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International Journal of Pharmaceutics 317 (2006) 14–18
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Stability of valacyclovir: Implications for its oral bioavailability

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Received 10 May 2005; received in revised form 28 December 2005; accepted 5 January 2006

Abstract

The absolute bioavailability of the prodrug valacyclovir, the L-valyl ester of acyclovir, after oral administration is ~54.5%. Since premature hydrolysis of this prodrug in the intestinal lumen may be a possible reason for its incomplete bioavailability and the chemical and enzymatic stability of the valacyclovir has been investigated. Release rates were investigated in both phosphate buffers with varying pH as well as in human and dog gastrointestinal fluids. The stability of the prodrug was found to be dependent on pH. This prodrug is chemically stable along the acidic pH side (under 4), while the prodrug degrades in alkaline medium through a base-catalyzed pseudo-first-order kinetics. The degradation of the prodrug valacyclovir progressed faster in intestinal fluid than in phosphate buffer at the same pH. There was no appreciable release of valacyclovir neither in the human and dog stomach contents nor in phosphate buffers at pHs fewer than 4, although its degradation was fastest in the human and dog stomach contents. In light of this result, we can conclude that the degradation of the valacyclovir in the upper intestinal lumen is probably one of the causes of its poor bioavailability.

Keywords: Valacyclovir; Stability; Luminal degradation; Oral availability; Acyclovir

1. Introduction

Acyclovir, a synthetic purine nucleoside analogue derived from guanine, is used mainly for the treatment of viral infections. Acyclovir has in vitro inhibitory activity against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), varicella-zoster, virus Epstein-Barr virus and cytomegalovirus (O'Brien and Campoli-Richards, 1989). The efficacy of acyclovir in the treatment and suppression of HSV-1, HSV-2 and varicella-zoster virus infections is well documented, and acyclovir has been shown to play a role in the suppression of cytomegalovirus infections (Meyers et al., 1988; Balfour et al., 1989; Prentice et al., 1994).

Despite in vitro activity against herpes viruses and a favorable toxicity profile, many potential applications of acyclovir are limited by its poor absorption. Acyclovir is absorbed slowly and incompletely from the human gastrointestinal tract. The exact mechanism of absorption of acyclovir is not fully characterized. Oral bioavailability is reported to be between 15 and 30% (Fletcher and Bean, 1985). The poor absorption is considered to be a result of characteristics of the drug itself and not its delivery vehicle (De Miranda and Blum, 1983). Thus, to achieve sufficient plasma acyclovir concentrations for the acute treatment or suppression of less susceptible herpesviral diseases, intravenous therapy may be necessary.

Several approaches initially were tried to improve after the oral bioavailability of acyclovir (Beauchamp et al., 1992; Purifoy et al., 1993). Experiments with esters of a number of amino acids were favorable, with the valine-esterified compounds demonstrating the best properties (Purifoy et al., 1993). Addition of the valine moiety to acyclovir results in a substrate for active transport mechanisms in the human intestine. The valine-esterified compound has similar polarity and ionization at physiological pH; thus, an improvement in passive diffusion-related uptake would not be expected (Sould-Lawton et al., 1995).

The prodrug, valacyclocir, synthesized by the addition of a naturally occurring amino acid, L-valine to acyclovir, results in the achievement of plasma acyclovir concentrations superior to those obtained with oral acyclovir, while requiring less frequent administration. Valacyclovir is at least as effective as oral acyclovir for a number of indications (Perry and Faulds, 1996; Acosta and Fletcher, 1997).

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doi:10.1016/j.ijpharm.2006.01.050

After orally administration of valacyclovir to humans, approximately 54% of the dose is absorbed. Of the absorbed valacyclovir, more than 99% is rapidly converted to acyclovir to give high plasma acyclovir concentrations and low plasma valaciclovir concentrations which became undetectable after 3 h postdose, with acyclovir and known acyclovir metabolites, CMMG and 8-OHACV, accounting for the absorbed dose.

The enhancement in oral valacyclovir bioavailability has been attributed to its enhanced permeation across the intestine compared with acyclovir. Following absorption valacyclovir is rapidly and nearly completely hydrolyzed to acyclovir and L-valine, an essential amino acid, by first-pass metabolism. This hydrolysis is mediated primary by the enzyme valacyclovir hydrolase, and occurs predominantly in the liver.

Approximately 46% of the administered dose is recovered in urine and 47% in feces, where no valacyclovir is detected in stool samples, containing only acyclovir. These founding indicate that, in addition to absorbed valacyclovir being converted to acyclovir, unabsorbed valacyclovir is also converted to acyclovir within the gut lumen; this may explain the less than 100% bioavailability of acyclovir following oral dosing with valacyclovir in humans (Weller et al., 1993).

The oral availability is defined as the fraction of drug administered that reaches the systemic circulation unmetabolised. Kinetically, oral availability can be described as the product of the fraction of drug absorbed from the gastrointestinal lumen, the intestinal presystemic metabolism and the hepatic first-pass availability. The possible contributing factors to poor oral biavailability are the diminished access for absorption because of chemical degradation, physical inactivation and insufficient contact time in transit through the gastrointestinal tract; poor permeability across the gastrointestinal mucosa; elimination during the first passage through the gut wall and the liver. Reliable estimates of the relative importance of these causative factors are essential as guides to chemical modifications aimed to optimize oral bioavailability.

Since premature hydrolysis of valacyclovir in the gut lumen is evidenced by the recovery of acyclovir in feces without evidence of valacyclovir, the aim of this study was to investigate the aqueous stability of valacyclovir and its in vitro metabolism in different gastrointestinal media arising from humans and dogs.

2. Materials and methods

2.1. Materials

Glaxo-SmithKline Inc. (Research Triangle Park, NC, USA) provided valacyclovir. Acyclovir was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All chemicals and solvents were of analytical grade.

2.2. Analytical method

High-performance liquid chromatography (HPLC) was performed with a system consisting of a Waters interface module system, a Waters WISP 712 Autosampler, a Waters 996 photodiode array detector and a Waters HPLC 515 pump (Waters, Milford, MA, USA). The analytical column used was a LiChroCART® column (250 mm × 4 mm i.d.) packed with LiChrospher® 100 RP-18, 5 μm particle size (EM Science, Gibbstown, NJ, USA) preceded by a LiChroCART® guard column (4 mm × 4 mm) of the same packing material. The mobile phase used was 0.1 M potassium phosphate monobasic buffer (pH 6.7), containing 25% (v/v) methanol. The flow rate used was 1 ml/min, and the UV detection wavelength was set at 254 nm. The HPLC system was controlled with Waters Millennium software (Version 3.0.1; Waters). Assays of gastrointestinal fluid samples were carried out as follows. In a typical assay, fluid samples were thawed at room temperature and 0.2 ml of 10% (v/v) trifluoroacetic acid in water was added to 0.2 ml of gastric or intestinal fluid and 1 ml of KH_2PO_4 buffer in an Eppendorf tube. The mixture was vortexed for 1 min and centrifuged at 12,500 rpm and 4 °C for 15 min. The supernatant was filtered using a 0.45 μm filter cartridge, and 15 μl of the filtered supernatant was injected directly onto the column for HPLC analyses. The aqueous buffer solutions were injected directly onto the column for HPLC analyses. The retention times were ∼3.2 and ∼5.8 min for acyclovir and valacyclovir, respectively. Standard curves using solutions of acyclovir and valacyclovir in aqueous buffer solutions were constructed over the concentration range of 20–90 μg/ml and were found to be linear ($r^2 = 0.999$). Additionally, the standard reference curves for gastrointestinal fluids were obtained by adding known amounts of diluted stock standard of valacyclovir to blank gastric or intestinal fluid in a concentration range of 16–40 μg/ml. Each stability profile represents the average of at least three independent runs with the same sampling schedules. The standard deviation of each point is typically 5% or less.

2.3. Aqueous stability

The degradation of valacyclovir was studied in potassium monobasic phosphate buffer solution at 37 °C. The buffer used was adjusted to the corresponding pH value with hydrochloric acid or sodium hydroxide solutions over the range of 1.84–7.94. The ionic strength ($\mu = 0.5$) was maintained constant for each buffer by adjusting with a calculated amount of potassium chloride. The reaction was initiated by adding 500 μl aqueous stock solution to 5 ml preheated buffer solution to a final concentration of 8 μg/ml. During the experiments, the solutions were kept in a water bath at 37 °C and at various times samples were collected and chromatographed immediately or shortly after stopping the reaction with an equal volume of phosphate buffer at pH 4 or less (pH of maximum stability). Pseudo-first-order rate constants (k_{obs}) for the degradation were determined from the slopes of linear plots of the logarithm of residual compound against time.

2.4. In vitro metabolism

Hydrolysis of valacyclovir was studied in human and dog gastric juices and human and dog intestinal fluids. These fluids were collected using the Loc-I-Gut technique (Lindahl et al., 1997). The human study was approved by the Ethics Committee of the Universidad Nacional de Córdoba and followed the conventions

of the Declaration of Helsinki. The pH of the gastrointestinal secretions was determined using a pH meter (Piccolo Plus HI 1295 electrode).

The experiments were initiated by adding 200 μl of a stock solution of valacyclovir at a concentration of 80.63 μg/ml to 2 ml of preheated biological media. The mixture was stirred well and was incubated for 24 h in a temperature-controlled water bath at 37 °C. At appropriate intervals, samples of 200 μl were drawn and deproteinized with a double volume of acetonitrile. After mixing and centrifugation at 13,000 rpm at 5 °C for 7 min, the supernatant was analyzed by HPLC as describe above.

3. Results

3.1. Stability in aqueous media

The disappearance of intact valacyclovir was monitored by the HPLC method over the pH range 2–8 at 37 °C. Fig. 1 shows time course for disappearance of valacyclovir in the aqueous buffer solutions. The degradation rate constant (k_{obs}) was calculated by using linear regression analysis obtained from at least six time intervals. The degradation half-time ($t_{0.50}$) and the approximate shelf-life ($t_{0.90}$) were calculated as $0.693/k_{obs}$ and $0.1054/k_{obs}$, respectively (Table 1) (Kostenbauder and Bogardus, 1990; Martin, 1993). The influence of pH on the rate of hydrolysis of valacyclovir is shown in Fig. 2. The influence of buffer species on the rate of hydrolysis of valacyclovir by varying the buffer concentration was not determined. Valacyclovir was hydrolyzed according to first-order kinetics. At pHs higher than ~4, valacyclovir underwent a base-catalyzed reaction that lead to the active drug acyclovir and L-valine. The maximal stability was observed at pH under 4. At the pH of 1.84, valacyclovir is only 2% hydrolyzed after a period of 24 h (data not shown). The obtained results indicate that the prodrug was stable a low pH and the rate of decomposition was accelerated at higher pH.

Table 1
k_{obs}, $t_{0.50}$ and $t_{0.90}$ values for the degradation of valacyclovir at 37 °C in phosphate buffers at different pHs and dog and human gastric (GF) juice and intestinal (IF) fluid

	pH	$t_{0.50}$ (h)	$t_{0.90}$ (h)	[a] k_{obs} (h^{-1})
Phosphate buffer	4.11	39.60	6.00	0.0175
	4.92	20.20	3.60	0.0343
	6.32	15.54	2.35	0.0446
	7.39	10.06	1.52	0.0689
	8.09	7.48	1.13	0.0926
Dog gastric fluid	1.30	111.77	16.94	0.0062
	1.77	44.42	6.73	0.0199
	2.30	61.33	9.29	0.0113
	3.64	34.82	5.28	0.0232
Average ± S.D.	2.3 ± 1.0	63 ± 34	9.6 ± 5.2	0.015 ± 0.008
Dog intestinal fluid	7.09	7.29	1.11	0.0950
	7.23	5.72	0.87	0.1211
	7.77	2.96	0.45	0.2341
	8.00	8.08	1.22	0.0858
Average ± S.D.	7.5 ± 0.4	6 ± 2	0.9 ± 0.3	0.13 ± 0.07
Human gastric fluid	1.22	1386	210	0.0005
	0.97	990	150	0.0007
	1.37	990	150	0.0007
Average ± S.D.	1.2 ± 0.2	1122 ± 229	170 ± 35	0.0006 ± 0.0001
Human intestinal fluid	6.15	9.57	1.45	0.072
	6.32	9.38	1.40	0.074
	6.51	9.11	1.38	0.076
Average ± S.D.	6.3 ± 0.2	9.4 ± 0.2	1.41 ± 0.04	0.074 ± 0.002

[a] Values are an average of three determinations with R.S.D. values within 5%.

3.2. In vitro metabolism

The half-life and shelf-life of valacyclovir after incubation in gastrointestinal media are shown in Table 1. Figs. 3 and 4 show the time course for disappearance of valacyclovir in the contents of human and dog gastrointestinal tract.

The degradation rate for this drug was higher than in the buffers at the same pH, which suggests either a catalytic effect

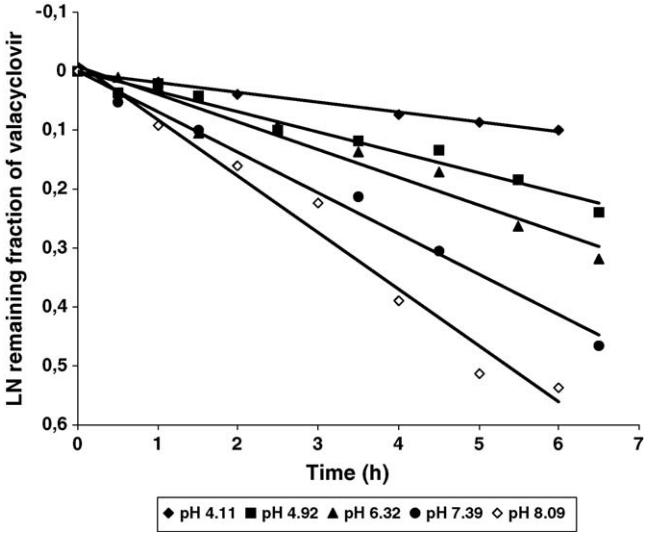

Fig. 1. First-order plots for hydrolysis of valacyclovir in phosphate buffer solutions at 37 °C.

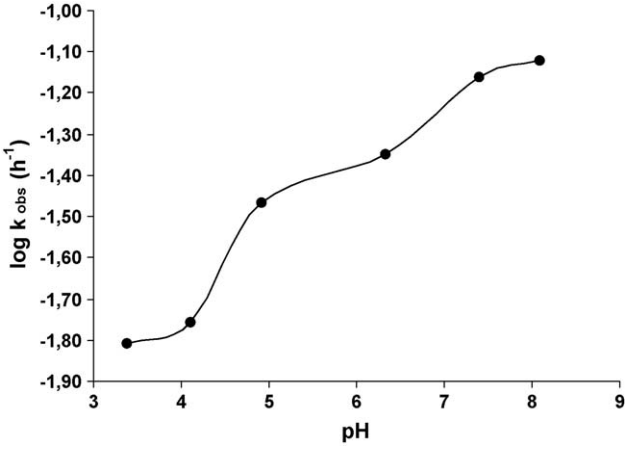

Fig. 2. The pH rate profile for the hydrolysis of valacyclovir in phosphate buffer ($\mu = 0.5$) at 37 °C.

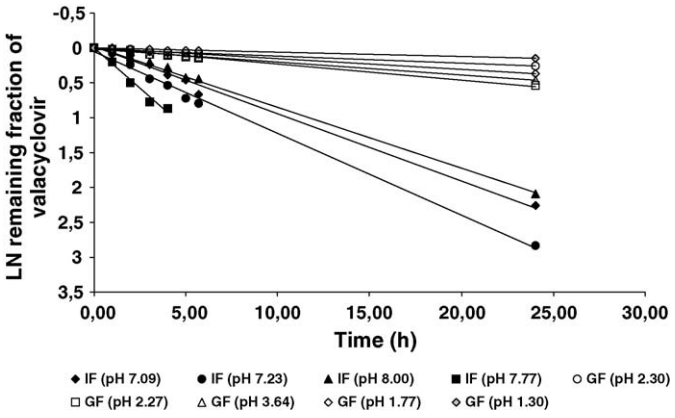

Fig. 3. First-order plots for hydrolysis of valacyclovir in dog gastric (GF) juice and dog intestinal (IF) fluid at 37 °C.

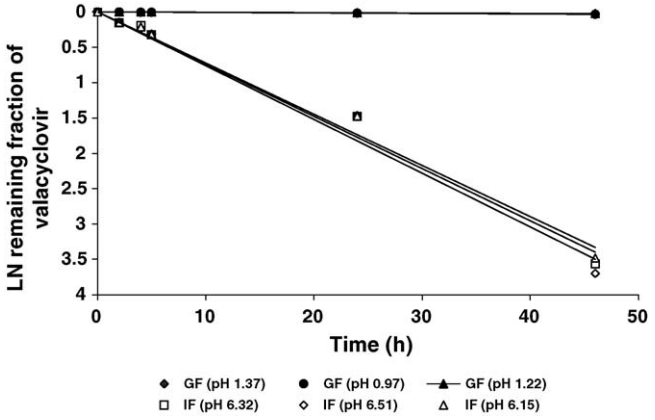

Fig. 4. First-order plots for hydrolysis of valacyclovir in human gastric (GF) juice and human intestinal (IF) fluid at 37 °C.

of enzymes or ions arising from the human and dog gastrointestinal tract. Valacyclovir demonstrated excellent stability in acidic phosphate buffers, as well as, in the human and canine gastric contents for many hours with minimal hydrolysis. Although, valacyclovir demonstrated good stability in the dog gastric content, with a half-life and shelf-life of 63.09 ± 34.26 and 9.56 ± 5.19 h, respectively, a faster degradation is seen for valacyclovir in this medium as compared to the phosphate buffer solution at the acidic pH side (∼25% of valacyclovir is hydrolyzed after 24 h), possible due to enzymatic activity in this media. The degradation of valacyclovir was very slow in the human gastric content, with a half-life and shelf-life of 1122 ± 229 and 170 ± 35 h, respectively. In human and dog intestinal fluids, the degradation followed first-order kinetics, and the half-lives were 9.4 ± 0.2 and 6.0 ± 2.3 h, respectively.

4. Discussion

The oral route is attractive for drug administration because it is associated with patient convenience and compliance and lower costs. However, it has been recognized that some drugs are much less effective when administered by this route than when given parentally. A number of causes for this phenomenon can be considered including instability of the drug in the gastrointestinal environment, presystemic metabolism and incomplete release of drug from the dosage form and poor intestinal permeability.

Valacyclovir demonstrates an oral bioavailability that is three to five times greater than acyclovir. Several carrier-mediated transporters in the intestine may facilitate valacyclovir absorption. The findings of Landowski et al. (2003) suggest that valacyclovir is a substrate for PEPT1 and HPT1 transporters and those appear to have similar valacyclovir transport abilities. Therefore, it is quite likely that in vivo, the much higher expression levels of HPT1 compared with PEPT1 may determine its predominance in valacyclovir transport. On the other hand, PEPT1 is predominantly expressed in the jejunum and ileum. HPT1 expression, however, is significant in all regions of the gastrointestinal tract (Herrera-Ruiz et al., 2001). In addition, Landowski et al. (2003) found a significant negative correlation of pharmacokinetic parameters of valacyclovir with expression levels of MDR1, MRP2 (cMOAT) and the cytochrome P450 IIIA subfamily member genes, which may indicate that these genes are involved in valacyclovir efflux and metabolism.

According to Guo et al. (1999), the optimum pH for valacyclovir uptake is 7.5, presumably because it exists predominantly as a mixture of neutral and cationic species at that pH. The uptake is almost two-fold higher than that at lower pH (5.5 and 6.0) and higher pH (8.0), which might be explained by its chemical structure. Valacyclovir has three pK_a values equal to 1.90, 7.47 and 9.43; correspond to the amine attached to the C_2 in the purine, the N-terminal amine in valine and the NH-acidic N_1 in the purine, respectively. At low pH conditions (6 or lower), valacyclovir would exist primarily as a cationic moiety. As the pH is increased from 6.0, the net cationic charge present on the drug becomes progressively less, reaching an almost neutral state.

On the other hand, the measurements of luminal pH in the normal gastrointestinal tract have shown a progressive increase in pH from the duodenum to the terminal ileum, a decrease in the caecum and then a slow rise along the colon to the rectum (luminal pH in the proximal small bowel ranges from 5.5 to 7.0 and gradually rises to 6.5–7.5 in the distal ileum. There is a fall in luminal pH from the terminal ileum to the caecum (range 5.5–7.5); pH then rises in the left colon and rectum to 6.1–7.5) (Nugent et al., 2001).

Taking in consideration that the T_{max} of valacyclovir after oral administration approximately is 1.71 ± 0.69 h (Sould-Lawton et al., 1995), the regional variation of the expression of the valacyclovir transporters along the intestine, the pH profile for all segments of the gut, that the optimal uptake of valacyclovir occurs at pH 7.5 and that, the efflux back of valacyclovir across the apical membrane into the lumen rises the amount of valacyclovir available for uptake; it is possible that this prodrug probably is mainly absorbed from jejunum and ileum, which suggests that some hydrolysis of valacyclovir must occur in the proximal small intestine, but most of the hydrolysis must occur distally.

Several interdependent processes may determine the overall absorption parameters of valacyclovir following oral administration undoubtedly. However, in view of the results reported here, within the valacyclovir overall absorption process, atten-

tion should be paid to the luminal degradation of this prodrug along the gastrointestinal tract, since we have found that the influence of pH on the bioavailability of valacyclovir is substantial. Approximately 15% of the dose of valacyclovir given orally is chemically hydrolyzed at pH ∼ 7.4, during the small intestinal transit (∼3 h). It is reasonable to assume that enzymes present in the intestinal lumen play a major role in the hydrolysis of valacyclovir. The results obtained here suggest that ∼25% of the dose of valacyclovir administrated orally is chemically and enzymatically hydrolyzed in the dog intestinal lumen and ∼16% in the human intestinal lumen, during the small intestinal transit (∼3 h). These results indicate that the drug degrades faster along the dog intestine, probably because of the different pH values in the gut lumen between these species, being the pH of the human intestinal fluid lower than 1 in the dog intestinal fluid.

In summary, this study provides new fundamental data on the processing of valacyclovir in the gastrointestinal tract. The results clearly show that there is no major hydrolysis of this prodrug in the stomach. Nevertheless, data obtained in this study provide evidence that an important proportion of valacyclovir might be hydrolyzed in the luminal gut.

Since one of the requirements of a successful oral delivery system is reasonable stability in the environment of the gastrointestinal (GI) tract, we can conclude that the luminal degradation of the prodrug valacyclovir is probably one of the causes of its poor bioavailability.

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