Transdermal Delivery of Antisense Oligonucleotides with Microprojection Patch (Macroflux®) Technology

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INTRODUCTION

In recent years, antisense oligodeoxynucleotide (ODN) technology has emerged as one of the most promising functional genomic therapies. Several clinical trials have demonstrated its therapeutic value and low toxicity (1). To date, parenteral infusion has been the primary mode of ODN delivery. However, efforts to develop more convenient routes of administration are being explored.

Transdermal iontophoresis increased ODNs across the skin; however, delivery of a therapeutically relevant dose was not achieved (2–5). The major barrier for transdermal delivery is the stratum corneum, the outermost "dead" layer of the skin. In human skin, the stratum corneum is 10–20 μm thick, whereas in mice and rats it is significantly thinner. Removal of the stratum corneum by mechanical abrasion, tape stripping, or chemical treatment has been shown to significantly enhance permeation through the skin for a wide range of pharmaceuticals, including phosphorothioated (PS) ODNs (5–8). However, these approaches may be limited due to the lack of control and reproducibility, as well as the irritancy potential (9).

Microprojection patch is a novel microfabrication technology for controlled transdermal drug delivery. The patch system incorporates a stainless steel or titanium microprojection array. When applied onto the skin manually or by an applicator, microprojections penetrate and create superficial pathways through the skin barrier layer to allow drug delivery. The array can be combined either with passive or iontophoretic delivery systems. In this study, we demonstrate that microprojection patch technology can facilitate the controlled transdermal ODN delivery.

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ABBREVIATIONS: FITC, fluorescein isothiocyanate; HGP, hairless guinea pig; ODN, oligodeoxynucleotide; PS, phosphorothioated.

MATERIALS AND METHODS

Materials

ISIS 2302 is a 20-mer PS ODN; 5'-GCCCAAGCTGGCATCCGTCA-3', that was synthesized in-house (ISIS Pharmaceuticals, Carlsbad, CA, USA). A randomized 20-mer PS ODN (5'-TTATAGAAGACCATTGTTCT-3') and its corresponding fluorescein isothiocyanate (FITC)-labeled version were custom-synthesized by Zeneca Life Science Molecules (Wilmington, DE, USA). ^{3}H-labeled versions of both ISIS 2302 and the randomized PS ODN were synthesized by Trilink BioTechnologies (San Diego, CA, USA). Hydroxyethyl cellulose was purchased from Union Carbide Corporation (Danbury, CT, USA).

Drug Reservoir and Patch Systems

ODNs, spiked with the ^{3}H-labeled version, were dissolved in 2% (w/v) hydroxyethyl cellulose gel to final concentrations of 1.6–200 mg/ml. The ODN gel, pH 7.4, was contained within an adhesive foam ring with a volume of approximately 350 μl and a skin-contacting area of approximately 2 cm^2. In the present study, microprojection arrays (Macroflux®, AIZA Corporation Mountain View, California) were fabricated from a 30-μm thick foil of stainless steel with projections perpendicular to the plane of the foil. The array, 2 cm^2, has a microprojection density of 240/cm^2 and a length of 430 μm. Four patch systems, which included two iontophoretic systems (Fig. 1, A and B) and two passive systems (Fig. 1, C and D), were used in the study. In the former, the foam ring with the ODN gel was placed under the cathode. In the latter, an occlusive backing membrane was placed directly on the top of the ring to seal the drug compartment. An array was adhered to the skin side of the foam ring with the ODN gel to form an integrated system (Fig. 1, A and C). Systems without the array (Fig. 1, B and D) were used as pretreatment systems or as the control.

Animals

Hairless guinea pigs (HGPs), ibm:GOHI-hr (Biological Research Labs. Fullinsdorf, Switzerland), were fasted the night before the study, but with free access to water. Animals were allowed food and water *ad libitum* after system application. All animals were housed individually and were maintained in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care according to the "Principles of Laboratory Animal Care" of the National Institutes of Health.

Patch Application

HGPs were anesthetized with ketamine/xylazine. The skin site was gently cleaned with isopropyl alcohol pads before application. A polypropylene disc was used to press the microprojection array into the skin by finger force. The ODN gel was placed either on top of the array (integrated systems) or on top of the treated skin after the array removal (pretreatment systems). HGPs then were wrapped with Vetwrap (3M, St. Paul, Minnesota) and returned to their cages until the end of the specified wearing time.

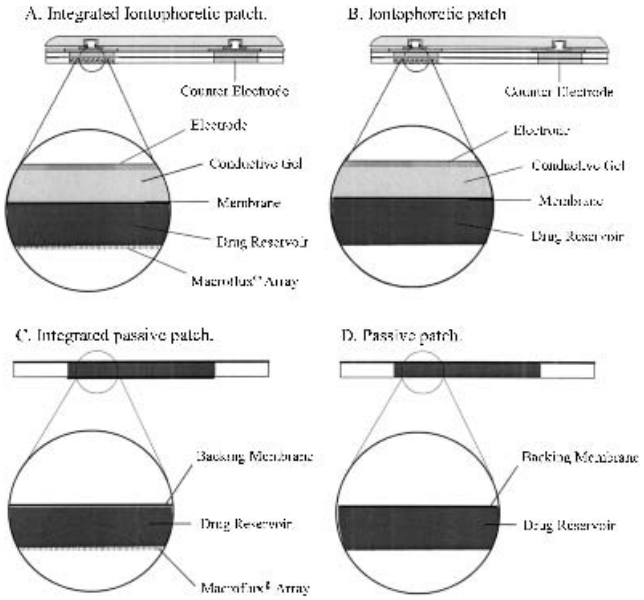

Fig. 1. Schematic drawings of delivery systems are shown. Iontophoretic patches (A and B) have a power source in the back. The foam ring with the drug reservoir is placed under the cathode. (A) An integrated iontophoretic patch with a microprojection array is shown. (B) An iontophoretic patch without the array is shown. In passive patches (C and D), an occlusive backing membrane was directly placed on the backside of the foam ring to seal the drug reservoir. (C) An integrated passive patch with a microprojection array is shown. (D) A passive patch without the array is shown.

Sample Analysis

PS ODNs are cleared slowly from the liver (elimination $t_{1/2}$, 40–50 h) with minimal differences between species and between sequences (10,11), and are relatively stable in the skin (4,12). The liver accumulates about 50% of the compound after intravenous administration (data not shown). Therefore, in our study, the amount of ODN delivered was determined by measuring the liver ^{3}H-ODN content, and the skin distribution was examined by using both ^{3}H-ODN and FITC-ODN. In delivery studies, animals were euthanized 2 h after the patch removal. The livers were weighed, immediately frozen at −80 °C, and stored until analysis. Triplicate samples per liver were taken and oxidized by a biological oxidizer (OX500, R. J. Harvey Instrument Corp., Hillsdale, New Jersey) before scintillation counting. The oxidizing efficiency of the instrument was determined at each time of use and was approximately 90%. Some liver samples were also extracted and analyzed for 20-mer ISIS 2302 content by capillary gel electrophoresis as described previously with the exception that a phenyl-bonded solid-phase extraction column (Supelco Inc., Bellefonte, PA, USA) was used for extraction (13). The amount of ^{3}H-ODN remaining in the skin was also measured following the sectioning of skin biopsies in 20-, 40-, 100-, and 200-μm increments. Each slice was dissolved in Hyamine (Baker, Phillipsburg, New Jersey), and its radioactive content was evaluated by scintillation counting. Alternatively, the entire biopsy sample, without cryotoming, was dissolved and its radioactivity was determined by scintillation counting. Each data point represents the mean of three determinations with its associated standard error. When FITC-ODN was used, skin or organ biopsy specimens were cryotomed, which was followed by counter staining with propidium iodide. The sections were evaluated visually by fluorescence microscopy.

RESULTS AND DISCUSSION

HGP has been used successfully as an experimental model for skin research and drug transport (14–17). Their skin is not only histologically more similar to human skin, but also showed similar mechanical properties, such as extensibility and elasticity (data not shown), which are desirable for evaluating microprojection patch technology.

The penetration of microprojections on the skin of HGPs indeed enhanced transdermal ODN delivery. Following 4 h of iontophoresis at 100 μA/cm^2, the flux of ISIS 2302 (5 mg/ml donor concentration) with an integrated microprojection ar-

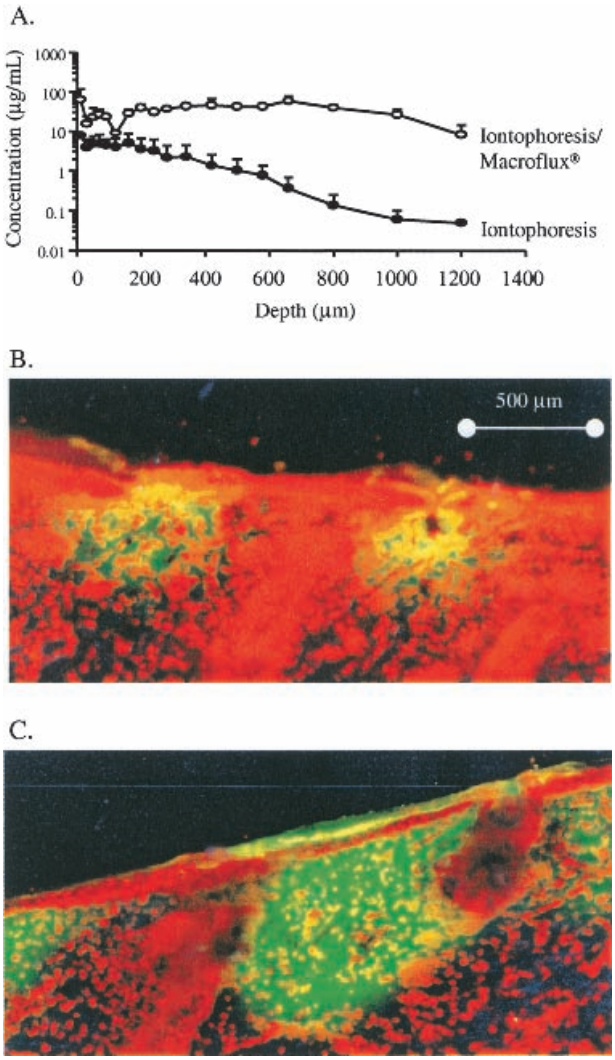

Fig. 2. Skin ODN distribution is shown. (A) Skin ISIS 2302 concentrations following topical delivery by iontophoresis alone or iontophoresis with the integrated microprojection array are shown. Skin biopsy specimens were taken immediately after the patch removal, and the amount of ^{3}H-ODN was determined in sections at various depths as described in the Methods section. (B) A cross-section of the skin following 1-h delivery with a passive patch integrated with a microprojection array is shown. (C) A cross-section of the skin following 1-h delivery with an iontophoretic patch (at 100 μA/cm^2) integrated with a microprojection array is shown.

ray was 8.08 ± 0.60 μg/cm^2/h as compared to 0.08 ± 0.02 μg/cm^2/h without the array. Following 4 h of passive delivery, microprojection patches delivered 16.18 ± 1.84 μg systemically, although there was no detectable amount of ISIS 2302 without the arrays (data not shown). ODN concentrations in the skin were mapped by measuring radioactivity in plane sections of skin biopsy specimens taken from iontophoretic delivery sites (Fig. 2A). A steep decrease of drug concentrations from the stratum corneum to the dermis was observed in the sites receiving iontophoresis alone, while concentrations of the drug were higher and remained almost constant to 700–800 μm deep in the sites receiving iontophoresis that had been integrated with microprojection arrays. The transport pathways were visualized by using a randomized 20-mer FITC-ODN at a donor concentration of 5 mg/ml. Following passive diffusion with an integrated microprojection array for 1 h, sections parallel to the skin surface revealed that a high intensity of yellow-green FITC-ODN was superimposed on the pathways created by the microprojections. There was no observable ODN in the area where the skin was intact. In the cross-sections, there was an enhanced lateral diffusion underneath the stratum corneum, as well as an ODN gradient from the skin surface to the dermis (Fig. 2B). Following 1 h of iontophoresis with an integrated microprojection array, observations were similar except that the ODN seemed to penetrate deeper into the dermis and diffused more around the pathways (Fig. 2C). Skin samples were also examined at different time points after the patch removal. In all cases, a decrease in ODN intensity was observed as a function of time. In animals receiving iontophoresis, skin ODN intensity lasted longer than it did in animals receiving passive patches, probably indicating a larger skin depot. Skin appendages did not exhibit FITC fluorescence (data not shown). The samples taken from the liver, on the other hand, showed a rather homogenous distribution of fluorescence that was not observed in samples from control animals (data not shown). These results demonstrate that pathways created by microprojections were the primary routes of ODN transport, and that skin appendages played a minimal role in ODN delivery.

The enhanced delivery by the microprojection array could be regulated by current density, time duration, and donor concentration. At a donor concentration of 5 mg/ml with an integrated iontophoretic patch, a current-density-dependent delivery of ISIS 2302 was observed from 0 μA/cm^2 (16 ± 1.5 μg) to 400 μA/cm^2 (355 ± 35 μg) for a delivery period of 4 h (Fig. 3A). Similarly, the delivery of the randomized ODN increased from 12 ± 5.7 μg at 0 μA/cm^2 to 451 ± 96 μg at 400 μA/cm^2. When current density (100 μA/cm^2) and donor concentration (5 mg/ml) were constant, a 24-h delivery gave a total of 348 ± 55 μg ISIS 2302 systemically, compared to 25.7 ± 3.4 μg following a 2-h delivery (Fig. 3B). This demonstrates that near-sustained delivery was achieved for 24 h, suggesting that skin pathways created by microprojections remain open throughout the 24-h period. The delivery was also donor-concentration-dependent (Fig. 3C). At a 50-mg/ml donor concentration, approximately 480 μg of ISIS 2302 was delivered during a 4-h period, which was an amount about 16 times higher than that of the 1.6-mg/ml donor concentration. In contrast, iontophoresis without a microprojection array did not seem to be affected by donor concentration (data not shown).

The results shown in Fig. 3A suggest that neither nucleotide sequence nor composition seem to have a large impact on ODN delivery. However, others have shown that the nucleotide sequence, composition, and the three-dimensional structure of ODN played important roles in the iontophoretic delivery of this class of compound (2–4). A possible explanation is that the pathways created by microprojections may be dif-

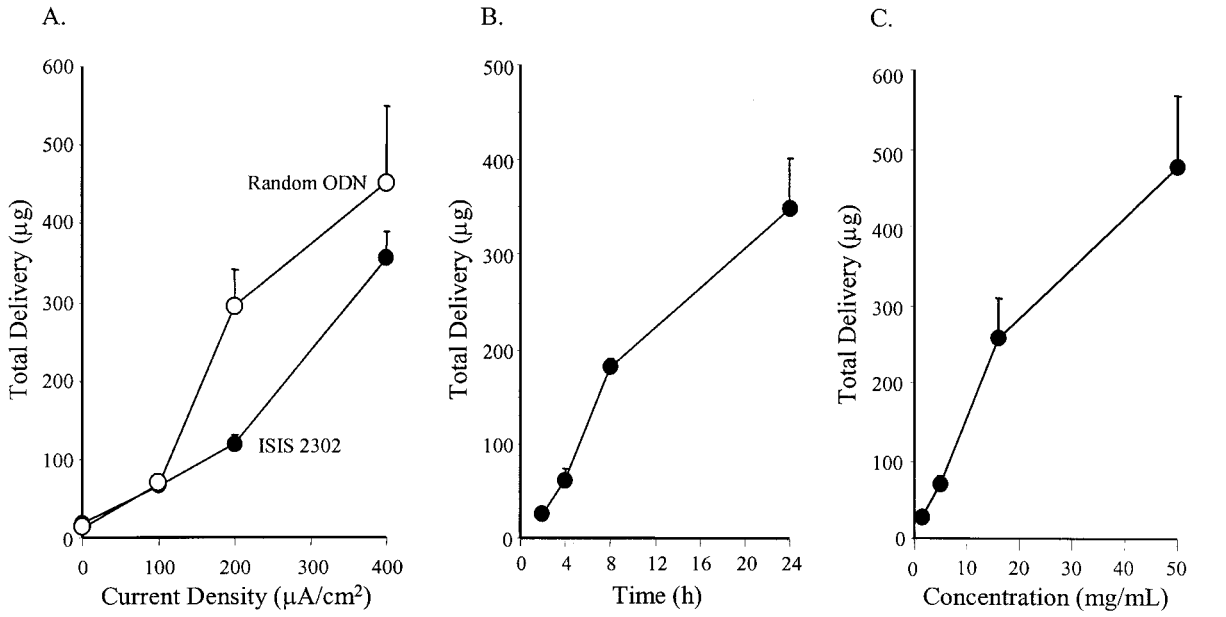

Fig. 3. The regulation of the transdermal ODN delivery is shown. In these experiments, the microprojection array was integrated with the delivery system. Animals were euthanized 2 h after the patch removal. Total delivery was assessed as described in the Methods section. (A) The effect of current density is shown. ISIS 2302 or the randomized ODN was formulated at 5 mg/ml. The duration of delivery was 4 h. (B) Time-dependent delivery is shown. ISIS 2302 was formulated at 5 mg/ml. The current density was at 100 μA/cm^2. (C) Concentration-dependent delivery is shown. The duration of delivery was 4 h at 100 μA/cm^2.

ferent from iontophoretic pathways. The latter favors small and positively charged species (18,19), whereas the former may have different properties and may be insensitive to sequence or composition, and perhaps to molecular weight, at least within a certain range.

As shown in Fig. 1, there were four system configurations. Table 1 shows that in all configurations, the total amount of ISIS 2302 delivered in 24-h period was concentration-dependent. It is noteworthy that at a donor concentration of 200 mg/ml, there was a minimum difference between configurations. The delivery of ISIS 2302 was up to 16 mg/day for a 2-cm^2 patch. The ODN remaining in the delivery site of the skin increased from about 0.1 to 1 mg, with donor concentrations increasing from 5 to 50 mg/ml. However, no further accumulation of ISIS 2302 in the skin was observed when the donor concentration increased to 200 mg/ml (results not shown). The local amount of drug achieved in our study is known to be more than adequate biologically for pharmacological activities (20). The results suggest that the microprojection array could be useful not only in systemic delivery but also for local ODN treatment.

Some liver samples from representative animals were analyzed for 20-mer ISIS 2302 by capillary electrophoresis. About 20–50% of the radioactivity in the liver was accounted for by intact 20-mer ISIS 2302, which was similar to that obtained following intravenous administration (data not shown). These results demonstrated the stability of transdermally delivered ODN and the validity of our measurements.

Although ODNs are highly negatively charged, the fluxes of these compounds using iontophoresis alone were found to be orders of magnitude below the target dose for therapeutic efficacy. Doses of the first generation PS ODNs being tested in the clinic for systemic disorders are in the range of 0.5–5 mg/kg/day. It is estimated, however, that the second-generation chemistries of ODNs will require 1/10 of the effective first-generation dosage due to their superior tissue half-life. Using the microprojection patch technology, we delivered as much as 16 mg/day with a 2-cm^2 array. Finally, even at the highest delivery rate achieved, erythema at the application site was mild and transient, indicating that both the array and ODNs were well-tolerated. Therefore, the therapeutic delivery target is now achievable. Increasing the potency of ODNs or improving the delivery conditions could bring practical implications to the final system development.

In conclusion, the microprojection patch technology, used in conjunction with iontophoresis or passive delivery systems, is capable of delivering therapeutically relevant amounts of ODNs into and through the skin. Transdermal delivery can be controlled by duration of the delivery, donor drug concentration, current density, and active patch area. Therefore, the technology provides a viable delivery method for this class of drugs. In addition, the microprojection patch technology may provide a needle-free transdermal delivery option for other large hydrophilic drugs and biopharmaceuticals that are currently only administered by injection.

TABLE I. Effects of Donor Concentrations and System Configurations on ISIS 2302 Delivery[a]

	Macroflux® pretreatment (mg)		Integrated macroflux (mg)	
Donor (mg/ml)	Passive diffusion	Iontophoresis	Passive diffusion	Iontophoresis
5	0.2 ± 0.02	0.5 ± 0.05	0.2 ± 0.04	0.3 ± 0.05
50	2.6 ± 0.7	6.4 ± 0.5	7.4 ± 1.5	8.3 ± 1.4
200	10.0 ± 3.0	15.6 ± 3.8	14.0 ± 3.2	15.2 ± 1.8

[a] ISIS 2302 was formulated in HEC gel and applied to each HGP through a 2-cm^2 patch for 24 h. In the iontophoretic system, the current density was 100 $\mu A/cm^2$. Animals were euthanized 2 h after system removal. Total delivery was assessed as described in the Methods section.

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