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Influence of some preservatives on the corneal permeability of pilocarpine and dexamethasone, in vitro

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Summary

The influence of some commonly used preservatives on the corneal permeability and uptake of pilocarpine and dexamethasone were investigated in vitro. Isolated corneas were exposed to benzalkonium chloride (0.01%), chlorobutanol (0.5%), metagin (0.04%) + propagin (0.02%), and chlorhexidine digluconate (0.01%), all in clinically used concentrations, for 4 h at 35°C. Benzalkonium chloride and chlorobutanol significantly increased the uptake and permeability of the two drugs. The influence of the parabens was of lower magnitude and the effect of chlorhexidine digluconate resulted in small or insignificant changes of the studied parameters. The barrier function of the corneal epithelium was strong. Both drugs showed a significant increase in permeability and uptake when de-epithelized corneas were used. Abrasion of the epithelium or preservatives showed a more pronounced influence on the corneal permeability and uptake for dexamethasone than pilocarpine HCl.

Introduction

It is well-known that ophthalmic formulations in multi-dose containers have to be sterile according to the requirements of pharmacopoeias, e.g. USP and BP. To fulfill this criteria "suitable and harmless" preservatives are added to prevent the growth of micro-organisms. The most frequently used preservatives in ophthalmic formulations are benzalkonium chloride (BAC), chlorhexidine digluconate (CDG), organic mercurials, esters of parahydroxybenzoic acid such as metagin (methyl hydroxybenzoate) and propagin (propyl hydroxybenzoate) (M + P), and chlorobutanol (CB). The ohoice is often based on pharmaceutical, technical and microbiological considerations, e.g. compatibility, antimicrobial spectrum, stability, irritancy and toxicity. Often little attention has been paid to the effect of preservatives on the ocular bioavailability of drugs.

The cornea is the main pathway for ocular permeation of topically applied drugs (Patton, 1980). Studies have shown that corneal epithelial alterations can be induced by compounds often used in eye drops (Pfister and Burstein, 1976). As the epithelium has barrier properties to permeation into the eye, epithelial alterations have been shown to influence the corneal permeability of different substances. For example, benzalkonium chloride, the most common preservative, increases the corneal permeability of a variety of compounds, e.g. small ions (Burstein and Klyce, 1977), fluorescein (Green and Tonjum, 1971; Burstein,

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1984), inulin (Keller et al., 1980), prednisolone phosphate (Green and Downs, 1974) and horseradish peroxidase (Tonjum, 1975). This effect of BAC is generally attributed to its adverse effects on the epithelium, as it causes cell damage and widening of the intercellular spaces of the superficial epithelial cell layers (Pfister and Burstein, 1976). Treatment of the cornea with cetylpyridinium chloride (0.02%) was as effective as total removal of the corneal epithelium in enhancing the corneal absorption of penicillin G (Godbey, 1979). CDG (0.01%) has been shown in rabbits to increase the permeability rate of sodium fluorescein by a factor of 1.5 (Burstein, 1984).

The objective of this in vitro study was to measure and compare the effect of some commonly used preservatives on the corneal uptake and permeability of pilocarpine and dexamethasone.

Materials

The corneas used in this study were obtained from pigs (Yorkshire and Swedish Landrase, Farmek, Uppsala), aged 6–7 months. ^{3}H-labelled pilocarpine hydrochloride was obtained from Amersham International plc, Amersham, U.K. The compound had a radiochemical purity higher than 96% and a specific radioactivity of 2.06 GBq/mmol (55.7 mCi/mmol). [^{3}H]dexamethasone (1.70 TBq/mmol), with a radiochemical purity of 96.2% was purchased from Amersham, U.K. Unlabelled pilocarpine hydrochloride and dexamethasone were obtained from Sigma Chemicals Company, U.S.A.; BAC, CB and M + P were of pharmaceutical quality (Ph.Eur.). CDG was obtained as a 20% solution (Arlacide G) from ICI. All other chemicals used were of analytical grade.

Methods

Unlabelled and labelled pilocarpine hydrochloride (0.405 mM) or dexamethasone (0.230 mM) were dissolved in glutathione bicarbonate Ringer's (GBR) solution (Schoenwald and Huang, 1983; O'Brien and Edelhauser, 1977). To the experimental solutions one of the following preservatives was added; BAC (0.01%) CDG (0.01%), CB (0.5%), and M (0.04%) + P (0.02%). These concentrations of the preservatives are often used in commercial ophthalmic formulations. The corneal permeability of pilocarpine and dexamethasone was studied in perfusion models (Camber, 1985). Paired corneas not older than 1 h from death of the animals were used. In each pair, the experimental cornea was exposed to preservative or de-epithelized and the contralateral one served as control. To the donor side of the apparatus, the above-mentioned solutions were added and 100 μl were withdrawn from the receiving side every 40 min for a period of 4 h. The samples were mixed with a scintillation cocktail (Opti Phase 'MP', FSA Laboratory Supplies, U.K.) before being counted in a 1214 Rackbeta liquid scintillation counter, Wallac Oy, Finland.

The apparent permeability coefficients (P_{app}, cm/s) were determined as described previously (Camber, 1985). The corneal uptake of the substances was determined upon completion of the experiment. The remaining scleras of the corneas were removed and the trimmed corneas were placed in scintillation vials and weighed. After solubilization with a tissue solubilizer (Soluene-350, Packard) the resulting solutions were treated with isopropanol and hydrogen peroxide before the scintillation cocktail (Dimilume-30, Packard) was added.

Results

The P_{app} for pilocarpine and dexamethasone during the different perfusion conditions are shown in Table 1. For the interval 80–240 min, the increase of radioactivity on the endothelial side was a linear function irrespective of the presence or absence of preservatives and corneal epithelium. The preservatives could be divided into two groups according to their promoting effect on the corneal uptake and permeability of pilocarpine and dexamethasone. Fig 1a and 2a show clearly that BAC and CB form one group, whereas CDG together with M + P give the other. Benzalkonium chloride and CB had the largest effect on the

TABLE 1

Corneal permeability and uptake of pilocarpine and dexamethasone

Substance	Perfusion condition	Permeability coefficient $(cm \cdot s^{-1}) \times 10^6$, mean (S.D.)	Mean difference (S.D.) [2]	Corneal uptake $\frac{(DPM/g \text{ of cornea})}{(DPM/ml \text{ of solution})} \times 100$ mean (S.D.)	Mean difference (S.D.) [2]	Number of paired corneas
Pilocarpine HCl	Normal	6.42 (0.68)	–	11.31 (0.98)	–	30 [1]
	BAC	10.85 (0.91)	4.44 (1.20) ***	19.77 (1.13)	8.08 (1.25) ***	6
	CB	11.71 (0.72)	5.04 (1.20) ***	16.70 (2.97)	6.22 (2.73) **	6
	CDG	7.15 (0.99)	1.21 (1.22)	13.40 (2.18)	2.12 (1.81) *	6
	M + P	7.89 (0.63)	1.55 (0.93) *	12.17 (1.30)	1.10 (1.32)	6
	De-epithelized cornea	16.78 (2.00)	10.07 (1.88) ***	23.82 (2.79)	11.78 (2.53) ***	6
Dexamethasone	Normal	0.62 (0.14)	–	10.19 (1.97)	–	30 [1]
	BAC	2.07 (0.49)	1.54 (0.50) ***	25.50 (5.30)	16.25 (5.66) ***	6
	CB	2.91 (0.52)	2.22 (0.56) ***	21.47 (5.96)	10.50 (5.68) ***	6
	CDG	0.91 (0.53)	0.29 (0.40)	13.93 (3.87)	3.27 (5.59)	6
	M + P	0.93 (0.22)	0.43 (0.22) **	12.90 (1.44)	3.60 (1.40) **	6
	De-epithelized cornea	9.48 (0.63)	8.75 (0.79) ***	37.33 (8.57)	26.50 (7.49) ***	6

[1] The corneas used are the same as those used as controls in the paired experiments.

[2] Differences in corneal permeability and uptake between corneas perfused during normal and test conditions tested by Student's *t*-test for paired observations. Significances denoted by: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

permeability with a factor of 1.7 for pilocarpine and 4.0 for dexamethasone. The influence of CDG and M + P were of lower magnitude, giving a 1.2-fold increase of pilocarpine, while the influence on the dexamethasone permeability was more pronounced, resulting in a 1.5- and 1.9-fold increase, respectively. The P_{app} increased markedly when the epithelium was removed, about 2.5 times

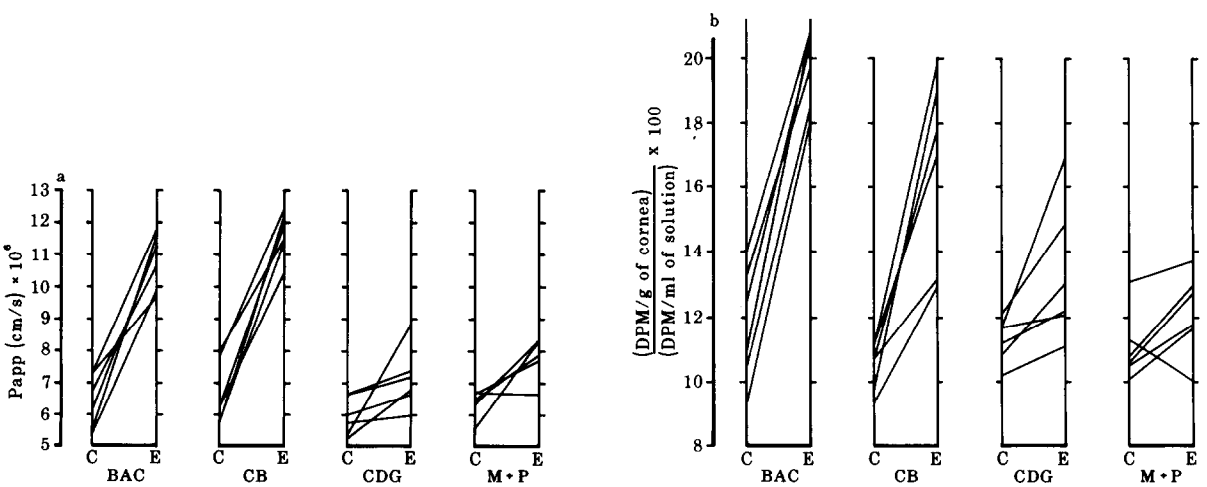

Fig. 1. Influence of preservatives on the corneal permeability (a) and uptake (b) of pilocarpine HCl. Paired corneas were used: control cornea (C), and experimental cornea (E).

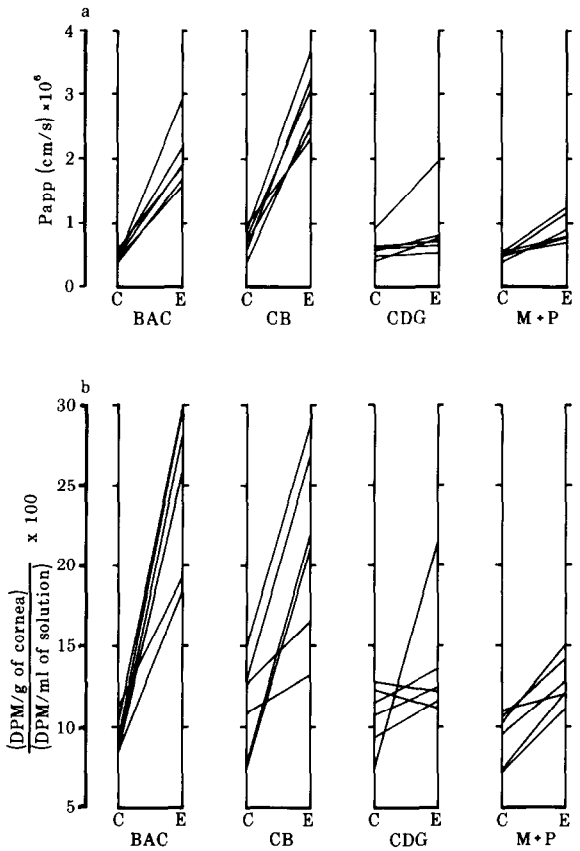

Fig. 2. Influence of preservatives on the corneal permeability (a) and uptake (b) of dexamethasone. Paired corneas were used: control cornea (C), and experimental cornea (E).

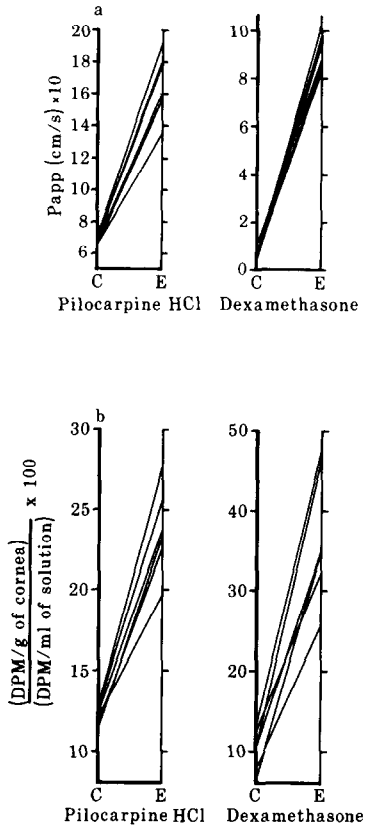

Fig. 3. Influence of removal of the corneal epithelium on the corneal permeability (a) and uptake (b) of pilocarpine HCl and dexamethasone. Paired corneas were used: control cornea (C), and experimental cornea (E).

for pilocarpine and 13 times for dexamethasone (Fig. 3a). The corneal uptake of pilocarpine and dexamethasone after 4 h of perfusion is demonstrated on Fig. 1b, 2b and 3b. A correlation is seen between the corneal permeability and uptake. The influence of the uptake of pilocarpine increased in the following order; M + P and CDG < CB and BAC < de-epithelized cornea. Dexamethasone was in both respects, P_{app} and corneal uptake, more susceptible to preservatives and deepithelization. The following rank order could be given when grouping the promoting effects of preservatives and de-epithelization; CDG < M + P < CB < BAC < de-epithelized cornea.

Discussion

This in vitro study confirmed previous laboratory investigations that preservatives, in clinically used concentrations, increase the corneal permeability and uptake of ophthalmic drugs, e.g. pilocarpine and dexamethasone. In this study BAC and CB had a significant influence on these parameters for the drugs investigated, whereas M + P and CDG had a more modest effect.

The effect mediated by preservatives is due to toxic or damaging effects on the corneal epithelium. Since it is well documented that the epithelium constitutes the main part of the transoc-

ular barrier (Klyce, 1972), an alteration of this membrane will result in increased permeability and corneal uptake. Both in vivo and in vitro studies on the corneal integrity have shown that BAC is considerably more toxic than CDG, giving morphological alterations (Burstein, 1980) and increased permeability of fluorescein (Burstein, 1984). Chlorobutanol and M + P have not been so well investigated, but from studies reported, it is obvious that CB in a concentration of 0.5% gave an almost total loss of the surface epithelial layer after 1 h incubation (Burstein and Klyce, 1977). It is also well documented that corneal damage increases with increased concentration and exposure time.

In this study the cornea was exposed to the preservative for 4 h, which is not comparable with the in vivo situation, where the contact time is much shorter. This has to be kept in mind when evaluating the results obtained. However, the experimental set-up allows a relative comparison between the tested preservatives and their effect on the corneal permeability and uptake of ophthalmic drugs.

This study shows that the preservatives have the largest effect on the corneal permeability and uptake for dexamethasone. Even if dexamethasone has a moderate liphophilicity, removal of the epithelium had a pronounced effect on the permeability. Similar results have been reported by Kupferman and Leibowitz (1974). They showed that de-epithelization increased the aqueous humor concentration of dexamethasone about 4-fold. It is not surprising that a hydrophilic compound such as pilocarpine showed increased permeability and uptake after removal of the corneal epithelium. This has been shown both in vitro (Mitra, 1983), and in vivo (Sieg and Robinson, 1976). But it is rather remarkable that the more liphophilic dexamethasone is relatively more susceptible to removal or alteration of the liphophilic epithelium.

This fact has to be considered when dealing with steroid therapy locally in the eye. An undesirable side effect of corticosteroids is their ability to induce an ocular hypertension (Becker and Mills, 1963; Armaly, 1963; Armaly, 1966). It has been reported that the corneal and aqueous humor concentrations of prednisolone were higher after addition of BAC to an Adsorbobase vehicle with prednisolone phosphate (Green and Downs, 1974). The authors concluded that BAC would be contraindicated in ophthalmic steroid preparations with increased corneal retention time. Our study shows that CDG would be a better alternative to use with respect to its much lower influence on the uptake and permeability of dexamethasone.

However, for drugs with their active site inside the eye globe the permeability enhancing effect of preservatives is beneficial e.g. carbachol (Smolen et al., 1973).

The influence of preservatives on the bioavailability has to be considered, especially when drugs are reformulated, e.g. from a multi-dose formulation with preservatives to a single-dose formulation without preservatives. This means that bioequivalence studies should be conducted when ophthalmic preparations are reformulated with the exclusion of preservatives.

Consideration should also be given when dealing with preservatives and corticosteroids for the eye to vehicles giving an extended corneal contact time.

In summary, BAC and CB had a significant effect on the corneal permeability and uptake of pilocarpine and dexamethasone, whereas CDG had no or only minor influence on these parameters. These results imply that CDG is the preservative which maintains the original or initial permeability of the cornea and in that respect it seems that CDG could be the preservative of choice, especially in therapy where the drug should act locally.

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