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Enhancement of Nasal Absorption of Acyclovir via Cyclodextrins

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Abstract

The objective of this work was to increase the nasal absorption of acyclovir by using cyclodextrins as absorption enhancers. Acyclovir was selected as a model drug. A rat in situ nasal perfusion technique was utilized in the investigation to examine the rate and extent of absorption of acyclovir. The remaining analyte concentrations in the nasal perfusate were quantitated by reversed phase high performance liquid chromatography. In vitro enzymatic drug degradation study was carried out with rat nasal washings. Various experimental conditions were optimized such as nasal perfusion rate, pH of the perfusion medium, and concentrations of cyclodextrins such as α, β, γ, methyl-β, hydroxypropyl-β-cyclodextrins. Nasal absorption of acyclovir was pH dependent. Initial absorption rate constants were determined by the plot of log % remaining amount of drug in perfusate vs time. It was maximum at pH 7.4 and decreases at lower and higher pH conditions. The uptake of analyte by flow circuit component was assessed prior to the animal study to minimize system artifacts. In in-vitro enzymatic degradation study, no measurable degradation was observed during first week. The extent of drug absorption was increased via absorption enhancers. From the above absorption enhancers, hydroxypropyl-β-cyclodextrin appeared to be more effective for enhancing the nasal absorption of acyclovir than the other absorption enhancers. The order of increasing absorption of acyclovir caused by the enhancers was hydroxypropyl-β-cyclodextrin (5%) > methyl-β-cyclodextrin (5%) > α-cyclodextrin (1.3%) > γ-cyclodextrin (1.5%) > β-cyclodextrin (2%).

Introduction

Systemic drug delivery by the nasal route is currently receiving considerable attention because this route offers many advantages, such as a rapid absorption and onset of pharmacological effect, avoidance of liver first pass metabolism and high systemic availability and an easy administration route particularly suitable for self- medication [1]. Their are, however, limitations; for example, low permeability across the mucosa, degradation of drug by enzymes in the nasal cavity, and drug loss by rapid mucociliary clearance. To improve systemic bioavailability through nasal administration, two strategies are commonly employed; these are, structural modification and formulation manipulation [2].

Acyclovir (ACV), a cyclic analogue of the natural nucleoside 2′-deoxyguanosine, is clinically used in the treatment of herpes simplex, varcella zoster, cytomegalovirus, and Epstein- Barr virus infections [3]. Absorption of orally administered acyclovir is slow, variable and incomplete, with a bioavailability of ∼15–30% [4]. An in vitro study using porcine buccal tissue indicated that buccal transport of acyclovir occurs predominantly by a passive diffusion mechanism, probably through the paracellular route [5]. Therefore, this compound may serve as a good model drug to study nasal absorption enhancement via cyclodextrins.

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In this study, effect of cyclodextrins such as α, β, γ, methyl and hydroxypropyl β-cyclodextrin on nasal absorption of acyclovir was studied. A rat in situ nasal perfusion technique [6, 7] was utilized to examine the nasal uptake of the drug, its chemical stability and enzymatic hydrolysis were evaluated. Possible mechanisms involved in the nasal absorption of the drug are discussed.

Materials

High performance liquid chromatography (HPLC) grade acetonitrile and Na_2HPO_4 were obtained from s. d. Fine chemicals, India. α, β, γ, methyl and hydroxypropyl beta cyclodextrin (Cerestar) were obtained from S.A. Chemicals, India. Acyclovir was a gift sample from Cipla, India.

Methods

Preparation of nasal solutions

Absorption enhancers were dissolved into the phosphate buffer saline solution to obtain desired concentration. Drug was dissolved into the above solution (0.8 μg/ml) and pH of the solution was adjusted to 7.4. Osmolarity of the solution was adjusted to 295 mOsm by using sodium chloride solution.

Animal studies

The rat in situ nasal perfusion technique developed by Hirai *et al.* [6] and Huang *et al.* [7] was used. Male albino rats, weighing 250–350 gm, were anesthetized with an intraperitoneal injection of thiopental sodium (0.1 ml/100 gm body weight). After an incision was made in the neck, the trachea was cannulated with a polyethylene (PE-200) tube to maintain respiration. Another PE-200 tube was inserted through the oesophagus towards the posterior part of the nasal cavity to the mouth. The cannula served to deliver the solution to the nasal cavity. The perfusion medium, which was prepared with isotonic phosphate buffer saline solution, pH 7.4, was circulated by means of peristaltic pump at a flow rate of 2 ml/min. The perfusate was recollected into a reservoir, which was maintained at a temperature of 37 ± 0.5 °C through out the course of an experiment. A constant perfusate volume of 5 ml was maintained throughout with constant stirring and an aliquot (100 μL) was sampled at predetermined time interval.

Separate experiments were conducted to measure the loss of analyte from the solution due to adsorption and/or absorption to system components (tubing, pump or glasswares) or due to volatilization. Each perfusion solution was circulated for two hours through the system without the rat included. The reservoirs were sampled at 30 min intervals to measure disappearance of analyte with time.

In vitro enzymatic degradation study with rat nasal washing

Isotonic phosphate buffer saline solution, pH 7.4, was perfused through the rat nasal cavity for 2 h. The perfusate at the end of the experiment was collected and stored at −70 °C until further use. One volume of drug stock solution was mixed with nine volumes of prewarmed nasal washing solution (37 °C) and vortexed. A zero time sample (100 μL) was taken and mixed with 10 μL of perchloric acid and vortexed for 30 s to precipitate proteins. The mixture was incubated at 37 °C, and 100 μL samples were withdrawn at predetermined time intervals and subjected to the same treatment.

Analytical procedure

The concentration of acyclovir was quantitated by reversed-phase HPLC. Prior to analysis, all the samples were treated with perchloric acid (70% solution) to precipitate the proteins. After centrifugation at 10000 × g for 15 min. The supernatant was injected onto the HPLC column (Waters C_{18} spherisorb, 5 μm, 250 mm), the signal was monitored at 254 nm. Mobile phase prepared with acetonitrile and phosphate buffer (pH 2.5) (5 : 95 v/v). The flow rate was maintained at 1 ml/min.

Results and discussion

In control experiments performed without animals, drug loss by adsorption on to or absorption into the tubes of the perfusion system was found to be insignificant. However, this loss was not taken into account for data processing.

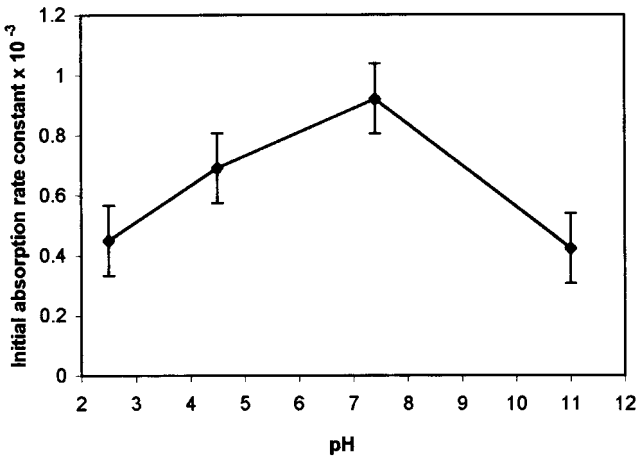

Figure 1. Effect of pH on nasal absorption of acyclovir in rats (mean ± SEM; n = 5).

Effect of pH on nasal absorption of ACV

Initial absorption rate constants were determined by the plot of log % remaining amount of drug in perfusate Vs time. It showed that absorption rate was pH dependent and reached maximum values at pH 7.4, decreases at lower and higher pH values as shown in Figure 1. It depends on the ionization state of the diffusion molecule, in agreement with the pH partition theory. Acyclovir, a guanine derivative with molecular weight of 225 and pKa values of 2.27 and 9.25. Octanol: (0.2 M) phosphate buffer partition coefficient, which is an index of lipophilicity, is 0.018 indicating the drug is hydrophilic in nature. This may be because of the structure containing ether, alcohol, phenolic and amino group. Since this drug is of low molecular weight and shows satisfactory solubility in water (1 in 400), it is expected that the drug will pass mainly by passive diffusion through aqueous pores i.e. tight junctions [8]. By the presence of acidic, phenolic-OH group and basic amino group in guanine, the molecule will be affected by environmental pH. The absorption of acyclovir was expected to continue in unionized form i.e., absorption could be via transcellular as well as paracellular route. Existence of aqueous pores or channels in nasal mucosa through which water soluble drugs permeates has been speculated by several authors [9–12]. In rats, used as a model here the estimated range of the pore size of nasal mucosa is 0.4–0.8 nm and the number of pores is four times than that of present in jejunum. The fact indicates that the nasal epithelium barrier is less tight than the intestinal barrier. It is thus clear that zwetterion (which is unionized), small in molecular size and weight and has significant water solubility will be better absorbed from the nasal mucosa as compared to gastrointestinal mucosa.

Input rate

For all the input rates, 1, 2 and 4ml/min, input rate seemed to have no effect on the ACV nasal absorption phase, which

was confirmed by the non-significant difference between percent remaining amounts of drug in perfusate after 2 h.

Enzymatic drug degradation studies and solution stability

In case of nasal absorption studies, it is important to study whether the drug degrades by the enzymes which are present in the rat nasal cavity. In enzymatic drug degradation studies, no measurable enzymatic drug degradation was occurred during first week. Drug was found to be stable to the enzymatic activity of the rat nasal mucosa.

Nasal solutions were kept for stability studies as per ICH guidelines for three months. The drug was found to be stable for three months. At 40 °C/75%R.H., the drug content was found to be 97.56% as shown in Table 1.

Effect of cyclodextrins on nasal absorption of ACV

Figures 2, 3 and 4 shows % remaining amount of drug in perfusate- time profile of ACV after in situ perfusion studies of ACV in the presence of various absorption enhancers. In case of pH 7.4 buffer, only 10% of the drug absorbed into the nasal cavity. In order to improve the nasal absorption of ACV, various absorption enhancers such as α, β, γ, methyl and hydroxypropyl β-cyclodextrins (HPBCD) were studied. In case of parent cyclodextrins, α cyclodextrins (1.3%) and to a lesser extent β (2%) and γ cyclodextrins (1.5%) are able to improve the nasal absorption of ACV. These results was in good agreement with the previous results shown by Hirai *et al*. α-cyclodextrin showed 24% nasal absorption of ACV whereas β and γ-cyclodextrin showed 15 and 20% nasal absorption of ACV respectively. To improve further nasal absorption of ACV, derivatives of cyclodextrins were studied. The cyclodextrin derivative, HPBCD, at a concentration of (5% w/v) strongly improved nasal ACV absorption in rats. HPBCD showed 38% of nasal absorption of ACV whereas methyl-β-cyclodextrin showed 30% of nasal absorption of ACV. In the present study, the effect of different concentrations of HPBCD on the nasal absorption of ACV was studied. The effect was concentration dependent. The order of increasing absorption of ACV caused by the enhancer was HPBCD (5%) > methyl β-cyclodextrin (5%) > α-cyclodextrin (1.3%) > γ-cyclodextrin (1.5%) > β-cyclodextrin (2%). Optimum concentrations of cyclodextrin were used for the present study. Above these concentrations, cyclodextrins were found to be caused nasal damage [13].

Our present data demonstrated that cyclodextrins especially HPBCD was effective in enhancing the nasal absorption of ACV. The mechanism whereby the nasal absorption of drugs was improved by these absorption enhancers is not still understood. Generally penetration enhancers acts via one of the following mechanisms: they increases membrane fluidity, inhibit enzyme activity, reduces mucus viscosity or elasticity, open up tight junctions or they solubilized the drug.

Cyclodextrins are biocompatible cyclic oligosaccharides. Cyclodextrin has shown to solubilise specific membrane lipids from human erythrocytes through the formation of inclusion complexes, leading to an increase in membrane

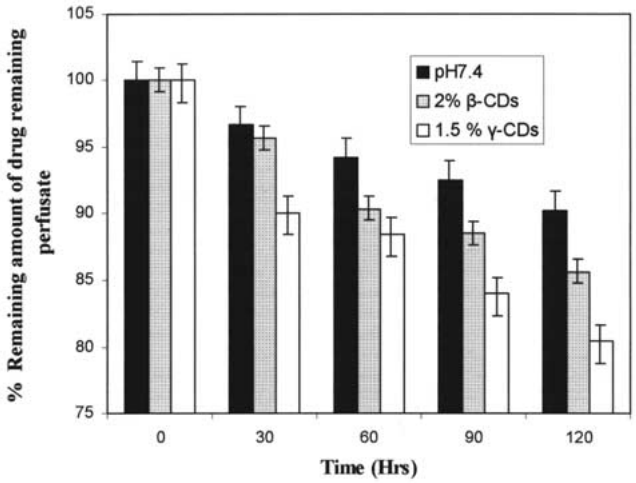

Figure 2. Effect of beta and gamma cyclodextrins on nasal absorption of acyclovir in rats (mean ± SEM; n = 5).

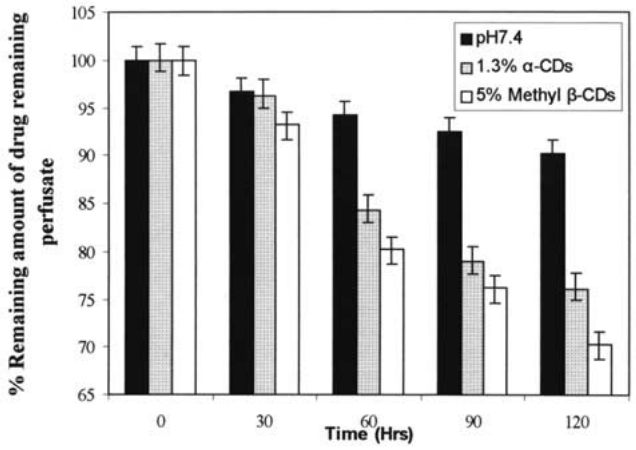

Figure 3. Effect of alpha and methyl beta cyclodextrins on nasal absorption of acyclovir in rats (mean ± SEM; n = 5).

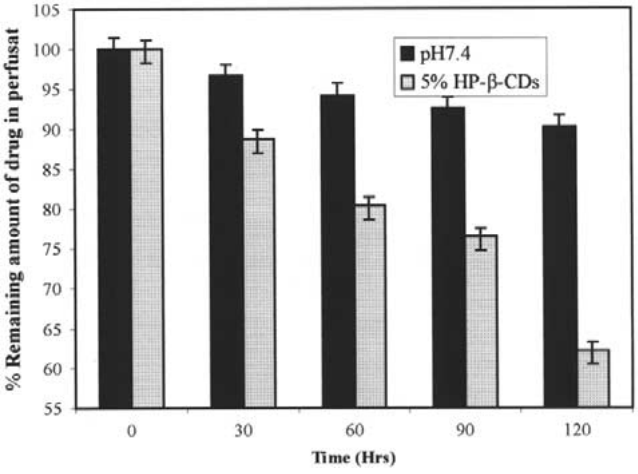

Figure 4. Effect of HPBCD on nasal absorption of acyclovir in rats (mean ± SEM; n = 5).

Table 1. Stability (mean ± S.D; n = 5) of acyclovir nasal solutions after three months storage

Compound	% Drug content Temperature conditions			Observations
Acyclovir	R.T 95.22 ± 1.254	30 °C/60%R.H. 97.89 ± 0.894	40 °/75%R.H. 97.56 ± 1.176	No measurable degradation was observed during three months

permeability. This occurs without the entry of the cyclodextrins into the membrane: they extract the lipids from the membrane into a new compartment located in the aqueous phase. Cyclodextrin may also affect nasal mucosal membrane in same manner [13]. Therefore it may be considered that cyclodextrin increased the nasal absorption of ACV by some of these mechanisms in the same manner as described above. Cyclodextrin derivative, HPBCD found to be potent absorption enhancers as compared to the other Cyclodextrin concentrations used in the present study. This might be because HPBCD extract more lipid from the nasal mucosa as compared to the parent cyclodextrins.

The integrity of the paracellular pathway is known to depend on extracellular Ca^{++} ions and thus, the integration of cyclodextrin with Ca^{++} resulted into increase in paracellular permeability of the complexes [13].

Conclusion

Nasal in situ perfusion technique can be used to study the nasal absorption of acyclovir. We can conclude that hydroxypropyl β-cyclodextrin (5%) appeared to be more effective for enhancing the nasal absorption of acyclovir than the other cyclodextrin concentrations (1.3–5%) used in the present study.

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