Studies about monoamine oxidase inhibitory activities on Korean Green Tea.

Keum Hee Hwang*, Sang-Hee Byun, Sun-Hee Lim

STC NARA Co. STC Life Science Center , 173 Dohwa 2-Dong, Mapo-Gu, Seoul, Korea, 121-745

Summary

To examine the nervous sedative effects of Green Tea, the monoamine oxidase(MAO) inhibitory activities were determined in the brain and liver of rat *in vitro* on green tea with the different region and harvested period. The MAO plays a central role in the metabolism of many amines including the neurotransmitter monoamines. MAO is a flavoprotein found exclusively in the mitochondrial outer membrane, occuring in the MAO-A and MAO-B subtypes. MAO-A deaminates serotonin and noradrenaline, whereas MAO-B prefers penylethylamine and benzylamine as substrates. Brain MAO-A activity was inhibited by green tea methanol extracts. Especially lately harvested green tea (中雀)extract showed potential inhibitory activities. And the liver MAO-B activity was also inhibited by all of the green tea extracts with strong intensity. Whereas (-)catechin which is well known one of the main bioactive component of green tea did not show inhibitory activity on MAO-A and -B at all. So we will continue to study the active compound of the nervous sedative activity of green tea on chemical and biochemical effort by activity guided isolation.

Key words : monoamine oxidase (MAO), Serotonin, Korean green tea, MAO inhibitor

Introduction

The green tea means the processed young leaves of *Camelia sinensis* L.(Theaceae) which was harvested with different periods in early spring. Various bioactivities of constituents of green tea have been reported. Anticancer activities, antioxidant and antimutagenic activities, protective effects on UVA- and UVB- induced skin damage, neuroprotective effects, antiinflammatory activities, induction of apoptosis, cardiovascular disease and other pathologies were studied with isolated compounds and crude extracts of green tea. catechins from green tea were studied about the activities of anticarcinogenic, antioxidant, prevention of skin cell injury, apoptotic, modulation of lipid metabolism, regulation of intestinal glucose transport. Epigallocatechin-3-gallate(EGCG) and (-)-epigallocatechin(EGC), major components of green tea were reported the anticancer activities, antioxidative effect, protective effect on UV light-induced skin

damages, apoptosis activity. Polyphenols of green tea were also reported antiinflmmatory activity, chemopreventive activities, antioxidative effects, brain cell preventive effect. green tea extracts has been studied as the therapeutic purpose of inflammation, brain protection, mitogenic, and cardiovascular disease. However, the papers about anti-hypertension and dementia treatment were rare.

Serotonin is important neurotransmitter in central nervous system. The MAO plays a central role in the metabolism of many amines including the neurotransmitter monoamines. MAO is a flavoprotein found exclusively in the mitochondrial outer membrane, occuring in the MAO-A and MAO-B subtypes. MAO-A deaminates serotonin and noradrenaline, whereas MAO-B prefers penylethylamine and benzylamine as substrates. MAO inhibitors have been used for the purpose of therapeutics of Parkinson's disease, depressant and hypertension.

In the course of our studies on the inhibitory activity of the monoamine oxidase(MAO), the MEOH extract of korean green tea demonstrated inhibitory activities against MAO-A and MAO-B. Especially, among the green tea harvested with the different region and period, late harvested green tea (中雀) produced region A in Korea extract showed potential inhibitory activities.

Materials and Methods

Materials

The green teas harvested and processed in the different periods and different regions were purchased from each other companies(BS and CH) in Korea. The voucher specimens were kept in the research laboratory. Sprague-Dawley male rats were purchased from Daehan Experimental Animal Co. (Yeumsung, Korea). (+)Catechin, (-)catechin and (-)EGCG were obtained from Sigma.(Sigma Co. U.S.A.).

Isolation of samples

The dried green tea (81.2 g) was minced by domestic mixer and added 10 parts of 80% MeOH. After stand in room temperature for 7 days, MeOH extracts were filtered and concentrated by vacuum pump evaporator on 50°C water bath. Concentration of the solution afforded an extract of about 40.3 g, which was suspended in H_2O and partitioned with $CHCl_3$ (15.4 g), EtOAc (26.3 g) and BuOH(14.0 g), successively. Each fraction was screened about inhibitory activities on MAO-A and MAO-B. At the same time, epigallocatechin-3-gallate(EGCG), (-)catechin and (+)catechin, which were well known the major components of green tea were also measured about inhibitory activities on MAO-A and MAO-B. The EtOAc soluble fraction was subjected to column chromatography over silica gel 60 (70-230 mesh), preparative TLC eluted under gradient conditions with $CHCl_3$ in MeOH and Hexane in EtOAc. The EtOAc soluble fraction was compaired of its TLC pattern to some known compounds.

Preparation of Monoamine Oxidase-A (MAO-A)

Enzyme sources were prepared from brain of Sprague-Dawley male rat by the routine procedures. In other words, The rat was anaesthetized with ethyl-ether and was lost blood with 3.13% sodium citrated syringe from heart. The brain tissue was obtained from decapitated brain immediately. The brain was washed with 0.01M phosphate buffered saline(PBS, pH 7.0), and homogenate at 4°C for 1 minute followed by added cold 0.25M sucrose by 9 parts of wet weight of tissue. Centrifuged at 700 g in 4°C for 20 min. Supernatant was centrifuged at 18,000 g for 20 min immedeately. Pellet was suspended in 5 parts of PBS, and used for crude enzyme preparation.

MAO-A assay

Prepared crude MAO-A 0.5 mL was added to test tubes with took 1.0 mL of green tea extracts. Incubated at 37.5°C for 15 min. in shaking incubator. As a substrate, 0.5 mL of 1.0 mM serotonin was added and incubated at 37.5°C for 90 min. To terminate the enzyme action, test tubes were heated at 95°C water bath for 3 min. and centrifuged at 700 g for 20 min., immediately. Supernatants were poured in prepared Amberlite CG-50(H^+ form) column(0.6 x 4 cm). After washed with distilled water thoroughly(over 40 mL), eluted with 3 mL of 4N acetic acid, the eluate was determined of absorbance at 277 nm. Instead of samples, same volumes of distilled water were added in control. In the sample controls, the substrates were added on the time of activity termination instead of initiation of action. Each group was performed with duplicates and calculated the inhibition percentages of samples by proper expression.

Preparation Monoamine Oxidase-B (MAO-B)

Enzyme sources were prepared from liver of Sprague-Dawley male rat by the routine procedures. In other words, The rat was anaesthetized with ethyl-ether and was lost blood with 3.13% sodium citrated syringe from heart. Obtained liver tissue was washed with 0.01M phosphate buffered saline(PBS, pH 7.0), and homogenate at 4°C for 1 minute followed by added cold 0.25M sucrose by 5 parts of wet weight of tissue. Centrifuged at 700 g in 4°C for 20 min. Supernatant was centrifuged at 18,000 g for 20 min immediately. Pellet was suspended in 5 parts of PBS, and preserved at freezer before the treatment of samples.

MAO-B assay

Enzyme assay methods were performed by McEwen's methods. Prepared crude MAO-B 0.5 mL was added to test tubes with took 1.0 mL of green tea extracts. Incubated at 37.5°C for 15 min. in shaking incubator. As a substrate, 0.5 mL of 4.0 mM benzylamine was added and incubated at 37.5°C for 90 min. To terminate the enzyme action, added

0.2 mL of 60% perchloric acid and added 4 mL of cyclohexane, simultaneously. Mixed immediately with voltex mixer and centrifuged at 700 g for 20 min. Cyclohexane layer was determined of absorbance at 242 nm. In the same manner as in MAO-A, instead of samples, same volumes of distilled water were added in control. In the test controls, the substrates were added on the time of activity termination instead of initiation. Each group was performed with duplicates and calculated the inhibition percentages of samples by proper expression.

Assay of MAO Activity and Inhibition by Green Tea Extracts and Standard Compounds

Preparation of test samples: Each fraction was screened about inhibitory activities on MAO-A and MAO-B. At the same time, epigallocatechin-3-gallate(EGCG), (-)catechin and (+)catechin, which were well known the major components of green tea were also measured about inhibitory activities on MAO-A and MAO-B.

Results and Discussion

By the activity guided separation, we compared the activities of MeOH extract and various solvent fraction with pure components which were well known major components of green tea. Brain MAO-A activity was inhibited by green tea MeOH extracts. And the liver MAO-B activity was also inhibited by all of the green tea extracts with strong intensity. Whereas (-) catechin which is well known one of the main bioactive component of green tea did not show inhibitory activity on MAO-A and -B at all. Epigallocatechin-3-gallate(EGCG) and (+)catechin did not show inhibitory activities against MAO-A, But MAO-B activity was inhibited by these two compounds in low intensity. So we compared the TLC patterns of above three compounds with each fraction of various solvent extracts. We found that the above three compounds have different Rf values from active fraction of green tea in our bioassay system. $CHCl_3$, BuOH, aqueous fraction of green tea were showed inhibitory activities against MAO-A and EtOAc fraction inhibited MAO-B activity. EGCG and (+)catechin were water soluble compounds. They were showed strong inhibitory activities against MAO-B relatively. But they were showed weak inhibitory activities on MAO-A.

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