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EFFECT OF VARIOUS OPHTHALMIC OINTMENT BASES ON CARBENICILLIN AND GENTAMICIN STABILITY

Hamed Hamed El-Shattawy

Department of Pharmaceutics, Division of Pharmacy, Faculty of Medicine, Al-Azhar University, Nasr-City, Cairo, Egypt

ABSTRACT

The stability and relative availability of each carbenicillin and gentamicin in eighteen ointment bases, for possible ophthalmic use, when stored at $5^{o}C$ for eight months were investigated by a microbiological agar plate method. The effect of some additives on the activity of these antibiotics in fresh and stored ointments was also investigated. The activity of carbenicillin has been decreased after storage in ten of the investigated bases while gentamicin retained its activity in all the bases. It appears that methylparaben, propylparaben and tocopheryl acetate have no significant effect on the activity of both antibiotics except in case of stored carbenicillin ointments which contain either Span 40 or Span 80.

INTRODUCTION

The extent of diffusion of an antibiotic from an ointment base, as measured by inhibition zones, has been utilized for assessing the stability of the incorporated antibiotic[1-6].

The loss in activity was determined by many authors[1,4,6] by comparison of the size of the zone produced at a given time with that of the zone produced by the ointment when first prepared.

Plaxco et al[2] determined the bacitracin potency of the ointments by extraction of the antibiotic from the base and the extracted solutions were appropriately diluted and placed on the seeded plates using Vincent disks. The zones produced were compared with a standard curve obtained from standard bacitracin solutions. They found a limited correlation between bacitracin potency as determined by this method and by the agar plate diffusion method.

Plein and Plein[3] found that by plotting the squares of the diameters of the inhibition zones produced by varying concentrations of antibacterial drugs against the logarithms of the concentrations resulted in straight line curves. They presented a cylinder-plate method for quantitative determination of diffusion of antibacterial drugs from ointments by reference to the standard curves. The results of simple cylinder-plate-inhibition zone diffusion tests in six different ointment bases were compared with the quantitative data obtained in testing diffusion from the six ointments by the cylinder-plate standard curve method.

Yousef[5] reported that the potency of the stored ointment can be estimated by referring its rate of diffusion, as measured by the inhibition zone, to a standard curve representing various concentrations of the antibiotic ointments and the corresponding zones of inhibition. An alternative method was to compare the diffusion equivalents, corresponding to the inhibition zones produced by the freshly prepared and stored ointments. Diffusion equivalents were calculated from a standard curve representing the inhibition zones produced by serial dilutions of the antibiotic.

The author[7] previously compared the release of each carbenicillin and gentamicin from eighteen ointment bases for possible ophthalmic use. The purpose of this investigation was to assess the ability of these 18 ointment bases to retain the activity of each carbenicillin and gentamicin after storage and to assess the effect of some additives on the antibacterial activity of these antibiotics.

EXPERIMENTAL

Materials

Carbenicillin disodium (Pfizer) and gentamicin (Schering) were supplied as dry sterile powders and were used as received. All other chemicals were USP, USNF or analytical grade and were used without further purification.

Preparation of Antibiotic Ointments

The compositions of the eighteen ointment bases as given in Table 1 and the methods of preparation of the sterile bases and ointments were the same as in the previous investigation[7]. Thirty six different ointments were compounded from carbenicillin, gentamicin and eighteen ointment bases so that each base contained one of the antibiotics in 0.3% concentration. Another 36 different ointments contain in addition 0.1% mixture (2:1) of methylparaben and propylparaben as preservatives and 0.1% tocopheryl acetate as antioxidant were also prepared. These 72 ointments were stored at 5°C for eight months. After this period, 72 different fresh ointments were prepared in the same way, i.e., from each antibiotic 18 different fresh ointments were prepared without additives and another 18 different fresh ointments were prepared with preservatives and antioxidant.

Stability of Antibiotic Ointments

One hundred fourty four different ointments were microbiologically tested to assess the stability of the contained antibiotic. A micro-

TABLE 1 The Composition of Ophthalmic Ointment Bases

Constituents	Constituent. % for Bases																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Aerosil 200	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	5
Castor Oil	-	-	-	-	-	-	75	85	84.6	80.8	-	90	-	-	85	-	-	93
Cetyl Alcohol	-	-	-	-	5	-	-	-	-	5	-	-	-	-	-	-	-	-
Cetostearyl Alcohol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	5	5	-
Glyceryl Monostearate	-	-	-	-	-	0.5	-	-	0.5	-	0.5	-	-	-	-	-	-	-
Hard Paraffin	-	-	-	-	-	-	-	-	-	-	19.5	-	4	5	-	-	-	-
Hydrogenated Castor Oil	-	-	-	-	-	-	15	15	14.9	14.2	-	5	-	-	-	-	-	-
Liquid Paraffin	-	10	10	20	19	19.9	-	-	-	-	60	-	10	20	-	5	10	-
Span 40	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-
Span 80	-	-	-	-	-	-	10	-	-	-	-	-	-	-	-	-	10	2
White Wax	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	-	-
Wool Alcohols	-	-	-	-	-	-	-	-	-	-	-	-	-	5	5	-	-	-
Wool Fat	10	10	-	-	-	-	-	-	-	-	20	-	4	-	-	5	-	-
Yellow Soft Paraffin	90	80	90	80	76	79.6	-	-	-	-	-	-	82	70	-	85	75	-

biological agar plate method as described by Yousef[5] was employed using Escherichia coli (ATCC No. 10536) as the test organism for the two antibiotics. The medium was adjusted to pH 6 for carbenicillin and to pH 8 for gentamicin[8]. Four holes were made in each plate and were filled with four different ointments made from one antibiotic and one and the same base, but with different compositions and storage conditions, i.e., each plate contains fresh and stored antibiotic ointments made from the same base with and without preservatives and antioxidant. Four plates were prepared for every set of one antibiotic and one base. The extent of growth inhibition was measured from the edge of the hole to the periphery of the zone to the nearest 0.1 mm. The results of zones of inhibition produced by the ointments represent the average of 16 readings made from four separate experiments, with a standard deviation of not more than ± 0.5 mm in each instance, and are given in Table 2.

RESULTS AND DISCUSSION

An ointment base may interact with the active medicament to form a product which may be less active than the original medicament[6]. A reduction in antimicrobial activity of an antibiotic may reveal subtle changes not demonstrable by chemical methods, therefore, microbiological methods remain generally the standard for resolving doubt with respect to possible loss of activity.

In this study, no attempts were made to construct standard curves made from different concentrations of each antibiotic in every base with and without the addition of preservatives and antioxidants. This is because it is not the aim of this work to estimate the actual amount of the diffused antibiotic, but to assess, comparatively, the ability of the investigated bases to retain the activity of the antibiotic after storage.

TABLE 2

Effect of Storage on the Stability of Carbenicillin and Gentamicin in Ointments

	Zones of inhibition (radius segment) in mm produced by carbenicillin				Zones of inhibition (radius segment) in mm produced by gentamicin			
	Without Additives		With Additives		Without Additives		With Additives	
Base	Fresh	Stored	Fresh	Stored	Fresh	Stored	Fresh	Stored
1	2.1	2.1	2.2	2.2	2.1	2.1	2.3	2.2
2	2.1	2.1	2.2	2.2	2.2	2.1	2.2	2.2
3	2.0	2.0	2.1	2.1	2.3	2.3	2.4	2.4
4	2.0	2.0	2.2	2.2	2.0	2.0	2.2	2.2
5	2.4	2.2	2.4	2.2	2.8	2.8	3.0	3.0

6	2.3	2.3	2.3	2.3	4.5	4.5	4.5	4.5
7	5.9	4.5	5.9	3.5	4.7	5.7	4.7	4.7
8	5.1	4.1	5.0	4.1	4.8	4.9	5.0	4.9
9	4.6	4.6	4.6	4.6	4.6	4.7	4.6	4.5
10	5.2	3.0	5.1	3.0	5.1	5.1	5.1	5.1
11	2.0	2.0	2.2	2.2	4.0	4.0	4.1	4.1
12	3.1	2.5	3.1	2.0	5.0	4.9	5.0	5.0
13	3.0	3.0	3.1	3.1	4.9	4.9	5.0	4.9
14	3.2	2.5	3.1	2.5	4.5	4.5	4.6	4.6
15	4.5	1.5	4.5	1.5	2.7	2.7	2.8	2.8
16	2.1	1.5	2.1	1.5	2.5	2.5	2.6	2.6
17	2.3	1.0	2.5	1.0	2.2	2.2	2.3	2.3
18	4.3	1.0	4.4	1.0	4.6	4.6	4.6	4.6

It is apparent from Table 2 that the activity of carbenicillin in Bases 9, 13, 6, 1, 2, 3, 11 and 4 held up very well for eight month's storage at 5°C in absence or presence of additives (methylparaben, propylparaben and tocopheryl acetate). From Table 2, it can be computed that carbenicillin retained 91.7, 80.6, 80.4, 78.1, 76.2, 71.4, 57.7, 43.5, 33.3 and 23.3 percent activity in Bases 5, 12, 8, 14, 7. 16, 10, 17, 15 and 18 respectively after storage for the same period and at the same temperature without additives while retained 21.7, 64.5, 80.4, 80.6, 59.3, 71.4, 58.8, 40, 33.3 and 22.7 percent activity respectively in the same bases in presence of additives.

Referring the above results to the compositions of the mentioned bases as seen in Table 1, it is evident that the activity of carbenicillin has been decreased after storage in all those bases which contain Aerosil, cetyl alcohol, cetostearyl alcohol, Span 40, Span 80, white wax and wool alcohols. It is evident also that carbenicillin activity has been significantly decreased after storage in those bases which contain more than one of the above mentioned ingredients (Bases 18, 15 and 17).

The decrease in activity of carbenicillin in Base 8 after storage may be attributed to a change in the rheological properties of the stored base rather than an unstability. This is because Base 9 which contains the same constituents as Base 8, in addition to glyceryl monostearate, showed excellent keeping qualities.

It appears that the presence of methylparaben, propylparaben and tocopheryl acetate as additives has not significant effect on the activity of carbenicillin in fresh and stored ointments except in stored ointments of those bases which contain Spans (Bases 18, 17, 7 and 12) where a significant decrease in activity was traced.

This effect can be attributed to the complex formation between hydroxybenzoates and nonionic surfactants[9].

All of the ointments containing gentamicin showed excellent stability after storage for eight months at 5°C with or without additives. The investigated ointment bases can be arranged in decreasing order with respect to their ability to release gentamicin it contained after storage as follows: Bases 10, 12, 13, 8, 7, 9, 18, 14, 6, 11, 5, 15, 16, 3, 17, 2, 1 and 4 respectively.

It appears that the presence of methylparaben, propylparaben and tocopheryl acetate as additives has no significant effect on the activity of gentamicin in fresh and stored ointments even in those bases which contain nonionic surfactants. A possible explanation is that the presence of intact gentamicin covers any loss in the paraben's activity due to complexation with nonionic surfactants, a case which is not found with carbenicillin in stored Bases 18, 17, 7 and 12 as the latter is already inactivated in these bases.

From Tables 1 and 2 it is evident that the best base for carbenicillin is that which is composed of castor oil gelled by hydrogenated castor oil and contains glyceryl monostearate as self-emulsifying agent. The other castor oil gelled bases failed to retain full activity of carbenicillin during storage. However, these bases did retain the ability to release carbenicillin more than some of the stable ointments. For gentamicin, the best eight bases include six bases composed of castor oil gelled by hydrogenated castor oil or Aerosil and may contain cetyl alcohol, Span 40, Span 80 and glyceryl monostearate as wetting and spreading agents. It is evident also that the poorest bases for both antibiotics include those bases which have been chosen as reference bases. This is generally in agreement with the results of the previous study[7].

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