Inhibition of the pigmentation process using N-glycosylation inhibitors loaded pH-sensitive liposomes

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Abstract

Inhibition of the early endoplasmic reticulum N-glycosylation process induces the inactivation of tyrosinase, the key enzyme for melanin biosynthesis. It has been known that alpha-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.

This work aims at evaluating the increased activity of N-glycosylation inhibitors delivered by the nano-carriers, pH-sensitive liposomes into human melanoma cells. Multi-lamellar vesicles (MLV) were prepared by the hydration method. Liposomes were composed of cholresteryl hemisuccinate (CHEMS) and lipids with the appropriate mixing ratio. Note that CHEMS can stabilize lipids such as dioleoylphosphatidylethanolamine (DOPE) at neutral pH. But, these liposomes become unstable due to the protonation of CHEMS at acidic pH and show fusogenic behaviors. A mean diameter of the prepared liposomes was measured by dynamic light scattering. Relatively small amount of N-glycosylation inhibitors such as castanospermine, deoxynojirimycin (DNJ) and N-butyl deoxynojirimycin (NB-DNJ) were loaded into pH-sensitive liposomes. Drug loaded liposomes were incubated with HM3KO (human melanoma cell line) for following 5 days. Culture medium containing drug loaded liposomes was renewed every two days. Inhibitory effect on N-glycosylation process in HM3KO was evaluated by the EndoH & PNGaseF digestions and the western blotting. In addition, melanin synthesis was monitored by the absorbance at 490 nm. Efficacy of drugs to interfere with the pigmentation processing in mammals was increased by the intracellular delivery using nano-sized pH-sensitive liposomes. In the presence or absence of CHEMS incorporated into

liposomes, intracellular delivery of fluorescein (Ex/Em = 496/519) labeled liposomes loaded dextran-rhodamine B (10,000 MW, Ex/Em = 572/589) was observed after the incubation with HM3KO, using a confocal laser scanning microscope to confirm the major rule of CHEMS for the pH-sensitive intracellular carrier. These results imply that the entrapment of N-glycosylation inhibitors in pH-sensitive liposomes is particularly attractive for therapeutic applications in pigmentation processes and pH-sensitive liposome, itself is promising for the efficient intracellular drug delivery vehicles. (Financial supports: IMT-2000 R&D, project No. 01-PJ11-PG9-01NT00-0050 in Republic of Korea)

References

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