European Journal of Pharmaceutical Sciences 42 (2011) 503–508

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Effect of formulation factors on the trans-scleral iontophoretic and post-iontophoretic transports of a 40 kDa dextran in vitro

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ARTICLE INFO

Article history:
Received 4 October 2010
Received in revised form 21 January 2011
Accepted 16 February 2011
Available online 23 February 2011

Keywords:
Trans-scleral iontophoresis
Macromolecule
Ocular delivery
Ocular barriers
Post-iontophoretic transport
Electroosmosis

ABSTRACT

The aim of the work was to study in vitro, across isolated porcine sclera and across the trilayer sclera–choroid–Bruch's membrane (SCB), the effect of iontophoresis on the permeation of a 40 kDa dextran (FD-40), chosen as model compound of high molecular weight neutral drugs.

In particular, the effect of vehicle composition (in terms of buffering agent and ionic strength) and current intensity (from 0.3 to 4.2 mA, corresponding to 0.5–7 mA cm^{-2}) was investigated. Additionally the post-iontophoretic transport of FD-40 through SCB was studied.

The results obtained in the present paper confirm the importance of formulation parameters during transscleral iontophoresis of a neutral high molecular weight hydrophilic compound transported by electroosmosis. In particular, ionic strength seems to be the more relevant parameter, while the buffering agent (phosphate vs HEPES) is not relevant. The enhancement obtained increases – although in a stepwise way – with current intensity, after a threshold value of approximately 1.5 mA. However, the real variable to be considered is probably current density (threshold value 2.5 mA cm^{-2}) more than intensity, in analogy with transdermal iontophoresis. The inclusion of further static barriers besides the sclera, such as choroid and Bruch's membrane, reduces, as expected, the permeation of FD-40, but iontophoresis is able to significantly promote FD-40 transport also through this more complex barrier, without altering its permeability. Finally, the study of the post-iontophoretic transport highlights the formation of a pronounced FD-40 reservoir inside the sclera. This reservoir permits to obtain in vitro a sustained transscleral flux up to 3 h after current stop. This result could be of interest in the case of a real application, prolonging the enhancement effect also after iontophoresis stop.

1. Introduction

Different strategies are under investigation to sustain or enhance drug transport across the sclera (Barocas and Balachandran, 2008; Del Amo and Urtti, 2008; Gaudana et al., 2009; Janoria et al., 2007) in order to target the posterior eye segment. Among these strategies, an interesting approach is represented by iontophoresis (Myles et al., 2005) (i.e. the application of low-intensity electrical current to the sclera to enhance the delivery of drugs (Kalia et al., 2004)), which has been shown to be potentially applicable to small molecules (Eljarrat-Binstock and Domb, 2006; Eljarrat-Binstock et al., 2007, 2008; Gungor et al., 2010; Hayden et al., 2006; Li et al., 2005; Myles et al., 2005). Nevertheless, most of the new drugs are high molecular weight compounds, such as proteins and oligonucleotides (Sharif and Klimko, 2006), whose iontophoretic behavior is at present mostly unknown: a very few papers are present in the literature (Chopra et al., 2010; Davies et al., 2003; Molokhia et al., 2009; Nicoli et al., 2009) and the mechanistic understanding of high molecular weight iontophoresis remains a challenge awaiting elucidation. In fact, the relative contribution of the mechanisms involved, primarily electrorepulsion and electroosmosis but also increased passive permeability, can be different according to the molecular weight and the charge of the permeant, as can be deduced from skin transport studies (Kalia et al., 2004; Schuetz et al., 2006).

In a previous paper, the transscleral iontophoresis of neutral dextrans with MW from 4 to 120 kDa has been investigated in vitro through isolated porcine sclera (Nicoli et al., 2009). The obtained results indicated that current application at pH 7.4 generates an electroosmotic flow (Pikal, 2001) in the anode-to-cathode direction that was able to increase 2–6.5 times the transscleral flux of neutral dextrans. Obviously the contribution of electrorepulsion was negligible.

In this paper we focus our attention on vehicle composition (buffer type and ionic strength) and current intensity on the iontophoretic and post-iontophoretic transports of a dextran of

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doi:10.1016/j.ejps.2011.02.008

Table 1
Permeation parameters of FD-40 through isolated sclera.

Donor buffer ionic strength (M)	Iontophoresis	J ($\mu g\,cm^{-2}\,h^{-1}$)	$P \times 10^7$ ($cm\,s^{-1}$)	EF	Ref.
HEPES[a,b,c] 0.154	–	0.57 ± 0.32	3.15 ± 1.70	–	Nicoli et al. (2009)
HEPES[b,a,c] 0.154		0.48 ± 0.19	2.68 ± 1.05	–	This work
HEPES 0.154	Anodal 1.75 mA	2.70 ± 0.36	15.0 ± 1.90	3.9 ± 0.5	Nicoli et al. (2009)
HEPES 0.194	Anodal 1.75 mA	0.54 ± 0.07	3.01 ± 0.01	1	This work
HEPES 0.154	Anodal 0.3 mA	0.025 ± 0.005	0.14 ± 0.03	1	This work
HEPES 0.154	Anodal 1 mA	0.13 ± 0.04	0.70 ± 0.23	1	This work
HEPES 0.154	Anodal 3 mA	2.48 ± 0.71	13.8 ± 3.94	3.6 ± 1.0	This work
HEPES 0.154	Anodal 4.2 mA	5.96 ± 1.37	33.1 ± 7.60	8.7 ± 2.0	This work
PBS 0.194	Anodal 1.75 mA	0.69 ± 0.14	3.84 ± 0.79	1	This work
PB 0.043	Anodal 1.75 mA	1.71 ± 0.42	9.51 ± 2.34	3.5 ± 0.9	This work

[a] Fresh sclera.
[b] Previously frozen (−80 °C) sclera.
[c] Passive permeation parameters in HEPES, PB and PBS are not statistically different ($p = 0.27$).

40 kDa (FD-40), selected as a model molecule of potential drugs used for the treatment of posterior segment eye diseases, such as pegaptanib (50 kDa), ranibizumab (48 kDa) or PEDF (pigment epithelium-derived factor, 50 kDa).

The experiments were performed across isolated pig sclera, trilayer sclera–choroid–Bruch's membrane (SCB) and, only preliminarily, on freshly excised pig eye bulbs, to evaluate the effect of barrier complexity on iontophoretic and post-iontophoretic transports.

2. Materials and methods

2.1. Materials

HEPES ((4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) was purchased from Sigma (St. Louis, USA) as well as fluorescein-isothiocyanate (FITC)-dextran (FD-40; MW = 39 kDa; molecular radius = 4.5 nm (Sigma–Aldrich)). All other chemicals used were of analytical grade.

Buffers composition: Phosphate buffered saline (PBS): 0.19 g L^{-1} KH_2PO_4, 5.98 g L^{-1}, $Na_2HPO_4{\cdot}12H_2O$, 8.8 g L^{-1} NaCl pH 7.4 with phosphoric acid (ionic strength: 0.194 M). Phosphate buffer (PB): 0.19 g L^{-1} KH_2PO_4, 5.98 g L^{-1}, $Na_2HPO_4{\cdot}12H_2O$ pH 7.4 with phosphoric acid (ionic strength: 0.043 M). HEPES buffered saline (HEPES): 5.96 g L^{-1} HEPES, 9.0 g L^{-1} NaCl pH 7.4 with NaOH 5 N (ionic strength: 0.154 M). Additionally, HEPES buffers with a lower or higher NaCl content (ionic strength: 0.019 and 0.194) were also prepared.

2.2. Pig eye bulb and tissue preparation

Porcine globes came from pigs (Large White, Landrance; 10–11 months; weight 145–190 kg) obtained from a local slaughterhouse. Freshly excised pig eyes were transported in an ice bath to the laboratory and, after removal of the muscular tissue, were weighed and measured with a caliper. Their weight was in the range 7.1–9.1 g (axial length 20–26 mm).

For permeation experiments with the trilayer composed of sclera, choroids and Bruch's membrane (SCB), the eye bulb was sectioned within 2 h from the explant: the anterior segment was removed and then vitreous and retina were eliminated. The retinal pigmented epithelium (RPE) was removed (application of a NaCl 5% (w/v) solution for 2 min (Cheruvu and Kompella, 2006)), and the trilayer SCB was cut to obtain pieces 1.5 cm × 1.5 cm.

For permeation experiments through isolated sclera, choroids and Bruch's membrane were gently removed with a forceps.

For permeation experiment with the whole eye bulb, the eyes were cleaned from the muscular tissue and mounted on the diffusion system (see Section 2.5) within 2 h from explant.

2.3. Permeation experiments through isolated tissues

Permeation experiments were performed in Franz-type diffusion cells (0.6 cm^2) using either porcine sclera or the trilayer SCB as barrier. In case of bare sclera, both fresh and frozen tissues were used. Preliminary data (Table 1) indicate that the freezing procedure (−80 °C) did not modify the permeability toward FD-40. Similar results were obtained by Olsen et al. (1995) with other high molecular weight compounds. However, it is worth mentioning that, if the freezing procedure is conducted more slowly (i.e. at −20 °C), a significant increase in the permeability toward macromolecules can be obtained (data not shown).

The receptor compartment contained 4 ml of HEPES or PBS or PB, thermostatted at 37 °C and magnetically stirred to avoid any boundary layer effect. The donor compartment contained 250 μl of FD-40 500 μg ml^{-1} dissolved in HEPES, PBS or PB. In the iontophoretic experiments, silver–silver chloride electrodes and salt bridges (agar 2% (w/w); 1 M KCl) were used. The current (intensity: 0.3–1–1.75–3–4.2 mA; anodal) was applied for 2 h by a constant current generator (Iono1, Cosmic, Pesaro, I). At predetermined time intervals, the receptor solution was sampled for the determination of the drug permeated. The stability of FD-40 upon current application in the presence of salt bridges had been previously demonstrated (Nicoli et al., 2009).

Experiments were also performed to evaluate the possible damaging effect (recorded as an increase of permeability) of current application on the trilayer. The current was applied for 2 h, followed by a passive permeation experiment.

Additional experiments were performed to evaluate the possible role of the sclera as a drug reservoir: in this case after 2 h of passive or iontophoretic permeation, the donor solution was removed and the sampling of the receptor solution was continued up to 5 h.

2.4. Data processing

The transscleral flux (J, $\mu g\,cm^{-2}\,h^{-1}$) was calculated as the slope of the regression line at steady state, while the permeability coefficient (P, $cm\,h^{-1}$) was calculated as:

$$P = \frac{DK}{H} = \frac{J}{C_D} \quad (1)$$

where K is the partition coefficient between the sclera and the donor solution, H is the thickness of the membrane, D is the diffusion coefficient ($cm^2\,s^{-1}$) and C_D ($\mu g\,ml^{-1}$) represents the concentration of the donor solution.