



## APPROACHES FOR ENHANCING THE BIOAVAILABILITY OF ACYCLOVIR: A CRITICAL REVIEW

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### ABSTRACT

Acyclovir, a purine nucleoside analogue, is a promising antiviral compound effective against Herpes Simplex Virus (HSV), Varicella-Zoster Virus (VZV), Epstein-Barr virus (EBV), and Cytomegalovirus (CMV). From the past 3 decades, it has been extensively exploited by the scientists for the diseases caused by these infective viruses. Upto a certain extent they are able to make dosage forms which can treat the infections caused by these viruses but, cannot perfectly/completely cure the ailments. In spite of its effective antiviral activity, oral bioavailability of Acyclovir is low (15 to 30%) and highly variable. This review discusses various possible reasons for the poor oral bioavailability of Acyclovir. In addition, it also focuses on various formulation approaches investigated by formulation scientists for the improvement of its oral bioavailability such as self microemulsifying drug delivery system, gastroretentive mucoadhesive microspheres, microemulsions, niosomes etc. Though the reasons for the poor oral bioavailability of Acyclovir are conflicting and inconclusive, many authors have reported widely varying formulation approaches to circumvent this problem and results suggested that these dosage forms can take place in the market. However detailed clinical studies are still needed to reconfirm these findings. The present review critically analyzes the pharmacokinetic and clinical limitations of conventional therapy of Acyclovir and different formulation strategies taken by the research scientists to overcome its formulation and clinical limitations.

**KEYWORDS:** Acyclovir, Bioavailability, Clinical limitations, Formulation strategies, Clinical studies.



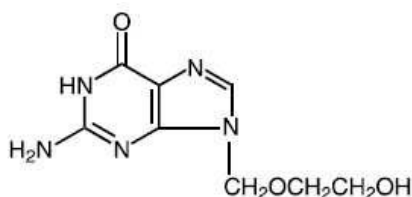
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## 1. INTRODUCTION

Acyclovir, chemically known as 2-amino-1, 9-dihydro-9-[(2-hydroxyethoxy)methyl]-6H-purin-6-one, is a purine nucleoside analogue, initially known for its potent and selective antiviral activity against viruses of the herpes group<sup>1,2</sup>. Acyclovir is a structural analog of deoxyguanosine (Figure 1). Acyclovir (first known as acycloguanosine) was synthesized in the U.S.A. as part of the Burroughs Wellcome programme for the development of

guanosine nucleosides resistant to phosphorylase degradation with the first observation of antiviral activity being made by Collins & Bauer in the U.K. at the Beckenham laboratories of the former Wellcome Foundation<sup>3</sup>. In addition to Herpes Simplex Virus (HSV), it has *in vitro* activity against Varicella-Zoster Virus (VZV), Epstein-Barr Virus (EBV), and Cytomegalovirus (CMV)<sup>4</sup>.



**Figure 1**  
**Structure of Acyclovir**

Selective toxicity of Acyclovir to cells infected with herpes virus is due to its anabolism to the monophosphate by a virus-coded thymidine kinase and subsequent conversion to the di- and triphosphates, presumably by cellular kinases<sup>5,6,7</sup>. The drug has been shown to be of clinical benefit when administered topically, orally, or parenterally for the prophylaxis and treatment of certain herpes virus infections<sup>4</sup>. Table 1 summarizes the indications in which Acyclovir is used clinically. In spite of its effective antiviral activity, the use of

Acyclovir still has biopharmaceutics and pharmacokinetic limitations. Absorption of Acyclovir in the gastrointestinal tract is slow, variable and incomplete<sup>8</sup>. This article summarizes various reports published on poor and variable bioavailability of Acyclovir and studies conducted in an attempt to improve the same. In addition, emphasis is also laid on studies conducted on oral extended release formulations of Acyclovir to reduce the dosing frequency.

**Table 1**  
**Comparison of indications and dosage of Acyclovir and Valacyclovir**

Indication	Dosage		Remarks
	Acyclovir	Valacyclovir	
Acute Treatment of Herpes Zoster	800 mg every 4 hours orally, 5 times daily for 7 to 10 days (specifically mentioned as "Acute treatment")	1 gram 3 times daily for 7 days (not mentioned as "Acute treatment")	Daily dose of Acyclovir is 4 grams and Valacyclovir is 3 grams
Treatment of Chickenpox	800 mg four times a day for five days.	20 mg per kg of body weight, taken 3 times a day for 5 days.	Daily dose of Acyclovir is 3.2 grams and Valacyclovir is based on body weight
<b>Genital Herpes</b>			
Treatment of Initial Genital Herpes	200 mg every 4 hours, 5 times daily for 10 days	1 gram twice daily for 10 days	Daily dose of both drugs is 1 gram
Chronic Suppressive Therapy for Recurrent Disease	400 mg 2 times daily	1 gram once daily	Daily dose of Acyclovir is 800 mg and Valacyclovir is 1 gram
Intermittent Therapy	200 mg every 4 hours, 5 times daily for 5 days	---	

## 2. PHARMACOKINETICS OF ACYCLOVIR

Pharmacokinetics of Acyclovir has been extensively studied in various experimental animals and in man and has been well established after administration through various routes<sup>9,10,11,12,13</sup>. Absorption of oral Acyclovir is slow and variable, with a bioavailability of only 15 to 30%<sup>14</sup>. This necessitates administration of high dose of the drug to maintain therapeutically required blood levels. Further the elimination half-life of Acyclovir is very low (2.5 to 3.3 h),

and hence it must be administered frequently to maintain required blood levels throughout the course of therapy. The requirement of frequent administration of Acyclovir (five times a day) leads to poor compliance among the patients, in turn leading to reduction in therapeutic efficacy and development of resistance. The section 3 explores the possible reasons resulting in the poor bioavailability of Acyclovir. Table 2 summarizes the pharmacokinetic parameters of Acyclovir

**Table 2**  
**Pharmacokinetic parameters of Acyclovir**

Oral Bioavailability	15-30%
Elimination Half-life	2.5-3.3 h
Administration	5 times a day
Time to maximum plasma concentration ( $t_{max}$ )	1.4 h
Area under plasma concentration profile ( $AUC_{inf}$ )	3.3 $\mu\text{g}\cdot\text{h}/\text{ml}$

## 3. POSSIBLE REASONS FOR POOR BIOAVAILABILITY

### 3.1 Polymorphism & pseudopolymorphism

Kristl et al.<sup>15</sup> prepared anhydrous forms of Acyclovir and compared its intrinsic dissolution with that of hydrated Acyclovir. The anhydrous form dissolved relatively slowly than the hydrated form. Apart from this work, not much of the work has been published on the details physicochemical properties and pharmacokinetics of various polymorphic / pseudopolymorphic forms of Acyclovir. This research area still remains a potential consideration for improving solubility, in turn its bioavailability.

### 3.2 Absorption mechanism

There are conflicting reports on the existence of saturable absorption process for Acyclovir in the gastrointestinal tract (GIT). Karsnyet al.<sup>16</sup> suggested that the gastrointestinal absorption of Acyclovir is a saturable process, based on the non linearity in absolute bioavailability after oral administration of increasing doses of Acyclovir. They investigated pharmacokinetics of Acyclovir in dogs after administration of capsule and gavage. The absolute bioavailability decreased with increasing dose in both modes of

administration. Absolute bioavailability was 91, 80 and 54% for 5, 20 and 50 mg/kg, respectively after capsule administration and corresponding figures were 89, 61 and 67% after gavage dosing. But no mechanistic studies were performed to conclude the hypothesis that absorption of Acyclovir is a saturable process. In another study performed by Lewis and his co-workers in 1986<sup>11</sup>, bioavailability of Acyclovir was found to be significantly higher when the drug was administered as intraduodenal infusion or sipped solution as compared to conventional tablets. In case of conventional tablets, 2 x 200 mg tablets of Acyclovir were administered to healthy male volunteers under fasted condition with 200 ml of 5% dextrose solution and followed by another 100 ml of 5% dextrose solution at exactly 1 hr, 2 hrs and 3 hrs intervals. As a different form, 400 mg of Acyclovir in 500 ml of 5% dextrose solution was infused into the duodenum through a nasoduodenal tube at a constant rate of 2.08 ml/min over 4 hrs. In the third method, volunteers sipped a similar Acyclovir solution over 4 hrs at a sipping rate of 10.4 ml every 5 min. Mean areas under the plasma concentration time curves (AUCs)  $\pm$ s.d. for tablet, intraduodenal infusion and sipping were,

14.7 ± 5.1; 24.6 ± 5.1 and 28.4 ± 9.5 (n = 6),  $\mu\text{mol l}^{-1} \text{ h}$ . AUCs for infusion and sipping were significantly greater than that for tablets ( $p < 0.05$ ). These results showed that reducing the delivery rate of Acyclovir to its absorption site in the gut does significantly improve the amount absorbed and based on these results the authors suggested existence of capacity limited absorption for Acyclovir.

Conversely, the pharmacokinetic parameters including bioavailability were not affected significantly as compared to oral bolus administration when Acyclovir was administered as gastric infusion over 4 hrs period in rats<sup>17</sup>.  $C_{\text{max}}$  values after administration of 120 mg/kg of Acyclovir as oral bolus and gastric infusion were 1200 ± 109 ng/ml and 1591 ± 156 ng/ml, respectively. AUCs for corresponding treatments were 464.5 ± 68.2  $\mu\text{g}\cdot\text{min}/\text{ml}$  and 616.3 ± 60.2  $\mu\text{g}\cdot\text{min}/\text{ml}$ . Though  $C_{\text{max}}$  and AUC increased for gastric infusion as compared to oral bolus, but the increase was not statistically significant at  $p < 0.01$ . It is possible that these results could be statistically significant at  $p < 0.05$ , but such analysis was not performed by the authors. In another study, the mechanism of uptake of Acyclovir in rat jejunum using *in vitro* and *in situ* methods was investigated<sup>18</sup>. Uptake of Acyclovir was linear in the concentration range of 0.01 to 5 mM with *in vitro* intestinal ring method. While the carrier-mediated uptake of Uracil was inhibited to the extent of 31% by Acyclovir, Uracil did not significantly influence the uptake of Acyclovir. When the single-pass perfusion was performed for Acyclovir over a 500 fold concentration range of 0.1 to 5 mM, there was no overall decrease of permeability seen with increase in initial perfused drug concentration. Moreover, when 0.05 mM Acyclovir was coperfused with 1 mM DNP (2,4-dinitrophenol), the wall permeability of Acyclovir did not decrease, but resulted in elevation from 0.098 ± 0.031 to 0.212 ± 0.052. All the above findings led to the suggestion that the uptake mechanism of Acyclovir in the rat jejunum is predominantly via passive diffusion.

The results of Kristl and Tukker<sup>19</sup> were in agreement with the above results. The authors studied the permeability of Acyclovir in S-G cells

(Sweetana-Grass diffusion cells) both in m-to-s and s-to-m direction, but no polarization in transport was observed leading to the suggestion that Acyclovir was absorbed passively and not by active transport. But there were two observations in their study. Firstly, comparison of apparent permeability coefficient ( $P_{\text{app}}$ ) with lipophilicity ( $\log P$  n-octanol/water) of the tested compounds showed a decrease of permeability with increase in  $\log P$  values. Secondly the study could not derive any explanatory conclusion on the higher bioavailability of deoxyacyclovir (a prodrug of Acyclovir, at least 75% of the administered dose of deoxyacyclovir is absorbed) than that of Acyclovir. In another study conducted by Wilson et al. in 1987<sup>20</sup>, delay in the gastric emptying and prolonged intestinal transit time induced by a heavy breakfast significantly decreased the absorption of Acyclovir. Wilson et al.<sup>20</sup> investigated the theory that reduction of the rate of gastric emptying may increase the absorption of Acyclovir by two mechanisms, firstly by increasing the contact time with gastric acid and secondly by slow delivery from the stomach to the sites of absorption in the intestine. Acyclovir suspension containing anion exchange resin radiolabelled with (99mTc)perchnetate was administered to healthy subjects immediately following heavy and light meals, and blood levels of the drug were measured over a 24 h period. Transit of the marker was followed by gamma scintigraphy. Followed by a heavy meal, gastric emptying were significantly greater and small intestinal transit time was slower as compared to lighter meal, though the latter effect was unexpected. Mean peak plasma concentration and the AUCs were significantly greater when the drug was administered with lighter meal compared to the heavy meal, in spite of higher gastric emptying time and slower small intestinal transit time followed by heavy meal. Though this study could not prove the intended theory, it has to be noted that the influence of food on drug absorption is a very complex process and involves multiple factors<sup>21</sup>.

In a recent study the regional absorption of Acyclovir in a rat GIT model was investigated by Liu et al.<sup>22</sup>. The absorption of Acyclovir in

different segments of GI tract for 3 hrs were  $9.46 \pm 0.62\%$ ,  $20.22 \pm 1.50\%$ ,  $15.7 \pm 1.33\%$ ,  $9.15 \pm 1.01\%$ , and  $4.59 \pm 0.48\%$  from stomach, duodenum, jejunum, ileum and colon, respectively. Though these results suggest that the absorption of Acyclovir is predominant from upper parts of gastrointestinal tract, the drug does get absorbed from colon to a greater extent. Hence there could be reasons other than regional absorbability that are responsible for poor bioavailability of Acyclovir. In a study conducted by Merzlikine et al.<sup>23</sup>, Acyclovir exhibited poor apical to basolateral permeability ( $P_{app,A \rightarrow B} = 2.84 \pm 0.8 \times 10^{-6}$  cm/sec), which was similar to the standard probe for the paracellular absorption, mannitol ( $P_{app,A \rightarrow B} = 2.53 \pm 0.18 \times 10^{-6}$  cm/sec). This observation, in addition to the increase in  $P_{app,A \rightarrow B}$  of Acyclovir in the presence of tight junction modulators, lead to the conclusion that this drug is absorbed via paracellular transport.

### 3.3p-glycoprotein efflux

A characteristic feature of P-gp substrates is that they show a higher transport from the basolateral to the apical (B→A) than from the apical to the basolateral (A→B) side of an intestinal membrane. Palmberger et al.<sup>24</sup> studied the transport of Acyclovir from A→B (absorptive) and B→A (secretory) across rat intestine and Caco-2 cell monolayers. Permeability coefficients ( $P_{app}$ ) were determined for Acyclovir in buffer and in the presence of verapamil, a calcium channel antagonist that is known to have inhibitory effect on p-glycoprotein. The  $P_{app}$  of secretory transport was 2.3 and 2.5-fold higher than absorptive transport for Acyclovir in rat intestine and Caco-2 cell monolayers, respectively. In the presence of Verapamil, the absorptive and secretory transport of Acyclovir was not significantly different due to the p-glycoprotein inhibitory effect of Verapamil. These findings provided further evidence to the earlier reports that p-glycoprotein inhibitors could improve absorption of Acyclovir<sup>25, 26</sup>.

## 4. APPROACHES FOR ENHANCEMENT OF ORAL BIOAVAILABILITY

### 4.1 Self microemulsifying drug delivery system (SMEDDS)

Patel and Sawant<sup>27</sup> prepared and optimized SMEDDS with high loading of Acyclovir with an objective to improve the oral bioavailability of Acyclovir. Comparative bioavailability study of optimal formulation and drug solution was performed in male albino rats. As compared to the drug solution, the SMEDDS formulation exhibited 2.2-fold and 3.5-fold higher  $C_{max}$  and AUC, respectively. Various modes by which drug absorption is enhanced from the SMEDDS have been listed by the authors<sup>28</sup>, but in the case of Acyclovir, following could be probable modes:

- Inhibition of gastric motility due to lipid phase of emulsion, which allowed more time for drug dissolution and absorption
- Increase mucosal permeability via incorporation of lipid from mixed micelles and enhanced mesenteric lymph flow
- Increased dissolution due to the large surface area offered by the emulsion

### 4.2 Niosomes

Attia et al.<sup>29</sup> prepared Acyclovir niosomes by the conventional thin film hydration method in an attempt to improve its oral bioavailability. Niosomes contained a lipid mixture of cholesterol, span 60, and dicetyl phosphate in the molar ratio of 65:60:5, respectively. Niosomal dispersion was found to have sustained release characteristics *in vitro* as compared to free drug. Pharmacokinetic study was performed in male New Zealand white rabbits. The niosomal drug dispersion showed significantly ( $p < 0.005$ ) higher values for  $C_{max}$ ,  $t_{1/2}$ ,  $AUC_{0 \rightarrow \infty}$ , and MRT; and significantly ( $p < 0.005$ ) lower values for absorption ( $K_a$ ) and elimination ( $K$ ) rate constants compared with free drug solution. The increase in the MRT and  $AUC_{0 \rightarrow \infty}$  values and the decrease in the  $K_a$  value reflected the sustained release effect of the niosomal formulation and were in line with *in vitro* drug release study. The sustained release effect could be due to the penetration of the

drug into deeper layers of intestinal mucosa facilitated by the carrier effect of niosomes, followed by slow release of encapsulated drug. The relative bioavailability of niosomal formulation compared with drug solution was found to be  $2.55 \pm 1.82$ . The improved bioavailability could be due to lipophilic nature of the niosomal formulation, which enhances partitioning to the mucosa, effect of the nonionic surface-active agent (span 60) on the barrier function of the intestinal mucosa of the gastrointestinal membrane and prolonged localization of drug-loaded niosomes at the site of absorption.

#### 4.3 Beta-cyclodextrin

Rosset al.<sup>30</sup> prepared inclusion complexes of Acyclovir with beta-cyclodextrin (ACY- $\beta$ CD) in solid and solution state. Formation of 1:1 stoichiometric complex was investigated and confirmed by various characterization techniques such as <sup>1</sup>H-NMR, X-ray diffraction, differential scanning calorimetry and thermogravimetry. Dissolution of ACY- $\beta$ CD complex was much higher than the pure drug alone or its physical mixture with  $\beta$ CD. The increase in dissolution in the case of complex was attributed to the increased solubility due to decrease in crystallinity of the inclusion complex. Though the authors did not conduct *in vivo* studies, Luengoet al.<sup>31</sup> studied the pharmacokinetics of ACY- $\beta$ CD complex. Acyclovir, its 1:1 inclusion complex with beta cyclodextrin and 50:50 mixture of pure drug and the inclusion complex were administered intraintestinally to male Sprague-Dawley rats in doses equivalent to an Acyclovir dose of 75 mg/kg. Both the ACY- $\beta$ CD complex and the Acyclovir/complex mixture had a higher bioavailability than Acyclovir in terms of AUC. By comparing the AUCs by the Friedman Test, there was no statistically significant difference between Acyclovir and the complex ( $p > 0.050$ ). However, there were statistically significant differences between Acyclovir and the Acyclovir/complex mixture ( $p = 0.0126$ ) and between the complex and the Acyclovir/complex mixture ( $3p = 0.0003$ ). Small number of animals

used in this study (4 each for Acyclovir and complex, and 2 for the mixture) could be responsible for such an observation. Further detailed studies would be required on the effect of Acyclovir inclusion complex with  $\beta$ CD on bioavailability of Acyclovir.

#### 4.4p-Glycoprotein inhibition

Salamaet al.<sup>25</sup> found that the apical tobasolateral (A $\rightarrow$ B) apparent permeability coefficient ( $P_{app}$ ) of Acyclovir was significantly increased by 23% ( $p < 0.05$ ) when the drug was incubated with an enaminone, DM27, which is a P-gp inhibitor. This increase was obtained only with  $10^{-8}$  M concentration of DM27, but not at lower or higher concentrations ( $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$ ,  $10^{-7}$  &  $10^{-10}$  M) and possible reasons for this observation were not discussed. There are no other reports available relating poor bioavailability of Acyclovir with P-gp efflux. Palmberger et al.<sup>24</sup> also studied the effect of P-gp inhibition by novel thiolated chitosans on permeability of Acyclovir. Effect of three chitosan-4-thiobutylamidine (Chito-TBA) conjugates with increasing molecular mass (Chito-9.4 kDa-TBA, Chito-150 kDa-TBA and Chito-600 kDa-TBA) on transport of Acyclovir across rat intestinal mucosa and Caco-2 cell monolayer was studied. In comparison to buffer only, the transport of Acyclovir in presence of 0.5% (m/v) unmodified chitosan, 0.5% (m/v) Chito-150 kDa-TBA and 0.5% (m/v) Chito-150 kDa-TBA with 0.5% (m/v) reduced glutathione (GSH), was 1.3, 1.6 and 2.1-fold improved, respectively. Transport studies across Caco-2 cell monolayers showed that P-gp inhibition is dependent on the average molecular mass of thiolated chitosan showing following rank order: 0.5% (m/v) Chito-150 kDa-TBA/GSH > 0.5% (m/v) Chito-9.4 kDa-TBA/GSH > 0.5% (m/v) Chito-600 kDa-TBA/GSH. Tablets of Acyclovir prepared from thiolated polymers showed sustained *in vitro* drug release. The degree of retardation was dependent on the average molecular mass of polymer. Based on the results of permeability studies and *in vitro* release characteristics Chito-150 kDa-TBA was identified to have the most appropriate polymeric chain length.

#### 4.5 Microemulsions

Ghosh et al.<sup>32</sup> designed a microemulsion based drug delivery system to improve poor oral bioavailability of Acyclovir. Quaternary microemulsion system of water/Labrasol/Plurol Oleique/Labrafac containing 5% concentration of Acyclovir was developed and optimized through a pseudoternary phase diagram. The rate of diffusion of the drug from microemulsion was found to be faster than that from tablet in intestinal permeability studies using rat duodenum. After 5 h of diffusion the fraction of drug diffused from microemulsion was 85%, which was much higher than the fraction diffused from tablet (69%). Pharmacokinetics of the microemulsion was studied in male albino Sprague-Dawley rats in comparison with intravenous injection and tablets. Microemulsion formulation resulted in more sustained absorption of Acyclovir than tablets.  $t_{max}$  of microemulsion formulation and tablets was 3 hrs and 0.5 hrs, respectively.  $C_{max}$  and AUC of Acyclovir from microemulsion were 1.9-fold and 12.8-fold higher than tablets. When compared with intravenous administration, absolute bioavailability of tablet and microemulsion were 2.2 and 27.9 %, respectively. The improvements seen with respect to microemulsions were statistically significant ( $p < 0.01$ ). Enhanced absorption of Acyclovir from microemulsion was explained in terms of huge surface area, increase in the intestinal permeability due to the presence of surfactants and stability of the formulation in the gastrointestinal tract.

#### 4.6 Multiple emulsions

Paul et al.<sup>33</sup> developed water-in-oil-in-water type multiple emulsions (w/o/w emulsions) of Acyclovir and investigated its oral bioavailability in comparison with drug solution. Multiple emulsions (MEs) were prepared by using different concentrations (15 and 20%) of Span-80 and Span-83 as lipophilic surfactants and Brij-35 as hydrophilic surfactant. Optical photomicrographs confirmed multiple nature of MEs and that the samples belonged to type C containing many small droplets in the internal phase of the multiple globules<sup>34</sup>. Drug release

studies through dialysis bag and rat intestine revealed initial rapid release followed by a much slower release. MEs were found to be more stable in refrigerator than in room temperature. *In vivo* studies of most stable MEs were conducted on albino rats. MEs achieved much higher  $C_{max}$  ( $12.98 \pm 0.98$  and  $14.82 \pm 1.11$   $\mu\text{g/ml}$  for 15% Span-80 and 20% Span-83, respectively) than plain drug solution (8.05  $\mu\text{g/ml}$ ). ME containing 15% Span-20 showed 4.25 times and ME containing 20% Span-83 showed 4.6 times increase in the AUC than plain drug solution indicating better absorption from gastrointestinal mucosa and higher bioavailability as compared to plain solution. Plasma concentrations achieved by MEs were more consistent and sustained than plain drug solution.

#### 4.7 Gastroretentive mucoadhesive microspheres

Gastric mucoadhesive drug delivery systems have been widely studied to prolong the gastric residence time of drugs possessing absorption window in the upper parts of gastrointestinal tract. The advantage of improving bioavailability of Acyclovir by prolonging gastric residence was first established in a study by Groning et al.<sup>35</sup>. The authors prepared a press coated magnetic depot tablet formulation containing carnauba wax coated disc shaped magnet as the inner core. The inner core was surrounded by two compression coated layers containing Acyclovir along with a release controlling polymer hypromellose (Methocel K4M). The two coats contained different amounts of Acyclovir and about 10% polymer. The formulation used in the pharmacokinetic study contained 160 mg and 40 mg of Acyclovir per tablet in the inner layer and outer layer, respectively. *In vitro* drug release of this formulation showed a sustained drug release profile over 12 hrs. Magnetic tablets and immediate release tablets were administered as two treatments of a three way cross-over study in five healthy male subjects. In case of the third treatment, an external magnet was placed at the stomach level of the subjects for 12 hrs in an attempt to prolong the gastric residence. The plasma concentration

profiles showed that higher and longer lasting plasma concentrations can be achieved after administration of magnetic depot tablets when the external magnet is placed at stomach level as compared to the same formulation administered without the external magnet. The differences in plasma concentrations were statistically significant at 7, 8, 10 and 12 hrs after the ingestion of the magnetic depot tablet with an extracorporeal magnet present. In three out of five subjects the AUCs, which were obtained with the depot tablets, are increased after administration in presence of an extracorporeal magnet. Though the AUC of Acyclovir in presence of extracorporeal magnet was not higher than immediate release tablets, the AUC was much lower in the absence of the magnet. This study established the potential of gastroretention to improve bioavailability of Acyclovir as compared to non-gastroretentive dosage forms.

Dhaliwal et al.<sup>36</sup> developed mucoadhesive microspheres using Chitosan, Thiolated Chitosan, Carbopol 71G and Methocel K15M as mucoadhesive polymers for gastroretentive delivery of Acyclovir. Microsphere formulations containing chitosan and Thiolated Chitosan were prepared by emulsion-chemical crosslinking method, whereas those containing Carbopol and Methocel were prepared by spray drying method. The mucoadhesion time of microspheres in pig intestine followed the rank order of Thiolated Chitosan ( $8.0 \pm 0.8$  h) > Chitosan ( $3.1 \pm 0.4$  h) > Carbopol 71G ( $1.1 \pm 0.2$ ) > Methocel K15M ( $0.2 \pm 0.1$  h). The highest mucoadhesion time observed with Thiolated Chitosan was attributed to formation of strong covalent bonds (disulfide bonds) with mucin due to the presence of thiol groups. All microsphere formulations exhibited rapid swelling and sustained *in vitro* drug release as compared to plain drug. Microspheres containing Thiolated Chitosan had prolonged retention in upper gastrointestinal tract. In the GI distribution study using 6-Carboxyfluorescein loaded Thiolated Chitosan microspheres, 22.3±3.1% formulation was recovered from stomach after 2 h. 41.6±2.9% and 26±2.1%

formulation were recovered after 4 h and 10 h, respectively, from duodenum and jejunum portions of intestine. These were about 2-fold higher in comparison to Chitosan, Carbopol 71G and Methocel K15M microsphere formulations and was attributed to the better mucoadhesive properties of Thiolated Chitosan at pH 5–6, the pH of duodenum and jejunum regions of intestine. All microsphere formulations were able to maintain higher plasma concentrations than drug solution, with exceptionally high levels from Thiolated Chitosan microspheres. It exhibited nearly four times higher AUC<sub>0–24</sub> value of Acyclovir ( $1091 \pm 51$  ng·h/ml) as compared to drug solution ( $282 \pm 28$  ng·h/ml). It also maintained the plasma concentration up to 24 hrs, but the drug solution could maintain only for 5 hrs.

Liu et al.<sup>22</sup> evaluated the *in vivo* bioavailability of Acyclovir from mucoadhesive microspheres in comparison with conventional tablets in beagle dogs. The mucoadhesive microspheres had sustained *in vitro* drug release up to 12 hrs. The same was reflected as increased  $t_{max}$  *in vivo* from 2.33 hrs (conventional tablets) to 5 hrs (mucoadhesive microspheres). Most of the microspheres were retained in stomach even after 6 hrs of oral administration to rats and beagle dogs. Though the  $C_{max}$  of microspheres did not differ significantly from conventional tablets, relative bioavailability was found to be 145%. Tao et al.<sup>37</sup> prepared mucoadhesive microspheres of Acyclovir using ethyl cellulose as matrix former and Carbopol 974P as mucoadhesive polymer. Emulsion solvent evaporation method was used for preparation of microspheres. *In vitro* drug release of the drug from microspheres was faster when the concentration of Carbopol was increased. This was attributed to the formation of hydrophilic channels on the water-insoluble Ethyl cellulose leading to increased diffusion of drug. The increase in Carbopol concentration also altered the release mechanism from fickian to anomalous (non-fickian) mechanism by accelerating erosion of the swelling matrix resulting in a combination of diffusion and erosion mechanism of drug release. On the



other hand, increase in Carbopol concentration increased the mucoadhesion and gastric retention due to its numerous carboxyl groups which facilitate the formation of hydrogen bonds with mucus. Oral administration to rats demonstrated significant advantages of mucoadhesive microspheres (Carbopol to Ethylcellulose ratio of 1:3) over suspension. Microspheres had more sustained plasma concentrations than suspension but the peak plasma concentration was slightly lower (627.2 ng/ml for microspheres vs 750.5 ng/ml for suspension). The  $AUC_{0-t}$  (6055.9 ng.h/ml) and mean residence time (MRT) (7.2 hrs) for microspheres were significantly higher than that of suspension (2335.6 ng.h/ml and 3.7 hrs, respectively) ( $p < 0.05$ ).

#### 4.8 Absorption enhancers

Shah et al.<sup>38</sup> investigated effect of absorption enhancers, Dimethyl  $\beta$ Cyclodextrin (DM $\beta$ CD), Chitosan Hydrochloride (CH) and Sodium Lauryl Sulfate (SLS) on transepithelial permeation of Acyclovir across Caco-2 and Madin-Darby canine kidney (MDCK) cell monolayers. Permeation of Acyclovir was studied for the drug alone, drug with different concentrations of individual absorption enhancers and combination of absorption enhancers.  $P_{app}$  values of Acyclovir in the absence of absorption enhancers were  $0.352 \pm 0.07 \times 10^{-6}$  cm/sec and  $0.523 \pm 0.011 \times 10^{-6}$  cm/sec in Caco-2 and MDCK cell monolayers, respectively. These values increased significantly in presence of 1%, 3% and 5% DM $\beta$ CD, 0.1%, 0.3% and 0.5% CH and 0.009%, 0.012% and 0.015% SLS ( $p < 0.05$ ). Similarly, increase in  $P_{app}$  in presence of combination of absorption enhancers, 5% w/v DM $\beta$ CD+0.5% w/v CH, 0.5% w/v CH+0.015% w/v SLS and 5% w/v DM $\beta$ CD+0.015% w/v SLS were also significant ( $p < 0.05$ ).  $P_{app}$  values of Acyclovir in the presence of combination of absorption enhancers were higher than that in the presence of individual agents indicating synergistic effect of combinations. Highest enhancement of  $P_{app}$  was observed with the combination of 5% w/v DM $\beta$ CD+0.5% w/v CH. This was attributed to the extraction of

phospholipids from the biomembrane by DM $\beta$ CD, once the tight junctions are opened upon interaction of CH.

Merzlikineet al.<sup>23</sup> investigated the impact of the ability of Chitosan Glutamate and Carbomer 974P to modulate the tight junctions in the intestinal wall on the bioavailability of Acyclovir. The authors evaluated the influence of Chitosan Glutamate, Carbomer 974P, alone and in combination with EDTA-Na<sub>2</sub>, on the *in vitro* Caco-2 permeability and oral rat pharmacokinetic profile of Acyclovir. The presence of chitosan glutamate (1%) increased the apical to basolateral permeability ( $P_{app,A \rightarrow B}$ ) of Acyclovir from  $2.84 \pm 0.8 \times 10^{-6}$  cm/sec to  $11.6 \pm 0.37 \times 10^{-6}$  cm/sec, a significant 4.1-fold increase ( $p < 0.05$ ). The increases in  $P_{app,A \rightarrow B}$  observed in presence of Chitosan Glutamate (1%)/EDTA-Na<sub>2</sub> (0.01%) and Chitosan Glutamate (3%) were 4.6- and 3.4-folds, respectively, relative to control ( $p < 0.05$ ). Carbomer 974P (1%) and Carbomer 974P (1%)/EDTA-Na<sub>2</sub> (0.01%) increased  $P_{app,A \rightarrow B}$  by 1.2- and 1.5-folds, respectively, relative to control, but the results were not statistically significant ( $p > 0.05$ ). In rats, Chitosan Glutamate (1–3%) and Chitosan Glutamate (1%)/EDTA-Na<sub>2</sub> (0.01%) formulations led to 1.7- to 2-fold increase in AUC and absolute bioavailability of Acyclovir and 1.5- to 3.1-fold increase in the renal recovery of Acyclovir compared to control ( $p < 0.05$ ). These results also supported the findings of Shah et al.<sup>38</sup> that modulation of tight junctions can be a viable approach to overcome the limited oral bioavailability of Acyclovir.

#### 4.9 Sustained release formulations

Sustained release formulations of Acyclovir have been studied for two reasons. First reason is to improve patient compliance by reducing the dosing frequency of the drug. The second reason is to improve the bioavailability based on the reports that the absorption of Acyclovir is a carrier mediated saturable process and slow release of drug would replenish the carriers. Yang and Hu<sup>39</sup> studied the single dose pharmacokinetics of sustained release and immediate release tablets of Acyclovir in dogs. Sustained release tablets resulted in much

prolonged absorption. Sustained release tablets exhibited later  $t_{max}$ , lower  $C_{max}$  and higher MRT and AUC as compared to immediate release tablets. The relative bioavailability of Acyclovir sustained release tablet was 152% with reference to immediate release tablets. The pharmacokinetic differences between sustained and immediate release tablets were statistically significant ( $p < 0.01$  for AUC and  $p < 0.05$  for other parameters). Though this study provided encouraging results, use of dogs as animal model for this study has to be reviewed. Absorption of Acyclovir in dogs is very high; as much as 75% of administered dose was recovered in urine<sup>16, 40</sup>. Further studies in human will be required on this subject. Interestingly an earlier study performed on human volunteers compared 200 mg sustained release tablets and 100 mg conventional tablets did not result in significant improvement in bioavailability<sup>41</sup>. The bioavailability of sustained release tablets relative to conventional tablets following single and multiple dosing was  $105.9 \pm 12.0\%$  and  $95.2 \pm 8.4\%$ , respectively. These results were not significantly different when analyzed using ANOVA and two sided t-test procedures.

## 5. CONCLUSION

Acyclovir is an effective antiviral compound active against a broad range of viruses Herpes Simplex Virus (HSV), Varicella-Zoster Virus (VZV), Epstein-Barr virus (EBV), and Cytomegalovirus (CMV). Conventional therapy of Acyclovir including tablets, creams etc. is associated with higher relapse rates because the viruses are generally considered to be refractory. So development of Acyclovir formulations is a challenge which can provide drug release in controlled and a sustained manner to completely treat the infections caused by dangerous viruses. The formulation and biopharmaceutics based approaches mentioned in the present article seem to have conquered the drawbacks of conventional systems of drug delivery and provide an opportunity for optimizing the delivery of Acyclovir in a desired manner to cure the infections and completely eradicate them from the body because of enhanced bioavailability along with better patient compliance.

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