Transdermal Use of Phosphorodiamidate Morpholino Oligomer AVI-4472 Inhibits Cytochrome P450 3A2 Activity in Male Rats

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Purpose. To determine if dermal absorption of an antisense phosphorodiamidate Morpholino oligomers (PMO) can inhibit target gene expression in the liver *in vivo*.

Method. Antisense PMO targeted to cytochrome P450 (CYP) 3A2 was applied topically to adult male rats at doses of 0.03, 0.3, and 3.0 mg. CYP3A enzyme activity in the underlying skin and liver was evaluated 24 h following application.

Results. Systemic PMO bioavailability was determined by detection of full-length PMO in liver and fluorescence micrography in underlying skin. CYP3A enzyme activity were measured by hydroxylation of 7-benzyloxy-4-(trifluoromethyl)-coumarin and data were expressed as nanomoles of product/ 100 μg S9 protein/h. A topical dose of 0.03 mg inhibited enzyme levels from 576 ± 17 (vehicle) and 564 ± 20 (control PMO) to 432 ± 20 in the antisense-treated liver ($p < 0.05$). Increasing the dose to 0.3 mg further inhibited enzyme level to 278 ± 13 ($p < 0.005$). The inhibition did not increase further when the dose was increased to 3 mg. In the skin, starting enzyme levels were approximately one third of the liver (171 ± 9) and maximum inhibition was reached at a lower dose. Topical delivery of 0.03 mg led reduced skin enzyme levels in half to 89 ± 32 ($p < 0.05$). Increasing the dose to 0.3 mg and 3.0 mg did not produce any further inhibition, at 73 ± 8 and 72 ± 17 respectively.

Conclusion. Topical application of antisense PMO in rats is a feasible delivery strategy for gene targets in liver and underlying skin.

KEY WORDS: CYP3A2; antisense; phosphorodiamidate morpholino oligomers.

INTRODUCTION

Antisense technology offers the premise of gene-specific therapeutics as it advances from research-based applications to the clinic (1). The sequencing of the human genome has further increased interest in this technology. Phosphorodiamidate Morpholino oligomers (PMO) represent multiply modified DNA molecules in which the deoxyribose sugar is replaced with a six-member morpholine sugar, and the backbone is comprised of non-ionic phosphorodiamidate linkages (2). PMO's have a host of highly desirable properties that make them particularly suitable as potential antisense therapeutic agents: well-tolerated biologically with no apparent side-effects, not degraded in the body, high aqueous solubility, high duplex stability with RNA, and long half-life following systemic administration (3).

Cytochrome P450 3A2 (CYP3A2) is a constitutively expressed gene that codes for a heme-containing enzyme in the rat that is orthologous to human CYP3A4 and is involved in the phase I oxidative metabolism of a large variety of pharmaceutical agents (4,5). This important enzyme is largely present in liver, small intestines and kidney, but significant amounts have also been reported in the skin (6,7). CYP3A2 makes a particularly good target to study transdermal activity of PMOs in an *in vivo* setting because: (a) the largest site for distribution of systemically administered PMOs in rats is liver (8); (b) commercially available assays make it simple to precisely measure reduction in the functional activity of the enzyme; and (c) there are potential therapeutic implications of developing methods to inhibit this drug metabolizing enzyme (9).

Passive dermal penetration is a highly desirable delivery strategy for antisense compounds (10). Several *in vitro* studies have demonstrated the ability of oligonucleotides to enter and cross the skin using either chemical or physical penetration enhancers (11–13). Recently, we demonstrated that iontophoretically delivered C5-propyne modified phosphorothioate oligonucleotide, with the same sequence as the active PMO used in these studies could alter targeted liver CYP3A2 enzyme levels in the intact rat (14). Lin *et al.* (15) delivered phosphorothioate oligonucleotides to hairless guinea pigs using a microprojection array patch. The data presented in this paper build on our previous studies and demonstrate the feasibility of passive transdermal delivery of non-ionic PMO compounds to alter CYP3A2 expression in rat liver *in vivo*. The potential for passive delivery should offer significant advantages over iontophoretic delivery of antisense agents.

MATERIAL AND METHODS

Oligomer Synthesis

All PMO were synthesized at AVI BioPharma (Corvallis, OR, USA) as previously described (16). Purity was greater than 90% full length as determined by reverse phase HPLC and MALDI TOF mass spectroscopy. The antisense 22-mer PMO targeted to rat CYP3A2 mRNA (Genbank accession number U09742) translation initiation region and was named AVI-4472. It has the following base sequence: 5′-GAGCTG-AAAGCAGGTCCATCCC-3′.

Animals

Male Sprague Dawley rats (Zivic Miller, Porterville, PA, USA) with jugular vein ports and weights between 240–260 grams were housed in plastic cages in the Laboratory Animal Resources Facility at Oregon State University (OSU) in Corvallis, OR, USA. The animals were maintained in a climate-controlled room with 12 h light/dark cycle and allowed access to a commercial rat chow and tap water *ad libitum*. Hairless mice CRL:SK1 ages 8–20 weeks were maintained in an AAALAC approved facility at the VA Medical Center, Omaha, NE, USA. All animal protocols conformed to "Prin-

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ciples of Laboratory Animal Care" (NIH publication #85-23 revised 1985).

In Vitro Experiments

Dorsal skin was removed from male hairless mice after the animals were killed by CO_2 asphyxiation. Full thickness skin patches were placed in a Bronough style, flow-through diffusion cell system (Permegear, Riegelsville, PA, USA). The receiver chamber (0.2 ml) was perfused with 25 mM Hepes, 133 mM NaCl at pH 7.4 (1 ml/h), which then passed to a fraction collector. The skin was allowed to equilibrate for 180 min. The oligonucleotide was dissolved in 95% propylene glycol and 5% linoleic acid to a final concentration of 38 μg/ml. Three hundred micro liters of flouresceinlabeled PMO were placed in the donor chamber and penetration was monitored by assaying the receiver fluid for fluorescence.

PMO Administration *in Vivo*

A portion of the rat's right rear flank was shaved with electric clippers (model A5, Oster, Milwaukee, WI, USA) such that no bruising, swelling or inflammation was evident upon visual inspection of the shaved region. 3.0, 0.3 or 0.03 mg of PMO was applied in a 100 μl volume of vehicle (95% propylene glycol + 5% linoleic acid). For the purpose of consistency, all transdermal applications were made using a plastic ring with an internal area of 2 cm^2 as a guide. Transdermal application of PMO in rats was made 24 h prior to euthanization for the purpose of organ collection.

Photomicrography

Organ slices were placed in plastic molds filled with embedding medium for frozen tissue processing (Sakura Finetek, Torrance, CA, USA). The molds were then wrapped in tin foil to protect from light and frozen in a −80 degree centigrade freezer. Five micron cryostat sections were cut at Oregon State University Veterinary Diagnostic Laboratory (Corvallis, OR, USA). Slides were air-dried in dark for 30 min and cover slip mounted using Fluoromount-G mounting medium (Southern Biotechnology Associates, Birmingham, AL, USA). The photomicrographs were taken with a Nikon Diaphot 300 microscope connected to an Olympus (Melville, NY, USA) Magnafire SP-brand digital camera. The exposure times were kept constant for all fluorescent pictures at 30 s.

CYP3A Enzyme Activity Assay

This assay was modified from Gentest Technical Bulletin, version 3, dated 09/25/1998 (Woburn, MA, USA) and is a tool for determining CYP3A activity (17). A one hundred micro gram aliquot of protein from the post-mitochondrial supernatant of tissue homogenate (S-9 fraction) was diluted in 0.1 M potassium phosphate (Sigma) to a volume of 500 μl. This was followed by addition of 7.5 μl of 5.0 mM substrate 7-benzyloxy-4-[trifluoromethyl]-coumarin (BFC) (Gentest) and β-NADPH regenerating reaction mixture (Gentest). All tubes were incubated for 60 min in a 37°C waterbath. The reaction was stopped by addition of 100 μl of stop solution (80% acetonitrile, 20% tris buffer pH 7.4). All tubes were centrifuged at 15,000 Xg for 5 min and the supernatants were collected for determination of the fluorescent product, 7-hydroxy-4-[trifluoromethyl]-coumarin (HFC), at the excitation wavelength of 409 nm and emission wavelength of 530 nm. All readings were compared to a standard curve prepared from 0.25 mM stock solution of HFC (Gentest).

Immunoblot Analysis

Levels of CYP3A2 and β-actin proteins were determined by western immunoblots in liver S-9 fractions. Fifty micro grams total protein was separated on a 12% sodium dodecylsulfate/acrylamide gel and immunoblotted according to standard techniques. Polyclonal primary antibodies for CYP3A2 were from Gentest (Woburn, MA, USA) and primary monoclonal antibodies for β-actin (clone AC-40) were from Sigma. β-actin immunodetection was performed to confirm that all lanes were loaded with similar amounts of protein by stripping the same blot in 100 mM 2-mercaptoethanol, 2% SDS, 62.5 mM tris-HCl, pH 6.7 for 1 h at 50°C followed by the washing and blocking steps as described. Densitometry was performed on a Kodak Image Station 440 (NEN, Boston, MA, USA).

HPLC Detection of PMO in Rat Tissue Samples

This assay was performed as previously published (18). Briefly, an internal standard PMO (15-mer whose sequence was derived from a 5′ truncation of AVI-4472) was added to each 250-μl aliquots of plasma and liver lysates. The samples were methanol extracted and heated in a water bath at 70°C for 10 min. Samples were then lyophilized and reconstituted by adding 100 μl aliquot of 5′-fluoresceinated DNA (1.0 O.D. units/ml) whose sequence was complimentary to that of AVI-4472 PMO. A set of AVI-4472 standards prepared by spiking appropriate amounts (10, 25, 50, 100, 250, 500, and 1000 ng/250 μl of plasma) into blank rat plasma along with the internal standard (500 ng) were similarly extracted. The samples were analyzed by injecting on to a Dionex DNA Pac PA-100 column (4 × 250 mm column; Dionex Corporation, Sunnyvale, CA) using a Varian autosampler (AI-200) connected to a Varian HPLC pump (model 9010 inert) equipped with a Varian fluorescent detector (model 9075). Mobile phase A was 0.025 *M* Tris (pH 8). Mobile phase B was 0.025 *M* Tris (pH 8)/1 *M* NaCl. The gradient program employed was (90% A–10% B at 0 min, 55% A–45 %B at 20 min, 0% A–100% B at 25 min) and the pump flow rate was 1.5 ml/min. The runs were monitored at excitation and emission wavelengths of 494 nm and 518 nm respectively.

Statistical Analysis

All data are presented as mean ± standard deviation. Statistical analysis compared groups by one-way analysis of variance (Prism v3 from Graphpad, San Diego, CA, USA). Differences of $p < 0.05$ and $p < 0.005$ are denoted as * and **, respectively.

RESULTS

The feasibility of using topical antisense to induce metabolic changes in the liver was determined prior to initiation of *in vivo* studies. Previous studies have demonstrated that hairless mouse provides a reasonable model for dermal penetra-

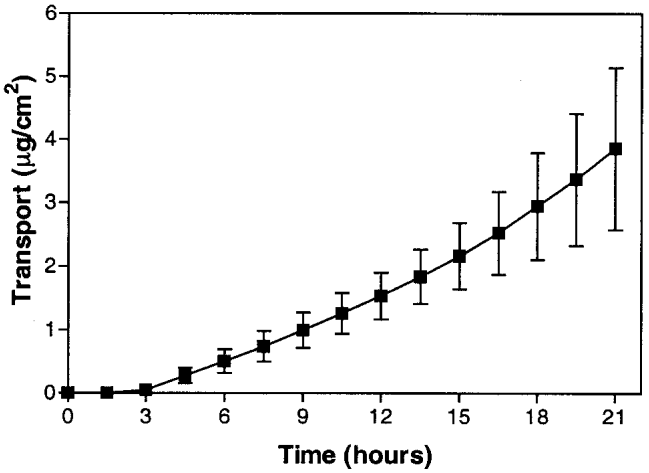

Fig. 1. Passive delivery of 38 μg PMO AVI-4472 dissolved in 5% propylene glycol and 5% linoleic acid across hairless mouse skin. N = 3.

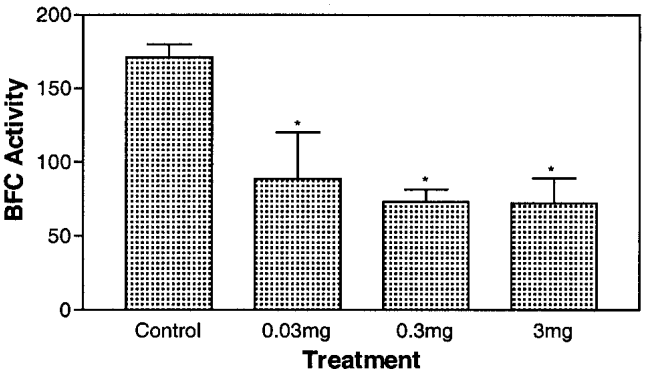

Fig. 3. Rat skin CYP3A enzyme activity 24 hours following transdermal treatment with increasing doses of AVI-4472. Control PMO was used at the highest concentration (3 mg/rat) only. Skin tissue immediately underlying the application site was used. CYP3A enzyme activity was measured by hydroxylation of BFC to HFC and data is expressed as nanomoles of product/ 100 μg S9 protein/ hour. N = 4. Data for vehicle control group is not available. Differences of $p < 0.05$ from control are denoted as *.

tion through rat skin (14). *In vitro* transdermal studies were performed using the PMO AVI-4472. A donor solution of 38 μg/ml was placed on isolated skin from hairless mouse, with a steady state flux of 0.197 ± 0.076 μg/cm^2 h, lag time of 4.28 h and a total delivery of 3.9 ± 1.3 ug/cm^2 in 24 h (Fig. 1). Arora *et al.* (8) have shown that intraperitoneally delivered PMO at the dose of 25 μg has antisense activity against the c-myc gene in rat liver. Delivery is linearly related to both donor concentration and surface area. Assuming 4 μg/cm^2 * 2 cm^2 applied surface area = 8 μg for 38 μg/ml (or 11.4 μg), then a dose of approximately 30 μg should produce a therapeutic response and was therefore selected as a starting dose for *in vivo* transdermal studies.

Antisense PMO was applied on shaved rat skin for *in vivo* studies (see Methods section for details). Liver and skin samples were harvested 24 h following application. CYP3A2 activity was examined in S9 fractions from liver and skin at the application site. Fig. 2 and Fig. 3 demonstrate microsomal CYP3A2 activity in liver and skin tissues, respectively, with enzyme activity presented as nanomoles product/100 μg S9 protein/hour. In the liver, a PMO dose as low as 0.03 mg/rat was able to reduce enzyme activity to 432 ± 20 vs. 576 ± 17 saline ($p < 0.05$) and 564 ± 20 control PMO sequence. Increasing the dose by a factor of ten to 0.3 mg PMO/rat led to a significantly inhibited enzyme level of 278 ± 13 ($p < 0.005$). The reduction of CYP3A2 activity remains significant at 326 ± 34 ($p < 0.005$), with no additional inhibition observed by further increasing the dose by a factor of ten to 3 mg PMO/rat. In the skin, where starting enzyme levels were approximately one third of the liver (171 ± 9), the inhibition reached a maximum at a lower dose. Topical delivery of 0.03 mg reduced skin enzyme levels by half to 89 ± 32 ($p < 0.05$), while increasing the dose to 0.3 mg and 3.0 mg did not produce any further inhibition 73 ± 8 and 72 ± 17, respectively.

Figure 4 shows the immunoblot analysis of rat liver (A) and skin (B) following topical delivery of CYP3A2 antisense PMO at 0.03, 0.3 and 3.0 mg/rat. The protein expression data are consistent with enzyme activities presented if Fig. 2 and Fig. 3. CYP3A2 levels were slightly inhibited at 0.03 mg/rat for both skin and liver. The enzyme was strongly inhibited in

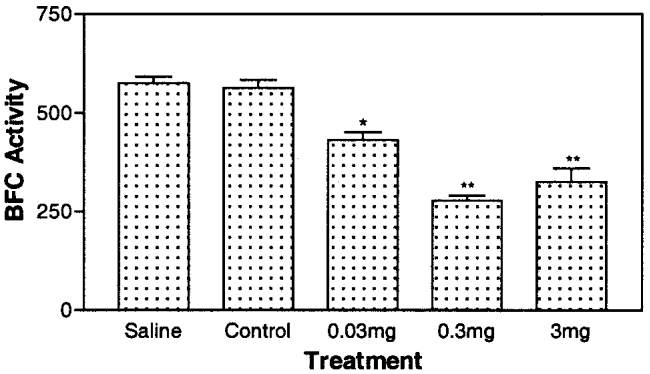

Fig. 2. Rat liver CYP3A enzyme activity 24 hours following transdermal treatment with increasing doses of AVI-4472. Control PMO was used at the highest concentration (3 mg/rat) only. CYP3A enzyme activity was measured by hydroxylation of BFC to HFC and data is expressed as nanomoles of product/ 100 μg S9 protein/ hour. N = 4. Differences of $p < 0.05$ and $p < 0.005$ are denoted as * and **, respectively.

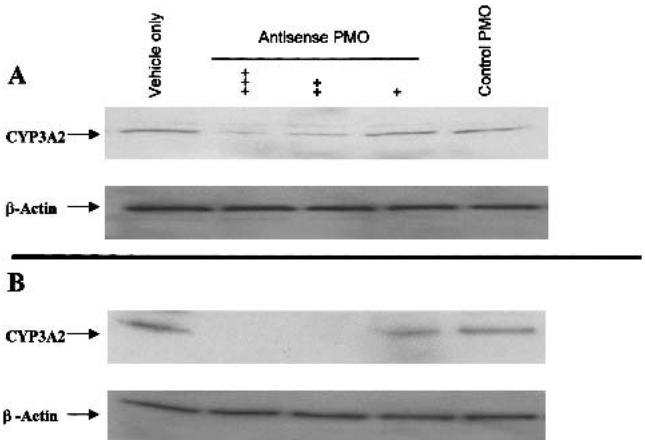

Fig. 4. Rat liver CYP3A immunoblots from S9 fractions 24 hours following transdermal treatment with increasing doses of AVI-4472. β-Actin serves as a control. +, ++, and +++ indicate 0.03, 0.3 and 3.0 mg topical PMO treatments, respectively.

the skin at the 0.3 and 3.0 mg doses. These doses also reduce levels in the liver, but not to as great an extent as in the skin. The immunoblot control (β-actin) demonstrates that this is not a function of different quantities being placed on the gel and that the inhibition is specific to antisense PMO treatment. Thus, inhibition of CYP3A2 was dose-dependent and sequence-specific.

Figure 5 demonstrates that intact PMO was detectable in skin and liver tissue lysates following transdermal delivery. This technique is capable of detecting cleaved oligomers, including (N-1)-mers, but none were observed. Figure 6 examines the tissue distribution of fluorescein-labeled PMO 24 h following similar application. A diffused cytoplasmic pattern of distribution was observed, with more intense nuclear distribution. The darker round spots observed in the pictures are air bubbles. These artifacts are an inherent limitation of the technique and should be disregarded.

DISCUSSION

This article presents the first evidence that passive transdermal delivery of antisense compounds can produce systemic changes in targeted gene expression in the liver without the use of physical penetration enhancement techniques such as iontophoresis.

Transdermal flux of PMO *in vitro* was approximately twenty-fold greater than the same sequence containing a phosphorothioate backbone and C5 propyne. This is consistent with another report in which modifying an uncharged methylphosphonate by adding a phosphate linkage that created a single negative charge reduced dermal penetration by a factor of ten (19).

The functional CYP3A2 enzyme assay data is presented in Fig. 2 and Fig. 3 and indicate a dose-dependent inhibition in liver and underlying skin, respectively, following topical

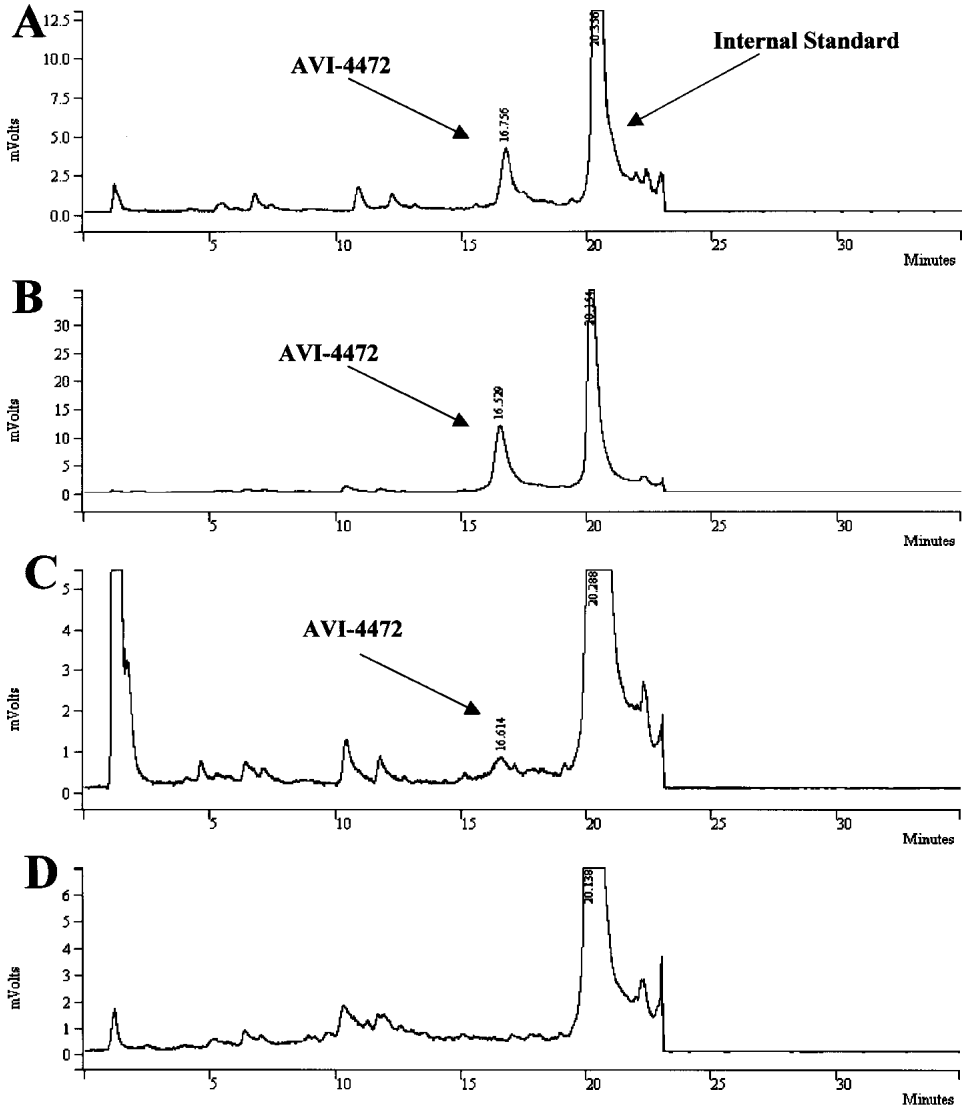

Fig. 5. Representative HPLC chromatogram showing detection of full-length PMO AVI-4472 in rat tissues following transdermal treatment with 3.0 mg/rat PMO. Chromatogram A is AVI-4472 quality control reference standard. Chromatogram B is the skin tissue underlying PMO application site 24 h after PMO application. Chromatogram C is liver tissue 24 h after PMO application. Chromatogram D is plasma sample 120 min after PMO application. Note that no PMO was detected in plasma samples at any timepoints tested. Also note that the limit of PMO detection by this assay is 100 ng/ml.

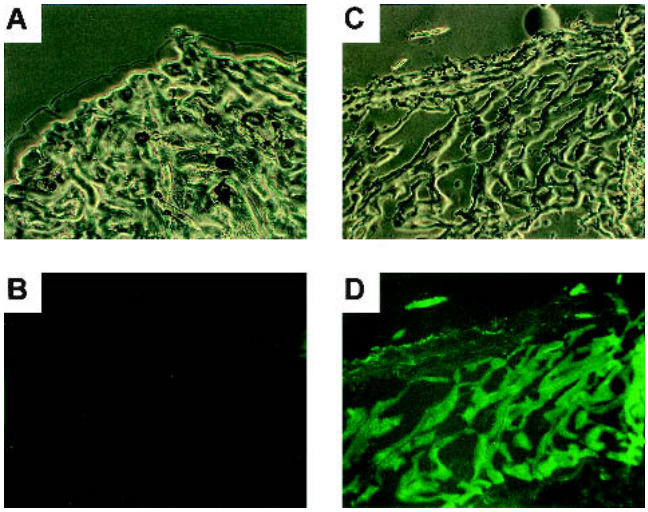

Fig. 6. Representative ×200 photomicrographs of cryostat-cut rat skin sections at application site, 24 hours following application of vehicle 0.3 mg flourescein-labeled PMO. The darker round spots observed in the pictures are air bubbles. These artifacts are an inherent limitation of the technique and should be disregarded. Panels (A) and (B) are identical fields form a vehicle-treated rat under phase contrast and fluorescence conditions, respectively. Panels (C) and (D) are identical fields form a PMO-treated rat under phase contrast and fluorescence conditions, respectively. The exposure time for both fluorescence micrographs was kept constant at 30 s.

administration of 0.03 mg and 0.3 mg doses. The highest dose of 3 mg dose offers no further inhibitory activity when compared with the 0.3 mg dose. The likely cause of this observation is that the 0.3 mg dose has already achieved the maximal enzyme inhibition. This is better appreciated by putting the experimental model in context. The half-life of the target enzyme CYP3A2 is estimated to be between 20 and 24 h. Since the tissue samples for analyzing enzyme activity were harvested 24 h following antisense PMO application, the maximal anticipated inhibition of enzyme activity was approximately 50%. The authors speculate that the lack of linearity of the dose-response at the highest dose is a limitation of the model used and not a phenomenon associated with transdermal use of antisense PMOs. The potential to use PMO antisense to modulate gene expression in the skin can be explored further based on these results. Several other studies have also demonstrated the feasibility of using topical antisense to treat dermal disorders (20). One used posphorothioate antisense on *c-fos* to skin that had been exposed to UV radiation. Epidermal *c-fos* expression was almost completely blocked 18 h post irradiation (21). Furthermore, a phosphorothioate antisense oligonucleotide to TGF- β1 was able to decrease scarring of incisional wounds when applied topically to mice (22). A chimeric oligonucleotide was able to restore melanin synthesis in albino BALB/c mice when given either topically or intradermally. The gene correction was observed for three months after oligonucleotide application (23). Furthermore, topical delivery of a cream containing phosphorothioate antisense to ICAM-1 effectively inhibited mRNA levels in the skin (24).

The ability of topically applied AVI-4472 to act systemically was evident by inhibition of CYP3A2 in the liver. A single topical application of 0.03 mg led to a target enzyme reduction of 25% after 24 h and increasing the dose to 0.3 mg improved inhibition to 50%. Previously we have demonstrated that a C5 propyne modified phosphorothioate oligonucleotide targeted to CYP3A2 could be delivered transdermally *in vivo* with iontophoretic enhancement. A dose of 0.24 mg decreased CYP3A2 enzyme levels in the liver by 30% after 24 h (14).

The studies presented demonstrate that a transdermally delivered non-ionic antisense PMO can reach concentrations sufficient to inhibit cytochrome P450-3A2 expression *in vivo.* The importance of this work is reflected in the answer to the key issue of whether transdermal antisense therapy is feasible in humans. Doses can be converted from rat to human using the method of Freidreich *et al.* (25). Assuming that the 0.3 mg topical dose is necessary in rats, a donor dose of (0.3 mg/0.25 kg * 7 kg/m^2 * 11.8 m^2) = 15 mg would be required for man. Drugs are usually considered transdermal delivery candidates if their daily delivery requirements are less than 50 mg. The authors, however, acknowledge the inherent limitation of the rat model in making such a dose extrapolation, as the permeability of shave rat skin is likely higher than human skin.

Feasibility of passive transdermal delivery for therapeutics offers tremendous advantage. Like the oral route (18), the transdermal delivery route should ensure patient compliance. Percutaneous penetration is likely to be particularly useful in patients with gastroporesis or interrupted gastric mucosa, where oral delivery would be unpredictable. Dermal delivery indicates that local modulation of gene expression in the skin is also feasible.

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REFERENCES

1. J. Goodchild and P. Iversen. Oligonucleotides – from design to the clinic. *Curr. Opin. Mol. Therap.* **3**:223–223 (2001).
2. J. Summerton and D. Weller. Antisense properties of morpholino oligomers. *Nucleosides Nucleotides* **16**:889–898 (1997).
3. P. L. Iversen. Phosphorodiamidate morpholino oligomers: favorable properties for sequence-specific gene inactivation. *Curr. Opin. Mol. Ther.* **3**:235–238 (2001).
4. F. P. Guengerich. Cytochrome P450 3A4: regulation and role in drug metabolism. *Ann. Rev. Pharmacol. Toxicol.* **39**:1–17 (1999).
5. K. E. Thummel and G. R. Wilkinson. In vitro and in vivo drug interactions involving human CYP3A. *Annu. Rev. Pharmacol. Toxicol.* **38**:389–430 (1998).
6. F. K. Jugert, R. Agarwal, A. Kuhn, D. R. Bickers, H. F. Merk, and H. Mukhtar. Multiple Cytochrome P450 Isozymes in Murine Skin: Induction of P450 1A, 2B, 2E, and 3A by Dexamethasone. *J. Investig. Dermatol.* **102**:970–975 (1994).
7. M.I. Singer, L.E. Shapiro, and N.H. Shear. Cytochrome P-450 3A: interactions with dermatologic therapies. *J. Am. Acad. Dermatol.* **37**:765–771 (1997).
8. V. Arora, D. C. Knapp, B. L. Smith, M. L. Statdfield, D. A. Stein, M. T. Reddy, D. D. Weller, and P. L. Iversen. c-Myc antisense limits rat liver regeneration and indicates role for c-Myc in regulating cytochrome P-450 3A activity. *J. Pharmacol. Exp. Ther.* **292**:921–928 (2000).
9. V. Arora and P. L. Iversen. Redirection of drug metabolism using antisense technology. *Curr. Opin. Mol. Therap.* **3**:249–257 (2001).

10. R. M. Brand. Topical and transdermal delivery of antisense oligonucleotides. *Curr. Opin. Mol. Ther.* **3**:244–248 (2001).
11. V. V. Vlassov, V. N. Karamyshev, and L. A. Yakubov. Penetration of oligonucleotides into mouse organism through mucosa and skin. *FEBS* **327**:271–274 (1993).
12. R. M. Brand, A. Wahl, and P. L. Iversen. Effects of Size and Sequence on the Iontophoretic Delivery of Oligonucleotides. *J. Pharm. Sci.* **87**:49–52 (1997).
13. V. Regnier, N. De Morre, A. Jadoul, and V. Preat. Mechanisms of a phosphorothioate oligonucleotide delivery by skin electroporation. *Int. J. Pharm.* **184**:147–156 (1999).
14. R. M. Brand, T. L. Hannah, J. Norris, and P. L. Iversen. Transdermal delivery of antisense oligonucleotides can induce changes in gene expression in vivo. *Antisense Nucleic Acid Drug Dev.* **11**:1–6 (2001).
15. W. Lin, M. Cormier, A. Samiee, A. Griffin, B. Johnson, C.-L. Teng, G. E. Hardee, and P. E. Daddona. Transdermal Delivery of Antisense Oligonucleotides with Microprojection Patch (Macroflux) Technology. *Pharm. Res.* **18**:1789–1793 (2001).
16. J. Summerton and D. Weller. Morpholino antisense oligomers: design, preparation and properties. *Antisense Nucleic Acid Drug Dev.* **7**:187–195 (1997).
17. D. M. Stresser, A. P. Blanchard, S. D. Turner, J. C. Erve, A. A. Dandeneau, V. P. Miller, and C. L. Crespi. Substrate-dependent modulation of CYP3A4 catalytic activity: analysis of 27 test compounds with four fluorometric substrates. *Drug Metab. Dispos.* **28**:1440–1448 (2000).
18. V. Arora, D. C. Knapp, M. T. Reddy, D. D. Weller, and P. L. Iversen. Bioavailability and efficacy of antisense morpholino oligomers targeted to c-*myc* and cytochrome P450 3A2 following oral administration in rats. *J. Pharm. Sci.* **91**:1009–1018 (2002).
19. H. W. Nolen III, P. Catz, and D. R. Friend. Percutaneous penetration of methyl phosphonate antisense oligonucleotides. *Int. J. Pharm.* **107**:169–177 (1994).
20. R. M. Brand and P. L. Iversen. Transdermal delivery of antisense compounds. *Adv. Drug Deliv. Rev.* **44**:51–57 (2000).
21. F. Gillardon, I. Mill, and E. Uhlmann. Inhibition of c-Fos expression in the UV-irradiated epidermis by topical application of antisense oligodeoxynucleotides suppresses activation of proliferating cell nuclear antigen. *Carcinogenesis* **16**:1853–1856 (1995).
22. B. M. Choi, H. J. Kwak, C. D. Jun, S. D. Park, K. Y. Kim, H. R. Kim, and H. T. Chung. Control of scarring in adult wounds using antisense transforming growth factor-beta 1 oligodeoxynucleotides. *Immunol. Cell Biol.* **74**:144–150 (1996).
23. V. I. O. Alexeev, A. Domashenko, G. Cotsarelis, and K. Yoon. Localized in vivo genotypic and phenotypic correction of the albino mutation in skin by RNA-DNA oligonucleotide. *Nat. Biotechnol.* **18**:43–47 (2000).
24. R. C. Mehta, K. K. Stecker, S. R. Cooper, M. V. Templin, Y. J. Tsai, T. P. Condon, C. F. Bennett, and G. E. Hardee. Intercellular Adhesion Molecule-1 Suppression in Skin by Topical Delivery of Anti-Sense Oligonucleotides. *J. Invest. Dermatol.* **115**:805–812 (2000).
25. E. J. Freidreich. Qualitative comparison of toxicity of anticancer agents in mouse, rat, dog, monkey and man. *Cancer Chemother. Reports* **50**:219–244 (1966).