ELSEVIER

International Journal of Pharmaceutics 107 (1994) 169–177

international journal of pharmaceutics

Research Papers

Percutaneous penetration of methyl phosphonate antisense oligonucleotides

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(Received 8 February 1993; Modified version received 6 December 1993; Accepted 23 December 1993)

Abstract

A series of antisense methyl phosphonate oligonucleotides (MPOs) were evaluated in vitro for skin penetration and retention using either hairless mouse or human cadaver skin. Several skin penetration enhancers were used to promote uptake of MPOs ranging in molecular weight from 1834 to 5500. In general, as the molecular weight of the MPOs increased, the penetration rate decreased. Tape stripping experiments with both hairless mouse skin and human cadaver skin indicated that the stratum corneum is the primary barrier to penetration. Comparison of a 14-mer MPO and the same MPO modified by the introduction of one negative charge (phosphate linkage) per molecule reduced the skin permeability by about 10-fold. The amount of MPO retained in the dermis at the end of the 24 h permeation experiments indicated that most of the compounds were able to reach a nominal target concentration of 1.0 μM. The largest MPO tested (18-mer, Mol. Wt 5500) gave the least amount of material penetrating through human cadaver skin, but depending on the vehicle, was retained in nearly sufficient amounts in the target tissues (dermis). Thus, the 18-mer represents a logical candidate for further evaluation due to the potential for delivery into the target tissue with limited systemic exposure.

Key words: Antisense oligonucleotide; Methyl phosphonate oligonucleotide; Dermal delivery

1. Introduction

Antisense oligonucleotides can potentially inhibit or modify the expression of individual genes (Ts'o et al., 1987). Should these agents function in vivo as hypothesized, they could be developed into unique, highly specific therapeutic drugs. In some cases, antisense oligonucleotides are designed to bind to specific mRNA molecules and suppress translation, thus inhibiting protein synthesis. In other cases, DNA is the target for antisense oligonucleotides. In the latter case, a triple helix (triplex) is formed on binding to DNA preventing the replication or transcription through hybridization of single-stranded DNA regions. Diseases for which the antisense strategy is being pursued are viral infections (HIV, herpes simplex, human papilloma, cytomegalo), cancer (leukemia, lymphoma, breast, squamous cell carcinoma), other infections (fungal, protozoal, bac-

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SSDI 0378-5173(94)00015-W

terial), and genetic disorders (hemophilia, cystic fibrosis, muscular dystrophy).

A key aspect to the successful development of antisense oligonucleotides is drug delivery. Like peptides/proteins, most antisense compounds are relatively large molecules (ranging from about 500 to over 10000 Mol. Wt) and are relatively polar. These properties tend to reduce the ability of these molecules to permeate through biological membranes. In addition, the body produces nucleases that can potentially degrade oligonucleotides (although therapeutic antisense compounds are designed to resist enzymic degradation). In addition to problems related to reaching the target cell or organism, the antisense compounds must be taken up by the cell and reach target DNA or mRNA strands (Jaroszewki and Cohen, 1991). Several delivery methods currently under investigation involve the use of liposomes (Ropert et al., 1992; Thierry et al., 1992) and nanoparticles (Chavany et al., 1992).

A number of the diseases mentioned above are best treated by topical drug therapy. In this regard, a series of experiments were performed to evaluate the ability of a specific class of antisense oligonucleotides (methyl phosphonate oligonucleotides; MPOs) to permeate into and through hairless mouse and human cadaver skin. These compounds are referred to herein by their nucleotide base number, which ranged from 6 (6-mer) to 18 (18-mer). A number of putative skin permeation enhancers were examined for their ability to enhance the topical delivery of MPOs.

2. Materials and methods

2.1. Materials

MPOs were provided by Genta Inc. (San Diego, CA); these compounds were used as received. Five different MPOs of varying molecular weight were assessed in this study: 6-mer (1834 Mol. Wt), 10-mer (3057 Mol. Wt), 14-mer (4270 Mol. Wt), and an 18-mer (5500 Mol. Wt); a 14-mer with a single phosphonate linkage in place of one of the methyl phosphonate groups was also examined. Decylmethyl sulfoxide (DMS; technical grade; m.p. 46–49°C) was purchased from Columbia Organic Chemical Co. (Camden, SC). Oleic acid (OA; N.F.) was purchased from Mallinckrodt, Inc., Paris, KT. Propylene glycol (PG; U.S.P.), ethanol (EtOH; U.S.P.), and ethyl acetate (EtAc; U.S.P.) were purchased from Spectrum Chemical Mfg. Co. (Gardena, CA). Glycerol monooleate (GMO) was a gift of Lonza, Inc., Long Beach, CA. Azone was a gift of Nelson Research (Irvine, CA). All other chemicals were reagent or HPLC grade and were used as received.

The human cadaver skin was acquired from the International Institute of Advanced Medicine, Exton, PA. In all cases the skin was removed from the abdomen with a dermatome and frozen until use. The human cadaver skin was generally 300–500 μm thick. Therefore, the skin contained complete epidermis (stratum corneum and viable epidermis) along with a portion of the dermis. The hairless mice (male HRS/J strain; 8–10 weeks old; 20–25 g) were obtained from Jackson Labs, Bar Harbor, ME.

2.2. Permeability experiments

A system with nine glass Franz diffusion cells was used for the permeability experiments. The hairless mice were killed in a CO_2 chamber, and an approx. 3–6 cm^2 area of full-thickness skin was excised from the abdomen. After removal of subcutaneous fat, the skins were washed with physiologic saline and used within 1 h in the permeability experiment. The human skin was thawed, rinsed in physiologic saline and mounted on the diffusion cells. The surface area of the diffusion cells was 2 cm^2. The skin was mounted and clamped between the cell body and the cell cap with the epidermal side facing upward (donor side). The cells were then allowed to equilibrate for 1 h. The donor phase (0.1 ml/cm^2) was placed directly on the skin through the cell cap, which was then sealed with a glass stopper. The receptor solution in contact with the dermal side of the skin was phosphate-buffered saline, pH 7.4, with 0.05% sodium azide added to prevent bacterial growth. The diffusion cells were maintained at 37°C by thermostatically controlled water which

was circulated through a jacket surrounding the cell body. Mixing of the receptor solution was accomplished by a small magnetic stirring bar driven by an external 600 rpm motor. At each time point, sample aliquots (200 μl) were removed from the receptor phase with a syringe needle and replaced with an equal volume of receptor solution. Dilution effects were accounted for in the analysis of drug concentrations in the receptor solution. The concentration of MPO in the receptor solution was measured by HPLC (see below). Each donor vehicle/MPO combination was tested in triplicate and all vehicle compositions are reported as volume fractions.

2.3. Methyl phosphonate tissue levels

At the conclusion of the permeation experiment the skins were rinsed with 1–2 ml of water, then rinsed with several milliliters of acetonitrile/water (1:1) to remove any residual solid MPO from the surface of the skin. The skins were then rinsed with several additional milliliters of water. Regions of skin not exposed to the donor vehicle were cut away and discarded. The epidermis (including the stratum corneum) was removed by rubbing the surface of the skin, then lifting off the epidermal layer using tweezers (the vehicles, after remaining in contact with the skin under occluded conditions, appeared to facilitate separation of the epidermis from the dermis). The dermis was cut into small pieces using scissors and soaked in 2.0 ml of 0.02 M sodium phosphate buffer, pH 7.4. After 6 h of soaking, the tissues were homogenized with a Brinkmann Polytron for 60 s. Aliquots of the homogenized samples were centrifuged in 1.5 ml microcentrifuge tubes for 2 min at $10000 \times g$. A small amount of the supernatant (150 μl) was then injected directly on the HPLC column for analysis (see below). Known amounts of MPO were added prior to homogenization of untreated skin samples to assess the recovery of the MPO. These recovery experiments confirmed that measurement of MPO was essentially quantitative using the procedures described.

In the case of the 18-mer, the samples were handled in a different manner to increase its detectability in tissue samples. Aliquots from either the receptor solution or tissue homogenates (1.5 ml) were evaporated to dryness on a rotary evaporator (Savant SVC 200). The dry samples were then reconstituted in 200 μl of the mobile phase (see below) and of this, 150 μl was injected on the HPLC column. The retention time was 13.2 min. Recovery of the 18-mer using the evaporation/reconstitution technique was quantitative as determined by a spiking experiment.

2.4. Chromatographic analysis

Concentration of the MPOs in the various solutions was measured using a Waters 840 HPLC system consisting of two model 510 pumps, a model 481 UV detector, a model 710B WISP (sample processor), and a Digital computer model 350 microprocessor/programmer. The column used was a 3.9 mm × 15 cm, 4 μm, Waters Nova-Pak C18.

The HPLC gradient was varied according to the MPO analyzed. The following is an example for the measurement of the 14-mer. Solvent A was 0.05 M ammonium acetate buffer adjusted to pH 7.0; solvent B was acetonitrile/solvent A (0.75:0.25). A gradient was used to elute the 14-mer as shown in Table 1. Similar gradients were used to elute the other MPOs. In all cases, the flow rate was 1.1 ml/min and the detection wavelength was 260 nm.

Table 1
HPLC gradient system used to elute the 14-mer MPO [a]

Time (min)	% solvent A [b]	% solvent B [c]
0	100	0
5	70	30
14	55	45
15.5	2	98
32	2	98
35	100	0
42	100	0

[a] Flow rate, 1.1 ml/min; wavelength, 260 nm; retention time, 11.1 min.
[b] Solvent A: 0.05 M ammonium acetate buffer, pH 7.0.
[c] Solvent B: acetonitrile/solvent A (0.75:0.25).

3. Results and discussion

3.1. Permeation and retention of MPOs using hairless mouse skin

A number of vehicles were used to study their effect on skin penetration of MPO. Table 2 gives the cumulative amount of a 14-mer MPO permeating though hairless mouse skin over a 24 h period from water, EtOH, and EtOH/DMS (0.95 : 0.05). The results indicate that addition of DMS to EtOH increases the penetration of the 14-mer relative to that from water and EtOH, which were both ineffective at promoting penetration of this compound. The two EtAc-containing vehicles (EtAc, EtAc/DMS (0.975 : 0.025)) proved to be only slightly more effective than water at increasing the penetration of the 14-mer MPO (see Table 2).

The effect of removing the stratum corneum on the permeation of a 14-mer MPO through skin was also studied. Fig. 1 shows the cumulative amount of 14-mer delivered through hairless mouse skin from several vehicles with the stratum corneum intact and with the stratum corneum removed by tape stripping. There was a marked increase in the amount of 14-mer permeating over 24 h when the stratum corneum was removed compared with that using intact skin. This finding was expected and suggests that the stratum corneum is largely responsible for controlling the permeation rate of these types of compounds.

A 10-mer MPO was also examined for its ability to penetrate through hairless mouse skin

Table 2
Cumulative amount of 10-mer and 14-mer MPOs permeated through hairless mouse skin after 24 h

MPO	Donor vehicle	Cumulative amount delivered at 24 h (nmol/cm^2)
10-mer	EtOH	0.69 ± 0.34 [b]
	EtOH/DMS (0.95 : 0.05)	4.1 ± 0.74
	EtOH/H_2O/DMS (0.80 : 0.15 : 0.05)	4.0 ± 0.79
14-mer	H_2O	0.16 ± 0.12
	EtOH	0.07 ± 0.03
	EtOH/DMS (0.95 : 0.05)	1.3 ± 0.64
	EtOH/DMS (0.975 : 0.025)	1.0 ± 0.24
	EtOH/OA (0.95 : 0.05) [c]	0.29 ± 0.08
	EtOH/OA (0.975 : 0.025)	0.21 ± 0.03
	EtAc	0.28 ± 0.03
	EtAc/DMS (0.95 : 0.05)	0.24 ± 0.12
	EtAc/OA (0.95 : 0.05)	0.13 ± 0.04
	EtOH [d]	44 ± 21
	EtOH/DMS (0.95 : 0.05) [d]	43 ± 6.4
	EtOH/H_2O/DMS (0.80 : 0.15 : 0.05)	0.82 ± 0.25
	EtOH/H_2O/DMS (0.80 : 0.15 : 0.05) [e]	0.63 ± 0.09
	EtOH/H_2O/DMS (0.80 : 0.15 : 0.05) [f]	0.48 ± 0.09
	EtOH/H_2O/DMS (0.80 : 0.15 : 0.05) [g]	0.054 ± 0.01
14-mer-1A	H_2O	0
	EtOH	0
	EtOH/DMS (0.95 : 0.05)	0.14 ± 0.06

[a] Unless indicated in footnotes, all vehicles were saturated with MPO.
[b] Mean ± S.D. ($n = 3$).
[c] OA, oleic acid.
[d] These skins were free of stratum corneum, which was removed by tape stripping.
[e] MPO concentration in vehicle was 1.0 mg/ml (below saturation).
[f] MPO concentration in vehicle was 0.5 mg/ml (below saturation).
[g] MPO concentration in vehicle was 0.05 mg/ml (below saturation).

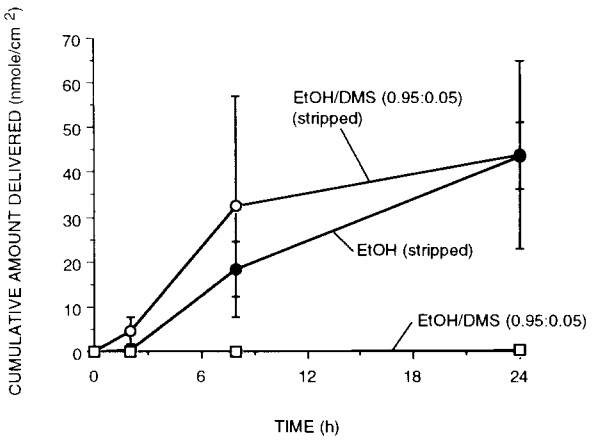

Fig. 1. Cumulative amount of the 14-mer (nmol/cm^2) delivered through intact hairless mouse skin or stratum corneum-free (tape stripped) hairless mouse skin over 24 h. The vehicles tested were EtOH/DMS (0.95:0.05) and EtOH as indicated. Each vehicle contained 2.5 mg/ml (in excess of saturation in all cases). Exposed skin area was 2.0 cm^2 and 200 μl (100 μl/cm^2) was applied to each skin. Error bars are SE ($n = 3$).

from EtOH, EtOH/DMS (0.95 : 0.05), and EtOH/H_2O/DMS (0.80 : 0.15 : 0.05). Compared with the penetration data gathered with the 14-mer, the 10-mer was considerably more permeable on a mole basis: depending on the vehicle, between 5 and 10 times more 10-mer permeated over 24 h when compared with the 14-mer. This finding was also expected based on the smaller size of the 10-mer (3057 Mol. Wt) compared with the 14-mer (4270 Mol. Wt).

The effect of different concentrations of 14-mer MPO in the donor vehicle was examined using an EtOH/H_2O/DMS (0.80 : 0.15 : 0.05) solvent system. Lowering the concentration of MPO in this donor vehicle from saturation to below saturation reduced the total amount of MPO delivered through hairless mouse skin over 24 h (see Table 2). The saturated vehicles had a concentration of 2.0 mg/ml; lowering the concentration to 1.0 or 0.5 mg/ml lowered the total amount delivered from 3.5 to 2.7 or 2.1 μg/cm^2, respectively. Reducing the concentration to 0.05 mg/ml substantially reduced the amount delivered (0.23 μg/cm^2) over 24 h.

A second 14-mer MPO was tested with hairless mouse skin. This compound (14-mer-1A) contained a single phosphonate linkage to introduce one negative charge per molecule at physiologic pH. This charged 14-mer is slightly more hydrophilic than its uncharged counterpart as demonstrated by its shorter HPLC retention time (11.5 min (14-mer-1A) vs 13.2 min (14-mer)) under the same conditions. The nucleotide sequence of the two 14-mer compounds was the same. Three vehicles were examined (H_2O, EtOH, and EtOH/DMS (0.95 : 0.05)). The results are shown in Table 2 (cumulative amount of MPO permeated through skin over 24 h) along

Table 3
Recovery of 10-mer and 14-mer MPOs in the dermis after 24 h permeation study using hairless mouse skin [a]

MPO	Donor	Concentration	
		μg/gm [b]	μM [c]
10-mer	EtOH	140 ± 86	45.7 ± 28
	EtOH/DMS (0.95 : 0.05)	26.4 ± 2.7	8.6 ± 0.90
	EtOH/H_2O/DMS (0.80 : 0.15 : 0.05)	51.7 ± 1.06	16.9 ± 0.35
14-mer	EtOH/DMS (0.95 : 0.05)	30.2 ± 11.9	7.1 ± 2.8
	EtOH/DMS (0.95 : 0.05) [d]	112 ± 67	26.3 ± 15.7
	EtOH/H_2O/DMS (0.80 : 0.15 : 0.05)	77 ± 45	17.9 ± 10.7
	EtOH/H_2O/DMS (0.80 : 0.15 : 0.05) [e]	18.4 ± 8.8	4.3 ± 2.1

[a] Unless indicated, all vehicles were saturated with excess solid MPO.
[b] Amount (μg) of MPO recovered per g of wet dermal tissues.
[c] Concentration of MPO assuming a density of 1.0 for the dermis and a molecular weight of 4270 and 3057 for the 14-mer and 10-mer, respectively.
[d] Stratum corneum-free skin (tape stripped).
[e] Concentration of MPO was 0.5 mg/ml (below saturation).

with a summary of the other 14-mer-containing vehicles tested with hairless mouse skin. The introduction of one negative charge into the oligonucleotide backbone substantially reduced the amount permeated over 24 h through hairless mouse skin. 14-mer-1A was undetectable in the receptor solution from both the H_2O and EtOH vehicles at 24 h. There was some 14-mer-1A detected in the receptor solution at 24 h from the EtOH/DMS (0.95:0.05) vehicle; however, the total amount detected in the receptor solution was about 10 times less than that detected using the same nonionic sequence.

Several of the vehicles were examined for their ability to deliver MPO into hairless mouse skin after a 24 h application period. The penetration studies were performed for 24 h after which the epidermis was removed and the amount of MPO in the dermal tissues was measured. The amount of MPO in the dermis after 24 h is shown in Table 3. The concentration (μM) of MPO in each section was estimated by assuming a density of 1.0 for both the stratum corneum and the viable tissues (the dry density of stratum corneum is variable with a mean value of slightly greater than 1.0 (Anderson and Cassidy, 1973)). In these experiments, the concentration of MPO in the dermal tissues is the most important parameter to measure when considering topical pharmacotherapy with MPOs. In general, a concentration in excess of 1.0 μM, and preferably over 10 μM, is desirable based on dose/response data collected in vitro (Goodchild, 1989).

3.2. Permeation and retention of MPOs using human cadaver skin

A series of permeation experiments was also performed with human cadaver skin in place of hairless mouse skin. In addition to the 10-mer and 14-mer, a 6-mer and an 18-mer were also examined with human cadaver skin.

The cumulative amount (nmol/cm^2) of 6-mer MPO (Mol. Wt 1834) permeating through human cadaver skin after 24 h from MPO-saturated vehicles is listed in Table 4. In general, EtOH-containing vehicles yielded higher cumulative amounts of the 6-mer MPO delivered compared with the PG-containing vehicles. An Azone-containing vehicle (PG/Azone, 0.95:0.05) was relatively ineffective at promoting the penetration of the 6-mer through human cadaver skin compared with the other vehicles. The cumulative amount of 10-mer, 14-mer, and 18-mer MPOs permeated through human cadaver skin was generally dependent upon the molecular weight of the MPO when the EtOH, EtOH/DMS, and EtOH/H_2O/DMS vehicles were used (see Table 4). For

Table 4
Cumulative amount of MPOs (6-mer, 10-mer, 14-mer and 18-mer) MPOs permeated through human cadaver skin after 24 h [a]

Vehicle	Cumulative amount delivered at 24 h (nmol/cm^2)			
	6-mer	10-mer	14-mer	18-mer
EtOH	3.4 ± 0.28 [b]	4.0 ± 6.7	0.37 ± 0.48	NP [c]
EtOH/DMS (0.95:0.05)	21.1 ± 5.1	6.0 ± 4.2	0.83 ± 1.0	0.05 + 0.14
EtOH/H_2O/DMS (0.80:0.15:0.05)	13.8 ± 5.7	0.94 ± 1.3	0.18 ± 0.17	NP
PG	0.21 ± 0.37	NP	NP	0.05 ± 0.29
PG/DMS (0.95:0.05)	0.57 ± 0.50	NP	NP	ND [d]
PG/GMO (0.91:0.09)	NP	NP	NP	ND
PG/GMO/DMS (0.65:0.30:0.05)	NP	NP	NP	ND
EtOH/Azone (0.95:0.05)	ND	NP	NP	NP
EtOH/GMO (0.95:0.05)	ND	NP	NP	NP
EtOH/H_2O/GMO (0.80:0.15:0.05)	ND	NP	NP	NP

[a] All vehicles were saturated with excess oligomer.
[b] Values are mean ± SD (n = 3).
[c] NP, experiment not performed.
[d] ND, not detected.

example, very little if any 18-mer (Mol. Wt 5500) was able to permeate through human cadaver skin over the 24 h period (see Table 4).

A comparison of the cumulative amount of MPO permeating through hairless mouse skin or through human cadaver skin shows that for the different MPOs tested, hairless mouse skin was only slightly more permeable than was human cadaver skin. For example, the amount of 14-mer permeating through hairless mouse skin after 24 h from EtOH/DMS (0.95:0.05) was 1.3 ± 0.64 nmol/cm^2 compared with 0.83 ± 1.0 nmol/cm^2 through human cadaver skin. In the case of pure EtOH as the vehicle, hairless mouse skin yielded a lower cumulative amount of MPO permeated compared with permeation through human cadaver skin (see Tables 2 and 4).

The retention of MPO in the epidermis (including the stratum corneum) and dermis was also measured in human cadaver skin. Again, it was assumed that the density of both the epidermis and dermis was 1.0 g/cm^3. The data collected in the retention studies are summarized in Table 5. The 6-mer MPO was found to exceed the preliminary target value from each donor vehicle. The concentration of all four MPOs was always greater in the epidermis compared with the dermis. Removing the stratum corneum by tape stripping prior to measuring skin penetration from the EtOH/DMS (0.95:0.05) vehicle led to a high concentration of 6-mer (27 ± 15 μM) in the viable tissues. At the same time, the EtOH/H_2O/DMS (0.80:0.15:0.05) vehicle also produced a high concentration of the 6-mer (66

Table 5
Retention of MPO in the epidermis and dermis of human cadaver skin [a]

MPO	Vehicle	Concentration in epidermis [b] (μM)	Concentration in dermis [c] (μM)
6-mer	EtOH/H_2O/DMS (0.80:0.15:0.05)	1020 ± 1330 [d]	66 ± 78
	EtOH/DMS (0.95:0.05)	190 ± 30	27 ± 15
	EtOH	88 ± 65	6 ± 1
	PG	47 ± 10	6 ± 6
	PG/DMS (0.95:0.05)	138 ± 4	6 ± 3
	EtOH/Azone (0.95:0.05)	10 ± 2	2 ± 2
	EtOH/GMO (0.95:0.05)	9 ± 1	4 ± 1
	EtOH/H_2O/GMO (0.80:0.15:0.05)	48 ± 5	6 ± 1
10-mer	EtOH/H_2O/DMS(0.80:0.15:0.05)	72 ± 39	4 ± 2
	EtOH/DMS (0.95:0.05)	360 ± 280	11 ± 2
	EtOH	780 ± 1190	6 ± 4
14-mer	EtOH/H_2O/DMS (0.80:0.15:0.15)	70 ± 78	8.6 [e]
	EtOH/DMS (0.95:0.05)	150 ± 120	6 ± 2
	EtOH	160 ± 180	5 ± 4
18-mer	EtOH/H_2O/DMS (0.80:0.15:0.05)	31 ± 27	ND
	EtOH/DMS (0.95:0.05)	60 ± 43	2.6 ± 2.5
	EtOH	230 ± 43	ND
	EtAc/DMS (0.95:0.05)	95 ± 84	0.61 ± 0.05
	PG	ND	0.73 ± 0.30
	PG/GMO (0.95:0.05)	11.1 ± 4.0	1.35 ± 0.22
	PG/GMO/DMS (0.65:0.30:0.05)	5.7 ± 6.8	0.73 ± 0.68
	PG/DMS (0.95:0.05)	18.4 ± 13.4	2.3 ± 2.3

[a] The samples were processed at the end of the permeability experiments following separation of the epidermis from the dermis.
[b] The calculated concentration (μM) of MPO in the epidermis.
[c] Concentration (μM) of MPO in the dermal tissues.
[d] The values are means ± S.D. ($n = 3$).
[e] $n = 1$ for this vehicle.

$\pm$ 78 μM) in the dermis despite an intact stratum corneum. However, the 6-mer MPO dermal concentration measured from the EtOH/H_2O/DMS (0.80:0.15:0.05) vehicle was quite variable between the three skins examined as demonstrated by the large standard deviation. Most vehicles gave a concentration of 6-mer MPO in the dermis ranging from 2 to 6 μM. Both the 10-mer and 14-mer MPOs led to similar concentrations in the dermal tissues. Despite the inability to detect 6-mer in the receptor solution after 24 h from the EtOH/Azone and EtOH/GMO vehicles, there were measurable levels of 6-mer in the epidermis and dermis from all the vehicles tested.

For the EtOH/DMS (0.95:0.05) vehicle, there was a trend in which more MPO was retained in the dermis as the chain length was reduced. The relationship between MPO molecular weight and amount of MPO retained in the viable tissues is shown in Fig. 2.

The relationship, if any, between the amount of drug permeating through skin and the amount of the drug retained within the skin is important in the development of topical dosage forms (Shah et al., 1992). The relationship between the cumulative amount of 6-mer MPO permeating through human cadaver skin from various vehicles and the amount of drug retained in the dermis after 24 h

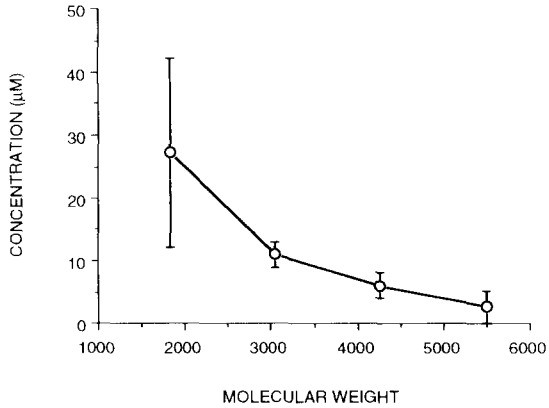

Fig. 2. Relationship between the amount of MPO retained in the viable tissues after the 24 h permeation experiment and the molecular weight of the MPO. The vehicle for all the MPOs was EtOH/DMS (0.95:0.05).

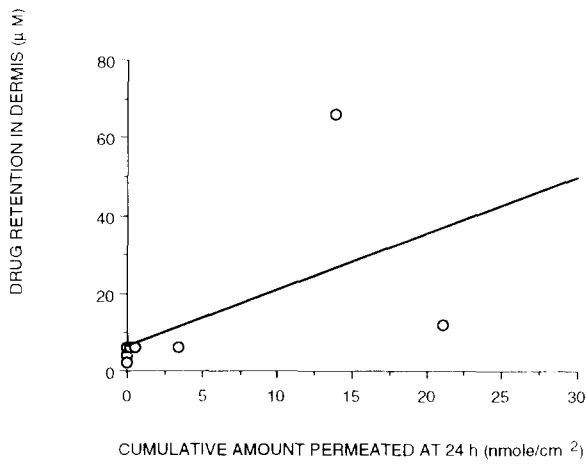

Fig. 3. Relationship between cumulative amount of 6-mer permeating (nmol/cm^2) through human cadaver skin after 24 h from various vehicles (see Tables 4 and 5 for vehicles used with the 6-mer) and the amount of drug retained (μM) in the dermal tissues. The correlation coefficient is 0.30.

is shown in Fig. 3. There is a poor correlation between the two variables (coefficient of correlation, 0.30) indicating that the amount of 6-mer MPO permeating through skin is a poor indicator of retention. In order to minimize potential side effects, it is desirable to keep permeation through the skin and into the systemic vasculature to a minimum.

In general, the 18-mer MPO resulted in low concentrations in the dermal tissues, and in some cases did not exceed the minimum target of 1 μM (see Table 5). However, the PG/DMS (0.95:0.05) and EtOH/DMS (0.95:0.05) vehicles led to relatively high concentrations of the 18-mer MPO in the viable tissues (2.3 ± 2.3 and 2.6 ± 2.5 μM, respectively). Overall, the data indicate that all the MPOs tested are retained at concentrations which may provide adequate tissue levels to control the target disease or infection. The highest molecular weight compound tested (18-mer; 5500 Mol. Wt) was generally retained less, compared with the other MPOs tested. However, very little of the 18-mer was found to permeate through the skin, suggesting that topical administration of the 18-mer may lead to lower systemic delivery as compared with the lower molecular weight MPOs

examined. Often, there was considerable variability in the permeability and retention data collected from each vehicle. While such variability is often expected, more experiments are required to confirm the relative effectiveness of the vehicles tested.

Both hairless mouse skin and human skin allowed measurable amounts of MPO to reach the dermal tissues, and in most cases, pass through into the receptor solution. The addition of putative skin penetration enhancers such as DMS and GMO, when added to the base vehicles of EtOH or PG, generally led to higher permeation rates and to higher tissue retention. However, there was generally a poor correlation between the amount of MPO permeated over 24 h and that retained in the viable tissues. There may be relationships between the solubility of the MPOs in the various vehicles tested or the partition coefficients and the amount of drug penetration rate or in tissue retention. Additional experiments should determine if indeed such relationships exist.

In conclusion, the results indicate that development of topical dosage forms using MPOs for treatment of dermal viral and other infections should be possible with minimal systemic exposure as indicated in this study with the 18-mer MPO.

Acknowledgment

The financial support of Genta, Incorporated, San Diego, CA is gratefully acknowledged.

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