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Research paper

# Conversion of cyclosporine A prodrugs in human tears vs rabbits tears

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**Abstract**

The aim of this study was to evaluate the rate and mechanism of conversion of two water-soluble prodrugs of cyclosporine A (CsA) intended for topical delivery to the eye. The new molecules were designed according to the double prodrug concept: a solubilizing moiety was grafted onto CsA via an ester function, which could be hydrolysed via a two-step process (enzymatic and chemical). Prodrug solutions were prepared extemporaneously in an isotonic and neutral aqueous medium compatible with ophthalmic use. The rates of conversion into the parent molecule were determined by incubating the prodrugs in fresh rabbit or human tears or in a phosphate buffer solution (PBS) at pH 7.4. Both prodrugs were converted into CsA within the first minute in the presence of rabbit tears with rate constants of $k=5.9\times10^{-3}$ min$^{-1}$ and $k=3.8\times10^{-3}$ min$^{-1}$, respectively, for UNIL088 and UNIL089, whereas chemical conversion in PBS was negligible ($k=0.5\times10^{-3}$ min$^{-1}$ for both molecules). Incubation of UNIL088 in human tears showed a significantly high conversion rate. It is concluded that the developed double prodrugs underwent a bioconversion in physiological media and thus represent promising candidates for topical delivery of CsA to the eye.


*Keywords:* Cyclosporine A; Prodrug; Ex vivo model; Ocular drug delivery; Conversion; Rabbit tears; Human tears

## 1. Introduction

The immunosuppressive drug cyclosporine A (CsA) is nowadays commonly used in the management of several ocular conditions with an immune component such as uveitis [1], dry eye syndrome [2] and in the prevention of corneal graft rejection [3]. These pathological states can be treated by i.v. or oral administration but systemic levels of CsA induce severe side effects such as nephrotoxicity and cardiotoxicity [4]. The use of local formulations such as collyria should circumvent these side effects. However, due to its lipophilic nature, CsA cannot be formulated as an aqueous solution. Hence, numerous approaches have been investigated for topical CsA administration but, so far, only an emulsion for human use (Restasis®, Allergan Inc., Irvine, CA) has been approved by the Food and Drug Administration (FDA) [5]. Emulsions, however, present the major disadvantages of causing blurred vision and providing relatively low drug availability due to the high affinity of CsA for the oily phase of the formulation. The water-soluble prodrug approach is a very efficient way to solubilize CsA in aqueous media avoiding these drawbacks. This concept has already been applied to several molecules in ophthalmology to modify the hydrophilicity of drugs [6] (e.g. esters of steroids [7] and amino acid esters of acyclovir [8]). As CsA possesses (on position 1) a residue with a free hydroxyl group, the synthesis of ester prodrugs is a feasible option. A series of CsA ester derivatives have been synthesized [9] and among

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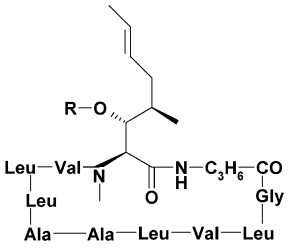


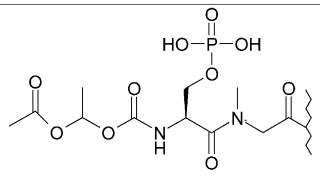
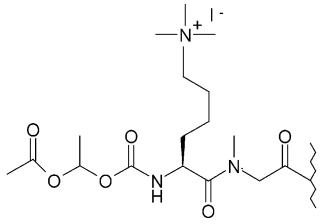

| Compound | Mw | R |
|---|---|---|
| CsA | 1202 | H |
| UNIL088 | 1570 |  |
| UNIL089 | 1574 |  |

Fig. 1. Chemical formula of CsA, UNIL088 and UNIL089.

them, UNIL088 and UNIL089 (Fig. 1) were selected for further investigation. The chain of the prodrugs to be removed to release the drug is the dipeptide sarcosine-serine (or lysine)-(acyloxy)alkyl-oxy-carbonyl, carrying a phosphate group (UNIL088) and an ammonium group (UNIL089) as solubilizing moieties. These two molecules have been designed according to the double prodrug theory: the prodrugs are converted into the active parent molecule via a two-step enzymatic and chemical mechanism. The rapid elimination from the precorneal area of any topically applied solution requires the prodrug derivatives to undergo quantitative conversion within the first minute after application. The conversion rate of a prodrug into the parent molecule is commonly evaluated by incubating the compound in the presence of commercial enzymes [7,10], human plasma [11] or homogenates of ocular tissues [12–14]. However, these models are not representative of the tear composition in enzymes emphasizing the need for a reliable tool able to evaluate the potential of the prodrugs to be converted into their parent molecule under clinical conditions and able to determine the nature of the underlying mechanism (enzymatic or chemical).

The aim of the present study was firstly to demonstrate that after solubilization at a concentration equivalent to 0.2% (w/v) of CsA in an aqueous medium, UNIL088 and UNIL089 could generate CsA when incubated in the presence of rabbit tears using a new and reliable ex vivo model. Secondly, the prodrugs were incubated in a phosphate buffer solution at pH 7.4 to determine whether or not the conversion was purely chemical or enzymatic. Finally, the prodrugs were incubated in human tears to test the validity of the rabbit tear model. The prodrug with the fastest kinetics of transformation would then be chosen for further investigations.

## 2. Materials and methods

### 2.1. Materials

Prodrugs (UNIL088 and UNIL089) were synthesized and characterized according to Wenger et al. [9]. Mannitol for isotonic solutions was purchased from Acros Organics (Geel, Belgium). Isotonic phosphate buffer at pH 7.4 was composed of 179 mg of monopotassium phosphate, 953 mg of disodium phosphate, 415 mg of sodium chloride and distilled water to 100 ml. Chemicals of analytical grade were obtained from Fluka (Buchs, Switzerland) and all solvents (water and acetonitrile (SDS, Peypin, France)) were of analytical grade.

### 2.2. Methods

#### 2.2.1. Preparation of prodrug solutions

Prodrug solutions (UNIL088 and UNIL089) were extemporaneously prepared at a concentration equivalent to 0.2% (w/v) of CsA, in an aqueous 5% (w/v) mannitol solution adjusted to pH 7 with 1 N NaOH. The prodrug powder is solubilized immediately at room temperature and under slight mechanical agitation. Isotonicity (Automatic osmometer type Digital/L, Knauer, Germany) and pH (Metrohm 691 pH meter, Herisau, Switzerland) were assessed prior to experiment.

#### 2.2.2. Rabbit and human tear collection

Animal tears were collected from non-anaesthetized New Zealand albino rabbits (weighing approximately 4.0 kg) with disposable 2 μl glass micro-capillaries (Microcaps Drummond, Thomas Scientific, New Jersey). No artificial tear secretion stimulation was performed. Immediately after collection, capillaries were blown into a vial under a gentle nitrogen flow. Kinetics of conversion of the prodrugs into CsA were performed within 5 min after tear collection so that tear proteins and enzymes are still intact. Human tears were self-collected by volunteers using the same method as for rabbit tears. The experiments were approved by the local Ethics Committee.

#### 2.2.3. Analytical method

CsA and prodrugs were analysed and quantified by high performance liquid chromatography (HPLC). This method was developed specifically to quantify in the same run hydrophobic CsA and hydrophilic prodrugs. Analytical separations were conducted using a C4 column