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Note

Effect of chitosan, benzalkonium chloride and ethylenediaminetetraacetic acid on permeation of acyclovir across isolated rabbit cornea

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Abstract

The overall objective of this study was to evaluate the effect of chitosan, benzalkonium chloride (BAK) and disodium ethylendiaminetetraacetic acid (EDTA), alone and in combination, on permeation of acyclovir (ACV) across excised rabbit cornea. Corneas of male New Zealand White rabbits were used in these studies. Transcorneal permeation studies were conducted at 34 °C using a side-bi-side diffusion apparatus. In the presence of 0.01% BAK, transcorneal permeability of ACV was observed to increase almost 10.5-fold, from 3.5×10^{-6} to 37.4×10^{-6} cm/s. At 0.005% BAK, permeability of ACV was almost 3-fold higher than control. Combination of BAK 0.005% and EDTA 0.01% increased transcorneal penetration of ACV by 2.5-fold. Chitosan 0.2 and 0.1% increased corneal permeability of ACV by 5.8- and 3.1-fold, respectively, whereas, at 0.02%, chitosan did not exhibit a statistically significant effect. BAK at 0.005%, in combination with 0.01% EDTA and 0.1% chitosan, increased transcorneal ACV permeation by 5.5-fold. This study suggests that a judicious combination of chitosan, BAK and EDTA can lead to a significant increase in ACV's transcorneal permeability and that chitosan can enhance diffusion of hydrophilic agents across the corneal membrane. Further in vivo evaluation is warranted.

Keywords: Chitosan; Cornea; Permeability; BAK; EDTA; Acyclovir; Hydrophilic

In recent years, several polymers have been identified that can safely and reversibly disrupt cellular tight junctions (Majumdar and Mitra, 2006). Among these, chitosan appears to be a very promising candidate. In vitro and in vivo studies demonstrate chitosan's ability to increase passive diffusion of compounds across biological membranes, probably through its effect on the tight junction proteins (Luessen et al., 1996; Schipper et al., 1997; Dodane et al., 1999; Senel and Hincal, 2001; Ranaldi et al., 2002; Sinswat and Tengamnuay, 2003; Sandri et al., 2004; Smith et al., 2004; Yu et al., 2004; Kerec et al., 2005; Kos et al., 2006). In the past, utility of absorption enhancers in ophthalmic formulations has been limited by their potential adverse effects (Di Colo et al., 2002; Kaur and Kanwar, 2002). In contrast, chitosan appears to be well tolerated by the corneal cells (Felt et al., 1999; de Campos et al., 2004; Enriquez de Salamanca et al., 2006), and also possesses wound healing and antimicrobial properties (Felt et al., 2000), making it a promising transcorneal absorption promoter. Additionally, chitosan may increase retention of topically instilled ophthalmic formulations. Another significant advantage of chitosan is that its action appears to be reversible (Dodane et al., 1999; Ranaldi et al., 2002; Smith et al., 2004).

Effect of chitosan on corneal tight junctions has lately started attracting attention. A recent report by Zambito et al. suggests that *N*-trimethyl chitosan (TMC) fails to disrupt the tight junctions of the corneal epithelium and increase transport of tobramycin (a paracellular marker) (Zambito et al., 2006). The results are intriguing since in all other epithelial tissues chitosan has been observed to disrupt cellular tight junctions and enhance paracellular transport.

In view of chitosan's potential in ocular drug delivery, a closer look at its effect on transcorneal permeability of hydrophilic therapeutic agents is necessary. The purpose of this study was to evaluate, in vitro, chitosan's effect on transcorneal diffusion of a small hydrophilic molecule, acyclovir (ACV), known to traverse the corneal epithelium by passive diffusion (Majumdar et al., 2003). Another objective of this project was

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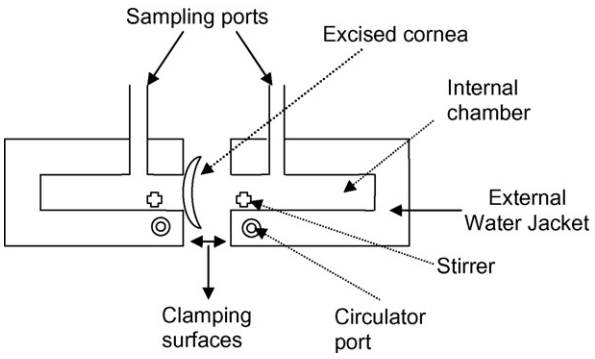

Fig. 1. Schematic representation of the diffusion apparatus.

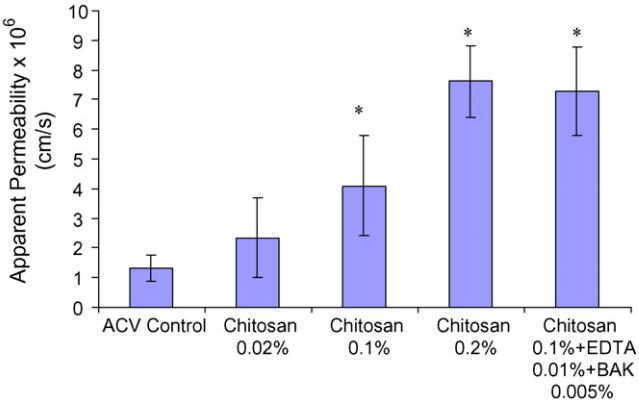

Fig. 2. Transcorneal permeability of acyclovir (ACV), alone (control) or in the presence of chitosan and EDTA. Values represent mean ± S.D. ($n = 4$); (*) indicates $p < 0.05$ (statistically significant difference from the control).

to delineate the effect of benzalkonium chloride (BAK) and ethylenediaminetetraacetic acid (EDTA), commonly included in ophthalmic formulations as preservatives but also known to act as penetration enhancers (Green and Tonjum, 1971; Green and Downs, 1974; Tonjum, 1975; Grass et al., 1985; Camber et al., 1987; Flach et al., 1989; Ashton et al., 1990; Citi, 1992), on the absorption promoting properties of chitosan. Literature reports indicate that BAK up to a concentration of 0.01% and chitosan up to a concentration of 1.5% is well tolerated by the corneal cells (Felt et al., 1999; Pawar and Majumdar, 2006; Rathore and Majumdar, 2006). The concentrations of BAK, EDTA and chitosan in this study were selected on the basis of these and other reports.

Acyclovir was purchased from Hawkins Inc. (Minneapolis, USA). Solvents were of analytical grade and were obtained from Fisher Scientific (St. Louis, MO, USA). Chitosan (catalog number 448869) and all other chemicals were procured from Sigma (St. Louis, MO, USA).

New Zealand albino rabbits (Myrtle's Rabbitry, Thompson Station, TN) were used for the determination of in vitro corneal permeability. Experiments using rabbits conformed to the tenets of the Association for Research in Vision and Ophthalmology statement on the Use of Animals in Ophthalmic and Vision Research. A side-bi-side diffusion apparatus (Fig. 1) from PermeGear Inc. (Bethlehem, PA) was used in this study and protocols as published previously were followed (Majumdar et al., 2003). Chitosan solutions were prepared in Dulbecco's phosphate buffered saline (DPBS) containing 2% acetic acid (pH 6.0 ± 0.2). Receiver chamber contained DPBS pH 7.4 in all cases. Samples were analyzed using a Waters HPLC-UV system at a wavelength of 254 nm (Majumdar et al., 2003).

Corneal membrane permeability was determined by normalizing the FLUX to the donor concentration, C_d, according to Eq. (1)

$$\text{Permeability } (P_{app}) = \frac{\text{FLUX}}{C_d} \quad (1)$$

where FLUX = (dM/dt)/A, M the cumulative amount of drug transported in time t and A is the corneal surface area exposed to the permeant.

All experiments were conducted in quadruplicate and results are expressed as mean ± standard deviation. Data obtained was subjected to statistical analysis using Student's t-test. A difference between mean values was considered to be statistically significant when the p-value was ≤0.05.

Transcorneal ACV permeability (Fig. 2) in the presence of 0.2 and 0.1% chitosan were almost 5.8-fold (7.61×10^{-6} cm/s) and 3.1-fold (4.1×10^{-6} cm/s) greater, respectively, compared to that of ACV alone (1.32×10^{-6} cm/s). However, chitosan, at a concentration of 0.02%, did not produce a statistically significant increase in ACV's corneal permeability. These results thus demonstrate that, contrary to the earlier report by Zambito et al., chitosan can increase corneal permeability of hydrophilic molecules. Considering that chitosan produces marginal effect on transcellular permeation and that ACV is a relatively hydrophilic molecule, the results suggest an interaction between chitosan and the corneal tight junctions.

BAK also enhanced corneal permeation of ACV. In the presence of 0.005 and 0.01% BAK apparent permeability of ACV increased from 3.56×10^{-6} to 10.4×10^{-6} and 37.4×10^{-6} cm/s, respectively (Fig. 3). On the other hand, the difference in transcorneal permeability of ACV in the presence of BAK 0.005% or BAK (0.005%) and EDTA (0.01%) was not statistically significant. Lack of any detectable effect of EDTA on tight junctions, in this study, as compared to some earlier reports (Pawar and Majumdar, 2006; Rathore and Majumdar,

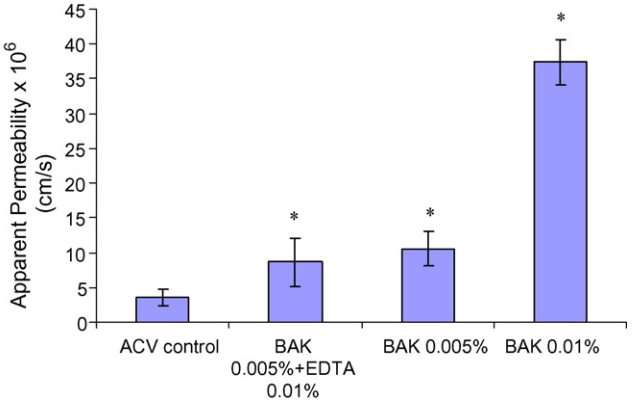

Fig. 3. Transcorneal permeability of acyclovir (ACV), alone (control) or in the presence of BAK and EDTA. Values represent mean ± S.D. ($n = 4$); (*) indicates $p < 0.05$ (statistically significant difference from the control).

2006) is probably due to the presence of calcium in the transport buffer limiting EDTA's chelating action on intracellular calcium. Also, any membrane fluidization property of EDTA would probably be overshadowed by the activity of BAK. However, our results are in agreement with those of Scholz et al. wherein EDTA was not observed to influence transcorneal permeability of another hydrophilic molecule, pilocarpine-HCl (Scholz et al., 2002).

A synergistic effect was observed when chitosan, BAK and EDTA were used in combination. Whereas, 0.1% chitosan could generate only a 3.1-fold increase in ACV's corneal permeability, chitosan at the same concentration in the presence of BAK and EDTA generated a 5.5-fold increase, from 1.32×10^{-6} to 7.3×10^{-6} cm/s (Fig. 2).

Interestingly, osmolality of the transport buffer that was employed for the chitosan studies was 328 ± 6 mosmoles (Osmette S, model 4002, Precision Systems Inc., Natick, MA), whereas, that of DPBS (used for the BAK and EDTA studies) was 270 ± 6. This increase in osmolality led to a 2.7-fold decrease in transcorneal ACV permeation (from 3.5×10^{-6} to 1.3×10^{-6} cm/s), which is consistent with earlier findings (Scholz et al., 2002).

Experiments were also conducted to evaluate whether the corneal epithelial integrity was re-established following withdrawal of the permeation enhancing agents. For this purpose, at the end of 3 h, donor and receiver solutions were removed and both chambers were washed twice with the respective transport buffer. Following this, drug solution was added to the donor chamber and transport buffer was added to the receiver side. The transport studies were continued for another 3 h at 34 °C. Similar protocols were followed for the control set (no penetration enhancers added) also to ensure that the experimental procedure was not damaging the corneal epithelial cell layer integrity. The observed permeability coefficients of ACV during the initial 3 h transport study, in the presence or absence (control) of the permeation enhancers, and during the second 3 h study period, in the absence of any enhancer, were not significantly different (data not shown). The results clearly indicate that a 3 h period was not sufficient for the corneal epithelial cellular integrity to be re-established. Lack of essential cellular nutrients in the buffer systems and duration of the recovery phase may be responsible for the observed lack of recovery. Further studies are being carried out to design a suitable model/protocol to monitor corneal tight junction recovery.

In conclusion, this in vitro study demonstrates that chitosan can increase transcorneal diffusion of hydrophilic agents, probably through disruption of tight junctions, and may be a useful adjunct in ophthalmic formulations. BAK exhibits a synergistic effect on the absorption promoting property of chitosan. Chitosan's mechanism of action, extent of absorption enhancement and reversibility of effect, alone and in combination with BAK and EDTA, warrants further investigation in vivo.

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