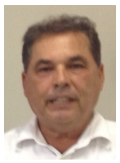
In vitro permeation study of microemulsions containing cidofovir



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INTRODUCTION

The human papillomavirus (HPV) is a virus of the genus Papillomavirus family Papovaviridae and can lead to a variety of benign, premalignant and malignant mucosal and skin surfaces. There are approximately 200 types of HPV have been identified, as the potential for malignancy, classified into types of low (types 6, 11, 13, 32, 34, 40, 42, 44, 53, 54, 55 and 63) and high risk of malignancy (types 16, 18, 31, 33 and 35) (De Villers 1989). HPV 6 and 11 are responsible for 90% of genital warts (condylomata acuminata) (Lowy 2006).

As options for the treatment of warts exist imiquimod, podophyllin, fluorouacil, trichloroacetic acid and cidofovir. Cidofovir 1 - [(S)-3-hydroxy-2-(phosphonomethoxy) propyl] cytosine dihydrate is a nucleoside analogue with potent activity against a broad spectrum of viruses (De Clerq 2007). Studies have shown that topical application of cidofovir inhibited or prevented the development of papillomas (Duan 2002).

The application of microemulsion vehicles for cutaneous drug delivery is becoming increasingly popular due to their high solubilization potential for both lipophilic and hydrophilic drugs. It was demonstrated that permeation rates from microemulsions were significantly higher than from conventional emulsions. The vehicles frequently act as penetration enhancers, depending on the oil/surfactant constituents, which involves the risk of inducing local irritation (Garti 2006).

The aim of this work is the characterization and evaluation of microemulsions containing cidofovir and evaluate the permeation profile of them.

MATERIALS AND METHODS

Materials

The formulations used in the preparation of microemulsions were selected from the diagram developed by Silva (2008).

Cidofovir was obtained from Boyle Chem (China). Labrasol® and Plurol oleique® were obtained from Gattefossè (France). Isopropyl myristate was purchase fom Vetec (Brazil). Polawax®, isodecyl oleate, propylene glycol, EDTA, BHT and menthol were puchased from Fagron (Brazil). Cineol was obtained from Biodinâmica (Brazil).

Table 1: Composition of microemulsions

Composition (%)	F4CDV	F4C	F4M	F5CDV	F5C	F5M
Labrasol®	45	45	45	30	30	30
Plurol Oleique®	15	15	15	10	10	10
Isopropyl myristate	10	7,5	7,5	10	7,5	7,5
Polawax®	-	-	-	-	-	-
Isodecyl oleate	-	-	-	-	-	-
Propylene glycol	-	-	-	-	-	-
EDTA	-	-	-	-	-	-
BHT	-	-	-	-	-	-
Cineol	-	2,5	-	-	2,5	-
Menthol	-	-	2,5	-	-	2,5
Cidofovir	1	1	1	1	1	1
Buffer 7,4 q.s.p.	100	100	100	100	100	100

Methods

For the characterization of the formulations was performed polarized light microscopy (DM 750P, Leica ®), determining the droplet size and zeta potential (Zetasizer Nano-ZS90, Malvern Instruments ®) and viscosity (Anton Paar ®).

For tests of penetration and permeation were used pig ear skin as a model membrane, obtained from the local slaughterhouse. A Franz diffusion cell with an effective diffusion area of 1,15 cm² was used for the experiment. The experiments were conducted for 24h. The collected samples were filtered and analyzed by HPLC/UV (Shimadzu, Japan) at 274 nm, mobile phase buffer pH 6,0 / acetonitrile (97:3).

RESULTS AND DISCUSSION

Characterization

All microemulsions prepared showed rheological behavior of Newtonian type, characteristic of microemulsions.Microemulsions formulations were observed as completely dark under cross polarizer, wich implied its optically isotropic nature.

The average droplet size, PDI and zeta potential of microemulsion are described in table 2.

Table 2: Characterization of the formulations

Formulation	Droplet size (nm)	PDI	Zeta potential (mV)
F4CDV	35,05	0,217	-7,71
F4C	36,21	0,248	-7,90
F4M	59,42	0,164	-8,75
F5CDV	25,75	0,453	-8,80
F5C	28,40	0,448	-9,370
F5M	48,72	0,257	-17,80

In vitro skin studies

The permeation profiles (fig. 1) shows that the formulation F5M demonstrated the highest skin permeation values, followed by F5C, and the another formulations do not showed significantly different statistically.

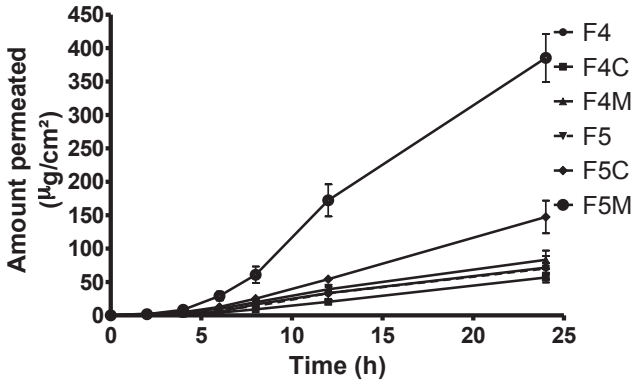

Figure 1: Skin permeation of cidofovir.

The figure 2 shows the amount penetrated in the stratum corneum and the figure 3 represents the amount penetrated in the epidermis and dermis.

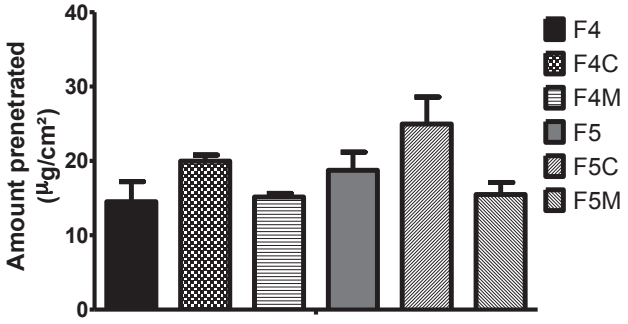

Figure 2: Total amount of cidofovir in the SC.

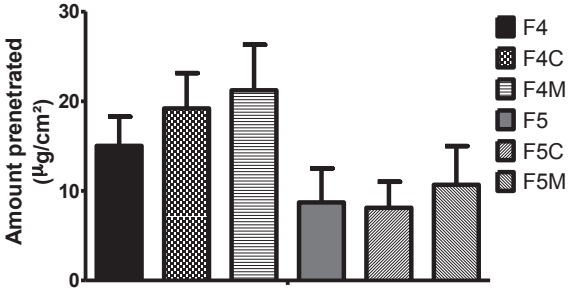

Figure 3: Total amount of cidofovir in the epidermis and dermis.

CONCLUSION

Among the microemulsions, the F4M was one that showed better skin retention. This greater amount retained in the epidermis and dermis can be credited to the high concentration of surfactants in the formulation, disrupting more intensely barrier lipids that comprise the stratum corneum (Oliveira 2004; Silva 2010). This result may be a positive indicator, making these the most appropriate preparations for a possible treatment of HPV, since the virus has tropism for the viable epidermis, the basal cell layers of the epidermis (Souto 2005, Stanley 2008).

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