Formulation and clinical evaluation of some new nalidixic acid topical formulations

S.S. Tous[1], A.M. El Sayed[1], M.G. Abd El Mohsen[1], E.M.K. Youssef[2], M.N. Agban[3], M.F. Boushra[1]*

[1]Pharmaceutics Department, Faculty of Pharmacy, Assiut University, 71526, Assiut, Egypt
[2]Dermatology and Venereology and Andrology Department, [3]Microbiology and Immunology Department, Faculty of Medicine, Assiut University, Assiut, Egypt
*Correspondence: mariamceutics84@yahoo.com

In a novel attempt, nalidixic acid was formulated in different topical 1 % (w/w) gel and cream bases. The viscosity, pH and drug content of preparations were investigated. The highest in vitro release was achieved by (Na-CMC) gel base. The presence of isopropanol as a cosolvent and nalidixic acid sodium benzoate solid dispersion (1:8) in the selected formulae had enhanced both the in vitro release and antibacterial activity of nalidixic acid. Both DSC and FTIR spectroscopy had shown that the drug is compatible with selected excepients. All cream formulations had shown weaker antibacterial activity therefore only gel bases were further investigated for their stability. No significant change in pH or drug content had been observed in stored gel formulations. The best clinical result in impetigo was achieved after 4-7 days with treatment by gel containing isopropanol and nalidixic acid - sodium benzoate solid dispersion (1:8).

Key words: Nalidixic acid – Topical – Gel – Release – Impetigo.

Nalidixic acid or 1-ethyl-1,4 dihydro-7-methyl- 4 oxo-1,8 naphthyridine-3 carboxylic acid is considered to be the first clinically applied quinolone derivative which brought the quinolone group to center of attention [1]. The discovery of nalidixic acid in 1962, and its introduction for clinical use in 1967, marked the beginning of five decades of quinolone development and use [2]. Nalidixic acid is available in the market in the form of oral tablets and oral suspension and the usual adult dose is 4 g daily by mouth in 4 divided doses for at least 7 days in acute infection [3]. Since no topical formulations of nalidixic acid are yet available in the market, it is of tremendous benefit to formulate it topically and make benefit of its antibacterial activity in treatment of skin infections like impetigo. Worldwide, impetigo is the most common bacterial infection in children, although it can occur in patients of any age [4]. The treatment of impetigo has always been challenging, and a review of the literature over the last 200 years indicates the lack of success with a wide variety of treatment options [5]. Nowadays, treatments for impetigo include topical and systemic antibiotics and topical antiseptics. In most cases topical antibiotics are probably as effective as oral antibiotics [6]. Therefore the present work aims to formulate nalidixic acid for the first time in different gel and cream bases and evaluate its *in vitro* release characters and its antibacterial activity. Then the selected formulations were further investigated for their clinical efficacy in patients with impetigo.

I. EXPERIMENTAL

1. Materials

Nalidixic acid was kindly provided by Memphis Co. (Egypt). Standard cellophane membrane (molecular cut of range = 12000) and Pluronic F127 were purchased from Sigma Chem. Co. (United States). White soft paraffin, liquid paraffin, wool fat, beeswax, sodium carboxymethy cellulose (Na-CMC), cetostearyl alcohol, stearic acid, potassium hydroxide, sodium lauryl sulfate, Tween 80, and borax were obtained from Adwic, El-Nasr Pharmaceutical Chemicals Co. (Egypt). Carbapol 934 was bought from C.P. Evans Co. (United Kingdom), sodium alginate was purchased from Judex laboratories (United Kingdom) and span 60 from Fluka chemical, Buch (Switzerland).

2. Methodology

2.1. Preparation of different nalidixic acid topical formulations

In all preparations, nalidixic acid was sieved through mesh No. 100. Then the calculated amount of the drug was incorporated into the formed base by the mechanical method in order to obtain 1 % (w/w) of nalidixic acid topical formulation.

2.1.1. Preparation of nalidixic acid cream

The composition of different cream (emulsion based ointment) formulations is illustrated in *Table I*. For cream preparation, all aqueous phase ingredients and the oil phase ingredients were placed into separate beaker and heated to 70 ± 2 ºC at water bath [7]. Then the two beakers were mixed and stirred till emulsification.

2.1.2. Preparation of nalidixic acid gel

Hydrophilic cellulose derivative gel bases at 3 and 6 % w/w concentration (formulae G1 and G2) were taken in a 100-mL beaker and wetted by water for 24 h [8] .They were then homogenized by magnetic stirring in order to obtain a uniform gel.

To prepare Carbopol gel 0.5 and 1 % w/w (formulae G3 and G4) Carbopol dispersions in water were homogenized by magnetic stirring for 30 min and left to equilibrate for 24 h. After this period, the pH was adjusted to 5-7 with triethanolamine [9].

Aqueous gel formulations containing 20 and 25 % (w/w) Pluronic (formulae G5 and G6) were prepared by the cold method. The polymer was added slowly to cold water with continuous agitation. The mixture was then stored overnight at 4° C. Aqueous Pluronic dispersions are solution at low temperature and they are converted to semisolid gel at room temperature [10].

For formulae (G7 and G8), sodium alginate was utilized as gelling agents (7 and 10 % w/w) where the required quantity of sodium alginate was weighted and dispersed in a small quantity of distilled water to form a homogeneous dispersion [11].

2.2. Evaluation of different nalidixic acid topical preparations

2.2.1. Cosmetic and aesthetic criteria

Physical appearance of different formulations was observed visually while spreadability was observed by spreading 1 g of formulation on a clean even glass surface [12].

2.2.2. pH measurements

A digital pH meter was used to determine the pH of the selected

Table I - Composition of different cream (E) bases containing 1 % (w/w) nalidixic acid.

Ingredients	Percent of ingredients of each formulation (% w/w)				
	E1	E2	E3	E4	E5
Span 60	6	-	-	-	-
Beeswax	-	16	-	-	-
Borax	-	1	-	-	-
Liquid Paraffin	-	50	-	-	6
Cestostearyl alcohol	-	-	25	-	8
Stearic acid	-	-		15	-
Sodium lauryl sulfate	-	-	-	-	1
Propylene glycol	-	-	12	-	-
Glycerin	-	-	-	5	-
Potassium hydroxide	-	-	-	0.72	-
Tween 80	-	-	5	-	-
White soft paraffin	64		25	-	25
Water to	100	100	100	100	100

Table II - Composition of nalidixic acid cream and gel formulations containing cosolvent and/or solid dispersion.

Ingredients	Percent of ingredients of each formulation (% w/w)					
	E6	E7	E8	G14	G15	G16
Nalidixic acid	1	-	-	1	-	-
Nalidixic acid-sodium benzoate solid dispersion (1:8)	-	equivalent to 1 % drug	equivalent to 1 % drug	-	equivalent to 1 % drug	equivalent to 1 % drug
Isopropanol:water 30 % (v/v)	1	-	1	1	-	1
Emulsion based ointment (o/w) E3 to	100	100	100	-	-	-
Na-CMC gel base (G1) to	-	-	-	100	100	100

formulations [13, 14]. The obtained data were further checked by pH papers. The results are the mean of three determinations.

2.2.3. Viscosity measurements

The viscosity of the different formulations was determined using Brookfield DV-III viscometer [15]. T-bar spindles were used to measure the viscosity and were rotated at 25 rpm. The average reading of three determinations was recorded.

2.2.4. Determination of drug content

An accurately weighed amount of each formulation was dissolved in 5 mL 0.1 N NaOH, filtered using a nylon membrane filter disk (0.45 μm).Then they were suitably diluted and assayed spectrophotometrically for drug content at λ_{max} 257 nm against a similarly treated blank.

Drug content was calculated according to the following equation:

(actual nalidixic acid amount * 100)/theoretical amount = % w/w

2.3. *In vitro* release study

2.3.1. *In vitro* release of nalidixic acid from different topical formulations

The *in vitro* release of the drug from different formulations was studied by dialysis method using cellophane membrane. Of each formulation, 500 mg was placed on a circular area of cellophane membrane previously soaked in the receptor medium for at least 30 min. The loaded membrane was then firmly stretched using a rubber band over one of the open ends of a glass tube with a surface area of 1 cm^2. The tube was then immersed in 250 mL beaker containing 25 mL of phosphate buffer pH 7.4 serving as a receptor media. The receptor medium was kept at 32 °C reflect the usual skin temperature [11, 16] in a thermostatically controlled water bath adjusted at 25 spm. At predetermined time intervals of 30, 60, 90, 120, 150 and 180 min, aliquots of 2 mL were withdrawn from the receptor media and immediately replaced by equal volume of fresh phosphate buffer kept at the same temperature. Aliquots at different time intervals were assayed spectrophotometrically at 257 nm for drug content against a suitable blank and the cumulative amount of drug released was calculated. The results are the mean of three determinations.

2.3.2. Effect of drug concentration on nalidixic acid release.

Among all prepared formulations, the highest release of nalidixic acid was achieved by formula (G1) which is Na-CMC gel base. Therefore it was selected to study the effect of drug concentration on its release. Sodium carboxymethyl cellulose (3 % w/w) gel bases containing different concentration of the drug were prepared (0.5, 2, 3, 4, 7 % w/w) and the effect of drug concentration on its release was evaluated according to the previously mentioned method.

2.3.3. Effect of cosolvency and solid dispersion on nalidixic acid release

In a previous work about nalidixic acid solubility [17], 30 % (v/v) isopropanol have given the highest rise in nalidixic acid solubility among different cosolvents. While for solid dispersions, nalidixic acid-sodium benzoate solid dispersion in ratio 1:8 had increased nalidixic acid solubility most. Therefore they were selected to study their effect on drug release from selected formulations. Of each category (cream and gel bases) formulae E3 and G1 had given the highest release therefore they were reformulated as shown in *Table II* to investigate the effect of both cosolvency and solid dispersion on nalidixic acid release.

To study the effect of cosolvency (formulae E6 and G14), nalidixic acid was first mixed with 1 mL of 30 % (v/v) isopropanol solution and then incorporated into the base to obtain 1 % (w/w) nalidixic acid