Pakistan Journal of Pharmaceutical Sciences
5(1) 25-36 (1992)

EVALUATION OF PRESERVATIVES IN EYE-DROP PREPARATIONS

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ABSTRACT

A systematic study of commonly used commercial eye-drop preparations has been made by identification and assay of the added preservatives, i.e. benzalkonium chloride, parabens, phenylmercuric nitrate and thiomersal, hi forty sit samples. The commercial samples with known preservatives contain an amount, almost in agreement with the values stated on label. Twenty fie samples with identified preservative (not stated on label), contain the added compound 50-150% w/v in excess of the recommended limits and thus may be harmful to the eyes. U.K., U.SA. and many other countries has strict regulations regarding the use of preservatives in eye preparations, because of their potential toxicity. Similar regulations should be promulgated in Pakistan for locally manufactured products.

Introduction

The eye is particularly vulnerable to infection and ophthalmic preparations are required to be sterile. Since 1940s several reports warning the hazards of using contaminated eye-drops have appeared (Allen, 1959; Dale *et al*, 1959; Crompton, 1962). Among these occurrence of a number of unfortunate incidents (Savin, 1967) and loss of eye after the use of solutions contaminated with *Pseudomonas aeruginosa* (Gunn and Carter, 1965; Ayliffe *et al.*, 1966) are noteworthy.

The use of antimicrobial preservatives in eye-drop preparations provides assurance that products are not contaminated with organisms which affect their safety, efficacy or acceptability to the patient. An ideal preservative for a particular formulation should possess certain antimicrobial and physicochemical characteristics as well as a low toxicity profile (Beveridge, 1983; Bloomfield, 1988). Factors influencing the choice of preservatives for pharmaceutical products including eye-drop preparations are reviewed in detail (Bean, 1972; Russell, 1974; Block, 1977; Cowen and Steiger, 1977).

In the pharmaceutical industry, there appears to be a tendency to rely entirely on chemical preservatives to protect the product against microbial contamination. It is, therefore, important to take into consideration the increasing concern over possibilities of toxic effects associated with preservatives, in particular, when the added amounts exceed the usual concentration limits.

The present study has been conducted to make a systematic survey of forty six commonly used commercial eye-drop preparations containing benzalkonium chloride, parabens, phenylmercuric nitrate and thiomersal as preservative and to point out whether a manufacturer is add[i]ng these compounds at higher than the preservative levels to prove toxic enough to cause damage to the eye.

Materials and Methods

Eye-drop preparations

The following freshly prepared eye-drop preparations were procured directly from the manufacturer or through distributor:

(A) Eye-drops containing preservative not stated on label (Locally manufactured).

1; b) Atropine 1% w/v (two brands);
2a, b) Atropine 2% w/v (two brands);
3a, b) Atropine sulphate 1% w/v (two brands);
4a, b) Atropine sulphate 2% w/v (two brands);
5a., b, c) Sulphacetamide 10% w/v (three brands);
6a, b, c) Sulphacetamide 20% w/v (three brands);
7a, b, c) Sulphacetamide 30% w/v (three brands);
8) Betamethasone 0.1% w/v,
9) Dexamethasme 0.1% w/v
10a, b, c) Chloramphenicol;
11) Dexamethasone-Neomycin;
12) Trimethoprim-Polymyxin;
13) Polymyxin-Gramicidin.

(B) Eye-drops containing preservative (Labelled amount, % w/v, given in parenthesis) along with the country of manufacture.

i) Benzalkonium chloride:
14) Atropine 1% w/v, USA (0.01%);
15) Dexamethasone 0.1% w/v, USA (0.01%);
16) Carbachol 3% w/v, USA (0.01%);
17) Fluorometholone 0.1% w/v, USA (0.004%);
18) Dexamethasone-Neomycin, USA (0.004%).

ii) Parabens (Methyl paraben, MP; Propyl paraben, PP):
19) Sulphacetamide 10% w/v, USA (MP 0.002%, PP 0.05%);
20a, b) Sulphacetamide 10% w/v, Pak (MP 0.05%, PP 0.01%) (two brands);
21) Sulphacetamide 20% w/v, USA (MP 0.002%, PP 0.05%);
22) Sulphacetamide 20% w/v, Pak (MP 0.05%, PP 0.01%).

iii) Phenylmercuric nitrate:

23) Atropine 0.25% w/v, UK (0.002%);
24) Atropine *05% w/v*, UK (0.002%);
25) Atropine 1% w/v, UK (0.002%);
26) Atropine 2% w/v, UK (0.002%);
27) Antazolin Compound, Swiss (0.002%).

iv) Thiomersal:

28) Sulphacctamide 10% w/v, USA (0.01%);
29) Sulphacetamide 20% w/v, USA (0.01%);
30) Chloramphenicol 05% w/v, USA (0.01%);
31) Amphotericin 0S% w/v, Germany (0.004%);
32) 5-Fluorocytosine 1% w/v, Germany (0.005%);
33) Betamethasone 0.1% w/v, Pak (0.005%).

Preservatives

Benzalkonium chloride solution 50% w/v (BP), methyl hydroxybenzoate (methyl paraben), propyl hydroxybenzoate (propyl paraben), phenylmercuric nitrate and thiomersal were obtained from BDH. They were used as analytical standards.

Reagents and solvents

All reagents and solvents were analytical grade or of the purest form available from BDH/Merck. The solvents (e.g. chloroform and ethanol) were redistilled before use.

Identification of preservatives

The unknown preservatives present in eye-drop preparations were identified by applying British Pharmacopoeia (1988) tests and screening methods reported by Jacobs (1958) and Rangana (1977).

Assay of preservatives

Benzalkonium chloride, parabens and phenylmercuric nitrate/thiomersal were assayed spectrophotometrically by the methods of Ballard *et al.* (1954), Blaugh and Grant (1974) and Joint A.B.C.M.-SA.C. Committee (1956) respectively.

Results and Discussion

Identification and assay of preservatives

The pharmaceutical manufacturers in the U.K. and U.S.A. are requ[i]red to state on label the identity and concentration of preservative added to an eye-drop

preparation to maintain sterility and to caution the physician of its possible harmful effects. However, the local eye-drop manufacturers do not state the preservative and its concentration on the label of their products. It was, therefore, necessary to identify the added preservative in these preparations. The identification of preservatives in twenty five eye-drop samples alongwith the determined concentration of each compound is reported in Table 1. Atropine (la,b; 2a,b), betamethasone (8), dexamethasone (9) and demethasone-neomycin (11) contain benzalmnium chloride (0.019-0.030% w/v, 25-50% in excess of recommended limit), sulphacetamide (5a,b,c;6a,b,c;7a,b,c) contains parabens (0.142-0.218% w/v, 42-110% in excess of recommended limit), atropine sulphate (3a,b;4a,b) contains phenylmercuric nitrate (0.012-0.016% w/v, 20-60% in excess of recommended limit) and chloramphenicol (10a,b,c), trimethoprim-polymyxin (12) and polymyxinneomycin-gramicidin (13) contain thiomersal (0.019-0.025% w/v, 90-150% in excess of recommended limit). Thus the preservatives present in these preparations are much in excess (50-150% w/v) of the recommended limits and could prove to be highly toxic and harmful to the eye.

As the various preservatives in eye-drop preparations are present at very low concentrations, highly sensitive and specific analytical methods are requ[i]red for the[i]r assay. The pharmacopoeial methods (BP, USP) are not sensitive enough to determine the preservatives at the level added to eye-drop preparations and are thus unsuitable for assay. The spectrophotometric methods used in this study were found to be reliable for the assay of individual compounds. All determinations were carried out in duplicate and results presented in averages. When the duplicate results showed a variation of more than five percent, the assays were repeated until satisfactory data obtained. The assay results of various preservatives in eye-drop samples 14-33 are given in Table 2. All these samples were found to contain the preservative concentration almost in agreement with the label value and thus within the recommended limits (Mullins and Cadwalladar, 1976; Talman, 1977; Parrott, 1979). These preparations may be considered safe for ophthalmic use.

Preservative safety and regulatory aspects

The preservative system is a vital part of a formulation in assuring that a product will maintain predetermined microbiological standards from the date of manufacture until it is used (Raiman, 1970). Antimicrobial preservatives are added to eye-drop preparations because they are toxic to certain microorganisms whose pathogenic actions are unacceptable or which cause product spoilage or deterioration. At the same time these substances should be totally safe to the eye. The commonly used preservatives have recommended limits for effective antimicrobial concentration above which they can be toxic to the patient. It is always advisable to use the lowest effective concentration of a preservative, however, its chemical concentration may not be a true measure of the effective concentration (Ansel, 1969). The various problems associated with the use and development of preservatives for pharmaceuticals are discussed by Wedderburn (1964). New preservatives are subject to a number of mandatory tests to provide toxicity data as a legal requ[i]rement before approval (Hayden, 1988).

Table 1

Identification/content of preservatives in commercial eye-drops

Sample No.	Product	Preservative** (percent w/v)			
		Benzalkonium chloride	Parabens	Phenylmercuric nitrate	Thiomersal
1a	Atropine 1% w/v	+ (0.025)	-	-	-
b	Atropine 1% w/v	+ (0.026)	-	-	-
2a	Atropine 2% w/v	+ (0.019)	-	-	-
b	Atropine 2% w/v	+ (0.030)	-	-	-
3a	Atropine sulphate 1% w/v	-	-	+ (0.014)	-
b	Atropine sulphate 1% w/v	-	-	+ (0.013)	-
4a	Atropine sulphate 2% w/v	-	-	+ (0.012)	-
b	Atropine sulphate 2% w/v	-	-	+ (0.016)	-
5a	Sulphacetamide 10% w/v	-	+ (0.190)	-	-
b	Sulphacetamide 10% w/v	-	+ (0.155)	-	-
c	Sulphacetamide 10% w/v	-	+ (0.142)	-	-
6a	Sulphacetamide 20% w/v	-	+ (0.205)	-	-
b	Sulphacetamide 20% w/v	-	+ (0.162)	-	-
c	Sulphacetamide 20% w/v	-	+ (0.185)	-	-

(Table 1 .... cont'd)

Sample No.	Product	Preservative** (percent w/v)			
		Benzalkonium chloride	Parabens	Phenylmercuric nitrate	Thiomersal
7a	Sulphacetamide 30% w/v	-	+ (0.182)	-	-
b	Sulphacetamide 30% w/v	-	+ (0.181)	-	-
c	Sulphacetamide 30% w/v	-	+ (0.210)	-	-
8	Betamethasone 0.1% w/v	+ (0.021)	-	-	-
9	Dexamethasone 0.1% w/v	+ (0.020)	-	-	-
10a	Chloramphenicol	-	-	-	+ (0.025)
b	Chloramphenicol	-	-	-	+ (0.019)
c	Chloramphenicol	-	-	-	+ (0.025)
11	Dexamethasone-Neomycin	+ (0.022)	-	-	-
12	Trimethoprim-Polymyxin	-	-	-	+ (0.019)
13	Polymyxin- Neo-Gramicidin	-	-	-	+ (0.020)

*Identity and concentration not stated on label.
** + Sign indicates the presence of a particular preservative.

Table 2

Content of preservatives in commercial eye-drop.

Sample No.	Product	Preservative	Labelled amount percent w/v	Determined amount percent w/v	Recommended Limit** percent w/v
14	Atropine 1% w/v	Benzalkonium chloride	0.01	0.010	0.004-0.02
15	Dexamethasone 0.1% w/v		0.01	0.011	
16	Carbachol 3% w/v		0.01	0.009	
17	Fluorometholone 0.1% w/v		0.004	0.004	
18	Dexamethasone-Neomycin		0.004	0.003	
19	Sulphacetamide 10% w/v	Parabens	0.052	0.054	0.001-0.1
20a	Sulphacetamide 10% w/v		0.060	0.063	
b	Sulphacetamide 10% w/v		0.060	0.062	
21	Sulphacetamide 20% w/v		0.052	0.053	
22	Sulphacetamide 20% w/v		0.060	0.062	

(Table 2 ..... cont'd)

Sample No.	Product	Preservative	Labelled amount percent w/v	Determined amount percent w/v	Recommended Limit** percent w/v
23	Atropine 0.25% w/v	Phenylmercuric nitrate	0.002	0.002	0.002-0.005
24	Atropine 0.5% w/v		0.002	0.003	
25	Atropine 1% w/v		0.002	0.002	
26	Atropine 2% w/v		0.002	0.003	
27	Antazolin Compound		0.002	0.003	
28	Sulphacetamide 10% w/v	Thiomersal	0.01	0.011	0.001-0.01
29	Sulphacetamide 20% w/v		0.01	0.011	
30	Chloramphenicol 0.5% w/v		0.01	0.010	
31	Amphotericin 0.5% w/v		0.004	0.005	
32	5-Fluorocytosine 1% w/v		0.005	0.005	
33	Betamethasone 0.1% w/v		0.005	0.006	

*Identity and concentration stated on label.
**Jaconia, 1972; Mullins and Cadwallader, 1976; Talman, 1977; Parrott, 1979; USP, 1985.

If the preservatives are present at the lowest effective concentrations or within the recommended limits as observed in eye-drop samples 14-33, there is no cause for concern about their possible toxic effects. However, the use of preservatives at concentrations much higher than the preservative levels or recommended limits, as determined in eye-drop samples 1-13 (50-150% in excess of recommended limits), and their degradation products (Connors *et al.*, 1986), constitute a toxic hazard and the patient is at potential risk for damage to the eye. Some local eye-drop manufacturers do not seem to consider the serious implications of use and consequent harmful effects of the preservative at concentrations higher than the safe limits (Carter, 1972; Jaconia, 1972; Mullins and Cadwallader, 1976; Talman, 1977; Boylan, 1986; USE, 1985).

The eye is very sensitive to chemicals particularly those which are toxic on contact with the eye tissues. Since the preservatives are potentially irritating to the posterior surface of cornea, iris and anterior chamber, the eye-drops containing preservatives are not officially allowed for use to the injured eye (USP, 1985). Several harmful and irritating effects of benzalkonium chloride (Riegehnan *et at*, 1956; Fisher and Stillman, 1972; Davies, 1973; Honigman, 1975), parabens (Nathan and Spear, 1961; Hugo and Foster, 1964; Reynolds, 1982), phenylmercuric nitrate (Foster, 1965; Abrams and Majzout, 1970), and thiomersal (Honigman, 1975) have been reported. The type and extent of corneal damage caused by preservatives (King et at, 1956) and the nature of their allergic reactions (Smith, 1987) are well known. It is, therefore, advisable to exercise great care and control in the use of right concentration of preservatives, to avoid any harm to the eyes. The preservatives cannot and should not be used as a means of achieving sterility. The intending function of the preservative is to prevent the growth of microorganisms, inadvertently introduced during use, and not to serve as a manufacturing aid (Mullins and Fleming, 1979).

The use of preservatives is regulated in many countries. In the U.K. and U.S.A., preservatives are regulated by the Department of Health and Social Security under the Medicines Act 1968 and by the Food and Drug Administration under the Federal Food, Drug and Cosmetic Act, respectively. In the EEC countries, control of the use of preservatives in pharmaceuticals is defined in Directive 83/570/EEC or under individual national legislation. The regulation of preservatives is because of their potential toxicity rather than for inadequacy as preservatives.

World Health Organization has emphasized the need for regulation of pharmaceuticals in developing countries in order to carry out proper implementation of national drug policies (Jayasuriya, 1985). An important aspect of this need has arisen out of the indiscriminate use of additives such as preservatives and stabilizers in pharmaceuticals, for which there is no legal requirement of disclosure on the label (Hassan, 1985). The use of excessive amounts of preservatives in eye-drop preparations is of particular concern from the safety point of view and warrants measures for its control. The control of preservatives is also necessary to provide

assurance of quality, safety and efficacy of our pharmaceutical products exported to the international markets. The use of preservatives in pharmaceuticals should be regulated in Pakistan to ensure patient safety and to bring it at par with practices in developed countries.

Acknowledgement

The authors wish to thank Dr. G. Hassan, former Director, Quality Control, Merck Sharp & Dohme of Pakistan Ltd., for helpful suggestions.

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