Abstract

Our research objective is to determine underlying oxidative stress mechanism of endothelial dysfunction in atherosclerosis. We hypothesize that intracellular ROS and RNS play a crucial role in endothelial dysfunction. Various doses of 40 and 80 µg/ml oxLDL in mildly, moderately and fully degrees are used as oxidant in umbilical artery culture system. To investigate ROS, we used 10 µM DFO, ferric (Fe^{3+}) chelator in Haber-Weiss reaction and 0.3 mM EDTA, ferrous (Fe^{2+}) chelator in Fenton's reaction and also 100 µg/ml BHT for peroxyl radical scavenger of oxLDL in pretreatment groups.

We found that LOX-1 expression was increased significantly as dose-degree fashion when activated by oxLDL. Surprisely, upregulation of LOX-1 expression induced by iron chelator or even BHT pretreatment followed by oxLDL in various doses and degree was documented. These finding indicates the vascular enzymatic sources of intracellular ROS such as NAD(P)H oxidase at the endothelium. Metal-transition driven ROS does not the major vascular source generated ROS. Remarkably increased inducible expression of LOX-1 may be affected by ROS and RNS in the system. It is noteworthy that the expression of eNOS and NO production were reduced in 40 and 80 µg/ml oxLDL of moderately to fully oxidation. Moreover, reduced activity of SOD was seen clearly resulting from effects of ROS/RNS and low level of $O_2^{\bullet-}$ in the system. Dismutation rate is slower than transformation rate of $O_2^{\bullet-}$ and NO to be peroxynitrite ($ONOO^-$). Loss of SOD activity indicates the endothelial damage since extracellular SOD (ecSOD) is one of isoform. Both ROS and peroxynitrite, RNS, overwhelm and damage any protein, lipid, cell membrane etc. especially those of endothelium leads to reduction of NO production and eNOS expression. Notably, increased p38 MAPK activity is demonstrated from redox signalings to redox – sensitive protein kinase.

It is noticed that endothelium is still intact as seen in 40 µg/ml mildly and 10 µg/ml moderately oxLDL activation. Obviously, eNOS expression is upregulated and NO is produced indicating normal vascular function and confirmed by normal

morphology. Activation of higher doses and degrees of oxLDL leads to endothelial damage, scanty collagen and fibroblast including disoriented smooth muscle cells.

Obviously, increased mean density of p38 MAPK is presented as dose-degree dependent of oxLDL. In iron chelator (DFO, EDTA) and BHT pretreatment groups, those mean density slightly reduced especially in fully oxidation. These finding indicates the redox-sensitive protein of p38 MAPK through redox signalings.

In conclusion, activation of more than 40 μg/ml in moderately to fully oxidation through LOX-1 receptor mediated intracellular ROS and RNS leads to endothelial dysfunction in atherosclerosis.