CHARACTERISATION OF FLUORESCENT NBD-CHOLESTEROL EFFLUX IN THP-1 DERIVED

Wei Wang, Wei Song, Xiaowei Yan. Peking Union Medical College Hospital

Objectives

Macrophage cholesterol efflux plays an important role in maintaining cellular lipid homeostasis, and preventing cells from formation of lipid-laden foam cells. Although radioactive [3H]-
cholesterol was widely used as a tracer in cholesterol efflux assay, time- and labour-consuming assay procedure and radioactivity disposal procedure may limit its use in high-throughput screening. Here, we developed a new method using fluorescent NBD-cholesterol as a substitute for [3H]-cholesterol to measure cholesterol efflux in THP-1 derived macrophages.

Methods THP-1 cells were cultured in RPMI 1640 with 20% FBS, and differentiated into macrophages under incubation with 100 ng/ml of phorbol myristate acetate (PMA) for 72 h. NBD-cholesterol uptake and metabolism in THP-1 derived macrophages were characterised using fluorescent microscope and spectrophotometer. Cholesterol efflux in THP-1 derived macrophages was measured using either 22-NBD-cholesterol or [3H]-cholesterol as a tracer. The correlation data was obtained after compared percentage efflux of NBD-cholesterol with that of [3H]-cholesterol. NBD-cholesterol efflux was also measured in THP-1 cells compared with human peripheral blood mononuclear cells (PBMCs).

Results NBD-cholesterol distributed rapidly into cell organelles except nucleus. Uptake of NBD-cholesterol in THP-1 macrophages was concentration- and time-dependent, and reached a plateau after 4-h incubation. Next, we measured cholesterol efflux in THP-1 derived macrophages using either 22-NBD-cholesterol or [3H]-cholesterol as a tracer. The correlation data was obtained after compared percentage efflux of NBD-cholesterol with that of [3H]-cholesterol. Our results showed that percentage efflux of NBD-cholesterol was significantly correlated to that of [3H]-cholesterol using either apoA-1 or HDL as lipid acceptor (R²=0.882 for apoA-1, and R²=0.887 for HDL, respectively, p<0.001). Furthermore, NBD-cholesterol efflux in THP-1 cells showed similar trend with that in human peripheral blood mononuclear cells (PBMCs).

Conclusions Fluorescent NBD-cholesterol can be used as a sensitive and specific probe for cholesterol efflux assay in THP-1 derived macrophages.