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Deformable Liposomes for Topical Skin Delivery of Arbutin

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ABSTRACT – The aim of this study was to investigate the effect of deformable liposomes with sodium cholate on the skin permeation and skin deposition of arbutin, a hydrophilic skin-whitening agent. Various compositions of liposomes were prepared by the extrusion method. Particle size distribution and entrapment efficiency were determined by the laser light scattering and the gel permeation chromatography, respectively. The *in vitro* rat skin permeation and deposition of arbutin in various skin layers were investigated using the Keshary-Chien diffusion cells at 37°C. The average particle size of the deformable liposomes ranged from 217.4 to 117.4 nm, depending on the composition. The entrapment efficiency was dependent on surfactant concentration and loading dose of arbutin. The permeation rate of 5% arbutin in deformable liposomes was 8.91 (±1.33) μg/cm^2/h, and was not significantly different from 5% arbutin aqueous solution [9.82 (±0.86) μg/cm^2/h]. The deposition of arbutin was 43.34 (±12.13) and 16.99 (±7.83) μg/cm^2 in stratum corneum layer and epidermis/dermis layer, respectively, after 12 h of permeation study. These results are consistent with several earlier studies for the localization effect of liposomal formulations in stratum corneum, and demonstrated the feasibility of the deformable liposomes as a promising carrier for the skin deposition of hydrophilic skin-whitening compounds.

Key words – Arbutin, Deformable liposomes, Topical delivery, Skin permeation, Skin deposition

Melanogenesis is a normal process in human skin to protect itself from the damage of UV-light in nature. However, abnormal hyperpigmentations including melasma, freckles and senile lentigines and other skin hyperpigmentation disorders could be a serious aesthetic problem.[1] Melanin is produced in melanocytes mediated by several enzymes among which tyrosinase is essential in catalyzing the oxidation of tyrosine into dopa and subsequently dopaquinone. Inhibition of the tyrosinase activity or suppression of the expression and synthesis of the enzyme itself would be effective pathways to hinder melanogenesis.[2]

Numerous depigmentating agents have been investigated for the treatment of skin hyperpigmentation, including hydroquinone,[3] gentisic acid,[4] ascorbic acid,[5] kojic acid,[6] retinoids[7] and arbutin.[8] However, some of them have been proven to be very toxic to melanocytes, and some other chemicals need further research to conform their skin whitening effect. Recent studies showed that arbutin (hydroquinone-(-D-glucopyranoside) is an effective and safe skin whitening agent which has an inhibition effect on melanosomal tyrosinase activity.[8] Arbutin (Figure 1) is an active ingredient found at high concentration in certain plants, especially in bearberry (Arctostaphylos uva-ursi). It was also reported that the depigmentation effect of arbutin works through an inhibition of the melanosomal tyrosinase activity, rather than suppression of the expression and synthesis of tyrosinase in human melanocytes. It is a competitive inhibitor of tyrosinase and its inhibitory concentration is nontoxic to melanocytes.

Since melanocytes locate between epidermis and dermis layers, it is important for topically delivered arbutin to penetrate

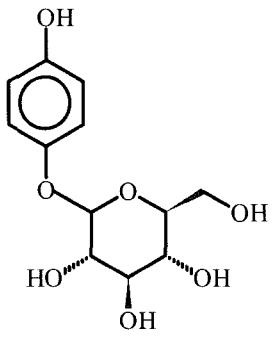

Figure 1–Chemical structure of arbutin.

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through the stratum corneum and to maintain an effective concentration near the melanoctyes for a certain period of time.[9] However, the formidable barrier property of the stratum corneum and the high hydrophilicity of arbutin (logP value, -1.49) make it difficult to permeate through the skin and reach to its site of action (i.e., melanoctyes). In many cases, traditional formulations, such as ointment and lotion, failed to achieve a sufficient skin deposition of arbutin. However, liposomal formulations have emerged as attractive alternatives for topical delivery due to their biocompatibility, non-toxicity, suitability for both hydrophilic and lipophilic compounds. These versatile characteristics of liposomes enable them to be applied in a wide area as drug carriers. Also, several groups have shown that liposomes enhance the penetration of drugs into the skin.[10,11]

Recently, a novel type of liposome named as "deformable liposome" has been reported as a proper carrier for topical delivery of skin whitening agents and other therapeutic agents for the treatment of skin disorders.[12-15] Enhanced skin permeation and deposition of chemicals was also reported when surfactants, such as span 80, tween 80, PEG-8-L (octaoxyethylene laurate ester), or dipotassium glycyrrhizinate, were included, thereby the elasticity and/or deformability of the deformable liposomes was increased.[16-18] Although its morphology is similar to the conventional liposomes, it is known to be more effective in the transdermal delivery of peptides and other chemicals, especially when it is incorporated with surfactants such as sodium cholate.[19] Thus, herein we investigated the characteristics of the arbutin deformable liposomes, and their *in vitro* skin permeation and skin deposition behavior in comparison to aqueous solution.

Experimental

Materials

Arbutin, sodium cholate and sodium dodecyl sulfate (SDS) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Soybean phosphatidylcholine (S_{75}, Lot No. 776075-4) was a generous gift from Lipoid Co. (Ludwigshafen, Germany). Sephadex LH-20 was obtained from Amersham Pharmacia Biotech (Uppsala, Sweden). Triple distilled water was used in the experiments. All other chemicals used were of analytical grade or higher.

Preparation of arbutin liposomes

Various compositions of deformable liposome prepared in this study were summarized in Table I. Soybean phosphatidylcholine (435 mg) was first dissolved in 0.5 mL of ethanol, and then was added with sodium cholate (0-120 mg). Arbutin (50-250 mg) was dissolved in distilled water, and then two solutions were mixed thoroughly. This suspension was sonicated for 30 seconds using the Sonics Vibra Cell (VC 750, Sonics & Materials Inc., Newtown, CT, USA) and passed through a high pressure extruder (Northern Lipids Inc., Vancouver, Canada) with serial pore size membranes (Isorpore™ membrane filter, County Cork, Ireland) from 3.0 mm to 0.2 mm. Deformable liposomes were homogenized by passing through the final membrane five to ten times, and were kept in the refrigerator until used.

Particle size measurement

The mean particle size and particle size distribution of deformable liposomes were determined by NICOMP 370 Submicron Particle Sizer (Particle Sizing Systems, Santa Barbara, CA, USA) after dilution with distilled water. Each sample was determined in triplicate.

Entrapment efficiency

The entrapment efficiency (EE) of arbutin in the deformable liposomes was determined after separating non-entrapped arbutin by using the gel permeation chromatography. An aliquot (100 μL) of liposome solution was loaded to sephadex column (Sephadex™ LH-20), and the diluents were washed by water with a flow rate of 1.0 mL/min, and collected in the tubes which were changed every 5 min. Then, optical density of each tube was determined with UV-spectrometry at 280 nm (Gene Spec, Naka Instruments, Japan) and was plotted as a function of tube numbers. Tubes of the first and second peaks were separately collected, which contained liposome and free arbutin, respectively. Free arbutin concentration was directly analyzed by HPLC, while liposome was first dissolved in 0.5% triton X-100 solution before analyzed by HPLC. Entrapment efficiency of arbutin in deformable liposome was calculated by the following equation:

$$EE(\%) = \frac{C_{lipo} \times V_{lipo}}{C_{lipo} \times V_{lipo} + C_{free} \times V_{free}} \times 100$$

where, C_{lipo} and C_{free} represent arbutin concentration in liposome and free solution, respectively, while V_{lipo} and V_{free} represents the volume of liposome and free solution, respectively.

In vitro skin permeation and skin deposition studies

Male Sprague-Dawley rats (220-250 g), purchased from Dae-Han Laboratory Animal Research Center (Taejon, Korea), were euthanized by CO_2 asphyxiation. The dorsal hairs were shaved with clippers and the full-thickness skin was surgically removed. The skin specimen was carefully cleaned with nor-

mal saline, then was cut into square pieces (2 cm×2 cm). *In vitro* skin permeation was conducted using Kershary-Chien diffusion cells with a permeation area of 2.14 cm^2. The temperature of the diffusion assembly was maintained at 37°C. Arbutin (5%, w/w) in deformable liposomal solution (1 mL) or in distilled water (as control) was applied to the donor cells facing the stratum corneum side of the skin, and was occluded with parafilm. The receptor half-cells facing the dermis side was filled with phosphate buffer saline (PBS, pH 7.4, 12 mL) containing 0.01% gentamicin to prevent bacterial degradation of arbutin during the permeation process. At each predetermined time interval, an aliquot (400 μL) of receptor solution was taken, and was refilled with the same volume of fresh receptor solution. Samples were kept in the freezer until analyzed by HPLC.

At the end of permeation experiments (12 h), the skin was detached from the diffusion cells and was carefully rinsed five times with distilled water to remove excess arbutin on the surface. Skin specimen of various time (0.5, 3, 6, 9 h) were obtained in separate permeation studies. The stratum corneum of the skin was surgically separated from the variable epidermis/dermis layer. The two layers of the skin were separately cut into small pieces, and homogenized with 2 mL of receptor solution for 2 min at 10,000 rpm (Polytron-Aggregate® PT3100, Kinematica, Switzland). The homogenized suspension was centrifuged for 20 min at 3,100 g and then the supernatant was filtered through the micropore filter (0.45 μm, Sartorius Co., Germany). The concentrations of arbutin in the filtrate were determined by HPLC.

HPLC analysis of arbutin

A Merck RP-8 column (LiChrospher® 250×4 mm, 5 μm particle size, Darmstadt, Germany) was used in the analysis of arbutin concentrations. The HPLC system was equipped with a binary pump (Gilson Model 305 and 306) and automatic injector (Gilson Model 234). The wavelength of the UV detector (Gilson Model 118) was set at 280 nm. The mobile phase was 50 mM acetate buffer adjusted to pH 2.5 with acetic acid at a flow rate 0.8 mL/min. All samples to be analyzed were injected at a volume of 20 μL. The retention time of arbutin was about 10.9 min.

Results and Discussion

Particle size distribution

The effect of composition on the average particle size of deformable liposomes containing arbutin is showed in Table I. When the sodium cholate concentration increased from 0.6 to 2.4%, the mean particle size of liposome showed a decreasing tendency from 151.9 nm to 117.4 nm, although they were not significantly different. For liposomes prepared with different final pore size extruder filter from 1.2 μm to 0.2 μm, the data showed an mean diameter from 217.4 nm to 136.5 nm. The increase of arbutin concentration has no effect on the particle size of liposomes.

Entrapment efficiency

The entrapment efficiency (EE) of arbutin in various liposome formulations is shown in Table I. Theoretically, it is dif-

Table I–*Particle Size and Entrapment Efficiency of Arbutin in Various Formulations of Deformable Liposomes Fabricated by Soybean Phosphatidyl Choline (PC) Containing Sodium Cholate as a Surfactant*

Formulation of liposomes					Particle size (nm)	EE (%)	Loading of arbutin (μg/mg lipid)
Arbutin (%, w/w)	Soybean PC (%, w/w)	Ethanol (%, v/v)	Sodium cholate (%, w/w)	Extrusion pore size (μm)			
0.5	8.7	10.0	1.2	1.2	217.4 (±84.6)	21.81	12.53
0.5	8.7	10.0	1.2	0.6	181.6 (±59.7)	18.54	10.66
0.5	8.7	10.0	1.2	0.2	136.5 (±43.1)	15.65	8.99
1	8.7	10.0	0.6	0.2	151.9 (±38.6)	12.85	14.77
1	8.7	10.0	1.2	0.2	138.7 (±45.8)	7.74	8.89
1	8.7	10.0	2.4	0.2	117.4 (±47.4)	1.52	1.75
3	8.7	10.0	1.2	0.2	129.2 (±26.0)	7.31	25.20
5	8.7	10.0	1.2	0.2	131.6 (±40.0)	4.26	24.48

Each data is the mean± standard deviation of three determinations.

$EE(\%) = \frac{C_{lipo} \times V_{lipo}}{C_{lipo} \times V_{lipo} + C_{free} \times V_{free}} \times 100$ where, C_{lipo} and C_{free} represent arbutin concentration in liposome and free solution, respectively, while V_{lipo} and V_{free} represents the volume of liposome and free solution, respectively.

ficult for arbutin, a highly hydrophilic drug, to achieve high entrapment efficiency. The maximum EE of 21.81% was obtained when 0.5% arbutin was incorporated with 1.2% sodium cholate. However, EE decreased as the increase of arbutin and sodium cholate concentration, and as the decrease of the filter pore size. Decrease of EE from 12.85% to 1.52% was observed when the sodium cholate concentration increased from 0.6% to 2.4% (w/v), which is consistent with a previous report where the EE of estradiol decreased with the increase of surfactant concentration, such as cholate, span 80 and tween 80.[17] It was suggested that this effect might be related to the displacement of drug by cholate, or the coexisting micelles formed at higher than critical micelle concentration of sodium cholate (4 mM). Some fraction of arbutin entrapped in mixed micelles may result in the reduction of entrapment efficiency.

EE decreased with the increase of arbutin concentration due to the change of the ratio between arbutin amount and phosphatidylcholine amount, as expected. However, the absolute amount of entrapped arbutin increased from 8.99 μg/mg lipid to 24.48 μg/mg lipid when the arbutin content increased from 0.5% to 5% with the 1.2% of sodium cholate. These results imply that the increased arbutin concentration in the inner aqueous phase resulted in the increase of absolute arbutin amount entrapped in the liposome.

Skin permeation profiles of arbutin

Skin permeation of arbutin in liposomes was performed compared with its aqueous solution at the same concentration. A liposomal formulation with 5% arbutin, 1.2% sodium cholate was selected for the *in vitro* skin permeation studies due to its relatively high encapsulated amount per lipid (24.48 μg/mg lipid). The concentration of sodium cholate [1.2% (w/v)] was chosen based on a literature that recommended the optimal concentration of surfactant.[20] It has been also reported that too low or too high concentrations of surfactants are not beneficial in vesicular delivery of oestradiol through skin.[17] As shown in Figure 2 and Table II, the permeation rate of 5% arbutin in liposomes solution was (8.91±1.33 μg/mL), which was not significantly different from that of 5% arbutin in solution (9.82 ±0.86 μg/mL), probably due to the low EE of arbutin

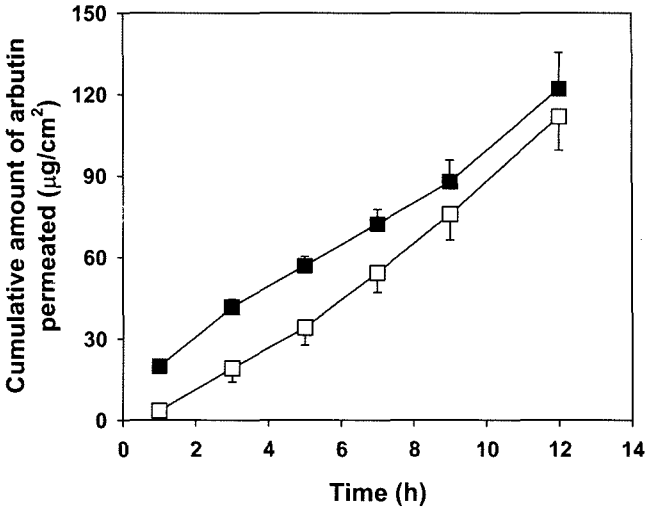

Figure 2–*In vitro* rat skin permeation profiles of arbutin (5%) at 37°C. (□) 5% arbutin in aqueous solution; (■) 5% arbutin in deformable liposomal solution. The bars represent the standard deviation (n=5).

(4.26%, Table I). However, it was interesting to note that the lag time of the permeation profile of arbutin in liposomes was -1.39 (±0.77) h, while that of arbutin solution was 1.11 (±0.38) h, which implies that the liposomes can deliver much more amount of arbutin at the initial stage than aqueous solution, but its driving force seems to decrease after 5 h. Thus, the permeated amount of arbutin showed no significant difference between the liposomal fomulation and aqueous solution at 12 h ($p>0.05$).

However, many reports support that deformable or elastic vesicles can achieve higher skin permeability of lipophilic drugs, such as oestradiol, hydrocortisone, pergolide, than their aqueous solutions.[15-17] It is well known that surfactants in deformable liposomes enhance the skin permeability of chemicals, yet the mechanism(s) is debatable. One proposed mechanism is that the fusion of vesicles with skin lipid in stratum corneum or sebaceous structures of the skin achieved the enhancement of skin permeation.[21,22] Another mechanism is that liposomes with edge-activators can be deformable and can penetrate across the interstices of stratum corneum.[13] On the basis of these hypotheses, deformable liposome was expected

Table II–*Comparison of Parameters After 12-h of in vitro Skin Permeation Study of Arbutin Formulations (5% Arbutin) at 37°C*

Formulation	Permeation rate (μg/cm²/h)	Lag time (hr)	Skin deposition after 12 h (μg/cm²)	
			Stratum corneum	Epidermis/dermis
5% solution	9.82 (±0.86)	1.11 (±0.38)	43.94 (±6.30)	16.84 (±2.76)
5% liposomes	8.91 (±1.33)	-1.39 (±0.77)	43.34 (±12.13)	16.99 (±7.83)

Each data is the mean± standard deviation of five determinations.

to enhance the skin permeability of arbutin. However, there was no significant enhancement of skin permeation of arbutin, which is inconsistent with the results mentioned above. It can be speculated that the EE of arbutin in liposome was only about 5% (Table I) due to its hydrophilicity (logP=−1.49), implying that 95% of loaded arbutin exists in the solution. In the initial stage of skin permeation, liposomes seem to synergistically enhance the arbutin permeation, which resulted in higher driving force for permeation than solution (Figure 2). However, after arbutin in the liposome was depleted, the permeation rate seems to be almost the same as that of arbutin solution. Controversial data were reported that conventional liposomes delayed skin permeation compared with their solutions.[22,23] They claimed that an extra lipid barrier formed by phospholipids on the skin surface may decrease the permeability. The slow drug release from the liposomes or the interaction between the drug and the lipid molecules may be the reason for the decreased permeability. The controversial results may be due to different physicochemical properties of compounds and surfactants in the liposome, which needs further investigation.

Skin deposition of arbutin

The change of arbutin deposition in different skin layers with the various application time is shown in Figure 3. When liposomal solution of arbutin was applied, its deposition in stratum corneum rapidly increased for 3 hours, and maintained the plateau for at least 12 h. Compared with the deformable liposomes, arbutin solution caused the steady increase of deposition both in stratum corneum and epidermis/dermis with the increase of application time. The amount of arbutin in stratum corneum deposited by liposomes were significantly higher than arbutin solution ($p<0.05$) before 6 h, which formed high arbutin depot thereby resulting in higher permeation rate in initial stage (Figure 2). However, the aqueous solution of arbutin gradually increased arbutin concentration in the stratum corneum during the permeation process, and the amount of deposition was not significantly different from that of liposome after 12 h. In epidermis/dermis layer, however, two formulations showed no significant difference, which is due to the sink condition of the receiver solution. Thus, the deformable liposomes showed a special effect on the localization of arbutin in the stratum corneum layer, but not in epidermis/dermis layer. It was proposed that liposomes work as localizers rather than transporters since the deposition of liposomes into the stratum corneum create a reservoir.[24] These results are consistent with several earlier studies for the effect of localization of drugs in stratum corneum by liposomal formulations.[10,25]

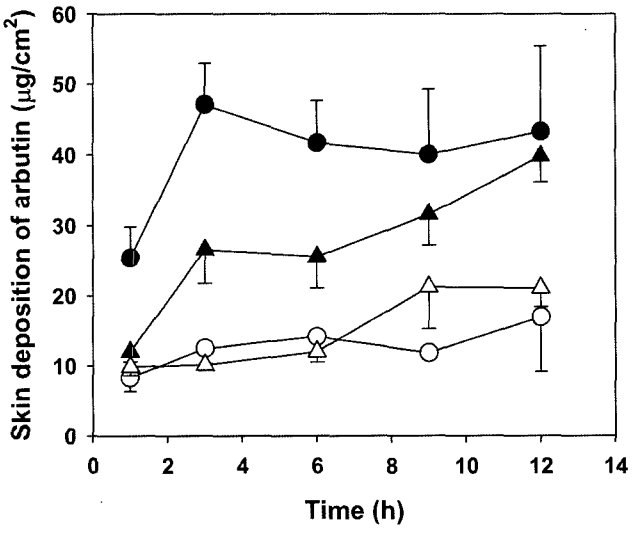

Figure 3–Skin deposition of arbutin at different time points of rat skin permeation at 37°C. 5% arbutin in aqueous solution, (▲) in stratum corneum layer, (△) in epidermis/dermis layer; 5% arbutin in deformable liposomal solution, (●) in stratum corneum layer, (○) in epidermis/dermis layer. The bars represent the standard deviation (n=5).

Conclusions

The deformable liposomes containing sodium cholate significantly enhanced skin deposition of arbutin in stratum corneum compared with its aqueous solution, while the skin permeability for 12 h was not significantly different from aqueous solution. High deposition of arbutin in topical skin layers by deformable liposomes may act as a reservoir for prolonged biological activity. Thus, the deformable liposomes prepared in this study seem to be a promising carrier for skin deposition of hydrophilic skin-whitening agents, such as arbutin.

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