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Studies about Monoamine Oxidase Inhibitory Activities of Korean Green Tea (*Teae sinensis* L.) Harvested from Different Time and Location

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Abstract – This study was designed to investigate the nervous sedative effects of green tea. The sedative effect was evaluated by examination of Monoamine oxidases (MAOs) inhibitory activity *in vitro* in the brain and liver of rat fed on green tea cultivated and harvested from the different regions and periods. It showed that methanol extracts of green tea inhibited significantly the brain MAO-A activity. Especially late harvested green tea extracts showed potential inhibitory activity. The liver MAO-B activity was also inhibited by all of the green tea extracts with strong intensity. This study confirmed that major compounds of green tea such as catechin, epigallocatechin-3-gallate (EGCG) and L-theanine, which were well known for the main bioactive components in the tea plants, were not associated with the MAO inhibitory activities of green tea. These results suggested that a MAO inhibition activity comes from other minor tea components we have to search in the future.
Keywords – Monoamine oxidase (MAO), Korean green tea, MAO inhibitor, Catechin, EGCG

Introduction

The green tea means the processed young leaves of *Teae sinensis* L. (Theaceae), which harvested in early spring. Various bioactivities of green tea have been reported. Anticancer (Wang *et al*., 2002, Yang *et al*., 2002, Hsu *et al*., 2001), antioxidant (Lau *et al.,* 2002, Liebler *et al.,* 2001 Nagai *et al.,* 2002, Cai *et al.,* 2002), and antimutagenic activities (Gupta *et al*., 2002), protective effects on UVA- and UVB- induced skin damage (Tobi *et al.,* 2002), neuroprotective effects (Kakuda, *et al*., 2002, Pan, *et al*., 2003), anti-inflammatory activities (Das *et al*., 2002, Tedeschi *et al*., 2002), induction of apoptosis (Vergote *et al*., 2002) were studied with isolated compounds as well as crude extracts of green tea. Catechins from green tea were extensively studied about the activities of anticarcinogenic (Yang *et al*., 2002), antioxidant (Liebler *et al*., 2001). EGCG and (−)-epigallocatechin (EGC), other two major components of green tea, were also reported for the anticancer activities (Wang *et al*., 2002), antioxidative effects (Liebler *et al.,* 2001), protective effects on UV light-induced skin damages (Tobi *et al.,* 2002), apoptosis activities (Vergote *et al*., 2002). Polyphenols of green tea were also reported for anti-inflammatory activities (Tedeschi *et al*., 2002), chemo-preventive activities (Hsu *et al*., 2001), antioxidative effects (Cai *et al.,* 2002), and brain cell preventive effects (Choi *et al*., 2002). Green tea extracts has been studied as the therapeutic purpose of inflammation (Das *et al*., 2002), brain protection (Pan, *et al*., 2003), and cardiovascular disease. However, studies about anti-hypertension, nervous sedative effect and dementia treatment (Choi *et al*., 2002) were rare. Monoamine oxidase (MAO; EC1.4.3.4) is the most extensively studied enzyme associated with central monoamine transmitter systems. Pharmacologically, MAO can be divided into two forms, termed MAO-A and MAO-B. Monoamine oxidases (MAOs) play a central role in the metabolism of many amines including the neurotransmitter monoamines. MAOs are flavorproteins found exclusively in the mitochondrial outer membrane, occurring in MAO-A and MAO-B subtypes. MAO-A delaminates serotonin and norepinephrine, whereas MAO-B prefers phenylethylamine and benzylamine as substrates. MAO inhibitors have been used for the purpose of therapeutics of Parkinson's disease, depressant and hypertension (Blaschko *et al*., 1974, Cooper *et al*., 1996). In this study we determined the inhibitory activity of Korean green tea obtained from the different regions and harvested periods

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on MAO activity in the brain and the liver of rat by *in vitro* system.

Experimental

Plant materials and reagents – The green teas harvested and processed in the different periods and different regions in Korea were purchased. The voucher specimens (#KM120925) were kept in the research laboratory in Kookmin university (Seoul, Korea). Sprague-Dawley male rats were purchased from Bio Genomics, Ind., which was licensed by Charles River Technology Experimental Animal Co. (Seoul, Korea). Serotonin, benzylamine, (+)-catechin, (−)-catechin, L-theanine, (−)-epigallocatechin-3-gallate [(−)-EGCG] and Amberlite CG-50 (H^+ form) were obtained from Sigma Co. (St. Louis, U.S.A.). This research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in for the US guidelines (NIH publication #85-23, revised in 1985). The green tea samples were classified according to harvested period, in April moderate (Apr-M), in April terminal (Apr-T), in May early (May-E), and in May moderate (May-M). Each 10 g of eight dried green tea was minced by domestic mixer and added 10 parts of 80% methanol. Following stood in room temperature for 7 days, methanol extracts were filtered and concentrated by vacuum pump evaporator on 45°C water bath. Each extract was examined for the inhibitory activities on MAO-A and MAO-B.

Preparation and assay of MAO-A – Enzyme sources were prepared from brain of Sprague-Dawley male rat by the routine procedures (Hwang *et al*., 2003). The rats were anaesthetized with ethyl-ether and were lost blood with 3.13% sodium citrated syringe from heart. The brain tissue was obtained from decapitated brain immediately. The brain was washed with 0.01 M phosphate buffered saline (PBS, pH 7.0), and homogenate at 4°C for 1 minute followed by added cold 0.25 M 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 immediately. Pellet was suspended in 5 parts of PBS, and used for crude enzyme preparation.

Prepared crude MAO-A (0.5 mL) was added to test tubes with 1.0 mL of green tea extracts. It was incubated in shaking incubator at 37.5°C for 15 min. 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 × 4 cm). After washed with distilled water thoroughly (over 40 mL), eluted with 3 mL of 4 N acetic acid, elute 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 duplicated and calculated for the inhibition percentages of samples by proper expression.

Preparation and assay of MAO-B – Enzyme sources were prepared from liver of Sprague-Dawley male rat by the routine procedures. 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 0.01 M phosphate buffered saline (PBS, pH 7.0), and homogenate at 4°C for 1 minute followed by added cold 0.25 M sucrose by 5 parts of wet weight of tissue and 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. Enzyme assay methods were performed by McEwen's methods (Lyketsos *et al*., 2002). Prepared crude MAO-B 0.5 mL was added to test tubes with 1.0 mL of green tea extracts. It was 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 vortex mixer and centrifuged at 700 g for 20 min to precipitate the protein. 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 for the inhibition percentages of samples by proper expression.

The inhibitory activity of Green tea on the MAO – The methanol extracts of Korean green tea harvested from the different region and period were measured about inhibitory activities on MAO-A and MAO-B. At the same time, EGCG, (−)-catechin and (+)-catechin, which were well known the major components of green tea, were also examined. Enzyme sources were prepared from brain and liver of Sprague-Dawley male rats by the routine procedure and enzyme assay methods were performed by previous report (Hwang *et al*., 2003). Since the methanol extract of <region A May-E> green tea showed potent

inhibitory activity against MOA-A, the TLC patterns of the two groups, one is green tea extract group and another is already known pure compound group such as EGCG and catechin, were examined weather or not the MAO activities were from the known compounds.

Statistical analysis – All experiments were conducted in independent triplicate (n = 3) and data were expressed as mean ± SD. Statistical significance was evaluated by one-way analysis of variance using SPSS Win program (Version 19.0, Cary, NC), and individual comparisons were determined using Duncan's multiple range tests at the $p < 0.05$ level.

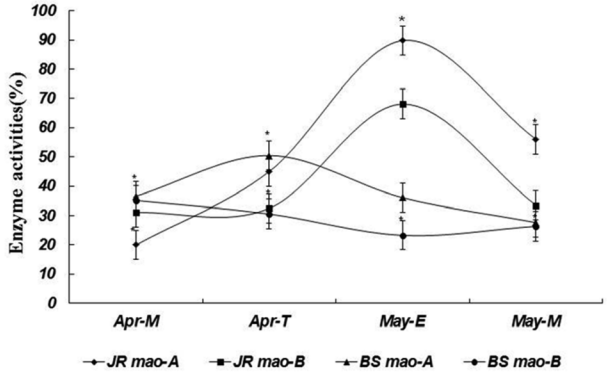

Fig. 1. Inhibitory activities of green tea harvested in different region and period on MAO. Data represent the means ± S.D. Different subscript letters indicate a significant difference at the $p < 0.05$ by Duncan's multiple range tests.

Results and Discussion

The methanol extracts of green tea from two different locations and four different harvesting times were examined on the inhibitory activity of MAO-A and MAO-B. All of the green tea extracts showed inhibitory activities about MAO-A and MAO-B. In general, it is recognized that early harvested green tea was thought to have high product value in markets. But it did not show strong inhibitory activity on MAOs. We found that the inhibitory activity of green tea on MAOs gradually increased with harvest period until May early, and decreased after (Fig. 1.). All of them, May-E extract showed the most potent inhibitory activity in both enzymes. For the purpose of determining the MAO inhibitory activities of the green tea, harvested in different period, examined the inhibitory activities in various concentrations (0.05, 0.50 and 5.0 mg/ml). A (region A) and B (region B) green teas were also compared. Region A green tea showed a potent inhibitory activity in dose-dependent manner. All of them, May-E green tea showed the most potent inhibitory activity on MAO-A and MAO-B (Fig. 2.). And the methanol extract of green tea extract especially from <region A May-E> also showed inhibitory activity in a dose dependent manner in MAO-A and MAO-B (Fig. 2.). In order to study the active compound of green tea, the activity guided isolation was considered. First of all, the activities of methanol extract of <region A

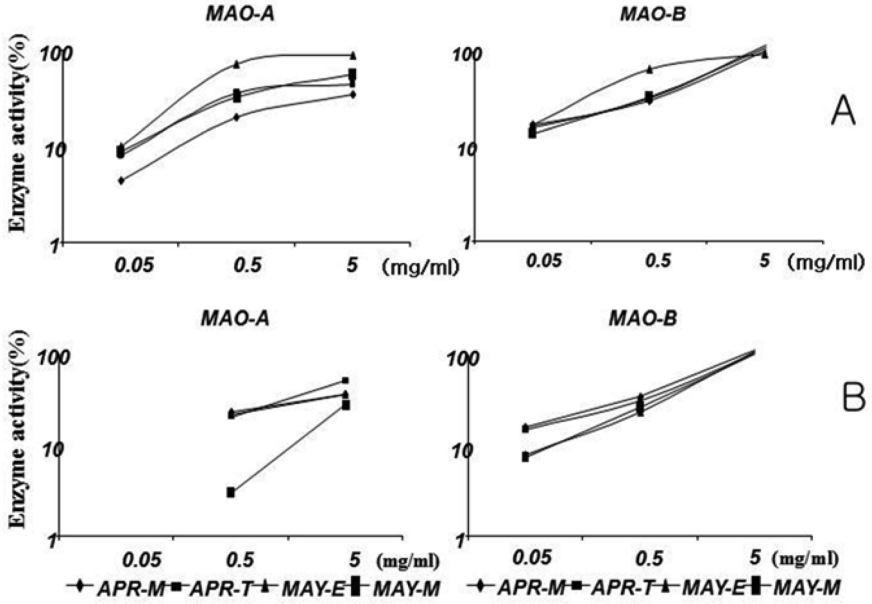

Fig. 2. Inhibitory activities of green tea harvested in different period on MAO-A and MAO-B. Prepared crude MAO-A (0.5 mL) was added to test tubes with 1.0 mL of green tea extracts. It was incubated in shaking incubator at 37.5°C for 15 min. As a substrate, 0.5 mL of 1.0 mM serotonin was added and incubated at 37.5°C for 90 min. Prepared crude MAO-B 0.5 mL was added to test tubes with 1.0 mL of green tea extracts. It was incubated at 37.5°C for 15 min in shaking incubator.

Table 1. IC_{50} value and specific activity of green teas on MAO

Fractions	Amounts (g)	IC_{50} Values (mg/ml)		Specific activity	
		MAO-A	MAO-B	MAO-A	MAO-B
A Apr-M	0.179	5 <	4.1	0.20×10^3	0.24×10^3
A Apr-T	0.202	5 <	3.8	0.20×10^3	0.26×10^3
A May-E	0.141	0.33	0.33	0.30×10^4	0.30×10^4
A May-M	0.201	3.6	3.8	0.28×10^3	0.26×10^3
B Apr-M	0.173	5 <	1.7	0.20×10^3	0.86×10^3
B Apr-T	0.198	5	1.6	0.20×10^3	0.64×10^3
B May-E	0.173	5 <	1.9	0.20×10^3	0.52×10^4
B May-M	0.177	5 <	1.85	0.20×10^3	0.54×10^3
L-theanine		–	–	–	–
Caffeine	1.817	0.22	0.71	0.45×10^4	0.14×10^4
Catechin		1.44	0.60	0.14×10^4	0.45×10^4
EGCG		0.66	3.34	0.30×10^4	0.60×10^3

May-E> green tea and well known tea compounds, such as EGCG and catechins, were compared. Brain MAO-A was inhibited by methanol extracts of green tea. And the liver MAO-B was also inhibited with strong intensity. Especially <A May-E> showed potent inhibitory activities on both enzymes. The IC_{50} values on MAO-A and MAO-B were 0.33 and 0.33 mg/ml, respectively. But they showed weak inhibitory activities on MAO-A as much as methanol extract of <region A May-E>. L-theanine did not show inhibitory activity on any of enzymes (Table 1). Based on the TLC patterns of above three compounds with methanol extracts of <region A May-E> green tea, it turned out there were many spots methanol extract besides standard major compounds in our chromatographic solvent system. These results suggested that a MAO inhibition activity comes from other minor tea components we have to search in the future. And we will continue to study active compounds of green tea on MAO inhibition. Psychiatric disturbances affect as many as 90% of patients with Alzheimer's disease (AD) and are a major focus of treatment. Depression is one of the most frequent psychiatric complications of AD, affecting as many as 50% of patients (Lyketsos *et al*., 2002, Lyketsos *et al*., 2003, Olin *et al*., 2002). In recent studies, MAO inhibitor is focused as the drug on the treatment of depression associated with Alzheimer disease. Irie *et al*. have reported in a recent communication that eugenol exhibits an antidepressant-like activity in mice comparable to that of imipramine, the classical tricyclic antidepressant (Irie *et al*., 2004). Tao et al. have also reported eugenol isolated from the botanical *Rhizoma acori graminei* for inhibitory activity toward the MAO-A and MAO-B activities in a rat brain mitochondrial fraction. They discussed on their results that the antidepressant-like action of eugenol could be mediated by its MAO-A inhibitory activity. Their findings provide for the first time a scientific rationale for the traditional use of RAG for the treatment of one of the most prevalent neuropsychiatric comorbidities of AD: depression (Tao *et al*., 2005). Fusar-Poli *et al*. reported psychologic therapy using MAOIs offers further advantages after heart transplantation (Fusar *et al*., 2006). Traditionally, MAO inhibitors were used for treatment of Parkinson's disease (Gutschow *et al*., 2006, Olanow *et al*., 2006, Siderowf *et al*., 2006).

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