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Research Article

PERMEABILITY ASSESSMENT OF GRISEOFULVIN MICROEMULSION THROUGH RAT SKIN

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ABSTRACT

Griseofulvin is an antifungal agent that inhibits fungal mitosis. It has been recommended orally for the treatment of fungal disease. It is a highly lipophilic, poorly soluble drug with low oral bioavailability. Therefore the aim of the present investigation was to increase its solubility by using microemulsion system for topical drug delivery. Microemulsion (ME) formulations were prepared by mixing of appropriate amounts of surfactants including span 20, labrasol, co-surfactant such as pleurololeic and oil phase including oleic acid and transcutul P (10:1). The prepared MEs were evaluated regarding their particle size, viscosity and permeation of the drug through skin of male Swiss Albino rats. The mean droplets sizes of MEs formulations were in the range of 37.2 to 94.31 nm and the mean of viscosities of MEs formulations were in the range of 264.3 to 385.2 cps. Comparison between J_{ss} of simple solution control group and MEs indicated that formulation of the drug in form of me may significantly improve drug permeation through rat skin. It was concluded that formulation factors including water and oil percentages, and also surfactant to co-surfactant ratio had an impact on drug permeability through biologic membranes.

Keywords: griseofulvin, microemulsion, permeability.

INTRODUCTION

Microemulsions (MEs) are transparent, isotropic, thermodynamically stable and easily manufactured systems that are composed of water, oil and amphiphilic compounds, including surfactants and co surfactants[1]. Their droplet size lies between 10-200 nm, which makes the microemulsion transparent liquids. The formation of microemulsions occurs only when the tension at the oil/water interface is very low and the region is highly flexible and soft[2]. MEs can be classified into oil in water (o/w), water in oil (w/o) and bicontinuous systems[3]. Due to greater solubility of drugs and possibility of altering affinity of drugs to *stratum corneum*, MEs are able to increase the cutaneous absorption of lipophilic and hydrophilic drugs in topical drug delivery that this is[2].

Griseofulvin (GF) is a heterocyclic benzofuran antifungal agent that was firstly isolated from *penicillium spp*. in 1939. Its molecular weight is 352.77 Dalton and has a logP of 2.15. Griseofulvin inhibits fungal mitosis by disrupting the mitotic spindle through interaction with polymerized microtubules. The drug has poor water solubility and a variable bioavailability, with numerous systemic side effects due to its long duration of treatment after oral administration[4-7]. The aim of the present study was to increase GF solubility by using a ME system for topical drug delivery.

MATERIALS AND METHODS

Griseofulvin(GF) was gifted by Tolidaru pharmaceutical company (Iran). Labrasol and Plurololeic were kindly donated by Gattefosse (France). Transcutul and pandoleic were obtained

from Merck (Germany). Water was distilled deionized and filtered in our lab. All chemicals were of analytical grade.

Microemulsion preparation

Various microemulsions were chosen from the pseudoternary phase diagram with 3:1, and 5:1 weight ratios of span20/labrasol/Pluorololeic. Griseofulvin (0.2%) was added to oleic acid/transcutul P (10:1) as oil phase and vortexed, and then the surfactant–cosurfactant (S-C) mixture was added. Finally an appropriate amount of double distilled water was added to the mixture drop by drop until the GF containing MEs were obtained by stirring the mixtures at ambient temperature[8]. Oil and water percentages and surfactant to cosurfactant ratio (S/C) were considered as independent variables and their effects on physicochemical properties, stability and permeability of MEs were evaluated. Different amount and proportions of ME compositions are tabulated in table 1.

Particle size analysis

The average droplet sizes of samles of MEs were measured at 25 ºC by SCATTER SCOPE 1 QUIDIX (South Korea), and their refractory indices (RI) were also calculated[9].

Viscosity measurement

Rheologic behavior of samples was evaluated at 25 ºC by a Brookfield viscometer (DV-II+Pro Brookfield, USA) using No.34 spindle under shear rates of 50 and 100 rpm. Each measurement was performed in triplicate[10].

Table 1: Compositions (w/w percent) and proportions of microemulsion formulations. S/C and S-C are surfactant to cosurfactant ratio and their total amount, respectively

Formulation	S/C	Oil	S-C	water
ME1	5:1	30	65	5
ME2	5:1	30	67	3
ME3	5:1	5	90	5
ME4	5:1	5	92	3
ME5	3:1	5	92	3
ME6	3:1	5	90	5
ME7	3:1	30	67	3
ME8	3:1	30	65	5

***In vitro* permeation study**

A specially designed Franz diffusion cell with a effective diffusion area of 3.45 cm^2 was utilized to study the permeability of GF formulations through rat skin. . The whole thickness samples of shaved abdominal male Swiss albino rat skin were removed and any extraneous subcutaneous fats were cleaned from the dorsal using cooled acetone solution. The excised samples were stored at -20 ˚C. Before the experiment, they were kept at room temperature and then were hydrated for 16 h in water at room temperature, then dried and their thicknesses were measured. Then they were placed between the donor compartment (containing 5 mL MEs) and the receiver compartment which was contained 30 mL of a mixture of phosphate buffer (pH 7.2) and methanol (1:2 ratio). Their position was so that while facing up, the epidermal side covered the diffusion cell completely. The receptor medium was stirred with a small magnetic bead at 200rpm continually. At predetermined time intervals (0.5,1,2,3,4,5,6,7,8, 10,24,28,32,48,52 and 56 h) 2ml samples were withdrawn from the receiver compartment and immediately replaced with an equivalent volume of the receiver solution to maintain sink condition. The permeated amount of GF was determined by UV spectroscopy method at 294 nm. Aqueous solution of GF was utilized as control[11-13].

Statistical methods

All the experiments were repeated three times and data were expressed as the mean value ± SD. Statistical data were analyzed by one-way analysis of variance and paired T-Test and P value of less than 0.05 was considered to be significant with 95% confidence intervals.

RESULTS AND DISCUSSION

Particle size and viscosity analysis

The Viscosity and mean Particle size of Griseofulvin MEs are illustrated in Table2. ME formulations showed particle sizes in the range of 37.2 to 94.31 nm. The ME8 and ME7 formulations had the smallest and largest particle sizes, respectively. Also the formulations exhibited viscosity values in the range of 264.3 to 385.2 cp. The ME2 and ME5 formulations had the lowest and highest viscosities, respectively. Table 2 shows the particle size and viscosity values of GF containing and drug free formulations.

Table 2: viscosity and particle size of microemulsion with and without GF

Formulation	Viscosity (cps)		Particle size (nm)	
	GF microemulsions	Drug free microemulsions	GF microemulsions	Drug free microemulsions
ME1	270.25±1.62	265±0.93	37.2	29.7
ME2	264.3±1.43	252.4±1.23	49.24	36.6
ME3	363.3±0.98	353.24±1.1	58.28	29.82
ME4	372.8±1.23	361±1.32	63.24	25.5
ME5	385.2±1.33	374.3±1.41	94.31	39.94
ME6	375.3±0.89	349±1.3	77.81	53.3
ME7	283±1.11	273.1±0.97	67.7	56.8
ME8	276.4±1.2	266.2±0.84	36.9	23.79

There was a significant difference between viscosity and particle size of ME formulations with drug and ME formulations without drug ($p<0.05$). The relations between viscosity and particle size with independent parameters (S-C, oil and water content) are shown by equation 1 and 2, respectively.

Viscosity= 443-1.69 (S-C) - 4.64 oil% -12.3 W% ---------- (Equation 1)

Particle size=122 – 5.44 (S-C) – 0.934 oil% -6.72 W% ---------- (Equation 2)

The results indicated that although the impacts of S-C and water content on viscosity were not significant ($p>0.05$), the oil percent of MEs significantly affected their viscosity ($p=0.000$). It may also be inferred that the effects of S/C, water and oil percentages on the ME particle sizes is significant ($p<0.05$). In accordance with the previous reports[12], it was showed that an increase in S/C, oil% and W% may lead to decrease in viscosity and particle size of GF containing formulations.

Permeation study

Figure 1 shows the cumulative amounts of permeated GF from different formulations through excised rat skin. As can be inferred from the figure, although all of the formulations controlled the drug permeation, the presence of higher amounts of oil significantly lessened the rate of GF permeation in comparison with simple solution control group.

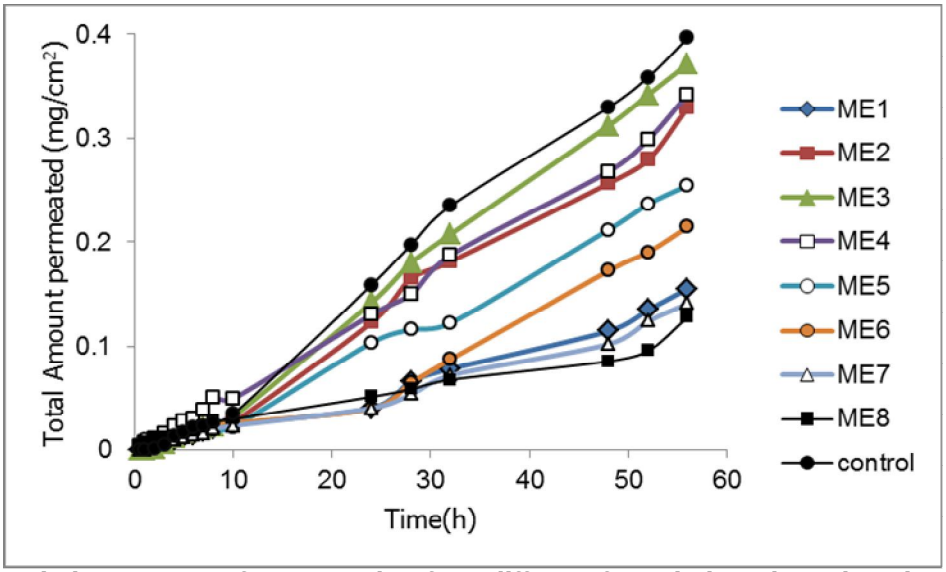

Fig. 1: Cumulative amounts of permeated GF from different formulations through excised rat skin

Different parameters, including flux (J_{ss}), permeability coefficient (P), lag time (T_{lag}) and apparent diffusion coefficient (D_{app}) were calculated from permeation studies. The linear slop of accumulative drug amount against time curve was considered as J_{ss}. Also, by crossing the steady state section of permeation profile to the horizontal axis, D parameters were calculated. The rate of drug permeation (J_{SS}) was drawn from amount of drug permeated through area under permeation-time curve. T_{lag} was calculated from equation 3, in which h and D were membrane thickness diffusion coefficient, respectively.

$T_{lag}=h^2 / 6D$ ---------- (Equation 3)

The permeability coefficient (P) was calculated from the steady-state flux data and applied concentration in the donor compartment (C_o) as in equation 4.

$P= J_{SS} / C_0$ ---------- (Equation 4)

For simulation of skin into normal condition, skin samples were hydrated up to 10 to 20%. Mean sample thickness were about 345±50 micrometer. Table 4 shows J_{SS}, T_{Lag}, D_{app} and P of griseofulvin ME formulations. A comparison among J_{ss} in control group and MEs indicated that the ME1, ME4, ME7 and ME8 had P-values less than 0.05 which means that all of them were significantly different from control group. Permeation data also showed that number 2, 3, 5 and 6 formulations had P-values higher than 0.05 which means that these formulation were not significantly different from control group.

Table 3: Permeation parameters of griseofulvin microemulsions (mean±SD)

Formulation	J_{ss} (mg/cm .h)	T_{lag} (h)	D (cm^2/h)	P (cm/h)
Control	0.0091±0.00007	6.27±0.77	0.00013±0.00001	0.091±0.0007
ME1	0.0028±0.0002	2.66±3.2	0.001±0.0012	0.001±0.00009
ME2	0.0049±0.0030	2.131±0.18	0.00038±3.360	0.0022±.001
ME3	0.0072±0.001	5.44±0.008	0.00014±2.36	0.0032±0.0005
ME4	0.0063±0.00028	3.54±1.68	0.00028±0.00013	0.0028±0.00012
ME5	0.0054±0.0037	8.6±1.75	0.00008±1.810	0.0024±0.0016
ME6	0.0052±0.0031	14.4±3.94	0.000058±0.00002	0.0023±0.0014
ME7	0.0045±0.00049	20.4±10.05	0.000053±0.00003	0.002±0.0002
ME8	0.0044±0.0002	1.87±0.11	0.00037±0.000051	0.002±0.000062

Equation 6 shows relation between independent variables and J_{ss}, which indicate that increase in oil and water amounts may lead to decrease in J_{ss}, while (S-C) increase has a direct impact on GF flux. No significant difference between test and control groups was observed ($p>0.05$). The equations 7 to 9 show the impact of independent factors on T_{lag}, P, and D_{app} respectively. In general, any increase in three mentioned variables led to decrease of T_{lag}. The results indicated that oil and water amount of MEs did not significantly affect their T_{lag} ($p>0.05$) while the effect of (S-C) was significant ($p=0.038$). According to equation 8, all of the variables inversely affect the permeability coefficient of the drug from MEs. Equation 9 shows that any increase in (S-C) and oil% may lead to elevation of permeability coefficient, while the effect of water amount of formulations is contrary. Among them, only the oil percentage effect was significant ($p<0.05$). Table 4 shows J_{SS}, T_{Lag} ,D_{app}, P, of solvents that were used for preparion of GF microemulsions.

J_{ss}= 0.00505+ 0.000294 (S-C) - 0.000061 oil% - 0.000044 W% -------- (Equation 6)

T_{lag}= 27.2 – 3.93 (S-C) -0.030 0il% -1.03 W% ---------- (Equation 7)

P=0.00394 – 0.000119 (S-C) – 0.000018 0il% - 0.000293 W% ---------- (Equation 8)

D_{app} = 0.000193 + 0.000020 (S-C) +0.000008 0il% -0.000039 W% ---------- (Equation 9)

Table 4: Permeation parameters of GF from different solvents through rat skin (mean±SD)

Solvent	J_{ss}(mg/cm².h)	T_{lag}(h)	D (cm²/h)	P (cm/h)
Control	0.0091±0.00007	6.27±0.77	0.00013±0.00001	0.091±0.0007
Oleic acid	0.0072±0.0016	8.89±7.64	0.00013±0.00011	0.0036±0.0008
Transcutul-P	0.0039±0.0028	1.32±0.53	0.00069±0.00027	0.0019±0.0014
Labrasol	0.0033±0.00049	1.28±1.16	0.001±0.00093	0.0017±0.00025
Span20	0.003±0.00049	8.31±9.61	0.00026±0.0003	0.0015±0.00025
Pleurol oleic	0.0021±0.00028	1±0.67	0.0011±0.00079	0.0011±0.00014

A comparison between J_{ss} in control group and solvents indicated that labrasol, span 20 and pleurol oleic showed P-value less than 0.05 which means all of them are significantly different from the control group, while oleic acid and transcutul-P had P-values bigger than 0.05 which means these solvents have no significant impact on GF permeation.

CONCLUSION

According to the results of the present study, it can be concluded that physico-chemical properties and *in vitro* permeability of GF are considerably influenced by factors including the contents of surfactant and co-surfactant, oil and water percentages.

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