

EFFECT OF SOME ADDITIVES ON THE
ANTIMICROBIAL ACTIVITY OF KANAMYCIN AND
GENTAMICIN IN OINTMENTS

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Kanamycin and gentamicin ointments were prepared using four different ointment bases, namely oleaginous, absorption, emulsion and water-soluble bases. Glycerol, propylene glycol, polyethylene glycols 200, 400, 600 and 1000, water, alcohol, dimethylformamide, dimethylsulfoxide and polysorbate 80 were separately incorporated at 10 % concentration into all the ointments prepared. The effect of all these additives on the antimicrobial activity of gentamicin and kanamycin was investigated.

Water-soluble base was found to be the best for preparing kanamycin and gentamicin ointments. Of the additives tested, glycerol, dimethylformamide, dimethylsulfoxide and polysorbate 80 proved to have more effect in increasing the activity of kanamycin than the other additives. While for gentamicin, the best effect was with either glycerol, propylene glycol or polyethylene glycol in water-soluble base, and with 10% water in oleaginous base.

Since liquids greatly influence percutaneous absorption, and accordingly therapeutic effect, some studies on the effect of various liquids on drug release from different ointment

bases have been reported ¹⁻⁶. Whitworth et al ¹⁻³ reported that the diffusion of salicylic acid, atropine and atropine sulfate from various ointment bases was influenced by the presence of low concentrations of certain liquids. Wurster and Kramor ⁴ showed an increase in absorption of three salicylate esters accompanied by increase of moisture conditioning. Shelmire ⁵ has stated that hydration of the stratum corneum appears to increase the rate of passage of all substances which penetrate the skin. In an in-vitro study, Nakano and Patel ⁶ investigated the effect of organic solvents as dimethylsulfoxide, dimethylacetamide, and di-n-butylpropionamide on the release and permeation of salicylic acid from different ointment bases and found that these organic solvents increased the release of the drug. Barry et al ⁷ studied the effect of dimethylsulfoxide on percutaneous absorption of salicylic acid and found that dimethylsulfoxide enhanced absorption. Maibach and Feldman ⁸⁻¹¹ have demonstrated a considerable increase in the topical absorption of hydrocortisone when applied with dimethylsulfoxide. Perrier and Hlynka ¹² reported the same findings on the effect of dimethylsulfoxide on the intracutaneous absorption of ³H-labelled hydrocortisone.

The object of this study was to investigate the effect of different kinds of additives on the antimicrobial activity of kanamycin and gentamicin from various ointment bases.

EXPERIMENTAL

Materials:

- 1- Gentamicin sulphate, supplied by Memphis Chemical Co., CAIRO, Egypt.

- 2- Kanamycin sulphate (U.S.S.R.)
- 3- Analytical grades of glycerol, ethyl alcohol, dimethyl formamide, dimethylsulfoxide.
- 4- Polysorbate 80 (Atlas Chemical Industries, Inc. Wilmington, DE 19899).
- 5- Polyethylene glycols 200, 400, 600, 1000 and 4000 (Pure grades).
- 6- Pharmacopeial grades of white beeswax, hard paraffin, white soft paraffin, liquid paraffin, wool alcohols, propylene glycol.
- 7- Nutrient medium, Nutrient agar, Difco (23 g/l).
- 8- Organism: *Bacillus pumilus* N.C.T.C. 8241.

Formulation:

Ointment bases were generally best classified according to the type of composition into four classes: oleaginous, absorption, emulsion and water-soluble ointment bases¹³⁻¹⁴.

A simple formula was chosen and prepared as a stock.

The employed formulae had the following composition:

- 1- Oleaginous Base¹⁵: White beeswax 5g. and white soft paraffin 95 g.
- 2- Absorption Base¹⁶: wool alcohol 6 g., hard paraffin 24 g., white soft paraffin 10 g. and liquid paraffin 60 g.
- 3- Emulsion Base¹⁷: Cetyl alcohol 15 g., white beeswax 1 g., propylene glycol 10 g., sodium lauryl sulphate 2 g. and water 72 g.
- 4- Water-soluble Base¹⁵: Polyethylene glycol 4000, 40 g. and polyethylene glycol 400, 60 g.

The bases were prepared by fusion.

Using the previously prepared bases, the following general formulae were adopted:

A- Drug 0.5 g. and base 99.5 g.

B- Drug 0.5 g., additive 10 g. and base 89.5 g.

All the formulae were prepared by fusion.

Antimicrobial Test:

One ml of a 24 hours broth culture of *Bacillus pumilus* N.C.T.C. 8241 was inoculated into 100 ml of sterile molten nutrient agar and maintained at 45°. The inoculated medium was well mixed and poured in separate quantities into sterile 15 cm diameter petri dishes each receiving 25 ml. After setting six cups, each 11 mm in diameter were cut into each of inoculated plates using a sterile cork borer. The cups were then aseptically filled with the respective ointment under test, and then incubated for 24 hours at 37°. The diameters of the resulting inhibition zones were accurately measured. Results are compiled in Tables 1 and 2.

RESULTS AND DISCUSSION

The suggested liquid additives were found to increase the diffusion of kanamycin and gentamicin from the used four ointment bases to different extents. The results appear to be dependent on the characteristics of the base and the kind of liquid additive.

Table 1 shows that no diffusion of kanamycin sulphate occurred from the oleaginous base. The release from the other bases was in the following order: water-soluble base > emulsion base > absorption base. This may be due to miscibility of the water-soluble base and the miscibility of the external polar phase (water) in the oil-in-water base (emulsion base) with the diffusion agar medium.

This, facilitates drug transfer from this base. While

in the case of oleaginous and absorption bases, the external phase is non-polar and immiscible with the polar diffusion agar medium, and hence a retarded drug release was shown. Table 1 shows the effects of 10% of the suggested additives, on the antimicrobial activity of kanamycin. Kanamycin ointment prepared using oleaginous base gave no antibiotic release. All the used additives with the exception of polyethylene glycols and polysorbate 80 produced no enhancement for the release of the drug from oleaginous base. Table 1 shows the effect of different grades of polyethylene glycols, viz., 200, 400, 600 and 1000 on kanamycin release from oleaginous base. All increased the antibiotic release, the less the molecular weight of polyethylene glycol, the more its effect. This indicates the obvious effect of the reduction of viscosity of the base on increasing the release.

The effect of polysorbate 80 on enhancing the diffusion of kanamycin from both oleaginous base and absorption may be due to the fact that, surfactants besides their role in increasing the solubility of the drug, make available more channels for drug diffusion, thus increasing the effective porosity of the matrix¹⁸. Furthermore, Duemling¹⁹ reported that more rapid penetration of drug is obtained by addition of a wetting agent to the paraffin ointment. He pointed out that surface active agents improve topical vehicles and promote diffusion of the medicament from the base.

Water had a slight effect on the diffusion of kanamycin, in some instances it exerted a retarding effect. The observed weak effect of water on diffusion of kanamycin from absorption and oleaginous bases may be due to its nature as a hydrophilic solvent. It does not mix readily with the lipophilic bases, and thus it does not increase the diffusion

of the drug in the base. Addition of all the proposed additives to the medicated emulsion or water-soluble base except glycerol showed no effect on the release of kanamycin, but rather a decrease in certain cases.

Table 2 shows the effect of 10 percent of the previous additives on the release of gentamicin from different ointment bases. Bases containing 0.5 percent gentamicin gave release in the following order: water-soluble > emulsion > absorption > oleaginous. All the used additives with the exception of polysorbate 80 and polyethylene glycol increased the release of gentamicin from the medicated oleaginous base. In this case also as for kanamycin, the lower the molecular weight of polyethylene glycol, the more the release of gentamicin. 10% water in the oleaginous base gave a pronounced increase in the release of gentamicin. In case of the absorption base, all the suggested additives except polyethylene glycol 1000 increased the release of gentamicin from the ointment. This may be due to the increase in the viscosity of this base on the addition of polyethylene glycol 1000. Alcohol was found to produce a moderate enhancing effect on the diffusion of gentamicin from the oleaginous, and water-soluble bases and a fairly good result from absorption base. This may be due to the better incorporation of the drug with the base in the presence of alcohol or due to its reducing effect on the viscosity of the base. When the surfactant, polysorbate 80 is incorporated in the absorption base, the diffusion of gentamicin is higher than that from emulsion base and is equal to the diffusion from water-soluble base.

It was also found that all the proposed additives increased the release of gentamicin from water-soluble base. These results were found to be in agreement with the work done by Whitworth and Steptrenson³. The authors found that the inclusion of liquids in ointment bases enhanced the diffusion of atropine sulfate and also the oil-in-water base gave higher drug release than that obtained by either water-in-oil or oleaginous bases.

Table 1: Effect of Some Additives on the Antimicrobial Activity of Kanamycin Ointment.

Ointment Base	Minimum Inhibition Zones Diameter, mm,									
	Oleaginous		Absorption		Emulsion		Water Soluble			
Additive	Drug	Drug + Additive	Drug	Drug + Additive	Drug	Drug + Additive	Drug	Drug + Additive	Drug	Drug + Additive
Glycerol	-	-	18.75	19.50	23.00	24.75	28.00	30.75		
Propylene glycol	-	-	18.75	20.25	23.00	22.00	28.00	27.50		
Polyethylene glycol 200	-	15.00	18.75	19.00	23.00	20.25	28.00	28.50		
	400	-	15.00	18.75	23.00	21.25	28.00	26.00		
	600	-	14.50	18.75	23.00	22.25	28.00	27.00		
Water	1000	-	18.75	17.00	23.00	21.50	28.00	28.00		
	-	-	18.75	19.25	23.00	22.75	28.00	28.00		
Alcohol	-	-	18.75	18.00	23.00	22.50	28.00	27.75		
Dimethylformamide	-	-	18.75	19.75	23.00	22.75	28.00	28.75		
Dimethylsulfoxide	-	-	18.75	21.50	23.00	22.25	28.00	28.75		
Polysorbate 80	-	16.50	18.75	24.00	23.00	23.00	28.00	28.75		
	-	-	18.75	24.00	23.00	23.00	28.00	28.75		

Table 2: Effect of Some Additives on the Antimicrobial Activity of Kanamycin Ointment.

Ointment Base	Minimum Inhibition Zones Diameter, mm.									
	Oleaginous		Absorption		Emulsion		Water Soluble			
Additive	Drug	Drug + Additive	Drug	Drug + Additive	Drug	Drug + Additive	Drug	Drug + Additive	Drug	Drug + Additive
Glycerol	20.00	21.00	21.75	22.25	22.00	23.00	23.75	25.50		
	20.00	22.00	21.75	24.50	22.00	21.50	23.75	25.25		
	20.00	21.00	21.75	25.50	22.00	21.50	23.75	25.25		
Propylene glycol	20.00	21.00	21.75	22.25	22.00	23.00	23.75	25.50		
	20.00	22.00	21.75	24.50	22.00	21.50	23.75	25.25		
	20.00	21.00	21.75	25.50	22.00	21.50	23.75	25.25		
Polyethylene glycol	200	20.00	19.00	21.75	25.50	22.00	21.50	23.75	25.25	
	400	20.00	17.00	21.75	24.25	22.00	18.50	23.75	24.75	
	600	20.00	15.50	21.75	23.75	22.00	20.00	23.75	25.00	
Water	1000	20.00	15.00	21.75	17.25	22.00	19.00	23.75	25.00	
		20.00	32.00	21.75	25.25	22.00	21.00	23.75	25.00	
		20.00	21.50	21.75	25.00	22.00	22.00	23.75	24.50	
Alcohol		20.00	21.50	21.75	25.00	22.00	22.00	23.75	24.50	
		20.00	21.00	21.75	26.00	22.00	20.00	23.75	23.75	
		20.00	21.25	21.75	25.00	22.00	21.25	23.75	24.00	
Dimethylsulfoxide		20.00	21.25	21.75	25.00	22.00	21.25	23.75	24.00	
		20.00	21.25	21.75	25.00	22.00	21.25	23.75	24.00	
		20.00	21.25	21.75	25.00	22.00	21.25	23.75	24.00	
Polysorbate 80		20.00	21.75	21.75	24.00	22.00	20.50	23.75	24.00	
		20.00	21.75	21.75	24.00	22.00	20.50	23.75	24.00	
		20.00	21.75	21.75	24.00	22.00	20.50	23.75	24.00	

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تأثير بعض المواد المضافة للمراهم على
مفعول الكاناميسين والجنتاميسين المضاد للجراثيم
علاء الدين على قاسم - محمد على على قاسم - الشربيني احمد ابراهيم
احمد طلعت نوح

تم في هذا البحث تحضير مرهم الكاناميسين ومرهم الجنتاميسين في
أربعة قواعد مرهمية هي الدهنية ، الامتصاصية ، المستحلبة ، والذائبة في
الماء ثم دراسة تأثير بعض الاضافات على المراهم المحضرة
وهي الجلسرين ، بروبيلين الجليكول ، عديد ايثيلين الجليكول ٢٠٠ ، ٤٠٠ ، ٦٠٠ ،
١٠٠٠ وكذلك الماء ، الكحول ، ثنائي ميثيل الفورماميد وثنائي ميثيل
السلفوكسيد والبوليسوربيت ٨٠ على التأثير . المضاد للميكروبات لهذه
المراهم .

وكانت اهم النتائج التي تم التوصل اليها في هذا البحث هي أن
القاعدة الذائبة في الماء هي أحسن قاعدة مناسبة من حيث انطلاق
عقارى الكاناميسين والجنتاميسين
هذا وكانت افضل الاضافات التي ادت الى تحسين انطلاق الكاناميسين
من القاعدة الذائبة في الماء هي الجلسرين وثنائي ميثيل الفورماميد
وثنائي ميثيل السلفوكسيد والبوليسوربيت ٨٠ .

وكانت افضل الاضافات لتحسين انطلاق الجنتاميسين من القاعدة الذائبة
في الماء هي الجلسرين وروبيلين الجليكول وعديد ايثيلين الجليكول
على الترتيب . هذا مع الاشارة بان تحضير مرهم الجنتاميسين في قاعدة
مرهمية دهنية تحتوى على ١٠٪ ماء تعطى تحسن واضح في انطلاق الجنتاميسين
من هذه القاعدة .