

Formation of cholesterol ozonolysis products by PMA-activated neutrophil-like HL-60 cells

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Increased levels of cholesterol ozonolysis products, 3 β -hydroxy-5-oxo-5,6-secocholestan-6-al (secosterol-A) and 3 β -hydroxy-5 β -hydroxy-B-norcholestane-6 β -carboxaldehyde (secosterol-B) have been detected in atherosclerotic plaque and brain tissues of Alzheimer's disease patients. We recently found that both of secosterols exhibited the strong cytotoxic effects with $IC_{50} = 10 \mu\text{M}$ compared to four other known oxysterols such as 7 β -hydroxycholesterol and 7-ketocholesterol. These findings suggest that cholesterol ozonolysis products may contribute to the development of atherosclerosis and also other oxidative stress-related disorders followed by metabolic syndrome.

In this study, we have developed a highly sensitive method for analysis of cholesterol ozonolysis products. The method consists of (1) derivatization of secosterol with dansyl hydrazine, and (2) separation and quantification with HPLC-fluorescent detector. The method allowed to detect $< 1 \text{ fmol}$ secosterol-A and -B. Using this method, we could detect these secosterols, for the first time, in the activated neutrophil-like differentiated HL-60 cell culture. Higher levels of secosterol-B than secosterol-A were formed by the activated HL-60 cells. Molecular mechanisms for the formation of secosterols by these cells are under investigation.

