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Research paper

Selection of drug candidates for gastroretentive dosage forms: Pharmacokinetics following continuous intragastric mode of administration in a rat model

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Abstract

The purpose of the study was to evaluate the pharmacokinetic effects obtained by gastroretentive dosage form (GRDF) for drugs absorbed by passive paracellular diffusion (atenolol, acyclovir) or active transport (valacyclovir). Model drugs were delivered as gastric infusion (GInf) through an implanted catheter (resembling GRDF), intravenous, oral (PO), and colonic administration to rats. For atenolol (highly soluble drug), GInf resulted in a prolonged T_{max} and reduced C_{max} in comparison to PO, whereas bioavailability was similar. Bioavailability after colonic bolus was significantly lower. Results were also simulated by a pharmacokinetic model. For acyclovir, GInf and PO demonstrated almost the same pharmacokinetic profile with low bioavailability, most probably due to the solubility-limited absorption. Valacyclovir demonstrated the significant change in the shape of pharmacokinetic profile as a function of the rate of gastric delivery, without variation in bioavailability. Valacyclovir was not absorbed from colon. Experimental and theoretical methodologies to assess the pharmacokinetic influences of GRDF mode of administration were developed, avoiding the need to compound the drug in a dosage form. GRDF provides a mean for controlled release of compounds that are absorbed by active transport in the upper intestine. It also enables controlled delivery for paracellularly absorbed drugs without a decrease in bioavailability.

Keywords: Pharmacokinetic model; Controlled release; Absorption mechanism; Intestinal transit; Absorption window

1. Introduction

The gastro-intestinal (GI) tract is composed of several regions differing in anatomy, biochemical environment, microbial flora, expression of transporters, and absorption characteristics. There are several processes that may occur simultaneously following drug release from a dosage form (DF) in the GI tract, including: chemical/enzymatic/bacterial degradation, absorption (passive and/or active), precipitation, efflux by P-glycoprotein pump, and metabolism by Cyp450 enzymes. As a consequence the pharmacokinetic profile of a drug may be influenced by its delivery site.

Many clinically used drugs could benefit from controlled release dosage forms (CR). A common property of conventional CR technologies is that a large part of the drug load is released in the colon, where the DF stays for a relatively long time period. This delivery approach, while suitable for many molecules, was found to be inappropriate for drugs that are poorly absorbed from the lower part of the GI tract [1].

The concept of controlled release gastroretentive dosage form (GRDF) was introduced in order to enable continuous delivery to the upper part of the GI tract, while minimizing the limitation of poor absorption from the colon. These DFs are designed to be retained in the stomach for

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a prolonged time period while releasing their content in a continuous and controlled manner. The gastric retention is attained by preventing the DF from passing through the pyloric sphincter. Detailed discussion regarding the different technological approaches to achieve gastric retention can be found elsewhere [2–6]. A number of alterations in pharmacokinetic and pharmacodynamic profiles of drugs have been reported following drug administration in GRDFs [7–11]. However, while much effort has been invested by research laboratories in the technological development of different types of GRDFs, systematic research on appropriate drug candidates as presented in this work has not been performed, yet.

The GI tracts of rats and humans were found to be similar in their absorption properties [12–15]. In the present study we developed experimental and theoretical methodologies for evaluation of pharmacokinetic effects of GRDF in the rat model. The effects of this delivery mode on pharmacokinetics of three model molecules differing in their molecular and absorption characteristics (acyclovir (ACV), atenolol (AT), and valacyclovir (VACV)) were investigated.

2. Materials and methods

2.1. Chemicals

Atenolol, acyclovir, and metoprolol tartarate were purchased from Sigma–Aldrich, Rehovot, Israel. Acyclovir suspension (Zovirax®) was purchased in the local pharmacy. Valacyclovir was purchased from Eurotrade World Commerce, Madrid. All other chemicals were of analytical reagent grade, and solvents were of HPLC grade.

2.2. Animals

All surgical and experimental procedures were reviewed and approved by the Animal Experimentation Ethics Committee of The Hebrew University Hadassah Medical School, Jerusalem. Male Wistar rats (Harlan, Israel), weighing 280–400 g, were used for all surgical procedures. The anesthesia of animals for the period of surgery was initiated with 1 mL/kg solution of ketamine 20 mg/mL: xylazine 100 mg/mL (90:10, v/v) by intra-peritoneal injection and maintained by pure ketamine as needed. In all rats the right jugular vein was cannulated with a cannula made up of polyethylene tubing (PE50, Intramedic® Polyethylene Tubing, Becton–Dickinson, MD) to allow blood sampling. Furthermore, in groups that received gastric continuous infusion or colonic administration, an additional PE50 cannula was inserted into the appropriate part of the GI tract (stomach or colon) to allow drug administration. After the surgery, animals were transferred to metabolic cages and stabilized overnight (12–16 h), during which the animals were fasted, and water was available *ad libitum*.

2.3. Experimental procedure

In the present study the drugs were administered by several routes. Intravenous bolus (IV) dose was delivered through the jugular vein cannula and followed by 0.2 mL of heparinized saline (50 IU/mL) to ensure the delivery of the whole dose. Oral bolus dose was delivered by a gavage needle. Delivery to the stomach or colon (caecum) was conducted through the cannula in the corresponding region of the GI tract.

The following doses and dosing formulations were used in the study. Acyclovir: IV dosing – 5 mg/kg (2.5 mg/mL solution in water, at 37 °C); oral bolus – 120 mg/kg (suspension 40 mg/mL); gastric infusion – 120 mg/kg over 4 h (10 mg/mL solution in water, with pH adjusted to 1.2 with HCl). Valacyclovir: oral bolus – 20 and 40 mg/kg (20 or 40 mg/mL solution in water, respectively); gastric infusion – 20 mg/kg over 4 or 8 h (2 or 1 mg/mL solution in water, respectively); colon infusion – 20 mg/kg over 4 h (2 mg/mL solution in water). Atenolol: IV dosing – 2 mg/kg (2 mg/mL solution in water); oral bolus, gastric infusion, and caecum bolus – 10 mg/kg (5, 1, and 15 mg/mL solution in water, respectively). Sequential blood samples were collected into heparin-containing test tubes at predetermined time intervals. Plasma was separated by centrifugation for 8 min at 1000*g* and stored at −20 °C until analysis.

2.4. Analytical procedure

All tested compounds were analyzed using Waters 2695 Separation Module HPLC system with Waters 2475 Fluorescence Detector and Waters 2996 Photodiode Array Detector (Waters Corporation, Milford, MA).

The assay procedure for acyclovir in plasma samples was based on a method previously described by Mascher et al. [16]. In brief, plasma (120 μL) was mixed for 20 s with 35 μL of perchloric acid (3 N) and then centrifuged for 8 min at 10,000*g*. The supernatant was injected directly into the HPLC system. The volume of injection was 50 μL. The separation was achieved by Phenomenex Gemini C18 column (5 μm, 4.6× 250 mm) at ambient temperature. The mobile phase consisted of methanol (15%) and water (85%, adjusted to pH 1.2 with perchloric acid). The flow was set to 1 mL/min. Acyclovir was detected with a $\lambda_{excitation}$ of 260 nm and $\lambda_{emission}$ of 375 nm. Retention time of acyclovir was about 3.7 min. The method was linear between 20 and 20,000 ng/mL.

Analysis of atenolol was based on previously reported methods [17,18] that were modified to meet our requirements. Plasma samples (150 μL) were mixed with 150 μL NaOH (1 M) and 15 μL of internal standard (metoprolol tartarate, 1 μg/mL). Both materials were extracted with 4 mL of ethyl acetate, evaporated to dryness and reconstituted with 140 μL of water. The volume of injection was 90 μL. The separation was achieved by XTerra RP-18 (3.5 μm, 4.6× 100 mm, Waters). The mobile phase consisted

of methanol:acetonitrile:sodium phosphate buffer 0.02 M, adjusted to pH 3 (11:7:82, v/v/v). The flow was set to 0.5 mL/min. Atenolol and metoprolol tartarate were detected with a $\lambda_{excitation}$ of 275 nm and $\lambda_{emission}$ of 300 nm, and retention times were 3.1 and 9.3 min, respectively. The calibration curves were linear between 25 and 10,000 ng/mL.

2.5. Data analysis

The pharmacokinetic parameters of the drugs were determined from the experimental plasma concentration–time data by compartmental and non-compartmental methods. To assess the statistical significance of the differences between the results in case there were more than two groups, the ANOVA test, followed by Tukey Multiple Comparison Test, where appropriate were employed. In the case of comparison between two groups the two-tailed *t*-test was used. A p value of less than 0.05 was termed significant. All data are presented as means ± SEM, if not stated otherwise.

2.6. Pharmacokinetic modeling and simulation

In this work a mathematical model to describe pharmacokinetics of atenolol was developed and further utilized for simulation of pharmacokinetic profiles of different CR formulations of AT. The modeling was performed using WinNonlin® 5.2 software (Pharsight Corporation, Mountain View, CA, USA). Fig. 1 presents the scheme of the final model. This model is based on a two-compartment model and consisted of two components. We utilized gastrointestinal transit data reported by Sawamoto and coworkers, who followed the passage of phenol red (unabsorbable marker) through the GI tract following oral administration to rats [19]. However, contrary to their work, we divided the GI tract only into four regions: stomach, upper small intestine, lower small intestine, and caecum. This number of intestinal compartments was found to be optimal in comparison to higher or lower number of compartments. It provided a reasonably good fit of experimental data on predicted curve, while minimizing the number of model parameters, and thereby increasing the predictive power of the model. The transit model was combined with our data on atenolol absorption following different modes of administration in rats. The modeling was performed using the mean concentration–time profiles. The pharmacokinetic parameters obtained from IV administration were set as constants and absorption rates were determined. The differential equations used to describe the pharmacokinetic model were as follows:

$$\frac{d[S]}{dt} = -K_{t1} \cdot [S];$$

$$\frac{d[USI]}{dt} = K_{t1} \cdot [S] - (K_{t2} + K_{a2}) \cdot [USI];$$

$$\frac{d[LSI]}{dt} = K_{t2} \cdot [USI] - (K_{t3} + K_{a3}) \cdot [LSI];$$

$$\frac{d[LI]}{dt} = K_{t3} \cdot [LSI] - (K_{t4} + K_{a4}) \cdot [LI];$$

$$\frac{d[C]}{dt} = K_{a2} \cdot [USI] + K_{a3} \cdot [LSI] + K_{a4} \cdot [LI] - (K_{12} + K_{10}) \cdot [C] + K_{21} \cdot [P];$$

$$\frac{d[P]}{dt} = K_{12} \cdot [C] - K_{21} \cdot [P];$$

where [S], [USI], [LSI], [LI], [C], and [P] are the drug amounts in the stomach, upper small intestine, lower small intestine, large intestine, central compartment, and peripheral compartment, respectively. The rate constants are as follows: K_{10} is the elimination from the body, K_{12} is the transfer from the central to the peripheral compartment, and K_{21} is the transfer from the peripheral to the central compartment. K_a and K_t are the first-order absorption rate constant and first-order intestinal transit rate constant, respectively, for different regions of the GI tract. Administration of the drug to the various GI regions was modeled by an addition of the zero-order input rate constant to the corresponding equation for an appropriate time period.

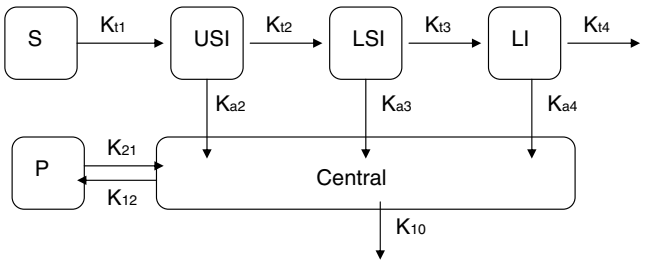

Fig. 1. Scheme of the model describing the pharmacokinetics of atenolol, where S, USI, LSI, LI, C, and P designate the stomach, upper small intestine, lower small intestine, large intestine, central, and peripheral compartments, respectively. Different K values represent first-order kinetic constants.

3. Results

The concentration vs. time profile of AT (2 mg/kg) following IV bolus administration to rats is presented in Fig. 2. The data fit the two-compartment model; the corresponding pharmacokinetic parameters are shown in Table 1. These parameters provided the basis for subsequent pharmacokinetic modeling. The parameters that were obtained by non-compartmental analysis (Table 2) were utilized for comparison with different enteral AT administrations and for calculation of bioavailability values. Fig. 3 presents the concentration vs. time profiles of AT following oral bolus, gastric infusion (over 4 h), and bolus administration into the caecum of rats (points represent the experimental data and lines values predicted by the pharmacokinetic model, as explained below). It can be seen that gastric infusion resulted in a longer absorption phase (T_{max} was significantly higher than for oral administration) and lower C_{max} values. Both oral bolus and gastric infusion lead to similar

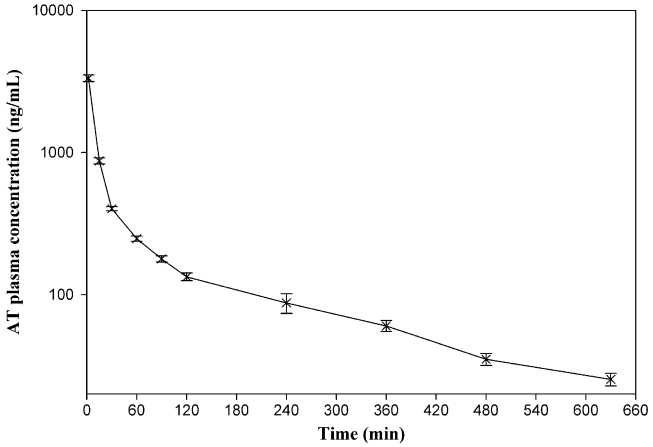

Fig. 2. Plasma concentration vs. time profile of atenolol (2 mg/kg, $n = 6$) observed following IV administration to rats (means ± SEM).

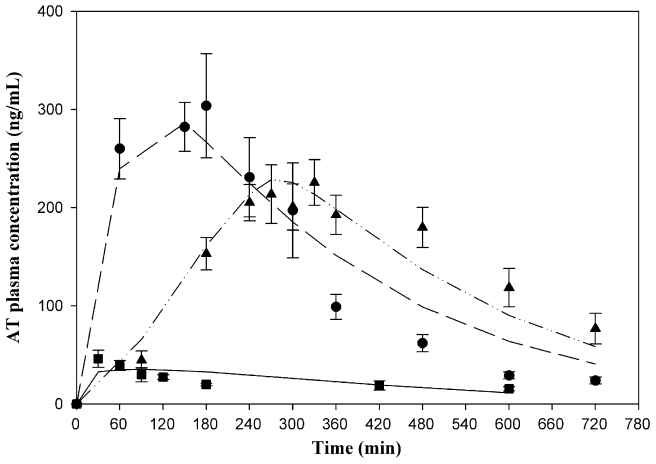

Fig. 3. Plasma concentration vs. time profile of atenolol (10 mg/kg) observed following administration as oral bolus (●, $n = 7$), gastric infusion over 4 h (▲, $n = 7$), and caecum bolus (■, $n = 4$) to rats (means ± SEM). The lines illustrate the concentrations predicted by the pharmacokinetic model described in the text and in Fig. 1.

Table 1
Pharmacokinetic parameters obtained for atenolol and acyclovir following IV administration by two-compartment pharmacokinetic model (means ± SEM)

Parameter	Atenolol	Acyclovir
AUC (μg·min/mL)	98.3 ± 3.5	123.2 ± 50.2
K_{10} (min^{-1})	0.040 ± 0.003	0.069 ± 0.013
K_{12} (min^{-1})	0.062 ± 0.003	0.067 ± 0.012
K_{21} (min^{-1})	0.011 ± 0.001	0.035 ± 0.005
Cl (mL/(min·kg))	20.5 ± 0.7	15.3 ± 1.8
V_{ss} (mL)	3314 ± 135	729 ± 104

bioavailability (Table 2). The administration of AT into caecum resulted in a much lower bioavailability (about 4.6%).

The concentration vs. time profile of ACV (5 mg/kg) following IV bolus administration to rats is presented in Fig. 4. The data fit the two-compartment model; the corresponding pharmacokinetic parameters are shown in Table 1. The parameters obtained by non-compartmental analysis (Table 3) were utilized for comparison with different enteral ACV and VACV administrations and for calculation of bioavailability values. Fig. 5 presents the concentration vs. time profiles of ACV following oral bolus and gastric infusion to rats. It can be seen that two concentration–time curves are almost identical; this means that in the case of

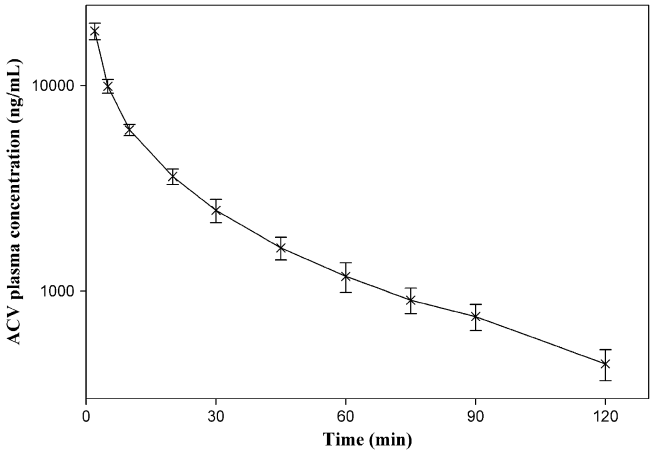

Fig. 4. Plasma concentration vs. time profile of acyclovir (5 mg/kg, $n = 6$) observed following IV administration to rats (means ± SEM).

ACV, the administration mode has no effect on the pharmacokinetic profile. The terminal half-lives (Table 3) were very similar for two enteral administrations but were significantly different from the terminal half-life calculated for intravenous ACV. This indicates that ACV elimination was absorption-rate limited, i.e. flip–flop pharmacokinetics.

Table 2
Pharmacokinetic parameters of atenolol following various modes of administration obtained by non-compartmental modeling (means ± SEM)

Parameter	IV ($n = 6$)	Oral bolus ($n = 7$)	Gastric infusion 4 h ($n = 7$)	Caecum bolus ($n = 4$)
Dose (mg/kg)	2	10	10	10
Terminal slope (min^{-1})	0.0036 ± 0.0002	0.0041 ± 0.0004	0.0043 ± 0.0004	0.0015 ± 0.0002[a]
Terminal half-life (min)	198 ± 9	177 ± 17	169 ± 15	487 ± 51[a]
AUC (μg·min/mL)	105.6 ± 3.0	102.9 ± 10.2	120.1 ± 10.0	24.1 ± 3.3
T_{max} (min)	–	184 ± 28	321 ± 48[b]	38 ± 8
C_{max} (ng/mL)	–	370 ± 42	255 ± 19[b]	46 ± 8
Bioavailability (%)	100	19.5 ± 1.9	22.7 ± 1.9	4.6 ± 0.6

[a] Significantly different from all other modes of administration (ANOVA, $p < 0.001$).
[b] Significantly different from oral bolus administration (t-test, $p < 0.05$).

Table 3
Pharmacokinetic parameters of acyclovir following various modes of administration (of acyclovir and valacyclovir) obtained by non-compartmental modeling (means ± SEM)

Parameter	Acyclovir			Valacyclovir			
	IV ($n = 6$)	Oral bolus ($n = 6$)	Gastric infusion 4 h ($n = 5$)	Oral bolus ($n = 6$)	Gastric infusion 4 h ($n = 6$)	Gastric infusion 8 h ($n = 4$)	Oral bolus ($n = 2$)
Dose (ACV equivalent), mg/kg	5	120	120	13.88	13.88	13.88	27.76
Terminal slope, min^{-1}	0.016 ± 0.001	0.005 ± 0.001*	0.004 ± 0.0006*	0.009 ± 0.002	0.011 ± 0.001	0.014 ± 0.002	0.008 ± 0.002
Terminal half-life, min	45 ± 4	180 ± 40*	181 ± 27*	101 ± 23	65 ± 4	54 ± 8	94 ± 18
AUC, μg·min/mL	348.3 ± 29.5	464.5 ± 68.2	616.3 ± 60.2	328.6 ± 23.1	366.2 ± 8.9	313.5 ± 44.4	915.2 ± 123.5
T_{max}, min	–	220 ± 40	240 ± 0	28 ± 5#	195 ± 24#	315 ± 15#	30 ± 10
C_{max}, ng/mL	–	1200 ± 109	1591 ± 156	4495 ± 331#	1364 ± 45#	715 ± 56#	10727 ± 332
Bioavailability, %	100	5.56 ± 0.82	7.37 ± 0.72	34.0 ± 2.4	37.9 ± 0.9	32.4 ± 4.6	47.3 ± 6.4

* Significantly different from IV administration (ANOVA, $p < 0.01$).
\# Significant differences among oral, gastric infusion (4h), and gastric infusion (8h) of valacyclovir (ANOVA, $p < 0.01$).

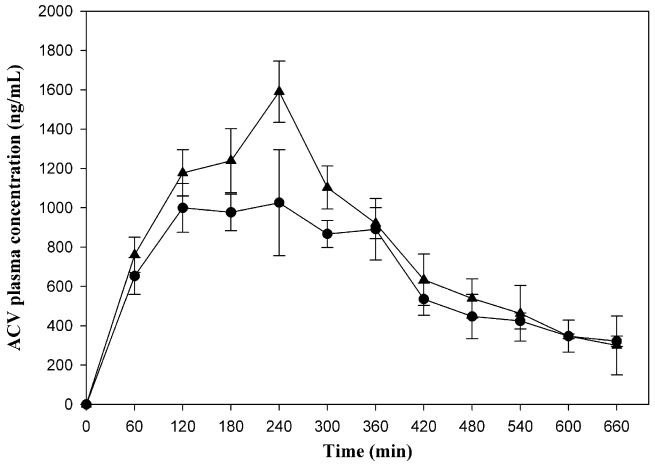

Fig. 5. Plasma concentration vs. time profile of acyclovir (120 mg/kg) observed following administration as oral bolus (●, $n = 6$) and gastric infusion over 4 h (▲, $n = 5$) to rats (means ± SEM).

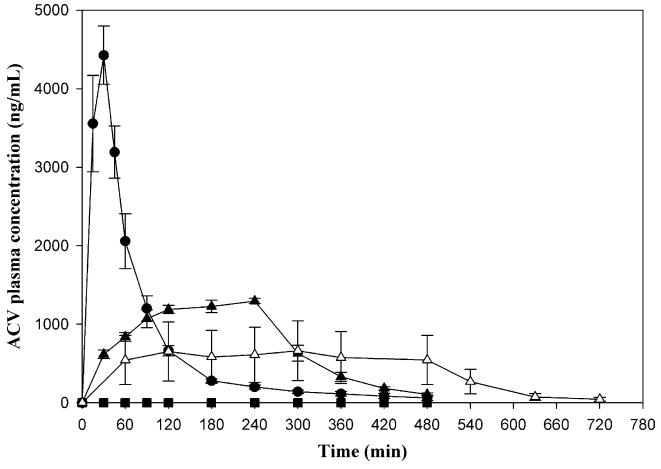

Fig. 6. Plasma concentration vs. time profile of acyclovir observed following administration of valacyclovir (20 mg/kg) as oral bolus (●, $n = 6$), gastric infusion over 4 h (▲, $n = 6$), gastric infusion over 8 h (△, $n = 4$), and colon bolus (■, $n = 8$) to rats (means ± SEM).

Bioavailability of ACV was low in both cases and not significantly different between the two groups.

ACV was also the entity that was measured in plasma following various modes of VACV administration. The prodrug VACV was shown to have a very short half-life in rats (about 7 min) [20]. The concentrations vs. time profiles of ACV in rat plasma following different VACV administrations (20 mg/kg, equivalent to 13.88 mg/kg of ACV) are shown in Fig. 6. Oral administration of the high dose (40 mg/kg) was performed only to two rats in order to verify that saturation of absorption does not occur. Following colonic administration of VACV there were no detectable concentrations of ACV in rats' plasma, as depicted by zero concentration at all time points along the x-axis in Fig. 6. The colonic administration experiment was performed with two groups of rats ($n = 4$ each) on two occasions to rule out any technical interference. All gastric VACV administrations resulted in similar ACV bioavailability (around 38%) and demonstrated a terminal ACV half-life that was not significantly different from intravenous ACV.

When the permeability of the drug through the intestinal wall varies at different parts of the GI tract, classic pharmacokinetic models cannot be applied. Instead there is a need for a model that combines absorption kinetics with the drug (or DF) transit along the GI tract. In addition, the model should follow the principle of parsimony and should not be too complicated to allow its practical use. The pharmacokinetic model developed in this work is composed of two components: gastrointestinal transit and absorption. Fig. 7 presents the experimental data points of percent of phenol red recovered at different regions of the GI tract as a function of time (adopted with modification from Sawamoto et al. [19]) along with predicted curves obtained by our GI transit model. It is evident that some lag-time exists before material is delivered from ileum to caecum ($T_{lag} = 101$ min). This probably occurs due to the effect of the ileo-caecal valve [21]. The following transit constants

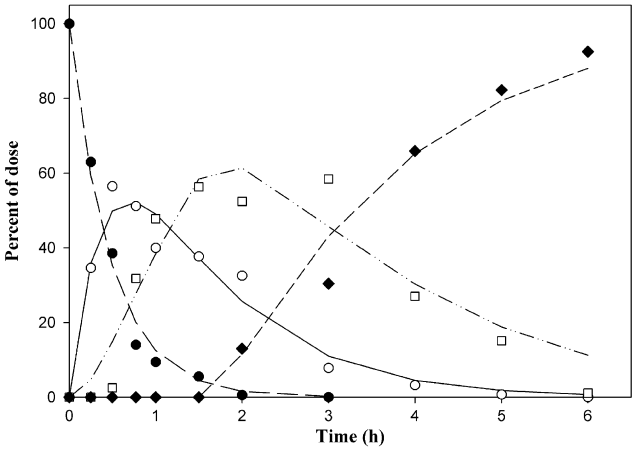

Fig. 7. Recovery of the phenol red in the different parts of the rats' GI tract following oral administration, data points (modified from Sawamoto et al. [19]) and lines as predicted by the transit model described in the text and in Fig. 1. ●, stomach; ○, upper small intestine; □, lower small intestine; ♦, caecum.

were obtained: $K_{t1} = 0.0347\ \text{min}^{-1}$, $K_{t2} = 0.0153\ \text{min}^{-1}$, $K_{t3} = 0.098\ \text{min}^{-1}$ and $K_{t4} = 0.0047\ \text{min}^{-1}$.

Fig. 3 depicts the observed plasma concentration-time profiles of AT after different modes of administration and the best fit provided by the pharmacokinetic model to the mean experimental data. Utilization of the transit parameters along with pharmacokinetic parameters calculated for IV administration allowed determination of absorption rate constants for upper ($K_{a2} = 0.00156\ \text{min}^{-1}$) and lower ($K_{a3} = 0.00117\ \text{min}^{-1}$) small intestine and for large intestine ($K_{a4} = 0.00018\ \text{min}^{-1}$).

Fig. 8 presents the results of a pharmacokinetic simulation for gastroretentive and conventional CR dosage forms (dose 10 mg/kg is released at a constant rate over 12 h) and an immediate release DF in rats. For simulation purposes it was decided that the conventional CR delivers its content

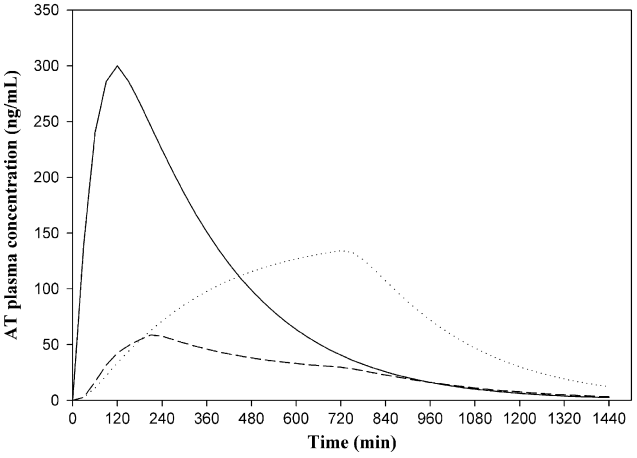

Fig. 8. Simulated plasma concentration-time profiles for atenolol delivered to rats as oral bolus (solid line), GRDF (dotted line), and a conventional CR (dashed line), as predicted by the pharmacokinetic model.

for 30 min into the stomach, followed by 60 min in the upper small intestine, 120 min in the lower small intestine, and the rest of the time in the colon. These transit data are in line with the transit time of chyme in small intestine (3–4 h) [22], and the fact that the transit rate in the upper intestine is higher than in lower regions. Alternatively, the GRDF delivers its entire payload in the stomach. The AUC values calculated by the non-compartmental method were 120, 106, and 38 μg min/mL for immediate release, GRDF, and CR, respectively.

4. Discussion

Different regions of the GI system vary in their absorption characteristics. Therefore, it seems reasonable that delivery of a drug to different regions along the GI tract will result in significant variations in the corresponding pharmacokinetic profiles. The GRDF approach provides an opportunity for continuous delivery of drugs to the upper part of the gut that cannot be achieved by other types of DFs. In the present study we evaluated the interrelation between drug absorption mechanisms and pharmacokinetic consequences that can result from a GRDF mode of administration. The findings of this study contribute to the rational selection of drug candidates for GRDF therapy.

The evaluation of drug candidates for GRDF in humans provides the most realistic estimate. However, it requires extensive preceding development, including compounding of a drug into the DF, and *in vitro* characterization and optimization of a release profile. Moreover, the interpretation of the pharmacokinetic data may be complicated by the variable (and not always known) retentivity of the GRDF in the stomach.

In our work, we have developed an experimental methodology that allows relatively simple assessment of absorption of drug candidates in rats. It was previously demonstrated by several research groups that the rat intestine provides a good prediction of the extent of absorption in humans [12–15]. For example, Zhao et al. have shown on a set of 111 drugs that for 94% of these drugs the absorption difference between humans and rats is less than 20% [14]. Chiou and Barve reported a similar very good correlation between humans and rats for 64 compounds [13]. On the other hand, in a similar comparison between humans and dogs (that are widely used as an animal model for drug absorption, and especially in the field of GRDF assessment) only poor correlation was reported [23]. In a more recent work Cao et al. have concluded that rat and human show similar drug intestinal absorption profile and similar transporter expression, while there are certain differences in the expression of metabolizing enzymes (Cyp450 or UDP-glucuronosyltransferase) [15]. Consequently, the effects demonstrated in this study in the rat model are largely expected to occur in humans, as well.

AT was chosen as a model of hydrophilic drug absorbed from the intestine by the paracellular route. It

demonstrates slow absorption and incomplete bioavailability in humans [24]. AT does not undergo first-pass metabolism for any significant degree, thus incomplete bioavailability is due to its low intestinal permeability. It was shown in different experiments that the permeability of AT is higher in the upper small intestine than in the colon. Ungell et al. reported the following permeabilities through the rat intestine in Ussing diffusion chambers: 5.95×10^{-6}, 5.08×10^{-6}, and 1.7×10^{-6} cm/s for jejunum, ileum, and colon, respectively [25]. It was also shown that permeability of AT through the Caco-2 monolayer (which has colonic origin) is lower than permeability of human jejunum *in vivo* [26]. Accordingly, it was hypothesized that the GRDF approach would allow controlled delivery of such drugs and would probably increase their bioavailability.

In our experiments, gastric infusion of AT led to an increase in T_{max} and a decrease in C_{max} in comparison to oral bolus, while both administrations resulted in the same bioavailability. The low bioavailability obtained following colonic administration demonstrates the inadequacy in developing a conventional CR formulation for this drug. On the other hand, the GRDF approach can be successfully implemented in the development of a controlled release formulation. Availability of the pharmacokinetic data from several modes of administration and the negligible metabolic transformation allowed us to develop the pharmacokinetic model that provides a good description of the experimental data. This model enabled us to simulate various drug release rates and gastric retention times. It clearly demonstrated that GRDF provides a way to develop CR formulations for drugs absorbed paracellularly without the need to increase the drug load of the dosage form to achieve the same bioavailability demonstrated by immediate release products (Fig. 8).

It should be noted that due to a long pharmacodynamic half-life of AT there is no practical benefit in developing a CR for the specific drug, and it was used in the study as a model molecule.

In our study, ACV represented a model for drugs absorbed paracellularly by passive diffusion with a limited solubility in the GI tract. Several researchers have demonstrated that ACV is absorbed from the GI tract mainly (or solely) by passive diffusion [27,28]. Meadows and Dressman demonstrated by the *in vitro* intestinal ring method and by *in situ* single-pass perfusion that the permeability of ACV was linear over a wide range of concentrations, and it was also unaffected by the addition of metabolic inhibitors [28]. Fujioka et al. [27] reported similar results from everted sac experiments. ACV, being zwitterion and having a negative log$P$ value, is unlikely to be absorbed transcellularly. The P_{app} of ACV in rat jejunum was reported to be 10.0×10^{-6} [29], which is similar to the permeability of other paracellularly absorbed molecules [25]. Moreover, our experiments showed that the permeability of ACV through rat jejunum is slightly higher than that of colon (unpublished data). Lewis and co-workers [30] have shown in a human trial that the AUC of ACV following administration as an intraduodenal infusion or sipping solution was significantly higher than following administration of tablets. From all these data it could be expected that continuous intragastric infusion of ACV would provide similar consequences as found for AT. However, the water solubility of ACV is significantly lower than the solubility of AT. This fact is especially important when taking into consideration the total daily dose of the two drugs (maximum daily dose for AT is 200 mg and for ACV 3200 mg). The less than proportional increase in bioavailability with elevation of dose was reported for ACV in animals and humans [31,32], which is explained by ACV precipitation in the gut. The bioavailability obtained in our study is in line with the previously published data [31].

In our experiments, oral bolus and gastric infusion of ACV over 4 h resulted in almost identical pharmacokinetic profiles. This can be explained by the solubility-limited absorption of the drug. Following oral gavage administration of the high dose of ACV suspension there was not enough fluid in the GI tract to allow full dissolution. In the case of gastric infusion an acidic solution of ACV was utilized to increase ACV solubility. The entry of this formulation into the GI tract of the rat caused, most probably, a change in pH and thereby ACV precipitation. Thus, in both cases the absorption of ACV occurred from a saturated solution and consequently there were no differences between the corresponding concentration–time profiles. The prolonged half-life obtained following both enteral administrations is the evidence for continuous absorption that occurs from the colon. However, the absorption rate from the colon is lower than that of the small intestine, and it results in an overall decrease in plasma concentration. Thus, in the case of ACV it can be concluded that due to low solubility of the molecule the majority of the formulation is transported to the lower regions of the GI tract with no opportunity to be absorbed. It can be further extrapolated to the delivery by GRDF as follows: if a drug is delivered from a GRDF at a rate that allows free dissolution in the acidic environment of the stomach but precipitates in the small intestine due to the pH change, the impact of such a delivery on pharmacokinetic profile is expected to be very minor.

VACV is the L-valyl ester prodrug that was developed to overcome the low oral bioavailability of ACV. We utilized VACV as a model for drug absorbed by an active absorption mechanism. It is commonly accepted that VACV is absorbed by a carrier-mediated transport. The two dipeptide transporters, hPEPT-1 and HPT-1, have been implicated in its absorption [33]. VACV was shown to inhibit absorption of other substrates of dipeptide transporters [34,35], and in turn VACV transport is inhibited by the presence of other substrates [36]. It was previously shown that VACV has an evident absorption window: the permeability coefficient of small intestine is much higher than that of the colon (1.522 vs. 0.09, respectively) [36].

We recently showed in a human study that GRDF can increase the bioavailability (as well as prolong the absorption phase) of riboflavin, a molecule that is absorbed in the upper GI tract by saturable transport [7]. In the present study we evaluated if these changes can also be achieved for molecules absorbed by non-saturable active transport (at a relevant dose range). Since VACV is a prodrug that is rapidly converted into ACV, the ACV concentration–time profile in plasma was used for comparison of the different modes of VACV administration. In contrast to riboflavin, it was demonstrated that continuous intragastric administration of VACV does not influence the AUC value as all groups (oral bolus and gastric infusions) demonstrated comparable bioavailability. However, the effect of administration rate on the shape of pharmacokinetic profile was dramatic (Fig. 6). The high sharp peak of concentration following oral bolus administration changed into a sustained plateau that was maintained for the duration of the gastric infusion. Moreover, the rate of drug administration enables a control over the concentration profile. It should be remembered that for the majority of drugs the high peak concentrations (high C_{max}) are not required for therapeutic effect and can sometimes be associated with adverse reactions. VACV was not absorbed from the colon. Thus the data demonstrate that only the GRDF approach enables true control release for drugs that are solely absorbed in the upper GI tract.

There is a certain misconception that prolonged gastric retentivity would lead to an increased bioavailability of any drug. The findings of this work combined with our previous report on riboflavin [7] clearly demonstrate that as long as the absorption of a drug follows first-order kinetics the bioavailability of a drug should not be expected to increase in comparison to immediate release formulation. Whereas in the case of saturable absorption process enhanced bioavailability can occur.

In the present study, we used GI site specific delivery in the rat model for assessment of advantages of gastroretentive mode of administration. However, this model can be seen more generally as a preclinical tool for evaluation of regional absorption properties of tested molecules. It may provide certain advantages, because it combines *in vivo* tissue permeability, drug solubility, tissue blood circulation, etc., and can be used together with other permeability assessment techniques (such as *in vitro* cell cultures, *ex vivo* diffusion chambers, and perfusion).

5. Conclusions

In the present study we developed experimental and theoretical methodologies that enable relatively simple evaluation of drug candidates for GRDF and avoid the need for the complex development of the formulation. GRDF provides a valuable addition to the CR formulation arsenal, which offers several advantages for drugs that are preferentially absorbed from the upper GI tract. The most significant influence was demonstrated on the pharmacokinetic profile of drugs that are absorbed solely in the upper part of the GI tract by an active transport mechanism. For these drugs the GRDF approach provides a way for controlled release, avoids the high peak concentration, and may reduce the required doses. The effects of GRDF on the profile of drugs that are absorbed by paracellular diffusion (such as AT) are similar but less profound. As long as no saturable transport is involved in drug absorption, the GRDF is not expected to increase the bioavailability in comparison to immediate release formulation. However, it prevents the bioavailability decrease, which is associated with conventional CR formulations due to a lower colonic absorption. Poor solubility characteristics may prevent desired changes in the pharmacokinetic profile. It should be noted that both pharmacokinetic and pharmacodynamic aspects must be taken into consideration in designing GRDFs.

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