Formulation and characterization of Phospholipon 90 G and tween 80 based transfersomes for transdermal delivery of eprosartan mesylate

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INTRODUCTION

Hypertension is a common risk factor for the development of other diseases such as cardiovascular diseases, stroke and renal failure. It is the second leading cause of chronic kidney disease. More than one billion adults are hypertensive world-wide and this number is expected to surpass 1.56 billion by the year 2025 (i.e. a 60% increase from the year 2000) (Jarari et al. 2016). Presently, many antihypertensive drugs are available in the market for the management of hypertension. However, oral administration of these drugs can cause many side effects and complications due to various physiological variables, food effects, etc. that are beyond the control of both patient and physician. Transdermal drug delivery is clearly the most reasonable means for delivering actives over a sustained period of time by avoiding the complications associated with oral delivery (Ashtikar et al. 2016). Several efforts were made to formulate the transdermal system for various antihypertensive drugs (Selvam et al. 2010; Gungor & Ozsoy 2012), including the angiotensin II receptor antagonists, an important antihypertensive class. For more information, the readers are directed to a comprehensive review on the transdermal delivery of angiotensin II receptor blockers (Ahad et al. 2016).

The main obstacle for the delivery of drug(s) via the transdermal route is the barrier nature of the stratum corneum. Many approaches have been assessed to overcome this barrier (Alexander et al. 2012). Amongst the newer approaches, liposomes were suggested as promising drug carriers for the delivery of drug(s) through transdermal route, but their usefulness in delivering of actives across the skin at therapeutic concentrations is questionable (Honeywell-Nguyen & Bouwstra 2005). Further improvements have been made in liposomes structures by incorporating edge activators (i.e. surfactants) which impart flexibility to the lipid vesicles. The new category of liposomes has been named as ultradeformable liposomes or transfersomes. These vesicles can squeeze through skin without disruption of vesicular structure. Transfersomes have exhibited improved in vitro and in vivo skin permeation compared to that of rigid liposomes, when applied for antihypertensives drugs (Mishra et al. 2007; Amin et al. 2013).

Many surfactants were used as edge activators for the preparation of transfersomes (El Zaafarany et al. 2010; Jacob & Kr 2013). Several reports have indicated the use of Tween® 80 as an edge activator for the formulation of transfersomes. Recently, methotrexate loaded transdermal ultradeformable liposomes were prepared using sodium cholate or Tween® 80 as an edge activator. Ultradeformable liposomes prepared with Tween® 80 were reported to be better with respect to flexibility and skin permeation than those prepared with sodium cholate (Zeb et al. 2016).

In 2015, Khan et al. developed 5-fluorouracil loaded transfersomes formulations using Tween® 80 and Span® 80 as an edge activator. Khan et al. concluded that, Tween® 80 was found better edge activator than Span® 80 and produced formulations with smaller particles sizes with higher drug entrapment efficiency (Khan et al. 2015). In another study, quercetin loaded deformable liposomes were prepared using Span® 20, Tween® 80,
and sodium cholate. The investigators reported that deformable liposomes prepared using Tween® 80 as an edge activator had higher flexibility and entrapment efficiency, particle in nano size and produced prolonged drug release than formulations prepared with Span® 20, and sodium cholate (Liu et al. 2013). Antecedently, Nava et al. had developed ketorolac tromethamine loaded elastic liposomes using Tween® 80. This formulation showed loading efficiency of approximately 73% with improved flexibility, optimum particle size, and stability (Nava et al. 2011). With the above knowledge, we have developed transfersomes formulation of eprosartan mesylate using Phospholipon® 90 G (a lipid bilayer-forming substance) and Tween® 80 as an edge activator. Eprosartan mesylate is an angiotensin II receptor blocker used to treat hypertension. It has an oral bioavailability of 13% in humans and half-life of 5–9 h (Israili 2000; Dangre et al. 2016). The eprosartan mesylate has a log p value of 3.9. These features of the drug, distinguished it as a suitable candidate for transdermal delivery.

Materials and methods

Materials

Eprosartan mesylate was procured from BASF, Germany. Phospholipon® 90 G was a gift sample from Phospholipid GmbH (Nattermannallee, Germany). Tween® 80 was obtained from Avonchem Limited (Cheshire, UK). Rhodamine 6 G was sourced from Sigma-Aldrich (St. Louis, MO). Methanol and chloroform were purchased from BDH (Dorset, UK) and Sigma-Aldrich (St. Louis, MO), respectively.

Animals

Wistar rats (150–180 g) were obtained from Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. Animals were kept in conformity with the instructions of the Guide for the Care and Use of Laboratory Animals approved by the center (NIH publications no. 80-23; 1996). The experimental guidelines of the animal care and use committee of King Saud University were adopted stringently in the experiment.

Preparation of transfersomes

Eprosartan mesylate loaded transfersomes were prepared using a thin film hydration technique (Mehanna et al. 2015). Phospholipon® 90 G and Tween® 80 (95–75:5–25% w/w) were dissolved in an organic phase (20 ml, chloroform:methanol, 2:1 v/v) and subsequently drug was added in the above mixture and then mixture was transferred to a round bottom flask. The organic phase was evaporated using a rotary evaporator (Buchi® Rotavapor®-210, Zurich, Switzerland) under reduced pressure. The thin, dried lipid film that was formed on the wall of the flask was then rehydrated with phosphate buffered saline (pH 7.4, 20 ml) (Dai et al. 2013). The coarse dispersions so formed were sonicated (Probe sonicator, Bandelin Electronic GmbH & Co.KG, Berlin, Germany) to produce small vesicles. The final dispersions were then filtered using polycarbonate membranes (450 nm and 200 nm, Chromafit® Xtra, Macherey-Nagel GmbH & Co. KG, Düren, Germany), and the final formulations were stored refrigerated (Al-Mahallawi et al. 2014).

Vesicles size, polydispersity index, and zeta potential

Before analysis, transfersomes were diluted with filtered phosphate buffered saline. The vesicles size, polydispersity index, and zeta potential of transfersomes were measured using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) at 25 ± 1°C (Dragicevic-Curic et al. 2009).

Entrapment efficiency determination

Transfersomes (1 ml) were transferred to eppendorf tubes and centrifuged for 1 h at 40 000 rpm and 4°C, using an ultracentrifuge (Optima™ Max-E, Beckman Coulter, Pasadena, CA) (Meng et al. 2013; Alomrani et al. 2014). After centrifuged, the supernatant was carefully collected and, after suitable dilution, samples were analyzed by HPLC (Ahad et al. 2015). The entrapment efficiency was calculated by using the following equation.

\[
\text{Entrapment efficiency} (\%) = \left(\frac{\text{Total amount of drug in the vesicles} - \text{Amount of drug detected only in the supernatant}}{\text{Total amount of drug in the vesicles}}\right) \times 100
\]

Transmission electron microscopy

The morphology of the optimized transfersomes formulation was evaluated by transmission electron microscopy (JEM-1011, JEOL, Tokyo, Japan), set at a voltage of 80 kV. The sample was negatively stained and allowed to dry at room temperature before being visualized with the electron microscope (Madheswaran et al. 2014).

In vitro skin permeation study

The in vitro skin permeation studies across rat skin were conducted using transdermal diffusion system (SFDC-6, Logan, UT). Rats were sacrificed by means of diethyl ether and skin samples were cut and removed. Hair from rat skin was removed by hair clipper. Fat and connective tissues were carefully excised and skin samples were checked for any cuts and holes (Sood et al. 2016). Thickness of the skin was measured and was in the range of 0.3 ± 0.1 mm. The rat skin samples were cut to the size of the Franz diffusion cell and fixed on it. Transfersomes formulations were placed in the donor chamber while the receptor chamber contained vehicle (12 ml, ethanol:phosphate buffered saline, 20:80 v/v) which was maintained at 37°C and stirred at 100 rpm with a magnetic stirrer. Samples (1 ml) were taken from the receptor chamber at predetermined time intervals and analyzed by HPLC (Ahad et al. 2015). The volume of vehicle in the receptor chambers were replenished with fresh vehicle.

Confocal laser scanning microscopy

For the confocal laser scanning microscopy study, drug solution in vehicle (control) and transfersomes formulation, both loaded with rhodamine 6 G (0.03%, w/v), were prepared and in vitro skin permeation was performed for 10 h, as described above. After 10 h, the rat skin was removed; surface cleaned, and fixed on a glass slide. The sample was evaluated for formulation penetration depth using an inverted Zeiss LSM 780 microscope (Carl Zeiss, Jena, Germany). The excitation wavelength was 528 nm, and the emission wavelength was 551 nm (Shi et al. 2012; Ma et al. 2015).

Skin irritation test

The skin irritation study was performed on Wistar rats using the Draize scoring method (Draize et al. 1944). Wistar rats were

\[
\text{Vesicles size} = \left(\frac{\text{Total amount of drug in the vesicles}}{\text{Amount of drug detected only in the supernatant}}\right) \times 100
\]
divided into two groups. Formalin (0.8% v/v), as a standard irritant and transfersomes were applied on the shaven dorsal sides of Wistar rats. After 24 h, the formulations were removed and the treated areas were evaluated for three days for indications of erythema and edema; responses were scored according to Draize et al. (1944). A formulation was considered to have skin irritant potential if the primary dermal irritation index was found to be ≥2 (Shara et al. 2005; Xu et al. 2008; Liu et al. 2014).

Results and discussion

Preparation of transfersomes

Transfersomes were prepared by thin film hydration technique. This technique is much frequently used owing to its simplicity and practicability, and its ability to yield small and uniform vesicles with high drug entrapment efficiency (El Zaafarany et al. 2010; Al et al. 2014). Developed transfersomes formulations, formulas (T-TW1 – T-TW3) were translucent and off-white in color (Al Shuwaili et al. 2016). The appearance of the prepared transfersomes formulations are presented in Figure 1(A). In previous studies phospholipid and surfactant have been investigated for the transfersomes formulations in various ratios for hydrophilic and lipophilic molecules, but the most investigations have concluded that the most beneficial proportion was found to be 85:15 (phospholipid:surfactant) (Nava et al. 2011). Further, it was stated earlier that entrapment efficiency decreased as the surfactant fraction rises, and that flexibility of vesicles reduced with lower surfactant concentrations (El Maghraby et al. 2000; Jain et al. 2003; Mishra et al. 2006; Mishra et al. 2007; Nava et al. 2011). Therefore, formulation (T-TW2) having Phospholipon® 90 G: Tween® 80 in 85:15 ratio was selected for morphology, confocal laser scanning microscopy, and skin irritation test study.

Vesicles size, polydispersity index, and zeta potential

The vesicles size distribution curve was unimodal in shape (Figure 1(B)). The prepared transfersomes showed vesicles sizes in the range of 71.18–85.66 nm (Table 1). The formulation with an 85:15 Phospholipon® 90 G:Tween® 80 ratio had a vesicles size of 74.88 ± 0.78 nm. There were substantial differences in the vesicles sizes among transfersomes comprising different Phospholipon® 90 G to Tween® 80 ratios. It was observed that the size of the vesicles decreased as the proportion of Tween® 80 increased. Further, transfersomes having smaller vesicles sizes are believed to be more effective than micron-sized vesicles for the delivery of actives across the skin. It was reported that vesicles having particle sizes ≤0.3 μm can efficiently deliver entrapped drug(s) into the deeper layers of the skin (Verma et al. 2003).

The polydispersity indexes of formulations were observed in the range of 0.13±0.021 to 0.25±0.007, representing a narrow size distribution. No substantial change in the polydispersity index was observed when the Tween® 80 concentration was increased from 5% to 15%. Further increasing the Tween® 80 to 25% significantly decrease the polydispersity index of the formulation T-TW3 (Table 1).

In the present study, the zeta potential analysis of eprosartan mesylate transfersomes showed a negatively charged vesicular surface. The zeta potentials of different formulations were in the range of −3.93 ± 0.038 mV to −6.19 ± 0.10 mV (Figure 1(C) and Table 1).
unilamellar, spherically shaped, sealed vesicles (Figure 1(D)). The electron microscope. Prepared transfersomes were found to be visualized using a transmission electron microscopy.

The transdermal delivery potentials of prepared transfersomes were assessed by in vitro skin permeation studies across rat skin. The prepared transfersomes showed transdermal flux for 24 h (mean ± SD). T-TW: transfersomes with Tween® 80; SD: standard deviation.

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<tr>
<th>Table 1. Composition and evaluation of eprosartan mesylate loaded transfersomes formulations (mean ± SD).</th>
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<td><strong>Formulation code</strong></td>
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<tr>
<td>T-TW1</td>
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T-TW: transfersomes with Tween® 80; SD: standard deviation.

Tween® 80 is a nonionic surfactant, so transfersomes comprised of Tween® 80 as an edge activator could have negatively charged surface due to the partial hydrolysis of polyethylene oxide head groups (i.e. (CH2-CH2-O)n) of the Tween® 80 (Zeb et al. 2016).

**Entrapment efficiency**

Entrapment efficiency is the portion of eprosartan mesylate that integrated into the transfersomes in comparison to the total amount of drug in the vesicles. The consequence of the Tween® 80 ratio on the eprosartan mesylate entrapment efficiency is presented in Table 1. The prepared transfersomes exhibited entrapment efficiency in the range of 83.00 ± 2.87% to 88.19 ± 1.40%. It was noticed that as we have increased the Tween® 80 ratio in formulation T-TW2 (85:15), the entrapment efficiency was found to be decreased in comparison to the formulation (T-TW1, 95:5) (Table 1). Additional increase in Tween® 80 ratio in formulation (T-TW3, 75:25), the entrapment efficiency further decreased in the formulation. Investigators explained that the presence of mixed micelles with vesicles or pore generation within the vesicles at higher surfactant concentration could be the reason for lower entrapment efficiency in the formulation (Edwards & Almgren 1990; Jain et al. 2003). The decrease in entrapment efficiency on increasing the ratio of Tween® 80 in formulation corresponds with the results of other researchers (Mishra et al. 2007; Khan et al. 2015; Moawad et al. 2017).

**Transmission electron microscopy**

The prepared transfersomes were visualized using a transmission electron microscope. Prepared transfersomes were found to be unilamellar, spherically shaped, sealed vesicles (Figure 1(D)). The vesicles sizes of the prepared transfersomes were in agreement with the results of the photon correlation spectroscopy.

**In vitro skin permeation study**

The transdermal delivery potentials of prepared transfersomes were assessed by in vitro skin permeation studies across rat skin. The skin permeation profile is plotted in Figure 2, and the permeation parameters are shown in Table 2. The skin permeation parameters were improved in the order of T-TW1 > T-TW2 > T-TW3. Prepared transfersomes showed transdermal flux in the range of 1.78 ± 0.16 to 5.02 ± 0.38 μg/cm²/h. The permeability coefficients of three prepared formulations were in the range of 0.59 ± 0.053 to 1.67 ± 0.128 cm/h.

The variance in Phospholipon® 90 G to Tween® 80 proportion pointed potential effect on the drug permeation rate. Maximum transdermal flux (5.02 ± 0.38 μg/cm²/h) was achieved with formulation T-TW1 having Phospholipon® 90 G to Tween® 80 in 95:5 ratio. Further increase of Tween® 80 in formulations T-TW2 and T-TW3 leads to decrease in transdermal flux of eprosartan mesylate to 3.06 ± 0.01 and 1.78 ± 0.16 μg/cm²/h respectively across rats skin. This could be due to the formation of mixed micelles (less flexible and less skin permeation ability than transfersomes) that revealed low permeation flux, permeability coefficient, and entrapment efficiency. The other reason for low transdermal flux at higher surfactant level could be due to the pore formation in the lipid bilayers (Lasch et al. 1990; Lopes et al. 2004). The three formulations presented a similar Tlag of 3.93 h. The skin permeation ability of these transfersomes could be explained by their ability to squeeze through skin pores, consequently allowing them to transport trapped actives through the skin (Jain et al. 2003).

**Confocal laser scanning microscopy**

Confocal laser scanning microscopy was performed to study the skin penetration depth of prepared rhodamine 6 G loaded transfersomes. The images obtained, showing drug solution and transfersomes skin penetration depth, are presented in Figure 3(A,B), respectively. Rhodamine 6 G loaded transfersomes (Figure 3(B)) depicted more evident fluorescence signals as compared to those of the rhodamine 6 G loaded drug solution (Figure 3(A)). The rhodamine 6 G loaded transfersomes exhibited dye fluorescence up to a 232 μm penetration depth in rat skin, while the rhodamine 6 G loaded drug solution showed a dye penetration of only 152 μm. This study distinctly demonstrated the transdermal potential of the eprosartan mesylate loaded transfersomes formulation.

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<th>Table 2. Transdermal permeation parameters for eprosartan mesylate loaded transfersomes across rat skin (mean ± SD).</th>
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<td><strong>Formulation codes</strong></td>
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T-TW: transfersomes with Tween® 80; Tlag: lag time; Kp: permeability coefficient; h: hours; SD: standard deviation.
Skin irritation

The positive control group treated with formalin (0.8% v/v), which had a primary dermal irritation index of 4.0, showed substantial skin irritation. In contrast, the primary dermal irritation index obtained after application of transfersomes was found to be 0.08 (Table 3). Since the prepared formulation did not produce detectable erythema or edema on the bare skin of Wistar rats, it was deemed safe to be used for transdermal application.

Conclusions

In the present study, transfersomes were formulated using Phospholipon® 90 G and Tween® 80 in varying ratios. Prepared transfersomes were negatively charged, nano-sized vesicles (71.18–85.66 nm), with entrapment efficiency of 83.00–88.19%, and transdermal flux of 1.78–5.02 μg/cm²/h. The confocal study demonstrated that the prepared transfersomes penetrated deeply into rat skin. Furthermore, no skin irritation was observed when the formulation was applied to shaven rat skin. Accordingly,
these developed transfersomes could be possible nano-sized drug carriers for the transdermal delivery of eprosartan mesylate to mitigate the adverse effects of oral and other parenteral routes.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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