Formulation, characterization, evaluation and in vitro study of transfersomal gel medroxyprogesterone acetate for transdermal drug delivery

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ABSTRACT

A hormonal contraception progestin such as medroxyprogesterone acetate (MPA) is used to help regulate ovulation thus as a part of contraception hormone therapy as a method of birth control. This study aimed to formulate, characterize, evaluated transfersomal gel containing medroxyprogesterone acetate and to increased subcutaneous penetration of medroxyprogesterone acetate. In this research, three transfersomes formulas were prepared and optimized, e.g. F1, F2 and F3 with phosphatidylcholine: tween 80 concentration were 90:10; 85:15; and 75:25, respectively. F2 was the best formula with the highest entrapment efficiency 81.20 ± 0.42 %, Average 81.35 ± 0.78 nm, morphology of vesicles were spheres, indeks polidispersity 0.198 ± 0.012 and zeta potential was -34.83 ± 0.64 mV. The transpersonal gel (FGT) containing F2, and non-transpersonal gel containing MPA in methanol(FG) were prepared. In vitro penetration test were conducted to both of them using Franz Diffusion cells. Analysis of medroxyprogesterone acetate used a high performance liquid chromatographic (HPLC) method with an ultraviolet detector on reversed-phase C18, 5µm; 150 x 4.6 mm column; using acetonitrile-0.1% formic acid (60:40/v:v) and was detected at a wavelength of 240nm with flow rate at 1.0 mL/min. Gel stability evaluation results showed that FGT was better than FG on pH stability, viscosity and rheological properties. Based on in vitro penetration study, cumulative subcutaneous penetration of medroxyprogesterone acetate from FGT was 2356.45 ± 197.73 ng.cm⁻² and from FG 359.15 ± 13.60 ng.cm⁻², respectively. Flux value for FGT and FG were 112.77 ± 6.47 ng.cm⁻².hr⁻¹ and 17.99 ± 4.81 ng.cm⁻².hr⁻¹, respectively. It could be concluded that transfersomal gel medroxyprogesterone acetate for transdermal drug delivery increased cumulative transdermal penetration of medroxyprogesterone acetate by six times more than non-transfersomal gel dosage form.

INTRODUCTION

A hormonal contraception progestin such as medroxyprogesterone acetate (MPA) is used to help regulate ovulation thus as a part of contraception hormone therapy as a method of birth control. Medroxyprogesterone acetate is also used to treat certain types of cancer, endometriosis, and abnormal sexuality in males. Medroxyprogesterone acetate is taken orally and by invasive injection into a muscle (Burkman, 2001).
tem for regulatory of birth control has some clinical advantages compared to conventional hormonal oral administration (Prausnitz et al., 2004). Based on a pharmacokinetic side, transdermal contraception overcome variability in intestinal absorption, due to factors such as pH in the stomach, gastrointestinal motility, gastro emptying rate and gastrointestinal transit time. The hormonal contraception is delivered directly into the systemic circulation, avoiding the hepatic first-pass metabolism that occurs with oral administration—transdermal contraception maintaining constant drug concentrations in the circulation (Sachan et al., 2013).

The critically challenge to subcutaneous drug penetration is crossing the stratum corneum as a barrier of the skin, which has a low permeability to molecules from the environment due to the lipid-rich composition (Sachan et al., 2013). Molecules passing through the stratum corneum must penetrate among cells and pierce between interfaces of cellular lipid bilayers (Hirva and Jenisha, 2016). Thus, a high degree of complexity and technical system approaches are required to allow continuous hormonal progestin delivery through the skin. The subcutaneous drug penetration must show the appropriate physical and chemical properties to facilitate passage (such as suitable lipophilicity, a low molecular mass, and high stability) (Hirva and Jenisha, 2016). To overcome the subcutaneous penetration problems, it can be conducted with nanovesicle such as lipid nanovesicular transfersomes.

Transfersomes are an ultra-deformable nanovesicle lipid which can be loaded with both of hydrophilic or lipophilic active pharmaceutical ingredients and applied to the skin in an aqueous formulation. Transfersomes consists of phospholipids as a vesicle forming and an edge activator to improve deformability of vesicle, which is generally a single chain surfactant (Scognamiglio et al., 2013). Transfersomes are strongly suspected cross the stratum corneum as a barrier of the skin under the influence of a trans-epidermal water activity gradient which makes the driving force for vesicle subcutaneous penetration through the skin (Benson, 2006). Surfactant is used to increase the subcutaneous penetration through improving the vesicle deformability via skin cellular layers by layers until the systemic circulation system (Gupta et al., 2012).

This study aimed to formulate, characterized, evaluated transfersosomal gel containing medroxyprogesterone acetate and to increased cumulative subcutaneous penetration of medroxyprogesterone acetate.

MATERIALS AND METHODS

Materials

Medroxyprogesterone acetate was purchased from Sigma Aldrich (Singapore), tween 80, Phospholipon® 90G (Soya Phosphotidylcolin ≥ 91%) was purchased from Lipoid (GMBH, German), phosphate buffer solution pH 7.4, gelling base, dichloromethane, methanol, and acetonitrile were of analytical grade.

Animals

The animal’s membranes used in the in-vitro study were from abdomen white female rats (Sprague Dawley strain), provided by Bogor Agricultural Institute. All of the methods for sacrificing and getting abdomen skin from the animals have been approved with No: KET-943/UN2.F1/ETIK/PPM.00.02/2019, by the ethic committee from Faculty of Medicine, Universitas Indonesia.

Formulation of transfersomes

Three transfersome formulas were prepared, e.g. F1, F2 and F3 with phosphatidylcholine: tween 80 concentration were 90:10; 85:15; and 75:25, respectively by thin-film hydration method. The formulas are shown in Table 1. Phospholipon® 90G was dissolved in dichloromethane, and tween 80 was dissolved in dichloromethane in another flask. Both of them were mixed in a round bottom flask, and the solvent was evaporated at 40±1°C using rotary evaporator with a speed of rotary was 150 rpm Wirant et al. (2017).

The thin-layer formed then was flowed by nitrogen and incubated for 24 hrs in the 40±1°C. After one day, the phosphate buffer solution pH 7.4 used to make it hydrated. The temperature was 40±1°C for the hydration process at a speed of rotary was 150 rpm. At the time transfersomes suspension was produced, ultrasonication for 10 minutes in 60 amplitude was conducted to reduced the particle size. It had been extruded by mini extruder with 200 nm pore of membrane polycarbonate (Iskandarsyah et al., 2017).

Characterization of particle size distribution, polydispersity index, and zeta potential

The characterization of the particle size distribution of transfersomes formulas was conducted by a dynamic light scattering method (DLS), using a particle size analyzer (Malvern Zetasizer). The suspension of transfersome was diluted up to 10 ml with distilled water; then the characterization of the particle size and zeta potential were determined. The characterization was conducted in 3 times to each formula (Sarmah et al., 2013).
Figure 1: Morphology of medroxyprogesterone acetate transfersomes (transmission electron microscope), 40,000 magnification, A=F1, B=F2 and C=F3

Figure 2: (A) Particle size distribution and polydispersity index of F2, and 2(B) zeta potential value of F2
Figure 3: Rheogram of FGT (A) and FG (B)

Figure 4: pH of FGT and FG at low temp, 4±2°C (A), at room temp, 25±2°C (B) and high temp, 40±2°C (C)
Morphology of the MPA transfersomes

In this research, the morphology characterization of transfersomes was observed using a transmission electron microscope (TEM) (JEOL 1010). The sample was dropped onto a copper grid size 400 nm; then the transfersomes were air-dried in room temperature. After drying, the transfersome had been seen using TEM at 40,000 magnification with a voltage of 80.0 kV.

Entrapment efficiency determination

The amount of medroxyprogesterone acetate that entrapped in the vesicle of transfersomes was estimated by the ultracentrifugation method. The transfersomes suspension (1 ml) in a centrifugation tube was centrifuged at 12000 rpm for 10 min. The supernatant was collected from the centrifuge and precipitated in microtube, then diluted with methanol. Then, the drug concentration was determined using HPLC method with an ultraviolet detector on reversed-phase C18, 5 µm; 150 x 4.6 mm column; using acetonitrile-0.1% formic acid (60:40/v:v) and was detected at a wavelength of 240 nm with flow rate at 1.0 mL/min (Patel et al., 2009).

Preparation and evaluation of gel

The composition of FGT and FG containing medroxyprogesterone acetate are shown in Table 2. An appropriate amount of HPMC was dispersed in 70-80°C demineralized water for 10 mins, and then the HPMC stirred until a homogenous gel was formed. Then, transfersomes of medroxyprogesterone acetate were mixed into the gel base in the homogenizer and stirred at 1500 rpm for 15 minutes. Similarly, a gel containing medroxyprogesterone acetate without transfersomes was prepared by the same method as a comparative standard (Malakar et al., 2012).

Physical evaluation and pH

Organoleptic tests, such as homogeneity, colour, and odour, were conducted. The pH value, viscosity, and rheology properties were determined using pH meter and Brookfield viscometer at the room temperature.

Physical stability of the gel study

Physical stability of the gel was observed in three different temperatures at low temperature 4±2°C, room temperature 25±2°C, and high temperature 40±2°C. Then, the gels were observed including the organoleptic, pH, viscosity, and rheology properties during storage for 12 weeks with interval two weeks of each observation (Mitkari et al., 2010).

In vitro penetration study

In vitro penetration study was conducted using Franz diffusion cell for this research, with a receiver compartment volume was 16 ml of phosphate buffer solution (pH 7.4) maintained at a temperature of 37±0.5°C and stirred by a magnetic bar and membrane diffusion area was 1.67 cm². The gel dosage forms (±1 g) from FGT and FG were applied on the membrane, then the top of each the diffusion cell was covered (Panwar et al., 2010). At the time intervals (1, 2, 3, 4, 5, 6, 8, 10, 12, and 24 hrs), 2 ml aliquots of the receptor medium were taken and replaced by 2 mL volume of fresh receptor phosphate buffer solution immediately to maintain sink condition. The sample was then analyzed using HPLC at wavelength 240 nm. Each measurement was conducted in triplicate.

RESULTS AND DISCUSSION

In this research, the transfersomes composition was soya phosphatidylcholine and tween 80. The function of phospholipid is to form the lipid bilayer arrangement in vesicles. While non-ionic surfactant
Table 1: Medroxyprogesterone acetate transfersome formulas, F1, F2 and F3

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<tr>
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<tbody>
<tr>
<td>MPA</td>
<td>300 mg</td>
<td>300 mg</td>
<td>300 mg</td>
<td></td>
<td>300 mg</td>
<td></td>
</tr>
<tr>
<td>Phospholipon® 90G</td>
<td>2.125 g</td>
<td>2.125 g</td>
<td>2.125 g</td>
<td></td>
<td>2.125 g</td>
<td></td>
</tr>
<tr>
<td>Tween 80</td>
<td>0.375 g</td>
<td>0.375 g</td>
<td>0.375 g</td>
<td></td>
<td>0.375 g</td>
<td></td>
</tr>
<tr>
<td>Phosphate buffer solution pH 7.4</td>
<td>Ad 25 ml</td>
<td>Ad 25 ml</td>
<td>Ad 25 ml</td>
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<td>Ad 25 ml</td>
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</tr>
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P = Phospholipon® 90G, T = Tween 80, F1= Transfersome Formula 1, F2= Transfersome Formula 2, F3= Transfersome Formula 3

Table 2: Composition of gels

<table>
<thead>
<tr>
<th>Substances</th>
<th>FGT (%b/b)</th>
<th>Formula</th>
</tr>
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<tbody>
<tr>
<td>Transfersome Suspension Containing</td>
<td>2.5 ml</td>
<td>-</td>
</tr>
<tr>
<td>12 mg/mL of MPA</td>
<td>-</td>
<td>0.03</td>
</tr>
<tr>
<td>Medroxyprogesterone acetate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gelling Base ad</td>
<td>100 g</td>
<td>100 g</td>
</tr>
</tbody>
</table>

such as Tween 80 was selected because this type of surfactant has a hydrophilic ethoxy group and a lipophilic hydrocarbon group to form a lipid. Phosphatidylcholine arranges into the lipid bilayer and closes itself to form a circular vesicle in an aqueous environment. Besides, a surfactant is responsible for deformability the vesicles’ lipid bilayers by increasing their flexibility through pores of the stratum corneum by squeezing (Benson, 2006). Surfactants improve the deformability of the vesicle, as well to improve the subcutaneous penetration of the drug into the stratum corneum and through the skin layer and finally reach the systemic circulation (Gupta et al., 2012). Three formulas were formulated and optimized, e.g. F1, F2 and F3 with phosphatidylcholine: tween 80 concentration were 90:10; 85:15; and 75:25, respectively. Desired medroxyprogesterone acetate transfersome was characterized with the spherical shape of vesicle morphology, high entrapment efficiency, Zaverage < 200 nm, polydispersity index < 0.2, and has zeta potential > ±30mV.

The summary results of this characterization are shown in Table 3.

Morphology of MPA transfersomes

The results showed that all the transfersome formulas had characteristics as a spherical-shaped particle, as shown in Figure 1.

Characterization of particle size distribution, polydispersity index, and zeta potential

The characterization of particle size distribution, polydispersity index, and zeta potential of transfersomes formulas were analyzed using a particle size analyzer (Malvern Zetasizer). Particle size distribution, polydispersity index. Figure 2, showed that the size of particles F2 was 81.357 ± 0.783 nm. Polydispersity index is a parameter that indicates the heterogeneity of particles. The lower value of Polydispersity index, the more homogenous the particle size was (Sharma et al., 2013). An excellent value of polydispersity index should be lower than 0.5. Based on this theory, the transfersomes F2 in this study showed the lowest polydispersity index at 0.198 ± 0.012. Another critical factor for a transfersomes suspension is zeta potential. Zeta potential represents the repulsive potential between particles. A stable suspension should have zeta potential lower than −30 mV or higher than +30 mV (Sharma et al., 2013). Figure 2 showed that the zeta potential value of the F2 was −34.83 ± 0.64 mV. It was indicated that this formula would have the best stability compared with the other.

The pH of the solvent used for the suspension, a charge of the drugs, and conductivity from the suspension can affect the zeta potential value (Bhalaria et al., 2009). Zeta potential value from this medroxyprogesterone acetate transfersome was a negative charge. It may be associated with the charge of medroxyprogesterone acetate entrapped (Sharma et al., 2013).

Entrapment efficiency determination

The percentage of entrapment efficiency is explained as the percentage entrapment of the...
Table 3: Characteristic of medroxyprogesterone acetate transfersomes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vesicles morphology</td>
<td>Sperical shape</td>
<td>Sperical shape</td>
<td>Sperical shape</td>
</tr>
<tr>
<td>Zaverage (nm)</td>
<td>81.81 ± 6.18</td>
<td>81.357 ± 0.78</td>
<td>73.180 ± 1.23</td>
</tr>
<tr>
<td>Polydispersity index</td>
<td>0.203 ± 0.034</td>
<td>0.198 ± 0.012</td>
<td>0.230 ± 0.004</td>
</tr>
<tr>
<td>Zeta Potential (mV)</td>
<td>-26.57 ± 2.37</td>
<td>-34.83 ± 0.64</td>
<td>-27.47 ± 1.71</td>
</tr>
<tr>
<td>Entrapment efficiency (%)</td>
<td>73.09 ± 1.79</td>
<td>81.20 ± 0.42</td>
<td>52.09 ± 8.11</td>
</tr>
</tbody>
</table>

All values were represented as means ± SD (n=3).

active pharmaceutical ingredient added to the transfersome suspension. The percentage of entrapment efficiency for transfersome suspension was determined by an indirect method (M et al., 2015). The percentage of entrapment efficiency of F2 was the highest, with the value was 81.20 ± 0.42 % (n=3).

Gel evaluation

Organoleptic properties were evaluated on the gel, and the results have shown that FGT and FG colour were transparent, and pH value were 5.74 and 5.72. Both of the formulas shown had the same homogeneity.

Viscosity and rheological properties of the gel

The viscosity and rheological properties for any gel dosage form were an essential aspect in determining the ability of the gel in delivering the active ingredients across the skin. Viscosity and rheological properties mainly induce spreadability, determine adhesiveness, and affect drug release from the semisolid dosage form and subcutaneous penetration through the skin (Sharma et al., 2013). The viscosity values of FGT was 27200 cps, and FG was 25600 cps. Rheology properties both of gels were pseudoplastic thixotropic, as shown in Figure 3.

The gel physical stability

The result showed that both gels appearance and colour were stable during storage condition at 4±2°C and at 25±2°C for 12 weeks at two temperatures, but and 40±2°C temperature there were colour changes on FGT became slightly yellow than the beginning. This changing occurred because of the phospholipid colour from the transfersome substance. This phenomenon showed that transfersome couldn’t keep stability at 40±2°C temperature.

The other properties such as viscosity, rheological properties value decrease slightly at three temperatures. The pH of FG shown decrease more than FGT at three temperature, as shown in Figure 4, this indicates that vesicle of transfersome could keep the stability of gel transfersome. The decrease of viscosity, particularly in the FG, can be caused by the reduction in the pH of the gels.

In vitro penetration study

Figure 5 showed the penetration profile of medroxyprogesterone acetate from both gels. The cumulative amount of medroxyprogesterone acetate penetrated from FGT was 2356.45 ± 197.73 ng.cm⁻² and from FG was 359.15 ± 13.60 ng.cm⁻², respectively. Based on this data, it was indicated that gel transfersomes could increase the penetration and amount of active substance.

Another important parameter of in vitro penetration study is flux. Flux is the amount of active ingredient passed through a membrane per unit area membrane into the circulating system per unit. (Kulkarni et al., 2011). Figure 6 shown that flux of FGT was 112.77 ± 6.47 ng.cm⁻².hrs⁻¹ and FG was 17.99 ± 4.81 ng.cm⁻².hrs⁻¹. It indicated that the medroxyprogesterone acetate penetration rate from transfersomal gel was faster than non-transfersomal one.

Another critical parameter is the lag time. Lag time is the time for an active ingredient takes to cross away through the skin membrane and diffuse into the receptor fluid until reach a steady-state condition. In this study, the lag time of FGT was 7.96 ± 0.16 hrs, while FG was 1.72 ± 0.42 hrs. It showed that the lag time of FGT was faster than FG.

Penetration deformability of the transfersome reached five times smaller than the diameter of the transfersome itself. These transfersomes are composed of phospholipid which will self-arranges into lipid bilayer in an aqueous solution and closes itself to form a nanovesicle. Transfersomes in the formula had an edge activator function which can create deformability of transfersomes by decreasing the surface tension. Therefore, transfersomes could penetrate through the smaller pores (Bhalaria et al., 2009). The transfersomes penetration mechanism into the skin follows the osmotic gradient mechanism that evaporates water when the transfersomes
are applied on the skin surface (Sharma et al., 2013). The osmotic gradient is formed by the skin’s ability as a penetrating barrier, preventing loss of moisture from the skin and maintaining a water content difference of 75% in the epidermis and almost dry or about 15% on the skin surface. The polar part of the lipid bilayer arrangement of the skin may draw water on the skin surface and the lipid bilayer generally spontaneously withstands dehydration of the skin. Therefore, vesicles that have a lipid bilayer arrangement will move from areas that have low water content to areas with high water content. As a result, when vesicles are placed on the surface of the skin, the lipid vesicles will be attracted to the skin. As an elastic vesicle, vesicles can enter through the pores of the stratum corneum and deform if they are of a small enough size. As with liposomes, the vesicle system cannot deform form because it has a lower penetration ability than transfersomes (Gupta et al., 2012).

CONCLUSIONS

In conclusion, this study showed that FGT significantly increased the cumulative amount by six times more than FG in vitro penetration of medroxyprogesterone acetate, through the abdomen membrane of a rat.

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Conflict of interest

The authors declare that they have no conflict of interest for this study.

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