J. Soc. Cosmet. Scientists Korea
Vol. 30, No. 1, May 2004, 29-32

pH 감응형 나노입자를 이용한 멜라닌 합성저해 연구

박 주 영† · 최 현 정 · 심 종 원 · 안 수 미 · 김 준 오 · 장 이 섭

태평양 기술연구원 피부과학연구소

Inhibition of Melanin Synthesis by Enhanced Cytosolic Delivery of N-glycosylation Inhibitors Using pH-Sensitive Nano-carrier

Ju Young Park†, Hyun-Jung Choi, Jong Won Shim, Soo Mi Ahn, Junoh Kim, and Ih-Seop Chang

Skin Research Institute, Amore Pacific R&D center, 314-1, Bora-ri, Giheung-eup, Yongin-si, Gyeonggi 449-729, Korea

요 약: 비형질 세망 조직에서 N-글리코실레이션 과정의 초기 단계를 차단하면 멜라닌 생 합성의 주 효소인 티로시나제의 활성이 저해된다. 본 연구에서는 *in vitro* 환경에서 N-글리코실레이션 저해제의 활성을 증가시키고자 전달체로 pH 감응성을 갖는 나노 크기의 지질구조체를 제조하고 이를 평가하였다. 이 pH 감응성 지질구조체 Melexsome은 일반적인 지질성분인 포스포리피드와 콜레스테롤 기반의 지질안정 성분으로 구성되며, 통상적인 리포좀 제조법에 따라 제조되었다. 글리코실레이션 저해 성분물질을 포집시킨 Melexsome의 효과는 EndoH & PNGaseF 분해와 western blotting 방법에 의해 평가하였고, 멜라닌 합성량 또한 측정되었다. 이 결과, pH 감응성을 갖도록 제조된 Melexsome이 N-글리코실레이션 저해제의 효능을 효과적으로 증진시킴을 알 수 있었다. 또한, 공초점 주사 현미경에 의한 세포관찰 결과에 따르면 Melexsome은 여타의 전달체에 비하여 세포질 내에 보다 효과적으로 전달되는 것으로 보여지며, 따라서 이같은 양친성 지질성분 기반의 pH 감응성 나노 전달체는 N-글리코실레이션 저해제의 전달 시스템으로서 미백 화장료 제품이 가져야 하는 침착된 색소에 의해 어두워진 피부톤의 개선 효과를 극대화 시키는데 적합하다고 여겨진다.

Abstract: Inhibition of the early N-glycosylation process in the endoplasmic reticulum prevents the activation of tyrosinase, a key enzyme for melanin biosynthesis. This work aims at evaluating the increased activity of N-glycosylation inhibitors *in vitro* by employing a nano-sized pH-sensitive liposome as a delivery carrier. Melexsome, a pH-sensitive nano carrier loaded with glycosylation inhibitos, was prepared by the hydration method with phospholipids and cholresterol-based amphiphiles. Inhibitory effects of Melexsome on the N-glycosylation process were evaluated by EndoH & PNGaseF digestion and the western blotting. Melanin synthesis was also monitored after treatment with Melexsome. Interestingly, Melexsome effectively increased the efficacy of N-glycosylation inhibitors. Melexsome was also much more efficiently translocated into the cytoplasm as observed in CLSM. These results demonstrated that the amphiphilic lipid-based pH-sensitive nano-carriers could be used as an efficient delivery system for N-glycosylation inhibitor to enhance the effects of skin whitening cosmetics.

Keywords: *melanin synthesis, N-glycosylation inhibitor, pH-sensitive liposome, pigment-lightening*

1. Introduction

It has been known that α-glucosidase I activity is interfered and glycans are arrested as glucosylated structures and undergo no further pigmentation process in the presence of N-glycosylation inhibitors. N-glycosylation process occurs in early endoplasmic reticulum of melanocytes. Therefore, N-glycosylation inhibitors should be delivered to endoplasmic reticulum in order to suppress the maturation of tyrosinase. Melexsome, a nano-sized and pH-sensitive lipid-based carrier was designed to efficiently deliver N-glycosylation inhibitors to the intracellular active site and to minimize the degraded amounts of N-glycosylation inhibitors by the endosomal and lysosomal process.

† 주 저자 (e-mail: jypark731@amorepacific.com)

2. Experiments

Deoxynojirimycin (DNJ) and N-butyldeoxynojirimycin (NB-DNJ) (Figure 1) were used as N-glycosylation inhibitors. To deliver N-glycosylation inhibitors to endoplasmic reticulum, a pH-sensitive Melexsome incorporated with cholesterol-based amphiphiles was used. Multi-lamellar vesicles (MLV) were prepared by the hydration method. Melexsome was composed of the appropriate amount of phospholipids and cholesterol-based amphiphiles. A usually used molar ratio of lipid and amphiphiles was 3 : 2. Briefly, a mixture of lipids in chloroform/methanol (95 : 5) was dried using a rotary evaporator under reduced pressure. Dried lipids were hydrated with PBS containing active compounds to be loaded. Hydrated lipid films were sonicated using a bath-type sonicator at room temperature. PEG-5 rapeseed sterol was used instead of cholesterol-based amphiphiles as a compound of control liposome. pH-sensitivity of Melexsome was evaluated at various pH by the measurements of turbidity using their absorbance at 550 nm. Size distribution of the resulted Melexsome was examined by dynamic light scattering (Zetasizer 3000 HS, Malvern, UK).

HM3KO, a human melanoma cell line was cultured with Minimum Essential Medium (MEM) supplemented with 1% (v/v) antibiotics (streptomycin, 10,000 g/mL; penicillin, 10,000 IU/mL) and 10% (v/v) fetal bovine serum (Gibco BRL, Gaitthersburg, MD) at 37℃ in a humidified atmosphere containing 5% CO_2. Pigment-lightening effects were measured by the method of EndoH & PNGaseF digestions and the western blotting. Melanin synthesis was monitored by the absorbance at 490 nm.

Cells grown to 80% confluency were incubated with the Dextran-rhodamine B (Ex/Em = 572/589) loaded Melexsome and control liposomes containing 1% Fluorescein-DHPE (Ex/Em = 496/519) for 1 hr. After the incubation with liposomes, the cells were washed with PBS, incubated with 2 g/mL DAPI (Ex/Em = 358/461) in PBS for 2 min to stain the nucleic acids and again washed with excess PBS. For the fixation, pre-cooled methanol at -20℃ was added to the cells for 5 min. After removal of methanol, slides were air-dried for 30 min and treated with ProLong Antifade solution (Molecular Probes, Eugene, OR, USA) to prevent a photo bleaching of fluorescent materials. Mounted slides were kept in the dark at 4℃ until microscopic observation. The cells were examined by using a confocal laser scanning microscope (CLSM, Radiance 2000/MP, Bio-rad) to monitor the cellular delivery enhanced by Melexsome.

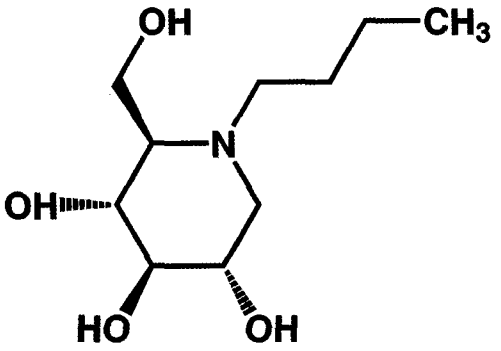

Figure 1. Chemical structure of NB-DNJ.

3. Results and discussion

The size distribution of pH-sensitive Melexsome was evaluated by dynamic light scattering as shown in Figure 2. The mean diameter of the prepared Melexsome was in the range of 150~300 nm depending on the concentration of the loaded glycosylation inhibitors.

pH-sensitive characteristics of Melexsome was evaluated at various pH by the turbidity measurements using their absorbance at 550 nm. Turbidity of Melexsome was suddenly increased below pH 6.0 that is early endosomal pH in cytoplasm. After then, liposomal structure of Melexsome was collapsed below pH 5.0, lower range of the endosomal pH. Note that a cholesterol-based amphiphile renders the vesicles pH-sensitive; it stabilizes the lipid vesicles above neutral pH, while induces Melexsome's structural instability via its own protonation at acidic pH and even shows a fusogenic behavior.

As following the examination by the EndoH & PNGaseF digestions, the western blotting (Figure 4(a)) and the measurement of melanin biosynthesis (Figure 4 (b)), pigment-lightening effects (Glycosylation inhibiting; GI) were efficiently enhanced by the incorporation of Melexsome. Figure 4(a) shows that GI effects of 50 M NB-DNJ loaded Melexsome was superior to that of the 50 M intact NB-DNJ. Interestingly, it was found

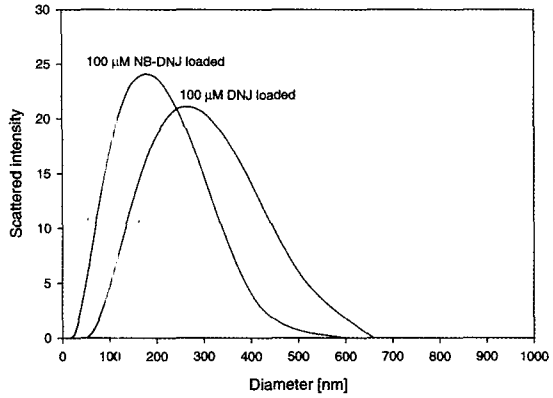

Figure 2. Size distribution of Melexsome.

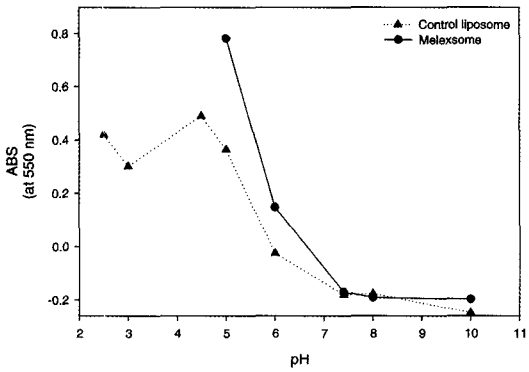

Figure 3. Turbidity of Melexsome and control liposome at various pH.

that the pH-sensitive Melexsome effectively increased the efficacy of N-glycosylation inhibitors. In addition, with the same drug-loading yield, the inhibitory effects of the drugs loaded within the pH-sensitive Melexsome were superior to those of conventional liposomes. The pH-sensitive Melexsome was also found to much more efficiently translocat into the cytoplasm, as observed in CLSM study using fluorescein (Ex/Em = 496/519) labeled liposomes loaded dextran-rhodamine B (10,000 MW, Ex/Em = 572/589). These results indicate that the improved efficacy of the N-glycosylation inhibitors within the Melexsomes might be due to the increased translocation efficiency to the cytoplasm.

4. Conclusions

It was demonstrated that cholesterol-based amphiphiles incorporated Melexsome could be used as an efficient delivery system for N-glycosylation inhibitors to target

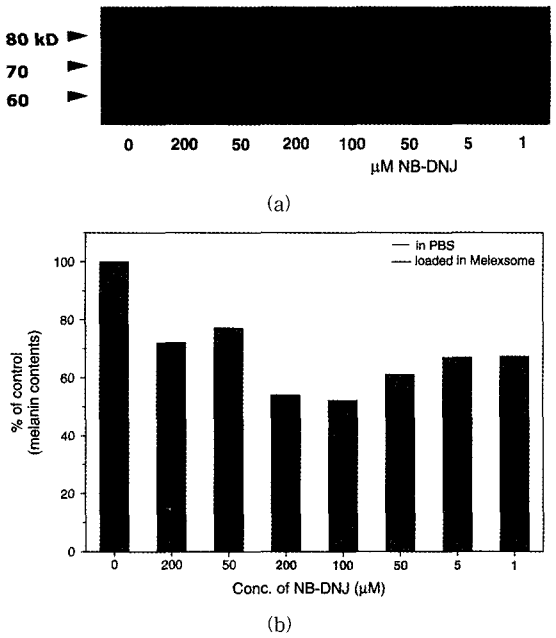

Figure 4. Pigment-lightening effects evaluated by the western-blotting (a) and reduced melanin biosynthesis (b).

cytoplasmic site. Thus, Melexsome has promising potentials as a nano-sized delivery carrier for functional cosmetic formulations to enhance the pigment lightening effects of whitening-active ingredients.

5. Acknowledgment

This work is supported in part from the National Research Laboratory (NRL) program (Project No. 2000N-NL-01-C-270) by the Ministry of Science and Technology, Republic of Korea. The authors thank S. H. Kwon for the CLSM analysis (NICEM, Seoul National University).

References

1. S. Simões, V. Slepushkin, N. Düzgünes, and M. C. Lima, On the mechanisms of internalization and intracellular delivery mediated by pH-sensitive liposomes, *Biochimica et Biophysica Acta,*, **1515**, 23 (2001).
2. I. M. Hafez and P. R. Cullis, Cholesteryl hemisuccinate exhibits pH sensitive polymorphic phase behavior, *Biochimica et Biophysica Acta,* **1463**, 107 (2000).

3. G. E. Costin, M. Trif, N. Nichita, R. A. Dwek, and S. M. Petrescu, pH-sensitive liposomes are efficient carriers for endoplasmic reticulum-targeted drugs in mouse melanoma cells, *Biochemical and Biophysical Research Communications*, **293**, 918 (2002).
4. N. Düzgüne and S. Nir, Mechanisms and kinetics of liposome-cell interactions, *Advanced Drug Delivery Reviews,* **40**, 3 (1999).
5. O. V. Gerasimov, J. A. Boomer, M. M. Qualls and D. H. Thompson, Cytosolic drug delivery using pH- and light-sensitive liposomes, *Advanced Drug Delivery Reviews,* **38**, 317 (1999).