to solubility

The time necessary to attain equilibrium solubility depends clearly on the particle size. Cs was achieved much faster for the micronised than for the coarse powder. Initially (at 0.25 h) concentrations were nearly identical for both powder sizes. A possible explanation for this observation is that in the coarse fraction, obtained by sieving the bulk powder, smaller particles may have adhered to the surface of the larger particles. This was confirmed by microscopic observations (data not shown). Subsequent to the initial burst phase, dissolution continued from the core fraction. In this phase the micronised powder exhibited a much faster approach to equilibrium, behaviour that can be attributed to its greater surface area and (probable) higher surface energy.

Mathematical evaluation of dissolution results

Fitting of different mathematical functions was used to describe the in-vitro performance of the two powder fractions under varying hydrodynamic conditions. The best fit was obtained for the Weibull distribution, in which the factor β describes the shape and the factor τ_d characterizes the overall rate of dissolution (Langenbucher 1976). Changes in τ_d indicate a dependence of the dissolution process on the hydrodynamics.

Shape factor β

The Weibull equation reduces to a simple first-order exponential if β equals 1; $\beta > 1$ reflects a sigmoidal profile, usually observed if disintegration of the dosage form is a limiting factor to dissolution. $\beta < 1$ is characteristic for a steeper initial slope followed by a flattened tail in the final part (Langenbucher 1976). For both powder sizes, a faster initial dissolution rate ($\beta < 1$) was observed at all rotational speeds. For the micronised powder, the fast initial rate may be attributed to the amorphous spots at the surface of the particle, resulting in regions with higher surface energy (Feeley et al 1998; Newell et al 2001); for the coarse fraction, very small particles adhering at the surface of the powder may contribute to the faster initial rate.

The lower β value within a powder fraction at 50 rev min^{-1} was linked to the observation of coning effects (more pronounced for the coarse grade) in the vessel.

Rate constant

Rate constants calculated from τ_d revealed minimal differences among paddle speeds for the micronised felodipine, indicating little or no sensitivity of the micronised powder to hydrodynamics. In contrast, the dissolution rate constants of the coarse-grade felodipine showed a 45-fold increase when the paddle speed was increased from 50 to 150 rev min^{-1}, indicating a pronounced sensitivity to hydrodynamics. These results concurred with those of the in-vivo studies.

Dissolution rate constants at all paddle speeds were much smaller for the coarse powder than for the micronised powder, indicating the importance of formulation parameters to the dissolution rate and hence absorption of poorly soluble drugs. These results are in agreement with previous studies in which the dependency of hydrodynamic effects on formulation was addressed using matrix tablets (Lindner & Lippold 1995; Abrahamsson et al 1998).

Why is the dissolution of the coarse, but not the micronised felodipine dependent on the hydrodynamics? Bisrat & Nyström (1988) reported a dependence of the boundary layer thickness on the particle size. Their results indicated that for particles $< 5\,\mu$m in diameter, the thickness of the boundary layer is so small that a change in stirring rate has essentially no effect on the dissolution rate. For larger particles (like the coarse-grade felodipine), the boundary layer thickness decreases with increasing stirring rate, resulting in the observed dependency of dissolution on hydrodynamics. A further consideration is the role of surface area. During dissolution, the volume of a particle decreases at a faster rate than the surface area. Hence at relatively small particle sizes surface area is the dominant factor in the dissolution (Dali & Carstensen 1996).

In-vivo–in-vitro comparisons

Since the same lots of coarse and micronised felodipine were used in-vitro and in-vivo, and since the composition of the dissolution medium was adjusted to simulate in-vivo conditions, it was possible to selectively study the effect of hydrodynamics on the dissolution process in-vitro and to compare the results directly with the in-vivo data. In-vivo changes in hydrodynamics affected the dissolution, and hence absorption, of the coarse powder, as evidenced by the two-fold greater extent of absorption in the fed compared with the fasted state. Little or no effect was observed for the micronised powder. With respect to the in-vitro studies, a similar behaviour of the two powder fractions was observed in the Weibull parameters (i.e., a clear effect of hydrodynamic changes on the dissolution of the coarse powder but little or no effect for the micronised powder). The best agreement of the in-vivo and the in-vitro data resulted when paddle speeds of 75 and 125 rev min^{-1}, simulating fasted- and fed-state hydrodynamics

in-vivo, respectively, were used. The similarity factor data confirmed that at this rotational speed, combination profiles were similar for micronised but dissimilar for the coarse-grade felodipine.

A correlation of the rates of absorption and dissolution was hampered by the paucity of in-vivo data. Sampling time points before t_{max} were too sparse, especially for the micronised powder, to permit the calculation of a model-dependent rate constant. Although a variety of model-independent parameters exist, such as C_{max}/AUC, partial AUC until t_{max} or exposure (Chen 1992; Macheras et al 1994; Tothfalusi & Endrenyi 1995; Tozer et al 1996), all were likewise compromised by the sparse sampling and subsequent uncertainty in C_{max} and t_{max} values.

Conclusions

This study indicated that, using 500 mL of an appropriate dissolution medium, paddle speeds in the range 75–125 rev min^{-1} can be used to simulate hydrodynamic conditions in the upper gastrointestinal tract under certain dosing conditions. Predictions of the in-vivo performance of a drug and its formulation may be improved by adjusting the hydrodynamics, as well as the composition and volume, of the dissolution medium appropriately. However, further studies with other compounds and various formulations will need to be conducted before a general recommendation of rotational speeds in the 75–125 rev min^{-1} range can be made.

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