Transdermal Delivery of Haloperidol by Proniosomal Formulations with Non-ionic Surfactants

Anahita Fatih Azarbayani, a En Hui Tan, a Yew Weng Chan, b and Sui Yung Chan *. a

a Department of Pharmacy, National University of Singapore; Block S4, level 2, Science Drive 4, 117543, Singapore; and
b Department of Anesthesiology, Singapore General Hospital; 169608, Singapore.

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Proniosomal formulations with non-ionic surfactant were studied. The effect of hydrophilicity and hydrophobicity of one or two surfactants on drug solubility, proniosome surface structure and stability and skin permeation of haloperidol from different formulations were investigated. Haloperidol (HP) was entrapped in proniosomes with very high efficiency for all formulations. Stability studies performed at 4 °C and 25 °C for a period of 6 weeks did not reveal any significant drug leakage (p>0.05). Formulations with single surfactants were found to increase the skin permeation of HP more than formulations containing two surfactants. The number of carbons in the alkyl chain of the non-ionic surfactant influenced the in vitro permeation of HP though the epidermis and the skin permeation was increased with increase in hydrophilic–lipophilic balance (HLB) value of the surfactant. Interfacial tension and surfactant hydrophobicity appeared to be useful for elucidating mechanism of skin permeation and for comparing drug fluxes from different proniosomal formulations.

Key words proniosome; haloperidol; hydrophilic–lipophilic balance; non-ionic surfactant; skin permeation

Stratum corneum (SC) is the main barrier for transdermal delivery of drug molecules. An ideal drug for transdermal delivery should be lipophilic enough to partition into the SC layer. It is known that maximum skin permeability occurs at log P of approximately 3 to 4. Haloperidol (HP) is a hydrophobic molecule (log P=3.49) with low molecular weight of 375.9 Da, hence making it a suitable transdermal candidate. HP is a neuroleptic drug for schizophrenia and mania. The therapeutic HP plasma concentration ranges from 0.8 to 5.15 ng/ml. A long-acting formulation would be appropriate for maintenance therapy since frequent dosing may lead to non-compliance. The clinical need for and the low maintenance dose of haloperidol (3—10 mg/d) began the search for the alternative transdermal route.1)

Suitable mixtures of lipids and surfactants, known as proniosomes, have the potential to be used as carriers for drug delivery across the skin. They have similar structure and properties to liposomes, but often have improved chemical stabilities with the incorporation of surfactants.2–5) Numerous studies have been conducted on the effects of drug concentration, the type of surfactant and the contents of alcohol and lipids on the in vitro drug permeation profile across skin.2,4,6) Formulations containing lecithin increased the entrapment efficiencies of the drug compared to formulations containing cholesterol only. Presence of surfactants in the formulations has shown to increase the drug flux rate across the skin.6,7)

The HLB number (hydrophilic–lipophilic balance) defines the polarity of nonionic surfactants in terms of an empirical quantity. Balance of hydrophobicity and hydrophilicity has been found to be important for effective drug transportation through the skin.8) The HLB number for the formulation determines the stabilities of the emulsion and microemulsion.9,10) HLB values of surfactants were also reported to influence the bioavailability and intestinal absorption of the drug.11)

The objective of this work was to examine the effects of different HLB values of non-ionic surfactants, Span and Tween, in proniosomal formulations. Single or blends of non-ionic surfactants were used and their effect on drug solubility, vesicle stability, surface tension, and skin permeation of HP from pronosome formulations were studied.

MATERIALS AND METHODS

Materials Span 40, Span 60 and Span 85, Tween 80, sodium phosphate monobasic monohydrate, phosphate-buffered saline, cholesterol and haloperidol, fluorescein and rhodamin 6G were from Sigma (Singapore). Lecithin (Lipoid E 80) was a gift from Lipoid GMBH (Germany). Other chemicals used were of analytical grade.

HPLC Analysis Drug concentrations were determined by a HPLC from Shimadzu (Japan) 2010A. The analysis was carried out by using reverse phase Agilent C18 analytical column, (4.0×250 mm, 5 µm) at detector wavelength of 245 nm. The mobile phase was a 50:50 volume ratio of acetonitrile and 0.05 M phosphate monobasic, with the pH adjusted to 3 using phosphoric acid. Flow rate of 1.5 ml/min at 40 °C and the injection volume of 50 µl gave a retention time of approximately 4.9 min. Standard solutions of HP (0.05 to 2.00 µg/ml) were prepared in 0.03% (v/v) lactic acid solution.

Phase Solubility and Surface Tension Studies Saturated solutions of HP in water–glycerol (50:50, v/v) containing 225 mg/ml of various surfactant mixtures of HLB (1.8, 2, 6, 6.7, 10, 16, 16.7) were prepared. The amount of the surfactants used was calculated using the weight fractions of the surfactant (A and/or B) as shown in the following equation10):

\[ \text{HLB (mixture)} = \text{HLB}_a \times 0.014 + \text{HLB}_b \times 0.018 \]  

(1)

where \( A\% + B\% = 100 \).

The samples were then placed on a rotary shaker, without light for 5 d and finally filtered through Millipore membrane filters (0.215 µm pore, 25 mm diameter). The concentrations of HP were determined by HPLC. Experiments were done in triplicates. Solubility enhancement ratios (ER) of HP were calculated using the equation below:
The concentration of total HP, and warmed in a sonicator (120 rpm) water bath at 65 °C for 10 min. Then phosphate buffer saline was added and the mixture was further warmed in the water bath for 5 min, so that a clear solution was obtained. The mixture was allowed to cool to room temperature.

**Proniosome Formulations** Proniosomes were prepared using a modified literature method. The compositions of different proniosomal formulations are listed in Table 1. HP, surfactant, cholesterol and lecithin, in the ratio of 9:9:1 were mixed with isopropyl alcohol. The jar was capped and placed in the receptor compartment with the stratum corneum facing upwards. The exposed surface area for the drug permeation in the skin was 0.785 cm². The receptor compartment was filled with isotonic phosphate-buffered saline (pH 7.4). A 1 ml of proniosomal formulation was placed in the donor compartment and covered with paraffin and aluminum foil. Samples were collected at 4-h intervals for 36 h. The temperature was kept at 37 ± 0.5 °C throughout the experiment. The samples were analyzed by the HPLC method. All experiments were performed in triplicate.

In Vitro Skin Permeation of Drug from Proniosomes

The permeation of HP from proniosomal formulations was determined by flow-through diffusion cell. The epidermis was mounted on the receptor compartment with the stratum corneum facing upwards. The exposed surface area for the drug permeation in the skin was 0.785 cm². The receptor compartment was filled with isotonic phosphate-buffered saline (pH 7.4). A 1 ml of proniosomal formulation was placed in the donor compartment and covered with paraffin and aluminum foil. Samples were collected at 4-h intervals for 36 h. The temperature was kept at 37 ± 0.5 °C throughout the experiment. The samples were analyzed by the HPLC method. All experiments were performed in triplicate.

**Scanning Electron Microscopy Imaging of Proniosomes** Proniosomal formulations were mounted on a thin glass slide and left to dry. Slides were mounted on an aluminium stub using silver paint. The vesicles were sputter-coated with gold at 10 mA and 1.2 kV for 4 min, using a vacuum evaporator (Jeol Fine Coat Ion Sputter JFC-1100, Japan). Electron micrographs were obtained with a field-emission SEM (Jeol JSM-T220A Scanning Microscope, Japan) equipped with a digital camera, operating at 15.0 kV alternating voltage.

**Preparation of Human Epidermis** The abdominal skin of an Indian female was obtained with patient consent post plastic surgery. Full-thickness skin with epidermis facing downwards was immersed in water of 60 °C for 2 min, and then the epidermis was carefully peeled off and stored at −80 °C. Prior to permeation studies, the epidermis was thawed and hydrated in an aqueous 0.9% (w/v) sodium chloride and 1% (v/v) antibiotic antimycotic solution under room conditions for 2 h.

**Table 1. Composition and Appearance of Proniosomal Formulations**

<table>
<thead>
<tr>
<th>Proniosomal Formulations</th>
<th>Surfactant (% of 225 mg)</th>
<th>Composition</th>
<th>Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Span 85</td>
<td>Span 60</td>
<td>Span 40</td>
</tr>
<tr>
<td>1.8</td>
<td>100</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>93.75</td>
<td>6.25</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>—</td>
<td>41</td>
<td>59</td>
</tr>
<tr>
<td>6.7</td>
<td>—</td>
<td>—</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>—</td>
<td>—</td>
<td>60</td>
</tr>
<tr>
<td>16</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>16.7</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

C=cholesterol, L=lecithin, B=phosphate buffer saline, IPA=isopropyl alcohol. All formulations contained 2 mg/ml HP.

ER = \frac{C_o}{C_i} \quad (2)

where $C_o$ is the HP solubility in the formulations with surfactants and $C_i$ is the saturation solubility of HP in the control sample without surfactant. Surface tensions of these solutions were measured using Sigma Tensiometer 701 from KSV. A Du Noury ring was used and measurements were carried out at room temperature.

**Encapsulation Efficiency and Stability of Proniosomes** A 10 mg of each proniosome formulation was diluted with 1 ml of phosphate buffer pH 7.4. HP-containing proniosomes were separated from the free un-entrapped drug by centrifuging the samples at 12000 rpm for 30 min at room temperature. The supernatant was recovered and assayed by an HPLC method. All experiments were performed in triplicate.

The permeation of drug across the epidermis can be described by Fick’s 2nd law of diffusion:

\[ Q = A K D C_o \left( e^{-K D L t} - \frac{1}{6} e^{-\frac{K D L t}{J_o^2}} \right) \quad (4) \]

Permeation parameters are interpreted from the cumulative amount of released drug per unit skin area ($Q/A$ versus time ($t$) plot. The gradient and $x$-intercept of the linear portion of the plot yield steady-state flux ($J_o$) and lag time ($t_l$) respectively.

The drug permeability ($KD/L$) is derived as shown in Eq. 5:

\[ KD = \frac{J_o}{C_o} \quad (5) \]

where $C_o$ is the initial drug concentration in the donor cell. The diffusion parameter, $D/L^2$ reflecting the mobility of the drug solute in the skin was calculated using the following equation:

\[ D = \frac{1}{6} \frac{J_o}{t_l} \quad (6) \]

The partition parameter, $KL$, reflecting the distribution of...
the drug between the skin and the donor solution is derived from:

$$K_L = \frac{KD/L}{D/E}$$  \hspace{2cm} (7)

The enhancement index, $EI$, a ratio of the drug permeability from the control and the proniosomal formulations, measures the enhancement in drug penetration. \(^1\)

$$EI = \frac{(KD/L)_{	ext{proniosome}}}{(KD/L)_{	ext{control}}}$$  \hspace{2cm} (8)

**Confocal Laser Scanning Microscopy (CLSM)** Skin penetration of rhodamine-loaded proniosomes was viewed using a CLSM. The CLSM was a Nikon A1R laser scanning spectral confocal and digital camera from Japan. All samples were excited at 488 and 561 nm and X–Z sectioning has been used to determine the depth of permeation using an objective of ×60. Following the in vitro skin permeation studies, the skin samples were placed on a glass slide and covered with a glass cover-slip. The slides were inverted and images were captured through the cover-slip side of the prepared samples. The full epidermis was scanned at different increments through the z-axis of the microscope.

**Statistics** The values are expressed as mean±S.D. of $(n=3)$. Comparisons were made using one-way analysis of variance, ANOVA (Graph Pad Prism, Version 2) followed by Tukey’s multiple comparison post-test to determine the differences between treatment groups. The differences were considered statistically significant when $p<0.05$.

**RESULTS AND DISCUSSION**

**Solubility and Surface Tension** The solubilities of HP in different surfactant mixtures and also the surfactant surface tensions in water: glycerol solutions are presented in Fig. 1. The drug has a relatively low solubility in the control solution. The addition of surfactants enhanced the solubility of HP significantly. The formulation with a surfactant mixture corresponding to HLB 10 resulted in the highest drug solubility, almost 13-fold compared to that in the control solution. Surface tension measurements showed higher interfacial tensions for formulations with HLB values of 10 and formulations with 1.8 and 16.7 had the lowest surface tensions values.

**Encapsulation Efficiency and Vesicle Stability** Proniosomes with non-ionic surfactants of alkyl ester including Span (sorbitan esters) and Tween (polyoxyethylene sorbitan esters) with encapsulated HP were prepared. As shown in Fig. 2 the encapsulation efficiencies were high and there was no significant difference amongst the formulations $(p>0.05)$. After the formulations were stored at room temperature (25°C) and fridge (4°C) for 6 weeks, no statistical significance in drug encapsulation efficacies was observed $(p>0.05)$.

**SEM Imaging** The scanning electron microscopy images of the proniosomes are shown in Fig. 3. Proniosomes with lower HLB values seemed to be mostly spherical and discrete with sharp boundaries with smooth and rigid surfaces (Figs. 3a—g). Figures 3f and g show the surface characteristics of vesicles made with surfactants of HLB 16 and 16.7, and the spheres have deformable structures which may be attributed to the type of surfactant in the formulation. The main difference between deformable and rigid vesicles was thought to be due to fluidity of the lipid bilayer of the deformable vesicles. \(^{14}\)

**In Vitro Drug Permeation Studies** Figure 4 shows the release profiles of HP over a 36-h period. There is a significant increase in the permeation of HP from formulations containing surfactants with HLB value 16.7 and as shown in Table 3, the HP flux rate was almost 2.8-folds higher than that of the control $(p<0.05)$. Formulations with single surfactants were found to increase the permeation of HP more than mixtures of surfactants. Thus these surfactants when used together in proniosomes did not show any synergism with regards to permeation of the drug through the skin.

The mechanism of surfactant mixture on skin permeability is not well understood. Mixture of surfactants results in complex structures, after mixing of the two surfactants a new type of mixed micelle may be formed that could behave differently than the two single surfactants. Previous findings show that synergy in penetration enhancing effect is concentration-dependent and occurs when nearly equal fractions of the surfactants are present in the mixture. \(^{15–18}\) In this work the concentration of the two surfactants were calculated to

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**Fig. 1.** Solubility and Surface Tension Measurements of Haloperidol in Formulations with Respective HLB Values $(n=3)$

**Fig. 2.** Entrapment Efficiencies (%) of Proniosomal Formulations

Sampling was carried out at day 1, week 3 and week 6. Samples were kept at 4°C and at 25°C, $n=3$.
obtain the required HLB value and therefore are not equimolar. This could explain the non-synergistic effect of the two surfactants in the skin penetration of haloperidol.

The lag time was increased probably due to the rate-limiting membrane barrier of the lipid bilayers and drug-reservoir characteristics of the proniosomes, however the cumulative drug amount permeated from all formulations was higher than that of the control.3,4) Therefore, both the lipids and the non-ionic surfactants of the vesicles could have enhanced the drug permeation through the skin. Lecithin and cholesterol helped to adsorb and fuse the formulation on to the skin surface and then disrupt the lipid structure of the skin. Most investigators agreed that vesicle size did not influence the drug permeation profiles. Phospholipids do not penetrate to the deeper layers of the skin but instead fuse with the skin and disrupt the lipid structure of the stratum corneum.19—24) The concentrations of surfactants in these formulations are in excess of their critical micelle concentrations and micelles are

<table>
<thead>
<tr>
<th>Surfactant trade name</th>
<th>Chemical description</th>
<th>Ethylene oxide units</th>
<th>HLB</th>
<th>Carbons in the alkyl chain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tween 20</td>
<td>Polyoxyethylene (20) sorbitan mono-laurate</td>
<td>20</td>
<td>16.7</td>
<td>12</td>
</tr>
<tr>
<td>Tween 80</td>
<td>Polyoxyethylene (20) sorbitan mono-oleate</td>
<td>20</td>
<td>15</td>
<td>18 (unsaturate)</td>
</tr>
<tr>
<td>Span 40</td>
<td>Sorbitan mono-palmatate</td>
<td>—</td>
<td>4.7</td>
<td>16</td>
</tr>
<tr>
<td>Span 60</td>
<td>Sorbitan mono-stearate</td>
<td>—</td>
<td>6.7</td>
<td>18</td>
</tr>
<tr>
<td>Span 85</td>
<td>Sorbitan trioleate</td>
<td>—</td>
<td>1.8</td>
<td>54 (unsaturate)</td>
</tr>
</tbody>
</table>

Table 3. Permeation Profiles of Different Proniosomal Formulations (n = 3)

<table>
<thead>
<tr>
<th>Proniosomal formulations</th>
<th>$J_w$ ($\mu$g/cm²·h)</th>
<th>$KD/L$ (cm/h)</th>
<th>$t_L$ (h)</th>
<th>$D/L^2$ (h⁻¹)</th>
<th>$KL$</th>
<th>$EI$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.792±1.232</td>
<td>1.465±0.205</td>
<td>2.792±0.46</td>
<td>0.061±0.011</td>
<td>24.061</td>
<td>—</td>
</tr>
<tr>
<td>1.8</td>
<td>15.223±1.441</td>
<td>2.537±0.24</td>
<td>4.336±0.497</td>
<td>0.039±0.005</td>
<td>65.394</td>
<td>1.732</td>
</tr>
<tr>
<td>2</td>
<td>10.406±0.543</td>
<td>1.734±0.091</td>
<td>4.612±0.376</td>
<td>0.036±0.003</td>
<td>47.772</td>
<td>1.184</td>
</tr>
<tr>
<td>6</td>
<td>17.635±1.953</td>
<td>2.939±0.325</td>
<td>4.28±0.155</td>
<td>0.039±0.001</td>
<td>75.403</td>
<td>2.006</td>
</tr>
<tr>
<td>6.7</td>
<td>22.392±1.337</td>
<td>3.732±0.223</td>
<td>4.057±0.207</td>
<td>0.041±0.002</td>
<td>90.693</td>
<td>2.547</td>
</tr>
<tr>
<td>10</td>
<td>19.459±0.868</td>
<td>3.243±0.145</td>
<td>3.155±0.549</td>
<td>0.054±0.01</td>
<td>60.051</td>
<td>2.213</td>
</tr>
<tr>
<td>16</td>
<td>10.337±6.683</td>
<td>1.723±1.114</td>
<td>2.788±0.978</td>
<td>0.066±0.027</td>
<td>26.096</td>
<td>1.176</td>
</tr>
<tr>
<td>16.7</td>
<td>24.532±0.961</td>
<td>4.089±0.16</td>
<td>4.299±0.213</td>
<td>0.039±0.002</td>
<td>105.295</td>
<td>2.79</td>
</tr>
</tbody>
</table>
too large to diffuse into the skin layers. Therefore direct contact and adherence of the vesicles with skin surface is important for the drug to penetrate and partition between the stratum corneum and the formulation. Surface tension values will give us idea of the extent of the contact of the drug-loaded vesicle with the skin surface. From Fig. 1 the lowest interfacial tension was observed for formulations containing HLB 1.8 and 16.7. Formulations with Span 85 (HLB 1.8) did not change drug permeation significantly when compared to that of the control, indicating that there is an optimal balance between hydrophilicity and lipophilicity. HLB 1.8 could be too hydrophobic to enhance the permeation rate of HP, a hydrophobic drug. Due to the high affinity of the drug to such vesicle, diffusion to the skin may be limited. Although formulations with HLB 16 had higher HLB value, however due to the formation of large aggregate of the two mixed surfactants used in this formulation, the skin permeation was not enhanced. The low skin permeation observed from formulations with HLB 16 is due to the non-synergistic effect of the mixture of the two surfactants in formulations drug permeation across the skin was comparable to the control samples.

Drug permeation may be correlated to the physicochemical properties of the surfactant. Table 2 demonstrates some characteristics of non-ionic surfactants including number of carbons in the alkyl chain. The smaller the chain length of the fatty alcohol, the higher the drug release rate. In our study, the rate of drug release from proniosomal formulations based on the carbon chain lengths can be ranked as: Tween 20 (C12) > Span 60 (C18) > Span 85 (C54). Therefore physicochemical characteristics of surfactants such as surface tension length of the alkyl chain and HLB value of the non-ionic surfactants influence the rate of drug permeation from various proniosomal vesicles.

To support the above hypothesis, confocal laser scanning microscopic studies were conducted. For this purpose proniosome formulations containing single surfactants with the highest and the lowest skin permeation rates were used. The extent of vesicular penetration was measured by CLS microscopy after the in vitro skin permeation studies of proniosome formulations with HLB 16.7 and HLB 1.8 each containing 0.03% rhodamine 6G. Proniosomes with HLB values of 16.7 resulted in increased permeation of rhodamine-label which corresponded to more rhodamine transport across the stratum corneum and into the deeper layers of the skin, whereas rhodamine-labeled proniosomes of HLB 1.8 had limited penetration onto the skin (Fig. 5).

**CONCLUSION**

The effect of HLB values of surfactants on the permeation
rate of HP from these formulations was studied. HP was encapsulated in proniosomes with very high efficiency after 6 week storage in room temperature (25 °C) and in the fridge (4 °C). In vitro skin permeation studies revealed that formulations with single surfactants increased the permeation of HP more than those with mixtures of surfactants. Surface tension, hydrophobicity, and the structure of the individual surfactant appeared to be useful for elucidating mechanism of drug permeation and for comparing drug fluxes from different proniosomal formulations across the epidermis.

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