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In-vitro Absorption Studies of Mucoadhesive Tablets of Acyclovir

Remeth Dias[1]*, Sfurti Sakhare[2] and Kailas Mali[1]

Satara College of Pharmacy, Satara[1], 415004*

Gourishankar Institute of Pharmaceutical Education & Research, Satara[2]

Author for Correspondence: rjdias75@rediffmail.com

Abstract

In this work, the dissolution-absorption studies were conducted on marketed tablets of acyclovir (ACIVIR-200mg) and the mucoadhesive tablets of acyclovir using varying concentration of sodium lauryl sulfate as a permeation enhancer. The purpose of this study was to improve the absorption of acyclovir using sodium lauryl sulfate as permeation enhancer. The everted and perfused intestine model was used to study the permeation of mucoadhesive tablets of acyclovir. The studies yielded dissolution-absorption relationship that can be used to predict dissolution or permeation-rate-limited absorption for formulated and marketed formulations. The results showed that marketed tablets of acyclovir had less permeability coefficient (0.778×10^{-9} cm/sec) as compared to mucoadhesive tablets with varying concentrations of sodium lauryl sulfate. The permeability increased with increasing concentration of sodium lauryl sulfate and permeability coefficient for mucoadhesive tablets with 4% sodium lauryl sulfate was found to be 5.231×10^{-9} cm/sec.

Amongst the varying concentrations of sodium lauryl sulfate used, 4% of sodium lauryl sulfate in the dissolution medium of mucoadhesive tablet of acyclovir showed highest increase in permeation of acyclovir therapy increasing the bioavailability of acyclovir.

Keywords: *Acyclovir, bioavailability, sodium lauryl sulfate, mucoadhesion, permeability.*

INTRODUCTION

Oral route of drug administration is the most convenient and preferred route by patients but the main aim in the process of drug development is to obtain a drug product with a good oral bioavailability. The problem of bioavailability is dependent upon the formulation, the physiological variables and the physicochemical characteristics of the drug itself. In reality; drug absorption is a complex process dependent upon drug properties such as solubility and permeability, formulation factors, and physiological variables including regional permeability differences, pH, luminal and mucosal enzymology, and intestinal motility[1]. The rate limiting barriers for the absorption of orally administered drugs are aqueous solubility and intestinal permeability[2]. Thus, intestinal permeability becomes one of the essential parts in predicting oral bioavailability of new drug candidates. Various in vitro methods for assessing the intestinal permeability of a given drug have been developed and reviewed recently[3]. Different in vitro methods are available such as use of excised tissues, epithelial cell culture models and artificial membranes[2].

In the present work, chicken's small intestine was used for intestinal absorption studies of mucoadhesive tablets of acyclovir, based on the assumption that membrane permeability of drugs is not species-dependent since the composition of plasma membrane of intestinal epithelial cells is similar across the species[3].

Acyclovir [9-(2-hydroxyethoxylmethyl) guanine], a synthetic purine nucleoside analog derived from guanine, is the most widely used antiviral agent. It is effective in the treatment of herpes simplex virus (HSV) mainly HSV-1 and HSV-2 and varicella zoster virus. The pharmacokinetic parameters of acyclovir following oral administration generally are highly variable. It has a plasma half-life of about 3 hours on average in adults with normal renal function[4, 5]. According to the

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Biopharmaceutical Classification System, acyclovir is categorized as class- III drug i.e. having high solubility and less permeability[6]. Its absorption in the GIT is slow, variable and incomplete. The bioavailability of acyclovir after oral administration ranges from 10-30%. Approximately 80% of an oral dose is never absorbed and excreted through feces. Also the frequency of administration of acyclovir is high, that is 200 mg 5 times a day and 400 mg 5 times a day depending upon the type of infection[4,5].

One of the approaches to improve the permeability of poorly absorbed drugs from the gastrointestinal tract is co-administration of absorption enhancers, including surfactants, bile salts, calcium chelating agents, fatty acids, cyclodextrins, chitosan and other mucoadhesive polymers[7]. These substances promote the permeability of poorly permeable drugs mainly by opening the tight junctions between the intestinal epithelial cells or by inhibiting the efflux transporters located at the apical membranes of the enterocytes [7, 8]. The modulation of efflux systems is of particular interest, since many pharmaceutically acceptable excipient can inhibit secretory transporters, including P-glycoprotein and several multi-drug resistance associated proteins at very low concentration[8].

The aim of the present work was to study the enhancement of permeability of mucoadhesive tablets of acyclovir by using sodium lauryl sulfate (SLS) as permeation enhancer and compare it with the marketed tablets of acyclovir.

MATERIALS AND METHODS

Materials:

Acyclovir was provided exgratis by Biochem Laboratories Ltd, Mumbai, India. Carbopol-934P was a gift from Ind- Swift Laboratories, Chandigarh; HPMC K100M was gifted by Flamingo Pharmaceuticals, Mumbai, India. All other chemicals used were of analytical grade. The instruments used are single punch compression machine (Cadmach, Ahmedabad), six stage tablet dissolution apparatus (Electrolab, Mumbai, Model TDT-06P), UV spectrophotometer (Schimadzu, Japan, Model 1700).

Methods:

Preparation of mucoadhesive tablets:

Table 1 enlists the composition of mucoadhesive formulation prepared using polymers Carbopol-934P and HPMC K100M. Dibasic calcium phosphate was added as pore forming agent along with directly compressible dried lactose as the diluent, with fixed quantity of talc as lubricant. Drug and the excipient were homogeneously blended and subsequently compressed into flat-faced tablets (450 mg, 10 mm diameter) using single punch tablet compression machine.

Isolation of everted intestine:

Male white Leghorn chicks weighing between 500 and 600 g were bought from the local market. The Krebs–Ringer solution was prepared by combining 6.3 g NaCl, 0.35 g KCl, 0.14 g $CaCl_2$, 0.16 g KH_2PO_4, 0.15 g $MgSO_4 \cdot 7 H_2O$, 2.1 g $NaHCO_3$, and 5 g glucose in one liter of distilled water. For isolation of everted intestine, the chicks were slaughtered, a median incision of the abdomen was performed, and the small intestine was freed. The lumen was carefully cleared from mucus by rinsing with a pH 7.4 buffer solutions (Krebs–Ringer solution). An intestinal segment of the first 6- cm length was removed and transferred to oxygenated Krebs–Ringer solution. It was washed thoroughly with warm Krebs–Ringer solution. The proximal extremity of the intestine was turned back and ligated on a glass rod to form an everted bag.

Procedure for absorption studies in the continuous dissolution–absorption aystem:

The dissolution–absorption studies were performed using Continuous Dissolution Absorption System[3] as shown in Fig 1, in two parts.

Part 1: A marketed tablet of acyclovir was used. The dissolution medium consisted of 900 mL phosphate buffer pH 5.0 maintained at 37 ± 0.5 °C. A fresh intestinal segment was clamped to the perfusion apparatus. The total volume of the absorption compartment (tube A and tube B of perfusion apparatus) was 30 mL of Krebs–Ringer solution. The drug diffused from dissolution medium (mucosal side) to the serosal side (absorption compartment). The marketed tablet was transferred to the dissolution basket of the designed system. The tablet was rotated at 50-rpm speed. Dissolution samples (2 mL) were withdrawn at preselected time intervals up to 3h. The dissolution samples were taken with replacement at 10, 20, 30, 40, 50, 60, 90, 120, 150 and 180 minutes, and the released

acyclovir was determined spectrophotometrically at 251 nm. The transported drug from the absorption compartment was sampled with replacement (Krebs–Ringer solution) at 13, 23, 33, 43, 53, 63, 93,123, and 183 minutes and analyzed spectrophotometrically for transported acyclovir at 254 nm. To allow time for drug to circulate from the dissolution vessel to the everted intestine surface, absorption samples of formulated tablet were collected 3 min later than their corresponding dissolution samples. The whole experiment was repeated in triplicate (n=3) using fresh dissolution medium as well as fresh intestinal segment each time.

Part 2: The same procedure was repeated for mucoadhesive tablets except that, sodium lauryl sulphate was added at three varying concentrations of 2%, 3% and 4% in the dissolution medium and the studies were repeated in triplicate for each concentration of sodium lauryl sulphate using fresh dissolution medium as well as fresh intestinal segment each time.

Data analysis:

Three experiments, each in triplicate were performed for all the tablets tested. The apparent permeability coefficient (Papp) of the tablets was calculated from the linear portion of a plot of the total amount of drug transported versus time, and determining the slope of the straight line.

The Papp of the drug across the intestinal mucosa, expressed as Papp (cm/sec) was calculated according to the equation:

$$Papp(cm/sec) = (dQ/dt) \times 1/(60 \times A \times C_o) \quad \text{(Eq.1)}$$

Where, dQ/dt is the steady state appearance rate (mg/min) namely the amount of a compound traversing the tissue in time t (min).

A is the exposed area of the tissue.

C_o is the initial concentration of the drug in the donor compartment.

The enhancement ratio was calculated according to the equation:

$$\text{Enhancement ratio (ER)} = \frac{\text{Permeability coefficient of drug with enhancer}}{\text{Permeability coefficient of drug alone}} \quad \text{(Eq.2)}$$

The statistical comparisons were made by utilizing analysis of variance (ANOVA) followed by the Dunnett's multiple comparison test.

RESULTS:

Evaluation of the Acyclovir marketed tablet and mucoadhesive tablet with varying concentrations of sodium lauryl sulfate:

The permeability coefficient values of acyclovir marketed tablets (ACIVIR-200mg) across the excised intestinal segment and the mucoadhesive tablets with varying concentrations of sodium lauryl sulfate are shown in table 2. Fig.2 shows the percent of drug released during the permeability study. For the permeation enhancement studies, different concentrations of sodium lauryl sulfate were used i.e. 2% to 4% in the dissolution medium of mucoadhesive tablets. It was found that permeability coefficient Papp increased with increasing SLS concentration from 2% to 4%. The permeability coefficient of marketed tablet was very less (0.778×10^{-9} cm/sec) as compared to mucoadhesive tablet with 4% SLS (5.231×10^{-9}cm/sec).

DISCUSSION:

According to the Biopharmaceutical Classification System, acyclovir is categorized as class- III drug i.e. having high solubility and less permeability[6]. Its absorption in the GIT is slow, variable and incomplete. The bioavailability of acyclovir after oral administration ranges from 10-30%. Approximately 80% of an oral dose is never absorbed and excreted through feces. Also the frequency of administration of acyclovir is high, that is 200 mg 5 times a day and 400 mg 5 times a day depending upon the type of infection[4,5].

Mucoadhesive drug delivery system of acyclovir was formulated so as to ensure sustained release of acyclovir for ten consecutive hours in stomach. As acyclovir is absorbed through absorption window, it was postulated that the slow but complete release will ensure increased absorption of acyclovir but the absorption found was very low, due to its low permeability.

Surfactants are often included in the oral solid dosage forms in order to improve their wetting or dissolution properties. But it is important to know whether these surfactants at concentrations that are achieved in the intestinal lumen enhance the permeability of the active ingredient.

According to the literature, acyclovir absorption occurs predominantly by passive diffusion[8]. In an attempt to increase oral absorption of acyclovir, use of permeation enhancer was made in this study. In this work, chicken intestinal segment was used as a model for permeability studies. Three different concentrations of sodium lauryl sulfate were used. Among the tested concentrations, sodium lauryl sulfate at 4% concentration i.e. 0.04 mg/ml significantly increased permeability of acyclovir. The increased permeability was found due to the ability of permeation enhancer (sodium lauryl sulfate) to promote permeability in the absorptive direction by opening the tight junctions and/or by inhibiting the active efflux system [8, 10]. Thus, by use of sodium lauryl sulfate the problem of less permeability can be solved which may lead to increased absorption of acyclovir which in turn may increase its bioavailability.

From the permeability coefficients, it was found that the mucoadhesive tablet showed negligible absorption due to less amount of drug released, from which negligible amount was absorbed. The permeability of marketed tablet was high as compared to mucoadhesive tablet as the entire drug was released and hence a large amount was available for absorption. The permeability of mucoadhesive tablets with varying concentrations of sodium lauryl sulfate in their dissolution medium was found to be in increasing order with increase in concentration of sodium lauryl sulfate.

The enhancement ratio shows that as compared to marketed tablet the permeability of mucoadhesive tablets with varying concentrations of sodium lauryl sulfate i.e. 2%, 3% and 4% was increased to near about 5 times, 6 times and 7 times respectively. When the permeability of marketed and formulated tablets with varying concentrations of sodium lauryl sulfate was analysed using one way ANOVA followed by the Dunnett's multiple comparison test, the increase in permeation due to SLS was found to be statistically significant ($P<0.01$).

CONCLUSION:

The absorption of acyclovir in humans is lower than 90%, which classifies acyclovir among the low permeability drugs according to Biopharmaceutical Classification System. This is in agreement with the permeability measurement in this study, since the Papp value of mucoadhesive tablet and marketed tablet of acyclovir is much lower than Papp of mucoadhesive tablets using different concentrations of permeation enhancer sodium lauryl sulfate in the dissolution medium.

The above study suggests that the problem of less bioavailability of acyclovir cannot be solved by merely formulating mucoadhesive tablets, but use of permeation enhancer can lead to increased permeation through the gastrointestinal lumen and hence might increase its bioavailability.

In the nutshell, the following conclusions can be drawn from this study:

1. Merely formulating mucoadhesive tablets cannot increase absorption of acyclovir; using permeation enhancers can increase its permeability, which in turn can increase its absorption.
2. Comparison between the marketed tablet and mucoadhesive tablet showed that permeability of marketed tablet was more as compared to mucoadhesive tablet, but by using varying concentrations of sodium lauryl sulfate, the permeability of mucoadhesive tablet can be increased which was more than that of the marketed tablet. Hence, sodium lauryl sulfate (highest at 4% concentration) increased the permeability of mucoadhesive tablet.

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Table No.1 Composition of Acyclovir Mucoadhesive tablet

Sr. No.	Ingredients	Amount (mg)
1	Acyclovir	200
2	Carbopol-934P	55
3	HPMC K100M	45
4	Dibasic calcium phosphate (DCP) (5%)	22.5
5	Talc (1%)	4.5
6	Directly compressible lactose (DCL) qs to	450

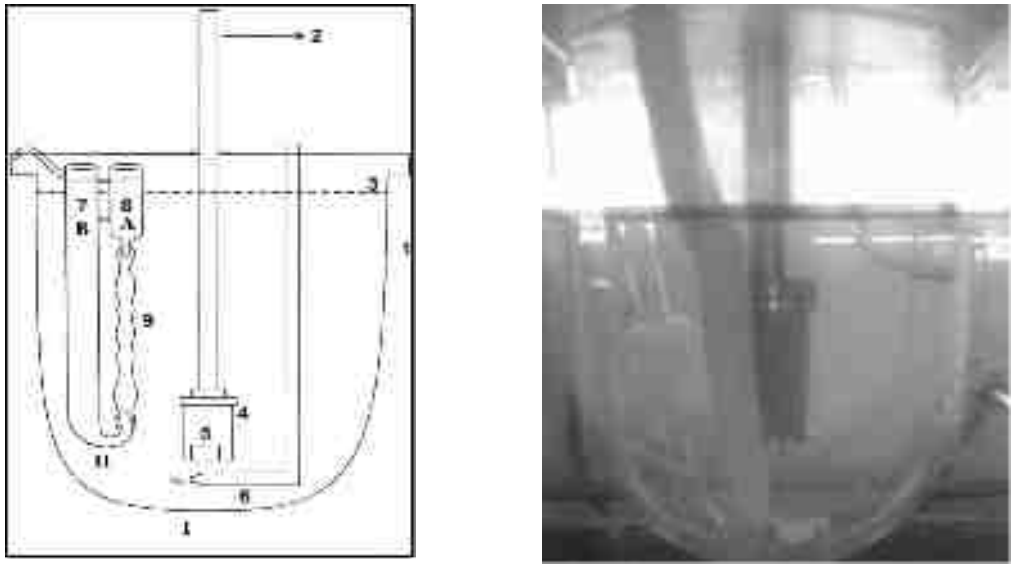
**qs indicate quantity sufficient.*

Table No. 2 Permeability coefficient values of tested tablets:

Sr. No.	Formulation	Permeability Coefficient P app (cm/sec)	Enhancement ratio
1.	Marketed Tablet (ACIVIR- 200 mg)	0.778×10^{-9}	-
2.	Mucoadhesive Tablet with 2% SLS	$*4.104\times10^{-9}$	5.275
3.	Mucoadhesive Tablet with 3% SLS	$*4.662\times10^{-9}$	5.992
4.	Mucoadhesive Tablet with 4% SLS	$*5.231\times10^{-9}$	6.723

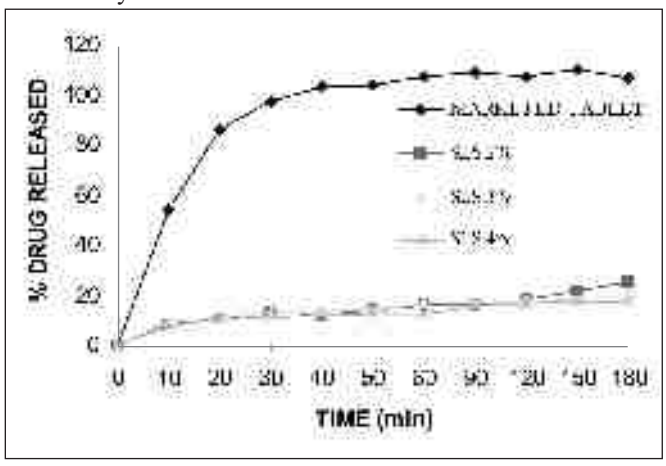
SLS-Sodium Lauryl Sulphate, ** significantly different from control formulation (P<0.01).*

Fig. 1 Continuous Dissolution–Absorption System using isolated everted intestine system.



(Labels:1.Dissolution flask, 2.Rotating shaft, 3. Dissolution medium, 4.Basket, 5. Tablet, 6.Oxygen tube, 7. Tube B, 8.Tube A, 9. Everted intestine, I. Dissolution –absorption system, II. Absorption (perfusion) apparatus.)

Fig. 2 Percent of drug released vs. time relationship for marketed and mucoadhesive tablets of acyclovir with varying concentrations of sodium lauryl sulfate.



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