Ezetimibe decreases cholesteryl ester content in human monocyte-derived macrophages and modifies cellular distribution of CD13

Evelyn Orsó[1], Horst Robenek[2], Gerhard Liebisch[1], Werner Kramer[3] and Gerd Schmitz[1]
([1]UKR Inst. f. Clin. Chem. Labor. Med., Regensburg; [2]Leibniz Inst. f. Atheroscler. Res., Muenster; [3]Sanofi-Aventis Germany Ltd., Frankfurt am Main, Germany)

Ezetimibe (EZ), an intestinal cholesterol absorption inhibitor was initially developed in non-glucuronidated form as an acyl-coenzyme A:cholesterol acyltransferase (SOAT1)-blocker, which in co-administration with 'statins' (3-hydroxy-3-methylglutaryl-coenzyme A-reductase inhibitors) provides a greater reduction in plasma cholesterol concentration, than monotherapy. The major molecular target of glucuronidated EZ (EZg) is NPC1L1 (Niemann-Pick C1-like 1) in enterocytes and hepatocytes, but other targets, such as CD13 (aminopeptidase-N) or annexin II/caveolin-1, have also been demonstrated.

Cellular effects of EZ and EZg have been investigated in human monocyte-derived macrophages, devoid of NPC1L1 and caveolin-1, loaded with atherogenic enzymatically modified low-density lipoproteins (eLDL).

Ezetimibe, but not EZg, disturbed the co-localization (confocal microscopy) and co-association (fluorescence resonance energy transfer (FRET)) of CD13 and its co-receptor CD64 (Fcγ receptor-I) in membrane microdomains. Immunogold-labelling of freeze-fracture replicas revealed the presence of CD13 in diverse compartments, e.g. plasma membrane, cytoplasma, *trans*-Golgi region and endo-lysosomes. Loading of cells with eLDL led to increased CD13 staining in the plasma membrane, and it was reversed by EZ. Administration of EZ, but not EZg, was associated with decreased cellular cholesteryl ester content, consistent with the SOAT1-inhibition of EZ. These mechanisms may contribute to the anti-atherosclerotic effects of EZ through a decrease of net cellular cholesterol content.

Immunogold-labeling of freeze-fracture replicas from human monocyte-derived macrophages. Representative electron micrograph.

The intracellular CD13-containing vesicles are co-stained with the endo-lysosomal marker CD107a (LAMP-1, lysosomal-associated-membrane-protein-1), indicating the presence of CD13 in this compartment. Note that CD107a is also detected on the E-face of the plasma membrane.

Scale bar: 0.2 μm

CD13: 18 nm colloidal gold, thick arrow;
CD107a: 12 nm colloidal gold, thin arrow;