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Surfactant-induced membrane dynamics and skin irritation

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Introduction:

Background: We have been studying clarification of the correlation between the irritation of surfactants and the membrane dynamics of cell-sized liposomes. However, regarding the surfactant-induced membrane dynamics, the characteristics of each contributing surfactant and the specific mechanism at the molecular level have not yet been clarified, and an alternative method for the Draize test [1,2] has not been developed yet.
Method: Cell-sized liposomes composed of unsaturated phospholipids (1,2-dioleoyl – sn-glycero-3-phosphocholine) were prepared, and the membrane dynamics of liposomes after the addition of various surfactants was observed using laser confocal microscopy and types of morphological changes of liposomes were classified.
Results and Discussion: Eight different types of morphological changes could be classified dependent on intensity of stimuli induced by various surfactants.
An increase in excess surface area of liposomes occurred for the addition of strongly irritating surfactants (ex. Triton X-100), while they shrinkage was observed by adding low or no irritating surfactants (ex, Tween20, amino acid surfactants).
Therefore, we concluded that our method is applicable as an alternative method to animal test such as Draize test[1]. We considered that membrane dynamics depends on the rate of Vflip-flop, and Vout. We further used our method to characterize low irritating mixed systems of surfactants such as sodium cocoyl glutamate and mixture of anionic surfactant and amphoteric surfactant by comparing the result with stinging test.
Key words: Liposome, Membrane dynamics, Surfactants, Irritation, Alternative method

Materials & Methods:

Chemicals :Phospholipid, 1,2-dioleoyle-sn-glycero-3-phosphocholine (DOPC) was from Avanti Polar Lipids. Rhodamine B, 1,2-dihexadecanoyl-sn-glycero-3-phospho-ethanolamine was used as a fluorescent probe,. TritonX-100, Tween20, and Tween80 were from Nacalai Tesque, Santa Cruz Biotechnology, Kanto Chemical Co., respectively. The amino-acid surfactants and amphoteric surfactant, cocamidpropyl betaine were from Asahi Kasei Chemicals.
Preparation of liposomes: Liposomes were prepared by the natural swelling method. DOPC and Rhodamin-DHPE were dissolved in chloroform/methanol, and the concentrations were 2 mM and 0.1 mM, respectively. Glucose was dissolved in methanol and the concentration was 10mM. Chloroform 10μl, DOPC 20μl, Rhodamine-DHPE 8μl, and glucose 12μl were mixed and the solution was dried under vacuum for 3 hours to form thin lipid films. The films were hydrated with 200 μl water at 37 ℃.
Microscopic observation: The prepared liposomal solution was placed in the lower compartment, and 32 μl of surfactant solution was added to the upper compartment. The confocal laser microscope was used for observation.
Stinging test: The test was conducted by (1) Wipe off the back of the neck with a tissue soaked in purified water. (2) Apply non-woven gauze permeated with 1 mL of the sample with surgical tape. (3) Hearing was conducted every minute immediately after application. (4) Record the situation up to 10 minutes later.

Results & Discussion:

Membrane dynamics: Eight different types of morphological changes could be classified dependent on intensity of stimuli by various surfactants (Fig. 1)[3].

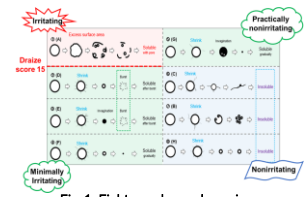


Fig. 1: Eight membrane dynamics

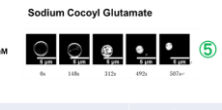
An increase in excess surface area of liposomes occurred for the addition of strongly irritating surfactants (ex. Triton X-100), while their shrinkage was observed by low and non-irritating surfactants (ex, Tween 20, amino acid surfactants depending on the intensity of stimula. Theref we concluded that our method is applicable as an alternative method to animal tests [3,4].

In order to acquire excess surface area, the surfactant molecules added from outside of liposome should be included in the inner leaflet of bilayers. If the surfactants in the outer leaflet moved to the inner leaflet (flip-flop), the bilayer obtained the excess surface area. Therefore, membrane dynamics depends on the rate of Vflip-flop, and Vout (Fig. 2).

Fig. 2: Three velocities in membrane dynamics

Comparison between sodium cocoyl-glutamate and sodium lauryl-glutamate:
We further used our method to characterize low irritating mixed systems of surfactants such as sodium cocoyl glutamate and mixture of anionic surfactant and amphoteric surfactant by comparing the result with stinging test.
Coconut oil is composed of about 90% saturated fatty acids, most of which are "medium-chain" fatty acids mainly lauric acid (C12). Sodium cocoyl-glutamate is an anionic surfactant composed of coconut oil fatty acids and glutamic acid.
This biodegradable surfactant is excellently suited for sensitive and allergic skin and has an extremely low irritation potential.

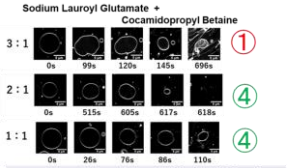
In order to clarify low irritation potential of sodium cocoyl-glutamate, irritation induced by sodium lauroyl-glutamate was evaluated by both membrane dynamics and human stinging test (Fig. 3).



Stinging test	Maximum Stimulus score	Stinging test	Maximum Stimulus score
Sodium Cocoyl Glutaminate (28mM)	17.0	Sodium Lauroyl Glutamate (28mM)	17.5

Fig. 3: Comparison between sodium cocoyl glutamate and sodium lauroyl glutamate

Mixture of anionic surfactant and amphoteric surfactant:
Cocamidopropyl betaine is amidopropyl dimethylamino acetate betaine chemically synthesized from fatty acids obtained from coconut oil. It is an amphoteric surfactant with a molecular weight of 342.5. Cocamidopropyl betaine is often used in cosmetics to increase detergency and improve foaming in combination with anionic surfactants and also to relieve irritation from strong anionic surfactants.
We tested irritation caused by mixture of sodium lauroyl glutamate and cocamidopropyl betaine at different mixing ratio, 3:1. 2:1 and 1:1 using both membrane dynamics and stinging test (Fig. 4). Results of membrane dynamics fit very well to the results of stinging test.



Stinging test	Maximum Stimulus score
Sodium Lauroyl Glutamate +Cocamidopropyl Betaine (3:1)	11.0
Sodium Lauroyl Glutamate +Cocamidopropyl Betaine (2:1)	10.0
Sodium Lauroyl Glutamate +Cocamidopropyl Betaine (1:1)	9.0

Fig. 4: Characterization of mixed system with amphoteric surfactant

The membrane dynamics of high-irritating surfactant and four relatively higher-irritating surfactants in low- and non-irritating surfactants showed the solubilization at the final state (Dynamics 2-5). On the other hand, we could not find the solubilization in the cases of three dynamics of low- and non-irritating surfactants (Dynamics6-8). Therefore, the solubilization process is important to evaluate the degree of irritation roughly. Additionally, two dynamics out of four membrane dynamics of relatively higher-irritating surfactants in low- and non-irritating surfactants (Dynamics 2 and 3) showed the burst process. The surfactants which showed such burst behavior might have higher irritation. Therefore, the burst can be an important process in the evaluation of the surfactant irritation. We considered that the solubilization and the burst processes will become crucial indicators to evaluate the degree of irritation of surfactants.

Conclusions:

【1】 There are two types of membrane dynamics, inflate (Draize score higher than 15) and shrinkage (Draize score less than 15).

【2】 Membrane dynamics can be characterized by 3 velocities (Vin, Vout and VFlip-Flop).

【3】 There was a good correlation between the results of the stinging test and the dynamics of liposome

【4】 Lauroylglutamate (C12) (main composition of cocoyl glutamate) is more irritating than sdium cocoyl glutamate, indicating that surfactants other than C12 may be effective to reduce irritation.

【5】 Amphoteric surfactants is effective to reduce irritation of an anionic surfactant, possibly because of ionic interaction and enlargement of apparent molecular weight (Coacervation?)

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