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Effects of Transcutol P on the corneal permeability of drugs and evaluation of its ocular irritation of rabbit eyes

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Abstract

Our purpose was to explore the use of Transcutol P (Trans) in an ocular drug delivery system. The effect of Trans on the corneal permeability of drugs was investigated in-vitro, using isolated rabbit corneas. The ocular irritation of Trans was also tested in rabbits in-vivo. In the presence of Trans, at a concentration of 0.005–0.03%, the maximum increase in the apparent permeability coefficient (P_{app}) was 1.5, 1.5, 3.0 and 3.3 fold for ribavirin, gatifloxacin, levofloxacin hydrochloride and enoxacin, respectively. However, the P_{app} value of oxaprozin was reduced in the presence of Trans. The maximum reduction was found to be 2.8 fold at a concentration of 0.03% Trans. The results of the ocular irritation studies showed that Trans was non-irritant at the concentrations studied (0.005–0.03%), while it produced slight irritation at a concentration of 0.05%. It was also found that Trans did not cause any visible ocular damage or abnormal clinical signs involving the cornea, iris or conjunctivae at all concentrations. We concluded that Trans may have potential clinical benefits in improving the ocular drug delivery of hydrophilic compounds.

Introduction

The successful delivery of drugs to the eye is extremely difficult because the eye is protected by a series of complex defence mechanisms that make it difficult to achieve an effective concentration of drug within the target area of the eye. These defence mechanisms lead to poor bioavailability of drugs delivered in classical ophthalmic dosage forms (eye drops) into the lower cul-de-sac. Drugs systemically administered for their ocular action also have a poor access to eye tissue. This is due to the presence of the blood–aqueous barrier, which prevents drugs from entering the aqueous humour, and the blood–retinal barrier, which prevents drugs from entering the extravascular retinal space and the vitreous body (Stjernschantz & Astin 1993).

The ease of drug penetration across biological membranes generally depends on the relative lipophilicity of the drug and the membrane. A parabolic relationship between the drug penetration rate and its lipophilicity has been observed for the cornea and other biological membranes (Mosher & Mikkelson 1979; Schoenwald & Huang 1983; Gershon et al 1984). Hence, the drug penetration usually increases with drug lipophilicity until a maximum is reached and then it ceases to increase or decreases with a further increase in drug lipophilicity. Although multiple pathways for drug transport across biological membranes have been proposed, the design of drugs with a suitable lipophilicity is of great importance to optimize drug penetration via the nonpolar pathways (corneal epithelium).

One alternative approach is to increase the transcorneal passage of drugs by incorporating absorption promoters/penetration enhancers into the drug formulations. This approach involves increasing transiently the permeability characteristics of the cornea using penetration enhancers. Diane et al (1994) studied the effects of hexamethylene louramide, hexamethylene octanamide, Azone and decylmethylsulfoxide on the corneal permeability of eight drugs in-vitro. However, the ocular irritation produced by these four penetration enhancers was not studied. Fabrizio et al (1996) studied the effects of benzalkonium chloride, EDTA, non-ionic surfactants, surface-active heteroglycoside and bile salts on the corneal permeability of four β-blockers in-vitro. Among these enhancers, sodium deoxycholate, digitonin,

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escin and polyoxyethylene 9 lauryl ether had a significant irritant effect at concentrations of 1.0% (w/w), 0.25% (w/w), 0.25% (w/w) and 2.0% (w/w), respectively. Hence, it is very important to carry out ocular irritation studies when employing new penetration enhancers and to evaluate their suitability for use in ocular pharmaceutical preparations.

Transcutol P (diethylene glycol monoethyl ether, Trans), an oil water-soluble liquid, is extensively used in topical, transdermal and oral pharmaceutical preparations as a solubilizer (a substance added to a solution to increase the solubility of the other substances) and an absorption enhancer. Compared with the control formulation, the skin permeability of nimesulide from a Carbopol-934-based gel formulation was significantly increased ($P < 0.05$) by the combination of oleic acid (3%) with Trans (30%) (Gungor & Bergisadi 2004). Trans showed excellent permeation-enhancing effects on ciclopirox across porcine hoof membrane when administered in a styrene-isoprene-styrene adhesive (Myoung & Choi 2003). Susana et al (1997) developed an albendazole liquid formulation using Trans (40% w/w) as a cosolvent, which increased the bioavailability of albendazole by over 82%.

To our knowledge, no report has described the use of Trans as a penetration enhancer for an ophthalmic drug delivery system. The aim of this study was to explore the use of Trans in an ocular drug delivery system. Five drugs, ranging from hydrophilic to lipophilic (ribavirin, levofloxacin hydrochloride, enoxacin, gatifloxacin and oxaprozin) were used as model compounds to study the effect of Trans on the corneal permeability. The mechanism of ocular permeation enhancement of drugs by Trans was also investigated. In addition, the ocular irritation produced by different concentrations of Trans was evaluated.

Materials and Methods

Materials

Enoxacin was purchased from Wuhan Pharmaceutical Manufacture (Wuhan, China). Gatifloxacin and ribavirin were purchased from HuBei Qianjiang Pharmaceutical Manufacture (Qianjiang, China). Oxaprozin was purchased from HuBei Hengdi Pharmaceutical Manufacture (Wuhan, China). Levofloxacin hydrochloride was kindly donated by Dandong Pharmaceutical Manufacture (Dandong, China). Transcutol P (Trans) was kindly gifted by Gattefosse (France). All the other chemicals were of analytical grade.

Animals

New Zealand White rabbits, 2.5–3.0 kg, were provided by the Animal Experimental Center of Shenyang Pharmaceutical University. The rabbits, housed in standard cages in a light-controlled room (19 ± 1°C, 50 ± 5% relative humidity), were given free access to a standard pellet diet and water. All studies were conducted in accordance with the Principles of Laboratory Animal Care (NIH publication no.92–93, revised in 1985), and were approved by the Department of Laboratory Animal Research at Shenyang Pharmaceutical University. The procedures with animals were reviewed and approved by the Animal Ethical Committee at Shenyang Pharmaceutical University.

Ocular irritation studies of Trans

Ocular irritation studies were performed according to the Draize technique (Draize et al 1944). A total of 36 rabbits, divided into 6 groups based on the concentrations of Trans, were used. The Draize technique was used to assess acute, intermediate and chronic exposure by applying compounds to the skin, penis and eyes of rabbits. This technique has been used by the FDA to evaluate the safety of several substances (Draize et al 1944; Fitzhugh et al 1946). The solutions, consisting of 0, 0.005, 0.01, 0.02, 0.03 and 0.05% (v/v) Trans in phosphate buffer at pH 7.4, were instilled into the left eye, 0.1 mL every 3 h, five times a day for a period of seven days. The condition of the ocular tissue was monitored after 1, 4 12, 24, 48 and 72 h after the end of the last instillation. The conjunctival congestion, swelling and discharge were graded on a scale of 0–3, 0–4 and 0–3, respectively. Iris hyperaemia and corneal opacity were graded on a scale of 0–4. The mean values from six treated eyes were calculated for each solution. The evaluation criteria used in accordance with the Draize technique were non-irritant from 0 to 3.9, slightly irritant from 4 to 8.9, moderately irritant from 9 to 12.9 and seriously irritant from 13 to 16, respectively.

Corneal permeation studies

After the rabbits were sacrificed by injecting intravenous air through a marginal ear vein, freshly excised rabbit corneas were immediately mounted in Franz-type diffusion cells, which were maintained at a constant temperature (35 ± 1°C), under mixing conditions using a magnetic stirrer at a rotating speed of 600 rev min^{-1} (79HW-1; Zhejiang, China). The corneal area available for diffusion was 0.70 cm^2 (the cornea was mounted within half an hour of sacrifice). Preheated (35°C), pH = 6.85, glutathione bicarbonate Ringer (GBR) buffer was added to the epithelial (2.0 mL) and the endothelial (7.8 mL) compartment. This buffer solution closely simulates tears and is referred to as simulated tear fluid (STF). This medium is known to preserve the integrity of the cornea for up to 6 h (O'Brien & Edelhauser 1977). To ensure oxygenation and agitation, a O_2–CO_2 (95:5) mixture was bubbled through each compartment at a rate of 3–4 bubbles/s. After 10 min, the solution on the epithelial side was withdrawn and replaced with 2.0 mL of different concentrations of drugs in phosphate buffer at pH 7.4, with and without Trans at various concentrations. Samples of medium from the endothelial side were withdrawn every 40 min from the sampling port and were replaced with an equal quantity of fresh GBR to maintain a constant volume. Each experiment was continued for 4.0 ± 0.67 h and repeated four times.

The apparent corneal permeability coefficients (P_{app}) were calculated according to equation 1 (Schoenwald & Huang 1983; Camber 1985).

$$P_{app} = \Delta Q/(\Delta t \times C_0 \times A \times 60)\ (cm \cdot s^{-1}) \quad (1)$$

Where $\Delta Q/\Delta t$ is the steady-state slope of the linear portion of the plot of the amount of drug in the receiving chamber (Q) vs time (t), A is the exposed corneal surface area (0.7 cm^2) and C_0 is the initial concentration of drug in the donor cell and 60 represents the conversion of minutes to seconds.

Chromatographic analysis

Ribavirin, levofloxacin hydrochloride, gatifloxacin, enoxacin and oxaprozin were assayed by reversed-phase HPLC (Shimadzu LC-10Atvp; Japan). The chromatographic conditions are given by Liu (2001), Zhang et al (2003), Hu et al (2002), Liu et al (2004) and Guo et al (2001), respectively. The peak area correlating linearly with the concentrations was in the range of 1.000 ~ 24.000 $\mu g \cdot mL^{-1}$ ($r^2 = 1$), 0.200 ~ 16.000 $\mu g \cdot mL^{-1}$ ($r^2 = 0.9997$), 0.212 ~ 15.900 $\mu g \cdot mL^{-1}$ ($r^2 = 0.9997$), 0.500 ~ 20.000 $\mu g \cdot mL^{-1}$ ($r^2 = 1$) and 0.1002 ~ 1.002 $\mu g \cdot mL^{-1}$ ($r^2 = 0.9996$), respectively. The sensitivities were 20, 10, 10, 15 and 20 ng, respectively.

Determination of corneal hydration levels (HL)

To determine the wet corneal weight, W_a, each corneal sample was carefully removed from the scleral ring and weighed. It was then desiccated at 100°C for 6 h to determine the corresponding dry corneal weight, W_b (Fabrizio et al 1996).

The percentage corneal hydration level (HL%), defined as $[1 - (W_b/W_a)] \times 100$, was determined both for untreated corneas (removed no later than 30 min after the death of the rabbit) and for corneas recovered from permeation tests performed in the absence and presence of enhancers.

Measurement of partition coefficients

Partition coefficients between n-octanol and buffer were determined by the modified shake-flask method. Briefly, a known amount of compound was dissolved in phosphate buffer at pH 7.4 and agitated with n-octanol for 30 min. The two phases were allowed to equilibrate for at least 1 h. The material balance was verified by summing the quantity of compounds recovered from both phases. The partition coefficient is the ratio of the drug concentration in the octanol and aqueous phase. Both phosphate buffer and n-octanol were saturated with each other before starting the experiment. In the experiments, the partition coefficients between n-octanol and phosphate buffer were determined.

Statistical analysis

The data obtained were expressed as mean ± s.d. The ocular irritation data were statistically evaluated by Kruskal–Wallis test, and the other experimental data were subjected to statistical analysis using two-way analysis of variance followed by Tukey's test. Differences were considered to be significant at $P < 0.05$.

Results

Ocular irritation studies

The average scope of ocular irritation test was 0, 0, 0, 1 ± 0.6, 1 ± 1 and 5 ± 2 from 0% to 0.05% (v/v) Trans, respectively. The results of the ocular irritation studies showed that Trans was non-irritant at concentrations of 0.005–0.03% (v/v), and slightly irritant at 0.05% (v/v), with regard to the cornea, disperse opacity; iris, hyperaemia; conjunctiva, redness of palpebral conjunctivae, swelling and discharge with moistening of the lids. There was no visible ocular damage or abnormal clinical signs involving the cornea, iris or conjunctiva at all concentrations. Based on these results, the concentrations of Trans selected for the corneal permeation studies were 0.005, 0.01, 0.02 and 0.03% (v/v).

Corneal permeation studies

Based on the log P (o/w) values listed in Table 1, the compounds studied can be roughly categorized into two groups according to their lipophilicity: ribavirin, levofloxacin hydrochloride, enoxacin and gatifloxacin as hydrophilic, and oxaprozin as lipophilic. Figure 1 shows the effect of Trans on the P_{app} values of four hydrophilic compounds. The permeability of levofloxacin hydrochloride and enoxacin significantly increased as the concentration of Trans increased up to a maximum value. A further increase in Trans concentration reduced the permeability. The maximum enhancement in P_{app} was 3.0 and 3.3 fold for levofloxacin hydrochloride and enoxacin, respectively ($P < 0.01$). Concentrations of 0.005% (v/v) and 0.02% (v/v) Trans increased the P_{app} by about 1.5-fold for ribavirin and gatifloxacin ($P > 0.05$).

The corneal permeability of oxaprozin in the presence of various concentrations of Trans is shown in Figure 2. Trans did not enhance the corneal penetration of oxaprozin but actually

Table 1 Effect of Transcutol P on the corneal permeability of drugs in-vitro

Drug	ζ-potential (mV)	Log P (o/w)	$P_{app} \times 10^6$ ($cm \cdot s^{-1}$)				
			0%	0.005%	0.01%	0.02%	0.03%
Ribavirin	−48.8	−2.6	6.155 ± 1.31	9.039 ± 2.09	7.027 ± 1.11	6.328 ± 0.29	6.077 ± 1.11
Levofloxacin HCl	−6.25	−0.87	3.943 ± 2.82	9.500 ± 1.04**	11.65 ± 3.90**	9.243 ± 1.32**	7.434 ± 3.41
Enoxacin	−30.3	−0.65	4.051 ± 1.16	8.870 ± 2.72	13.45 ± 3.70**	8.796 ± 2.15*	7.441 ± 2.14
Gatifloxacin	−57.5	−0.32	8.555 ± 1.56	9.304 ± 0.33	9.986 ± 5.43	12.50 ± 4.37	9.880 ± 1.46
Oxaprozin	4.49	0.37	7.819 ± 0.35	6.585 ± 0.30	5.216 ± 0.34	4.591 ± 0.70	2.787 ± 1.78

P_{app} data are means ± s.d., n = 4. *$P < 0.05$, **$P < 0.01$ vs control. Transcutol: clear, colourless, hygroscopic liquid; miscible with water, with acetone and with alcohol, miscible in certain proportions with vegetable oils, not miscible with mineral oils.

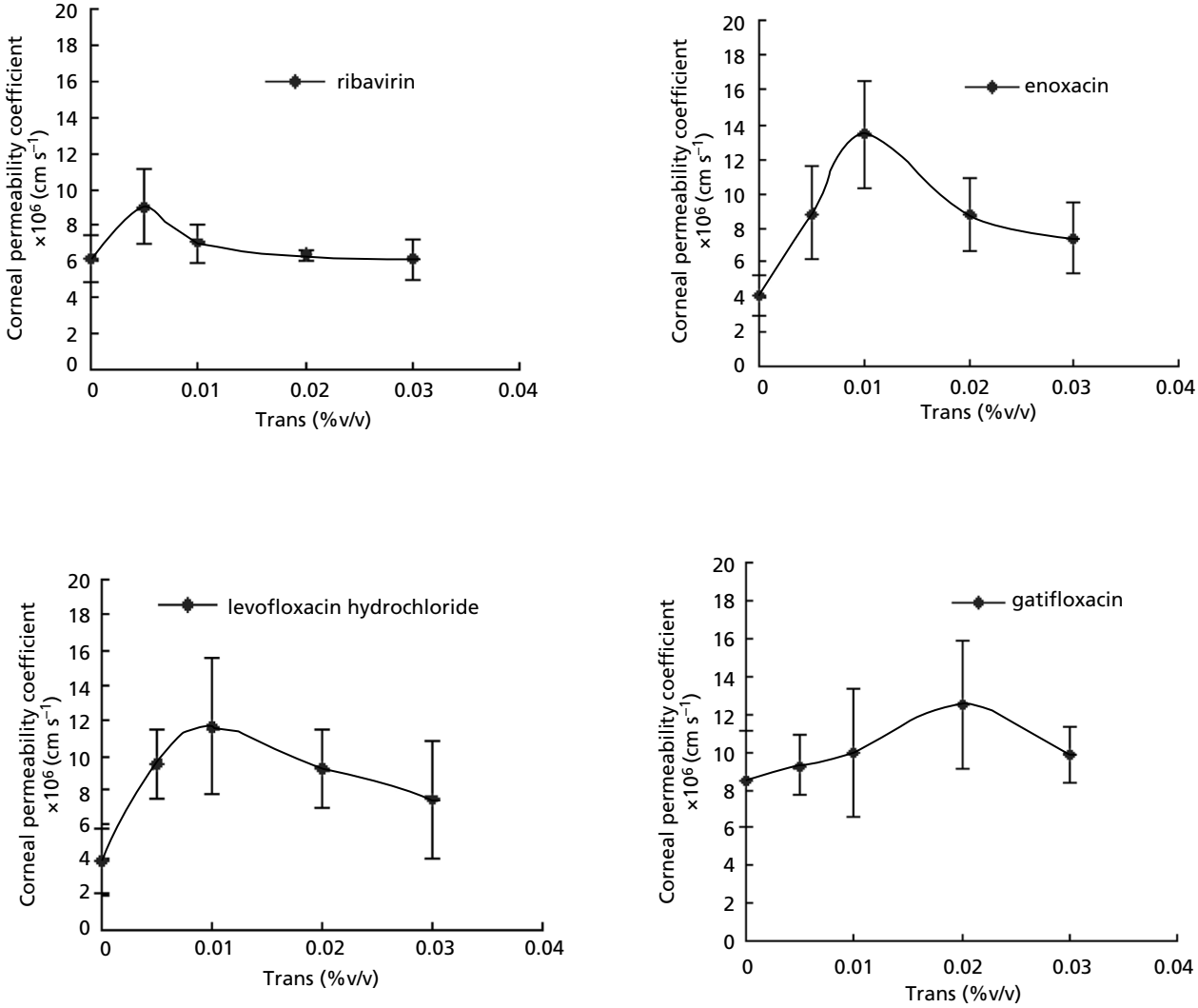

Figure 1 The corneal permeability coefficients of hydrophilic drugs (mean ± s.d., n = 4).

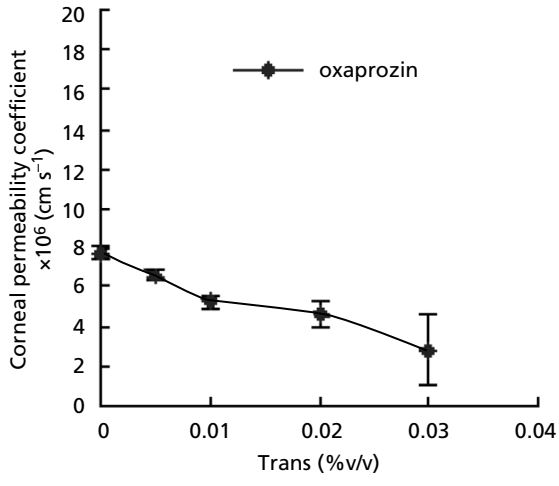

Figure 2 The corneal permeability coefficient of oxaprozin (mean ± s.d., n = 4).

reduced it. The P_{app} value of oxaprozin was reduced from 7.819×10^{-6} cm s^{-1} in the absence of Trans to 2.787×10^{-6} cm s^{-1} at 0.03% (v/v) Trans.

Different concentrations of Trans did not significantly change the lag-time of drugs, except for oxaprozin (Table 2). The lag-time of the appearance of oxaprozin in the receiver chamber increased from 14 to 29 min at a concentration of 0.03% (v/v) Trans, indicating a possible change in the absorption rate constant of oxaprozin in the presence of Trans (Diane et al 1994).

Effect of corneal hydration levels

The percentage corneal hydration is a parameter frequently used to evaluate damage to this tissue. According to Maurice & Riley (1970), the normal cornea has a hydration level of 76–80%. As is indicated by Schoenwald & Huang (1983), an 83–92% hydration level (i.e., 3–7 percent units or more over the normal value) denotes damage to the epithelium or endothelium. As shown in Table 3, the percentage corneal

Table 2 The lag-time of drugs in the presence and absence of Transcutol P (n = 4)

Drug	Lag-time (min)				
	0%	0.005%	0.01%	0.02%	0.03%
Ribavirin	33.94 ± 4.87	28.26 ± 5.66	23.40 ± 10.28	35.19 ± 7.84	26.29 ± 8.58
Levofloxacin HCl	53.29 ± 6.00	44.71 ± 10.84	45.65 ± 9.49	44.53 ± 11.25	44.43 ± 9.07
Enoxacin	41.77 ± 3.20	26.14 ± 22.07	42.73 ± 6.65	30.42 ± 16.06	43.43 ± 6.07
Gatifloxacin	46.85 ± 4.45	42.82 ± 6.94	41.98 ± 2.45	37.88 ± 5.39	49.46 ± 10.99
Oxaprozin	14.32 ± 7.63	19.44 ± 7.46	16.35 ± 5.45	25.51 ± 9.27	29.66 ± 5.11*

Data are means ± s.d., n = 4. *$P < 0.05$ vs control.

Table 3 The corneal hydration of drugs in rabbit eye in the presence and absence of Transcutol P

Drug	Corneal hydration (%)				
	0%	0.005%	0.01%	0.02%	0.03%
Ribavirin	79.48 ± 1.94	79.58 ± 1.18	79.09 ± 1.25	77.98 ± 2.15	78.80 ± 1.29
Levofloxacin HCl	79.13 ± 1.45	79.83 ± 1.25	80.07 ± 1.54	79.50 ± 1.50	80.74 ± 0.99
Enoxacin	78.67 ± 1.37	78.76 ± 1.22	82.94 ± 1.68	81.51 ± 0.61	82.09 ± 1.49
Gatifloxacin	80.77 ± 2.19	77.08 ± 0.46	80.31 ± 0.77	80.64 ± 0.84	81.46 ± 0.69
Oxaprozin	78.22 ± 1.16	78.94 ± 0.98	79.34 ± 0.45	77.85 ± 0.79	76.94 ± 3.51

Data are means ± s.d., n = 4.

hydration of drugs after the studies was not increased over 3%. This indicates that the Trans did not cause any damage to the epithelium or endothelium during the studies.

Discussion

The delivery of hydrophilic drugs to produce intraocular effects can certainly benefit from the use of penetration enhancers. The cornea is heterogeneous in structure and can be divided into three layers with different physicochemical properties. The epithelium, composed of five living cell layers, is the most lipophilic and there are tight attachments between the cells. It is the major penetration barrier for hydrophilic drugs. The stroma, the most hydrophilic, forms the bulk of the cornea both in terms of weight and thickness and is the major penetration barrier for lipophilic drugs. The epithelium and the monolayered endothelium restrict the passage of fluid and when their functions are compromised, the fluid will pass into the stroma and cause corneal swelling.

The results of this study show that Trans can increase or decrease the corneal penetration of drugs by producing different effects on the corneal barrier functions. Some studies (Indu & Smitha 2002) have found that corneal epithelial cells are surrounded by an outer cell membrane composed of a phospholipid bilayer with protein molecules embedded in the lipid membrane. When present at low concentrations, surfactants are incorporated into the lipid bilayer, forming polar defects, which change the physical properties of the cell membrane. When the lipid bilayer is saturated, mixed micelles begin to form, resulting in the removal of phospholipids from the cell membranes and, hence, leading to membrane solubilization. Trans is a surfactant and the results suggest that the mechanism of action of Trans on drug corneal transport may involve changes in the structure of the epithelium as a result of Trans producing micelles in the epithelial lipid bilayer. The micelles formed by Trans result in the removal of phospholipids from the epithelial cell membranes, thereby leading to an increase in the transcorneal passage of drugs. It has been suggested that there is a dose-dependent increase in permeability across different epithelia. It is also possible that Trans, at high concentrations in the epithelium, may loosen the epithelium cell junctions and facilitate the influx of hydrophilic compounds but retard the movement of lipophilic molecules by creating a hydration barrier (Diane et al 1994). This also contributed to the increase in the lag-time of oxaprozin.

The results of the ocular irritation and corneal hydration studies indicate that Trans is non-irritant at various concentrations (0.005, 0.01, 0.02 and 0.03% (v/v)).

Conclusion

This study evaluted the suitability and feasibility of using Trans as an ocular drug delivery system. Trans dramatically enhances the transcorneal penetration of hydrophilic drugs, which may be of potential clinical benefit. However, Trans does not improve, but actually retards, the penetration of lipophilic drugs across the cornea. The results of the ocular irritation studies indicate that concentrations of Trans from 0.005% to 0.03% (v/v) are non-irritant, except for 0.05% (v/v) Trans. In addition, none of these concentrations caused

visible ocular damage or abnormal clinical signs involving the cornea, iris or conjunctiva.

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