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Comparative *in-vitro* transcorneal permeation studies of aqueous drop of esmolol HCl through excised goat, sheep, and buffalo corneas

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Abstract

The present work focuses to evaluate the effect of formulation factors transcorneal permeation of aqueous drop of esmolol HCL through freshly excised goat, sheep, and buffalo corneas. Effect of concentration of esmolol HCl in aqueous solution on permeation of drug through excised goat, sheep, and buffalo corneas was study. Esmolol HCL ophthalmic solutions of different concentrations (pH 7.2) or 0.5% (w/v) solutions of different pH or 0.5% solutions (pH 7.2) containing different preservatives were made. Franz diffusion was used for measuring the drug permeated in the receptor by spectrophotometry at 280 nm, after 120 minutes. Compared with control formulation, esmolol HCL 0.5 %(w/v) solution at pH 7.2 containing benzalkonium chloride (0.01% w/v) in the formulation increased permeation to the maximum with all the corneas and the apparent permeability coefficient was found to be maximum 9.85±0.4 on goat cornea. The results of the ophthalmic aqueous drop optimized formulation containing BAC,control formulation (without BAC) and marketed cardioselective eye drop betoptic S (betaxolol HCl) through paired corneas showed near bit same result. It can be accomplished that from the present studies increase in concentration of esmolol HCL in aqueous drop causes a disproportionate increase in permeation.

KEYWORDS: PRESERVATIVE; CORNEA; PERMEATION; INFLAMMATIONS

Introduction

The introduction of beta blockers as ocular antihypertensive agents was a breakthrough for the medical management of glaucoma[1-2] and timolol was a milestone, being the first topical beta adrenergic antagonist approved in the US for glaucoma treatment. Beta blocker is ocular antihypertensive agent was a break through a medical management of glaucoma and timolol was a milestone being the first beta adrenergic antagonist used for glaucoma treatment.[3-6] In this work attempt has been made that esmolol hydrochloride is also used for the chronic clinical glaucoma treatment. Esmolol HCl eye drop is not available in market so in this we use to formulate esmolol HCl eye drop to reduce glaucoma disease. Most of the permeation studies reported have used rabbit cornea. Animal Ethics Committees are putting restrictions on experiments with rabbit cornea. Thus, it appears reasonable to look for alternate mammalian corneas, especially from those animals that are slaughtered every day for meat (eg, goat, sheep, and buffalo). In addition, such a study would also help in the development of veterinary ophthalmic formulation of the drug as goat, sheep, and buffalo constitute the bulk of the cattle population in the Indian subcontinent. Accordingly, the purpose of this investigation was to study the effect of formulation factors such as concentration of drug, pH, and presence of preservatives in aqueous drop on in vitro permeation of aceclofenac through excised goat, sheep, and buffalo corneas. To avoid biological variation, attempts were also made to evaluate the permeation characteristics of esmolol HCL formulation through freshly excised paired corneas of each species.

Methods

Corneal preparation

Freshly excised whole eyeballs of goat, sheep and buffalo were transported from local butcher's shop to laboratory in cold (4°C) saline within 1 h of slaughtering. The corneas were carefully dissected along with 2–4 mm of surrounding sclera tissue from the eyeball and washed with cold saline to remove any adhering pigments as shown in the figure 50 The washed cornea were preserved in freshly prepared balance base buffer (pH 7) with % w/v composition of NaCl—0.57, NaHCO3—0.361, KCl—0.04, K2HPO4—0.023, MgSO4—0.007 and CaCl2—0.08 in glass distilled water and bubbled with O_2 to keep the cornea in viable state.

Permeation experiment

Fresh corneas obtained by the above procedure were mounted on the modified Franz diffusion apparatus by sandwiching the scleral tissues between the clamped donor and the receiver chamber. Care was taken to maintain the convex surface shape of the cornea by suitable design of the clamp, receiver and donor chamber edge and also to ensure that the epithelial surface of the cornea is towards the donor side. Balance base buffer (composition same as given in previous section) was filled in receiver chamber after expelling all the air bubbles by inverting the diffusion cell and then allowing the bubbles to travel throughthe sampling port. The receiver fluid was maintained at 37±1°C with the help of circulating warm water and kept under stirring using a teflon coated magnetic bead. An aliquot (1 ml) of test sample was placed on the epithelial surface of each cornea in the donor chamber and covered with glass slip using silicone grease to prevent evaporation. In all experiments the permeation was continued for 120 min at predetermined time points of 30, 60, 90, and 120 min, a 1 ml sample was withdrawn throughthe sampling port, and analyzed by spectrophotometer method discussed earlier. The concentration of permeated drug at the defined time intervals was determined using standard curve.

The permeation (%) or *in-vitro* ocular availability was calculated as follows:

$$\text{Permeation } \% = \frac{Amount\ of\ drug\ permeated\ in\ recepter}{Initial\ amount\ of\ drug\ in\ doner} \times 100 \quad (1)$$

At the end of the experiment, each cornea (freed from adhering sclera) was weighed, soaked in 1-ml

methanol, dried overnight at 90°C, and reweighed. From the difference in weights, corneal hydration was calculated. Permeation characteristics of esmolol HCl formulations were also evaluated throughfreshly excised paired goat, buffalo, and sheep corneas as showed in figure 50. Statistical calculations of esmolol HCl ophthalmic aqueous solution were done by one-way ANOVA followed by Dunnett's test. Paired t-test was used for studies with paired cornea. A P value less than .05 was considered as criterion for significance.

Apparent permeability coefficient

Apparent permeability coefficient was also calculated using the following equation

$$\text{Papp} = \frac{\Delta Q}{\Delta t} \cdot \frac{1}{A.C0.60}$$

Where, $\frac{\Delta Q}{\Delta t}$ (ug/min) is the flux across the corneal tissue. A is the area of diffusion (cm^2) is the initial concentration of drug in donor compartment, and 60 is taken as the factor to convert minute into second. The flux across the cornea was obtained from the slope of the regression line obtained from the linear part of the curve between the amount permeated (Q) Vs time (t) plot.[7-10]

Preparation of test solutions of esmolol HCl ophthalmic solution of increasing concentration of pH 7.2

Esmolol HCl ophthalmic solution of different concentarion at pH 7.2

Required amount of esmolol was dissolved in sufficient isotonic phosphate buffer, pH of the solution was adjusted to 7.2 using 0.1N NaOH or 0.1N HCl, and final volume was made up to 100 ml with distilled water, to have solutions of 0.1, 0.2, 0.3, 0.4, and 0.5% (w/v) concentrations. All the prepared solution were filtered and packed in glass vials and sealed and these eye drop container were sterilized by autoclave at 121°C for 15 min.

Formulation of esmolol HCl ophthalmic solutions 0.5 % w/v, pH 7.2 containing preservative.

The drug esmolol (0.5g) was dissolved in 100 ml of isotonic phosphate buffer, pH 7.2 containing either benzalkonium chloride (BAC 0.01% w/v), or phenyl mercuric nitrate (PMN 0.001%w/v), or benzyl alcohol (BA 0. 5%v/v), etc were prepared and the final volume of each solution was made up to 100 ml with distilled water. All the prepared solution were filtered and packed in glass vials and sealed and these eye drop container were sterilized by autoclave at 121°C for 15 min.

Figure 1 Freshly excised paired corneas of each species

Results

The permeation data of esmolol HCl from ophthalmic solutions of increasing concentrations throughexcised goat, sheep, and buffalo corneas are shown in table 1. The data revealed an increase in the concentration of esmolol HCl from 0.1 to 0.5% increases in the amount of drug permeated at particular pH. It is worth mentioning that through an increase in permeaton was observed with incremental concentration of drug but at the same time there was a decrease in percentage permeation. The apparent permeability coefficient was found to be more in goat cornea compared with sheep and buffalo cornea for all concentration of esmolol HCl eye drop 0.1% to 0.5%.

The effects of different preservatives on permeation of esmolol HCl ophthalmic aqueous solution throughexcised goat, sheep and buffalo corneas are shown in tables 2. The study showed that in all the three corneas esmolol HCl ophthalmic aqueous solution, 0.5% (pH 7.2) containing benzalkonium

chloride as preservative produced significantly (P<.05) higher permeation, compared with control formulation containing no preservative.The highest percentage permeation of 72 %, 44 % and 30 % was obtained throughgoat, sheep and buffalo corneas for the formulations containing benzalkonium chloride and 65 %, 38 % and 25 % in case of formulations containing combination of methyl paraben and propyleparaben. In contrast to this control formulation containing no preservative percentage permeation showed an permeation of 28 % (goat), 18% (sheep) and 12 % (buffalo). Similarly, formulation containing benzyl alcohol showed marginal increase in permeation, while those with phenyl mercuric acetate and thiomersal, did not have any effect on permeation. It is evedent from result that use of benzalkonium chloride, a cationic surfactant, showed a significant increase in permeation. Combined presence of methyl paraben and propylparaben / benzalkonium chloride in the formulation, however, provided maximum permeation of the drug throughall the corneas. The increased permeation with formulation contaning benzalkonium chloride appears to be caused by emulsification of corneal epithelium and increased the solubility of esmolol HCl The corneal hydration of esmolol HCl eye drop throughgoat, sheep and buffalo corneas was found to be 75.5% to 77.9 % respectively. The combination of BAC and EDTA has also been reported to increase the permeation of moxifloxacin through excised goat, sheep, and buffalo corneas. [11-13] An attempt was also made to check permeation of optimized formulation containing benzalkonium chloride, control formulation (without benzalkonium chloride) and marketed cardioselective eye drop betoptic S (betaxolol HCl) throughpaired corneas of goat, sheep, and buffalo.By paired cornea we mean that from a single animal, one cornea was treated with optimized formulation containing benzalkonium chloride while the other cornea was treated with control formulation containing no additive. This procedure was adopted to minimize biological variation.

The permeation results of the ophthalmic aqueous drop optimized formulation containing BAC,control formulation (without BAC) and marketed cardioselective eye drop betoptic S (betaxolol HCl) throughpaired corneas of goat, sheep, and buffalo and the results are shown in table 3. The results indicate that formulation containing esmolol HCl with BAC increased the permeation of esmolol throughall the mammalian corneas compared with the control formulation, but in case of marketed cardioselective eye drop betoptic S (betaxolol HCl) our optimized formulation showed the near bit same result as showed in (figure 2). The thickness of the corneas was also observed as shown in the table 3. The thickness of goat, sheep and buffalo cornea were found to be 0.68±0.0003mm, 0.86±0.0003mm and 1.12±0.0006mm respectively.

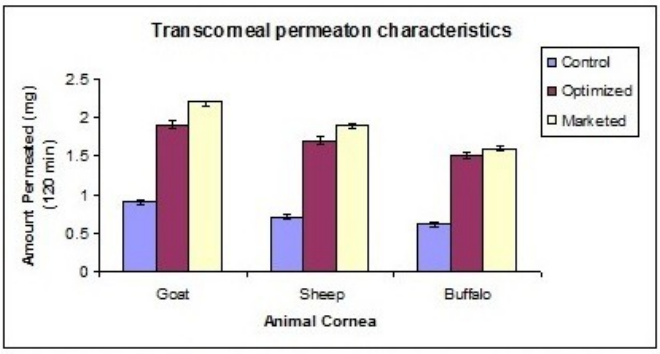

Figure 2 Transcorneal permeation characteristic of cardio selective control, optimized and marketed formulation (betaxlol HCl) betoptic® s through excised goat, sheep, and buffalo corneas

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Concentration % (w/v)	Amount Permeated (mg) (120 min)			Permeation (%) (120 min)			Corneal Hydration (%)			Papp cm/sec * 10^6		
	Goat	Sheep	Buffalo	Goat	Sheep	Buffalo	Goat	Sheep	Buffalo	Goat	Sheep	Buffalo
0.1	0.75±0.002	0.69±0.001	0.55±0.001	75.2	62.7	55.8	77.2±0.52	77.7±0.02	77.2±0.68	10.8±0.32	8.41±0.10	5.44±0.10
0.2	1.22±0.011	1.14±0.003	1.00±0.004	61.0	57.0	51.5	77.5±0.021	77.5±0.05	76±0.21	8.57±0.01	5.61±0.51	5.09±0.21
0.3	1.58±0.021	1.56±0.001	1.36±0.003	52.8	50.0	45.0	76.1±0.17	76.9±0.01	77.4±0.36	5.16±0.21	4.88±0.04	3.53±0.45
0.4	1.85±0.040	1.7 ±0.002	1.58±0.006	47.0	42.0	40.0	77.4±0.46	77.1±0.21	74±0.32	3.95±0.05	3.46±0.08	3.25±0.12
0.5	2.23±0.010	2.16±0.005	1.98±0.008	45.6	43.3	39.5	76.2±0.21	77.2±0.65	76.3±0.39	3.53±0.11	3.56±0.06	2.92±0.15

Table 1. Effect of concentration of esmolol HCl in aqueous solution on permeation of drug through excised goat, sheep, and buffalo corneas

Values are mean ± SE of Three corneas in each group. Statisticalsly significant (P < 0.5) determined by one-way ANOA followed by Dunnett's test

Preservative	Amount Permeated (mg) (120 min)			Permeation (%) (120 min)			Corneal Hydration (%)			Papp cm/sec * 10^6			Surface Tension (dyne/cm)
	Goat	Sheep	Buffalo	Goat	Sheep	Buffalo	Goat	Sheep	Buffalo	Goat	Sheep	Buffalo	
Control	1.4±0.02	0.9±0.05	0.6±0.04	28	18	12	76.1±0.42	77.4±0.01	76.2±0.5	4.23±0.3	2.74±0.01	2.31±0.12	66.4
BAC	3.8±0.03	1.7±0.08	1.5±0.05	72	44	30	77.2±0.02	77.1±0.24	76±0.34	9.85±0.4	7.49±0.08	4.60±0.25	41.1
MP-PP	2.6±0.04	1.8±0.04	1.2±0.03	65	38	25	77.5±0.14	75.5±0.54	77.4±0.3	8.62±0.1	6.91±0.02	4.0±0.01	48.6
BA	1.9±0.05	1.2±0.04	1.1±0.09	40	28	22	77.9±0.48	77.1±0.11	76±0.32	7.21±0.21	4.25±0.05	3.75±0.10	62.1
THM	1.1±0.09	1±0.07	0.9±0.01	22	21	18	76.2±0.12	77.2±0.65	77.3±0.3	3.86±0.10	3.53±0.06	2.74±0.24	65.8
PMA	1.2±0.01	1.1±0.06	1.0±0.01	28	22	18	77.27±0.1	77.2±0.44	77.6±0.2	4.25±0.42	3.71±0.04	2.74±0.20	62.1

Table 2. Effect of preservative on permeation of esmolol HCl from 0.5% aqueous solution through excised goat, sheep and buffalo cornea

BAK indicates benzalkonium chloride; MP-PP, combination of methyl paraben and propyleparaben; BA, benzyl alcohol; THM, thiomersal; PMA, phenyl mercuric acetate; Values are mean ± SE of Three corneas in each group. Statisticalsly significant (P < 0.5) determined by one-way ANOVA followed by Dunnett's test

Animal	Thickness of Cornea mm	Control Formulation			Optimized Formulation with BAC			Marketed formulation (betaxlol HCl) Betoptic® S		
		Amount Permeated (mg) (120 min)	Permeation (%) (120 min)	Papp cm/sec * 10^6	Amount Permeated (mg) (120 min)	Permeation (%) (120 min)	Papp cm/sec * 10^6	Amount Permeated (mg) (120 min)	Permeation (%) (120 min)	Papp cm/sec * 10^6
Goat	0.68±0.0003	1.4±0.02	28	4.23±0.3	3.8±0.03	72	9.85±0.4	4.7±0.05	84	11.41±0.4
Sheep	0.86±0.0003	0.9±0.05	18	2.74±0.01	1.7±0.08	44	7.49±0.08	3.21±0.08	51	8.43±0.1
Buffalo	1.12±0.0006	0.6±0.04	12	2.31±0.12	1.5±0.05	30	4.60±0.25	2.12±0.07	35	6.56±0.2

Table 3. Relative permeation characteristics of esmolol HCl from control and optimized formulation and marketed formulation (betaxlol HCl) betoptic® S through excised goat, sheep, and buffalo corneas

Values are mean ± SE of Three corneas in each group. Statisticalsly significant (P < 0.5) determined by one-way ANOVA followed by Dunnett's test