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Studies on extracellular vesicle cholesterol in systemic lupus erythematosus

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Cholesterol is a major neutral lipid of mammalian plasma membranes and is considered as essential for life. Circulating extracellular vesicles (EV) are known to contain cholesterol, although the role and metabolic fate of this cholesterol is unclear at present. Studies in this area have been hampered by the availability of accessible methods to visualize and track EV cholesterol. Although numerous probes are available, their behavior in membranes typically fails to adequately replicate that of cholesterol for general use as a cholesterol sensor. We have explored the use of 3-hexanoyl-nitrobenzoxadiazole-cholesterol (3NBDC), a cholesterol analog labeled at the C3 position and expected to display membrane orientation similar to that of cholesterol, to study EV cholesterol in health and disease. Using a classical sterol exchange system of erythrocytes and lipoproteins, 3NBDC was found to replicate the behavior of cholesterol, with detection by both spectrofluorimetry and flow cytometry possible for the samples. Incubation of differentiated THP-1 cells with 3NBDC labeled EV, followed by flow cytometry revealed a time dependent uptake of the labeled EV, which could be confirmed by confocal microscopy. Extracellular vesicles were purified from the plasma of both SLE patients and healthy controls using size exclusion chromatography. EV containing fractions were collected from each donor and immunophenotyping was done by using cell specific markers. The concentration of cholesterol present in EV isolated from both donor groups was determined using an enzyme-linked fluorescent assay and 3NBDC-based flow cytometry. Potential lipoprotein contamination was assessed by ELISA and immunoblotting techniques and was estimated to be minimal. This was further supported by transmission electron microscopy. The number of platelet (CD61) and endothelial cell (CD31+/CD42b-) derived EV was found to be considerably increased in SLE patients when compared to healthy controls, with no significance difference in the number of leucocyte (CD45) derived EV observed between both groups. In addition, EV cholesterol content was found to be considerably increased in the patient group compared to healthy controls. Our results support the use of 3NBDC as a viable cholesterol tracer that can be used to further investigate EV biology. In addition, it can also be used to detect cholesterol and an antigen in EV using standard flow cytometry approaches, a considerable advantage over current approaches. To our knowledge, this is first time report of the application of 3NBDC to EV and the first comparison of EV cholesterol content between healthy controls and SLE patients.

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