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 UV-A- 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), chemopreventive 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
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. Obtained liver tissue was washed with ethyl-ether and was lost blood with 3.13% sodium citrated syringe. 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 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.

**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