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KWANTA PITAKNANTAKUL : AN *IN VITRO* MODEL FOR THE STUDY OF Fe-CATALYZED OXIDATION OF LDL. THESIS ADVISOR : UDOM CHANTHARAKSRI Ph.D., YUPIN SANVARINDHA Ph.D., SUPEENUN UNCHERN Ph.D. 135 p. ISBN 974-661-409-6

Lipid peroxidation is one of the most commonly studied markers of oxidative damages when iron is presumed to play a pivotal role. Oxidative modification of lipids, low density lipoprotein (LDL) in humans has been taken as an important early marker in the pathogenesis of atherosclerosis. This study was aimed to explore in depth an *in vitro* model for the study of Fe-induced oxidation of LDL. Emphasis was on the delineation of basic underlying mechanisms of how iron-induced oxidative damages. The studies also provide an insightful understanding of the pathophysiology of thalassemia syndrome in which iron plays the pivotal role.

The LDL was prepared from serum of normal volunteers by a sequential-density gradient ultracentrifugation method. Conjugated dienes and TBARs were monitored as markers of the oxidized lipids. Incubation of LDL with $FeSO_4$ resulted in an extremely low level of TBARs in the medium, whereas the inclusion of ascorbic acid promoted the generation of TBARs in a dose-and time-dependent manner. The result clearly suggested that ascorbate was required for the Fe-induced oxidation of LDL. Interestingly, a complete reduction of iron by converting all iron into the Fe^{2+}-form with millimolar concentrations of ascorbate ceased the Fe-catalyzed oxidation of LDL. Thus, not just its level but the dynamic regeneration of the "active reduced form" of iron, Fe^{2+} was the essential component in mediating the oxidation of LDL. Therefore the role of ascorbate in the oxidation of lipids was partly due to its action as an iron reducing agent, and more importantly due to its ability in maintaining the "dynamic redox cycling" of iron. The latter was indeed the key factor in regulating the Fe-catalyzed oxidation of LDL. The results led us to conclude that the iron redox cycling was an essential step responsible for the regulation of Fe- induced oxidation of LDL *in vitro*.

Oxidation of LDL by Fe was mediated by a free radical mechanism, involving the Fenton reaction and decomposition of preformed lipid hydroperoxides in the LDL. The significant role of preformed lipid hydroperoxides (LOOH) within LDL in the initiation step of Fe-catalyzed oxidation of LDL was also evident as the oxidation of LDL could be completely abolished with ebselen, a glutathione peroxidase-mimetic selenoorganic compound. The evidence for the requirement of ascorbate in regenerating Fe^{2+} and the inhibitory data on LDL oxidation by SOD, catalase or hydroxyl radical scavengers (mannitol, thiourea, ethanol and DMSO) confirmed the validity of Fenton reaction in the oxidative process.

Protection against Fe-catalyzed oxidation of LDL by a number of antioxidants was demonstrated in this study. Antioxidant enzymes, SOD and catalase markedly inhibited Fe-induced oxidation of LDL. Iron chelators (desferrioxamine, CP 20 and CP 94) were also shown to have remarkable inhibitory effect in the oxidation of LDL whereby the sufficient amounts of chelators to bind all iron was necessary. The relative potency of chain-breaking antioxidant that inhibited TBARs formation was in the order of trolox > probucol > α-tocopherol. Interestingly, curcumin exerted antioxidative effect, while its action as an iron reductant was also observed. The protective action of these antioxidants against Fe-catalyzed oxidation of LDL *in vitro* rendered the possibility of their potential benefits in controlling the pathophysiological sequeleae of iron overload.