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ヒト内皮細胞中のアンジオテンシン II 誘発脂質過酸化の LC/ESI-MS 解析

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**【Objective】** Angiotensin (Ang) II (DRVYIHPF) has been implicated in various cardiovascular diseases. Ang II stimulates the generation of reactive oxygen species (ROS) that mediates cardiovascular remodeling. ROS can also induce lipid peroxidation with production of the genotoxic aldehydes, 4-oxo-2(*E*)-nonenal (ONE) and 4-hydroxy-2(*E*)-nonenal (HNE). However, there has been little attention given to the lipid hydroperoxide-derived damage to cardiovascular systems. Our previous studies have demonstrated that ONE and HNE modified N-terminus, Arg<sup>2</sup> and His<sup>6</sup> of Ang II and these modifications altered the biological activities of Ang II. The goal of the present study is to detect lipid-derived modifications to Ang II, glutathione (GSH) and proteins in the cell stimulated by Ang II to clarify how lipid peroxidation modulates cardiovascular system.

**【Methods】** EA.hy926 cells, the human vascular endothelial cell lines, were employed because of their expression of Ang II type I receptor (AT<sub>1</sub>R) and good sensitivity to Ang II stimulation. To detect extremely low-abundant modified-Ang IIs, -GSH and -proteins, conditions for clean-up and LC/ESI-SRM/MS were optimized. The cells were incubated with Ang II in the presence of ascorbic acid (AscA) and CuSO<sub>4</sub> at 37 °C for 24 h.

**【Results & Discussion】** Ang P (ONE-modified Ang II) and [His<sup>6</sup>(HNE)]-Ang II were detected in the cell culture medium incubated with Ang II, and further increased in the presence of AscA and CuSO<sub>4</sub>. A dose-dependent increase in TOG (ONE-modified GSH) was also observed upon treatment with Ang II. Losartan (AT<sub>1</sub>R blocker) was shown to inhibit the formation of TOG in a dose-dependent manner, supporting Ang II-induced lipid peroxidation and modification of various cellular molecules. Detection of lipid modified-proteins that are involved in cardiovascular system will improve understanding of the role that lipid peroxidation plays in cardiovascular diseases.