Formation of strong cytotoxic cholesterol ozonolysis products by neutrophil-like HL-60 cells activated with phorbol myristate acetate

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Cholesterol ozonolysis products, 3β-hydroxy-5-oxo-5,6-secocholestan-6-al (secosterol-A) and 3β-hydroxy-5β-hydroxy-B-norcholestane-6β-carboxaldehyde (secosterol-B), have recently been detected in atherosclerotic plaques and brain tissues of Alzheimer's disease patients. Our previous study showed that the secosterols exert much stronger cytotoxic effects on various cells than four other known oxysterols including 7β-hydroxycholesterol, 5β,6β-epoxycholesterol and 7-ketocholesterol. In this study, we have investigated the mechanism of secosterol production *in vivo* using human leukemia HL-60 cells. LC-MS/MS analyses revealed that significantly increased levels of secosterol-B, but not secosterol-A, were formed in the culture containing 10% heat-inactivated fetal bovine serum, only when HL-60 cells were differentiated to neutrophil-like cells and activated with phorbol myristate acetate (PMA). Vinylbenzoic acid (an ozone scavenger), apocynin (an NADPH oxidase inhibitor), allopurinol (a xanthine oxidase inhibitor), deferoxamine and taurine were shown to attenuate this formation of secosterol-B mediated by PMA-activated neutrophil-like HL-60 cells, whereas SOD, catalase, sodium azide and some hydroxyl radical scavengers such as DMSO and D-mannitol did not affect the formation of secosterols. On the other hand, significantly increased levels of secosterol-A were formed when PMA-activated neutrophil-like HL-60 cells were cultured in the serum-free medium only upon addition of exogenous cholesterol and IgG. These results suggest that secosterol-A is formed by an ozone-like oxidant(s) formed *in vivo* in the presence of antibodies by PMA-activated neutrophil-like cells and is converted to secosterol-B by serum components present in the culture medium. Taken together, these findings are indicating that cholesterol ozonolysis products are formed in vivo in inflamed tissues and may contribute to the development of atherosclerosis and also other oxidative stress-related disorders.