Transcorneal Permeation in a Corneal Device of Non-Steroidal Anti-Inflammatory Drugs in Drug Delivery Systems

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Abstract: This work is focused on the *ex vivo* study of corneal permeation of two anti-inflammatory drugs: diclofenac, and flurbiprofen (as a model of hydrophilic and lipophilic drug, respectively) loaded to cyclodextrins or polymeric nanoparticles in order to determine differences in their corneal permeation against free drug or commercial eye drops. These studies were carried out in a corneal device designed and developed in our laboratory. In this work the habitual conditions for the permeation studies were modified to reproduce the behaviour when eye drops were administered. For this reason a new tetracompartmental pharmacokinetic model was developed. The complex formation of diclofenac with cyclodextrins and the flurbiprofen loaded to polymeric nanoparticles has been shown as effective procedures to remarkably increase the bioavailability of the anti-inflammatory drugs. The efficiency of polymeric nanoparticles of Poly (D-L lactic-coglycolyc) acid and poly-ε-caprolacton as intraocular targeting of NSAIDs has also been proved, being the latter polymer more effective to increase the flurbiprofen corneal permeation. The apparent corneal permeability coefficient of samples has been calculated getting a low permeation values for free drugs.

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs), like diclofenac and flurbiprofen, have been found to be viable alternatives to steroids in treating ocular inflammation. This therapy requires, due to low permeation rates and a rapid pre-corneal loss, a frequent application or highly concentrated eye drop formulations. The drugs must penetrate across the cornea, to reach therapeutic targets within the globe. In general, no more than five percent of drug, present in the precorneal area, crosses the cornea and arrives to intraocular tissues. The rest of the administered drug is dragged by the tear to the nasal conduit where it is eliminated later by digestive tract.

The present research was focused on the *ex vivo* study of corneal permeation of two anti-inflammatory drugs (NSAIDs), frequently used in ocular pharmacology: diclofenac and flurbiprofen. The corneal permeation of these free drugs was compared to the one of the diclofenac forming complexes with cyclodextrins or the flurbiprofen loaded to polymeric nanoparticles.

MATERIALS AND METHODS

These studies were carried out in a corneal device designed and developed in our laboratory for this research [1], based in the others previously described [2-4].

The Fig. (**1**) shows the chamber used to measure corneal permeation in detail.

The principle of the apparatus is that the cornea of the rabbit is placed in a methacrylate chamber clamped between two pieces so that the epithelial surface faces one compartment (anterior or tear compartment) and the endothelial surface the other (posterior or aqueous humor compartment).

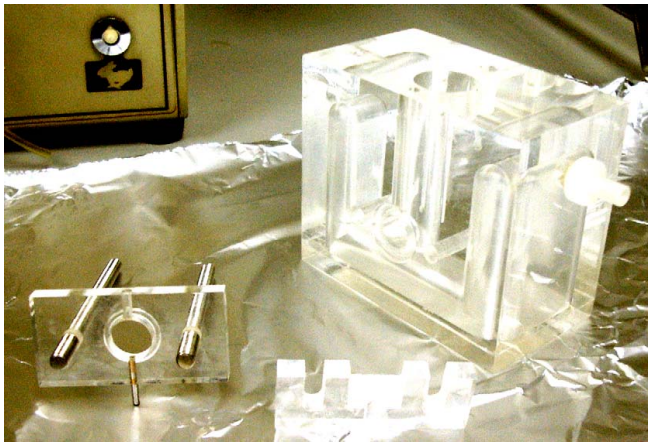

Fig. (1). Detail of the metacrylate chamber to clamp the cornea.

The Fig. (**2**) shows the entire setup of the system. The fluid contained in the anterior compartment can be exchanged continuously by means of a pump. The hydrostatic pressure in endothelial compartment can be chosen arbitrarily and independently. The experimental temperature can be controlled continuously.

The transcorneal electrical potential can be measured and registered also continuously to check the vitality of the rabbit cornea [5, 6].

Albino New Zealand rabbits weighing 1.8 to 2.2 kg, were used. After rabbits were sacrificed, the corneas were removed and immediately mounted and clamped in the chamber.

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Fig. (2). Entire setup of the corneal device for the study of corneal permeation.

The basic solutions were prepared from reagent grade chemicals as in the report of O'Brien and Edelhauser [7].

The artificial tears solution (ATS), for the epithelial side of the cornea, was a Bicarbonate buffered Ringer's solution prepared previously and maintained at 4º C. Previously to perform experiment the above solution was warmed and was added 0.9 g L^{-1} of glucose with magnetic stirring.

To prepare the artificial aqueous humor (AAH) for the endothelial side of the cornea takes the obtained ATS and add Glutathione 0.13 g L^{-1} and Adenosine 0.09 g L^{-1} at 37º C with stirring just before use.

The extraction of small volumes of the endothelial solution without loss of pressure was carried out by means of a syringe with a needle that crosses the cork of the endothelial compartment. Samples of 50 µL of the endothelial compartment were taken at different times (between 5 minutes and 2 hours) and then were analysed by Ultraviolet Absorption Spectroscopy.

Apparent permeability coefficients *(Papp)* were measured by previously described methods [8], according to this equation:

$$P_{app} = \frac{\delta Q}{\delta t \ \ 60 \ A \ C_0}$$

where $\delta Q / \delta t$ is the variation of drug amount, corresponding to the slope of the linear portion of the graphic (normally the first hour), 60 the conversion from minutes to seconds, A is the corneal surface (in this study 0,55 cm^2) and C_0 the initial drug concentration in the epithelial side.

CORNEAL PERMEATION WITHOUT WASHING PROCEDURE: TRI-COMPARTMENTAL MODEL

Most corneal penetration studies have been performed maintaining constant drug concentration in the artificial tear solution of the external or epithelial compartment. In this way the drug concentration at the internal or endothelial corneal side, increases regularly following an exponential slope like as reflected in Fig. (**4**).

When there exists a constant flux of drug a simplified, but very precise, tricompartmental model can been used. This model has been developed based on the one proposed by Maurice [9]. It works with the following elements (Fig. **3**).

A constant external concentration Ce, a variable corneal concentration Cc, a variable internal concentration Ci, a transfer rate constant from the exterior to the cornea k_1, and a transfer rate constant from the cornea to aqueous humor k_2.

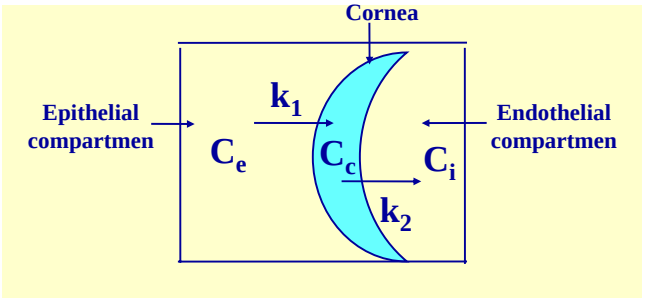

Fig. (3). Tricompartmental model. Ce: constant external or epithelial concentration, Cc: variable internal corneal concentration, Ci: variable internal or endothelial concentration, K_1 and K_2: transference constants from epithelial compartment to cornea and from cornea to endothelial compartment, respectively.

In this work Ce and Ci are known. k_1 and k_2 are unknown, while Cc is not needed for the calculation. The drug flow is considered to be passive and against the concentration gradient. The statement of apparent corneal permeability can be laid out with the following two kinetic equations:

$$\frac{dC_c}{dt} = k_1(C_e - C_c) - k_2(C_c - C_i)$$

$$\frac{dC_i}{dt} = k_2(C_c - C_i)$$

The system is reduced to the following equation:

$$C_i + (k_1 + 2 \cdot k_2) \cdot C_i + k_1 \cdot k_2 \cdot C_i = k_1 \cdot k_2 \cdot C_e$$

This equation has the solution:

$$y = A \cdot e^{m_1 \cdot t} + B \cdot e^{m_2 \cdot t} + C$$

where:

$$m_1 = \frac{-k_1 - 2k_2 + \sqrt{k_1^2 + 4k_2^2}}{2}$$

$$m_2 = \frac{-k_1 - 2k_2 - \sqrt{k_1^2 + 4k_2^2}}{2}$$

Using the Pythagoras theorem, it can be seen that m_1 is always negative, as m_2 where all terms are negative, so we can use n_1 and n_2 as m_1 and m_2. Taking also into account that $C_i(0) = 0$ and $C_i(t \rightarrow \infty) = C_e$ the model is simplified obtaining the equation in its final form:

$$C(i) = A \cdot [e^{-n_1 \cdot t} - e^{-n_2 \cdot t}] + C_e \cdot [1 - e^{-n_2 \cdot t}]$$

Were "t" is the variable time and n_1 and n_2 two pharmacokinetic parameters related to k_1 and k_2. A does not have an

immediate pharmacokinetic significance. It depends on the experimental conditions and on the product itself.

The apparent permeability for Sodium Diclofenac calculated by the slope of the graphic at sixty minutes was $9.55 \cdot 10^{-4}$ cm/h (Fig. **4**).

CORNEAL PERMEATION WITH WASHING PROCEDURE. TETRA-COMPARTMENTAL MODEL

When the drugs are administered as eye drops the tears wash quickly the corneal surface, so that the drug is pulled to the nasal conduit and its corneal permeation also diminishes quickly.

This behaviour was reproduced in our experiments subjecting the initial endothelial drug solution to a continuous wash of artificial tear during the permeation measures. The graphic so obtained is shown in Fig. (**4**).

The apparent permeability calculated in this graphic by the slope at sixty minutes in both procedures have the values shown in the figure.

This model requires a more complex treatment as a tetra-compartmental pharmacokinetic model.

In this model the statement of corneal permeability requires three differential equations:

$$\frac{dC_c}{dt} = k_1(C_e - C_c) - k_2(C_c - C_i)$$

$$\frac{dC_i}{dt} = k_2(C_c - C_i)$$

$$\frac{dC_e}{dt} = -k_1(C_e - C_c) - k_3 \cdot C_e$$

The main variation of Ce is to wash by ATS so it can approach $\frac{dC_e}{dt}$ to $\frac{dC_e}{dt} = -k_3 \cdot C_e$

So the anterior equation of the tricompartmental model is reduced to the following:

$$C_i + (k_1 + 2 \cdot k_2) \cdot C_i + k_1 \cdot k_2 \cdot C_i = k_1 \cdot k_2 \cdot C_e$$

And with a similar treatment that tricompartmental model let us to:

$$C_i(t) = A \cdot (e^{-n_1 \cdot t} - e^{-n_2 \cdot t}) + B \cdot (e^{-k_3 \cdot t} - e^{-n_2 \cdot t})$$

Were "t" is also the variable time and n_1, n_2, A and B, pharmacokinetic parameters related to k_1, k_2 and k_3.

TRANSCORNEAL PERFUSION OF DICLOFENAC β-CYCLODEXTRIN COMPLEX

In ophthalmology, local drug administration in the form of topically applied low viscosity aqueous eye drop solutions is generally preferred.

Topically applied drugs must be, at least to some degree, soluble in the aqueous tear fluid. NSAIDs used to treat ocular inflammation are lipophilic water-insoluble compounds that have to be introduced into aqueous eye drop formulations as water-soluble salts (like sodium diclofenac salt). However, they must also be somewhat lipid-soluble in order to penetrate the lipophilic corneal epithelium, through the corneal stroma and the lipophilic endothelium into the aqueous humor. In both cases, ocular bioavailability is seriously hampered by the low aqueous solubility or the hydrophilic properties of the penetrating molecules, respectively. In other words, for successful formulation in an aqueous eye drop solution a drug must be both water-soluble (that is hydrophilic) and lipid-soluble (that is hydrophobic).

Cyclodextríns are novel, chemically stable adjuvants that enhance ocular bioavailability of ophthalmic drugs without affecting the barrier function of the eye or increasing the viscosity of the aqueous, eye drop formulation.

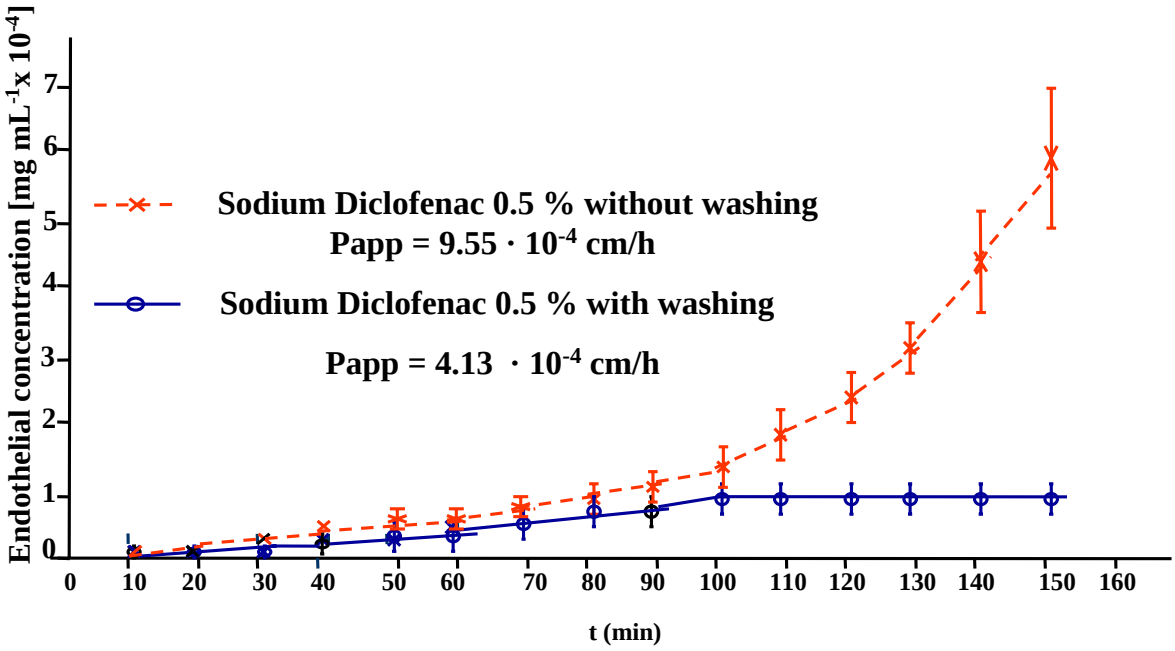

Fig. (4). Cornea permeation profile of Sodium Diclofenac 0.5 % solution without washing or with washing.