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学位論文内容の要旨

Main types of tea are green, oolong, and black tea, which are manufactured from the same plant species (Camellia sinensis), although degree of oxidation in fermentation and preparation process is different. Green tea is unfermented, oolong tea is a semi-fermented, and black tea is fully fermented tea. The fermentation of oolong tea is not mediated by microbes, but instead, it is mediated by oxidative enzymes such as polyphenol oxidase and peroxidase in the leaves. The fermentation process induces browning and generates unique flavors and tastes. Depending on the method of preparation including degree of oxidation, various oolong teas are available in Asian countries. Several studies have shown that oolong tea has many beneficial health effects such as antihyperglycemic, antiobesity, and mitigation of the risk of cardiovascular disease. The major components in oolong tea are alkaloids, saponins, polysaccharides, L-theanines, and polyphenols. The degree of oxidation in fermentation is affected to polyphenols of oolong tea. Oolong tea contains polymerized polyphenols derived from the oxidative polymerization of catechins such as oolonghomobisflavans. The polymerized polyphenols are not presented in green tea. Oolonghomobisflavan A (OFA) are most abundant oolong tea polymerized polyphenol, has a unique structure as a dimer of epigallocatechin gallate (EGCG). The biological effects of oolong tea polyphenols have been attracted considerable attention in recent years, although the mechanism remains unclear.

Low-density lipoprotein (LDL) is major cholesterol transporters, it has been suggested that the increasing of LDL in blood and the oxidation of LDL are positively associated with the onset of cardiovascular diseases. A high level of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in human body can inflict damage to the structure and function of LDL which may lead to atherosclerosis. LDL oxidation involves lipid peroxidation and modification of apolipoprotein. In the present study, we investigated antioxidant effect of OFA on LDL oxidation by ROS and RNS *in vitro*.

Radical scavenging capacity of OFA in solution was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Human LDL was prepared by discontinuous density

gradient ultracentrifugation using KBr. LDL oxidation was induced by peroxyl radical-generating reagent (AAPH), transition-metal ion (Cu^{2+}) or peroxynitrite generator 3-(4-morpholinyl)sydnonimine (SIN-1) in the presence of OFA. In oxidized LDL (oxLDL), lipids and apolipoprotein B-100 (apo B-100) may be main target molecules. As the indices of lipid peroxidation of oxLDL, cholesterol ester hydroperoxide (CE-OOH) and thiobarbituric acid reactive substances (TBARS) were determined using HPLC. Oxidative modification of apo B-100, protein carbonyl formation, and nitrotyrosine formation in oxLDL were analyzed by SDS-PAGE and western blotting. Heparin-binding activity of apo B-100 in oxLDL was analyzed by SDS-PAGE with Coomassie Brilliant Blue staining.

OFA, an oolong tea polymerized polyphenol, dose-dependently scavenged DPPH radicals. The IC_{50} of OFA has a higher radical scavenging capacity as compared to Trolox. OFA suppressed formation of CE-OOH in LDL oxidized by peroxyl radical and peroxynitrite, and formation of TBARS in LDL oxidized by Cu^{2+}. In addition, OFA inhibited fragmentation, carbonylation, and nitration of apo B-100 in the oxidized LDL, in which heparin-binding activity of apo B-100 was protected by OFA. Our results suggest that OFA exhibits antioxidant activity against both lipid peroxidation and oxidative modification of apo B-100 in LDL oxidized by ROS and RNS. Polyphenols in oolong tea may prevent atherosclerosis by reducing oxidative stress.

OFA is a unique polymerized polyphenol that has dimeric structure of EGCG, although information about the content of OFA in oolong tea is not enough so far. Therefore, the role of OFA in total antioxidant activity of oolong tea and comparison of antioxidant activity of OFA with EGCG would be important to elucidate. In addition, further studies using cells and animals are required to determine bioavailability of OFA including the absorption and metabolism because antioxidant activity of OFA may depend on their bioavailability *in vivo*.

論文審査結果の要旨

活性酸素種（ROS）および活性窒素種（RNS）による生体酸化ストレスは、さまざまな疾病に関与することが示唆されている。アテローム性動脈硬化症の発症因子の一つとして、ROS および RNS による低密度リポタンパク質（LDL）の酸化が示唆されている。本研究は、ROS および RNS による LDL の酸化に対するウーロン茶ポリフェノールの抗酸化効果を *in vitro* で明らかにすることを目的としたものである。

実験の結果、ウーロン茶ポリフェノールの一種であるウーロンホモビスフラバン A（OFA）は、ROS および RNS による LDL の脂質過酸化に対して濃度依存的な抑制効果を示した。また、LDL 代謝において重要なリガンドであるアポリポタンパク質 B-100 の ROS および RNS による酸化修飾に対しても OFA は抑制効果を示した。これらの結果は、ウーロン茶の摂取がアテローム性動脈硬化症の予防に寄与することを示唆するものである。本研究の成果は新知見であり、日本農芸化学会の英文誌への掲載が認められている。よって、SUKHBOLD ENKHTSETSEG 氏は、北見工業大学　博士（工学）の学位を授与される資格があるものと認められる。