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Effect of lipid composition on incorporation of trastuzumab-lipid-PEG micelles into liposomes and association of the immunoliposomes in HER-2 positive breast cancer cells

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Background and Aims: Regarding several advantages of active targeting against HER-2 receptor in breast tumor cells, we aimed to develop and characterize nano-immunoliposomes by incorporation of lipid-core polymeric micelle (PEG-PE) covalently attached to trastuzumab, humanized recombinant mAb against HER-2, into nanoliposomes of different compositions. The incorporation level and specific cell association were compared in breast tumor cells.
Methods: Different liposomal formulations at different mole ratios of DPPC, DPPG, PEG-PE, cholesterol and 1% rhodamine-PE fluorescent lipid probe were prepared by thin layer hydration followed by extrusion through 100nm double-stacked filter. PEG-PE-Maleimide/PEG-PE (1:4 mole ratio) micelles were prepared the same followed by vortexing and sonicating. Trastuzumab was monothiolated by Traut's reagent at controlled condition and purified by ultrafiltration. Thiolated antibody was conjugated with PEG-PE-maleimide micelles. Immunomicelles were incorporated into the nanoliposomes by post-insertion method. The incorporation level was determined by lipid mixing assay based on FRET of rhodamine-PE at self-quenching concentration. Specific breast cancer cell association was studied with and without pre-incubation with trastuzumab in MCF-7 (HER-2, 1+) and SKBR3 (HER-2, +3) cells by flow cytometry and fluorescent microscopy.
Results: Monothiolated trastuzumab was successfully bioconjugated (> 90%) to PEG- phospholipids micelle at maleimide/thiol mole ratio of 5:1 which was determined by Ellman's reagent. The micelles and the nanoliposomes had sizes of 50-80 nm. The level of incorporation of micelles into the liposomes was: DPPC/DPPG (55:45) > DPPC/PEG-PE/cholesterol (50:5:45) > DPPC/cholesterol (55:45) and DPPC/PEG-PE (95:5). The immunoliposomes specifically target SKBR3 than MCF-7 cells which was more pronounced for DPPC/PEG-PE/cholesterol > DPPC/DPPG and DPPC/PEG-PE > DPPC/cholesterol liposomes. The results of lipid mixing assay were in agreement with those of the cell association.
Conclusions: The extent of immunomicelle incorporation into liposome and the level of specific cell association depend on the liposome lipid composition.

Keywords: Trastuzumab; Nano-immunoliposomes; Lipid mixing