Background

Aside from water, tea is the most consumed beverage in the world (1, 2). In particular, green tea is consumed primarily in Japan, China, and some parts of the Middle East, North Africa, and North America. The benefits arising from drinking green tea have, therefore, been the focus of some studies during recent years.

The term green tea refers to the product manufactured from fresh leaf of the tea plant, *Camellia sinensis* (3). Green tea is an excellent source of water-soluble polyphenol antioxidants. In particular, green tea leaves are very rich in flavonoids, which are a group of polyphenols present in vegetables, fruits and beverages such as tea and wine (4). During manufacturing of green tea, the oxidation of polyphenolic components is precluded (1).

The flavonoids in green tea leaves are known as catechins, consisting of epicatechin (EC), epigallocatechin (EGC), catechin ((+)C), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) (5). These epicatechin isomers share a similar backbone, but contain varying number and location of hydroxyl groups. Catechins are colorless, astringent, water-soluble compounds that may constitute up to 30% of the dry leaf weight (1). They are powerful antioxidants, capable of rapid reduction of superoxide radical and alkyl peroxy radicals (2). Catechins are readily oxidizable, although their oxidation potentials vary (1). This property has been utilized through their use as food antioxidants. They retard rancidity in fats and oils by quenching free radical peroxide activity brought about by aerobic oxidation. In biological systems, oxygen is an important acceptor of electrons, leading to the formation of active oxygen and hydroxyl free radicals.

Introduction

Green tea has been found to possess antimutagenic properties. In animal experiments, green tea extract significantly inhibited the promotion of tumors and carcinogenesis (6). Another major area of research focus on green tea is its antioxidant properties.

Lipid peroxidation, free radical processes and oxidative modification of low-density lipoproteins (LDL) have strong relationship with the development of atherosclerosis (7). LDL oxidation can be catalyzed by heavy metals such as Cu^{2+} and promoted by activated cells. Oxidation of LDL is a free radical chain reaction and is separated into the processes of initiation, propagation and termination phase reactions (8). Antioxidants should efficiently inhibit LDL oxidation and reduce the biological consequences such as uptake by macrophages.

Activated oxygen species are thought to be involved in the damage of biomembrane. Several antioxidants including alpha-tocopherol have been shown to be effective against red blood cell hemolysis (5). Due to polyunsaturated fatty acids, the cell membrane may be most susceptible to free-radical attack.

Epidemiological studies show that drinking tea lowers the risk of heart disease. However, the mechanism of green tea's benefit for heart disease is not known (2). Oxidation of low-density lipoproteins (LDL) in the vessel wall is thought to be one of the steps involved in atherogenesis (3). In line with the oxidation hypothesis, dietary antioxidants are increasingly recognized as potentially important factors in the prevention of cardiovascular disease. Studies have

investigated the effects of drinking green tea on biomarkers of atherosclerosis and atherosclerotic lesion formation in hypercholesterolaemic rabbits.

Effects On Ldl Oxidation

Yokozawa and Dong tested in vitro the abilities of green tea extract and its three major components to inhibit lipid oxidation in low-density lipoprotein (LDL) catalyzed by copper. Their results show that green tea extract markedly delays oxidation (9). Of the three components, polyphenols had the strongest action. Similar action was also shown in the theanine-treated group but was weaker than in the former, whereas caffeine had a very limited effect. Based on this data, it is concluded that green tea extract can effectively inhibit oxidation and that this activity is due largely to the polyphenols it contains. According to the ultraviolet spectra, copper chelation is suggested to be one of the possible mechanisms of LDL anti-oxidation.

In a study that investigated the different phases of LDL oxidation, EC or EGC is found to inhibit the oxidation of LDL in the initiation phase when either isomer is added to the mixture of isolated human LDL and Cu^{2+} (8). However, depending on time and the concentrations used, EC and EGC have either accelerative or inhibitory effect in the propagation phase of Cu^{2+}-induced LDL oxidation. In the initiation phase, even low concentrations (5 nM-0.1 uM) of catechins inhibit oxidation, and EC has a longer inhibitory effect of LDL oxidation than EGC. A higher concentration of EC than EGC is needed to accelerate or inhibit LDL oxidation in the propagation phase. Higher concentrations of either catechin are needed to inhibit the oxidation than to accelerate. EC has a longer inhibitory effect than EGC in the initiation phase.

Experiments performed by Anderson *et al* show that green tea intake by rats in studies significantly prolongs the lag phase during the in vitro oxidation of LDL. The lag phase is a very sensitive indicator of resistance LDL to in vitro oxidation (7). The longer the lag phase, the more resistance the substance is to oxidation. The observations that green tea intake significantly increases the lag time suggest that the polyphenolic compounds such as certain catechins may also be transported in lipoproteins and inhibit their oxidation. On the other hand, the rate of conjugated diene formation (another indicator of lipoprotein oxidation) is only slightly slower with ingestion of green tea compared to the control diet (vitamin E deficient). In addition, green tea diet does not significantly decrease lipid peroxide values. The formation of thiobarbituric reactive substances (TBARS) is a nonspecific measure of oxidative damage to lipoproteins. Green tea diet shows a slightly lower TBARS value than the control group.

An in vivo study shows that green tea is a powerful inhibitor of plasma lipid peroxides and LDL oxidizability (2). It is found to be an in vivo antioxidant in both the normal and cholesterol-fed groups. As expected, the cholesterol feeding produced an increase in plasma lipid peroxides and LDL oxidizability; the latter as shown by the decrease in lag time and the increased maximal oxidation rate. Green tea significantly lengthened the lag phase in both the normal and cholesterol-fed groups. When compared to black tea, green tea is significantly better. Green tea increased the lag time 71% in normal animals and 63% in animals with high cholesterol. In fact, the green tea increased the lag time of the cholesterol-fed animals to the extent that it was not significantly different from the control group. In addition, the maximal oxidation rate in the high cholesterol-fed animals, but not the normal group, is decreased significantly by green tea. There is evidence that green tea polyphenols bind to LDL when spiked in plasma (2). This incorporation, subsequent to tea polyphenol absorption into the plasma, would explain the

increase in lag time after consumption. Green tea also protects human LDL from oxidative modification (5).

Conflicting evidences also exist and should be taken into consideration. One study demonstrates that green tea consumption has a potent antioxidant activity on LDL oxidation in vitro, but found no effect in vivo (10). In another study performed on humans, evidence shows that consumption of 6 cups of green tea per day did not affect resistance of LDL to oxidation, or markers of oxidative damage to lipids in vivo, but slightly increased total antioxidant activity of plasma (11). The in vitro experiment shows that resistance of isolated LDL to oxidation increased only after incubation of plasma with very high amounts of green tea. These amounts, when converted to tea catechin concentrations, were much higher than those expected in vivo.

Decrease In Hemolysis Of Red Blood Cells

Consumption of green tea is also associated with a significant decrease in susceptibility of red blood cells to hemolysis in rats (5). Jasmine green tea protects the polyunsaturated fatty acids from free radical-induced oxidation in red blood cell membrane in vitro. Catechin isomers may protect red blood cell membrane from free radical attack by either one or combination of following mechanisms. First, they may act as chelator to inactivate Cu^{2+} and catalytic cations involved in initiation of free radicals. Second, they may function as a free-radical chain reaction interrupter by trapping the free radicals. Third, they may work synergistically with alpha-tocopherol by donating a hydrogen to regenerate alpha-tocopherol when the latter is oxidized. The extent of hemolysis is related to the loss of alpha-tocopherol.

Of the four isomers, only EGC and EC, but not EGCG and ECG, are observed to be in aorta blood after an oral ingestion of green tea extracts (5). The study suggests that the active compounds are most likely EGC and EC instead of their derivatives, EGCG and ECG, although the former two isomers are quantitatively minor in green tea leaves. This may be explained by either one or combination of following possibilities. First, absorption of EGC and EC may be more efficient than that of EGCG and ECG. Second, EGCG and ECG may be hydrolyzed to form EGC and EC, respectively, before they are absorbed. Third, all four isomer may be absorbed via portal vein to liver where EGCG and ECG may be hydrolyzed to form EGC and EC, respectively. However, the mechanisms by which EGC and EC are circulated in blood but not EGCG and ECG remains a mystery.

The in vivo antioxidant activities of EGC and EC are found to be more important than EGC and EC because of their existence in the blood after ingestion (5). However, EGC and EC are less effective than the other two isomers. If ingestion of green tea is significantly associated with a decrease in susceptibility of red blood cells to hemolysis and other free-radical related diseases, part of the mechanisms may involve circulation of EGC and EC as free radical scavengers in blood stream.

Reduction In Atherosclerotic Plaque Formation

A study have found that green tea consumption tend to reduce atherosclerotic plaque formation (3). LDL is the major atherogenic lipoprotein in humans. Green tea reduces the maximum rate of LDL oxidation, but this may not fully explain the observed trend to inhibit atherosclerotic lesion formation, because the former effect is also found in animals fed black tea, whereas the latter is

not (3). There are several factors, including intrinsic LDL oxidizability, which may influence the initiation and progression of atherosclerosis. Green tea antioxidants may accumulate in the arterial wall and lower LDL oxidation in the arterial wall by increasing cellular antioxidant status or by inhibiting activities of oxidizing enzymes. Another factor mediating LDL oxidation in the arterial wall is the presence of free metal ions, and there is evidence that flavonoids are strong chelators of free metal ions (9).

It is clear that tea flavonoids have a number of activities with the potential to reduce atherosclerosis, which may not be directly related to and not detectable by determination of intrinsic LDL antioxidant status. Although the study showed no significant reduction of atherosclerosis, there was a trend towards reduced lesion formation in the green tea test group. Results from the study do not demonstrate that in markedly hypercholesterolaemic rabbits, a reduction of LDL oxidizability is directly causing a reduction of atherosclerosis. In addition, human and animal data suggest that green tea consumption may selectively decrease LDL and increase HDL concentrations (12). Since atherosclerosis may be attributable to lipoprotein oxidation, these factors may combine to contribute to a reduction in risk for atherosclerotic disease. Study performed by Vinson and Dabbagh demonstrates green tea as powerful in vivo inhibitor of lipid oxidation. The Syrian Golden hamsters used in the study show that teas in the concentration normally drunk by humans are antioxidant agents and thus provide the mechanism to explain green tea's epidemiological benefits for heart disease.

Conclusion

Worldwide studies conducted thus far present mixed evidences with regard to the effects of green tea on oxidation. Some studies conclude that green tea catechins have both in vitro and in vivo antioxidant activities while others show no antioxidant benefit in vivo. In particular, EC and EGC are the isomers contributing to the regulation of lipoprotein oxidation. The green tea diet is associated with increases in the lag phase but not with significant alterations in other measures of lipoprotein oxidation. Therefore, studies point toward some antioxidant capabilities by green tea, but they are not conclusive results like the results obtained for vitamin E using the same parameters. Since the in vivo experiments used the amount of green tea corresponding to that ingested by frequent tea drinkers, occasional tea drinking may not contain high enough level of antioxidants to confer benefits in humans.

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