

## Diffusion of metronidazole through human skin and synthetic membranes

### Metronidazolün insan derisi ve sentetik membranlardan difüzyonu

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#### Abstract

The aim of this study was to evaluate the diffusion rate of metronidazole through human skin and different synthetic membranes. Metronidazole is a drug used in the treatment of infections caused by anaerobic bacteria, also used in vaginal infections, antibiotic-associated pseudo-membraneous colitis, trichomoniasis and symptomatic amebiasis. Metronidazole has small molecular weight (171.16), low pKa value (2.62) and sparing solubility in different media and is chosen as a model drug in this study. Diffusion experiments were conducted using Franz diffusion cells and the related diffusion coefficients were calculated. The diffusion of metronidazole from aqueous and oily phases through human skin showed the same type of trendline as with the hydrophobic synthetic membrane (GVHP). Therefore, we can conclude that GVHP might be used to test drug permeation simulating the human skin.

**Keywords:** metronidazole, diffusion, human skin, synthetic membranes

#### Introduction

Metronidazole is a drug used in the treatment of infections caused by anaerobic bacteria, also used in vaginal infections, antibiotic-associated pseudo-membraneous colitis, trichomoniasis and symptomatic amebiasis (Rivera 1983, Martindale, 2007). It is a drug of first choice in the infections of *Helicobacter pylori* (Megraud, 2000) and it has also been reported to be of value in Crohn's disease (Achkar and Hanauer 2000, Remington 2006). Metronidazole is named as 2-methyl-5-nitroimidazole-1-ethanol or 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole. Its formula

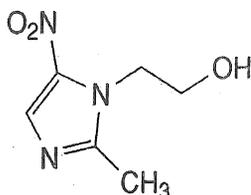


Figure 1. Chemical structure of metronidazole

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is  $C_4H_9N_3O$  and the chemical formula can be seen in Fig. 1 (Moffat 1986, Turgut and Özyazıcı 2004, Remington 2006). In this study we chose metronidazole as a model drug because of its small molecular weight (171.16), low pKa value (2.62) and sparing solubility in different media (Castela-Papin et al. 1999).

Mathematical predictions provide a useful indication of drug uptake and permeation across the skin, but refinement is necessary before there can be total confidence in the models. Prior to any clinical assessment, it is essential to have appropriate in vitro models available. Numerous studies have examined animal models, and none can be said to be perfectly predictive of human skin. Most vehicles have an effect on the barrier properties of the skin, and the effect varies with the animal species. Of all the species examined, the pig appears to be the most representative, but whenever possible, human skin should be the membrane of choice (Guy and Hadgraft 2003). According to this information, apart from the synthetic membranes, we evaluated the diffusion of metronidazole through human skin. Most common methods for evaluation of in vitro skin penetration use diffusion cells. The major advantage of in vitro investigations is that the experimental conditions can be controlled precisely, such that the only variables are the skin and the test material (Brain et al. 2002). Several diffusion studies conducted with Franz diffusion cells using synthetic membranes (Arellano et al. 1998, Dias et al. 1999, Müller and Kreuter 1999, Manosroi et al. 2005), natural membranes (Ansari et al. 2006) and human skin (Dias et al. 1999, Saija et al. 2000, Ansari et al. 2006, Reichling et al. 2006, Melero et al. 2009) were reported previously.

The present study aimed to evaluate the diffusion rates of metronidazole in aqueous and oily phases through human skin and different synthetic membranes. Metronidazole was used as a model drug throughout the study.

### Materials and Methods

Metronidazole as a pure drug was obtained from Selectchemie, Switzerland. Membrane filters with the codes of GNWP, HAWP, VCTP, VCWP and GVHP were purchased from Millipore, USA. Dialysis sacks were purchased from Sigma-Aldrich, Germany. Human skin samples were obtained from breast reduction operations. All solvents used were of pharmaceutical grade. The characteristics of the membranes are shown in Table 1.

**Table 1.** Characteristics of human skin and synthetic membranes used in diffusion studies

Type of membrane	pore size ( $\mu\text{m}$ )	thickness ( $\mu\text{m}$ )	nature
Human skin	-	600	-
GVHP (polyvinylidene fluoride)	0.22	125	hydrophobic
VCTP (polycarbonate)	0.10	20	hydrophilic
VCWP (mixed cellulose ester)	0.10	105	hydrophilic
GNWP (nylon)	0.22	170	hydrophilic
HAWP (mixed cellulose ester)	0.45	150	hydrophilic
Dialysis membrane (cellulose)	-	125	hydrophilic

### *Solubility studies*

Metronidazole in an amount in excess of its solubility was placed in pH 7.4 buffer in a bottle maintained at  $37 \pm 0.5$  °C and shaken in a constant temperature water bath (Certomat WRB, Braun, Biotech International) for 24 h. After ensuring that the equilibrium had been reached, the solution was filtered and the supernatant solution was diluted and assayed using UV spectrophotometer at the wavelength of 318.5 nm (UV Mini-1240 Shimadzu, Tokyo, Japan).

### *Diffusion studies*

Diffusion experiments were conducted using Franz diffusion cells that have a receptor volume of 31 mL and a diffusional area of about 3.14 cm<sup>2</sup>. pH 7.4 phosphate buffer solution was used as the receptor phase. All the diffusion studies were performed under sink conditions for metronidazole. Synthetic membranes and human skin sample without the subcutaneous fat (Saija et al. 2000) were used as a diffusion surface. Metronidazole in an aqueous phase (metronidazole and water) and in an oily vehicle (metronidazole and triglyceride) were introduced into the donor compartments and 3 mL of pH 4.0 buffer solution was added into the same compartment. The receptor compartment of the cell was maintained at 32°C. Teflon coated magnets were used to agitate the receptor compartments to provide uniform mixing. Sink condition was maintained throughout the experiment. The diffusion experiments were carried out using synthetic membranes and conducted over a 24 h period with predetermined time intervals of 0.5, 1, 2, 3, 5, 7, 24 h while the diffusion experiments using human skin were conducted over a 72 h period with predetermined time intervals of 1, 3, 5, 7, 24, 48, 72 h. Samples were taken from the receptor part and replaced with an equal volume of receptor phase and all samples were assayed using a UV spectrophotometer.

### *Skin sample preparation*

Full thickness human skin from a female volunteer (40 years old), who had undergone abdominal plastic surgery, was used. Immediately after excision, subcutaneous fat was removed by means of a scalpel and the skin was stored at -20°C until used (Wagner et al., 2000).

### *Data Analysis*

Apparent diffusion coefficients were calculated according to the following equation of Higuchi (Higuchi, 1962):

$$D_{app} = [(\text{slope})^2 \cdot \pi] / 4C^2$$

Where  $D_{app}$ : apparent diffusion coefficient; *slope*: when the amount of drug released to the receptor per unit area is plotted versus square root of time, a straight line should be obtained, the slope of which is related to the release rate of metronidazole. Regression analysis was used to calculate the slope;  $C$ : the initial concentration of metronidazole.

### *Statistical Analysis*

The results were expressed as means  $\pm$  standard deviations. Unpaired, two-tailed t-tests were performed at each time point. The threshold for statistical significance was at  $p < 0.05$ .

## **Results and Discussion**

The equilibrium solubility of metronidazole in pH 7.4 phosphate buffer solution was found to be  $10.092 \pm 0.317$  mg/mL. The effects of synthetic membrane types on the diffusion of metronidazole from oily phase are shown in Fig. 2. The results in Fig. 3 show the effects of synthetic membrane types on the diffusion of metronidazole from aqueous phase. It is apparent that the diffusion of metronidazole from the oily phase is significantly slower than the diffusion

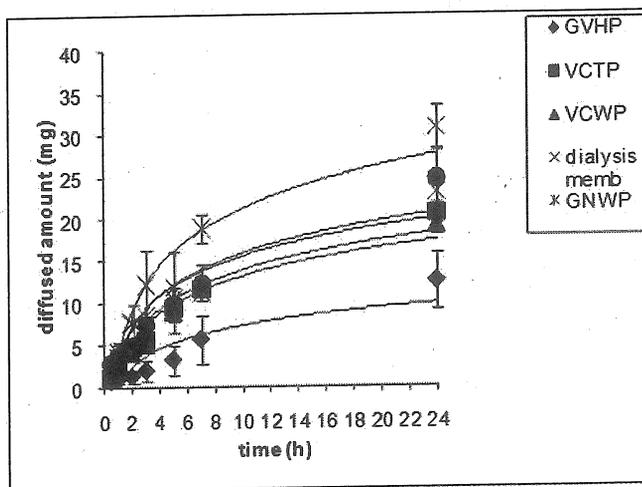
of metronidazole from the aqueous phase for all types of synthetic membranes used throughout the study ( $p < 0,05$ ). When the hydrophobic membrane (GVHP) was used, the diffusion rates of metronidazole were reduced compared with the diffusion rates from the hydrophilic membranes used. All the diffusional profiles of metronidazole from the membranes used, showed a logarithmic type of trendline except the diffusional profile of metronidazole from aqueous phase through GVHP synthetic membrane which showed a linear type of trendline (Fig. 2, 3).

The effect of human skin on the diffusion of metronidazole both from aqueous and oily phase is shown in Fig. 4. It is apparent that the diffusion of metronidazole through human skin is significantly slower than through all the synthetic membranes used ( $p < 0,05$ ). The diffusional profiles of metronidazole from aqueous phase through human skin and GVHP synthetic membrane showed a linear type of trendline (Fig. 2 and 4) while the diffusional profiles of metronidazole from oily phase through human skin and GVHP synthetic membrane showed a logarithmic type of trendline (Fig. 3 and 4). The apparent diffusion coefficients of metronidazole through human skin and synthetic membranes calculated according to the equation given in the data analysis part are shown in Table 2. The diffusion coefficients of metronidazole from aqueous phase through human skin and GVHP synthetic membrane being higher than the oily phase can be attributed to the linear diffusional profile of metronidazole from aqueous phase through both membranes. Similar results indicating the slower diffusion of drugs through human skin compared with their diffusion through the synthetic or natural membranes were indicated by a group of researchers. Dias et al. found out that the transport of caffeine from commercially available topical formulations through silicone and cellulose acetate membrane, mounted in modified Franz type diffusion cells, was faster than through human epidermis. They concluded that no correlation was found between transfer through the synthetic membranes and that observed through skin (Dias et al. 1999).

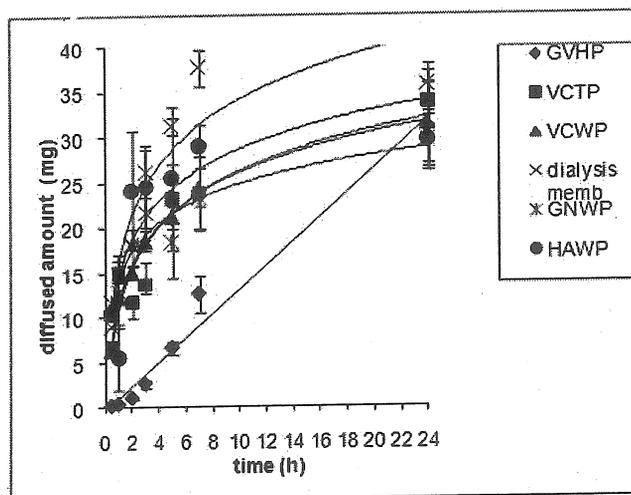
**Table 2.** Apparent diffusion coefficients ( $D_{app}$ : mean  $\pm$  SD) of metronidazole through human skin and synthetic membranes ( $\text{cm}^2/\text{s}$ )

Type of membrane	Aqueous phase	Oily phase
Human skin	$6.7 \times 10^{-8} \pm 4.8 \times 10^{-8}$	$1.2 \times 10^{-8} \pm 1.0 \times 10^{-9}$
GVHP	$114.0 \times 10^{-7} \pm 44.6 \times 10^{-7}$	$28.4 \times 10^{-7} \pm 36.0 \times 10^{-7}$
VCWP	$68.7 \times 10^{-7} \pm 14.2 \times 10^{-7}$	$47.2 \times 10^{-7} \pm 21.5 \times 10^{-7}$
VCTP	$87.0 \times 10^{-7} \pm 35.8 \times 10^{-7}$	$51.1 \times 10^{-7} \pm 11.0 \times 10^{-7}$
GNWP	$39.2 \times 10^{-7} \pm 21.8 \times 10^{-7}$	$52.1 \times 10^{-7} \pm 9.8 \times 10^{-7}$
HAWP	$48.6 \times 10^{-7} \pm 12.6 \times 10^{-7}$	$63.7 \times 10^{-7} \pm 12.0 \times 10^{-7}$
Dialysis membrane	$90.1 \times 10^{-7} \pm 14.8 \times 10^{-7}$	$105.0 \times 10^{-7} \pm 10.0 \times 10^{-7}$

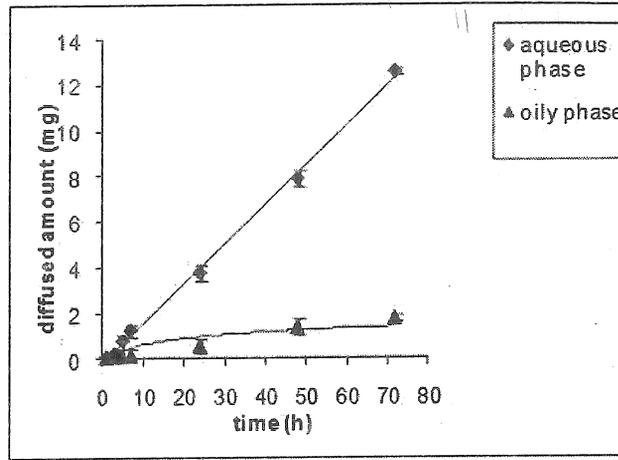
Ansari et al. worked with natural membranes such as the outer membrane of the peach and tomato, the inner layer of the egg and the middle membrane of the onion measuring permeation of diclofenac, metronidazole and erythromycin. The permeation of these drugs through natural membranes was compared to permeation through human skin and synthetic cellophane membrane using a modified Franz diffusion cell. They found out that the most similar diffusion profiles were those of onion and human skin for diclofenac, tomato and human skin for metronidazole, onion and cellophane membrane for erythromycin (Ansari et al. 2006).



**Figure 2.** The effect of synthetic membrane type on the diffusion of metronidazole from oily phase (n=3). The error bars show S.D.



**Figure 3.** The effect of synthetic membrane type on the diffusion of metronidazole from aqueous phase (n=3). The error bars show S.D.



**Figure 4.** Diffusion of metronidazole from aqueous and oily phases through human skin (n=3). The error bars show S.D.

## Conclusion

From the results we obtained, we can conclude that metronidazole in oily phase having a hydrophobic structure accumulated inside the skin. The diffusion of metronidazole from aqueous and oily phase through human skin showed the same type of trendline with the hydrophobic synthetic membrane (GVHP). Therefore GVHP might be used to test drug permeation simulating human skin. Further work should be done to support this conclusion.

## Acknowledgement

The authors would like to thank to Embil Pharmaceuticals for supplying metronidazole and synthetic membranes. The authors would also like to thank to Dr. Huseyin Karagoz from Department of Plastic Surgery, GATA, Haydarpaşa, Istanbul for supplying human skin samples.

## Özet

Bu çalışmanın amacı insan derisi ve farklı sentetik membranlardan metronidazolün difüzyon hızının değerlendirilmesidir. Metronidazol anaerobik bakteriler tarafından oluşan enfeksiyonların tedavisinde kullanılan bir ilaçtır ayrıca vajinal enfeksiyonlarda, antibiyotik ile bağlantılı psödomembranöz kolit, trikomonyaz ve semptomatik amebiyazis tedavisinde kullanılmaktadır. Metronidazol düşük molekül ağırlığı (171.16), düşük pKa değeri (2.62) ve farklı ortamlarda az çözünürlüğü olan bir ilaçtır ve bu çalışmada model ilaç olarak seçilmiştir. Difüzyon deneyleri Franz difüzyon hücreleri kullanılarak gerçekleştirilmiş ve difüzyon katsayıları hesaplanmıştır. Metronidazolün sulu ve yağlı fazdan difüzyonunda hidrofobik sentetik membran GVHP'nin insan derisi ile aynı eğilim çizgisini gösterdiği belirlenmiştir. Bundan dolayı, GVHP'nin ilaç permeasyonunda insan derisini simüle edebileceği sonucuna varılmıştır.

**Anahtar kelimeler:** metronidazol, difüzyon, insan derisi, sentetik membranlar

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Received: 01.02.2011

Accepted: 19.04.2011