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Antioxidant effects of green tea polyphenols

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Abstract **The unprecedented interest in the antioxidant activity of green tea polyphenols (GTP) is due to the apparent health benefits of tea drinking and the experimental results with the polyphenols. The results suggest that the galloylated catechins show stronger antioxidant effect than that of nongalloylated catechins and the double bond in C ring also plays an important role in this effect. There are synergic effects between different catechins and the antioxidant effect of (+)-epimers is stronger than that of (-)-epimers. The active site to scavenge free radicals mainly locates in p-hydroxyl of phenol in gallic acid and hydroxyl of phenol in the pyrane also shows activity. The large p bond in chromane and benzene ring of catechin is the structure base for their antioxidant effects. The prevent effect of GTP against diseases and their redox regulation in cell signal pathway are very important to be studied in the future.**

Keywords: green tea polyphenols (GTP), bioflavonoid, natural antioxidant, free radicals, ESR.

Throughout history, tea has been one of the most popular drinks all over the world. Epidemiological studies have shown that tea is beneficial to people's health by protecting the body from diseases[1,2]. The most benefit materials for human health in tea is green tea polyphenols (GTP). GTP can scavenge oxygen free radicals and lipid radical, prevent lipid peroxidation, it has also been shown to inhibit tumorgenesis and delay aging. Herewith presented is a discussion of recent studies of the scavenging effects of GTP on free radicals and the underlying possible mechanisms. It is hopeful to promoting the study and application of GTP for human health and anti-aging from the study.

1 GTP scavenges oxygen free radical

Oxygen is reduced to water through reception of 4 electrons. Superoxide, hydroxyl free radical, hydrogen peroxide and singlet oxygen are generated during this process, which cause lipid peroxidation of cell membrane. The ability to scavenge these active oxygen has been used to evaluate an antioxidant in recent years.

The oxygen free radicals generated from polymorphonuclear leukocytes (PMN) play an important role in immunology, but also damage normal cells[3]. We compared the scavenging effects of GTP, rosemary, curcumin, vitamin C and E on the free radicals and found that GTP had the strongest scavenging effects on the free radicals produced from PMA stimulated PMN. GTP's scavenging effect on $O_2^{\cdot-}$ was stronger than others except vitamin C in the systems of xanthine/xanthine oxidase and photoradiation of riboflavine/EDTA. In the Fenton reaction system and the photoradiation of hydrogen peroxide system, the GTP's scavenging effect on .OH was weaker than others except vitamin E[4].

2 Synergic effect of GTP on free radicals

Green tea extract usually contains 4 types of polyphenols. EC, ECG, EGC and EGCG (fig. 1).

The scavenging effects of these 4 components were studied by chemiluminoscence and ESR methods and it was found that the order of the scavenging effects on O_2^- was as EGCG>ECG>EC≈EGC[5]. The compound with more phenol-hydroxyl groups has stronger antioxidants.

Using pulse radiolysis, Jovanovic et al.[6] measured the reaction rate constants of GTP with O_2^-. Salah and Rice-Evans[7] measured the antioxidant potentials of the GTP using TEAC (trolox equivalent antioxidant activity) and got similar results.

The synergic scavenging effect of 2-catechin combination on O_2^- was studied and it was found that the

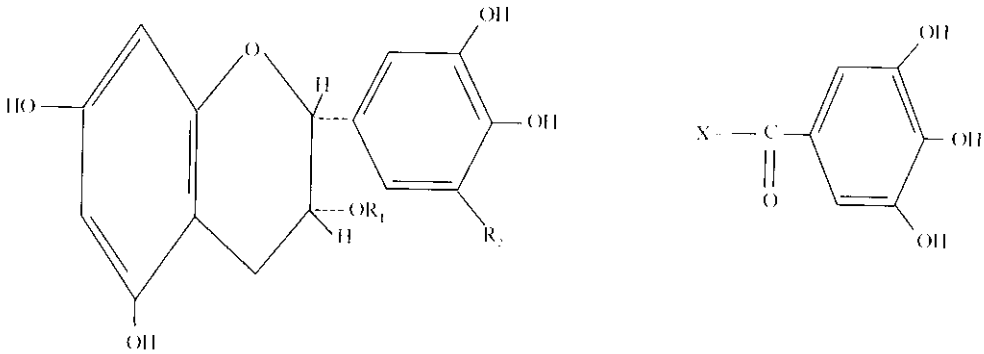

Fig. 1. The structures of green tea polyphenols. EC: (–)-Epicatichin R_1=H; R_2 =H; EGC: (–)-Epigallocatichin R_1=H; R_2 =OH; ECG: (–)-Epicatichin gallate R_1=X; R_2 =H; EGCG: (–)-Epigallocatichin gallate R_1=X; R_2 =OH.

combination of EGCG∶ECG was the strongest, then was ECG∶EC and EC∶EGC. The synergic effect was neither their addition nor their time, but the proportion to their mole concentration. The strongest synergic scavenging effect of 3-catechin combination on O_2^- was the combination of EGCG∶ECG∶EC. The strongest synergic scavenging effect of 4-catechin combination on O_2^- was the combination of EGCG∶ECG∶EC∶EGC=5∶2∶2∶1, which was similar to the ratio equivalent naturally found in tea. The effect was even stronger than the combination contained more amount of EGCG. This suggests that tea tree has successfully evolved in order to survive in a toxic environment[5].

The synergic effects of GTP with vitamin E were studied in solution, gel, LDL and red cell systems respectively in Liu Laboratory and it was found that GTP was an effective antioxidant in the above systems and the antioxidant ability was related with their polarities and the microenvironments[8]. They measured and calculated the effects of GTP and vitamin E on the inhibition time, rate of propagation, the kinetic chain length and stoichiometric factor and it was found that EGCG and ECG were effective as vitamin E and they had synergic effects with vitamin E and they could regenerate vitamin E in solution but different in SDS and CTAB gel systems. The inhibition time and stoichiometric factor were bigger but the reaction rate was smaller in CTAB than those in SDS[9]. This indicates that the charge on the surface of gel plays an important role for the antioxidant of GTP. The ESR result showed that there was a synergic effect between GTP and vitamin C in scavenging the free radicals in aqueous phase and breaking the chain reaction of lipid peroxidation in gel and biological membrane (fig. 2)[10]. In CTAB gel system, the order of the reaction of vitamin E with GTP was EGCG>ECG>EGC>EC[11].

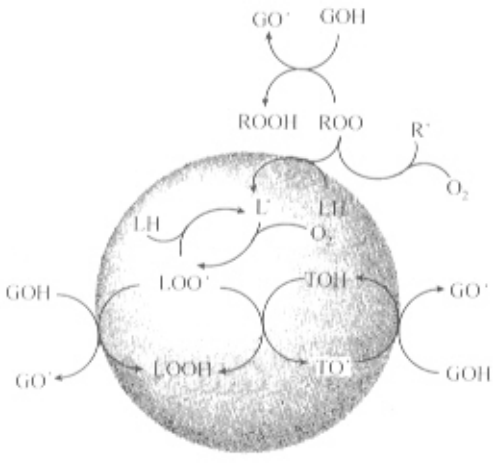

Fig. 2. The antioxidation mechanism of GTP in micelles [10].

3 Scavenging effect of GTP on oxygen free radicals generated from isolated ischemia-reperfused rat myocardium

The free radicals generated in ischemia-reperfused myocardium were measured directly by ESR and it was found that the isolated rat heart subjected to ischemia followed by reperfusion (Langendoff) induced a significant increase in the production of oxygen free radicals. The concentration of oxygen free radicals in ischemia-reperfused myocardium was about 188% of that in the normal myocardium. After pretreatment of the heart with GTP, the oxygen free radicals were significantly reduced and dose-dependent[12].

4 Inhibitory effect of GTP on the oxidant activity of $ONOO^-$

NO can react with superoxide with very high constant (6.4×10^9 mol • L^{-1} • s^{-1}) and generate peroxynitrite ($ONOO^-$), which is a very strong oxidant and can cause oxidation of cell membrane, protein and lead to cell damage and diseases. It can also generate hydroxyl radical and NO_2 in acid condition. Using spin-trapping method, the inhibitory effect of GTP on oxidation of $ONOO^-$ was studied and compared with that of quercertin and vitamin C. Although they could all effectively inhibit the oxidation of $ONOO^-$, the inhibitory effect of GTP was the strongest[13]. Pannala et al.[14] found that the scavenging effect of ECG and EGCG on $ONOO^-$ were more effective than that of EC and EGC.

5 GTP inhibits lipid peroxidation of synaptosomes

There are less catalase, glutathinperoxidase, glutathine and vitamin E but more unsaturated fatty acids, iron and copper ions in brain tissue, so it is easy to have lipid peroxidation damage for neuron. The protective effects of GTP on synoptosome against the lipid peroxidation damage were studied.

Using Fe^{2+}/Fe^{3+} to stimulate lipid peroxidation of synaptosomes, we found that the order of the protect effect of GTP on the lipid peroxidation of synaptosomes was EGCG>ECG>EGC>EC. The order of the scavenging lipid free radicals generated in this system was ECG>EGCG>EC>EGC[15]. Salah and Rice-Evans et al.[16,17] found similar result for inhibition effect of GTP on the lipid peroxidation of LDH.

The scavenging effect of GTP on lipid free radicals includes the free radical in the initiating process of lipid peroxidation by Fe^{2+}/Fe^{3+} and the propagation process of lipid peroxidation, which reflects the reaction mechanism of GTP with free radicals. The TBA result reflects the antioxidant effect of GTP on the terminal product of lipid peroxidation.

6 Protective effect of GTP on DNA damage

DNA is a main cell target for attacking of active oxygen, which causes mutation and cancer. (1,10-phenanthroline)$_2$Cu^{2+} and hydrogen peroxide was used to induce DNA damage and ESR and chemiluniscence was used to detect the protective effect of GTP on DNA. It was found that the order for protective effect of GTP on DNA damage was as: ECG>EC>EGCG>EGC. ESR spin trapping method was used to determine which free radical was involved in this process and it was found that hydroxyl radical played an important role and the order of the scavenging effect of GTP on hydroxyl radical in this system was also similar to the above[18].

7 Relationship between structure and activity of GTP epimers

Another aspect of the relationship between the structure and activity of an antioxidant that we looked at was the chemical structures, including steric structures, and the free radical-scavenging activities of catechins (EGCG, EGC, EC) and their corresponding epimers GCG (gallocatechin gallate), GC (gallocatechin), (+)-C (catechin).

In the irradiated riboflavin/EDTA system, the superoxide radical-scavenging activities of those catechins increased as follows: EC<(+)-C< EGC<GC<EGCG <GCG. The scavenging effects of GCG and EGCG with a gallate group at position 3 were stronger than those of nongalloylated catechins (GC, EGC, EC and (+)-C). EGC and GC, which have an orthohydroxyl group in the benzene ring, were more effective than EC and (+)-C. In addition, it was found that scavenging activities of GCG, GC and (+)-C were higher than those of their corresponding epimers respectively. The lower the concentrations of the polyphenols, the higher the differences of their scavenging effects[19].

Using photoradiation-hemoporphyrin system to generate $^1O_2$[20], it was found that $^1O_2$ generation decreased, the time to reach maximum shorted and the maximum decreased with the concentration of GTP. The scavenging effects of GCG, GC and (+)-C on $^1O_2$ were better than that of their epimers EGCG, EGC and EC, respectively. The scavenging activity of the catechins was in the following order: EC((+)-C)< EGC (GC) < EGCG (GCG)[19].

AAPH (2,2-azobis(2-amidinopropane) hydrochloride) can generate carbon-centered free radical in water solution at 37℃[20]. The dose-dependent scavenging effects of those catechins on the free radicals generated from AAPH were observed. The scavenging ability was as: EC<(+)-C< EGC <GC < EGCG <GCG. The scavenging abilities of GCG, GC and (+)-C were stronger than those of their corresponding epimers, respectively and the differences were amplified at lower concentration[19].

DPPH (1,1-diphenyl-2-picrylhydrozel) is an organic free radical and the scavenging effects of the catechins on the DPPH radical were EC<(+)-C< EGC <GC < EGCG <GCG. The scavenging abilities of GCG, GC and (+)-C were stronger than those of their corresponding epimers, respectively[19].

The above results indicates that different epimers have different abilities to scavenge different free radicals. This may play a significant role when GTP is used as natural antioxidant.

8 Mechanism of the scavenging effect of GTP on free radicals

In order to compare the antioxidant ability of GTP, we measured their scavenging effects on free radicals, abilities to chelate with iron ions, stabilities of semiquinone free radicals formed after reaction with free radicals and analyzed the active sites of GTP when react with free radicals.

Iron ions play an important role in inducing lipid peroxidation. The chelation effects of four compounds of GTP were studied by molar ratio method and it was found that the chelated ratio of GTP with Fe(Ⅱ) was as ECG 3∶2, EGCG 2∶1, ECG 3∶2, EC 3∶1[15]. This indicates that the chelating effects with iron ions play a role but were not the unique factor.

• OH free radical from photolysis of H_2O_2 without Fe^{2+}/Fe^{3+} was used to study the scavenging effect of GTP on hydroxyl free radicals, and it was found that except ECG other GTP did not exhibit any • OH scavenging property. This result indicates that • OH is not an important factor for inducing lipid peroxidation of synaptosomes[15].

Lipid free radical produced by lipid peroxidation of lecithin induced by lipoxidase without iron ions was used and it was found that the IC50 of scavenging lipid free radical for ECG, EGCG, EGC and EC was 0.37, 0.46, 2.7 and 3.0 mmol/L respectively. Those results were similar to the total inhibitory ability of GTP on lipid peroxidation of synoptosomes, suggesting that lipid radicals played a very important role in this process[15].

Another characteristic is the stability of the semiquinone free radicals formed from the polyphenols after reaction with oxygen free radicals. Using ESR technique, it was found that the stability of semiquinone free radicals decreased in the following order: EGCG>ECG>EC>EGC, suggesting that the stability of the semiquinone free radicals also play an important role in this process[15].

The active sites of different components of GTP need to be identified in order to understand the mechanism behind its actions. The ESR spectra of the semiquinone radicals formed from reaction with the oxygen radi-

cal and active sites were analyzed. It was found that the active site of EGCG was the orthohydroxyl group in the gallatic acid; the active site for ECG was the orthohydroxyl group in the galloyed gallatic acid; there were two active sites for EC, one was the hydroxyl group in chroman ring and the other was the hydroxyl group in pyrane ring; for EGC, there were also two active sites, one was the orthotrihydroxyl group in the galloyed gallatic acid and the other was the hydroxyl group in chroman ring[15].

9 Quantum chemical bases for scavenging effect of catechin on free radicals

From the calculation of quantum chemical theory (MNDO), the mechanism of scavenging effect of GTP and active center on free radicals were analyzed and discussed[21]. The structural characteristic of a molecular can be deduced from their eigenvectors. The electrons in Px and Py orbitals usually form σ bond and the electrons in Pz forms π bond. It was found that the electrons of carbon and oxygen atoms in the chroman ring were located in Pz orbital, suggesting that there was a large π bond in the chroman ring and the bonds of O-H in the chroman ring were easy to be broken. The hydroxyl groups were not real hydroxyl but were in the middle form between hydroxyl and quinone. The oxygen atom in pyrane ring enlarged this big conjugative system and increased the contribution of quinone. Other benzene ring was perpendicular to the chromane plane, so the electrons of Px also form a big π bond but smaller than that on chromane. The hydroxyl groups also had some components of quinone but the delocation of the electrons was smaller.

The more the electron multiken population and net charge difference between two atoms, the stronger the bond between the two atoms. The bond between O-H of the hydroxyl on the benzene ring was stronger than that of chromane, indicating that H atoms of O-H on the chromane were easy to be donated. So we suggested that the chromane ring could form quinone when reacted with free radicals. This is consistent with the ESR experimental results. This conclusion breaks the traditional idea, which only knew that the O-H on benzene ring could react with free radicals.

10 Suggestion for the future study

From the above it can be found that the polyphenols extracted from green tea have very strong antioxidant, which depend on their structures. But the study about the mechanism of their biological and pharmacological functions, for example, their receptor, signal pathway and their metabolism are not clear. In order to understand green tea's health benefit for human body, it is necessary to study these aspects.

There is close relationship between free radical damage and neuron degeneration diseases, for example, Parkinson's and Alzheimer's diseases are two typical degeneration diseases[22]. The oxidative damage is an important reason for the diseases although many factors can lead to the diseases. The mechanism of preventing these diseases by GTP and other antioxidants needs to be studied. We found that GTP could prevent apoptosis of PC12 cell induced by 6-OHDP[23] and Parkinson's disease in animal model. The mechanism of preventing these diseases by GTP needs to be studied.

Several studies indicated that GTP could enter into plasma and even pass through the brain-blood barriers[24]. But the concentrations, forms, staying times and their reactive mechanisms with free radical in different organs and tissues are not clear and need to be studied.

More and more research work indicates that active oxygen species act as signal and play an important role in the pathway of cell apoptosis[25,26]. But the mechanism is not clear and a lot of questions need to be studied. If the signal pathway of GTP in the regulation of redox and apoptosis of cell is found, it is very important theoretical progress.

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Combustion energies and standard molar enthalpies of formation for the complexes of the first-row transitional metal chlorides with *L*-a-histidine

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Abstract **Seven novel solid complexes of the first-row transitional metal with *L*-a -histidine were synthesized, and their compositions were determined. The constant-volume combustion energies of the complexes were measured by a precision rotation bomb calorimeter. The standard molar enthalpies of combustion and the standard molar enthalpies of formation were calculated. The results indicated that the plots of the standard enthalpies of formation against the atomic number of the metal show a regularity of zigzag.**

Keywords: transitional metal chloride, *L*-a -histidine, solid complex, standard molar enthalpies of formation.

The first-row transitional metals of chromium, manganese, iron, cobalt, nickel, copper and zinc, are essential trace elements in human body. The functions of metals in biology may be generally classified into two major groups: metalloproteins and metalloenzymes[1]. *L*-α-histidine basic units of proteins, which have to be absorbed from food because of not being synthesized by human body. Therefore, the understanding of the complexes of trace elements and *L*-α-His have received considerable attention. However, studies on their thermochemistry have not been reported anywhere. In refs.[2—4], phase chemistry and thermochemistry of coordination behavior of chromium nitrate with *L*-α-His, the phase chemistry of coordination behavior of zinc salts with *L*-α-His, and the standard enthalpies of formation and preparation of the solid complex of zinc with *L*-α-His were reported.

In this report, seven novel solid complexes of the first-row transitional metals with *L*-α-His were synthesized. The compositions of the complexes were determined by chemical and elemental analyses. The constant-volume combustion energies of the complexes were determined by a precision rotation bomb calorimeter. The