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Research paper

The influence of absorption enhancers on nasal absorption of acyclovir

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Abstract

The objective of this work was to increase the nasal absorption of acyclovir by using 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. In vitro enzymatic drug degradation study was carried out with rat nasal washings. Various experimental conditions such as nasal perfusion rate, pH of the perfusion medium and concentrations of absorption enhancers such as sodium deoxycholate, hydroxypropyl β-cyclodextrin, sodium caprate, sodium tauroglycocholate and EDTA were optimized. 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 found maximum at pH 7.4 and decreased at lower and higher pH conditions. In in vitro enzymatic degradation study, no measurable degradation was observed during first week. The extent of drug absorption was increased via absorption enhancers. In vivo studies were carried out for the optimized formulation in rabbits and the pharmacokinetics parameters of nasal solution were compared with oral solution. 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 > sodium deoxycholate > sodium caprate > sodium tauroglycocholate > EDTA.

Keywords: Acyclovir; Nasal solutions; Bile salts

1. Introduction

Systemic drug delivery by the nasal route is currently receiving considerable attention because it 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]. There 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 ACV 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 ACV 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 absorption enhancers.

In this study, effect of absorption enhancers such as sodium deoxycholate, hydroxypropyl β-cyclodextrin, sodium caprate, sodium tauroglycocholate and EDTA on nasal absorption of ACV was studied. A rat in situ nasal perfusion technique [6,7] was utilized to examine the nasal uptake of the drug and their chemical stability and enzymatic hydrolysis of drug.

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2. Materials and methods

2.1. Materials

High performance liquid chromatography (HPLC) grade acetonitrile and disodium hydrogen orthophosphate were obtained from s.d. Fine chemicals, India. Sodium deoxycholate, sodium caprate, sodium tauroglycocholate and EDTA were obtained from High Media Ltd, India. Hydroxypropyl β-cyclodextrin was obtained from S.A. Chemicals, India. ACV was a gift sample from Cipla, India.

2.2. 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 mg/ml) and pH of the solution was adjusted to 2.5, 4.5, 7.4 and 11.0. Osmolarity of the solution was adjusted by using sodium chloride solution.

2.3. 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 g, were anesthetized with an intraperitoneal injection of thiopental sodium (0.1 ml/100 g 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 esophagus 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 1, 2 and 4 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 absorption and/or adsorption to system components (tubing, pump or glassware) or due to volatilization. Each perfusion solution was circulated for 2 h through the system without the rat included. The reservoirs were sampled at 30 min intervals to measure disappearance of analyte with time.

2.4. 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 [8]. 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.

2.5. Analytical procedure

The concentration of ACV 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 $10\,000 \times g$ 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.

2.6. In vivo studies

Bioavailability studies were conducted in a group of six rabbits divided into two subgroups of three each. Each group was administered individually the nasal solution and an oral solution for comparative studies. The rabbits were weighed and the dose to be administered was calculated.

Three rabbits were given ACV solution (18.66 mg) by gauge using stainless steel bull tipped needle (13 gauge) attached to syringe. The needle was inserted into the back of mouth and to esophagus to administer oral dose. The other three rabbits were administered nasal solution containing calculated amount of dose was instilled in the nares of rabbits with the help of nasal spray.

Blood was collected from marginal vein at 0.5, 1, 2, 4, 6 and 8 h following administration of the drug in a heparinized centrifuge tubes. The samples were centrifuged immediately and plasma was stored at −20 °C till the time of analysis. The drug was extracted from plasma by a suitable extraction procedure. To 1 ml of plasma, 2 ml of acetonitrile was added in a stopper test tube. This was vortexed for 5 min. This was centrifuged at 5000 rev./min for 10 min. The organic phase was collected separately. It was evaporated under nitrogen gas and reconstituted with water. This was analysed using HPLC with UV detection. The mobile phase consisted of acetonitrile/phosphate buffer (pH 7.0) at a flow rate of 1.4 ml/min. The analysis was done at a wavelength of 254 nm. The parameters employed to evaluate were C_{max}, t_{max} and AUC values. AUC and other parameters such as K_{el} and $t_{1/2}$ were computed from observed plasma concentration against time profile.

3. 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.

3.1. Effect of pH on nasal absorption of ACV

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 Fig. 1. It depends on the ionization state of the diffusion molecule, in agreement with the pH partition theory. ACV, a guanine derivative with molecular weight of 225 and p*K*a values of 2.27 and 9.25. Octanol/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 [9]. By the presence of acidic, phenolic hydroxyl group and basic amino group in guanine, the molecule will be affected by environmental pH. The absorption of ACV was expected to continue in unionized form, i.e. absorption could be via transcellular as well as paracellular route. Existence of aqueous pores in nasal mucosa through which water soluble drugs permeates has been speculated by several authors [10,11]. 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 [8]. 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.

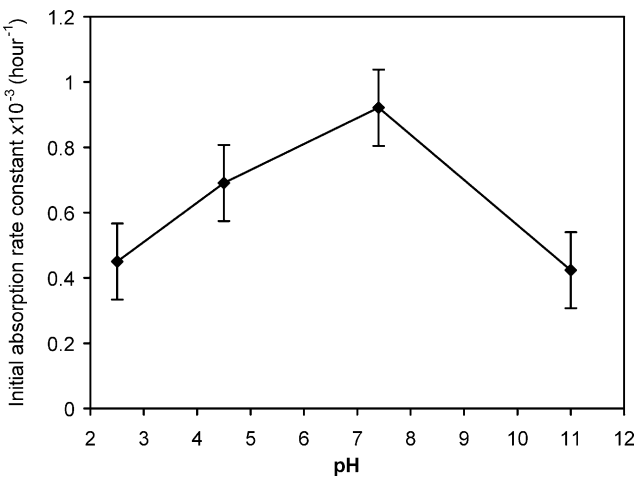

Fig. 1. Effect of pH on nasal absorption of acyclovir ($n = 6$).

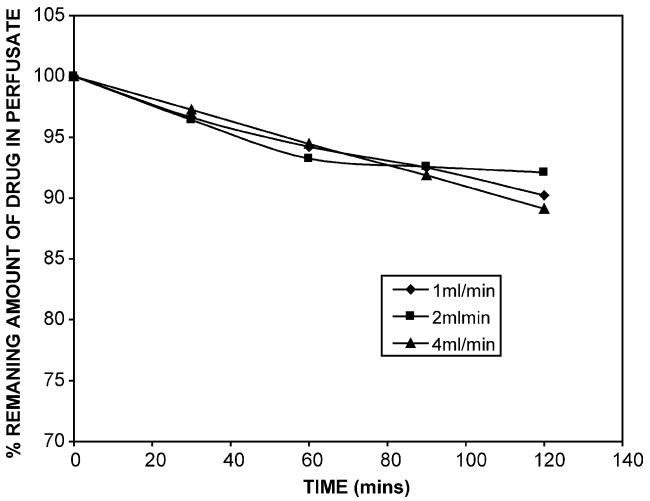

Fig. 2. Effect of input rate on nasal absorption of acyclovir ($n = 6$).

3.2. Input rate

Input rates of 1, 2 and 4 ml/min seemed to have no effect on the ACV nasal absorption phase as shown in Fig. 2, which was confirmed by the non-significant difference between percent remaining amounts of drug in perfusate after 2 h.

3.3. Enzymatic drug degradation studies and solution stability

Drug was found to be stable to the enzymatic activity of the rat nasal mucosa for 3 months.

Nasal solution (5% hydroxypropyl β-cyclodextrin) was kept for stability studies as per ICH guidelines for 3 months. The drug was found to be stable for 3 months. At 40 °C/75% RH, the drug content was found to be 97.56%.

3.4. Effect of absorption enhancers on nasal absorption of ACV

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 hydroxypropyl β-cyclodextrin, sodium deoxycholate, sodium caprate, sodium tauroglycocholate and EDTA were studied. In case of sodium caprate (1.3%) and to a lesser extent sodium tauroglycocholate (2%) are able to improve the nasal absorption of ACV. Sodium caprate showed 17.6% nasal absorption of ACV, whereas sodium tauroglycocholate showed 14.5% nasal absorption of ACV as shown in Fig. 3. To improve further nasal absorption of ACV, sodium deoxycholate and EDTA was tried. Sodium deoxycholate showed 37.65% of nasal absorption of ACV, whereas EDTA showed 12.04% of nasal absorption of ACV as shown in Fig. 4. The cyclodextrin derivative, HPBCD, at a concentration of (5%, w/v) strongly improved nasal ACV absorption in rats. HPBCD showed 38% of nasal absorption

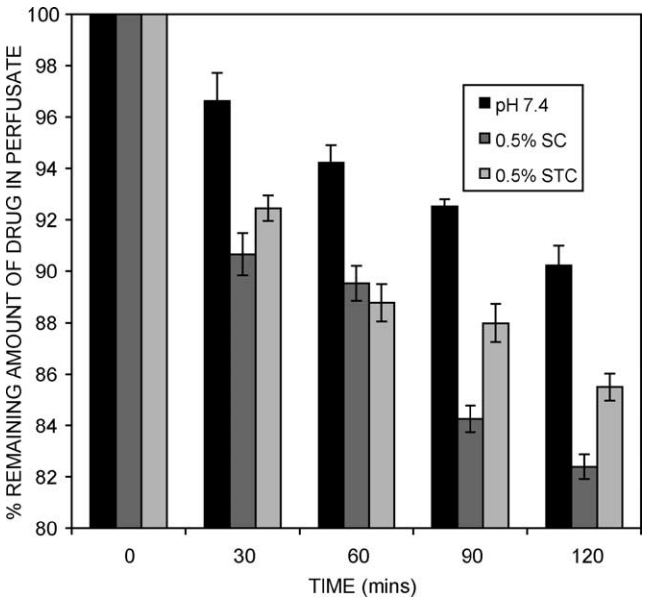

Fig. 3. Effect of sodium tauroglycocholate (STC) and sodium caprate (SC) on nasal absorption of acyclovir ($n = 6$).

of ACV as shown in Fig. 5. As the concentration of HPBCD and SDC increases, the nasal absorption of ACV also increases as shown in Figs. 6 and 7. The absorption enhancement was found to be concentration dependent. In the present study, the effect of different concentrations of absorption enhancers on the nasal absorption of ACV was studied. The effect was concentration dependent. The order of increasing absorption of ACV caused by the enhancers was HPBCD > sodium deoxycholate > sodium caprate > sodium tauroglycocholate > EDTA. Optimum concentrations of absorption enhancers were used for the present study. Above these concentrations, these absorption enhancers were found to be caused nasal damage.

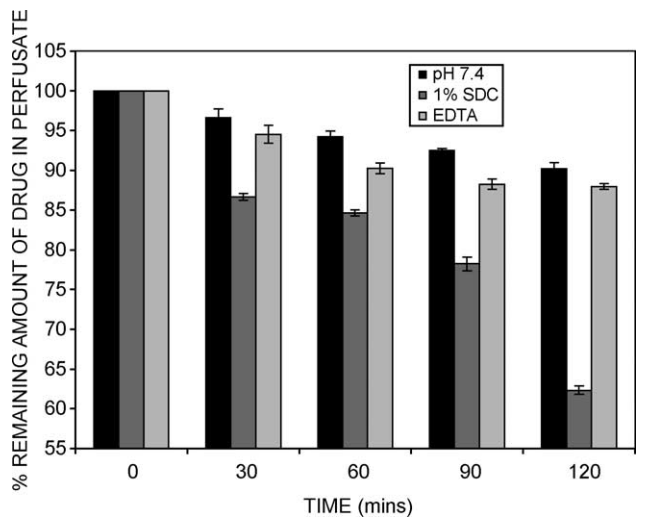

Fig. 4. Effect of EDTA and sodium deoxycholate (SDC) on nasal absorption of acyclovir ($n = 6$).

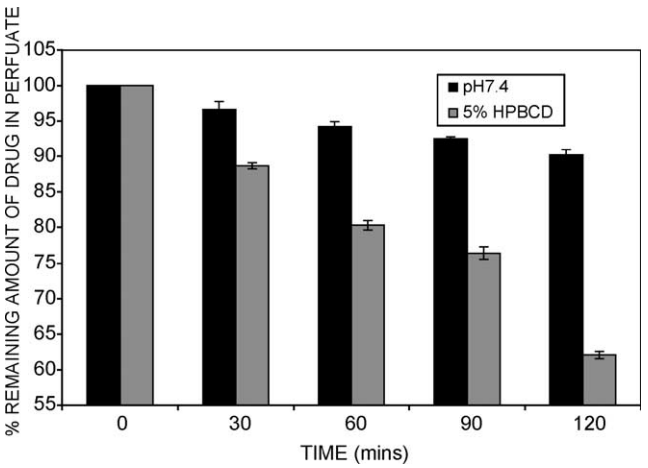

Fig. 5. Effect of HPBCD on nasal absorption of acyclovir ($n = 6$).

3.5. In vivo studies

In vivo studies were carried out for the 5% HPBCD formulations in rabbits. After administration of nasal solutions, the drug was observed to achieve plasma level rapidly. After 30 min, plasma concentrations of 3083.33 μg/ml were observed. However, in case of oral solution, a lag time of 1 h was seen with plasma concentration of 615 μg/ml achieved in 1 h. t_{max} values for nasal and oral solutions were 0.5 and 1 h, respectively, as shown in Fig. 8. K_{el} value for oral solution was 0.2396 h^{-1} and for nasal solution was 0.2328 h^{-1}. Plasma half-lives for nasal and oral solution was 2.975 and 2.891 h, respectively. AUC values for nasal and oral solution were 8713.75 and 1850.08 h μg/ml. A significant difference between the products was found for all the pharmacokinetics parameters. However, between the subjects the difference was non-significant indicating that there was less subject-to-subject variation.

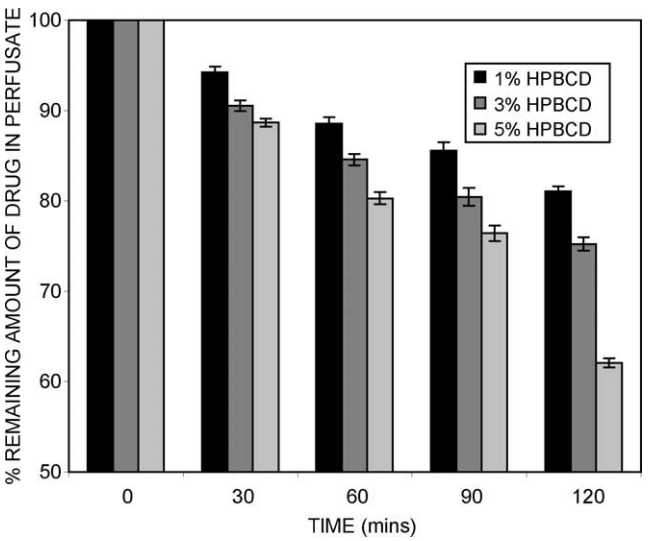

Fig. 6. Effect of various concentrations of HPBCD on nasal absorption of acyclovir ($n = 6$).

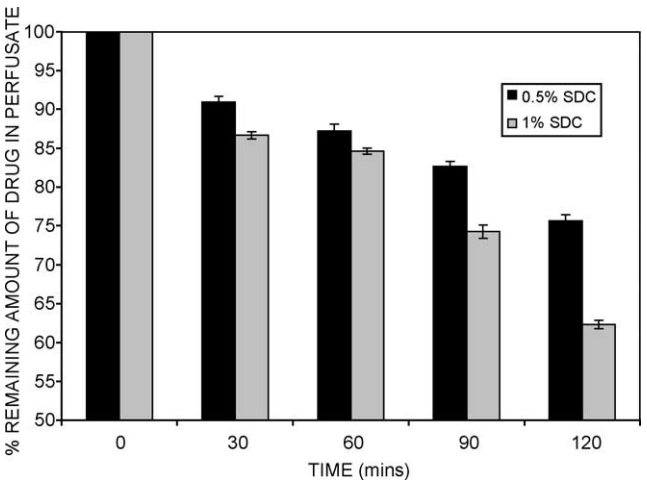

Fig. 7. Effect of various concentrations of SDC on nasal absorption of acyclovir ($n = 6$).

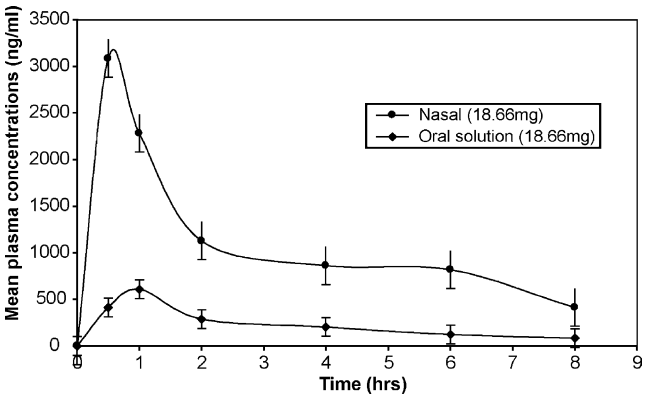

Fig. 8. Mean plasma concentration of ACV from the oral and nasal solutions ($n = 6$).

Our present data demonstrated that absorption enhancer especially HPBCD was effective in enhancing the nasal absorption of ACV. Generally cyclodextrin acts via one of the following mechanisms: they increase membrane fluidity, inhibit enzyme activity, reduces mucus viscosity or elasticity, open up tight junctions or they solubilized the drug [12]. It was reported that bile salts interact with cell membrane to form reverse miscell, which acts as a channel to increase permeation, by the test compound. It was also reported that bile salts enhance the permeation by removing epithelial cells, which constitute a major permeability barrier [13].

Na-caprate is reported to enhance the transcellular permeability by causing the membrane perturbation by interacting the protein region in the membrane and to enhance the paracellular permeability by some structural changes in the tight junction [14]. EDTA is known to be a chelating agent to form a chelating compound with the calcium ion existing at the tight junction in the membrane, resulting in increase permeability of the paracellular route [15].

4. Conclusion

Nasal in situ perfusion technique can be used to study the nasal absorption of ACV. We conclude that HPBCD appeared to be more effective for enhancing the nasal absorption of ACV than other absorption enhancers. In addition, sodium deoxycholate, sodium caprate, sodium taurocholate and EDTA are suitable adjuvants for improving the nasal absorption of ACV.

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