Effects of lipoproteins and insulin on phospholipid transfer protein content and mrna expression in human hepatoblastoma (hep G2) cells.

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Abstract

Human plasma phospholipid transfer protein (PLTP) was identified to promote phospholipid transfer from liposomes, very low density lipoprotein (VLDL) or low density lipoprotein (LDL) to high density lipoprotein (HDL). PLTP also plays an important role to facilitate HDL conversion. However, its physiological function is less known. The purpose of this study was to investigate the effects of lipoproteins and insulin on PLTP content and gene transcription in human hepatoblastoma (HepG2) cells. HepG2 cells were grown in 90 % MEM (minimum essential medium) supplemented with 10 % of fetal bovine serum, 1 mM sodium pyruvate, 50 U/mL penicillin, and 50 ug/ml streptomycin at 37℃ and in 5 % CO2 atmosphere. Upon 90 % confluency, the cells were switched to serum-free media without antibiotics for 24 hours. After 24-hour serum-free incubation, HDL (50 ug/mL), LDL (50 ug/mL), VLDL (50 ug/ mL) or insulin (1 ug/mL) was added to the media for 12-24 hours.
The control group was without any addition of lipoproteins or insulin. The conditioned media were collected at 12 and 24 hours for protein analysis. Cells were collected for the measurements of PLTP content using 10 % SDS-PAGE and Western blotting, and PLTP mRNA expression using slot blotting. The results showed that protein secretion into the conditioned media in LDL, VLDL and insulin treatment groups was significantly lower than the control group at both 12 and 24 hours ($p<0.05$). In addition, protein secretion was significantly increased at 24h than at 12h in all groups ($p<0.05$). The cellular PLTP exhibited one major band with molecular weight of 44.0 kilodaltons (kDa) in HepG2 cells. The cellular PLTP was not significantly different among the five groups. The level of PLTP mRNA was similar among the five groups. In conclusion, lipoproteins and insulin did not significantly affect PLTP content and gene transcription in HepG2 cells.