

Note

Development of an Auraptene-Loaded Transdermal Formulation Using Non-ionic Sugar Ester Surfactants

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Received November 19, 2023; accepted February 12, 2024

Auraptene (Aur) is a naturally occurring monoterpene coumarin ether that exhibits numerous therapeutic properties. Its high lipophilicity and low skin penetration, however, limit its potential application for local and transdermal delivery. Biocompatible non-ionic sugar esters (SEs) possess beneficial properties for the development of transdermal formulations in delivering pharmaceutically challenging molecules such as graphene and Aur. In the present study, we conducted a series of experiments to demonstrate the effect of several previously unstudied SEs on the skin permeation and distribution of Aur by preparing gel- and dispersion-type formulations. Skin permeation and deposition experiments were conducted using a Franz diffusion cell with rat skin as the membrane. The dispersion-type formulations prepared using SEs had higher entrapment efficiency, as well as better skin permeation and retention profiles. The dispersion-type formulation containing sucrose palmitate (sSP) exhibited the highest skin permeation over 8 h. Notably, the enhancement effects on Aur concentration in full-thickness skin after the application of the dispersion-type formulation was higher than those of the control formulation. These results indicated that the prepared formulation has potential for use in the transdermal delivery of Aur in pharmaceutical and cosmetic products.

Key words auraptene, sugar ester, transdermal delivery, skin concentration

Introduction

Biocompatible non-ionic sugar esters (SEs), which consist of sucrose as the hydrophilic component and fatty acids, such as stearic, palmitic, myristic, and lauric acids, as the lipophilic part, exhibit a broad range of hydrophilic-lipophilic balance (HLB) values from 1 to 16.¹⁾ These SEs have been used in pharmaceutical applications because they cause less damage to the skin barrier and with reduced irritation potential compared with other surfactants.¹⁻³⁾ Additionally, investigation with the use of SEs incorporated in the formulation as permeation and absorption enhancers increased the penetrating effect of a poorly water-soluble compounds^{1,4-6)} on their delivery through the skin.

Auraptene (Fig. 1, Aur; *MW* 298.38, log *Ko/w* 4.46, and estimated water solubility by EPISuite™ 2.5 mg/L) is a monoterpene coumarin ether that exhibits therapeutic effects.⁷⁾ However, its high lipophilicity and low skin penetration property limit its potential application for local and transdermal delivery. Thus, the value of biocompatible SE in the formulation development should be explored in delivering pharmaceutically challenging molecules such as Aur. In this study, we conducted a series of experiments to demonstrate the effect of several non-ionic SEs containing formulations on the skin permeation and distribution of Aur.

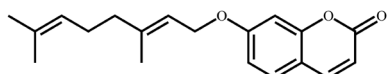


Fig. 1. Chemical Structure of Auraptene

Experimental

Materials Aur (98% purity) was obtained from LTK laboratories, Inc. (St. Paul, MN, U.S.A.). Non-ionic surfactant SEs with different grades: sucrose palmitate P-1670 (SP, HLB = 16), sucrose laurate L-1695 (SL, HLB = 16), sucrose oleate O-1570 (SO, HLB = 15) and sucrose myristate M-1695 (SM, HLB = 16) were purchased from Mitsubishi-Kagaku foods corporation (Tokyo, Japan). Cholesterol and all other chemicals used were of reagent grade and commercially acquired from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). The abbreviation used in the present study was summarized in the supplementary file.

Animals Hairless rats (male, 8 weeks old, 200 ± 15 g, WBN/ILA-Ht strain) were obtained from Josai University Life Science Center (Sakado, Saitama, Japan). The use of animals in this study adhered to the guidelines of the Institutional Animal Care and Use Committee of Josai University (Study Protocol Approval Code: JU20002).

Preparation of Formulations Appropriate amounts of surfactants and cholesterol were weighed out according to the compositions shown in Table 1. Aur was weighed and mixed with 400 μL of ethanol in a glass tube. Then, surfactant as well as cholesterol was added in the ethanol solution. The glass tube was covered and heated over a water bath (70 ± 5 °C) to dissolve the ingredients. Distilled hot water (100 μL) was then added and heated further for 5 min to obtain formulations amounting to 500 μL. After cooling to room temperature, a gel state formulation was obtained, and the formulation codes containing the surfactants SP, SL, SO, SM,

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Table 1. Composition of Aur Proniosomal Formulations

Formulation code	Formulation description	Aur	Sugar esters (SEs ^a)				Sugar ester solutions (SESs ^b) 0.9%				Cholesterol
			SP	SL	SO	SM	SPS	SLS	SOS	SMS	
gSP	Gel-type formulation	513	513	513	513	513	513	513	513	513	57
gSL											
gSO											
gSM											
sSP	Dispersion-type formulation	2.5	513	513	513	513	513	513	513	513	57
sSL											
sSO											
sSM											

Unit: mg. *a*) Melting point of SP, SL, SO, and SM are 48, 47, 43, and 40 °C, respectively. *b*) SESs: sucrose palmitate solution (SPS), sucrose laurate solution (SLS), sucrose oleate solution (SOS), and sucrose myristate solution (SMS).

and so were labeled gSP, gSL, gSM, and gSO, respectively. For the preparation of the dispersion-type formulations, 10 mL distilled water containing 90 mg of SE surfactant was used. The other procedures were the same as the gel state formulations. Briefly, Aur and cholesterol were dissolved in ethanol. Then, SE was added in the ethanol solution. The formulation contained 513 mg SEs, which was equivalent to the amount of SEs in the gel-state formulation. The obtained formulation was warmed to dissolve the ingredients. After cooling to room temperature, the obtained formulation was sonicated three times with a probe sonicator (VCX-750, Sonics & Materials Inc., Newtown, CT, U.S.A.) for 30 s each. The formulation codes containing the surfactants SP, SL, SO, SM, and so were labeled sSP, sSL, sSM, and sSO, respectively. A control formulation was also prepared with the same amount of water added instead of the surfactant (approx. 40% ethanol solution).

Vesicle Size Analysis The vehicle size for Aur-containing dispersion-type formulations underwent 10-fold dilution with distilled water prior to analysis. The vesicle size, polydispersity index (PDI), and zeta potential were measured using a Zetasizer (Malvern Panalytical, Worcestershire, U.K.). All analyses for vesicular size were conducted at 25 °C with three replicates.

Entrapment Efficiency Untrapped Aur was physically separated from Aur-containing dispersion-type formulations by ultracentrifugation (80000 rpm at 4 °C for 45 min) using an ultracentrifuge (Hitachi Koki Co., Ltd., Tokyo, Japan). The untrapped Aur in the supernatant was collected and diluted with ethanol and then quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Entrapment efficiency (*EE*) studies were performed in triplicates, and data were expressed as the mean $\pm$ standard error (S.E.).

Cryo-Transmission Electron Microscope (TEM) The morphology of the resulting formulations was observed using cryo-TEM. The formulation was placed on a hydrophilized copper grids (Akishima, Tokyo, Japan) and blotted. The sample was rapidly frozen using a freezing system (EM-CPC, Leica microsystems Japan, Tokyo, Japan), then visualized using an electron microscope (JEM-3100FEF, JEOL Ltd., Tokyo, Japan) at an accelerating voltage of 300 kV. The observation was carried out using a cooling holder (Gatan, CA, U.S.A.).

In Vitro Release Experiment *In vitro* release of Aur from formulations was evaluated using a diffusion cell with a sheet of dialysis membrane (molecular weight cut-off;

2000–14000 Da) (Sanko Junyaku Co., Tokyo, Japan) over 8 h at 32 °C. The sample (1.5 mL) was applied onto the membrane in the donor compartment, and the receptor compartment was filled with 6.0 mL of receiver solution composed of ethanol-water [50:50 (v/v)].

In Vitro Skin Permeation Experiment The excised hairless rat abdominal skin with a thickness of $630 \pm 33.9 \mu\text{m}$ (measured with a thickness gage, Teclock corporation, Nagano, Japan) was then used. Permeation of Aur from prepared formulations was investigated using a diffusion cell at 32 °C. The receiver solution was composed of ethanol-water [50:50 (v/v)] to maintain a sink condition. Formulations (1.5 mL) were applied to the donor compartment. Parafilm was used to cover the donor compartment to maintain the characteristics of the formulation.

After the permeation experiment, the membranes were de-mounted from the diffusion apparatus. The skin was rinsed with 1 mL of phosphate buffered saline (PBS) ten times from each side. The skin samples were halved, the other half was tape-stripped (20 times) to remove the stratum corneum (SC) for the quantification of Aur deposited in the viable epidermis and dermis (VED) layers. The skin samples were weighed to 0.05 g accuracy and cut with scissors before adding 450 μL PBS. The skin was processed on ice using a homogenizer. Then, acetonitrile (500 μL) was added to the homogenate, then mixed for 5 min using a vortex mixer and centrifuged at 15000 rpm at 4 °C for 5 min. The supernatant was collected and assayed using LC-MS/MS. All experiments were carried out with 4 replicates ($n = 4$).

Quantification of Aur by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) The concentration of Aur in samples were quantified using an LC-MS/MS system with positive ion electrospray ionization. The sample aliquot of 100 μL was added to 100 μL of acetonitrile and subjected to centrifugation at 15000 rpm at 4 °C for 5 min (Hitachi Koki Co., Ltd., Tokyo, Japan). The resulting sample was injected into the LC-MS/MS system, equipped with an autosampler and a LC-20 ad pump (Shimadzu, Kyoto, Japan), and a 3200 Q Trap[®] LC-MS/MS system (Applied Biosystems, Foster, CA, U.S.A.). Chromatographic separation was performed using shodex ODP2 HP-2B and ODP2 HPG-2A (2.0 mm i.d. $\times$ 50 mm and 2.0 mm i.d. $\times$ 10 mm, both from Showa Denko K.K., Tokyo, Japan) at 40 °C with a flow rate of 0.2 mL/min. The mobile phase was composed of 0.1% formic acid:acetonitrile [1:1 (v/v)]. Quantification of Aur was carried out in

Table 2. Size, PDI, Zeta Potential and Entrapment Efficiency (%)

Formulation code	Diameter ^{a)} (nm)	PDI ^{b)}	Zeta potential ^{b)} (mV)	EE (%) ^{a)}
sSP	1.48×10^2	0.80 ± 0.06	-30.5 ± 0.24	98.5
sSL	8.32×10^2	0.77 ± 0.19	-26.4 ± 0.87	99.0
sSO	8.32×10^2	0.72 ± 0.20	-30.9 ± 0.54	98.6
sSM	6.90×10^2	0.81 ± 0.09	-25.3 ± 0.70	98.3

a) Average in nm ($n=3$). b) Each value represents the mean $\pm$ standard deviation (S.D.) ($n=3$).

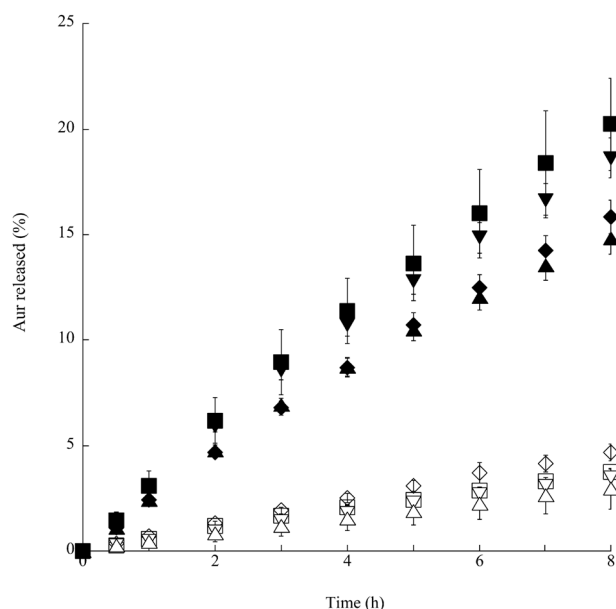


Fig. 2. Time Course of the Percentage of Aur Released from the Formulation

Each value represents the mean $\pm$ S.D. ($n=3-4$). When Aur solution (control) was applied, almost 100% release was observed within a few hours (data not shown). Symbols: $\square$: gSP, $\diamond$: gSL, ∇ : gSO, $\triangle$: gSM, $\blacksquare$: sSP, $\blacklozenge$: sSL, $\blacktriangledown$: sSO, $\blacktriangle$: sSM.

multiple reaction monitoring mode, monitoring the transition of precursor ions from 299.1 m/z to 163.1 m/z in an ion transfer tube temperature of 700 °C, a spray voltage of 5.5 kV and collision energy at 15 eV. The lower limit quantification of this assay was 100 ng/mL.

Statistical Analysis Tukey–Kramer HSD test and Dunnett's test after rejection test using JMP[®] pro 17.0.0 (SAS Institute Inc., NC, U.S.A.) were used in the analyses. The reported p -values were considered statistically significant at $p < 0.05$. All animal experiments with control formulation were always conducted to negate inter-experimental effects.

Results and Discussion

The particle size, PDI, zeta potential, and entrapment efficiency of prepared dispersion-type formulations are shown in Table 2. Figure 2 shows the Aur release profile from the prepared formulations. Aur release from gel-type formulations (gSP, gSL, gSO, and gSM) were lower than those from dispersion-type formulations (sSP, sSL, sSO, and sSM). The dispersion-type formulation of pSP showed 20.2% release of entrapped Aur over 8 h, which was the highest amount released among the formulations, but no significant difference in Aur release was confirmed compared with pSO, which had the second highest release (average 18.6%).

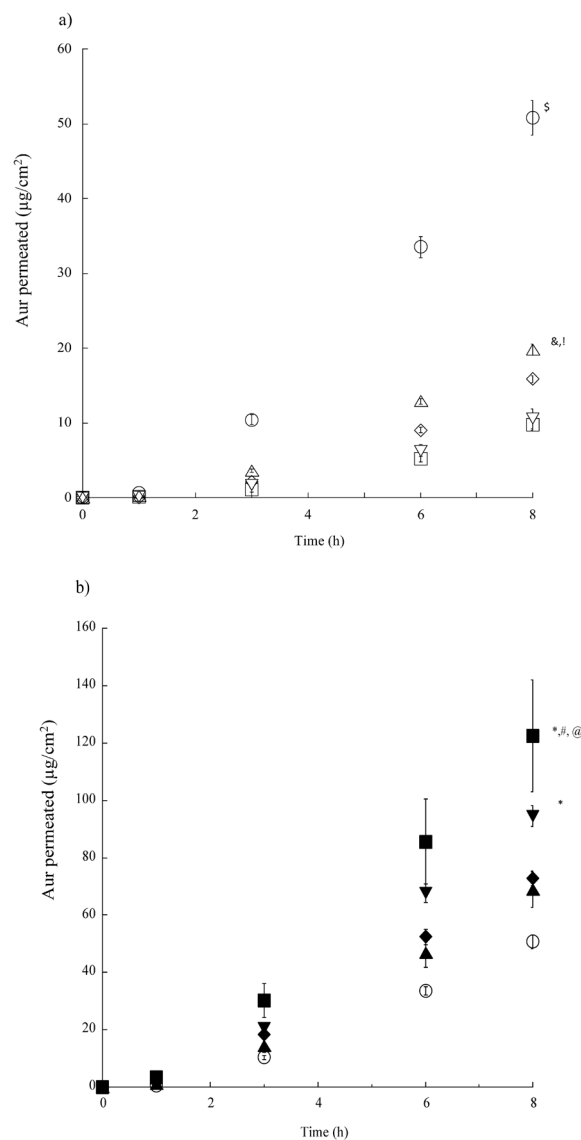


Fig. 3. Time Course of Aur Permeated through Hairless Rat Skin after Application of Gel-Type (a) and Dispersion-Type (b) Formulations

Each value represents the mean $\pm$ S.D. ($n=3-4$). $\circ$: control, $\square$: gSP, $\diamond$: gSL, ∇ : gSO, $\triangle$: gSM, $\blacksquare$: sSP, $\blacklozenge$: sSL, $\blacktriangledown$: sSO, $\blacktriangle$: sSM. $^{\circ}p < 0.05$: gel-type formulations vs. control, $^{\&}p < 0.05$: gSM vs. gSP, $^{\dagger}p < 0.05$: gSM vs. gSO, $^{\ast}p < 0.05$: sSP vs. control, $^{\#}p < 0.05$: sSP vs. sSL, $^{\textcircled{a}}p < 0.05$: sSP vs. sSM.

Figure 3 depicts the skin permeation profiles of Aur. The gel type formulations were initially investigated (Fig. 3a). These formulations showed a significantly lower skin permeation of Aur compared with Aur prepared by approx. 40% ethanol solution (control solution). When Aur permeation was compared among the gel-type formulations, following order was confirmed; gSL > gSP > gSO $\approx$ gSM. Subsequently, dispersion-type formulations were investigated. The cumulative amount of Aur that permeated from the formulations showed the following order: sSP > sSO > sSL $\approx$ sSM (Fig. 3b). Notably, these formulations showed higher Aur permeation compared with the control solution. The skin enhancement effect calculated with the cumulative amount of Aur permeated at 8 h was 2.4-, 1.8-, 1.4-, and 1.4-fold for sSP, sSO, sSL, and sSM, respectively.

Figure 4 shows the skin concentration of Aur. The dispersion-type formulations showed a higher concentration of Aur

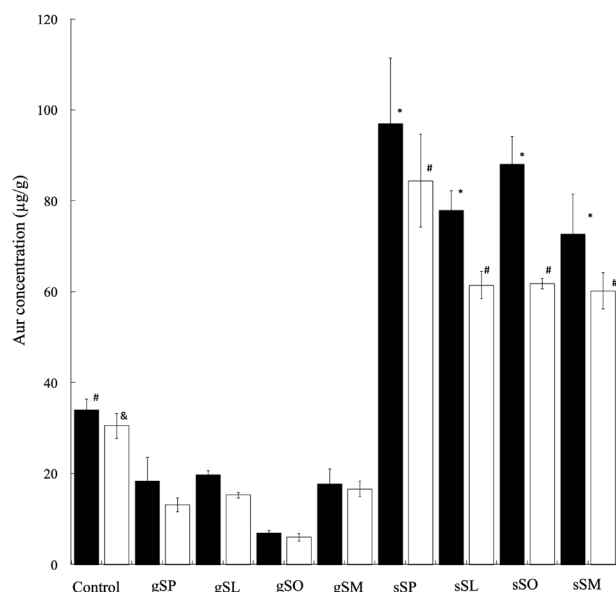


Fig. 4. Skin Concentration of Aur in Intact Skin (■) and Viable Epidermis and Dermis (VED) (□)

Each value represents the mean $\pm$ standard error ($n=4$). # and & $p<0.05$: gel-type formulations vs. control (intact skin and VED, respectively), * and # $p<0.05$: dispersion-type formulations vs. control ((intact skin and VED, respectively).

in intact skin as well as VED, compared with the control and gel-type formulations. The Aur concentration in VED obtained from the dispersion-type formulations was above $60\mu\text{g/g}$. The enhancement effects in the Aur concentration in full-thickness skin were 3.1-fold for sSP, 2.9-fold for sSO, 2.3-fold for sSL, and 2.0-fold for sSM. Almost the same enhancement effect of Aur concentration in VED was also confirmed (3.1-fold for sSP, 2.8-fold for sSO, 2.1-fold for sSL, and 2.0-fold for sSM). The skin concentration at the steady state (C_{ss}) is expressed with $KC/2$, where K is partition coefficient of chemical compounds from the formulation to the skin and C is applied chemical concentration.⁸⁾ This indicates that chemical diffusivity in the skin, D , is not related to the skin concentration at steady state. On the other hand, skin permeation is related to the product of the K and D values.⁹⁾ Therefore, the dispersion-type formulation would increase Aur permeability by increasing its K value in addition to increasing D value. Therefore, especially for SP and SP would have a potential as a chemical enhancer to increase skin permeation as well as skin concentration for lipophilic drugs. Although ethanol is known as a chemical permeation enhancer by disrupting of barrier function of the SC,^{10,11)} but reduced skin permeation as well as skin concentration were observed in the gel formulation. In the gel-type formulation, Aur release, which was related to diffusivity in the formulation, was lower than the dispersion-type formulation. Therefore, Aur release might be involved in the skin permeation enhancement effect.

Aur has biological activities such as inhibition effects on melanogenesis and anti-cancer effects. The obtained concentration in the VED (above $60\mu\text{g/g}$) is enough to expect these effects when the concentration data were referenced against reported data for median effective concentration and median inhibition concentration.^{12–15)}

The morphology of sSP is shown in Fig. 5. TEM observations revealed the presence of both several unilamellar vesicles. These nanosized particles are capable of interacting



Fig. 5. TEM Observation of sSP

with SC which widens the intercorneocyte gap and reduces the degree of corneocyte packing.^{16,17)} In addition, the contact area of the formulation on the skin surface generally increases with the application of vehicles.¹⁷⁾ Thus, a higher skin permeation enhancement effect may be obtained by the application of dispersion-type formulation.

Conclusion

Dispersion-type formulations were capable of increasing the skin permeation as well as the skin concentration of Aur, suggesting that formulation used in the present study have the potential for use in transdermal delivery of Aur in pharmaceutical and cosmetic products. However, further experiments are needed to clarify the mechanism of skin penetration enhancement of Aur.

Acknowledgments Kathrine Anne Flores is a recipient of a foreign graduate scholarships in specialized priority fields in science and technology by the Department of Science and Technology (DOST)-Science Education Institute (SEI), Republic of the Philippines.

Funding This research was supported by Josai University and has not received any external funding.

Conflict of Interest The authors declare no conflict of interest.

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