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ScienceDirect
International Journal of Pharmaceutics 336 (2007) 269–275
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Resealing of electroporation of porcine epidermis using phospholipids and poloxamers

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Received 4 October 2006; received in revised form 1 December 2006; accepted 4 December 2006
Available online 8 December 2006

Abstract

The resealing of porcine epidermis after electroporation is investigated. Porcine epidermis was subjected to electroporation (30 pulses at 100 V, 1 ms and at 1 Hz) in a vertical diffusion apparatus, in the presence of 2 mg/ml dimyristoylphosphatidylserine, to produce a long lasting permeable state. Resealing treatments include incubation in 0.0625–0.25 mM poloxamer 188 (P188), or incorporation of phosphatidylcholines (PC) and/or cationic lipids with additional pulses. The recovery of electric resistance of the epidermis samples after electroporation with or without resealing treatments was monitored. The transports of carboxyfluorescein and glucose were measured during the recovery process. Both P188 and PC were effective in resealing in terms of electric conductance and transport, with P188 reacting more rapidly and completely. P188 mediated lipid exchange between stratum corneum lipid particles was measured by fluorescence resonance energy transfer (FRET). Lipid reorganization facilitated by P188 and PC is suggested to be a major resealing mechanism of electroporation damage.

Keywords: Electroporation; Transdermal; Drug delivery; Lipids; Poloxamer; Recovery

1. Introduction

The transdermal path has become an increasingly popular route for delivering drugs. However, a major disadvantage to topical and transdermal drug delivery is that not every drug is capable of diffusing across the skin at a viable rate. Usually, only small, hydrophobic drug molecules can be effectively transported through the major barrier of the skin, the *stratum corneum* (SC). A number of methods have been devised to enhance the flux and to extend the molecular weight limitations of transdermal delivery. Both chemical and physical enhancement strategies have been explored for this purpose. Physical enhancing techniques such as electroporation (Denet et al., 2004), thermoporation (Bramson et al., 2003) and microneedles (Prausnitz, 2004) have the potential to create pores in the skin so that large, hydrophilic molecules may also pass through the SC, thereby extending the realm of deliverable drugs. By applying electroporation, new pathways (pores or local transport region, LTR) are created resulting in transient permeabilisation of the SC (Prausnitz et al., 1993; Gallo et al., 1997). It was found that these pathways remained open for minutes after the cessation of pulse trains, and that more drugs were transported during the post-pulse period than during the pulse application period (Murthy et al., 2003). Electroporation-mediated transport can be further enhanced if anionic lipids are present during pulsing, which extend the lifetime of the transport pathways (Sen et al., 2002a,b). The prolonged lifetime manifests as an extended period when the electric resistance of the skin remains almost as low as that immediately after the pulse train application, when the SC is permeated (Sen et al., 2002b).

Although a prolonged permeated state of the skin presents an advantage for drug transport, the slow recovery of the barrier function of the skin, nevertheless, causes a problem. A low resistive state suggests that the barrier property of the skin is compromised. Resealing of the skin after the completion of the drug delivery process to prevent further unwanted ingress of materials is therefore desired. However, very little information is available based on which to develop a skin resealing protocol.

A family of surface-active block copolymers known as poloxamers consisting of a hydrophobic poly(propylene oxide) group capped with hydrophilic poly(ethylene oxide) moieties at two ends, have been shown to seal damaged cell membranes (Lee et

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doi:10.1016/j.ijpharm.2006.12.006

al., 1992; Padanilam et al., 1994; Hannig et al., 2000; Orringer et al., 2001). These amphiphilic block co-polymers have a detergent-like property and tend to interact with lipid bilayers. The interaction depends on the hydrophilic–lipophilic balance (HLB) of the polymer, which in turn depends on the ratio of the lengths of its hydrophilic and hydrophobic blocks (Alexandridis, 1997). The adsorption/partition into lipid bilayers also depends on the structure of the lipid bilayer present. It was found that certain poloxamers such as P188 selectively partition into low density state or regions of the bilayer and render them to pack more tightly (Wu et al., 2004; Maskarinec et al., 2002). On the other hand, certain poloxamers adsorb/insert into lipid bilayers and disturb their structural integrity (Erukova et al., 2000; Demina et al., 2005). The former property has been utilized to facilitate the recovery of damaged cell membranes. Poloxamer 188 (P188) has been shown to reduce dye leakage from cells after electroporation (Lee et al., 1992) and improve cell survival after gene therapy using electroporation (Agarkova et al., 1987; Kusaoke et al., 1989). We hypothesize that P188 may help resealing skin/epidermis after electroporation, and there would be an increase in skin resistance as the damage is repaired.

Although anionic lipids are skin permeability enhancers in electroporation, neutral and cationic lipids seem to have an opposite effect (unpublished results). Phosphatidylcholine (PC) was found to be a damage retardant in electroporation and iontophoresis; PC liposomes applied during and after pulsing accelerated skin barrier repair (Essa et al., 2003, 2004). Thus, neutral and cationic lipids have potential to be used as skin resealants.

The study presented here is directed at the uses of lipids or poloxamers (P188) as skin resealing agents after anionic lipid-facilitated, electroporation-mediated transdermal transport of molecules. We studied the resistance recovery of epidermis permeabilized with dimyristoyl-phosphatidylserine (DMPS)-enhanced electroporation, and then infused with dioleoyl-phosphatidylcholine (DOPC) or a 1:1 ratio mixed dispersion of DOPC and dioleoyl-3-trimethylammonium propane (DOTAP), or after passive treatment with P188 to reseal the epidermis. The transport of glucose or 5- (and 6-)carboxyfluorescein (CF) following these resealing treatments was examined. The resealant functioning as activator for lipid reorganization was investigated.

2. Methods and materials

2.1. Chemicals

1,2-Dimyristoyl-sn-glycero-3-[phospho-L-serine] (Sodium Salt) (DMPS), 1,2-dioleoyl-3-phosphatidylcholine (DOPC), 1,2-palmitoyl-3-phosphatidylcholine (DPPC), 1,2-myristoyl-3-phosphatidylcholine (DMPC), dioleoyl-3-trimethylammonium propane (DOTAP), 1,2-dioleoyl-3-phosphatidylethanolamine-*n*-lissamine rhodamine B (rhodamine-PE) and egg phosphatidylethanolamine-fluorescein (fluorescein-PE) were obtained from Avanti Polar Lipids Inc. (Alabaster, AL); the concentrations were checked periodically by phosphate analysis to ensure they agree with manufacturer's specifications. 5- (and 6-)carboxyfluorescein (CF) was purchased from Molecular Probes (Eugene, OR); Dulbecco's phosphate buffered saline (PBS) was obtained from Gibco/BRL (Grand Island, NY). Poloxamer P188 is a generous gift from BASF Corp. (Mount Olive, NJ).

2.2. Porcine epidermis

Full thickness porcine abdominal skin was obtained fresh from the Center for Cardiovascular Medicine, SUNY Buffalo. Skin was heated to approximately 60 °C for 2 min and the epidermis was gently peeled off from the skin (Goldschmidt and Kligman, 1963). The fresh epidermis was placed onto filter paper, allowed to dry and stored at −20 °C until used. Before use the epidermis was placed in 0.9% saline solution and hydrated for 1 h.

2.3. Lipid formulation

SC was isolated from the epidermis by overnight trypsin treatment. The lipids of SC were extracted as previously described (Murthy et al., 2003). The lipids were stored at −80 °C until used. Given quantities of DMPS, DOPC or DOTAP were dried under a stream of dry nitrogen. Drying continued for 1 h in a vacuum desiccator to remove any trace amount of solvent. The samples were dispersed, by vortexing in PBS, at a concentration of 2 mg/ml lipid. Where both DOPC and DOTAP were used, 1 mg of each was used to give a combined lipid concentration of 2 mg/ml.

2.4. General experimental setup

Electric resistance and transport measurements were carried out using a Franz-type vertical glass diffusion apparatus (Crown Glass, Somerville, NJ). A piece of epidermis was placed between two compartments of the diffusion apparatus, one serving as the donor and the other as the receiver compartment. The area of epidermis separating the two compartments was 0.64 cm^2. Initially, the donor compartment contained 200 μl of either 2 mg/ml lipid dispersion or PBS. The receiver compartment was filled with PBS. Platinum wire electrodes were placed within 2 mm to the epidermis in both the donor and receiver compartments of the diffusion apparatus. The water jacket of the apparatus was connected to a circulating water bath and maintained at 37 °C. Electroporation was carried out using a pulse generator (Velonix 345, Santa Clara, CA, or AVTEC AVR-G1, Sonnenberg, NY) delivering 30 unipolar pulses of 90–100 V (1 ms pulse width at 1 Hz). To monitor the effect of resealants on the repair of the epidermis, the pulsed epidermis was washed with PBS, and treated with resealants (200 μl of either 2 mg/ml lipid suspension or 0.125 mM P188 solution for 30 s) after the initial pulses. Since some resealant lipids do not readily penetrate the epidermis, the lipid treatment was assisted by an additional train of 30 pulses. For DMPS and DOPC, the negative electrode was in the donor compartment while for DOPC + DOTAP the positive electrode was in the donor compartment. For comparison, an additional train of 30 pulses was applied in PBS prior to passive poloxamer treatments. Electric resistance

or transport measurements were taken immediately after the treatment.

2.5. Electric resistance measurements

Pre- and post-pulse epidermis resistances were measured using continuous low voltage (500 mV) 1 kHz bipolar square waves produced by a functional generator (Dynascan Model 3300, Chicago, IL). A load resistor, $R_L = 4.7\,k\Omega$, was placed in series with the epidermis, so that the voltage drop across the whole circuit, V_0, and across the skin, V_S, could be measured by a recording digital oscilloscope (Fluke 99 Scopemeter Series II, Holland). The electric setup had been given previously (Sen et al., 2002b; Gallo et al., 2002). The resistance of the skin, R_S, was approximated from the formula:

$$R_S = \frac{V_S \times R_L}{V_0 - V_S}$$

A piece of epidermis was used only if the resistance before treatment was greater than 5 kΩ. The resistance was monitored for 30–60 min following electroporation treatment.

2.6. Carboxyfluorescein transport measurements

CF transport through porcine epidermis was measured using a receiver cuvette adapted to contain electrodes and a donor chamber (Murthy et al., 2003). Following electroporation and resealing treatments (as described in Section 2.4), the epidermis was washed with PBS, and 200 μl 250 μg/ml CF solution was added to the donor compartment. The amount of CF transported across the epidermis into the receiver compartment was measured by monitoring the intensity increase over 1 h on a SLM 8000 Spectrofluorimeter (SLM Instruments, Urbana, IL). Excitation and emission wavelengths were 491 and 518 nm, respectively. The amount of CF was determined from the measured fluorescence intensity using a calibration curve prepared with known amount of CF.

2.7. Glucose transport measurement

Cumulative transport of glucose through porcine epidermis over a 30 min period was measured using the glass diffusion apparatus. Because glucose transport was much slower and the assaying method was less sensitive than those for CF, it was not practical to measure it in a shorter time interval. After electroporation and resealant treatments, the epidermis was washed in PBS and 0.2 ml of 1 mM glucose solution was added to the donor compartment and left for 30 min. The content of the receiver compartment was then removed, and the glucose concentration was measured using a Glucose (Trinder) 100 kit from Sigma Diagnostics (St. Louis, MO).

2.8. Lipid exchange measurement

Lipid exchange as a result of resealant treatment was examined using liposomes made from total lipid extraction of the SC. Liposomes were made from dried lipids by dispersing at 65 °C in phosphate buffer (pH 6.5) at a concentration of 1 mg/ml. The dispersions were then sonicated at 65 °C for a total of 5 min. These liposomes were doped either with 1 mol% of FITC-PE or with 1 mol% of rhodamine PE. Lipid exchange between liposomes, as a result of structural reorganization and fusion, was measured by fluorescence resonance energy transfer (FRET). The emission intensity of rhodamine at 590 nm resulting from excitation at 419 nm of fluorescein was measured as a function of time after rapid mixing of these two populations of liposomes in a cuvette in a spectrofluorimeter (SLM 8000, Urbana, IL).

2.9. Statistical analysis

Student's *t*-test was selected as the test of significance and a *p*-value less than 0.005 was considered statistically significant. The data points provided in the graphs are mostly an average of three to five trials. The error bars represent standard deviation.

3. Results

3.1. Recovery of electric resistance

A rapid way to monitor skin permeability is by electric resistance measurement. The electric resistance, representing mostly the transport of ions, is closely related to the skin permeability. The electrical resistance of intact SC is between 5 and 25 kΩ/cm^2 (Denuzzio and Berner, 1990) and has an electrical breakdown potential around 75–100 V (Pliquett et al., 1995; Edwards et al., 1995). The resistance recovery after one pulse was rapid, but recovery after multiple pulses was slower and easier to monitor (Pliquett et al., 2005). Because DMPS at a concentration of 1 mg/ml or higher further impeded resistance recovery (Sen et al., 2002b), we chose to study the resealing after the epidermis was subjected to 30–60 pulses in the presence of DMPS.

As shown in Table 1, immediately after applying the pulse train, the instant resistance of the epidermis, R, fell close to the residue value of the diffusion apparatus, typically 1–2 kΩ. This was less than 10% of its pre-pulse values R_i, i.e. the relative resistance $R_1 (= R/R_i)$ is <0.1. After the lipid pulse treatments, the relative resistance R_2 did not change immediately regardless

Table 1
Instantaneous relative resistance[a] after initial electroporation pulses (R_1) and after resealant treatment pulses (R_2)

Electroporation pulses	R_1	Resealant treatment pulses	R_2	n
DOPC, 30P	0.08 ± 0.05	PBS, 30P	0.05 ± 0.03	10
DMPS, 30P	0.05 ± 0.03	PBS, 30P	0.03 ± 0.02	11
DMPS, 30P	0.08 ± 0.06	DOPC, 30P	0.06 ± 0.05	10
DMPS, 30P	0.08 ± 0.05	DOPC + DOTAP, 30P	0.05 ± 0.03	7
DMPS, 30P	0.08 ± 0.03	PBS, 0P	0.83 ± 0.21	5
DMPS, 30P	0.12 ± 0.08	P188, 0P	0.95 ± 0.16	6

Errors represent standard deviation; P indicates number of applied pulses; differences of all R_1 values are not significant; difference among R_2 of the first four rows and among the last two rows are not significant ($p < 0.2$); difference between R_2 the first four rows and the last two rows are significant ($p < 10^{-7}$).

[a] Relative resistance is the instantaneous resistance value relative to the pre-pulse resistance value.

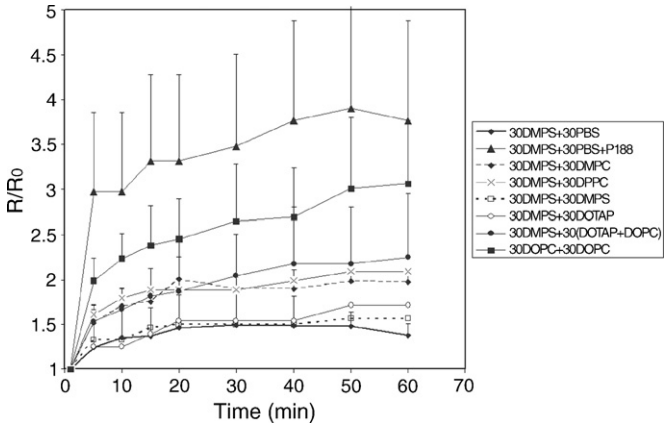

Fig. 1. Resistance recovery after 30 electroporation pulses in the presence of DMPS or DOPC, and 30 pulses of resealant treatment. The recovery is plotted as the ratio, R/R_0, of the time-dependent resistance value R relative to the resistance value R_0 measured immediately after the resealing treatment (at time zero). Pulse protocols are described in the legend block first as the number of electroporation pulses and the pulse media, followed by the number of treatment pulse and the treatment or control media. P188 (0.125 mM), when used, was added either during or after the treatment pulses in PBS, with similar results ($4 \geq n \geq 3$). Error bars represent standard deviation.

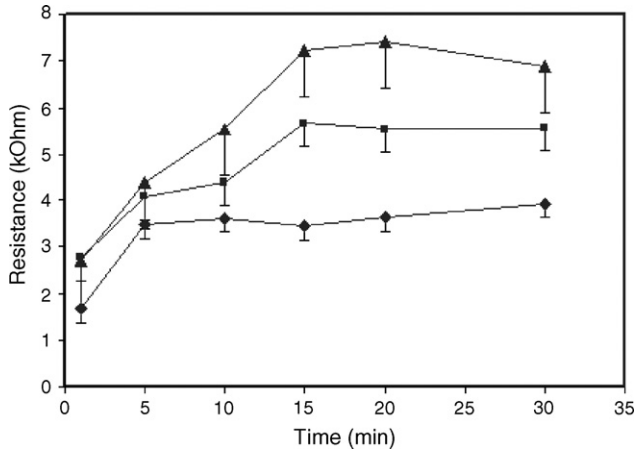

Fig. 2. Typical recovery of resistance after 30 pulses in DMPS and 30 pulses in PBS, and treated with different concentrations of P188. The concentrations are: 0.25 mM (triangles), 0.025 mM (squares) and 0.0025 mM (diamonds). Difference between treatment concentrations are significant ($p < 0.005$).

of whether or the type of lipids introduced. Without additional pulse treatment, the epidermis resistance regained to 83% of its pre-pulse value in 10 min after the cessation of the initial 30 pulse train application. If the poloxamer P188 (0.125 mM) was present during these 10 min, the resistance recovered almost completely to its pre-pulse value. The recovery in both cases were significant ($p < 10^{-7}$). The kinetics of long-term recovery effects of these reputed resealants were examined next. All long-term experiments were conducted using 60 pulses (30 porating pulse with DMPS and 30 treatment pulses either with or without resealing lipids). In the case of P188 treatment, the poloxamer was added either before or after 30 additional "treatment" pulses in PBS. The results are shown in Fig. 1. All epidermis resistances recovered to some extent in 60 min, with a more rapid recovering phase in the first 5–10 min. The effects of different resealants differed considerably. After an initial 30 pulses in DMPS and an additional 30 pulses with or without DMPS, the resistance recovered only slightly in 60 min, to less than 1.5 times from the value R_0 measured immediately after the treatment. The effect of the cationic lipid DOTAP alone at 2 mg/ml was minimal. There was no significant difference within this treatment group (control group). Treatments with either saturated (DPPC and DMPC) or unsaturated (DOPC) phosphatidylcholine (in some cases as 1 mg/ml:1 mg/ml mixture with DOTAP) after the initial 30 pulses in DMPS boosted the recovery in 60 min to about twice the R_0 value. Again, there was no significant difference within this treatment group (PC group) Treatment of P188 brought rapid and strong recovery in a concentration-dependent manner (Fig. 2). In 60 min, the resistance increased 3.5-fold from the R_0 value (Fig. 1). Interestingly, if DOPC was substituted for DMPS in the initial (porating) and/or treatment pulses, the recovery was also more pronounced (Fig. 1), although the effect was not noted immediately after the pulse application (Table 1). All treatment groups (except with DOTAP alone) recovered significantly faster and more complete than the control (DMPS only) group ($p < 0.005$). However, difference among various treatment groups are not as significant ($p < 0.01$).

3.2. *Restoration of the skin barrier function against carboxyfluorescein and glucose transport*

The kinetics of transport of a negatively charged, water-soluble compound, carboxyfluorescein (CF), over a period of 10 min was measured. The effects of various putative resealants on the transport were examined. Fig. 3 shows that the transport of CF after 30 pulses with DMPS followed by 30 pulses in PBS was significantly higher than that for any other groups. Transport of CF after 30 pulses with DMPS and left in PBS for 30 s without pulsing was still higher than those groups with further resealant treatments. Because the differences were very large, the cumulative transport was plotted in a logarithmic scale for clarity. The transport in the group treated with 30 pulses in DOPC was lower. The transport of all other treatment groups, including P188 treatment with and without additional pulse, and

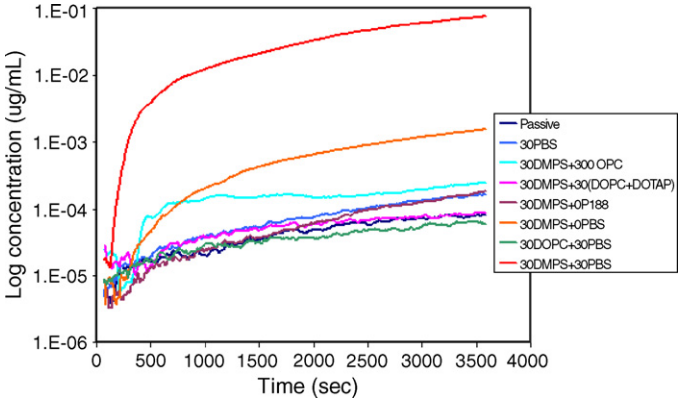

Fig. 3. Plot of log(concentration) of CF transport against time. Pulse and treatment protocols described in the legend box have the same connotation as those in Fig. 1 ($6 > n > 3$). Standard deviations, typically less than half a log range, are left out for clarity.

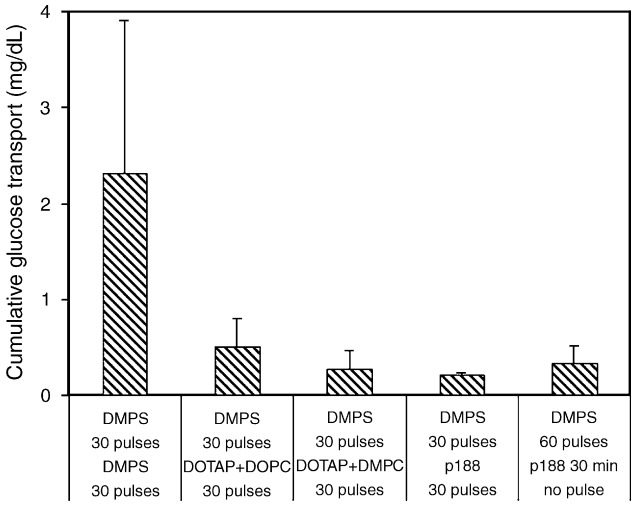

Fig. 4. Cumulative glucose transport in 30 min after various resealing treatments ($14 \geq n \geq 3$). Error bars represent standard deviation.

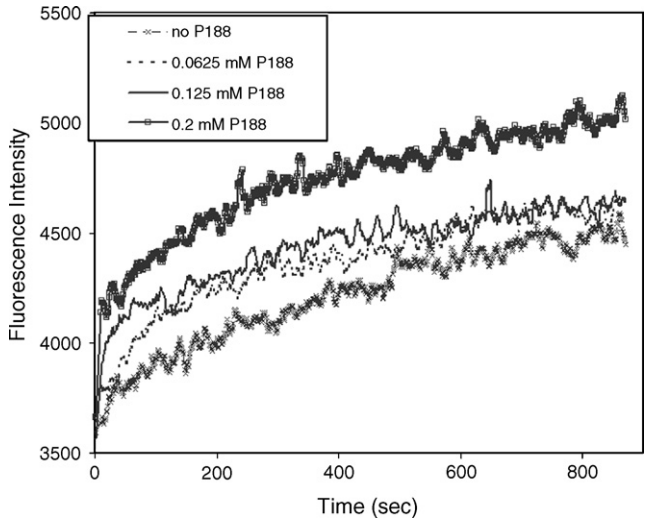

Fig. 5. Lipid exchange between stratum corneum lipid particles suspended in pH 6.5 buffer solution with different concentrations of P188, measured as FRET intensity against time. Traces of repeated samples superimpose within noise range.

those pulsed in DOPC + DOTAP, were practically the same as for passive CF transport or pulsed with PBS alone without DMPS, suggesting that the epidermis had been resealed.

Fig. 4 shows the cumulative transport of glucose in 30 min after pulsing in DMPS and resealant treatments. The transport after 30 pulses with DMPS, followed by another 30 pulses in DMPS was significantly higher than those followed by 30 pulses with DOTAP + DOPC or DOTAP + DMPC (Fig. 4, columns 1–3, $p < 0.005$). Treatment with P188 for 30 min without pulsing (column 5) had similar results ($p > 0.01$) as pulse treatment with P188 (column 4). The glucose transports in both P188 groups of samples were significantly ($p < 0.005$) lower than that in unsealed (DMPS only) samples (column 1). The results indicate that the epidermis resealed more rapidly against glucose transport by resealant treatments.

3.3. Repair mechanism of poloxamer P188

To study if the repair of the epidermis barrier function is related to lipid reorganization, the following lipid exchange experiments by fluorescence resonance energy transfer (FRET) were performed. Liposomes were made of SC total lipid extract with FRET tracers. Reorganization of lipid structure via lipid exchange or fusion between particles would be indicted by FRET. The FRET intensity as a function of time after liposome mixing is shown in Fig. 5. Lipid mixing, as indicated by FRET intensity, increased with time. There was a moderate increase of lipid mixing even in the absence of P188. Because the experiment was carried out in pH 6.5 buffer, a certain degree of liposome fusion contributing to FRET was expected (Murthy et al., 2003). With the increase of P188 concentration from 0.0625 to 0.125 mM, there was an initial increase in FRET intensity, but the endpoints were almost indistinguishable. Further increase the concentration to 0.2 mM significantly increased the FRET intensity. It was likely that a threshold of about 0.1 mM was needed to initiate a detectable recovery process with p188. It should be noted that, after the addition of 0.125 mM of P188 to the sample, a portion of sample particles was reduced from micrometer size to less than 20 nm, as measured by dynamical light scattering (results not shown). Lipid particle at this small size indicated micelle formation.

4. Discussion

Up to now, the investigation of transdermal drug delivery has been focused on methods to open up transport pathways to facilitate the passage of drugs. There has been relatively little effort to study the resealing of the pathways thus opened. With methods developed to prolong the opening time of pores or pathways, there is more need to develop methods to control the resealing of these openings once the delivery process is completed. The skin will reseal spontaneously, but the process is slow and gradual. Such long-term opening or partial opening of skin pores poses a risk of water loss and harmful substances influx if the skin is left to reseal spontaneously.

We found that most putative resealants such as PC and P188 accelerate resistance recovery, while the cationic lipid DOTAP alone at 2 mg/ml has little effect (Fig. 1). At 0.125 mM, the repair action of P188 is more effective and more rapid than that by PC. This finding parallels the short term CF transport kinetics which shows P188 treatment immediately impedes the CF transport to the extent of unpulsed epidermis (passive diffusion) or that pulsed in the absence of DMPS. The effect of PC is slower and less effective; the CF transport is noticeable at the first 600 s but then the resealing process catches on and levels the transport to slightly more than that for passive diffusion (Fig. 3). When mixed with PC at 1 mg/ml:1 mg/ml ratio, DOTAP helps resistance recovery and resealing against CF transport. The long-term cumulative transport of glucose echoes those for CF transport, i.e. P188 and PC + DOTAP are effective resealants, even after 60 pulses. Apparently, when mixed with PC at 1:1 ratio, DOTAP is effective in resealing. When used alone in

full strength (2 mg/ml), the positive charge of DOTAP may be excessive for the resealing function.

How do poloxamer and lipid resealants work? Electroporation creates sporadic local transport region (LTR) through which hydrophilic molecules can pass through (Sen et al., 2002b; Pliquett et al., 2005). Microscopically, these regions represent a temporary breakdown of the lipid lamellae in the SC, into zones of aggregates of lipid vesicles (Gallo et al., 2002). This type of structure is supposedly permeable to hydrophilic substance. In order for the lamellae to reform to repair the barrier function, a structural reorganization would be necessary. This process invariable involves lipid mixing. Since most SC lipids are amphiphilic with a very low critical micellar concentration (CMC), spontaneous regrouping through monomer exchange would be very slow especially at an experimental temperature (37°) which was much below the phase transition temperature of SC lipids (∼70°) (Murthy et al., 2004b). Introduced lipids are known to mix with SC lipids. We hypothesize that incorporating DMPS in the LTR lipids further stabilizes the vesicular structure by inhibiting the approach of lipid vesicles due to its charged nature, thereby prolonging the lifetime of the permeable state of the SC (Sen et al., 2002b). This was manifested as prolonged low skin resistance state when the epidermis was pulsed in DMPS (as compared to DOPC, Fig. 1). Detergent-like poloxamer P188 would facilitate the lipid exchange via defect and micelle formation, even breaking down vesicular structures to pave the way for lamellae reformation through lipid self assembly upon subsequent polymer dilution. Indeed, part of the population of particles of total lipid extraction of SC became much smaller upon P188 addition, implying vesicle breakdown and micelle formation. This hypothesis is supported by the lipid exchange measurement by FRET, showing P188 accelerates lipid exchange in a concentration-dependent manner as steps toward reorganization and lamellar formation. The detergency of poloxamer P188 must be sufficient to facilitate lipid exchange, while not strong enough to disrupt the existing lamellar structure of the SC lipids. Other detergents are known to be enhancers for transdermal transport with or without electroporation (Murthy et al., 2004a). The mechanism of action of poloxamers either as a transport enhancer, or a membrane resealer is believed to be their ability to interact with lipid bilayers. By altering the molecular packing of the bilayer, poloxamers lower the energy barrier of the bilayer to reorganize, either loosening a tightly packed, impermeable bilayer to a more permeable state, or to facilitate the pore-laden and vesicularized bilayer to reform into continuous lamellae. The reaction depends on the HLB and the concentration of the poloxamer, the packing state of the targeted bilayer, as well as the subsequent dilution of poloxamers though diffusion. A thorough study of the HLB of poloxamers and similar amphiphilic polymers for the best skin resealing property, such as that for their membrane destabilization property (Demina et al., 2005), is in order.

The neutral phospholipid PC has been shown to accelerate the repair of permeability induced by electroporation (Essa et al., 2003). The authors suggested that the repair mechanism was by released monomers impeding the transport pathway (Essa et al., 2003, 2004). In those reports, soybean PC, which had transition temperature below zero degree, was used. In this present study, all PC used also have lower phase transition temperature than that of the SC lipid. When mixed with the SC lipid in LTR, the transition temperature of the mixture would be lower than that of the unmodified SC lipids. Raising the experimental temperature closer to the transition temperature of the SC lipid is known to facilitate the permeation by and recovery from electroporation pulses (Gallo et al., 2002; Murthy et al., 2004b). At or near the phase transition temperature, defects in the bilayer lower the energy barrier for structural reorganization. By the same token, lowering the transition temperature of the lipids in the LTR, recovery could be accelerated. We postulate an alternative mechanism that the PC-induced recovery effect is due to the lowering of the transition temperature of the SC lipid in the LTR when low-melting PC is mixed with the SC lipids. Indeed, the effect of low transition temperature lipids such as DOPC is stronger than DMPC or DPPC. Being neutral, PC does not have the charge repulsive effect of DMPS, which inhibits mutual approaching of vesicles as a precursor for lamellar reformation. PC and PS have similar CMC; their monomer concentration in water would be similar but their effects on recovery are exactly opposite. In the case of DMPS, the charge repulsion effect of the PS headgroup dominates over the transition temperature lowering effect with regard to lipid reorganization.

In a broad sense, bilayer defects created by the detergent-like P188 or by lowering the phase transition temperature as a result of mixing PC with SC lipids in the LTR, facilitate lipid reorganization towards lamellar reformation in the LTR. Reformation of the lamellar structure is linked to the recovery of the barrier function of the epidermis (Gallo et al., 1999). Since PC treatment is less effective and requires additional pulses to incorporate the lipid into the SC, poloxamers are a more preferable choice as a skin resealing agent.

5. Conclusions

The results suggest that, following anionic lipid-facilitated electroporation-mediated transdermal drug delivery, it is possible to apply a passive solution of P188 or actively inserting PC to reseal the skin at the treatment site, restoring the skin barrier function.

Acknowledgements

This work is supported by a grant EB 001034 from the National Institutes of Health to S. Hui and in part by the NCI Cancer Center Support to Roswell Park Cancer Institute [CA016156]. We thank Maria Scholnick for the preparation of the total lipid extract from the SC.

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