ORIGINAL ARTICLE

DEVELOPMENT OF *IN VITRO IN VIVO* CORRELATION MODELS FOR CLOPIDOGREL TABLETS TO DESCRIBE ADMINISTRATION UNDER FASTING AND FED CONDITIONS

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Abstract

The dissolution profiles of clopidogrel 75 mg tablets in compendial gastric and intestinal media as well as in biorelevant simulated gastric and intestinal media mimicking fasting and fed conditions were determined. *In vivo* plasma concentration curves, that resulted following the administration of the same formulation of clopidogrel to healthy male and female volunteers in fasting and respectively fed state, were used for compartmental modeling of pharmacokinetic data. Data showed that *in vivo* exposure to clopidogrel increases significantly, when administration of the tablets is performed in fed state. *In vitro – in vivo* level A linear correlations were established for the intestinal fasting and fed data, using the Loo-Riegleman based deconvolution approach. The developed models confirm that the low solubility of clopidogrel in the biologically relevant absorption site medium is one of the sources of its increased PK variability. The robustness of the IVIVC recommends it as surrogate for *in vivo* bioavailability testing, being a cheaper and less time consuming initial screening tool for generic formulations.

Rezumat

S-a realizat determinarea profilelor de dizolvare a comprimatelor de clopidogrel 75 mg atât în mediu compendial gastric şi intestinal cât şi în medii biorelevante, simulând condiţii pre- şi post-prandiale la nivel gastric şi intestinal. Curbele de concentraţie plasmatică, ce au rezultat în urma administrării aceleiaşi formulări de clopidogrel unor voluntari sănătoşi de sex masculin şi feminin, în condiţii pre-prandiale şi respectiv post-prandiale, au fost utilizate pentru modelarea compartimentală a datelor de farmacocinetică. Datele *in vivo* arată că expunerea la clopidogrel creşşte semnificativ atunci când administrarea comprimatelor se realizează post-prandial. S-au stabilit corelaţii lineare *in vitro - in vivo* de nivel A pentru datele în mediu intestinal pre- şi post-prandial, utilizând modele de deconvoluţie bazate pe abordarea Loo-Riegleman. Modelele dezvoltate confirmă faptul că solubilitatea redusă a clopidogrelului în mediul de absorbţie biologic relevant reprezintă una din cauzele variabilităţii crescute a parametrilor farmacocinetici. Robusteţea modelului IVIVC îl recomandă ca surogat pentru testarea *in vivo* a biodisponibilităţii, fiind un test de *screening* iniţial pentru formulări generice mai ieftin şi mai rapid.

Keywords: clopidogrel, IVIVC, biorelevant dissolution media, PK profile, fasting, fed

Introduction

Clopidogrel is a thienopyridine derivative used as anti-platelet aggregation agent to inhibit blood clots formation in coronary artery disease, peripheral vascular disease, and cerebrovascular disease [6, 21]. Despite being the drug of choice for preventing patients that have undergone cardiovascular stent surgery from developing thrombotic events, nearly 20 to 40% of patients respond poorly to the standard regimen of therapy [25]. To date, the most cited causes for poor response to treatment include variability, drug resistance, and individual pre-treatment platelet reactivity [5, 15].

In terms of metabolism, it is known that two oxidative steps are required for the conversion of clopidogrel to its active metabolite (with oxo-clopidogrel being the intermediate in the metabolic pathway leading to the active compound). The reactions are mediated by multiple P450 cytochromes, with the main contributors being CYP2B6, CYP2C19, CYP3A4 and, to a lesser extent, CYP2C9 [10]. For CYP2C19 there are 3 major genetic polymorphisms: CYP2C19*1 (corresponding to normal function) and CYP2C19*2 and CYP2C19*3 (both loss-of-function alleles). Individuals who have 2 loss-of-function alleles can be considered poor metabolizers, while those who have 1 copy of a loss-of-function allele are regarded as intermediate metabolizers. Based on this fact it was postulated that genetic poly-morphism studies might be the key for

individualization of therapy and ultimately for surpassing the problem of poor responders [2]. Despite the fact that genetic testing has demonstrated a link between CYP2C19 genetic polymorphisms, altered clopidogrel metabolite concentrations and adverse clinical events, the correlation merely accounts for at best 12% of the overall variation in the response to clopidogrel therapy [11, 16, 18].

While great effort was made for elucidating the metabolic pathway of clopidogrel and into understanding the implications of distinct genetic variants, an important research area remains to date not pursued: the simple yet very relevant assessment of clopidogrel availability for further metabolizing. Stemming from biopharmaceutical considerations, following its poor solubility, it is expected that an important source of variability for clopidogrel might be the restricted, highly variable kinetic of its release in changing conditions along the gastro-intestinal tract, an aspect which has not been thoroughly studied until now. From variability in rate and extent of release of the active substance from pharmaceutical formulation would derive at least some of the large variability observed in pharmacokinetic studies. It is noteworthy to mention here that until recent years, pharmacokinetics of clopidogrel was also a white field in our knowledge, with the marketing authorization holder of the innovator product initially stating that plasma levels of the parent compound beyond 2 hours from administration are very low and below the quantification limit achievable at the time the product was launched on the market (0.00025 mg/L). Since then, progress has been slow and strenuous, with newly developed analytical methods having not only to surpass the issues of sensitivity (very refined LLOQ required to adequately quantify clopidogrel over a dosing interval) and selectivity (availability of adequate internal standards), but also the problem of stabilizing clopidogrel metabolites that are highly prone to back-conversion in standard sample processing conditions [19].

The main objectives of the present study were: to evaluate *in vitro* dissolution kinetics of clopidogrel in simulated gastric and intestinal media mimicking fasting and fed conditions, to reliably determine the pharmacokinetics of clopidogrel in healthy volunteers using state of the art HPLC-MS/MS methods and finally, to attempt a correlation between *in vitro* and *in vivo* data. Furthermore, depending on the observed correlations, we will try to identify if dissolution could be one of the sources of variability affecting the pharmacokinetics of clopidogrel and to select the most adequate biorelevant dissolution test that could be used as surrogate for *in vivo* bioavailability testing.

Materials and Methods

In vivo data

The in-vivo data used for these correlations were obtained from a single dose comparative bioavailability study of clopidogrel 75 mg administered in fasting state to 27 healthy male and female volunteers (original sample size was 28 volunteers, but one untreated drop-out was registered) and from a mixed gender pilot comparative bioavailability study of clopidogrel 75 mg in fed state conducted on a sample size of 12 subjects (in both cases, only plasma concentrations obtained after dosing with the commercially available reference standard (Plavix® 75 mg tablets) were considered for the purpose of this article). Both study protocols were reviewed and approved by the National Ethics Committee, by the National Agency for Medicines and Medical Devices and by the competent Institutional Review Board. All subjects enrolled were informed about the study medication and procedures and gave consent for the participation in the studies. Clinical investigations were conducted according to the Declaration of Helsinki principles and Good Clinical Practice. In Table I we presented the demographic characteristics of the subjects participating in the two studies.

Table I

Demographic characteristics of study subjects

Characteristic (method of data presentation)	FASTING comparative bioavailability study	FED comparative bioavailability study
Number of subjects	Enrolled: 28 Dosed: 27	Enrolled: 12 Dosed: 12
Gender distribution for dosed subjects (number, (% of study population))	Female: 15 (55.56%) Male: 12 (44.44%)	Female: 7 (58.33%) Male: 5 (41.67%)
Race of dosed subjects (number, (% of study population))	Caucasian: 27 (100.00%)	Caucasian: 12 (100.00%)
Age of dosed subjects (mean, (± standard deviation))	34.92 (± 14.80)	37.25 (± 15.04)
Body mass index of dosed subjects (mean, (± standard deviation))	24.10 (± 3.10)	25.84 (± 1.78)

Blood samples of 5 mL each were collected in tubes containing K_2EDTA as anticoagulant before dosing and at 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 12.0, 16.0, 24.0, 36.0 and 48.0 hours post dose. The collection tubes were then immediately immersed in water and ice bath until centrifugation. The samples were centrifuged for 10 minutes at 1500 g at a nominal temperature of 4°C. Separated plasma was divided into two aliquots of about 1 mL which were frozen at −70°C and maintained at this temperature until analysed. Clopidogrel content in plasma was determined according to a fully validated analytical method (as *per* European Medicines Agency (EMA) requirements) described in detail elsewhere [19, 20] and here shortly presented. After thawing the samples were vortexed for 3 minutes. Aliquots (0.2 mL) were transferred into polypropylene tubes, spiked with internal standard solution (0.02 mL d3-clopidogrel 100 ng/mL in acetonitrile) and then 0.4 mL of ice-cold acetonitrile was added as precipitation agent. Samples were then vortexed for 3 minutes and centrifuged for an additional 5 min (3000 g at 8°C). After protein precipitation, 0.4 mL clear supernatant aliquots were transferred from each sample into autosampler vials and submitted for analysis. The HPLC system consisted of two binary pumps model 1200SL Series from Agilent with a CTC-PAL HTS autosampler with thermostat, set at −5°C. Separations were carried-out on Ascentis Express RP-Amide columns (100 × 2.1 mm, 2.7 µm) employing as mobile phase a gradient of formic acid 0.1% in water and acetonitrile, at a flow rate of 0.2 mL/min; the volume of injection was 10 µL. The mass-spectrometer used was a quadrupole-linear ion trap model API 4000 QTrap (Applied Biosystems-Sciex) using an atmospheric pressure electrospray ionization source.

Quantitative data were acquired in multiple reaction monitoring (MRM) positive electrospray ionization mode and the MRM transitions considered for clopidogrel were 322.2/184.0 and for clopidogrel-d3 327.2/189.2. Nine-point spiked calibration curves were prepared and quality control samples were analysed during each analytical sequence.

Estimation of pharmacokinetic parameters by non-compartmental analysis was performed using subroutines of the KINETICA 2000 software (Innaphase Corp, Philadelphia, PA, USA). The maximum concentration (C_{max}) and the corresponding peak times (T_{max}) were evaluated from the individual plasma concentration - time profiles, whereas elimination rate constant (k_e) was obtained from the least-square fitted terminal log-linear portion of the profile. The elimination half-life ($t_{1/2}$) was calculated as $0.693/k_e$. The area under the curve to the last measurable concentration (AUC_{0-t}) was calculated by using trapezoidal rule method according to the formula $\Sigma[(C_{n-1} + C_n) \times (t_n - t_{n-1})/2]$, where C_{n-1} and C_n represent the concentrations determined at the subsequent sampling time points t_{n-1} and t_n (thus describing the area of one trapeze) and AUC_{0-t} represents the sum of areas of all trapezes calculated on non-zero values. The area under the curve extrapolated to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{0-t} + C_t/k_e$, where C_t is the last measurable concentration.

Compartmental analysis was performed using PK Solver Microsoft Excel data analysis Add-in [26].

In vitro dissolution

The *in vitro* drug release studies were performed on a DT 800 dissolution system (Erweka GmbH, Germany) using apparatus 2 (paddle method) at 50 rpm. A set of four biorelevant media representative for the fasted stomach (FaSSGF - Fasted State Simulated Gastric Fluid), postprandial stomach (FeSSGF - Fed State Simulated Gastric Fluid), (Table II), as well as intestinal fluid in fasted (FaSSIF - Fasted State Simulated Intestinal Fluid) and fed state (FeSSIF - Fed State Simulated Intestinal Fluid) (Table III) was used for the study of clopidogrel release kinetics. All media were prepared as presented in literature [4, 9, 24]. The corresponding compendial [23] media simulating the gastric (SGF – Simulated Gastric Fluid) and intestinal (SIF– Simulated Intestinal Fluid) environment were also used.

All experiments were run in triplicate. Samples of 2.5 mL were withdrawn after 5, 10, 15, 20, 25, 30, 45, 60, 90, 105 and 120 minutes using a glass syringe, and immediately replaced with an equal volume of preheated fresh medium. The resulting samples were filtered through a 0.45 µm Teflon® filter and immediately diluted with methanol. The quantitative determination of clopidogrel was performed by using a HPLC method, fully validated in accordance with the International Conference on Harmonization (ICH) regulations Q2(R1) [8].

Quantitative analysis of clopidogrel in the dissolution samples was performed by using a Waters HPLC system, consisting in a 600E Multisolvent Delivery System, a Waters 717 Autosampler and a Waters 486 Tunable Absorbance Detector (Waters, Milford, MA, USA). The detector was set at 220 nm.

The chromatographic separation was achieved on a Hypersil Gold column (150 x 4 mm, 5.0 µm) manufactured by Thermo Scientific, using as mobile phase an isocratic mixture of 0.1% trifluoroacetic acid: acetonitrile (85:15 v/v) delivered at a flow rate of 1.0 mL/min. The volume of injection was 5 µL for each sample.

The similarity factor (f_2) [13] was used for the comparison of clopidogrel dissolution rate in

different media, whereas the Area Under Curve (AUC) was used as measure of dissolution extent.

Table II

Composition of the Compendial and Biorelevant Gastric Dissolution Media

COMPONENT	SGF	FASSGF	FESSGF
NaCl (sodium chloride)	2.0 g	34,2 mM	237,02 mM
Pepsin	3,2 g	0,1 g	-
CH_3COOH (acetic acid)	-	-	17,12 mM
CH_3COONa (sodium acetate)	-	-	29,75 mM
Milk/Buffer	-	-	1:1
Sodium taurocholate	-	80 µM	-
Lecithin	-	20 µM	-
HCl (hydrochloric acid)	7 mL	q.s. ad pH 1,6	q.s. ad pH 5
Water (q.s. ad)	1 L	1 L	1 L
pH	**1.2**	**1.6**	**5.0**
Surface tension (mN/m)	70	42.6	52.3 ± 0.3
Osmolality (mOsm/kg)		120.7 ± 2.5	400 ± 10
Buffer capacity (mmol/L/ΔpH)			25
Dissolution volume (mL)	1000	500	500

Table III

Composition of the Compendial and Biorelevant Intestinal Dissolution Media

Component	SIF	FaSSIF	FeSSIF
NaH_2PO_4 (monobasic sodium phosphate)	6.8 g	3.438 g	-
NaCl (sodium chloride)	-	6.186 g	11.874 g
CH_3COOH (acetic acid)	-	-	8.65 g
NaOH pellets	-	-	4.04 g
Pancreatin	10 g	-	-
Sodium taurocholate	-	3 mM	15 mM
Lecithin	-	0.75 mM	3.75 mM
NaOH (sodium hydroxyde)	q.s. ad pH 6.8	q.s. ad pH 6.5	q.s. ad pH 5.0
Water (q.s. ad)	1 L	1 L	1 L
pH	**6.8**	**6.5**	**5.0**
Surface tension (mN/m)		54	48
Osmolality (mOsm/kg)		270 ± 10	670
Buffer capacity (mmol/L/ΔpH)		12	72
Dissolution volume (mL)	1000	500	1000

In silico models

Computer simulations performed using GastroPlus™ (version 8.0, Simulations-Plus Inc.; Lancaster, CA, USA) software.

The Advanced Compartmental Absorption and Transit Model *(*ACAT model*)* [1] implemented in GastroPlus™ software was used to predict the rate and extent of oral absorption. The model considers the gastrointestinal tract as being divided into nine consecutive compartments corresponding to the duodenum, jejunum (two compartments), ileum (three compartments), cecum and ascending colon, and enables predictions of rate and extent of drug absorption from the gastrointestinal tract, while taking into account intestinal drug efflux and metabolism [22].

The required input parameters, grouped under three categories, comprising three different sets of factors that influence oral drug absorption, namely "Compound", "Physiology" and "Pharmacokinetics", were either experimentally determined or *in silico* predicted. For the "Compound" tab, experimental values of clopidogrel solubility at pH 7.0 (0.0031 mg/mL) were used, whereas all other input variables (pKa, log *P*, Caco-2 or human jejunal permeability) were estimated *in silico* using the integrated ADMET Predictor™ module. When no information was available concerning a certain input parameter, default settings proposed by the program were used.

In the "Physiology" tab, the Opt logD Model SA/V 6.1 was used to estimate the changes in permeability throughout the GI tract. Simulations were performed in the GastroPlus™ Single Simulation Mode using the Opt logD SA/V 6.1 absorption scale factor (ASF) model, in the Human-Physiological-Fasted and respectively Human-Physiological-Fed mode.

For the "Pharmacokinetics" tab, experimental values of the pharmacokinetic parameters and

transfer rate constants obtained from the described *in vivo* studies were used.

The level A, point-to-point in vitro–in vivo correlation was established using the IVIVC Plus Module™ integrated into GastroPlus™ software. A deconvolution approach, based on Loo-Riegleman model was used for evaluation of clopidogrel absorbed fraction (FRA). The fraction of drug absorbed at the specified time points was further plotted against the percentage of drug dissolved at the same time points, and linear regression analysis was used to evaluate the obtained correlation.

Results and Discussion

In vitro dissolution

Gastric media

The dissolution profiles in compendial (SGF) and biorelevant (FaSSGF) simulated gastric media were practically superimposable, both exhibiting rapid, but somewhat variable release kinetics within the first 30 minutes and both resulting in complete dissolution (see Figure 1, left graph). This can be interpreted as a consequence of the sufficient solubility, correlated with dose, of clopidogrel in acidic conditions. The presence of bile salts in the FaSSGF media brought no additional benefit in terms of rate or amount of drug substance released (slightly inferior percentages of release can be observed for the first four evaluation time-points, as compared with SGF), thus suggesting that pH rather than the presence of surfactant agents is the rate limiting factor governing the release kinetics of clopidogrel.

However, in fed conditions, at a pH of 5.0, the solubility of clopidogrel is lower and consequently we see a dramatic decrease in percentage of drug released from the pharmaceutical formulation (only 5.8 % released within 120 minutes).

These results indicate a highly pH-dependent solubility profile, with a very rapid and complete dissolution in fasting gastric medium and a much slower and incomplete dissolution in fed gastric medium.

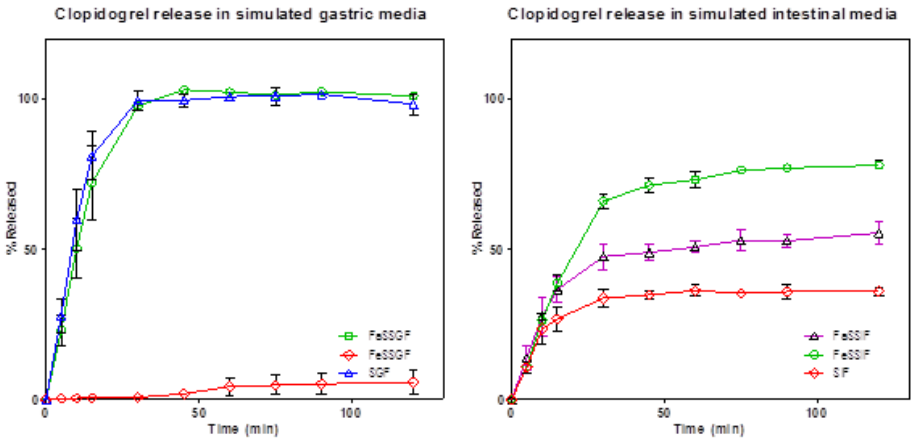

Figure 1.

In vitro dissolution of clopidogrel in gastric and intestinal simulated media, in fasting and fed conditions; a. gastric simulated media, b. intestinal simulated media

Intestinal media

The dissolution in intestinal medium was incomplete in both fed and fasting conditions (Figure 1). Although bile salts increased solubility by the intermediate of micelles, as is apparent when comparing SIF and FaSSIF, release in fasting intestinal medium remained still modest (60% within 2 hours). The amount released in fed state simulated conditions was significantly higher (80% drug released within two hours), thus permitting to conclude that, even in intestinal medium, the same principle of higher solubility at lower pH is applicable.

The differences between SIF and FeSSIF dissolution curves, already highly visible on graph, were further put into perspective by comparing the two extreme profiles in terms of similarity (calculated f_2 value of only 26.1) and AUC FeSSIF/SIF ratio (2.56).

Since clopidogrel is almost exclusively absorbed in the intestinal environment [15, 12], these differences in intestinal release will most likely have an impact on *in vivo* drug absorption, thus being expected that bioavailability of clopidogrel upon administration in fed conditions would be significantly higher than in fasted state (and not the opposite, as gastric dissolution data suggested).

The results in both gastric and intestinal simulated conditions indicate a highly pH-dependent solubility profile, with very rapid and complete dissolution in gastric medium (the dissolution profiles in compendial and biorelevant simulated gastric media were practically superimposable), followed by a significant tendency to precipitate

towards the higher pH of the intestinal media. The results are in accordance with the FDA available data [7], literature available solubility data [3], as well as *in silico*-generated solubility *vs.* clopidogrel pH (Figure 2). Stability of clopidogrel in all dissolution media used was assessed for 48 hours. In the acidic environment created by SIF, the clopidogrel degradation at room or body temperature (25 and 37ºC respectively), did not exceed 5% of the initial clopidogrel (3.4% and 4.6% respectively). In all other media, a less than 2% degradation of the drug was recorded.

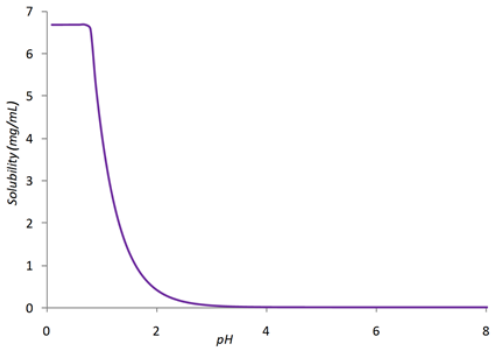

Figure 2.
Solubility *vs.* pH data for clopidogrel, generated using GastroPlus™ software

The gastrointestinal simulation of regional absorption distribution using the ACAT model indicated that absorption mainly occurs in the small intestine and colon, and is negligible in the stomach (Figure 3).

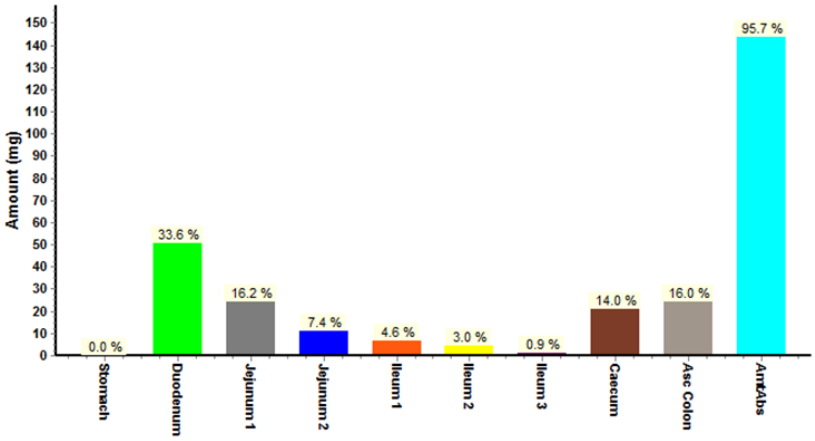

Figure 3.
Gastrointestinal simulation of regional absorption distribution of clopidogrel

The predictions are in complete agreement with the previously cited literature data and demonstrate that the clopidogrel fraction dissolved in gastric medium is not absorbed on site. Following this reasoning, it becomes clear that complete dissolution in gastric medium could implicitly indicate a good bioavailability, only if clopidogrel would remain dissolved until reaching its absorption site. However, our data have shown that the fraction released in the stomach would have to withstand a significant tendency to precipitate towards the higher pH of the intestinal media. This phenomenon was present but likely underestimated *in vitro,* where intestinal solubility reached its saturation point even in ideal volumetric and stirring conditions. Further considering that the volume of intestinal fluids *in vivo* (commonly accepted as about 80 mL [14] after drinking 240 mL of water, as per administration protocol in the PK studies) is much lower than the volume used for *in vitro* testing (500 or 1000 mL, depending on the simulated state), saturation and subsequent precipitation of the drug released at gastric level are unavoidable. It was therefore considered that an IVIVC model developed considering gastric medium would simply not be realistic.

Pharmacokinetics in healthy volunteers

Mean and individual plasma concentration *versus* time curves of clopidogrel, after administration of a single clopidogrel 75 mg tablet in fasting conditions to male and female healthy volunteers are presented in Figure 4. Upon reviewing the right graph (presentation of individual concentration curves in overlay mode), it can be noticed that clopidogrel exhibits great inter-subject variability in terms of both rate and extent of absorption.

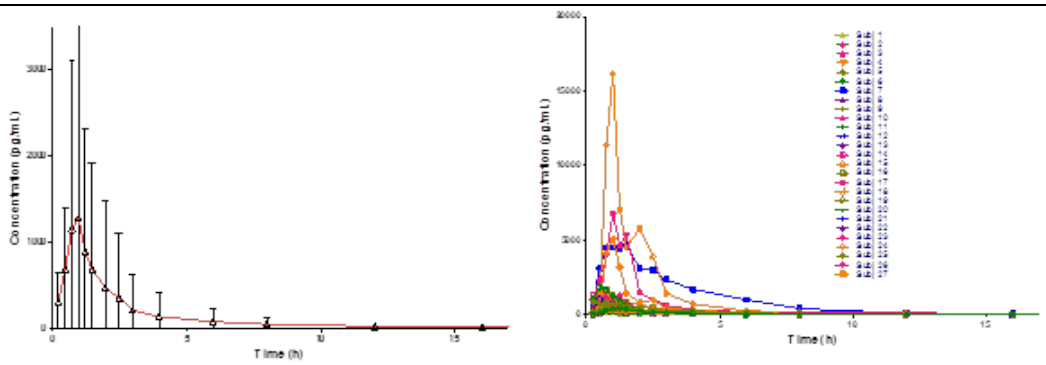

Figure 4.
Mean and individual pharmacokinetic profiles for clopidogrel (n = 27 subjects, dose = 75 mg, fasted state)

The intra-subject variation coefficient derived from an ANOVA model applied to replicate sets of ln-transformed C_{max} data was greater than 30 %, thus permitting to conclude that clopidogrel can be classified as a highly variable drug product.

In Figure 5, we presented the mean and individual plasma concentration versus time curves of clopidogrel, after administration of a single clopidogrel 75 mg tablet to healthy male and female volunteers, in post-prandial conditions.

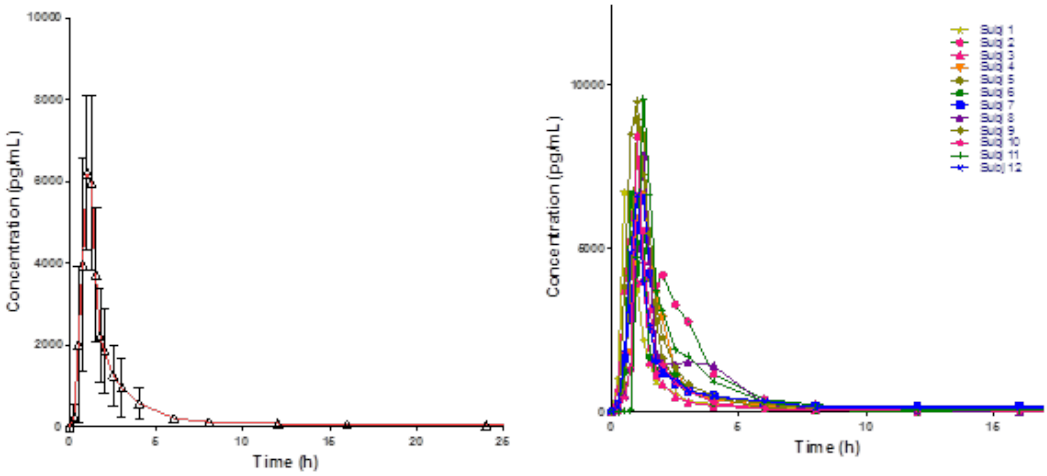

Figure 5.
Mean and individual pharmacokinetic profiles for clopidogrel (n = 12 subjects, dose = 75 mg, fed state)

The main pharmacokinetic parameters of clopidogrel, determined after the oral administration of a single clopidogrel 75 mg tablet in fasting and fed state, are presented in Table IV.

Table IV
Main pharmacokinetic parameters in fasting and fed state

	Fasting (N = 27) Mean data ± SD	**Fed (N = 12)** Mean data ± SD
C_{max} (ng/mL)	1.71 ± 3.23	7.48 ± 1.34
AUC_{0-t} (ng/mL x h)	2.99 ± 4.78	11.29 ± 3.0
$AUC_{0-\infty}$ (ng/mL x h)	3.09 ± 4.78	11.58 ± 3.38
K_{el} (1/h)	0.132 ± 0.063	0.071 ± 0.012

According to the data, administration of clopidogrel in fed conditions increases both rate (C_{max}) and extent (AUC) of absorption and reduces inter-subject variability.

Compartmental modeling of mean data, in spite of high variability of source data, proved a good fitting of plasma levels by the solution of bicompartmental models as can be seen in Figure 6 (fasting data) and in Figure 7 (fed data).

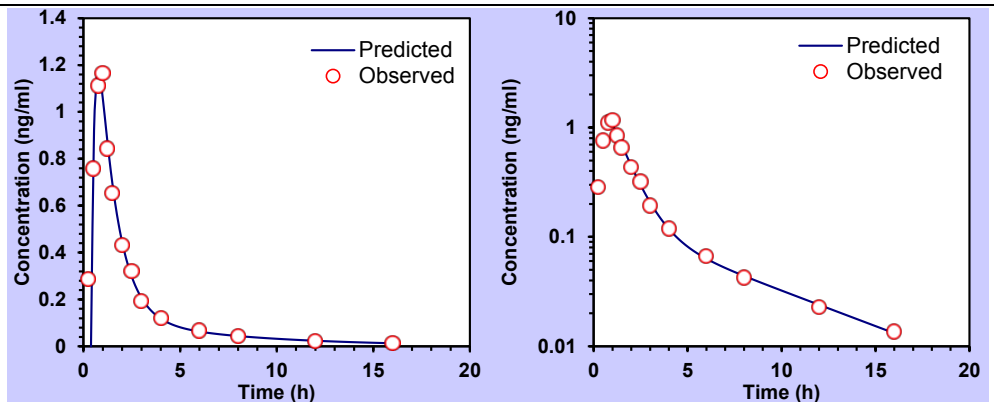

Figure 6.
Compartmental modeling of mean PK data for the fasting state; a. linear-linear, b. log-linear

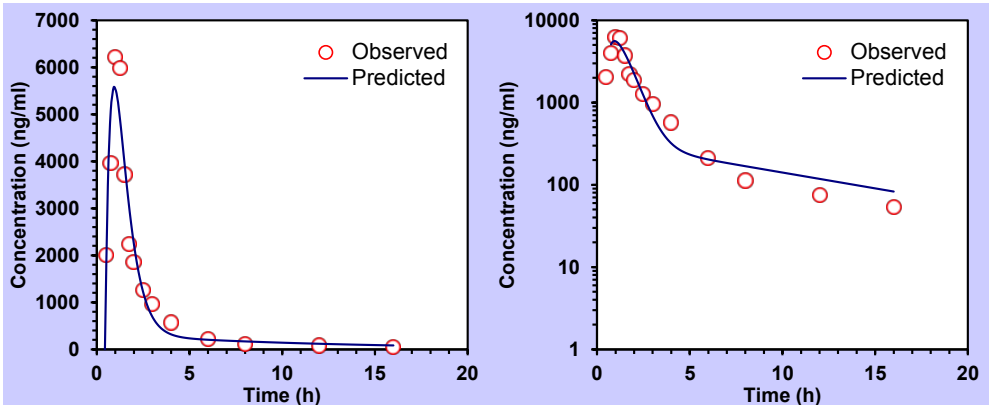

Figure 7.
Compartmental modeling of mean PK data for the fed state; a. linear-linear, b. log-linear

From the logarithmic representation of the data it can be seen that the tail of the curve is well defined through linear regression and consequently, the elimination rate constant can be reliably determined and used in further calculations of fraction absorbed (FRA). The transfer constants derived from the model are presented in Table V.

Table V
Transfer constants in fasted and fed state, obtained following bicompartmental modeling

Parameter	Unit	Value	
		Fasted	Fed
k_e	1/h	1.09	1.31
k_{12}	1/h	0.51	0.56
k_{21}	1/h	0.19	0.13
k_a	1/h	1.79	2.15

Where: k_e – first order elimination rate constant from central compartment; k_a – first order absorption rate constant; k_{12} and k_{21} - first order transfer rate constants between the central and peripheral compartment.

In vitro – in vivo correlations (IVIVC)

As previously discussed, only the intestinal dissolution profiles were considered relevant for clopidogrel *in vivo* absorption and were further evaluated in terms of IVIVC.

Since clopidogrel pharmacokinetics in both fasting and fed state were best described by bicompartmental models, a Loo-Riegleman based deconvolution approach was used for estimation of *in vivo* fraction absorbed (FRA). The results (Table VI) were used for developing a level A point-to-point correlation between the *in vitro* dissolution (FRD – fraction dissolved) and the in vivo absorption (FRA).

Table VI
Dissolved and absorbed fractions considered for IVIVC

Fasted state			Fed state	
FRA	FRD (FaSSIF)	FRD (SIF)	FRA	FRD (FeSSIF)
0.350	0.139	0.108	0.174	0.107
0.556	0.277	0.235	0.317	0.258
0.685	0.367	0.269	0.426	0.377
0.911	0.476	0.337	0.632	0.651
0.965	0.490	0.349	0.731	0.704
0.978	0.507	0.362	0.777	0.722
0.981	0.530	0.354	0.797	0.760
0.983	0.528	0.359	0.805	0.767
0.984	0.554	0.360	0.811	0.775

The correlations in biorelevant intestinal media (FaSSIF and FeSSIF) proved to be linear (Figures 8 and 9). Regarding the fed state, a remarkable aspect is that the slope of the linear correlation is very close to 1, suggesting a superposition between the *in vitro* dissolution and *in vivo* absorption. The calculated slope for the fasting correlation was higher than the unit (1.74), which reflects an overestimation of the absorbed drug fraction caused by the fact that absolute bioavailability data was not factored into the model due to the lack of availability of any such literature data.

Dissolution data in compendial medium (SIF) resulted in an exponential FRA/FRD relationship (Figure 8). The non-linear correlation is caused by the low solubility of clopidogrel in the dissolution medium. In the absence of physiological surface-active agents, SIF has the general tendency to underestimate the release kinetics of basic drugs in the gastro intestinal (GI) tract [17].

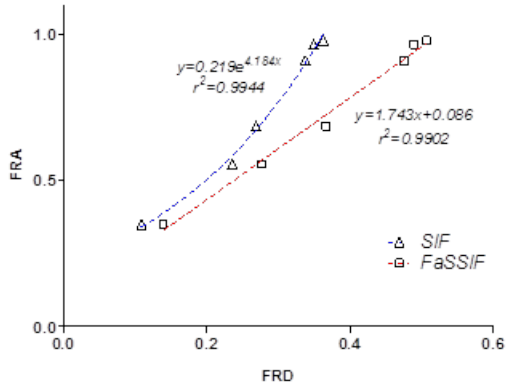

Figure 8.
IVIVC in intestinal biorelevant (FaSSIF) and compendial (SIF) media under fasting conditions

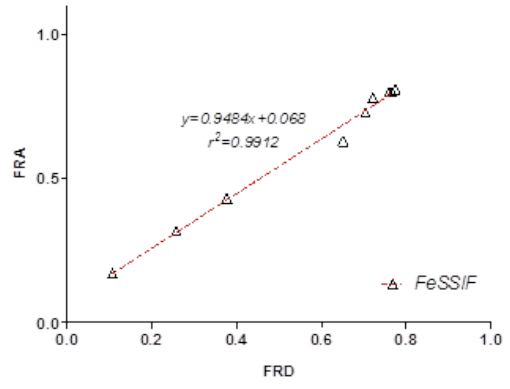

Figure 9.
IVIVC in intestinal biorelevant medium (FeSSIF) under fed conditions

Conclusions

The evaluation of *in vitro* dissolution kinetics of clopidogrel in simulated gastric media mimicking fasting and fed conditions, coupled with *in silico* data regarding the gastrointestinal simulation of clopidogrel regional absorption, have revealed that an IVIVC model developed considering gastric medium would simply not be relevant from a physiologic standpoint. Furthermore, this finding is confirmed by the inability of FeSSGF to predict that administration in fed state results in higher bioavailability of clopidogrel as compared with fasting conditions.

The main pharmacokinetic parameters of clopidogrel, determined after the oral administration of a single clopidogrel 75 mg tablet in fasting and fed state were determined using state of the art HPLC-MS/MS analytical methods. Data show that *in vivo* exposure to clopidogrel increases 4-fold when administration of the tablets is performed in fed state. It was also shown that administration with food reduces inter-subject variability with respect to both rate (C_{max}) and extent (AUC) of absorption.

Linear correlations were established between the actual PK data and the in vitro dissolution data in simulated intestinal media mimicking fasting (FaSSIF) and fed conditions (FeSSIF). The IVIVC under fed conditions revealed remarkable similarity (slope of the linear correlation very close to 1), thus demonstrating the validity of the correlation and demonstrating that the regression equations can be used for back-calculation of absorption profiles from *in vitro* dissolution data. The models may be used as surrogates for *in vivo* bioavailability testing, being a cheaper and less time consuming initial screening tool for generic formulations.

The increase of solubility in fed state, observed due to the fact that higher post-prandial physiological concentrations of surface-active agents compensate for the increase in pH levels, correlated with a significant decrease in variability in terms of absorption. Put into perspective, these data confirm that the low solubility of clopidogrel in the biologically relevant absorption site medium is one of the important sources of its increased PK variability.

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