Evaluation of antimicrobial effectiveness of ophthalmic drops according to the pharmacopeial tests criteria

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ABSTRACT

Background: In this study antimicrobial effectiveness test was performed on eye-drops which had high microbial contaminations in hospital practice to find out whether their antimicrobial efficacies affect the magnitude of microbial contamination during their uses.
Materials and Methods: Artificial tear, atropine sulfate, betamethasone, homatropine hydrobromide, phenylephrine hydrochloride, phenylephrine zinc, pilocarpine hydrochloride, tetracaine hydrochloride and tropicamide eye-drops were subjected to the United States Pharmacopeia (USP) and British Pharmacopeia (BP) antimicrobial preservative effectiveness tests.
Results: The results of this study showed that eight out of the nine products met the BP 'B' and USP criteria. The preservative employed in phenylephrine zinc eye-drop did not possess adequate antimicrobial activity against *P. aeruginosa*. Other eye-drops showed appropriate reductions in bacterial viability after 6 hrs, 24 hrs and 7 days, but showed a very low bacterial recovery after 28 days which didn't comply with the no recovery (NR) term of BP 'A' criteria. Since viable microbial counts were usually determined by plate count method, it seems that the term of NR should define an acceptable range.
Conclusion: The results indicated that there is not a clear correlation between antimicrobial efficacy testing of eye-drops and the rate of their microbial contamination while are being used. Other factors such as hygienic practices of eye-drops, proper bottle design and training of patients could influence their microbial contaminations. Regulation of in-use efficacy testing of eye-drops which is influenced by the environment, the frequency and technique of use, might be essential.

Keywords: Antimicrobial effectiveness test, challenge test, preservative, eye-drop, ophthalmic drop

INTRODUCTION

Ophthalmic drops are sterile preparations which are usually packed in multi-dose containers. In their uses, microbial contamination may lead to product degradation or result in ocular infection (1-4). Protection of these multiple dose products against microbial contamination is usually achieved by addition of a suitable preservative system (5-7). The antimicrobial effectiveness test is designed to provide a laboratory test that gauges the level of antimicrobial activity by a pharmaceutical product and to evaluate how well a product withstands microbial contamination while being used (8, 9). The method is similar in both British Pharmacopeia (BP) (10) and United States Pharmacopeia (USP) (11), but sampling times and logarithmic (log) reduction performance criteria of the BP are more stringent than those in the USP. It has been reported that there is a correlation between the performance of eye-drops according to the BP antimicrobial efficacy test and magnitude of microbial contamination during their uses (12), suggesting other investigators to extend similar studies on other multi-dose products. The aim of this study was to determine the antimicrobial efficacy of eye-drops produced by Iranian manufacturers according to the both United States and British Pharmacopeia to assess the correlation of antimicrobial performance of the eye-drops with magnitude of microbial contamination during their uses.

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MATERIALS AND METHODS

Test samples

The tested eye-drops were artificial tear, 1% atropine sulfate, betamethasone, 2%homatropine hydrobromide, 5% phenylephrine hydrochloride, phenylephrine zinc, 2% pilocarpine hydrochloride, tetracaine hydrochloride and 1% tropicamide which showed high microbial contamination during hospital uses (13). All products except phenylephrine zinc which had only benzalkanium chloride, contained benzalkanium chloride and ethylene diamine tetra-acetic acid (EDTA) as antimicrobial preservative system. All samples were produced by Iranian manufacturers.

Antimicrobial effectiveness testing

Possible antimicrobial effects of all samples were eliminated and validated following the method proposed by the USP under validation of microbial recovery from pharmacopeial articles using Fluid Casein Digest-Soy Lecithin-Polysorbate 20 medium (Merck) (14, 15). The unused eye-drops were subjected to the BP (10) and USP (11) preservative challenge tests using *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538, *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404 as test organisms. To determine the microbial killing rate, the eye-drops were inoculated with challenging microorganisms at a final concentration of 10^5-10^6 CFU ml^{-1} and the viable organisms were determined 30, 90 and 180 min after inoculation for bacteria and 24 hrs for fungi.

Aerobic viable cell count of 1:10 dilution of product in neutralizer was determined by plate count method and 0.5 log increase in colony forming units was accounted for variability.

RESULTS AND DISCUSSION

The antimicrobial preservative efficacy of the eye-drops challenged with *E. coli*, *S. aureus* and *P. aeruginosa* is shown in Table 1. After a contact time of 6 hrs all the eye-drops except phenylephrine zinc which showed only 1.7 logs reduction in *P. aeruginosa* initial count, reduced at least 2 logs of all bacterial counts.

Most of the eye-drops eradicated the inoculated microorganisms more than 3 logs in 24 hrs and also 7 days, except phenylephrine zinc. The number of *P. aeruginosa* in phenylephrine zinc was reduced 2 logs after 24 hrs of inoculation and was increased to about the initial count after 7 days.

After 14 days, all the eye-drops except phenylephrine zinc which showed 1 log reduction in *P. aeruginosa* count appeared well preserved against all the challenging organisms (≥3 logs reduction).

After 28 days, there was no bacterial recovery from betamethasone eye-drop, while the number of *P. aeruginosa* in phenylephrine zinc increased. Other eye-drops showed no increase in bacterial counts after 28 days which were about 10-50 CFU ml^{-1} of the products.

In all cases the number of fungi after 7 and 14 days were acceptable and those after 28 days were at least 2 logs lower than the initial counts (Table 2).

As shown in Table 3, more than 2 logs reduction in bacterial counts (after 30 min) and more than 3 logs reduction in fungal counts (after 24 hrs) were observed for all eye-drops except phenylephrin zinc.

The results of this study showed that eight out of the nine products met the BP 'B' criteria and USP while all of them except artificial tear were highly contaminated during hospital uses (Table 4). The preservative employed in phenylephrine zinc eye-drop did not possess adequate antimicrobial activity against *P. aeruginosa* to be able to bring about acceptable low levels of microbial contamination as demanded by regulatory bodies. Therefore another effective antimicrobial preservative system for this formulation should be employed.

Other eye-drops showed appropriate reductions in bacterial viability after 6 hrs, 24 hrs and 7 days, except a very low bacterial recovery after 28 days (10-50 CFU ml^{-1}) which didn't comply with the no recovery (NR) term of BP 'A' criteria. Viable microbial count, as recommended in both Pharmacopeia, was determined by plate count method using 1 ml of 1:10 dilution of product in neutralizer and no bacterial growth means that the number of challenging bacteria was reduced to lower than 10 CFU per ml of the product instead of NR. Therefore, it seems that the term of NR should define an acceptable range.

CONCLUSION

All the eye-drops except artificial tear were contaminated after 1, 2, 4 and 7 days of hospital uses with different rate of contamination from 23.5% for atropine sulfate to 84.4% for tetracaine hydrochloride (Table 4) while similar results were obtained for all the eye-drops except phenylehrine zinc when subjected to antimicrobial effectiveness testing according to the both Pharmacopeia. These comparisons indicate that there is not a clear correlation between antimicrobial efficacy testing of eye-drops and the rate of their microbial contaminations during their usage. Therefore in addition to preservatives,

Table 1. Antimicrobial preservative efficacy of the eye-drops challenged with *E. coli*, *S. aureus* and *P. aeruginosa*

Microorganism	Eye-drop	Sampling time/Viable count (CFU ml^{-1})					
		0	6 hours	24 hours	7 days	14 days	28 days
	Artificial tear	3.8×10^5	10^2	10^1	10^1	<10	<10
	Atropine sulfate	6.0×10^5	<10	10^1	10^1	<10	10^1
	Betamethasone	3.8×10^5	10^1	<10	10^1	10^1	<10
	Homatropine HBr	6.0×10^5	10^1	<10	10^1	<10	5×10^1
E. coli	Phenylephrine HCl	3.5×10^5	<10	10^1	<10	3×10^1	10^1
ATCC 8739	Phyenylephrine zinc	3.5×10^5	<10	<10	<10	8.1×10^2	3×10^1
	Pilocarpine HCl	3.8×10^5	<10	10^1	4×10^1	<10	2×10^1
	Tetracaine HCl	1.0×10^5	<10	10^1	2×10^1	7×10^1	10^1
	Tropicamide	3.8×10^5	<10	2×10^1	5×10^1	<10	2×10^1
	Artificial tear	7.1×10^5	<10	<10	<10	<10	10^1
	Atropine sulfate	4.9×10^5	<10	<10	<10	10^1	10^1
	Betamethasone	7.1×10^5	<10	<10	10^1	<10	<10
	Homatropine HBr	4.9×10^5	2×10^1	<10	<10	<10	3×10^1
S. aureus	Phenylephrine HCl	2.0×10^5	5×10^1	<10	<10	<10	<10
ATCC 6538	Phyenylephrine zinc	3.5×10^5	<10	<10	<10	<10	<10
	Pilocarpine HCl	7.1×10^5	<10	10^1	<10	<10	<10
	Tetracaine HCl	4.9×10^5	<10	2×10^1	10^1	<10	<10
	Tropicamide	7.1×10^5	<10	3×10^1	10^1	<10	10^1
	Artificial tear	3.3×10^5	<10	10^1	<10	<10	2×10^1
	Atropine sulfate	4.7×10^5	<10	<10	2×10^1	<10	2×10^1
	Betamethasone	3.3×10^5	2×10^1	<10	<10	<10	<10
	Homatropine HBr	4.7×10^5	<10	<10	3×10^1	<10	10^1
P. aeruginosa	Phenylephrine HCl	7.0×10^5	<10	<10	<10	4×10^1	<10
ATCC 9027	Phyenylephrine zinc	1.0×10^5	2×10^3	1×10^3	1.1×10^5	1.5×10^4	2.2×10^5
	Pilocarpine HCl	3.3×10^5	<10	1.6×10^1	10^1	<10	<10
	Tetracaine HCl	4.7×10^5	<10	<10	10^1	3×10^1	10^1
	Tropicamide	3.3×10^5	<10	5×10^1	<10	<10	<10

Table 2. Antimicrobial preservative efficacy of the eye-drops challenged with *C. albicans* and *A. niger*

Microorganism	Eye-drop	Sampling time/Viable count (CFU ml^{-1})			
		0	7 days	14 days	28 days
	Artificial tear	2×10^5	10^1	10^1	<10
	Atropine sulfate	1.1×10^5	<10	<10	<10
	Betamethasone	2×10^5	<10	<10	<10
	Homatropine HBr	1.1×10^5	<10	<10	<10
C. albicans	Phenylephrine HCl	1.1×10^5	<10	<10	<10
ATCC 10231	Phyenylephrine zinc	10^5	<10	<10	2×10^1
	Pilocarpine HCl	2×10^5	<10	<10	<10
	Tetracaine HCl	1.1×10^5	<10	<10	5×10^1
	Tropicamide	2×10^5	5×10^1	10^1	<10
	Artificial tear	2.5×10^5	<10	<10	<10
	Atropine sulfate	1.1×10^5	<10	<10	<10
	Betamethasone	2.5×10^5	<10	<10	<10
	Homatropine HBr	1.1×10^5	<10	<10	<10
A. niger	Phenylephrine HCl	1.1×10^5	<10	<10	<10
ATCC 16404	Phyenylephrine zinc	1.1×10^5	2.5×10^1	<10	<10
	Pilocarpine HCl	2.5×10^5	10^1	<10	<10
	Tetracaine HCl	1.1×10^5	6×10^1	<10	<10
	Tropicamide	2.5×10^5	<10	<10	<10

Table 3. Logarithmic reductions in challenging microorganisms viable counts after 30, 90 and 180 min for bacteria and 1440 min (24 hrs) for fungi

Eye-drop	Time (min)	Log reducations				
		E. coli ATCC 8739	*S. aureus* ATCC 6538	*P. aeruginosa* ATCC 9027	*C. albicans* ATCC 10231	*A. niger* ATCC 16404
Artificial tear	30	4	4	4		
	90	>5	>5	>5		
	180	4	>5	>5		
	1440				>5	3
Atropine sulfate	30	2	4	3		
	90	4	4	4		
	180	>5	>5	>5		
	1440				>5	4
Betamethasone	30	4	>5	>5		
	90	>5	>5	>5		
	180	>5	>5	>5		
	1440				4	4
Homatropine HBr	30	4	>5	>5		
	90	4	4	>5		
	180	4	>5	>5		
	1440				>4	5
Phenylephrine HCl	30	>5	>5	4		
	90	>5	>5	>5		
	180	>5	4	4		
	1440				>4	5
Phyenylephrine zinc	30	>5	2	<1		
	90	>5	4	2		
	180	>5	>5	4		
	1440				>4	1
Pilocarpine HCl	30	>5	>5	>5		
	90	>5	>5	>5		
	180	4	>5	>5		
	1440				>5	3
Tetracaine HCl	30	5	>5	>5		
	90	5	4	4		
	180	5	4	>5		
	1440				4	3
Tropicamide	30	4	3	>5		
	90	>5	>5	>5		
	180	4	>5	>5		
	1440				>5	5

Table 4. Antimicrobial preservative efficacy of the tested eye-drops according to the BP[1] and USP[2] criteria and their in-use microbial contaminations

Eye-drop	BP 'B' criteria	USP	Overall contamination after 1, 2, 4 and 7 days use (%), (13)
Artificial tear	Pass	Pass	0
Atropine sulfate	Pass	Pass	23.5
Betamethasone	Pass	Pass	80
Homatropine HBr	Pass	Pass	29.4
Phenylephrine HCl	Pass	Pass	43.58
Phyenylephrine zinc	Fail	Fail	50
Pilocarpine HCl	Pass	Pass	58.3
Tetracaine HCl	Pass	Pass	84.4
Tropicamide	Pass	Pass	40

[1]BP, British Pharmacopeia, A criteria for bacteria requires not less than 2 and 3 log reduction from the initial count after 6 and 24 hrs respectively and no recovery of viable cells after 28 days. B Criteria for bacteria requires not less than 1 and 3 log reduction from the initial count after 24 hrs and 7 days respectively and no increase from the 7 days count after 28 days. A criteria for yeast and molds requires at least 2 log reduction after 7 days and no increase from the 7 days count after 28 days. B criteria for yeast and molds require at least 1 log reduction after 14 days and no increase from 14 days count after 28 days.

[2]USP, United States Pharmacopeia, for bacteria requires not less than 1 log reduction from the initial count after 7 days, not less than 3 log reduction from the initial count after 14 days and no increase from the 14 days count after 28 days. For yeast and molds requires no increase from the initial count after 7, 14 and 28 days.

other factors could be responsible for microbial contamination of eye-drops. Hygienic practices of eye-drops especially in the hospitals, proper bottle design and training of patients could influence their microbial contaminations (16-17). It is essential to maintain and regulate the in-use efficacy testing which is influenced by the environment, the frequency and technique of use (18).

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