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Part I: Solid Dispersion Systems as a Means For Enhancing Rifampicin Release From Ointments. Clinical Evaluation of the Proposed Formulation

M.K. Youssef E.D. El-Sayed and M.A. Fouda
Pharm. Technology Dept., Faculty of Pharmacy, Tanta University, Tanta, Egypt

ABSTRACT

A trial was made to enhance the release of Rifampicin from topical preparations by incorporating it in the form of solid dispersion with water soluble carriers. The formula which showed the best in-vitro release was clinically tested. It was found that formulations containing Rifampicin in the form of solid dispersion with PEG have shown the best release characteristics of the antibiotic from oleaginous bases containing Tween 80. The clinical and bacterological data had confirmed the in vitro results and proved the superiority of the proposed formula to the conventional one.

INTRODUCTION

Rifampicin is one of the most famous antibiotics which is characterized by its limited dosage forms. It is mainly available in the form of suspensions and capsules. The antibiotic is distinguished by its low aqueous solubility. Absorption kinetics of the drug from GIT are irregular and dependent on gastric acidity and gastric contents (1,2),

as well as biliary and liver functions (3). Peak serum levels also show considerable variations (4).

Little informations in the literature regarding the use of Rifampicin in topical formulations (5).

The aim of the present work was to enhance the release of Rifampicin from certain ointment bases by incorporating the antibiotic with PVP, PEG or Urea in the form of solid dispersion. Also to optimize the release rate of the drug by including different types of surfactants in the tested formulations. Formulations which showed the best release results were clinically investigated.

EXPERIMENTAL

Materials:

Rifampicin: Lepetit, Italy, PEG 4000: Prolabo, France, Urea: El-Nasr Pharm. Co., Egypt, PVP 40000: Sigma, USA, Cetrimide: Searle, England, Sodiumlauryl Sulphate: Prolabo, France, Tween 80: Prolabo, France, Cetostearyl alcohol: BDH, UK, Hard Paraffin: Veb.Lab. Apolda, W. Germany, Yellow soft paraffin and Beeswax: Morgan Chem. Co. Egypt, Triolein: Matheson Coleman, USA, Dehymuls-K and Amphocerin-E: Henkel, W. Germany.

All solvents and reagents were of pure or analytical grade.

Equipment:

Spectrophotometer, Beckman model 24, USA,

Incubator shaker, GFL Type, West Germany.

Methodology:

The ointment bases used were: simple ointment B.P. 1973 (cetostearyl alcohol, hard paraffin, wool fat, yellow soft paraffin 5:5:5: 85% w/w), (Bees wax, Triolein 25:75% w/w), Dehymuls-K and Amphocerin-E. Rifampicin (particle size 80-100 micron) was incorporated in the ointment bases either alone or in the form of 20% w/w solid dispersion with either PEG 4000, PVP 40000 or Urea. Each formulation contained an amount of the solid dispersion equivalent to 5% w/w Rifampicin powder. Sodium lauryl sulphate, cetrimide or Tween-80 were individually included in each formulation in concentration of 1% w/w representing anionic, cationic and non-ionic surfactants respectively. Fusion method was the one of choice in the preparation of ointments (6), while the solvent technique produced the required solid dispersion systems (7).

I. In-vitro Experiments

Release Rate Studies:

Cast film was the technique adopted for determining the amounts of Rifampicin released from the proposed formulations (8,9). The technique involved spreading of 125 mg ointment on the bottom of a glass a beaker (Rasotherm type, with a cross sectional area of 30 cm^2). Seventy five ml McIlvaine's buffer of pH 7.4 were added to the beaker contents. Beakers were then loaded onto a shaking tray in a thermostatically controlled incubator at 37±0.1°C. Shaking

was continued for 90 min. at 150 rpm. Samples were withdrawn at predetermined time intervals, suitably diluted with fresh buffer and then measured spectrophotometrically at 475 (10). Blank experiments were conducted under the same conditions using similar release media and formulations without the drug. The withdrawn samples were compenstated with equal volumes of fresh buffer .

II. Clinical Investigation

Twenty three patients were selected from out-patient clinic of Dermatology Department, Faculty of Medicine, Tanta University. All patients were suffering from pyodermal infections which could be considered as impetigo clinical sympatoms. They were mostly from the city of Tanta and its surroundings where such infections are spread especially among children.

Selection of patients was based on clear clinical diagnostic features. On the other hand, patients received antibacterial drugs, either systemic or local one month prior to the first clinical examination, were excluded from this study. Male or female patients were randomly chosen in different ages, but they were mainly in childhood ages (0.5-6 years old). In addition, patient history, concerning previous dermatological diseases, was recorded. The parameters used in this investigation were selected so as to determine the efficacy of the proposed topical formula as well as application schudle. Bacteriological investiga-

tions were conducted parallel to the clinical studies. The formula used was the one which showed the best in-vitro release results (Rifampicin-PEG 4000 solid dispersion, Beeswax, Triolein 25, 19, 56% w/w respectively, in addition to 1% Tween 80).

During treatment investigations, some patients did not terminate the whole course. These unterminated cases were four patients. Accordingly, the actual cases studied were 19 patients. Two cases suffered from streptococcal. impetigo infections, while 17 cases were suffering from staphylococcal impetigo.

Ten patients received the proposed formula, 6 patients used the conventional treatment (gentian violet paint, in combination with chloramphenicol ointment), while 3 patients applied the ointment base only.

Methods:

Local treatment of both staphyloccocal and streptococcal impetigo infections was achieved by applying ointment at 12 hours intervals. Morning application was done in the clinic while evening application was done by the patient. The evening dose was supplied in a unit dose package (eye ointment collapsable tube). The dose quantity was calculated to be sufficient for covering the whole area. The dose was spread on the affected area starting from the periphery. The patients were requested to come daily to out-patient clinic for investigation and receiving the evening dose.

Patient Examination:

In case of non-bullous impetigo, the initial lesion is a very thin-walled vesicle on an erythematus base. The vesicle ruptures so rapidly that it is seldom seen as such. The exuding serum dries to form yellowish-brown crusts. Gradual irregular peripheral extension occurs without central healing and multiple lesions, which are usually present, may coalesce. The crusts eventually dry and separate to leave erythema which fade without scarring. The face, especially around the nose and mouth and limbs are the sites of predilection.

In Bullous impetigo, the bullae are less rapidly ruptured and become much larger; a diameter of 1-2 cm is common but they may be of very considerable size, and persist for 2-3 days. The contents are at first clear, but later these become cloudy. After rupture, thin, flat, brownish crusts are formed. Central healing and peripheral extension may give rise to circinate lesions. Although the face is often affected, the lesions may occur any where including the palms and soles, and may be widely and irregularly distributed. The sites of existing skin diseases, especially miliaria or trivial injuries such as insect bites are often the favouring sites of lesions. The buccal mucous membrane may also be involved.

Bacteriological examinations were carried out at every visit of the patients for the clinical investigations during the whole treatment period.

RESULTS AND DISCUSSION

I. In-Vitro Results

1- Effect of drug forms on the release of Rifampicin from different ointment bases:

Rifampicin alone formulated in different ointment bases was characterized by its low release in most cases. Some bases showed no antibiotic release. PVP solid dispersion systems showed the highest drug release magnitude (Table 1). Urea and PEG solid dispersion systems showed lower drug release characteristics compared to that of PVP systems. The amounts of drug released in case of urea and PEG systems differed according to the base type.

In all cases, the release of the antibiotic from the solid dispersion systems was greater than that from Rifampicin powder incorporated in the same bases (Tables 1-4).

The increase in the release of Rifampicin from its solid dispersion systems may be attributed to the enhancement of drug hydrophilicity caused by the presence of hydrophilic carrier. In addition, solid dispersion technique may lead to homogenous distribution of the drug in a fine state of subdivision near to that of its molecular range (9,10). So, the drug particles are reduced to the minimum size, this will increase their surface area exposed to the release medium. In the same time, the hydrophilic carrier will increase particles wettability leading to their faster release.

Table 1 Effect of Solid Dispersion system and Type of Base on the Release of Rifampicin from Ointment Bases

Time (min.)	Rifampicin released (μg/ ml) Simple ointment				Beeswax - Triolein				Dehymuls - k				Amphocerin - E			
	a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
10	0.02	10.90	3.21	3.10	1.93	36.31	7.21	8.17	0.28	11.52	3.82	4.82	0.06	7.71	7.66	4.22
20	0.02	14.82	5.28	5.19	2.47	41.93	12.90	14.21	1.81	17.29	6.02	6.01	0.10	10.12	10.21	6.06
30	0.02	16.73	6.17	6.08	3.17	46.19	14.71	17.51	2.26	21.16	7.62	6.52	0.12	14.08	12.10	8.09
40	0.03	17.82	7.12	7.08	4.61	48.02	16.11	19.71	3.12	25.82	8.28	8.07	0.16	17.61	14.06	10.00
50	0.03	18.09	8.92	8.82	5.13	48.61	17.91	22.10	4.52	27.81	10.92	8.71	0.2	20.12	15.91	11.82
60	0.06	18.21	9.29	9.18	5.99	48.99	18.10	24.13	5.09	33.78	11.29	991	0.30	23.61	18.10	13.02
70	0.06	18.42	10.51	10.63	6.63	50.61	19.28	26.11	5.92	36.29	12.21	12.20	0.9	27.91	19.80	14.21
80	0.10	18.82	10.90	10.92	7.22	52.19	23.07	29.81	6.32	38.22	14.82	13.50	1.1	30.12	21.90	15.80
90	0.10	19.06	12.20	12.13	7.98	54.21	23.96	30.16	6.79	40.09	16.21	14.10	1.9	32.20	23.61	16.07

a- Rifampicin alone
b- Rifampicin - PVP solid dispersion
c- Rifampicin - PEG solid dispersion
d- Rifampicin - Urea solid dispersion.

Table 2 Effect of Urea Solid Dispersion System and Different surfactants on the Release of Rifampicin

	Rifampicin released (μg/ml)											
	Simple ointment			Beeswax - Triolein			Dehymuls - K			Amphocerin - E		
	a	b	c	a	b	c	a	b	c	a	b	c
10	1.91	7.72	5.63	7.12	11.82	3.13	3.82	6.03	13.10	4.22	5.81	5.22
20	2.29	9.06	8.01	10.42	18.22	5.72	7.63	9.21	21.12	7.08	792	7.12
30	2.80	10.52	12.12	12.11	22.13	6.23	12.14	11.07	28.17	9.62	9.82	9.23
40	3.06	12.20	13.90	15.28	24.00	7.93	16.50	14.08	35.80	11.20	11.53	10.06
50	3.52	14.00	15.10	17.25	26.82	10.82	21.33	18.13	39.17	13.71	12.71	10.96
60	3.96	14.80	16.69	18.11	28.11	11.13	24.92	20.22	44.12	15.92	14.04	12.01
70	4.71	16.68	18.06	19.82	30.12	13.83	27.83	23.13	48.00	17.83	14.49	12.63
80	4.92	17.70	21.61	22.68	32.08	14.73	31.93	24.15	52.10	19.06	16.01	13.09
90	5.12	17.82	29.01	23.12	32.92	15.02	33.81	27.13	55.62	19.92	16.32	13.89

a- Cetrimide

b- Sodiumlauryl sulphate

c- Tween - 80

Table 3 Effect of PVP solid Dispersion System and Different surfactants on the Release of Rifampicin

	Rifampicin released μg/ml											
	Simple Ointment			Beeswax - Triolein			Dehymuls - k			Amphocerin - E		
	a	b	c	a	b	c	a	b	c	a	b	c
10	15.10	25.28	8.62	28.21	35.02	14.12	7.01	12.11	40.91	6.52	12.01	17.02
20	19.28	27.19	20.12	39.88	40.19	24.92	11.08	19.12	48.22	11.21	17.08	21.82
30	21.06	31.50	30.91	44.00	44.29	30.11	13.10	28.11	51.93	14.33	22.11	31.12
40	22.16	34.19	37.72	47.31	46.33	34.18	14.22	38.19	55.26	17.23	26.09	35.69
50	22.90	35.71	41.99	50.99	48.19	42.73	14.92	47.10	57.88	18.33	31.13	41.12
60	23.69	36.28	47.81	53.81	50.13	53.11	17.22	56.60	59.10	19.92	37.22	44.18
70	24.80	38.11	51.72	57.93	54.82	66.13	18.13	62.18	60.22	12.23	43.92	49.10
80	25.20	40.18	53.86	60.38	58.16	67.18	19.22	68.12	69.16	22.92	50.12	51.12
90	26.29	41.96	54.90	62.19	58.90	69.21	20.12	77.82	74.06	23.62	52.81	57.83

a- Cetrimide

b- Sodiumlauryl sulphale

c- Tween - 80

Table 4

Effect of PEG Solid Dispersion System and Different surfactants on the Release of Rifampicin

	Rifampicin released μg/ml											
	Simple oinlment			Beeswax - Triolein			Dehymuls - k			Amphocein - E		
	a	b	c	a	b	c	a	b	c	a	b	c
10	14.92	4.82	21.80	21.82	16.81	38.13	23.21	4.01	16.12	4.87	3.21	20.12
20	16.87	5.20	27.69	27.92	20.13	55.12	32.08	5.10	24.62	8.22	3.92	26.01
30	21.18	7.06	32.66	33.17	24.23	59.82	40.00	7.32	28.23	12.13	5.22	31.50
40	22.19	7.52	34.90	38.39	26.11	62.81	45.18	8.10	32.22	16.82	5.93	36.23
50	23.00	9.00	36.21	43.18	29.99	64.22	47.03	8.23	36.72	19.84	7.02	43.93
60	24.90	9.20	37.16	45.98	32.88	70.41	48.10	10.00	40.91	23.12	7.54	46.00
70	25.96	10.12	39.98	46.90	34.91	75.83	51.03	11.10	45.93	25.33	8.09	49.22
80	27.12	11.08	41.09	47.82	35.83	77.31	56.31	12.00	48.10	27.62	8.21	51.92
90	29.90	11.92	41.99	48.02	36.21	79.91	58.18	13.09	51.00	29.19	8.52	59.12

a- Cetrimide

b- Sodium lauryl sulphale

c- Tween - 80

The increase in the release rate of Rifampicin from the system containing PVP compared to the other tested systems may be due to the larger molecular size of PVP compared to that of the other carriers. The drug particles will be scattered on the large surface area occupied by the hydrophilic carrier. This hydrophilic area will be much higher in case ofPVP, so the penetration of the release medium through the ointment layer will be faster in case of PVP creating more channels for the drug release than those created in the case of PEG and urea. These results with PVP system were parallel to those reported by chiou and Riegelman (7).

On trying to explain the observed high holding capacity of the tested ointment bases towards drug molecules in the absence of soluble carriers, it is sensible to discuss the data in the light of the partitioning behaviour of drug molecules and its dispersions. The partition coefficient of Rifampicin and Rifampicin solid dispersion systems was determined and found to be 23.7, 17.5, 11.5 and 15.5 for the antibiotic, PEG, PVP and urea solid dispersions respectively (11). It is clear that the migration tendency of drug molecules towards the oil phase was significantly decreased in the presence of the tested carriers. Such decrease of partitioning of the antibiotic may be explained on the basis of the change in the hydrophilic properties of Rifampicin in the solid dispersion systems. This finding is strongly confirmed by comparing the release characteris-

tics of the drug from the oleagenous bases. Only four bases have released somewhat detectable amounts of the drug namely Dehymuls-K, Dehymuls-K with either Tween 80 or sodium lauryl sulphate, and Beeswax-Triolein with cetrimide. These bases are characterized by their relative high affinities towards water molecules (8).

2- Effect of base type on the release of Rifampicin from its formulations:

From the data presented in Tables 1-4, it is clear that Beeswax Triolein base showed the best release characteristics followed in the order by Amphocerin-E, Dehymuls-K, and simple ointment base respectively. The obtained results could be explained on the basis of composition and consistency of the ointment bases used.

Beeswax containes some of the natural surface active agents, thus facilitating the release of the drug from the ointment. Also, addition of Triolein to Beeswax forms microcrystalline structure in which the poorly soluble drug is suspended and sufficiently exposed to the release medium in a finely divided state. This will account for the higher release results obtained in the case of Beeswax-Triolein base.

Regarding Henkel bases (Dehymuls-K and Amphocerine-E), the enhancement of the release may be attributed to their structure. They are composed of triglycerides which contain ester groups. These ester groups act as polar centres which

can increase the base-water affinity with subsequent increase of the penetration of the release medium into the ointment bulk structure. This will result in higher release rates compared to those obtained in the case of hydrophobic bases (Table 1). In addition, the melting points of Henkel bases may play a significant role in increasing the antibiotic release (8). The higher release results obtained in the case of Amphocerine-E base than those obtained from Dehymuls-K base may be due to lower melting point of Amphocerine-E compared to that of Dehymuls-K (8).

The observed decrease in the release of the antibiotic from simple ointment base may be due to its composition which is mainly hydrocarbon in nature, in addition to its consistency which hinders the penetration of the release medium and consequently retarding the drug release.

In case of PEG-drug solid dispersion systems, Beeswax-Triolein and Amphocerine-E bases showed the highest release values. On the other hand, PVP and urea solid dispersion systems showed the following release order Beeswax-Triolein > Dehymuls-K > Amphocerin-E.

In all cases, Beeswax-Triolein base proved its superiority regarding its effect on enhancing the release characteristics of Rifampicin solid dispersion systems. This might be attributed to the high tendency of such base to attract water molecules inside its structure.

3- Effect of surface active agents on the release of Rifampicin from its formulations:

Surfactants are often incorporated in pharmaceutical formulations as emulsifying agents. When the epidermis is treated with compounds having significant surface activity, its water permeability is expected to be altered. One effect of surfactant action upon the skin may be changing of the physical state of water in the skin in such a way as to permit greater freedom to the passage of charged, hydrophilic substances.

It is obvious that the presence of surfactants in the tested ointment formulations showed a pronounced increase in the drug release. The effect of surfactants on the release of the antibiotic from most of the tested bases was found to be maximum in case of Tween 80, followed by sodium lauryl sulphate and cetrimide respectively.

The presence of surface active agents increased the wetting(Tables 2-4)and facilitated better fluid penetration into the base as a result of interfacial tension lowering effect. This effect of surfactants in increasing the wettability of the active medicament brings closer contact between the external diffusion medium and the medicament itself offering good release characteristics.

The variation in the extent of release due to presence of different types of surfactants may be explained on the

basis of formation of some sort of interaction between the Rifampicin and the surfactant molecules.

The higher release in the case of non-ionic surfactant Tween 80 (Tables 2-4) may be attributed to the increase in the hydrophilicity of the drug molecules in one hand and free migration of water molecules inside the base structure on the other hand.

Ionic surfactants will retard the drug release compared to non-ionic ones. Lower release was observed in case of cationic surfactant (cetrimide) than that in the case of anionic one (sodium lauryl sulphate). This may be due to the development of alkaline pH centres in the latter. In addition, acid-base reaction is expected to occur between the phenolic and amino groups of the drug with both of anionic and cationic surfactants. Regarding the case of Sodium lauryl sulphate, the interaction is expected to be dipole-dipole interaction between the surfactant anion and the piperazine nitrogen of Rifampicin molecule. This bond will be weaker than that formed in the case of cetrimide. Consequently, the release of drug will be faster in this latter case.

II. Clinical Investigation Results:

The patients personal data revealed that there is no significant correlations between such data and type or severity of the observed infections in the tested cases.

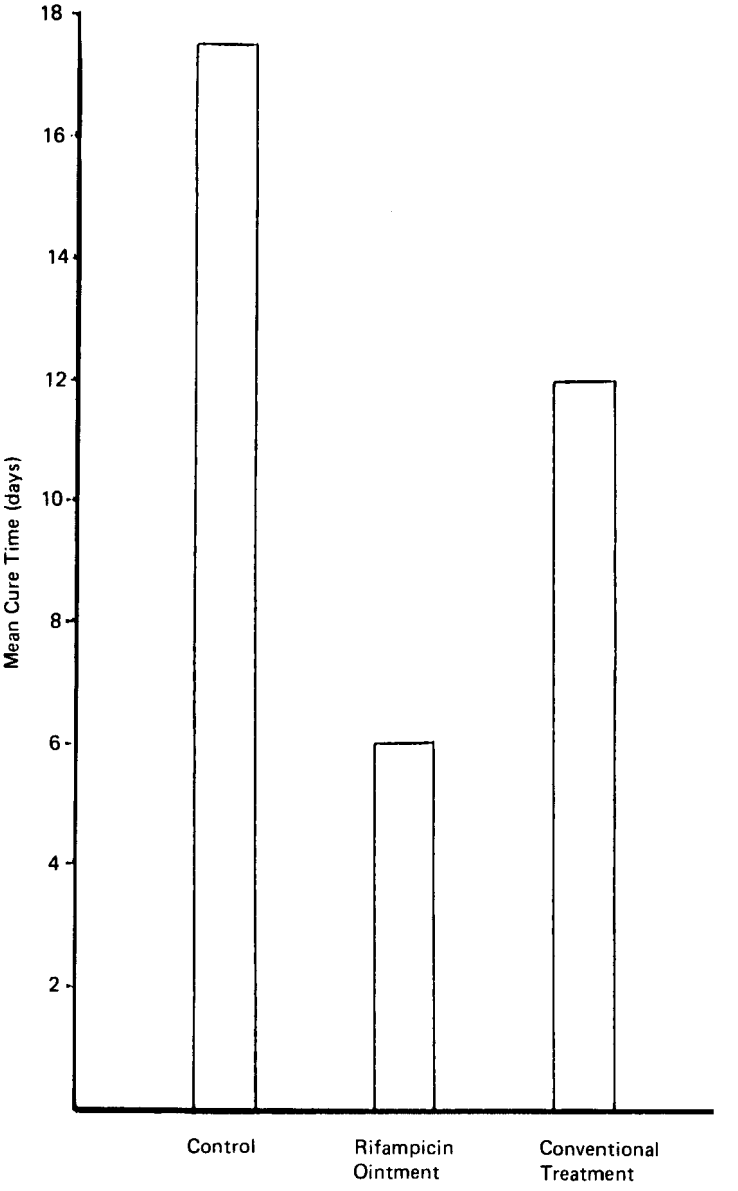

Fig. I : Mean Cure Time of Impetigo Case Using Various Types of Treatments.

The bacteriological examinations of the lesions indicated that negative growth of micro-organisms or bacteriological cure was almost identical to the clinical cure of the tested patients. The application of the proposed Rifampicin ointment proved its high efficiency in destroying the causative organisms.

Figure 1 presents the curing time (clinically detected) of control, proposed Rifampicin ointment and conventional treatment (gentian violet and chloramphenicol ointment). From the Figure, it is clear that the application of Rifampicin ointment formulation tested, reduced the cure time to about six days. On the other hand, the combination of both chloramphenicol and gentian violet induced cure during a period of 13 days.

On comparing the antibacterial activity of both tested treatments, it would be found that chloramphenicol in combination with gentian violet was characterized by high activity in-vitro compared with Rifampicin especially towards the causative organism isolated. Such high antibacterial activity of the conventional treatment was expected to reflect the increase in its efficacy. The unexpected results obtained with the proposed Rifampicin ointment may be attributed to the formulation design rather than the antibacterial efficacy of the applied antibiotic. Since the proposed Rifampicin ointment proved its superiority regarding the antibiotic release, its efficacy may be understood.

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