

GEL OF MICROPARTICLES TO TARGET CIDOFOVIR TO THE BASAL EPIDERMIS

Lydia Echevarría Zamacona¹, María J. Blanco-Príeto², Rocío Martínez de Grado², Pilar Ygartua Ayerra²

1 Laboratory of Research. Department of Research and Development. Italfarmaco, S.A. Madrid. Spain

2 Department of Pharmacy and Pharmaceutical Technology. Navarra University. Pamplona. Spain

Introduction and Purpose

Introduction: Cidofovir (CD), a monophosphated nucleotide similar to cytosine, represents a new class of antiviral agent with potent *in vitro* and *in vivo* activity against a broad spectrum of herpes viruses (1).

Topical administration of CD has been shown to be effective in the treatment of cutaneous infections of herpes simplex viruses HSV-1 and HSV-2 in animal models (2). Nevertheless CD is administered only intravenously.

Purpose: Our aim was to develop and characterize three formulations of CD: microparticles, hydrophilic gel and microparticles included in an hydrophilic gel in order to compare the percutaneous penetration profile of the three formulations and their distribution in the skin. Our main purpose was to obtain a prolonged therapeutic effect of CD in the basal epidermis, site of herpes virus infection lesions.

Materials and Methods

Materials: CD was supplied by Gilead Sciences, Carbopol ETD 2020 was obtained from BF Goodrich, propilenglycol, triethanolamine and disodium hydrogen phosphate were purchased from Roig Farma. Potassium phosphate was obtained from Probus and dichloromethane from Prolabo. The copolymer PLGA Resomer 502 was supplied by Boehringer Ingelheim. O.C.T. (Tissue Tek[®]) was purchased from Sakura. Tetrabutylammonium dihydrogenphosphate was supplied by Fluka. Finally, acetonitrile and other

solvents used were of analytical grade and were obtained from Merck.

Methods:

Hydrophilic gel of CD was formulated as a dispersion of Carbopol ETD 2020 into a CD suspension.

CD microparticles were prepared using the spray-drying method (3).

The formulation of CD microparticles included in an hydrophilic gel was prepared by dispersing the CD microparticles in a suspension of Carbopol ETD 2020 previously to the neutralization of the gel with triethanolamine.

Automatic Franz diffusion cells were used to perform the percutaneous penetration studies; porcine skin was mounted on the diffusion cells with the stratum corneum facing the donor compartment. 4,5 ml of phosphate buffer solution (pH 7,4) were used as the receptor medium. 400 μ l aliquots were collected from the receptor at designed time intervals. The receptor medium was maintained at $37\pm 1^\circ\text{C}$ and stirred at 300 rpm during 24 hours. After permeation experiments, ten slices of 40 μ m were cut at -19°C in parallel to the skin surface with a cryostat. CD was extracted from the skin and quantified by HPLC.

Results and discussion

24 hours after the percutaneous penetration study of, the three formulations tested could permeate through the porcine skin, nevertheless the quantity of CD permeated from the microparticles included in an hydrophilic gel is much lower than from the other two formulations (Table 1).

Time (h)	Quantity of CD permeated ($\mu\text{g}/\text{cm}^2$)		
	Gel	Microparticles	Microparticles in a gel
2	7,37	1,84	0,86
8	21,94	19,70	5,13
14	45,09	37,04	13,15
20	73,86	52,72	22,04
24	78,17	72,83	33,27

Table 1. Quantity of CD permeated per unit of surface from the three formulations during a percutaneous permeation study of 24h.

The Figure 1 represents the permeation profile of CD permeation for 24 hours.

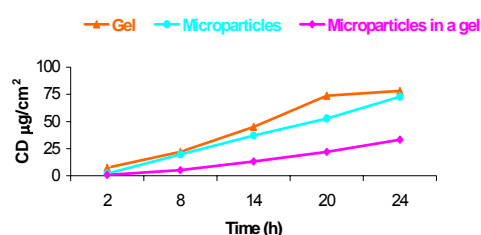


Figure 1. Percutaneous permeation profile of CD across porcine skin.

Thus, the topical application of CD microparticles included in an hydrophilic gel could avoid the renal toxicity of the CD, reducing the blood levels of the drug.

The percentage of CD dosis retained at the basal epidermis (between 120-160 μm , target site of the herpes virus) after the topical application of microparticles included or not in an hydrophilic gel is much higher than when the CD hydrophilic gel is applied.

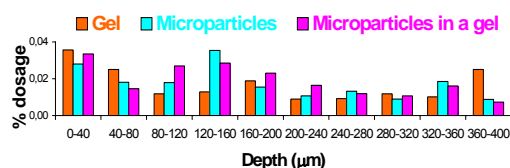


Figure 2. Distribution of CD in porcine skin layers after topical application of the three formulations during 24h.

In fact, there is a similarity between the microparticles included or not in an hydrophilic gel, but the limitation for the use of microparticles not included in the gel is that the CD permeation to the blood is too high while this inconvenient can be reduced or minimized if the CD microparticles are included in an hydrophilic gel.

So we can conclude that the CD concentration of $747,2 \mu\text{g}/\text{cm}^3$ (from the microparticles included in an hydrophilic gel) found at the basal epidermis is adequate to obtain an antiviral effect. And also, the quantity of CD found at the receptor side is lower when the microparticles are included in an hydrophilic gel. And this fact shows that the renal toxicity can be avoided.

Bibliography

- (1) Bronson, J.J., Ferrara, L.M., Hitchcock, M.J.M., Webb, R.R., Kern, E.R., Sokie, K.F., Martin, J.C. Plenum Press, New York, 277, (1990).
- (2) De Clercq, E., Holy, A. Antimicrob. Agents Chemother. 35, 701, (1991).
- (3) Santoyo, S., G^a de Jalón, E., Ygartua, P., Renedo, M.J., Blanco-Príeto, M.J. Int. J. Pharm. 242, 107, (2002).

Acknowledgements

The authors would like to thank Italfarmaco S.A. and "Asociación de Amigos de la Universidad de Navarra".