Journal of Controlled Release 67 (2000) 337–346

www.elsevier.com/locate/jconrel

Electroporation-mediated delivery of 3′-protected phosphodiester oligodeoxynucleotides to the skin

V. Regnier[a], A. Tahiri[a], N. André[b], M. Lemaître[c], T. Le Doan[b], V. Préat[a,*]

[a]*Université Catholique de Louvain, Unité de Pharmacie Galénique, Avenue E. Mounier, 73 UCL 73.20, 1200 Brussels, Belgium*

[b]*Université de Paris XI, Laboratoire de Biochimie des Transports Cellulaires, Bâtiment 430, 91405 Orsay, France*

[c]*Eurogentec Bel S.A., Parc Scientifique du Sart Tilman, 4102 Seraing, Belgium*

Received 21 May 1999; accepted 3 February 2000

Abstract

The feasibility of topical delivery in the skin of 3′ end modified phosphodiester oligonucleotides using electroporation was investigated. Experiments were performed in vitro, using hairless rat skin. Five pulses of (200 V, 450 ms) were applied. The 3′ end modifications of the 15 mer oligonucleotide were: (1) 3′-aminohexyl, (2) biotin, with a triethyleneglycol arm, (3) methylphosphonate links between nucleotides 13, 14 and 15, and (4) 2-*O*-methyl nucleotides at 13, 14 and 15 positions. All the modifications were efficient to protect the oligonucleotides against degradation in the skin. Electroporation increased the topical delivery of the 3′ end-modified phosphodiesters by two orders of magnitude compared to passive diffusion, without significant differences between the derivatives. Oligonucleotide concentrations in the range of 1 μm could be achieved in the viable skin. The delivery of a phosphorothioate congener was lower than phosphodiester delivery due to the interaction of phosphorothioate with the stratum corneum. Consequently, 3′ end-protected phosphodiesters could be an interesting alternative to phosphorothioate oligonucleotides for topical treatment of cutaneous diseases. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Electroporation; Phosphodiester, 3′ end-modified; Oligonucleotide; Skin; Topical delivery

1. Introduction

The striking advantage of the antisense approach is its potential specificity of action at the gene level (messenger RNA and genomic DNA). Nevertheless, antisense oligonucleotides (ON) must overcome several limitations before they can be widely applied as therapeutics. These limitations require (i) to improve their stability in biological systems, (ii) to optimize their affinity and efficacy without reducing their selectivity, and (iii) to target and deliver them across tissue barriers and cell membranes [1].

Electroporation is believed to create transient aqueous pathways in lipid bilayers by the application of short electric field pulses [2] However, the exact physical nature of these pathways remains unresolved. Until now, the most common use of high-voltage pulsing is the introduction of DNA into isolated cells (electrotransfection). Moreover, high-voltage electric field pulses have recently been shown to dramatically enhance the transdermal transport of moderate-sized compounds [3–5] or even

*Corresponding author. Tel.: +32-2-764-7320; fax: +32-2-764-7398.

E-mail address: preat@farg.ucl.ac.be (V. Préat)

0168-3659/00/$ – see front matter © 2000 Elsevier Science B.V. All rights reserved.
PII: S0168-3659(00)00223-6

macromolecules [6]. High-voltage pulsing can also permeabilize in vivo cells of various tissues, including the skin. Indeed, in vivo electroporation allows the transfection of plasmid DNA in the skin of mice [7,8] or the efficient local electropermeabilization of tumor cells to bleomycin [9].

Skin is a privileged target for antisense oligonucleotides given its accessibility, and also because many cutaneous diseases are potential candidates for antisense therapy (cutaneous carcinoma, psoriasis, xeroderma pigmentosum, viral diseases, etc.). We hypothesized that electroporation, which enhances stratum corneum and keratinocyte permeability, might be a useful method for cutaneous delivery of ON. We have recently shown that electroporation is particularly efficient in promoting the permeation of a 15-mer phosphorothioate ON inside the skin in vitro [10]. Zewert et al. [11] also demonstrated an increased transdermal delivery by skin electroporation. The electrical parameters of the pulses (pulse voltage, pulse time and number of pulses) and the oligonucleotide concentration in the donor compartment allow the control of the quantity of ON delivered to the skin. The concentration of oligomer in the viable skin reached therapeutic values (e.g., micromolar range) within minutes. Moreover, electroporated phosphorothioate ON were shown to be localized in the nucleus of the viable epidermis cells [12].

Nevertheless, despite their reasonable stability towards nuclease breakdown, the use of phosphorothioate oligonucleotides is still limited by some problems in vivo: non-sequence-specific effects, partly due to their affinity for proteins, reduced binding affinity to their RNA target, potential long-term toxicity, etc. [13]. Once criticised for their poor stability in living media, phosphodiester backbone ON could be reconsidered, provided that the 3′ end terminus of the oligomer is efficiently protected. Chemical groups such as hexanol, aminohexyl, acridine, and cholesterol [14], or detectable groups such as biotin [15] have been tethered to the 3′ end terminus of oligomers. For the same purpose, 3′-capped ON have been synthesized by modifying the nature of the three or four terminal sugars or phosphodiester linkages at the 3′ end of the oligomer molecule. The phosphate bonds have been modified in phosphorothioate or methylphosphonate [16], or methylphosphonothioate [17] structures. Other strategies consisted in building hairpin sequences at the 3′ end, yielding 3′-exonuclease-resistant ON [18,19].

The aim of this study was to assess the effect of skin electroporation on the topical delivery of 3′ end-modified PO (3′ PO). The stability of the PO in the donor compartment and the viable skin was first assessed. Topical delivery of the 3′ PO in the stratum corneum and the viable skin was evaluated and compared to the transport of the phosphorothioate congener (PS). The influence of stratum corneum removal by stripping was also evaluated.

2. Materials and methods

2.1. Oligonucleotides

Oligonucleotides were synthesized and prepurified on chromatography by Eurogentec Bel (Seraing, Belgium). Synthesis of the ON was carried out on an Expedite 8909 DNA synthetizer (Perceptive Bio, Framingham, MA, USA) using Glenn Research products (Sterling, VA, USA). ON were purified by reverse-phase HPLC after butanol extraction and ethanol precipitation. The purity was higher than 90%, as assessed by ion-exchange HPLC. The sequence ACC AAT CAG ACA CCA was chosen as complementary to a sequence of the UL 52 essential gene of HSV-1 [20]. Four different chemical modifications were selected to bring protection to the 3′ end of the phosphodiester oligomer (PO). The chemical structures are presented in Table 1. The corresponding thiolated compound (PS, see Table 1) was also used.

To evaluate the stability of the oligomers in the donor compartment or in tissue extracts, ON were labeled at the 5′ end position with [γ-^{32}P]ATP (ICN, Asse-Relegem, Belgium) using bacteriophage T4 polynucleotide kinase (Boehringer Mannheim, Brussels, Belgium) and purified by HPLC to ensure the complete elimination of residual ATP. Tritiated ON were used for the determination of the quantity of ON delivered to the skin. Therefore, ON were labelled at the C_8 position of purines by hydrogen exchange with tritiated water according to Graham et al. [22]. The purity of the ^{3}H-labeled ON was checked by denaturing PAGE [10].

Table 1

Code	Compound		MW[a] (Da)	z[b]
PO	ACC AAT CAG ACA CCA-R3	R3=H	4503	−14
Ami[c]	ACC AAT CAG ACA CCA-R1	$R1 = -P(=O)(O)-O-(CH_2)_6-NH_3^+$	4682	−14
Bio[d]	ACC AAT CAG ACA CCA-R2	$R2 = -P(=O)(O^-)-O-CH_2-CH(OH)-CH_2-O-(CH_2-CH_2-O)_3-$ ... $-NH-C(=O)-$ ... (biotin)	5071	−15
OMe	ACC AAT CAG ACA C◊C◊A◊	◊ = —O— (ribose with Base, 2′-OMe)	4593	−14
MeP	ACC AAT CAG ACA C#C#A	# = $-O-P(=O)(CH_3)-O-$	4501	−12
PS[e]	A*C*C* A*A*T* C*A*G* A*C*A* C*C*A	* = $-O-P(=S)(O^-)-O-$	4727	−14

[a] MW is the molecular weight calculated from the presented chemical structure (ionized form in aqueous solution).
[b] z is the net electric charge carried by the compound.
[c] Ami was synthesized using Glen Research's C6 amino modifier.
[d] Bio was synthesized using Glen Research's BioTEG phosphoramidite.
[e] PS was synthesized using Beaucage's reagent.

2.2. In vitro topical delivery of 3′ end-modified ON

The in vitro transdermal transport model was a vertical diffusion chamber made of two compartments separated by freshly excised rat skin, with epidermis facing the upper (donor) compartment. Samples of full-thickness abdominal rat skin (Mutant Iops hairless rat, Iffa Credo, St. Germain-les-Arbresles, France) were prepared by gently scraping off subcutaneous fat of freshly excised skin. Stripped skin, i.e., without stratum corneum, was obtained by stripping, 10 times, the skin surface with gummed paper (Scotch Cristal, Cergy Pontoise, France) before setting the skin in the diffusion cell. The skin area exposed was 1 cm^2. Pure platinum electrodes (0.25 cm^2, Johnson Matthey, Brussels, Belgium) were set in both compartments. The distance between both electrodes was 1 cm [3,4]. The cathode was located in the upper compartment and the anode in the lower compartment. The upper compartment was filled with 0.5 ml of ^{3}H-labeled ON solution (3.2 μM [^{3}H]ON, 3.4×10^{-4} mCi/ml or 3.2 μM [^{32}P]ON, 5.4×10^{-3} mCi/ml in 0.04 M Hepes buffer, pH 7, with EDTA 1 mM and 8%, w/v, sucrose for isotony), and the lower compartment with 2.5 ml of 0.024 M phosphate buffer, pH 7.4, made isotonic with 4% (w/v) glucose. The lower compartment was maintained at 37°C and continuously stirred. No shift in pH due to pulsing was observed [10,12].

If not mentioned, ON were allowed to permeate the skin during 4 h after pulsing. In passive diffusion

experiments, the ON solution was left in contact with the skin for 4 h without the application of electrical pulses.

2.3. Electroporation protocol

The electroporation device used was an Easyject Plus® (Cell One, Herstal, Belgium), delivering exponentially decaying (ED) capacitive discharge pulses, characterized by their initial voltage ($U_{electrodes}$) and their pulse time (τ). τ corresponds to the time required for the voltage to drop to 37% of its initial value [3,4,10,12]. The pulsing protocol consisted in applying five pulses of 200 V and τ=471 ms for intact skin (5×(200 V, 471±15 ms)), and five pulses of 200 V and τ=442 ms for stripped skin (5×(200 V, 442±21 ms)). The inter-pulse delay was 1 min.

2.4. Stability of 5′ end ^{32}P-labeled ON in rat skin

Four hours after topical delivery of the ^{32}P-labeled ON by electroporation, the skin was recovered from the diffusion chamber and was gently wiped clean. The area exposed to the donor solution was excised, pinned flat and tape-stripped 10 times to remove the stratum corneum. The remaining skin tissues, corresponding to the viable epidermis and dermis will be called hereafter 'viable skin'. The viable skin was immediately frozen in isopentane cooled by liquid nitrogen. As ON are mainly localized in the epidermis and in the uppermost layers of dermis [12], cryosectioning parallel to the skin surface was performed to a depth of 100 μm, the deeper tissues being discarded. Tissue sections were incubated for 1 h at 50°C with 2 mg/ml proteinase K (Boehringer Mannheim, Brussels, Belgium) in 0.5% SDS, 10 mM NaCl, 20 mM Tris–HCl, pH 7.6, and 10 mM EDTA [21]. The samples were analyzed by 20% PAGE in denaturing conditions, followed by autoradiography. Samples from the donor compartment were analyzed after 4 h of contact with the skin surface [10].

2.5. Quantification of the ^{3}H-labeled ON in the skin layers

After 4 h post-pulsing diffusion, the stratum corneum and the viable skin were separated as described above. Both were lyophilized to remove the tritium which might have exchanged with water and hydrolyzed in 1 ml NaOH 1 N at 45°C for 24 h. The homogenates were then counted for radioactivity (Beta Counter, Wallac 1410, LKB, Pharmacia). The quantity of ON, expressed as mean±standard error of the mean (n=3–8), were compared by one-way analysis of variance (ANOVA, $P<0.05$).

3. Results

3.1. ON stability in the donor compartment and inside the skin

As shown in Fig. 1A, the 3′ PO migrated differently in PAGE depending on their chemical modifications. Their migration was inversely proportional to the ratio between the MW and the net electric charge, except for MeP (see Table 1).

The integrity of the tested ON in the donor compartment was determined after 4 h of contact with intact or stripped skin, ON being in direct contact with the viable epidermis in this latter case. The data show that after 4 h of passive diffusion (left side of Fig. 1B1 and 1B2) or 4 h after pulsing (right side of Fig. 1B1 and 1B2), the 3′-protected phosphodiester ON as well as the non-protected compound remained fairly stable in the donor compartment. In the case of Bio, however a dark band corresponding to the de-biotinylated congener appeared after pulsing the stripped skin (Fig. 1B2).

The integrity of the ON in the viable tissues was analyzed after digestion of the collected tissues with proteinase-K (see Section 2 for details). Four hours after pulsing or after 4 h of passive diffusion, the amount of oligomers that reached the viable tissues of intact skin was limited, requiring concentration of samples to load a sufficient amount of radioactivity on the gel. Due to the high-salt content of these samples, band resolution was poor (Fig. 1C1). However, given the differential migration of the different derivatives as shown in Fig. 1A, the migration pattern observed suggested that the oligomers recovered under these conditions of delivery remained mostly intact. These results were subsequently confirmed by analysis of the ON recovered from stripped skin, after passive diffusion or pulsing (Fig. 1C2, left side and right side, respectively). The higher tissue accumulation of oligomers in the case

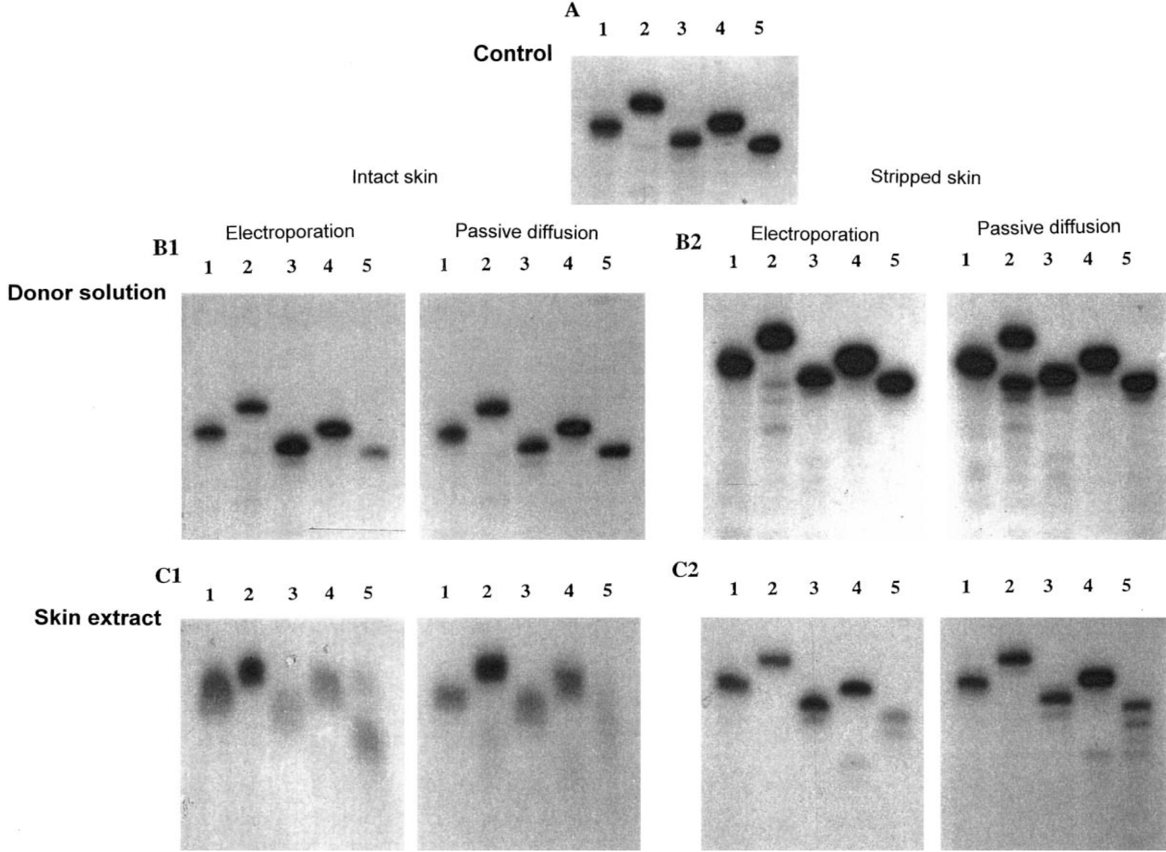


Fig. 1. PAGE analysis of the state of the 3′-modified phosphodiester ON (1, Ami; 2, Bio; 3, OMe; 4, MeP; and 5, PO; see Table 1 for details) before delivery to rat skin (A), after 4 h incubation on intact (B1) or 10 times stripped skin (B2), or extracted from the skin viable tissues 4 h after delivery of the ON to intact (C1) or 10 times stripped skin (C2). The skin samples were treated as described in Section 2. ON were delivered to the skin by 4 h passive permeation (left side) or by electroporation (5 (200 V~ 470 ms)) followed by 4 h passive permeation (right side).

of stripped skin allowed a much better gel analysis. The degradation pattern of Bio, observed in the donor compartment after delivery to stripped skin, was absent in the skin. The 3′ PO were stable in the skin. As expected, the non-protected compound (PO) exhibited significant degradation (Fig. 1C2, lane 5) within the viable tissues of stripped skin.

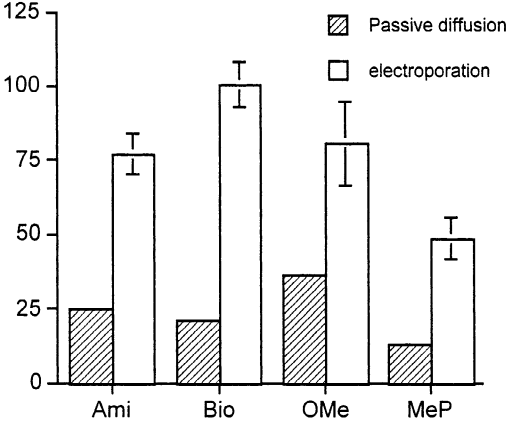
3.2. Quantification of 3′ end-modified ON in intact skin

3.2.1. Electroporation versus passive diffusion

We first investigated the ability of electroporation to increase the permeation of the 3′ PO in the skin. The quantity of ON was measured in the skin 4 h after pulsing or after 4 h passive diffusion.

As shown in Fig. 2A, electroporation significantly increased the transport of the 3′ PO in the stratum corneum. Upon electroporation, every congener was similarly transported (50–75 pmol/cm^2). The transport of Bio was, however, significantly higher than the transport of MeP. Similar results were obtained after 4 h passive diffusion, but the amounts transported were lower (~25 pmol/cm^2). Based on a stratum corneum thickness of 14 μm [22], i.e., 1.4 μl of tissue/cm^2 of skin, the concentrations of the 3′ PO in the stratum corneum fall in the range of 30–70 μM 4 h after pulsing, and 9–25 μM after 4 h of passive diffusion (Table 2).

Electroporation strongly promoted the delivery of the four derivatives in the viable skin as compared to the passive diffusion controls (Fig. 2B). Four hours



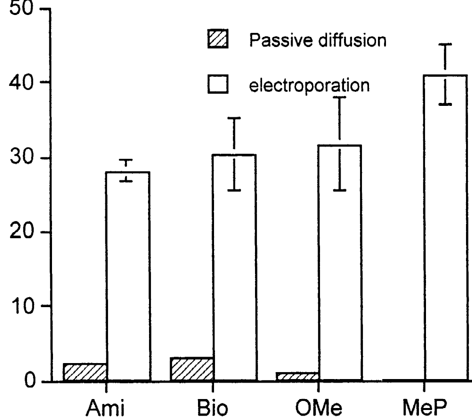


Fig. 2. Influence of 3′ end modifications on ON permeation in intact skin: mean quantities of 3′ end-modified phosphodiester ON (pmol/cm^2) recovered from the stratum corneum (A) or from the viable skin (B) 4 h after pulsing (5×(200 V, 471 ms)) or after 4 h passive diffusion. Donor solution: ON 3.2 μM, EDTA 1 mM, sucrose 8% in 0.04 M Hepes buffer, pH 7 (n=3–4).

after electroporation, approximately 30 pmol/cm^2 of each congener were detected in viable skin. In the absence of electric treatment, the quantities of Ami, Bio and OMe were approximately one order of magnitude lower, and the quantity of MeP, two orders of magnitude lower than upon electroporation. Based on a tissue thickness of 0.65 mm [10], the average ON concentration in the viable skin should be about 0.5 μM 4 h after pulsing, and less than 0.05 μM after 4 h of passive diffusion (Table 2).

3.2.2. Phosphodiesters versus phosphorothioate.

3.2.2.1. Persistence of the ON reservoir in the skin. The electrotransport of the 3′ PO and of PS was then compared. The experiment was carried out with only two 3′ PO (MeP and OMe). These were chosen given that their 3′ extremity is not derivatized with bulky protecting groups and hopefully their steric hindrance during transport within the skin structures should be more comparable to that of PS.

Fig. 3A shows that, when measured immediately after pulsing, the accumulation of PS in the stratum corneum was significantly higher than the accumulation of MeP and OMe. Four hours after pulsing, only one-third of the quantity of MeP or OMe was found in the stratum corneum right after pulsing remained in this tissue, while the quantity of PS was almost unchanged.

In the viable skin (Fig. 3B), measurements performed immediately after pulsing showed that the transport of MeP and OMe was more efficient than the transport of PS (concentration in the viable skin: about 1 μM for OMe and MeP, and less than 0.5 μM for PS (Table 2)). Four hours after pulsing, all oligomers were recovered in similar amounts (~35 pmol, i.e., ~0.5 μM).

3.2.2.2. Affinity for the stratum corneum. The accumulation of the 3′ PO in the stratum corneum was compared to that of PS, without application of electrical pulses. As displayed in Fig. 4, all derivatives accumulated in the stratum corneum, since ON concentration in the donor compartment was 3.2 μM. However, the passive accumulation of the 3′ PO oligomers in the stratum corneum was much lower than PS accumulation, indicating a much weaker interaction of the 3′ PO derivatives with the components of the stratum corneum.

3.2.3. Quantification of 3′ end-modified ON in stripped skin

Stripping the skin significantly increased the trans-

Table 2
Influence of stripping on 3′ end-protected ON topical delivery

		ON concentration in the skin (after 4 h)	
		Passive diffusion (μM)	Electroporation (μM)
Intact skin	Stratum corneum	9–25	30–70
	Viable tissues	0.003–0.05	0.5–0.6
Stripped skin	Viable tissues	0.5–1.2	2.5–3.5

port of the four derivatives in the viable skin (Fig. 5 versus Fig. 2). Approximately 190 pmol/cm^2 of each derivative were found in the viable skin 4 h after pulsing. After 4 h of passive diffusion, between 30 and 80 pmol/cm^2 of derivative were found. The ON concentrations inside the skin were above 2.5 μM 4 h after pulsing, and between 0.5 and 1.2 μM after 4 h passive diffusion (Table 2).

4. Discussion

The objective of this study was to investigate the ability of four 3′ end-modified phosphodiester oligonucleotides to permeate rat skin in vitro under a defined electroporation condition (5×(200 V, 450 ms)). Similar skin electroporation conditions were previously shown to deliver a 15-mer PS to the viable skin at therapeutic level and to promote a quick penetration of a FITC-labeled PS in the nucleus of the keratinocytes [10,12]. However, since PS have a strong affinity for the stratum corneum, PS were replaced by 3′PO ON.

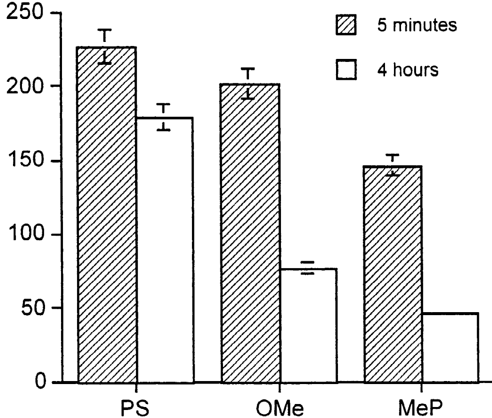
Because of the significant activity of skin nucleases and subsequent potential degradation of ON [23], the stability of the 3′ PO was investigated. The derivatized oligomers (Ami, Bio, Ome and MeP) were found to be fairly stable in the skin during hours, even when the skin was electroporated. In contrast, the unprotected congener (PO) suffered significant degradation by 3′ exonucleases (Fig. 1C) [10]. The selected 3′ end modifications were thus efficient to protect the ON against the nucleases present in the skin.

Electroporation enhanced the 3′ PO delivery in intact skin by two orders of magnitude as compared to passive diffusion. Similar data were previously reported for PS [10].

During pulsing, the 3′ PO were more efficiently transported in the viable skin than PS (Fig. 3). This is probably a consequence of the lower affinity of the PO for the stratum corneum: (i) even in the absence of electric treatment, lesser PO than PS accumulated in the stratum corneum (Fig. 4); (ii) the PO were able to diffuse out of the stratum corneum after pulsing in contrast to PS which remained for hours in this layer (Fig. 3A). Hence, the stronger affinity of PS for the stratum corneum might significantly decrease its delivery in the viable skin, even when pulses are applied. Another consequence of the lower interaction of PO with the skin (e.g., tissue proteins) [13] suggests that the free PO concentration in the skin could be more elevated than that of PS. This should make the PO more available for a specific interaction with their nucleic acid target. Besides the chemistry of the ON, the size and sequence of the ON could affect their delivery using skin electroporation as reported for iontophoresis [24,25].

However, even for the PO, the stratum corneum is an efficient barrier to permeation in the viable skin (Table 2). Therefore, we determined to what extent the stratum corneum removal can increase the quantity of ON delivered to the viable skin. 3′ PO permeation in stripped skin was one order of magnitude higher than in intact skin after electroporation. Stripping also increased the 3′ PO concentration in the skin by one or two orders of magnitude after passive diffusion (Table 2 and Fig. 5). Therapeutic levels were reached, as discussed by Regnier et al. [10]. As the stratum corneum regenerates rapidly [26], it could be removed by the patient. If a disease (e.g., psoriasis or herpetic lesions) impairs the stratum corneum barrier function, ON permeation will be facilitated, whereas hyperkeratosis could decrease ON topical delivery.

These studies were performed in vitro using



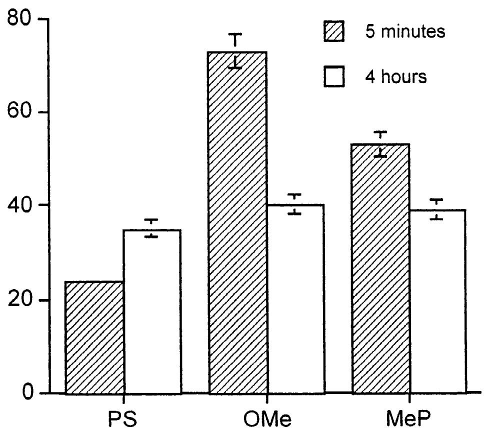


Fig. 3. Effect of the nature of the internucleotide linkage on the ability of the ON to diffuse in/out of intact skin after pulsing: mean quantity of PS or 3′ end-modified PO oligonucleotides (pmol/cm^2) recovered from the stratum corneum (A) or from the viable skin (B) of hairless rat skin immediately or 4 h after pulsing (5×(200 V, 471 ms)). Donor solution: ON 3.2 μM, EDTA 1 mM, sucrose 8% in 0.04 M Hepes buffer, pH 7 (n=3–4).

hairless rat skin as extensively used in literature reports. Histological, biochemical and non-invasive bioengineering studies demonstrate that electroporation does not modify the functionality of the skin. No histological lesions in the skin submitted to high-voltage pulses were observed after 4 h in a diffusion

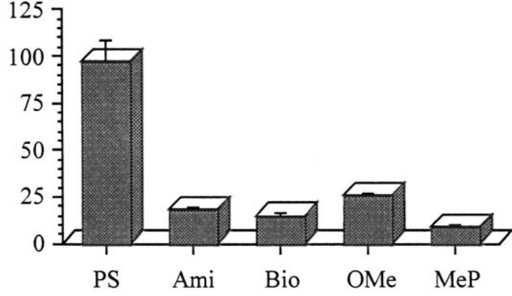


Fig. 4. Effect of the nature of the internucleotide linkage on the ON affinity for the stratum corneum of intact skin: mean concentration of PS or 3′ end-modified phosphodiesters (μM) in the stratum corneum after 4 h passive diffusion. Donor solution: ON 3.2 μM, EDTA 1 mM, sucrose 8% in 0.04 M Hepes buffer, pH 7 (n=3–5).

cell (data not shown). Lactate production in vitro by untreated skin and electroporated skin was similar (data not shown). Only a mild and reversible erythema was observed in vivo after skin electroporation [27]. Electroporation induces a disorganisation of the lipid bilayers similar to that observed after iontophoresis [28].

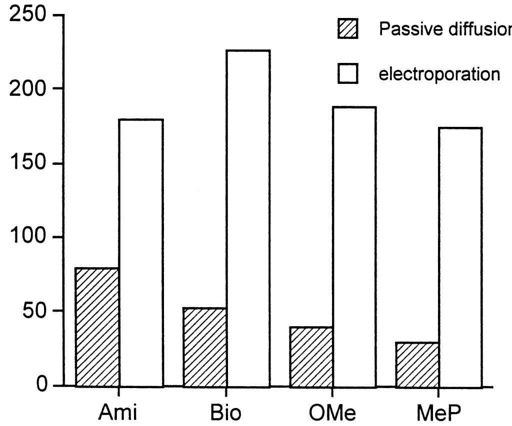


Fig. 5. Influence of 3′ end modifications on ON permeation in stripped skin: mean quantities of 3′ end-modified PO oligonucleotides (pmol/cm^2) recovered from the viable skin of hairless rat 4 h after pulsing (5×(200 V, 442 ms)) or after 4 h passive diffusion. Donor solution: ON 3.2 μM, EDTA 1 mM, sucrose 8% in 0.04 M Hepes buffer, pH 7 (n=3–4).

The transport of the 3′ PO during pulsing is dependent upon the osmolarity of the donor solution. When hypotonic donor solutions were prepared (i.e., without 8% sucrose), the transport of the PO in the viable tissues was twice as large as with isotonic solutions, whereas the transport of the PS oligomer was unchanged (data not shown). The lower affinity of PO for the stratum corneum, and therefore the smaller hindrance for their transport could be an explanation. Due to their weaker affinity for the stratum corneum, the PO might also be driven by osmotic flow towards the viable skin when the pathways are at their maximum size, i.e., during and between pulses.

In conclusion, this study shows that electroporation can efficiently deliver 3′ PO topically. Therapeutic levels were achieved in intact or stripped skin, with a low retention in the stratum corneum and a rather good stability when the 3′ end is protected. Consequently, PO could be an interesting alternative to their PS congeners for the potential treatment of cutaneous diseases by antisense therapy.

Acknowledgements

The authors thank Cell one (Seraing, Belgium) for lending the electroporation device Easyject Plus®, and Michel Dechamps and Michele Ledoux (Eurogentec) for the synthesis and analysis the oligonucleotides. This work was supported by Fonds de la Recherche Scientifique Médicale (FRSM, Belgium) and La Communauté Française de Belgique as part of Franco-Belgian CGRI-FNRS-CNRS cooperation programme. V. Préat is a senior research associate, Fonds National de la Recherche Scientifique (FNRS, Belgium). T. Le Doan is Director of Research at the Centre National de la Recherche Scientifique (CNRS, France).

References

[1] S. Akhtar, Antisense technologys:selection and delivery of optimally acting antisense oligonucleotides, J. Drug Target. 5 (1998) 225–234.

[2] J.C. Weaver, Electroporation theory. Concepts and mechanisms, Methods Mol. Biol. 47 (1995) 1–26.

[3] R. Vanbever, N. Lecouturier, V. Préat, Transdermal delivery of metoprolol by electroporation, Pharm. Res. 11 (1994) 1657–1662.

[4] R. Vanbever, Le Boulangé, V. Préat, Transdermal delivery of fentanyl by electroporation, I. Influence of electrical factors, Pharm. Res. 13 (1996) 557–563.

[5] M.R. Prausnitz, U. Pliquett, R. Langer, J.C. Weaver, Rapid temporal control of transdermal drug delivery by electroporation, Pharm. Res. 11 (1994) 1834–1837.

[6] M.R. Prausnitz, E.R. Edelman, J.A. Gimm, R. Langer, J.C. Weaver, Transdermal delivery of heparin by skin electroporation, Bio-Technology 13 (1995) 1205–1209.

[7] A.V. Titomirov, S. Sukharev, E. Kistanova, In vivo electroporation and stable transformation of skin cells of newborn mice by plasmid DNA, Biochim. Biophys. Acta 1088 (1991) 131–134.

[8] L. Zhang, G.A. Hoffmann, R.M. Hoffman, Depth-targeted efficient gene delivery and expression in the skin by pulsed electric fields: an approach to gene therapy of skin ageing and other diseases, Biochem. Biophys. Res. Commun. 220 (1996) 633–636.

[9] J.J.R. Belehradek, S. Orlowski, L.H. Ramirez, G. Pron, B. Poddevin, L.M. Mir, Electropermeabilization of cells in tissues assessed by the qualitative and quantitative electroloading of bleomycin, Biochim. Biophys. Acta 1190 (1994) 155–163.

[10] V. Regnier, T. Le Doan, V. Préat, Parameters controlling topical delivery of oligonucleotides by electroporation, J. Drug Target. 5 (1998) 275–289.

[11] T. Zewert, U. Pliquett, R. Langer, J.C. Weaver, Transdermal transport of DNA antisense oligonucleotide by electroporation, Biochem. Biophys. Res. Commun. 212 (1995) 286–292.

[12] V. Regnier, V. Préat, Localisation of a FITC labelled phosphorothioate oligonucleotides in the skin after topical delivery by electroporation or iontophoresis, Pharm. Res. 15 (1998) 1596–1602.

[13] A.M. Krieg, C.A. Stein, Phosphorothioate oligodeoxynucleotides: Antisense or anti-proteins ?, Antisense Res. Dev. 5 (1995) 241.

[14] H.B. Gamper, M.W. Redd, T. Cox, J.S. Virosco, A.D. Adams, A.A. Gall, J.K. Scholler, R.B. Meyer, Facile preparation of nuclease resistant 3′ modified oligodeoxynucleotides, Nucleic Acids Res. 21 (1993) 145–150.

[15] Y.S. Kang, R.J. Boado, W.M. Pardridge, Pharmacokinetics and organ clearance of a 3′-biotinylated internally ^{32}P-labeled phosphodiester oligodeoxynucleotide coupled to a neutral avidin/monoclonal antibody, Drug Metab. Disp. 23 (1995) 55–59.

[16] R.V. Giles, D.G. Spiller, D.M. Tidd, Detection of ribonuclease H-generated mRNA fragments in human leukemia cells following reversible membrane permeabilization in the presence of antisense oligonucleotides, Antisense Res. Dev. 5 (1995) 23–31.

[17] J. Temsamani, J.Y. Tang, A. Padmapriya, M. Kubert, S. Agrawal, Pharmacokinetics, biodistribution and stability of capped oligodeoxynucleotide phosphorothioates in mice, Antisense Res. Dev. 3 (1993) 277–284.

[18] J.Y. Tang, J. Temsamani, S. Agrawal, Self-stabilized antisense oligonucleotide phosphorothioates:properties and anti-HIV activity, Nucleic Acids Res. 21 (1993) 2729–2735.
[19] B. Poddevin, S. Meguenni, I. Elias, M. Vasseur, M. Blumenfeld, Improved anti-herpes simplex virus type I activity of a phosphodiester antisense oligonucleotide containing a 3′-terminal hairpin-like structure, Antisense Res. 4 (1994) 147–154.
[20] B. Roizman, Inhibition of Herpesviridae infection by antisense oligonucleotides, 1992, PCT WO 92/03051.
[21] J. Sambrook, E.F. Fritsch, T. Maniatis, in: Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989, p. B16.
[22] M.J. Graham, S.M. Freier, R.M. Crooke, D.J. Eckler, R.N. Maslova, E.A. Lesnik, Tritium labelling of antisense oligonucleotides by exchange with tritiated water, Nucleic Acids. Res. 21 (1993) 3737–3743.
[23] R. Marks, S.P. Barton, The significance of the size and shape of corneocytes, in: R. Maeck, G. Plewig (Eds.), Stratum corneum, Springer, Berlin, 1983, pp. 161–170.
[24] V.O.K. Oldenburg, G. Smith, H. Selick, Iontophoretic delivery of oligonucleotides across fullthickness hairless mouse skin, J. Pharm. Sci. 84 (1995) 915–921.
[25] R.M. Brand, A. Wahl, P.L. Iversen, Effect of the size and sequence on the iontophoretic delivery of oligonucleotides, J. Pharm. Sci. 87 (1998) 49–52.
[26] R. Vanbever, D. Fouchard, A. Jadoul, N. De Morre, V. Préat, J.P. Marty, In vivo non invasive evaluation of hairless rat skin after high voltage pulse exposure, Skin Pharmacol. Appl. Physiol. 11 (1998) 23–34.
[27] G. Menon, K. Feingold, P. Ellias, The lamellar body secretory response to barrier disruption, J. Invest. Dermatol. 98 (1992) 279–289.
[28] A. Jadoul, J. Bouwstra, V. Préat, Effects of iontophoresis and electroporation on the stratum corneum. Review of the biophysical studies, Adv. Drug. Del. Rev. 35 (1999) 89–105.