Mechanisms for formation of cholesterol ozonolysis products *in vitro* and *in vivo*

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Cholesterol ozonolysis products, 3□-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. These secosterols exert much stronger cytotoxic effects on various cells than four other known oxysterols including 7β-hydroxycholesterol and 7-ketocholesterol. These findings suggest that secosterols may contribute to the development of atherosclerosis and also other oxidative stress-related disorders. In the present study we have developed a sensitive method to analyze secosterol-A and -B on the basis of derivatization with dansylhydrazine and their detection by HPLC with a fluorescent detector and/or mass spectrometer. Using this method we studied the production of secosterol-A and -B in the cell culture of HL-60 cells. Significantly increased levels of secosterol-B, but not secosterol-A, were detected 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) were shown to attenuate formation of secosterol-B mediated by PMA-activated neutrophil-like HL-60 cells, whereas SOD, catalase, sodium azide and some hydroxyl radical scavengers did not affect 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 immunoglobulin G. Taken together, our 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 its conversion to secosterol-B by serum components present in the culture medium.