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Research Paper

Targeted intracorneal delivery—Biodistribution of triamcinolone acetonide following topical iontophoresis of cationic amino acid ester prodrugs

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ABSTRACT

The aim was to investigate intracorneal iontophoresis of biolabile triamcinolone acetonide (TA) amino acid ester prodrugs (TA-AA). Arginine and lysine esters of TA (TA-Arg and TA-Lys, respectively) were synthesized and characterized; quantification was performed by HPLC-UV and UHPLC–MS/MS. The aqueous solubility of the prodrugs (at pH 5.5) was ~1000-fold greater than TA. Anodal iontophoresis (10 min at 3 mA/cm^2) of TA-AA was investigated using isolated porcine cornea. Although no statistically significant difference was observed in total intracorneal delivery of TA (468.25 ± 59.70 and 540.85 ± 79.16 nmol$_{TA}$/cm^2, for TA-Arg and TA-Lys, respectively), the different susceptibilities of the prodrugs to hydrolysis influenced intracorneal biodistribution. Quantification of TA in twenty-five 40 μm thick corneal lamellae revealed significantly deeper penetration of TA following TA-Lys iontophoresis. Its superior resistance to hydrolysis enabled sustained electromigration into the deeper cornea suggesting judicious prodrug selection might enable targeted regioselective drug delivery. The intracorneal biodistribution following anodal iontophoresis of TA-Arg (2.3 mM; 10 min, 3 mA/cm^2) was visualized by full field optical coherence tomography providing qualitative confirmation of the extensive intracorneal penetration of TA. Short duration iontophoresis of TA-AA prodrugs may improve deep corneal bioavailability and efficacy in vivo, constituting a “single-shot” treatment option for corneal allograft rejection.

1. Introduction

Corneal graft allotransplantations are the most frequently performed organ transplantation (Niederkorn, 2013; Prendergast and Easty, 1991); their success rate is attributed to the low expression of histocompatibility antigens as compared to other organs (Medawar, 1948; Qazi et al., 2010). However, trauma or viral infection can induce corneal neovascularization (Gupta and Illingworth, 2011), which significantly increases the risk of corneal graft failure, with a decade survival rate of approximately 60% (Banerjee and Dick, 2003; Tan et al., 2012; Waldock and Cook, 2000). Most corneal graft rejections are caused by a T-cell mediated immune response against the major histocompatibility complex antigens of the donor tissue (Gebhardt and Shi, 2002). Leucocyte infiltration and neovascularization were shown to be especially severe when deeper corneal tissues such as stroma and endothelium were involved, leading to graft failure (Panda et al., 2007). Topical corticosteroid administration is the standard treatment for the prevention of corneal graft rejection (Banerjee and Dick, 2003). However, lacrimal drainage and absorption by periocular tissues constitute dynamic barriers that decrease ocular bioavailability and their impact is complemented by the properties of the corneal epithelium, which is a very effective static barrier to molecular transport (Prausnitz and Noonan, 1998;

Abbreviations: TA, triamcinolone acetonide; TA-AA, amino acid prodrugs of TA; TA-Arg, arginine ester prodrug of TA; TA-Lys, lysine ester prodrug of TA; EO, electroosmosis; EM, electromigration; OCT, optical coherence tomography; FFOCT, full field optical coherence tomography; CLSM, confocal laser scanning microscope; NMR, nuclear magnetic resonance; HPLC–UV, high-performance liquid chromatography with ultraviolet detection; UHPLC–MS/MS, ultrahigh-performance liquid chromatography with tandem mass spectrometry detection; LOD, limit of detection; LOQ, limit of quantification; ESI+, electrospray ionization in positive ion mode; MRM, multiple reaction monitoring; PBS, phosphate buffered saline; Boc-Arg(Boc)2-OH, N-Boc protected L-arginine; Boc-Lys(Boc)-OH, N-Boc protected L-lysine; DCHA, dicyclohexylammonium; 4-DMAP, 4-dimethylaminopyridine; DCM, dichloromethane; DCC, *N,N′*-dicyclohexylcarbodiimide; MES, 2-morpholino-ethanesulfonic acid monohydrate; NaCl, sodium chloride; TFA, trifluoroacetic acid; AgCl, silver chloride; ACN, acetonitrile; EtOAc, ethyl acetate; HEX, hexane; MeOH, methanol.

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Rojanasakul et al., 1992). Their combined effect means that treatment regimens necessitate hourly drug instillation over several days and this can result in poor patient compliance (Järvinen et al., 1995). Systemic administration of corticosteroids imposes the use of high dosages with the concomitant risk of severe off-target side effects.

Triamcinolone acetonide (TA) is routinely administered by intravitreal injection for the treatment of intraocular inflammatory diseases (Jermak et al., 2007) and sub-conjunctival injections have been employed to suppress corneal graft rejection (Costa et al., 2008; Mehrjardi et al., 2014). However, due to its low aqueous solubility (12 μg/ml (Mora et al., 2005)) the preparation of an injectable solution remains a challenge; indeed, the available TA formulations are usually drug suspensions, e.g. Kenacort A 40 (Bristol-Myers Squibb AG; Baar, Switzerland). Similarly, topical formulations such as ophthalmic ointments also contain TA as a suspension, e.g. Cidermex (UCB Pharma S.A.; Colombes, France) a previously commercialized TA and neomycin combination product. Given the formulation issues, poor bioavailability and the sheer inconvenience of using drug suspensions in the eye, it was decided to develop a series of water soluble biolabile amino acid prodrugs of TA (TA-AA) in order to facilitate formulation and to enable the use of short duration iontophoresis to improve intracorneal bioavailability of TA.

Iontophoresis is a noninvasive drug delivery technique based on the application of a mild electric potential gradient across a biological barrier, which drives ionized molecules into the membrane (Kalia et al., 2004). The combined effects of the concentration and potential gradients result in increased molecular transport and hence improve bioavailability, as compared to "simple" passive administration which relies on diffusion along a concentration gradient (Gratieri and Kalia, 2013).

At steady-state, the total flux (J_{TOT}) can be expressed as the sum of the passive and "active" contributions (namely, electroosmosis (EO) and electromigration (EM)):

$$J_{TOT} = J_P + J_{EO} + J_{EM} = \left[k_{p,x} + V_w + \left(\frac{i_d}{z_x F}\right) \times \frac{u_x}{\sum_1^i u_i c_i}\right] \times c_x = \left[k_{p,x} + V_w\right] \times c_x + \frac{i_d t_x}{z_x F} \quad (1)$$

where i_d is the applied current density, and u_i and c_i refer to the mobility and concentration of the ions carrying charge across the membrane and u_x, z_x, c_x and t_x are the mobility, valence, concentration and transport number of species x and $k_{p,x}$ is its permeability coefficient; F is Faraday's constant and V_w is the linear velocity of solvent flow. Neutral molecules are transported by passive diffusion and via EO; in addition to these two transport processes, cations and anions also benefit from EM which is a far more efficient transport mechanism and results in superior delivery.

Iontophoresis has been extensively studied for the delivery of water soluble ionizable species into and across the skin for local or systemic action. Given its ability to control drug delivery kinetics and its noninvasiveness, it has also drawn increasing interest in the field of ocular delivery. For example, corticosteroids have been shown to be successfully delivered into the anterior and posterior segments of the eye, using transcorneal and transscleral iontophoresis (Eljarrat-Binstock and Domb, 2006). Indeed, transscleral iontophoresis of methylprednisolone sodium succinate has been proposed as an alternative treatment for corneal graft rejection using the annular EyeGate® applicator (Halhal et al., 2004). Clinical response was seen after 24 h in 10 out of 18 patients although the applicator was not directly placed on the cornea. Lateral diffusion from the scleral limbus into the corneal tissue might be the main driving force for drug penetration.

Given its poor aqueous solubility and the lack of ionizable moieties at physiological pH, TA is a poor candidate for iontophoretic delivery and there are no water soluble derivatives available. To overcome this, a prodrug approach, similar to that used to improve topical iontophoretic delivery of aciclovir, was adopted (Chen et al., 2016a). It was previously shown that anodal iontophoresis of positively charged amino acid ester prodrugs of aciclovir could be used to increase the bioavailability of aciclovir in the skin – and more specifically, in the basal epidermis, where the virus resides (Chen et al., 2016b). Hydrolysis of the prodrug generated a non-toxic amino acid side by-product and the active drug moiety was released in the epidermis/dermis by the activity of endogenous enzymes (Abla et al., 2006).

The chemical modification of antiviral agents to produce amino acid ester prodrugs has previously been shown to facilitate topical corneal penetration via active transport since the prodrugs were substrates of amino acid transporters (Barot et al., 2012). The present study focused on a very different type of "active transport" since the delivery of the cationic prodrugs was facilitated by electrotransport upon application of an electrical potential rather than via transporter proteins.

The goals of this study were (i) to synthesize hydrosoluble, biolabile amino acid prodrugs of TA (TA-AA), (ii) to characterize the prodrugs and to evaluate their stability in contact with the cornea and (iii) to investigate the ability of short duration topical iontophoresis to enhance intracorneal TA bioavailability. Iontophoresis with cationic corticosteroid prodrugs would have the advantage of benefiting from both electromigration and electroosmotic transport mechanisms at the diseased tissue (in contrast to anionic phosphate or succinate analogues). Interestingly, TA has also been reported to be a contrast agent in optical coherence tomography (OCT), which is a frequently used technique in ophthalmology (Ehlers et al., 2010, 2013). OCT enables the precise visualization of the multilayered corneal epithelium, the collagenous stroma and endothelium because of the relatively low intrinsic light scattering properties of the tissue (Grieve et al., 2004). Therefore, in the last part of the study the post-iontophoretic intracorneal biodistribution of TA as a function of corneal depth was visualized by using full field optical coherence tomography (FFOCT) and correlated with the amounts quantified by UHPLC–MS/MS (Lapteva et al., 2014).

2. Materials and methods

2.1. Materials

TA and liquid paraffin were purchased from Haenseler AG (Herisau, Switzerland), N-Boc protected L-arginine and L-lysine (Boc-Arg(Boc)$_2$-OH and Boc-Lys(Boc)-OH DCHA salts) were sourced from Bachem (Bubendorf, Switzerland). 4-dimethylaminopyridine (4-DMAP), dry dichloromethane (DCM), sodium and potassium chloride, sodium and potassium phosphate were supplied by Sigma-Aldrich (Steinheim, Germany) and *N*,*N*′-dicyclohexylcarbodiimide (DCC) and 2-morpholino-ethanesulfonic acid monohydrate (MES) were purchased from Fluka (Buchs, Switzerland). Trifluoroacetic acid (TFA; 99% extra pure) and glycerol were obtained from Acros Organics (Geel, Belgium). ULC/MS grade formic acid was bought from Brunschwig (Basel, Switzerland). PVC tubing (ID 3.17 mm; OD 4.97 mm) used for the saline bridges and O.C.T. mounting medium (PVA, polyvinyl alcohol) were provided by VWR International AG (Nyon, Switzerland). Silver wire and silver chloride (AgCl) for the fabrication of electrodes were purchased from Sigma-Aldrich (Steinheim, Germany). All solvents (acetonitrile (ACN), ethyl acetate (EtOAc), hexane (HEX) and methanol (MeOH)) were HPLC grade (HiPerSolv Chromatonorm; Darmstadt, Germany).