Influence of Isotonic Agents on the Stability of Thimerosal in Ophthalmic Formulations

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Abstract □ Storage tests on the stability of thimerosal in trimethoprim-polymyxin B eyedrop formulations containing isotonic agents other than sodium chloride have been carried out. The HPLC assay of thimerosal in stored formulations containing boric acid and EDTA decreased with time and temperature, while formulations containing propylene glycol, glycerol, and mannitol showed no significant decrease in thimerosal assay compared with a formulation containing no isotonic agent. The latter group provide suitable alternatives to sodium chloride, which has been shown to have a seriously detrimental effect on thimerosal stability.

Keyphrases □ Thimerosal—stability in ophthalmic formulations, influence of isotonic agents, boric acid, EDTA, propylene glycol, glycerol, mannitol □ Isotonic agents—boric acid, EDTA, propylene glycol, glycerol, mannitol, effect on the stability of thimerosal, ophthalmic formulations □ Ophthalmic solutions—thimerosal stability with isotonic agents, boric acid, EDTA, propylene glycol, glycerol, mannitol

Thimerosal, ethyl (sodium *o*-mercaptobenzoato)mercury (I), is an effective antibacterial and antifungal agent widely used as a preservative in pharmaceutical formulations, particularly liquid formulations such as ophthalmic solutions. Ophthalmic solutions also frequently contain agents to ensure that the formulation is isotonic with lachrymal secretions. While such formulations may be presented in a nonisotonic form, it is generally preferred to render them isotonic in order to avoid patient discomfort, biological damage to the patient, or ineffective treatment. The most commonly employed isotonic agents are ionic, in particular sodium chloride. However, it has been reported (1-3) that the presence of halides in solutions of thimerosal can have an adverse influence on the stability of I.

This study was undertaken to examine the effects of several isotonic agent alternatives to sodium chloride on the stability of thimerosal in aqueous formulations when stored under realistic and moderately accelerated conditions.

EXPERIMENTAL

Compounds and Reagents—Thimerosal was obtained commercially[1], and the isotonic agents listed in Table I were "AnalaR" grade[1], with the exception of boric acid which was "Aristar" [1] and propylene glycol which was "GPR" [1]. Methanol used for chromatography was HPLC grade[2], and all water used was freshly distilled.

High-Performance Liquid Chromatography—A constant-flow pump[3] was used to deliver the eluant to two stainless steel columns (each 250 mm × 4 mm i.d.) connected in series and packed with 10-μm silica particles bonded with octadecylsilane[4]. Injections were made with a rotary valve injector[5] equipped with a 50-μL loop. No attempt was made to control the column temperature. A variable-wavelength detector[6] set at 222 nm was employed at an attenuation of 0.04 AUFS.

The chromatographic mobile phase consisted of a mixture of methanol–water–phosphoric acid in a ratio of 60:50:1. The pressure, at a flow rate of 4.0 mL/min was, 4800 psig. Separations were effected isocratically at ambient temperature, and quantification was carried out by comparison of the height of the peak obtained from test solutions with the height of the peak obtained from the control solution.

Preparation of Test Formulations and Analytical Procedure—A series of eyedrop formulations containing thimerosal (0.001% w/v) in water, trimethoprim sulfate, polymyxin B sulfate (as active ingredients), and an isotonic agent (as shown in Table I) were prepared and stored at 5°C overnight. Aliquots (5 mL) of each test formulation were transferred to 10-mL glass ampules, which were then sealed and placed in storage at 5°C, 25°C, 37°C, and 50°C for periods up to 6 weeks. Solutions in ampules were allowed to warm or cool to ambient temperature for 1 h prior to analysis. These solutions were sampled directly for assay. The mean of duplicate determinations was taken, the two results generally showing an agreement of better than 2%. The formulation containing no excipient was used as a control for the initial assay (the results being normalized to 100% in Table II), and the same formulation, stored at 5°C, was used as a control in the subsequent assays.

RESULTS AND DISCUSSION

The results shown in Table II indicate that thimerosal manifested a significantly better stability using all five alternative isotonic agents when compared with its stability in saline. Previous published work (4) has shown only 36% thimerosal to remain after storage at 25°C for 3 d in normal saline.

The five formulations readily fell into two groups. The first group containing the ionic excipients (boric acid and EDTA) showed a decreased level of I throughout the study, dependent on time and temperature. Quite early in the trial a decrease in the assay of I was observed with eventually only 35-45% I remaining after 6 weeks at 50°C. In contrast, the second group of formulations containing the nonionic polyhydroxy alcohols (propylene glycol, glycerol, and mannitol) showed the thimerosal to have good stability compared with the control formulation with no excipient. Here 80-84% of I remained after 6 weeks at 50°C compared with 85% for the control, reflecting an essentially insignificant difference. At a more realistic temperature of 25°C, the first group showed a loss of 10-20% of I after 6 weeks, while the second group showed no decomposition after the same period.

The exact reasons for the marked effect of sodium chloride on the decomposition of thimerosal are not clear at present. It is well known that chloride and other halides have a marked affinity to coordinate with mercury(II) compounds, and this could be a contributory factor in the detrimental effect of sodium chloride on the stability of thimerosal in solution. There is some evidence (5) to show that the decomposition of thimerosal in water to thiosalicylic acid and ethylmercuric hydroxide is a reversible reaction. The effect of chloride anions could be to disturb this equilibrium by reaction with the ethylmercuric hydroxide to give ethylmercuric chloride, which does not combine nearly as readily with thiosalicylic acid in neutral solution to give thimerosal by the back-reaction. A possible reason for the improved stability of thimerosal in the case of the first group of isotonic agents (boric acid and EDTA) is that their respective anions may not have such a marked affinity to coordinate with mercury(II), and so the back-reaction to thimerosal is not so readily suppressed.

In contrast, the second group of isotonic agents (propylene glycol, glycerol, and mannitol) are nonionic, and the equilibrium of thimerosal with its hydrolysis products is not affected by their presence, hence the observed insignificant differences in thimerosal assay between the formulations containing these polyhydroxy alcohols and the control formulation containing no isotonic

Table I—Concentrations of Isotonic Agents

Isotonic Agent	Concentration, % w/v
EDTA	4.10
Boric acid	1.75
Propylene glycol	1.84
Glycerol	2.40
Mannitol	4.68

[1] BDH Chemicals Ltd., Poole, England.
[2] Rathburn Chemicals Ltd., Walkerburn, Peeblesshire, Scotland.
[3] Model 740B; Spectra-Physics, Santa Clara, Calif.
[4] Spherisorb 10 ODS; Phase Separations Ltd., Clwyd, Wales.
[5] Rheodyne Inc., Cotati, Calif.
[6] Model LC3; Pye Unicam Ltd., Cambridge, England.

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Table II—Thimerosal Assay by HPLC after Storage in Trimethoprim–Polymyxin B Eyedrops with Alternatives to Sodium Chloride as Isotonic Agent [a]

Storage Temp.	Control [b] Week 1	Control [b] Week 3	Control [b] Week 6	Boric Acid Week 1	Boric Acid Week 3	Boric Acid Week 6	EDTA Week 1	EDTA Week 3	EDTA Week 6	Propylene Glycol Week 1	Propylene Glycol Week 3	Propylene Glycol Week 6	Glycerol Week 1	Glycerol Week 3	Glycerol Week 6	Mannitol Week 1	Mannitol Week 3	Mannitol Week 6
5°C	NA	NA	100	NA	NA	94	NA	NA	98	NA	NA	103	NA	NA	107	NA	NA	100
25°C	100	103	98	93	91	89	96	86	83	98	97	99	103	100	102	98	100	102
37°C	101	98	94	90	84	72	93	80	64	101	95	91	103	88	84	101	95	94
50°C	98.5	91	85	81	57	35	70	56	43	92	87	81	100	91	82	96	95	84

[a] Initial values at 5°C for each additive were 100%. NA = not assayed. [b] No isotonic agent.

agent. Nonionic compounds, such as these polyhydroxy alcohols, provide suitable alternatives to sodium chloride as isotonic agents in ophthalmic formulations containing thimerosal.

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Comparative Effects of Selected Phenothiazine Tranquilizers and Antihistaminics on Bacterial Cells and Possible Interactions with Antibiotics

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Received January 13, 1983, from the *College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.* Accepted for publication April 21, 1983.

Abstract □ Evaluation of the antibacterial effect of phenothiazine antihistaminics (trimeprazine, promethazine, and fonazine) and phenothiazine tranquilizers (promazine, chlorpromazine, triflupromazine, and propiomazine) on *Staphylococcus aureus* showed that tranquilizers were more active [minimum inhibitory concentration (MIC) 0.5–1.6 μg/mL] than antihistaminics (MIC > 1.6 μg/mL). The antibacterial activity was found to correlate with both the rate of adsorption of these drugs on the bacterial cells and the surface tension of their solutions. Phenothiazine tranquilizers caused rapid and extensive leakage of potassium ions from bacterial cells, while phenothiazine antihistaminics produced relatively slower leakage of these ions. A study of the effect of the phenothiazines on the antibacterial activity of some antibiotics showed that all phenothiazines produced a synergistic effect with erythromycin and an antagonistic effect with tobramycin. Variable effects were observed with chloramphenicol, and no effect was observed with penicillin. Results were explained on the basis of structural characteristics of the phenothiazines.

Keyphrases □ Phenothiazine tranquilizers—effects on bacterial cells, interaction with antibiotics, *Staphylococcus aureus* □ Phenothiazine antihistaminics—effects on bacterial cells, interaction with antibiotics, *Staphylococcus aureus* □ Antibiotics—interaction with phenothiazine tranquilizers and antihistaminics, *Staphylococcus aureus*

The phenothiazine group encompasses drugs of different therapeutic uses, most important of which are the antihistaminics and the tranquilizers. Most patients receiving these drugs are potential recipients of antibiotic therapy because of their increased susceptibility to infections (1). Synergism and antagonism between antibiotic combinations have been documented. Little attention, however, has been paid to possible interaction between antibiotics and phenothiazines. Some phenothiazines have measurable antibacterial activity *in vitro* (2–4), but are not used clinically for this purpose because of the high doses which would be required. The present work was undertaken to investigate the effect of a variety of combinations of antibiotics and phenothiazines against microorganisms, using *Staphylococcus aureus* as a model.

EXPERIMENTAL

Organism, Drugs, and Antibiotics—*Staphylococcus aureus* NCTC 6571 was used throughout this study. Cultures were maintained on blood agar slants. The drugs used were trimeprazine tartrate[1], fonazine mesylate[1], chlorpromazine hydrochloride[1], promazine hydrochloride[2], propiomazine hydrochloride[2], promethazine[3], and triflupromazine[3]. The antibiotics used were chloramphenicol[4], erythromycin[5], tobramycin[6], and penicillin[6]. Stock solutions of the phenothiazines and the antibiotics were prepared in sterilized distilled water to obtain concentrations of 1.0 mg/mL and 100 μg/mL, respectively.

Determination of Minimum Inhibitory Concentration (MIC)—The MIC values were determined by a standard twofold serial dilution method in brain-heart infusion (BHI) broth[7]. Tubes were inoculated with 10^5 colony forming units (CFU)/mL of *S. aureus*. The lowest concentration showing no visible growth after 18 h of incubation at 37°C was considered as the MIC.

Measurement of Surface Tension—Surface tension was measured by the ring method using a torsion balance[8]. All experiments were carried out in duplicate at 25°C. Distilled water was used as the reference ($\gamma_{25} = 72.4$).

Determination of Potassium Ion Efflux—The test organism was grown at 37°C for 18 h in BHI broth, and the cells were washed with saline to remove extracellular potassium ions before they were suspended in saline. Five milliliters of the suspension was boiled for 30 min, and the amount of potassium

[1] May & Baker, Dagenham, England.
[2] Wyeth, Andover, Mass.
[3] Squibb & Sons, Princeton, N.J.
[4] Parke-Davis & Co., Detroit, Mich.
[5] Abbott Laboratories, North Chicago, Ill.
[6] Eli Lilly & Co., Indianapolis, Ind.
[7] Oxoid Limited, England.
[8] Type OS; White Electronic Instrument Co., Worcestershire, England.

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