

Monoamine oxidase inhibitory activity of green tea produced in China, its processing and provincial differences.

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Summary

In ancient China, tea was used as medicine to cure the sicknesses. Chinese tea can be classified as no-fermented tea, semi-fermented tea and fermented tea according to pre-processing. And it has been made many shapes in appearance and styles in quality. Green tea is known to have enormous health benefits such as blood pressure lowering, anti-cancer and anti-aging effect. Those health benefits of tea might be considerable points in the process of new product development and also for the promotion of tea consumption.

The monoamine oxidase (MAO, EC 1.4.3.4) 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, occurring in the MAO-A and MAO-B subtypes. MAO-A deaminates serotonin and noradrenaline, whereas MAO-B prefers phenylethylamine and benzylamine as substrates.

The MAO inhibitory activity might be effective on the nervous sedative, antihypertensive and protective in dementia. The present study evaluated the MAO inhibitory activity of green tea produced in China, its processing and provincial differences.

Keywords

Monoamine oxidase inhibitor, Green tea, China

Introduction

The health-promoting and disease-preventing potential of naturally occurring substances in the diet is a major important scientific research area. Tea, which is processed from tea leaves (*Camellia sinensis* L.) is probably, the cheapest and the most popular beverage in the world. In China, green tea has been used as a herbal medicine for 5,000 years. There is no adverse effects in association with the medicinal use of tea. The objective of this study was to investigate monoamine oxidase inhibitory activities of Chinese green tea, and also the comparison of activities, according to the provinces and processing methods. The bioassay guided subfractionation of six kinds of green tea, which shown the strong MAO inhibitory activities has been achieved *in vitro*.

Materials and Methods

Sample preparation

The 36 kinds of green teas were purchased in commercial tea market in YounBian, KilLim province, China in July, 2000. It had been cultivated several provinces, BokGun; EunNam; KwangSeo; JeulKang and HaNam provinces, and also processed by the means of drying, roasting and fermentation. Fully ground green tea (100 g) was extracted with 70% ethylalcohol at room temperature, overnight (x 3) and filtered. The combined extracts were concentrated under reduced pressure to give a dark brown extract. This extract were partitioned with EtOAc and H₂O soluble fractions, and subfractions were dried at -70°C, 48 hours to give powder.

Bioassay

Sprague-Dawley male rats were purchased from Daehan Experimental Animal Co., Yeumsung, Korea. (+)-Catechin, (-)-Catechin and (-)-EGCG were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.

Monoamine oxidase A (MAO-A) inhibition assay

The MAO-A activity was determined using serotonin as a substrate. A reaction mixture containing 0.5 ml of enzyme solution in 0.01 M phosphate buffered saline (pH 7.0) and 1.0 ml of test solution was preincubated at

37°C for 15 min., after which 0.5 ml of 0.01 M serotonin creatine sulfate (Sigma Co.) in a buffer was added. Following incubation at 37°C for 90 min, the enzyme reaction was terminated by heating for 3 min in a 95°C water bath. After centrifugation, 1.6 ml of supernatant was loaded to an Amberlite CG50 (H⁺ form) column. The column was washed with over 40 ml of water and the unreacted substrate was eluted with 3.0 ml of 4.0 N acetic acid solution and subjected to spectrophotometrical measurement at 277 nm. Activity was calculated as follows:

$$\text{Inhibition \%} = (A_{\text{sample}} - A_{\text{compensate}} - A_{\text{control}}) / (A_{\text{blank}} - A_{\text{control}}) \times 100$$

Monoamine oxidase B (MAO-B) inhibition assay

The MAO-B activity was determined using benzylamine hydrochloride as a substrate. The reaction mixture containing 0.5 ml of enzyme solution in the buffer and 1.0 ml of test solution was preincubated at 37°C for 15 min., after which 0.5 ml of 0.04 M benzylamine hydrochloride (Tokyo Kasei Co.) was added. Following incubation at 37°C for 90 min., the enzyme reaction was terminated by adding 0.2 ml of 60% perchloric acid. The reaction product, benzaldehyde, was extracted with 4.0 ml of cyclohexane and subjected to spectrophotometrical measurement at 242 nm. In the control group, water was substituted for the test solution. In the blank group, the substrate was omitted, but was added after the incubation. To compensate the test solution's own absorbance, the substrate was omitted in the compensate group. Activity was calculated as follows:

$$\text{Inhibition \%} = (A_{\text{control}} - A_{\text{sample}} + A_{\text{compensate}}) / (A_{\text{control}} - A_{\text{blank}}) \times 100$$

For the comparison with subfractions of green tea on MAO inhibitory activities, (-)-catechin, (+)-catechin and (-)-epigallocatechin-3-gallate(EGCG), major bioactive components in green tea were also investigated.

Results and Discussion

In a dose-dependent manner with IC₅₀ values yielded of 6.5 to 0.1 mg of MAO-A and 3.0 to 0.1 mg of MAO-B, respectively (Table 1).

Table 1. Inhibitory Activities of Solvent Subfractions of Green Tea processed in China

Samples*			I	II	III	IV	V	VI	(-) catechin	(+) catechin	(-) EGCG
Aqueous fraction	MAO -A	IC ₅₀ (mg)	-	6.5	-	-	2.0	-	-	1.0	1.0
		Specific activity	-	20.4	-	-	20.6	-			
	MAO -B	IC ₅₀ (mg)	1.3	1.4	1.2	0.1	1.3	<0.1	-	0.6	0.7
		Specific activity	56.2	96.0	28.4	834.3	31.2	<1,262			
EtOAc fraction	MAO -A	IC ₅₀ (mg)	0.8	1.1	0.4	<0.1	-	0.8			
		Specific activity	20.9	75.7	77.8	<1,356	-	16.8			
	MAO -B	IC ₅₀ (mg)	-	1.4	-	3.0	0.3	<0.1			
		Specific activity	-	60.0	-	45.2	19.7	<141			

*I : Drying in a dugout, BokGun province; II : Drying in the sun, BokGun province;
 III : Drying in a dugout, BokGun province; IV : Fermentation, EunNam province;
 V : Drying in a dugout, BokGun province; VI : Drying in a dugout, BokGun province

Results of this investigation indicate that most of samples can attenuate peroxide production by inhibiting the deamination of monoamines as a scavenger, regardless of the methods of processing and provinces of cultivation. It was also shown to inhibit non-competitively both MAO-A and MAO-B with the IC₅₀ values. It is concluded that some Chinese green teas may be useful in the prevention/treatment of neurodegenerative diseases relating to *in vitro* MAO inhibitory activities. The structure-activity relationship examination should be followed for further research..

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