Summary

The Effects of Green Tea Catechins on Endothelial Function and Low-density Lipoprotein (LDL) Oxidation

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Atherosclerotic diseases, including heart diseases and cerebrovascular diseases, are the leading cause of death and they are now responsible for about a quarter of all deaths in Japan. The beginning of atherosclerosis is a complex process, but there is some evidence that the endothelial dysfunction play a key role in the early stage. Epidemiological studies suggest that high dietary intake of polyphenols is associated with decreased cardiovascular disease. Polyphenols are derived from plant materials, such as fruits and vegetables. Green tea contains abundant polyphenols, mainly catechins, and is a large source of polyphenols among the Japanese population.

The early stages of the atherosclerotic process are initiated by endothelial dysfunction. The purpose of this study was to investigate the effects of green tea catechins on endothelial function and low-density lipoprotein (LDL) oxidation which are known to play an important role in atherosclerosis.

1. Comparison of the effects of four major catechins of green tea on vascular endothelial cells

In the first section, the effects of four major catechins (epigallocatechin-gallate; EGCG, epicatechin-gallate; ECG, epigallocatechin; EGC, and epicatechin; EC) on endothelial nitric oxide syntase (eNOS) signaling and gene expressions in vascular endothelial cells were examined.

Atherosclerosis cause endothelial dysfunction with loss of nitric oxide (NO) bioactivity. Endothelium-derived NO synthesized by the endothelial NO synthase (eNOS). eNOS activation mediated by adenosine monophosphateactivated protein kinase (AMPK) and v-akt murine thymoma viral oncogene (Akt) phosphorylation. Firstly, whether catechins could regulate the eNOS signal pathway was investigated. Catechins were activated phosphorylation of eNOS, Akt, AMPK in HUVECs.

Next, Total RNA was extracted from HUVECs and subjected to microarray (Human Genome U133 Plus 2.0 Array, Affymetrix). Differentially expressed genes with ≥0.5 or ≤-0.5 log ratio were analyzed using DAVID (Database for Annotation, visualization and integrated discovery) tool and Ingenuity Pathways Analysis (IPA) software (Ingenuity Systems, Redwood City, CA, USA).

The numbers of genes determined to be differentially expressed were 780 (282 up-regulated and 498 down-regulated), 1109 (284 up-regulated and 825 down-regulated), 682 (261 up-regulated and 421 down-regulated) and 736 (277 up-regulated and 459 down-regulated) respectively, in EGCG-, ECG-, EGC- and EC-treated cells. Eighty-seven genes (32 up-regulated and 55 down-regulated) were identified as commonly regulated genes among four catechin treatment groups. Interestingly, I found that differentially expressed genes by catechins showed biological functions and signaling pathways associated with angiogenesis by using the bioinformatics DAVID and IPA tool.

These findings suggest that one of the underlying mechanisms of regulation of endothelial function by catechins is regulatory effect ageinst angiogenesis.

2. The effects of catechins on VEGF stimulated endothelial

The aim of this study was to investigate whether catechins (EGCG, ECG, EGC and EC) could improve vascular endothelial growth factor (VEGF)-induced endothelial activation. HUVECs were stimulated in the medium containing VEGF. Firstly, the effect of catechins on the angiogenesis related genes were examined. The mRNA expressions of matrix metalloprotease *(MMP)-14* and *MMP-2* in HUVEC were up-regulated by VEGF. Catechins showed significant inhibition against the VEGF-induced up-regulateion of *MMP-14* and *MMP-2* mRNA expression. Catechins showed significant inhibition on VEGF-induced *IL-6* and *COX-2* mRNA expression. The increases in the adhesion of THP-1 cells and the expression of adhesion molecules induced by VEGF were significantly suppressed by catechins. Further, catechins inhibited the proliferation assessed by using an *in vitro* scraping assay. VEGF stimulation increased the mRNA expression of *ETS-1*, a transcription factor that regulates inflammation and angiogenesis-related pathways, whereas catechins showed significant inhibition on VEGF-induced *ETS-1* expression.

ER stress is involved in the development and progression of a number of diseases including atherosclerosis. ER stress can lead to endothelial injury and subsequently to apoptosis and inflammation. Catechins also inhibited the VEGF-induced ER stress marker (C/EBP homologous protein; *CHOP* and glucose-regulated protein 78; *GRP78*) mRNA expression.

These findings suggest that catechins are effective in improving endothelial function by reducing VEGF-induced inflammation and angiogenesis in endothelial cells.

3. The effect of green tea catechin on endothelial function and LDL oxidation in in healthy subjects

The effects of green tea catechins on endothelial function in human studies were investigated to test the hypothesis that catechins effectively to endothelial function. In a randomized,

placebo-controlled, double-blind, crossover trial, 19 healthy men ingested green tea extract (GTE) as capsules in a dose of 1 g total catechin, of which most (>99%) was gallated type. One hour after ingestion, marked increases of the plasma concentrations of (−)-epigallocatechin gallate (EGCG) and (−)-epicatechin gallate (ECG) were observed. Accordingly, the plasma total antioxidant capacity was increased but flow-mediated vasodilation; FMD was not increased after GTE ingestion. I also investigated the effect of green tea catechins on low-density lipoprotein (LDL) oxidation which induces endothelial dysfunction. The LDL oxidizability was significantly reduced by the ingestion of GTE. Moreover, I found that gallated catechins were incorporated into LDL particles in non-conjugated forms after the pre-incubation of GTE with plasma *in vitro*. An additional human study with 5 healthy women confirmed that GTE intake sufficiently increased the concentration of gallated catechins, mainly in non-conjugated forms in LDL particles, enough to reduce the oxidizability of LDL. These findings suggest that green tea catechins are rapidly incorporated into LDL particles and prevented LDL oxidation.

Conclusion

This study demonstrated that green tea catechins improved multiple atherogenic processes, such as endothelial dysfunction and LDL oxidation. These findings suggest that green tea catechins can be effective to prevent atherosclerosis progression.