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Polymer effects on ocular bioavailability. II. The influence of benzalkonium chloride on the mydriatic response of tropicamide in different polymeric vehicles

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Summary

The effect of 0.01% benzalkonium chloride (BZ) on the mydriatic response of tropicamide in different vehicles was tested on rabbits and on humans. The vehicles were an isotonic buffered solution (AS) and four iso-viscous polymeric solutions: low molecular weight hydroxypropylcellulose (HPCL), medium molecular weight hydroxypropylcellulose (HPCM), poly(vinyl alcohol) (PVA) and poly(vinylpyrrolidone) (PVP).

In rabbits, the BZ-containing vehicles did not show any statistically significant activity difference respect to identical, BZ-free preparations. In humans, BZ did not produce any significant bioavailability increase in AS, even at the 0.05% concentration, while it appeared to depress the bioavailability enhancement produced by the polymeric solutions. This effect was statistically significant ($P < 0.05$) for PVA and PVP.

On the basis of some physicochemical evidence, the hypothesis is advanced that the reduced efficacy in humans of the polymeric vehicles in the presence of BZ may be due to a reduced adhesion tension (A_T) of the solutions to the corneal surface. This would interfere with the blinking-assisted spreading process of the vehicles over

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the precorneal area. The present experiments confirmed the poor performance of rabbits as models for investigations on polymeric ophthalmic vehicles, that may modulate the drug availability not by viscous effects alone, but also by dynamic surface spreading phenomena.

Introduction

A well-known disadvantage of medicated collyria is their short time of contact with the eye, resulting in a very poor bioavailability of the applied drugs. The relevant literature abounds with studies on possible methods for increasing the efficacy of liquid ophthalmic medications. This goal has been accomplished by two basically different approaches. The first consists of the addition of polymers, that may prolong the ocular time of residence of the medication by viscous and surface-spreading effects, thus augmenting the overall amount of drug penetrating into the anterior chamber. The other approach is based on the capacity of certain substances (e.g. the quaternary ammonium germicides) to reduce the diffusional resistance of the corneal structures, thereby increasing the rate of drug penetration. The most widely investigated compound of this type is benzalkonium chloride, an alkylbenzyldimethylammonium chloride mixture with C_8–C_{16} alkyl chains, often added to collyria as preservative at concentrations ranging from 0.004% to 0.03%.

Both in vitro tests and/or in vivo studies on humans (H) and on rabbits (R) have evidenced significant effects operated by benzalkoniun, or by analogous quaternary germicides, on transcorneal penetration of carbachol (O'Brien and Swan 1942, H; Mattila et al. 1968, H; Smolen et al., 1973, R), epinephrine (O'Brien and Swan 1942, H), fluorescein (Green and Tonjum, 1971, R), horseradish peroxidase (Tonjum, 1975, R), inulin (Keller et al., 1980, R), homatropine (Kassem et al., 1983a, H), penicillin (Godbey et al., 1979, R), phenylephrine (Mattila et al., 1968, H), physostigmine (Boyd, 1943, R; Mattila et al., 1968, H), pilocarpine (O'Brien and Swan, 1942, H; Green and Tonjum, 1971, R; Mikkelson et al., 1973, R; Green and Downs, 1975, R; Kassem et al., 1983b, H), and prednisolone (Green and Downs, 1974, R).

The effect of quaternary ammonium compounds is generally attributed to a physical disruption of the epithelial cell membranes and to a widening of the intercellular spaces (Pfister and Burstein, 1976), although other mechanisms, such as competitive inhibition of drug–protein binding in the tear fluids (Mikkelson et al., 1973) or, in the case of carbachol, in the corneal tissue (Smolen et al., 1973) have been proposed.

The effect of polymeric additives used in conjunction with quaternary ammonium derivatives appears to have been little investigated. Indeed, if these agents did operate by discrete, independent mechanisms, ophthalmic drug bioavailability might substantially benefit from their concomitancy. A previous paper from the present authors (Saettone et al., 1984) dealt with the influence of different polymeric additives on the mydriatic response of tropicamide, an important and widely used diagnostic agent in ophthalmology. An investigation on the effect of benzalkonium

chloride, alone or plus polymers, on the activity of the same drug was considered a useful extension of the previous work. Surprisingly, very little attention seems to have been paid to the action of quaternary derivatives on the activity of tropicamide. Mattila and co-workers (1968) reported that cetylpyridinium chloride (0.03%) significantly decreased the human mydriatic response of tropicamide (0.1%) in a methylcellulose solution, when compared with a solution containing the drug alone. The cycloplegic effect of tropicamide in the presence of the quaternary agent, on the contrary, was slightly greater than in its absence, but the difference was not statistically significant. It was hoped that additional investigation might shed more light on these rather peculiar results, adding to the knowledge of the complex biological and physicochemical mechanisms by which polymers and cationic surfactants may modulate the ophthalmic availability of tropicamide in liquid vehicles.

Experimental

Materials and methods

(a) Vehicles. Tropicamide (Prodotti Roche) solutions (0.2% w/w) in 0.2 M, pH 7.0 Sørensen isotonic phosphate buffer plus polymers and benzalkonium chloride (BZ) had the composition indicated in Table 1. All preparations were sterilized by autoclaving at 2 bars for 20 min, and were stored in 20-ml sterile plastic dropper bottles.

(b) Biological studies. The vehicles were evaluated for mydriatic activity both in human volunteers and in rabbits, following essentially the procedures outlined in the previous paper (Saettone et al., 1984). Each preparation was tested on groups of at least 10 subjects, by instilling 50 μl of a preparation in the lower conjunctival sac of one eye, the other serving as reference. When the same subject (human or rabbit)

TABLE 1

COMPOSITION OF THE VEHICLES USED IN THE STUDY

Vehicle	Ingredients
AS	Tropicamide 0.2% in buffer solution
AS/BZ_1	as AS, plus 0.01% w/w BZ
AS/BZ_2	as AS, plus 0.05% w/w BZ
HPCL	as AS, plus 4.5% w/w hydroxypropylcellulose, low MW [a]
HPCL/BZ	as HPCL, plus 0.01% w/w BZ
HPCM	as AS, plus 1.4% w/w hydroxypropylcellulose, medium MW [b]
HPCM/BZ	as HPCM, plus 0.01% w/w BZ
PVA	as AS, plus 5.0% w/w poly(vinyl alcohol) [c]
PVA/BZ	as PVA, plus 0.01% w/w BZ
PVP	as AS, plus 7.5% w/w poly(vinylpyrrolidone) [d]
PVP/BZ	as PVP, plus 0.01% w/w BZ

[a] Klucel LF, Hercules; [b] Klucel MF, Hercules: [c] Polyviol W 48/20, Wacker Chemie; [d] Plasdone K 90, GAF.

received more than one treatment, care was taken to instill the BZ-containing vehicle in the eye that had been previously (at least 2 weeks before) treated with the BZ-free preparation, and vice-versa. The mydriatic activity was evaluated by determining the variation in pupillary diameter vs time of the treated eye with respect to the diameter of the other, untreated eye. Lighting was maintained rigorously constant during the whole testing period, and, on each species, all measurements were carried out by the same operator.

(c) Physico-chemical measurements. Viscosity, surface tension, drug–polymer binding and surface-spreading determinations were performed as indicated previously (Saettone et al., 1984). For the latter test, glass slides wetted with a 0.05% w/w sodium fluorescein solution, instead of water, were used, since addition of the fluorescent marker to the BZ-containing vehicles resulted in formation of a precipitate. The critical micelle concentration (c.m.c.) of BZ in the different vehicles was determined from surface tension vs BZ concentration plots. Contact angle measurements were carried out at 30°C using a NRL contact angle goniometer (Ramè-Hart, Mountain Lakes, NJ, U.S.A.).

Results and Discussion

Biological data

A summary of the biological data (mydriatic activity) obtained with the vehicles under investigation is presented in Table 2. The data for the BZ-free vehicles, reported in Table 2, are the same as in the previous study. The effect of the BZ-containing vehicles was verified on the same groups of subjects (humans or rabbits) which had been submitted to the previous investigation, and the testing was practically simultaneous. The homogeneity of testing subjects and experimental conditions should therefore guarantee the validity of the reported comparisons. In rabbits, the BZ-containing vehicles did not show any definite trend of activity difference with respect to the reference vehicles. In the absence of polymers (AS vs AS/BZ) a reduced peak time was observed, and an increased ($P < 0.05$) duration, but the area under the activity–time curve (AUC) was not significantly influenced by BZ. In the presence of BZ plus polymers, the peak time showed an increase in all cases (20–50 min), possibly indicating a delayed absorption of the medicament, but all other activity parameters were not significantly altered with respect to the BZ-free preparations. The rabbit AUC data, also presented in graphical form in Fig. 1, confirm the absence of significant effects produced by BZ in the experimental animals.

The human data, reported in Table 2, show that the surfactant, both at the 0.01% (AS/BZ_1) and at the 0.05% (AS/BZ_2) concentration, did not substantially influence the activity parameters of the aqueous saline solution of tropicamide (AS); only a slightly decreased peak time (20–30 min) being apparent. In the presence of HPCL and HPCM, the addition of 0.01% BZ produced a slight peak time increase and a reduction of I_{max}, resulting in a light (not statistically significant) AUC decrease with respect to the BZ-free polymeric solutions. The reduction of the relative

TABLE 2

SUMMARY OF HUMAN AND RABBIT ACTIVITY DATA OF TROPICAMIDE IN THE VEHICLES UNDER STUDY

Vehicle	Peak time (min)	I_{max} [a] (mm, ±95% CL)	Duration [b] (min, ±95% CL)	AUC [c] (cm^2, ±95% CL)
Human data				
AS	90	2.3 (0.3)	240 (20)	71 (20)
AS/BZ_1	60	1.7 (0.3)	210 (20)	56 (24)
AS/BZ_2	70	2.4 (0.3)	250 (20)	79 (26)
HPCL	20	4.0 (0.4)	240 (10)	173 (21)
HPCL/BZ	30	3.1 (0.6)	260 (60)	128 (30)
HPCM	30	3.7 (0.3)	300 (20)	156 (36)
HPCM/BZ	40	2.5 (0.5)	250 (10)	108 (22)
PVA	40	4.5 (0.3)	420 (20)	270 (26)
PVA/BZ	60	3.5 (0.1)	400 (20)	202 (36)
PVP	20	4.0 (0.3)	360 (20)	245 (42)
PVP/BZ	45	3.5 (0.2)	340 (20)	195 (27)
Rabbit data				
AS	90	1.8 (0.3)	500 (30)	134 (28)
AS/BZ	30	2.0 (0.2)	640 (40)	157 (27)
HPCL	30	2.1 (0.2)	710 (40)	192 (24)
HPCL/BZ	60	2.6 (0.3)	645 (40)	252 (41)
HPCM	40	2.1 (0.1)	680 (60)	202 (32)
HPCM/BZ	90	2.4 (0.1)	700 (40)	230 (30)
PVA	40	2.5 (0.2)	630 (60)	216 (42)
PVA/BZ	60	2.3 (0.3)	740 (50)	223 (29)
PVP	60	2.5 (0.3)	670 (35)	221 (36)
PVP/BZ	90	2.2 (0.3)	670 (50)	210 (36)

[a] Peak mydriasis intensity. [b] Time for pupil to return back to normal. [c] Area under the mydriasis–time curve. AUC data were calculated from graphs where units are $mm \cdot min^{-1}$. On the vertical axis, 5.0 cm corresponded to 1.0 mm of pupil diameter increase; on the horizontal axis 5.0 cm corresponded to 100 min.

bioavailability, however, was statistically significant ($P < 0.05$) when the surfactant was present in the PVA and PVP solutions. In both cases, as in the previous ones, BZ increased the peak time and reduced the I_{max}. A graphical illustration of the human AUC data is presented in Fig. 2.

The lack of a positive influence of BZ on the ocular bioavailability of tropicamide in the rabbit, both in the absence and in the presence of polymers, appeared rather surprising, particularly in view of the numerous literature reports indicating the capacity of quaternary ammonium germicides to augment the transcorneal absorption of several drugs in this animal. The human results in the absence of added polymers were also unexpected, since they showed that BZ did not actually increase the mydriatic response of tropicamide even at the rather high concentration of 0.05%. The reduced relative bioavailability of the drug, observed when BZ was added to the polymeric vehicles (although not entirely unexpected on the premise of

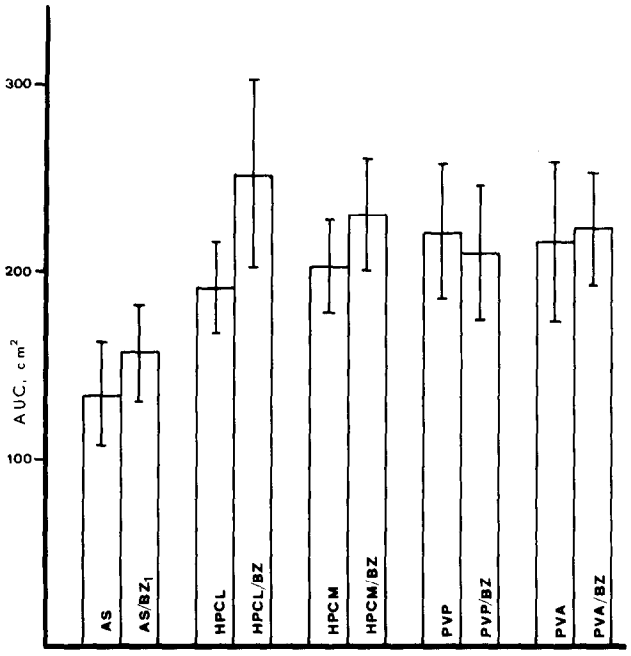

Fig. 1. Areas under the mydriasis response vs time curves (AUC) obtained in rabbits with the vehicles under study. Vertical lines over bars indicate 95% confidence limits.

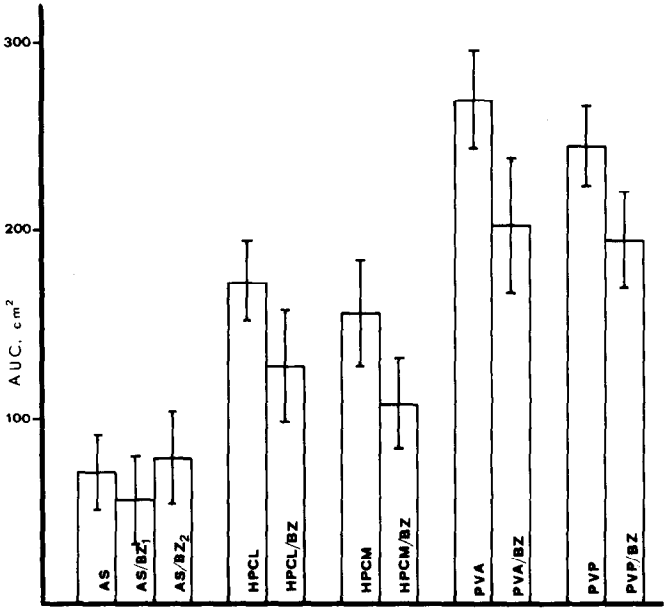

Fig. 2. Areas under the mydriatic response vs time curves (AUC) obtained in humans with the vehicles under study. Vertical lines over bars indicate 95% confidence limits.

the results of Mattila et al. (1968) mentioned in the introduction), was difficult to rationalize on the basis of the data at hand.

The previous study had substantially benefited from experiments, in which a fluorescent marker was added to the polymeric vehicles in order to permit an in vivo evaluation of the behaviour and of the retention time in the eye. The presence of BZ in the vehicles precluded these experiments, since on addition of sodium fluorescein the solutions became cloudy, probably as a result of formation of a BZ–fluorescein insoluble complex or salt. When instilled in the attempt to evaluate their spreading capacity and retention time, these turbid, highly fluorescent solutions gave erratic results, and the experiments were forsaken.

Physicochemical data

(a) Vehicle viscosity and rheological behaviour. The BZ-free vehicles had been purposely formulated in order to show very similar viscosities, so that the observed biological results would not be influenced by this variable. The relevant rheograms have been reported in the previous paper of this series (Saettone et al., 1984). HPCL, PVA and PVP showed an apparently Newtonian behaviour, and their viscosities were 66.3, 66.8 and 72.3 cps at 30°C, respectively. HPCM was pseudoplastic, with an apparent viscosity of 76.8 cps at a rate of shear of 700 s^{-1}. The addition of 0.01% w/w BZ resulted, in two of the polymeric vehicles, in a viscosity increase. In particular, the viscosity of PVA rose, in PVA/BZ, to 87.5 cps (+19 cps), that of PVP rose, in PVP/BZ, to 77.4 cps (+5 cps). The viscosity of HPCL was practically unmodified in the presence of the surfactant, and so was the apparent viscosity and the rheological behaviour of HPCM. The viscosity changes operated by 0.01% w/w BZ on PVA and on PVP should have produced, if any, a positive effect on the retention time of the vehicles in the eye, and hence a bioavailability increase, not a decrease. However, the viscosity changes were rather modest, and probably not capable as such of modifying the biological response of the preparations to a significant degree. On the other hand, the physicochemical significance of the phenomenon, which is indicative of formation of a polymer–surfactant 'complex' or polymer nucleated micelle (Tadros, 1974) is of particular interest. Such binding converts the initially non-ionic polymer into a polyelectrolyte, where the repulsions between the bound ions results in an increase in size, and hence in an increase of viscosity (Molyneux, 1984). It might be of interest to note that only the polymeric solutions that bound the surfactant to an appreciable degree, such as PVA and PVP, showed a significantly reduced human bioavailability of tropicamide in the presence of BZ. This may point to an influence of e.g. the conformational size of the polymer chains in solution on some parameter influencing transcorneal absorption in humans, such as the spreading characteristics and/or the thickness of the medicated precorneal film.

(b) Tropicamide binding by the vehicles. Table 3 reports the results of dynamic dialysis experiments, aimed at verifying if BZ would modify the binding capacity of the polymers for tropicamide. The data clearly show that the surfactant did not appreciably influence this parameter.

(c) Surface properties of the vehicles. Some of the main surface properties of the

TABLE 3

TROPICAMIDE BINDING BY THE VEHICLES

Vehicle	Tropicamide (% bound)
AS	0
AS/BZ	0
HPCL	28.7
HPCL/BZ	29.0
HPCM	31.1
HPCM/BZ	31.9
PVA	14.9
PVA/BZ	13
PVP	33.0
PVP/BZ	35.8

vehicles are summarized in Table 4. These include the surface tension, γ, the advancing contact angle on glass, θ, the product $\gamma \cos \theta = A_T$, corresponding to the adhesion tension of the solutions to glass, and the height of rise of the solutions on vertical, wet glass slides. Addition of 0.01% w/w BZ to the tropicamide solutions resulted in a strong decrease of the surface tension of AS (-29.2 dine/cm) and of PVP (-26.8 dine/cm). The surface tension of PVA, and of the two cellulose derivatives HPCL and HPCM was lowered to a smaller extent (-11.1, -7.5 and -6.3 dine/cm, respectively). The surface tension values of all BZ-containing vehicles fell into a relatively small range (30.6–38.8 dine/cm).

The contact angle on glass of AS and of all polymeric solutions, to the exception of PVP, was also decreased to some extent by BZ, thus indicating an improved wettability of the surface. In no case was complete wetting observed, on account of the fact that the surface (a microscope slide cover), not being pure silica, was slightly hydrophobic.

TABLE 4

SURFACE PROPERTIES OF THE VEHICLES

Vehicle	Surface tension, γ (dine/cm)	Contact angle on glass, θ (degrees)	$A_T = \gamma \cos \theta$ (dine/cm)	Height of rise on glass slide (mm)
AS	60.5	22	56.1	0
AS/BZ	31.3	15	30.2	0
HPCL	38.8	30	33.6	1.30
HPCL/BZ	31.3	27	27.8	0
HPCM	39.8	34	33.1	0
HPCM/BZ	33.5	29	29.3	0
PVA	45.2	26	40.6	8.10
PVA/BZ	34.1	19	32.2	4.05
PVP	57.4	30	49.7	5.50
PVP/BZ	30.6	31	26.2	3.30

The values of the product $\gamma \cos \theta$, representing the adhesion tension, A_T, of the solutions to the glass surface, are indicative of the affinity of the liquids for the solid. The ability of a liquid to form a continuous film over a solid surface depends on A_T, that corresponds also to the vertical component of the force, resulting from the surface tension, determining the rise of liquids in capillary tubes (Adamson, 1982). An inspection of the data shows that the A_T value of all solutions was depressed by BZ, thus indicating a reduced affinity for the solid surface. A confirmation of the influence of BZ on the adhesion tension of the solutions came from determinations of the climbing capacity of the vehicles on a glass slide. The relevant data, also reported in Table 4, show that this property, particularly evident in some polymeric solutions (PVA, PVP and HPCL), was significantly reduced in the presence of the surfactant. Previous work (Saettone et al., 1984) had shown, in agreement with the results of Benedetto and co-workers (1975), that the height of rise, in mm, of the polymeric solutions on a vertical glass slide from a small trough simulating the marginal strip reservoir could be directly related to the human ophthalmic bioavailability of tropicamide from the same solutions. This vehicle parameter, rather than the surface or interfacial tension or the contact angle, indicates the capacity of the polymer solutions of spreading at an air–tear interface, thus forming a stable film, whose thickness may vary depending on the chemical structure of the polymer, and possibly, on its conformation in solution. The relationship between this vehicle effect and human tropicamide bioavailability was rationalized assuming that only polymeric drug-loaded films endowed with good spreading properties at the air–tear interface might re-expand after each blink, due to the high surface pressure generated by the action of the descending lid, and rise again over the corneal (absorbing) surface. Thus, in humans, blinking would be instrumental in recoating the cornea with fresh drug solution from the marginal strip reservoir, provided that suitable polymeric agents be present in the vehicle to assist the process. For a more exhaustive discussion, the reader is referred to the original paper of Benedetto et al. (1975), and to the previous paper of this series (Saettone et al., 1984).

The reported data might provide a basis for understanding the reduced human bioavailability of tropicamide from the polymeric solutions in the presence of BZ. Indeed, if the spreading capacity on glass parallels, as it would appear, the spreading on the corneal surface, any factor interfering with spreading, as the presence of the surfactant, would influence the overall retention time of the vehicle over the precorneal area, and hence the drug bioavailability. The observed effect on A_T (and on corneal spreading) appears largely mediated by a strong reduction of the surface tension, operated by the surfactant. As pointed out by Holly (1978), an ideal tear substitute (or ophthalmic vehicle) should show a high adhesion tension to non-polar solids, but preferably a fairly high surface tension. A drastic lowering of the surface tension of the solution with a small molecular weight surfactant, besides increasing the risk of epithelial damage, may have a negative effect on the stability of the fluid film over the corneal surface. The effect of cationic surfactants like BZ to decrease the stability of thin aqueous films is well known (Padday, 1970), and a negative effect of BZ on tear film breakup time has also been reported (Wilson et al., 1975).

The latter observations might also explain the lack of influence of BZ on the

ophthalmic availability of tropicamide from the aqueous saline solution, AS. In the present experiments, contrary to many literature reports on other ophthalmic drugs, addition of BZ to an aqueous solution of tropicamide did not produce in either species (even at the relatively high 0.05% concentration in humans), any significant bioavailability increase. This may appear rather surprising, on consideration of the well-documented effect of cationic surfactants on corneal permeability. The following hypotheses might be advanced to explain the lack of influence of BZ on tropicamide absorption in the eye: (a) a reduction of the already poor stability of the precorneal film formed by the saline solution, AS; (b) an increased lacrimation, caused by the surfactant particularly at the higher concentration, resulting in an accelerated drainage of the instilled solution; and (c) an inclusion of the drug in surfactant micelles. Concerning the latter point, it should be observed that the critical micelle concentration (c.m.c.) of BZ in the different solutions, as measured from surface tension vs BZ concentration graphs, was (in g% w/w) 0.0027 for AS, 0.050 for HPCL, 0.025 for HPCM, 0.014 for PVA and 0.024 for PVP. Thus, only the AS/BZ solutions contained the surfactant at concentrations (0.01 and 0.05% w/w) greater than the c.m.c. In any case, the hypothesis of a reduced penetration of the drug resulting from micellar entrapment should be considered with some caution, in view of the relatively high concentration of tropicamide (0.2% w/w) with respect to the concentration of the surfactant. It is hoped that further in vitro and in vivo studies, now in progress, will clarify this point.

Conclusions

The following conclusions may be presented as the essential outcome of the present investigation.

Benzalkonium chloride is undoubtedly a very efficient preservative for ophthalmic solutions. However, at least in the case of tropicamide, it did not produce any bioavailability improvement in an aqueous solution, and it appeared to depress the bioavailability enhancement produced by some polymeric solutions. In view of these effects, and of the well known adverse effects of BZ on tear film stability, and on the integrity of the corneal epithelium, the use of this quaternary ammonium derivative as preservative for polymeric ophthalmic vehicles containing tropicamide (and possibly other drugs) should be carefully considered, and clinical tests should be carried out in order to define the actual advantages of the preparations.

The effects on tropicamide bioavailability operated by the different polymers and by BZ were evident on humans, but they went practically undetected on rabbits. This indicates further the poor performance of these experimental animals in investigations of effects, essentially mediated by surface spreading phenomena (cf. Saettone et al., 1982). The absence of blinking, and the different precorneal dynamics of instilled solutions, with respect to humans, may severely limit the usefulness of rabbits in studies, where information on the behaviour of polymeric solutions (drug vehicles, or artificial tears) is desired.

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