

GW23-e1087

THE EFFECT OF OXIDISED LOW-DENSITY LIPOPROTEIN ON NOTCH EXPRESSION IN THP1 MACROPHAGES

doi:10.1136/heartjnl-2012-302920a.3

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Objectives To explore the expression of Notch signal and cytokines by oxidised low-density lipoprotein (ox-LDL) in macrophages of human acute monocytic leukaemia cell line (THP1) and to search for possible mechanism of atherosclerosis (AS)

Methods Human macrophage from THP1 transform by phorbol 12-myristate 13-acetate (PMA) was cultured with final concentration of 50 mg/l ox-LDL for 6 h. Four receptors and five ligands of Notch signalling pathway were inspected. Dynamic changes in terms of cell shape were observed by phase contrast microscopy. Notch1, DIL4 and Jagged1 were given to 25 mg/l, 50 mg/l, 100 mg/l of three different concentrations of ox-LDL stimulation for 48 h. The best concentration was 50 mg/l. Real time-PCR (RT-PCR) detection of Notch1, DIL4 and Jagged1 mRNA expression levels of different time points after macrophages co-cultured with 50 mg/l ox-LDL for 0 h, 3 h, 6 h, 12 h, 24 h, 48 h. Notch1, DIL4 and Jagged1 protein expressions were determined by western-blot of different concentrations and different time points. Vascular cell adhesive molecule-1 (VCAM-1) and monocyte chemoattractant protein-1 (MCP-1) expression were determined by enzyme-linked immunosorbent assay (ELISA). Lipofectamine 2000 transfected Toll-like receptor 2 small interfering RNA (TLR2siRNA) which silence effect was obvious on THP-1 derived macrophages for 24 h. Macrophages with TLR2siRNA transfected previously co-cultured ox-LDL 50 mg/l for 48 h. RT-PCR were used to detect the blank control group and transfection of TLR2siRNA group Notch1, DIL4 and Jagged1 and the expression of inflammatory factor VCAM-1, MCP-1. Western-blot and ELISA detection of Notch1, DIL4, Jagged1, VCAM-1 and MCP-1 protein expressions respectively.

Conclusion Our data showed that with ox-LDL challenge the expressions of Notch1, DIL4, Jagged1 and the levels of VCAM-1, MCP-1 significantly increased in macrophages in a dose-time-dependent manner within some extent compared with that in the control group. Notch signal and TLR2 pathways had synergistic expression effect. Notch signalling was activated by ox-LDL stimulation and may partially mediate atherogenic effect macrophage functions.

Results Macrophages which induced by different concentrations of ox-LDL for 48 h occurred dendritic-like cell (DC) shape change. Compared with the control group DC shape change ratio were significantly increased ($p < 0.05$), 50 mg/l concentration effect was the highest. Macrophages joining ox-LDL stimulated of Notch1 expression was significantly elevated ($p < 0.05$) compared to control group. Notch2 and Notch3 expressions decreased slightly, the expression of Notch4 increased slightly, but not statistically significant. Ligands in DIL4 and Jagged1 expression were significantly elevated ($p < 0.05$), the expression of DIL1 mildly elevated, DIL3 and Jagged2 expressions decreased slightly, not reached statistical significance. Detected Notch receptors expression by western blot, we found that Notch1 expression was significantly elevated, Notch4 not change, Notch2 and Notch3 decreased. With different concentrations of ox-LDL stimulation of macrophage Notch1, DIL4 and Jagged1 expression were elevated ($p < 0.05$), the effect on the concentration of 50 mg/l increased the most obviously. At different time points after ox-LDL stimulation of macrophage Notch1, DIL4 and Jagged1 expression were elevated ($p < 0.05$), the most obvious effect was got at 6 h. Compared with the control group ox-LDL enhances VCAM-1 and MCP-1 expression ($p < 0.05$), the most obvious effect on the ox-LDL was got at concentration of 50 mg/l. Screening out TLR2siRNA-1 can appear obvious silencing effect on

the Notch1, DIL4 and Jagged1 expression with RT-PCR, western-blot and ELISA increased significantly suppressed in the transfection of siRNA group which THP-1 derived macrophages transfected by application of Lipofectamine 2000, the expression of inflammatory factor VCAM-1 and MCP-1 was elevated significantly suppressed.

Conclusions Our data showed that with ox-LDL challenge the expressions of Notch1, DIL4, Jagged1 and the levels of VCAM-1, MCP-1 significantly increased in macrophages in a dose-time-dependent manner within some extent compared with that in the control group. Notch signal and TLR2 pathways had synergistic expression effect. Notch signalling was activated by ox-LDL stimulation and may partially mediate atherogenic effect macrophage functions.