https://doi.org/10.31925/farmacia.2020.1.15 **ORIGINAL ARTICLE**

EVALUATION OF MICONAZOLE NITRATE PERMEABILITY THROUGH BIOLOGICAL MEMBRANE FROM DERMAL SYSTEMS

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Abstract

The development of pharmaceutical products with miconazole nitrate (MN) can bring various benefits to patients of whom fungal infections are resistant to classic antifungal formulas. In medical practice, dermal systems, in the form of polymeric films, represent an alternative to other medical products. The proposed dermal films contain hydroxyethyl cellulose (HEC) as a hydrophilic and bioadhesive matrix polymer, with the addition of polyethylene glycol (PEG) as a low-absorption promoter, so that the antifungal have a slow penetration towards the dermis. This study aims to evaluate the *in vitro* permeability of MN through biological membrane (pig ear skin) at pH 7.4, during its release from two polymeric films. *In vitro* pig ear skin permeation studies indicated that the amount of the drug released after 24 h was 40% in the case of formulation FI and 32% in the case of formulation FII from the initial dose (40 mg). The concentration of 40% MN released can be considered an appropriate antifungal dose, with the benefit of being accumulated in the *stratum corneum* where it is maintained for up to 4 days.

Rezumat

Dezvoltarea de forme farmaceutice cu nitrat de miconazol (MN) poate aduce numeroase beneficii pacienților care au dezvoltat rezistență clinică la formulările antifungice clasice. În practica medicală, sistemele dermice formulate ca filme polimerice reprezintă o alternativă de administrare față de alte produse medicamentoase. Filmele dermice propuse conțin hidroxietilceluloză, un polimer bioadeziv formator de matriță, în asociere cu polietilenglicol ca promotor cu proprietăți moderate de penetrare cutanată, astfel încât substanța activă să poată penetra lent țesutul cutanat. Acest studiu își propune evaluarea *in vitro* a permeației MN prin membrană biologică (piele de ureche de porc) la pH 7,4 din două tipuri de filme polimerice. Studiile de permeație *in vitro* prin membrană biologică indică o eliberare de 40% a substanței active după 24 de ore (FI), respectiv 32% (FII). Cantitatea de MN eliberată (40%) asigură eficiența antifungică, substanța activă acumulându-se în stratul cornos unde se menține până la 4 zile după prima aplicare.

Keywords: miconazole nitrate, dermal films, hydroxyethyl cellulose matrix, Franz cell method

Introduction

The increasing number of stress conditions caused by the diversification of activities conducted in modern society, associated with the occurrence of the corresponding pollution factors and the diversity of microbial agents, in particular caused by the increased mobility of people all over the world, led, in addition to the appearance of new diseases, to the occurrence and the diversification of fungal infections. The development of pharmaceutical forms with miconazole nitrate (MN) for external use can bring various benefits to patients presenting resistance to classic antifungal forms because of the fact that the active drug lasts longer in the tissue, acting as a slow release product [7, 8]. Repeated administration leads to an accumulating effect of multiple doses in the *stratum corneum*, which causes the disappearance of fungal infection [6, 28]. Miconazole nitrate is an antifungal drug that inhibits the synthesis and the incorporation of ergosterol in the fungal cell`s membrane as a result of blocking sterol-14α-demethylase, a cytochrome P450 dependent

enzyme, with a key role in biosynthesis of ergosterol. Accumulation of methyl-steroids affects the function of membrane phospholipids and inhibits certain enzymatic membrane dependant systems, like ATP-ase and enzymatic transport systems, with inhibition of growth and development of fungi. It also acts by affecting the permeability of the fungi membrane, selectively inhibiting RNA and DNA precursors and its mucopolysaccharides [6, 7, 19]. Transdermal forms offer several important advantages over conventional administration routes [2-4, 10, 14, 19, 21]. The skin acts as a barrier and the optimization of the release of the drug through this barrier is often the first step towards optimizing the efficiency of such pharmaceutical preparations [12, 23, 27]. The pharmaceutical product is placed on the skin for topical or systemic action, depending on the penetration properties (with local dermal effect through crossing the corneum layer of epidermis) and permeation capacity (with transdermal effect through absorption into the systemic circulation). It's known that the miconazole nitrate is retained in the skin layers up to 4 days after the first administration. The greatest challenge for such types of pharmaceutical forms with miconazole nitrate is to remain for as much as possible at the *stratum corneum* level. A transdermal administration can ensure an optimal concentration of the drug throughout the treatment period. The proposed dermal films contain hydroxyethyl cellulose (HEC) as a bioadhesive matrix forming polymer, with the addition of polyethylene glycol (PEG) as a low-absorption promoter so that the antifungal does not penetrate too much towards the dermis. The main reason for choosing HEC as a bioadhesive matrix forming polymer was its ability to adhere to mucous membrane and human skin, providing stability to the film [26], and the lack of toxicity [5, 15, 18]. HEC with an apparent viscosity of 4500 - 6500 mPa · s is highly water-soluble and practically insoluble in ethanol, being used as a hydrophilic gel former. HEC is a hydrophilic polymer with fast dissolution, and it forms gels at 1.5% - 2%, which exhibit good bioadhesiveness and yield capacity [5].

Although a series of mathematical assumptions have been issued describing drug diffusion through the skin, due to the complexity of the cutaneous organ, it is very difficult to describe a perfect mathematical model [20]. The unanimous acceptance is that the transportation of drugs through the skin takes place through passive diffusion. *In vitro* availability tests with the classical Franz cell can be performed under both static and dynamic diffusion conditions. Contact with the donor compartment is made through a diffusion membrane that simulates the transfer through the *stratum corneum*. According to FDA SUPAC-SS 1997, synthetic membranes with different compositions are accepted: polysulfone, cellulose acetate, cellulose nitrate or polyfluoroethylene, with a diameter close to that of the diffusion cell [11]. Nylon represents a polyamide derivative [24] very commonly used for preliminary tests. OECD Guidance (Organization for Economic Cooperation and Development) [22] considers that the use of a biological human skin membrane is the golden standard in assessing transcutaneous absorption in the design of the *in vitro* penetration study [9, 13]. Other types of biological membranes can be used instead, but it should be taken into account that they behave differently from human skin [16, 17].

The proposed study evaluates the *in vitro* permeation of the antifungal from dermal films through a biological membrane (Mb) using the Franz cell. The pig ear skin is a membrane more similar to human epidermis at pH of body fluids [1].

Materials and Methods

Materials

Miconazole nitrate (MN) was purchased from Sigma Aldrich Inc (Germany). Hydroxyethyl cellulose 250 M, NatrosolTM 250M (HEC, viscosity of 4500 - 6500 mPa · s) from Ashland (Germany), Polyethylene glycol 400 (PEG 400) from Sigma Aldrich Inc. (Germany), ethanol from Stireco LTH (Romania) (Table I).

Table I

Type and composition of samples proposed for analysis

Ingredient	Abbreviation	Formula %		Function
		FI	FII	
Miconazole nitrate	MN	5.00	5.00	Antifungal
Hydroxyethyl cellulose 250 M	HEC	2.00	3.00	Film forming
Polyethylene glycol 400	PEG_{400}	1.00	1.00	Plasticizer, drug solubilizer, humectant
Ethanol	-	10.00	10.00	Co-solvent
Ultrapure water	-	82.00	81.00	Solvent

Films preparation technique. MN was first dissolved in alcohol with stirring at 500 rpm for 5 minutes and after we added PEG, distilled water and HEC during constant stirring. The air bubbles were eliminated from the structured gel by maintaining the fluid phase for 25 minutes in the ultrasound bath. The resulting composition was poured into circular plates (diameter of 9.8 cm) which were then kept to dry at 40°C (24 h). The obtained films were used in the study after 48 hours of preservation at 20°C, protected from humidity [13].

Products in form of dermal systems, proposed in study. Samples of 2.54 cm^2 containing 40 mg MN in polymeric matrices of HEC (2% in FI and 3% in FII), with polyethylene glycol - PEG 400 were prepared in form of films with thickness of 0.23 mm (FI) and 0.30 mm (FII), by casting and solvent evaporation technique.

Preparation of biological skin used as the diffusion membrane. The biological membrane (Mb) consisted of skin from pig ears collected from a local abattoir. According to literature specifications, in order to preserve the integrity of the skin barrier function, the pig ears were removed immediately after slaughter from the carcass and washed with water [17]. After removing the hair yam, the ears were individually wrapped in aluminium foil and frozen at -26°C for a maximum of 6 months. It is known that the freezing preservation method will not influence the permeability properties of the biological skin subsequently used as the diffusion membrane in the *in vitro* tests [1, 7, 11]. The biological membranes were prepared by excising from the freshly defrosted pig ears spherical surfaces (1.8 cm diameter). The excess fat and the cartilage were carefully removed, and then the membranes were kept for 30 minutes in the buffer phosphate pH 7.4 [13].

Determination of in vitro release and diffusion through the biological membrane profiles. The *in vitro* permeation of MN (mg/cm^2*h) was determined by Franz cell method [12] in the following conditions: cell of 14 mL, phosphate buffer at pH 7.4 with 0.045% sodium lauryl sulphate, 32 ± 0.5°C, assessing the MN from acceptor sample of 5 mL, at 273 nm (Spectrometer UVD 3200, Labomed Inc., USA). The analysed sample consisted in disk shaped films with diameter of 1.8 cm (surface of 2.54 cm^2), deposited on the studied membrane (∅ 25 mm) and maintained in the donor compartment under occlusive conditions throughout the determination period.

Data interpretation and statistical analysis. GraphPad Prism 6 software was used running: linear regression followed by the runs tests, the Pearson correlation (r), area under the curve (AUC), unpaired t test followed by F test and Anova followed by Tukey's multiple comparison tests. Mean and standard deviation (SD) were calculated as statistical descriptors and statistical significance was set at $p < 0.05$ with confidence interval of 95% [25].

In a previous study, MN permeation was assessed *in vitro* through the Franz cell from dermal films using a synthetic membrane [7]. The values of antifungal permeation through synthetic membranes (Ms - Teknokroma, 0.45 µm) were compared with those of biological membranes (Mb - pig ear skin, < 1 mm).

Results and Discussion

The *in vitro* permeation curves of MN (40 mg/sample) released from two types of HEC matrices as new systems intended for dermal application (FI, FII) were determined over a period of 24 h (Figure 1), by using in the Franz cell samples of 2.54 cm^2 deposited on diffusion membranes.

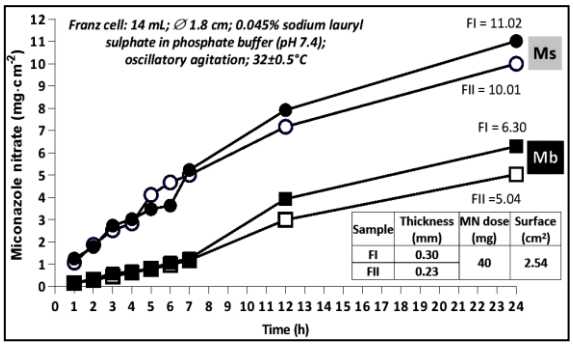

Figure 1.
The *in vitro* permeation curves determined from experimental data

The permeation determined through the two membranes used is the result of two initial successive and then simultaneous processes, namely: the release of MN by dissolution and diffusion from HEC/PEG matrix, followed by the membrane crossing of MN by molecular diffusion through the membrane pores, thus resulting in a cumulative process whose rate depends on both stages (Table II), but one of which is usually more limited.

Table II
The *in vitro* cumulative rate of MN dissolution and diffusion processes

Linear regression of release curves	FI - Mb	FII - Mb	FI - Ms	FII - Ms
Slope ± Standard Error (95% CI)	0.2848 ± 0.02041	0.2236 ± 0.01155	0.4335 ± 0.03616	0.3813 ± 0.03885
Y-intercept	-0.3419 ± 0.1995	-0.1989 ± 0.1129	1.371 ± 0.3535	1.655 ± 0.3797
X-intercept	1.20	0.89	-3.16	-4.34
R square	0.9653	0.9817	0.9535	0.9323
P value	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Deviation from zero	Significant	Significant	Significant	Significant
P value (runs test)	0.2619	0.3452	0.6429	0.0714
Deviation from linearity	Not significant	Not significant	Not significant	Not significant
Equation	Y = 0.2848 * X - 0.3419	Y = 0.2236 * X - 0.1989	Y = 0.4335 * X + 1.371	Y = 0.3813 * X + 1.655

Permeation through the biological membrane. The R square coefficient of the regression lines (Table II) indicates a good determination (of 96 - 98%), with the MN permeation rate of 0.28 (FI) and 0.22 (FII) mg/cm^2/h, through Mb. In quantitative terms, it was found that MN reached a flow through Mb of 6 mg/cm^2 (FI) and 5 mg/cm^2 (FII), respectively, which represents 40% (FI) and 32% (FII) of the initial dose (40 mg) of the sample. On a first evaluation of the results, 40% of MN released seems not to be a satisfactory percentage, but if we report to the published data in witch MN has been shown to accumulate in the *stratum corneum* where it is maintained for up to 4 days [24], this percentage can be considered an appropriate dose for a possible antifungal activity. Moreover, same published data show that repeated application multiplies the dose maintained under the *stratum corneum*, leading to the elimination of the mycotic infection [6, 19].

Permeation through the biological vs. synthetic membranes. The amount of MN permeated in 24 h through Mb was determined almost exclusively by the contact time between the sample and the membrane, as the Pearson r have the highest values (0.9825 - FI, 0.9908 - FII) which shows a strong correlation (h *vs.* mg/cm^2 MN permeated), compared with Ms. In these later cases, the Pearson r values of 0.9765 - FI and 0.9655 - FII indicate there are some other factors which affect the permeation process. Comparing all cases, it appears that the formulation FII (3% HEC) is more suitable than FI (2% HEC) for further studies which involve biological membranes.

Influence of polymeric matrix on permeation process. Area under the permeation curves (AUC) was calculated for the period of 1 h to 24 h and had the following values (mean ± SD, n = 3): 78.61 ± 4.3 (FI-Mb), 62.44 ± 5.4 (FII-Mb) and 164.5 ± 9.2 (FI-Ms), 152.7 ± 8.5 (FII-Ms). AUC-FI *vs.* AUC-FII shows statistically significant differences only for Mb ($p = 0.0154$; unpaired t test, confidence interval of 95%), case in which the variance was insignificant (Anova one way, Tukey's test) (Table III). The rate of MN permeation (mg/cm^2/h), expressed by the slope of the regression line (Table I), was significantly lower through Mb (0.28 - FI, 0.22 - FII) than through Ms (0.43 - FI, 0.38 - FII), with time-lag (h) only in the Mb case (1.2 - FI, 0.89 - FII), according to the calculated data in the Table IV.

Table III

Statistical comparison of the AUCs variance

Unpaired t test of AUC: FII vs. FI	Mb	Ms
p value	0.0154	0.1781
p value summary	* significant	not significant
Significantly different ($p < 0.05$)?	Yes	No
One- or two-tailed p value	two-tailed	two-tailed
Difference between means	-16.17 ± 3.985	-11.8 ± 7.232
95% Confidence interval	-27.24 to -5.105	-31.88 to 8.278
R squared (eta squared)	0.8045	0.3996
F, DFn, Dfd (F test)	1.577, 2, 2	1.171, 2, 2
p value	0.7761	0.921
p value summary	not significant	not significant

Table IV

Statistical comparison of the regression lines variance

Tukey's multiple comparisons test	Mean differences	95.00% CI of differences	Significant/Summary		Adjusted p value
FI-Mb *vs.* FI-Ms	-85.89	-104.6 to -67.19	Yes	****	< 0.0001
FI-Mb *vs.* FII-Ms	-74.09	-92.79 to -55.39	Yes	****	< 0.0001
FII-Mb *vs.* FI-Ms	-102.1	-120.8 to -83.36	Yes	****	< 0.0001
FII-Mb *vs.* FII-Ms	-90.26	-109 to -71.56	Yes	****	< 0.0001
FI-Mb *vs.* FII-Mb	**16.17**	**-2.527 to 34.87**	**No**	**no significance**	**0.0921**
FI-Ms *vs.* FII-Ms	**11.8**	**-6.897 to 30.5**	**No**	**no significance**	**0.2570**

**** = extreme significance

Conclusions

The biggest challenge for formulators is to transport the active substance to the desired site for a targeted action. Compared to the synthetic membrane, the biological membrane reduces to almost half the *in vitro* permeation of MN released from polymeric matrices consisting in HEC-PEG of 2:1 (FI) or 3:1 (FII). The differences found between the two analysed dermal systems are insignificantly influenced by HEC content, which means the permeation variation could be due to the thickness difference between the membranes (0.45 µm - Ms, < 1 mm - Mb), the PEG slightly content variation and/or the influence of PEG on the biological membrane, as it was used in formulations both as plasticizer and as promoter of absorption. The

formulation FII (3% HEC) is more suitable than FI (2% HEC) for further studies which involve biological membranes, to optimize the composition by design of experiment and the factorial analysis.

Conflict of interest

The authors declare no conflict of interest.

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