GW23-e0729 **CHARACTERISATION OF FLUORESCENT NBD-CHOLESTEROL EFFLUX IN THP-1 DERIVED**

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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 [^{3}H]-

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 [^{3}H]-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 [^{3}H]-cholesterol as a tracer. The correlation data was obtained after compared percentage efflux of NBD-cholesterol with that of [^{3}H]-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 [^{3}H]-cholesterol as a tracer. The correlation data was obtained after compared percentage efflux of NBD-cholesterol with that of [^{3}H]-cholesterol. Our results showed that percentage efflux of NBD-cholesterol was significantly correlated to that of [^{3}H]-cholesterol using either apoA-1 or HDL as lipid acceptor (R^2=0.882 for apoA-1, and R^2=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.