Arch. Pharm. Res. 13(2), 132-135 (1990)

Antioxidant Activity of Resveratrol Closely Correlates with Its Monoamine oxidase-A Inhibitory Activity

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(Received February 27, 1990)

Abstract□Polyhydroxystilbenes including resveratrol were reported to competitively inhibit monoamine oxidase-A without structural relation with substrates and synthetic inhibitors for the enzyme. We attempt to explore a plausible mechanism for their inhibitory activity on MAO-A. All the polyhydroxystilbenes tested showed the antioxidant activity on liver homogenate. Furthermore, the antioxidant activity turned out to closely correlate with the MAO-A inhibitory activity.

Keywords□Antioxidant, monoamine oxidase inhibitor, resveratrol, stilbene.

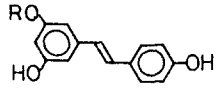
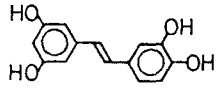
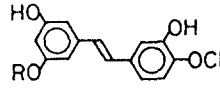
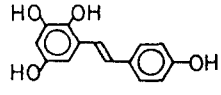
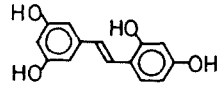
In search of natural products capable of inhibiting monoamine oxidase (MAO; EC 1.4.3.4), we isolated various kinds of phenolic substances as active principles[1,2]. Among them, resveratrol (**1**) proved to be the most potent inhibitor[2]. Its IC_{50} and Ki values were 2 μM and 2.5 μM, respectively, using serotonin as substrate and the mitochondrial MAO of rat brain as an enzyme preparation. A kinetic study revealed that resveratrol competitively inhibited MAO-A and thus was the selective MAO-A inhibitor[2].

Resveratrol belonging to polyhydroxystilbene never resemble synthetic inhibitors of MAO in the aspect of structural feature, since they are known to be all phenylethylamines and substrate analogues such as clorgyline, deprenyl, pargyline, phenethylhydrazine, amphetamine and harman[3,4]. In this paper, we describe that the inhibition of MAO-A by resveratrol is responsible for its antioxidant activity.

Antioxidant activity of resveratrol was evaluated by its inhibitory effect on lipid peroxidation induced when mouse liver homogenate was exposed to air. Lipid peroxide was measured by the 2-thiobarbituric acid (TBA) method[5]. As shown in Table I, resveratrol (**1**) was about eleven times as active as maltol[5] in comparison of ED_{50} value.

To study the relationship between the chemical structure and antioxiant activity, six polyhydroxystilbenes[2] that differ in the number and position of hydroxyl groups and in the substituted group were tested for their antioxidant activity (Table I). When an extra hydroxyl group presents at the *meta*-position on B ring of resveratrol like **3**, there was a slight diminution in antioxidant activity. As for **6** and **7** which have an extra hydroxyl group at the *ortho*-

Table I. Anti-oxidant activity of polyhydroxystilbenes and maltol

Compound Structure	R	No.	ED_{50}[a] (μM)	IC_{50}[b] (μM)
	H	**1**	10	2
	Glu[c]	**2**	20	30
		**3**	12	15
	H	**4**	24	24
	Glu[c]	**5**	120	1000
		**6**	180	100
		**7**	100	900
Maltol			110	

[a] Anti-oxidant activity on the auto-oxidation of mouse liver homogenate. Lipid peroxide was determined by TBA method[5].

[b] Inhibition of MAO-A[2]

[c] β-Glucopyranoside

position on A or B ring of resveratrol, the activities greatly decreased. Masking of hydroxyl proton with methyl and glycosyl groups diminished the activities (compounds **2**, **4**, and **5**).

The structure-activity relation for antioxidant activity seems to resemble that for MAO-A inhibitory activity[2], which is cited in Table I from reference 2. Fig. 1 shows a scatter diagram between both activities, which exhibited a complete correlation (coefficient, $r = 0.9287$).

It is already known that reveratrol inhibits lipid peroxidation by Kimura, *et al.*[6], who studied the effects of some stilbenes on liver in peroxided oil-fed rat. Stilbene derivatives are also reported to show antifugal activity[7] and inhibitory effect on prostaglandin synthetase[8], and compounds inhibiting PG biosynthesis exhibit inhibitory effects on platelet aggregation[8]. Thus, we examined inhibitory effect on platelet aggregation with the stilbene compounds that have the MAO-A inhibitory activity. The inhibitory effect on platelet aggregation was carried out through the measurement of malonyldialdehyde (MDA) generated from platelets by thrombin induction[9]. MDA is concomitantly released with 12-L-hydroxy-5,8,10-heptadecatrienoic acid (12-HHT) and thromboxane A_2 when platelets are treated with thrombin or other reagents[10]. As shown in Table II, resveratrol (**1**) and rhapontigenin (**4**) were as active as aspirin, but another stilbenes were inactive. However, closer correlation between the inhibitory activity on platelet aggregation and the MAO-A inhibitory activity of polyhydroxystilbenes was not found (correlation coefficient, $r = 0.57$). These results indicate that the inhibition of MAO-A by resveratrol and its derivatives is only reponsible for their antioxidant activity. Other investigators also isolated MAO inhibitors from natural products. These are phenolic substance such as chalcone[11] and anthraquinone[12], which are expected to possess antioxidant activity.

MAO catalyzes the oxidative deamination of a wide variety of amines[13], and is an intergral protein found in the mitochondrial outer membrane[14]. The enzyme active site contains flavin adenine dinucleotide (FAD) as a cofactor and also depends on the presence of sulfhydryl groups andd lipids for full activity[15]. The cyclopropylamines such as tranylcypromine irreversibly inactivate MAO by alkylating a sulfhydryl group in the enzyme active site, thereby blocking access of oxygen to the reduced flavin and preventing its reoxidation[16]. The acetylenic inhibitors such as clorgyline, pargyline, and deprenyl covalently bind the 8 α -5-cystein-FAD cofactor at the active site[17]. The hydrazines such as phenyl hydrazine, and isocarboxazid appear to form an irreversible adduct with the flavin in addition to alkylating sulfhydryl groups[18]. All of these suicide inhibitors are actually substrates for the enzyme, and initially react competitively before forming the irreversibe covalent linkage[4]. Since resveratrol, although it is not related with substrates

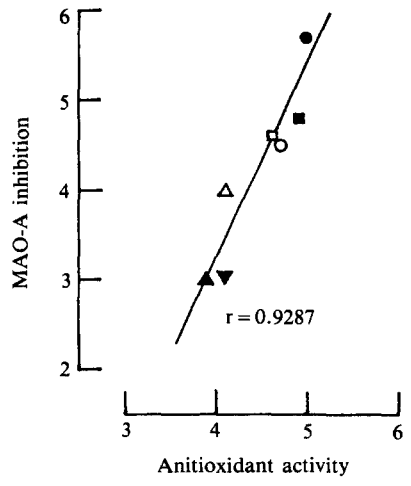


Fig. 1. A closer relationship between MAO-A inhibitory and anti-oxidant activities of polyhydroxystilbenes. Each activity is represented as $-\log IC_{50}$ or $-\log ED_{50}$, in the axis of co-ordinates. ●, resveratrol (**1**); ○ piceid (**2**); ■, 3,3'4',5-tetrahydroxystilbene (**3**); □, rhapontigenin (**4**); ▲, rhaponticin (**5**); △, 2,3,4'5-tetra-hydroxystilbene (**6**); ▼ oxyresveratrol (**7**).

Table II. Inhibitory effects of some polyhydroxystilbenes on malonyldialdehyde generation during thrombin-induced platelet aggregation

Compound	IC_{50} (M)[a]
1	3.8
2	>1000
4	3.0
5	>1000
Triacetate of **1**	35
Aspirin	3.5

[a] One m*l* of washed rat platelets (1×10^9 cells/m*l*) was pre-incubated with 0.1 m*l* of inhibitor in 0.01 M phosphate buffered saline (pH 7.4) at 37°C for 30 min and then 50 μl (10 units) of bovine thrombin was added to aggregate platelets. After further incubation of the reaction mixture at 37°C for 30 min, amount of MDA generated was determined by TBA method[9].

for the enzyme[2], possesses antioxidant activity, it may react competitively with the reduced flavin or the sulfhydryl group to block access of oxygen.

EXPERIMENTAL METHODS

Materials

Isolation of various stilbenes tested was described in a previous work[2]. TBA and bovine thrombin were purchased from Sigma Co.

Antioxidant activity

The activity was measured according to the method previously described[5]. In brief, liver of ddD mouse maintained with a normal chow diet was homogenized in ten volumes of 0.9% saline. To 0.3 m*l* of the homogenate, 0.1 m*l* of sample solution (DMSO below 0.2% used) was added and incubated at 37°C for 5 hr. TBA reagent (3.6 m*l*) was added and heated at 95°C for one hr. The TBA-pigment was extracted with 4 m*l* of n-butanol and the absorbance at 535 nm was recorded.

MDA generation during thrombin-induced aggregation of rat platelets

Washed platelets of rats[9] (0.85 m*l*) was warmed to 37°C in a small polypropylene tube and inhibitor solution (DMSO below 0.5% used) (0.1 m*l*) was added. The mixture was preincubated at 37°C for 30 min and then bovine thrombin (0.05 m*l*, 10 U) was added. After further incubation at 37°C for one hr, MDA generated was assayed by the TBA method previously described[9].

ACKNOWLEDGMENTS

This study was supported by a Basic Research Grant from the Korean Science and Engineering Foundation (KOSEF 86-0405-08)

LITERATURECITED

1. Han, Y.N., Noh, D.B. and Han, D.S.: Studies on the monoamine oxidase inhibitors of medicinal plants I. Isolation of MAO-B inhibitors from *Chrysanthemum indicum, Arch. Pharm. Res.* **10,** 142 (1987).
2. Ryu, S.Y., Han, Y.N. and Han, B.H.: Monoamine oxidase-A inhibitors from medicinal plants, *Arch. Pharm. Res.* **11,** 230 (1988).
3. Ho, B.T.: Monoamine oxidase inhibitors, *J. Pharm. Sciences,* **61,** 821 (1972).
4. Murphy, D.L., Garrick, N.A., Aulakh, C.S. and Cohen, R.M.: New contributions from basic science to understanding the effects of monoamine oxidase inhibiting antidepressants, *J. Clin. Psychiatry,* **45,** 7 (Sec.2)-July, 37 (1984).
5. Han, B.H., Park, M.H. and Han, Y.N.: Studies on the antioxidant components of Korean ginseng (V): The mechanism of antioxidant activity of maltol and phenolic acid, *Korean Biochem. J.* **18,** 337 (1985).
6. Kimura, Y., Ohminami, H., Okuda, H., Baba, K., Kozawa, M. and Arichi, S.: Effects of stilbene components of roots of Polygomum ssp. on liver injury in peroxidized oil-fed rats, *Planta Media,* **49,** 51 (1983).
7. Inamori, Y., Kubo, M., Kato, Y., Yasuda, M., Baba, K. and Kozawa, M.: The antifugal activity of stilbene derivative, *Chem. Pharm. Bull.* **32,** 801 (1984).
8. Goda, Y., Shibuya, M. and Sanakawa, U.: Inhibitors of the arachidonate cascade from *Allium chinense* and *in vitro* platelet aggregation, *Chem. Pharm. Bull.* **35,** 2668 (1987).
9. Han, Y.N., Baik, S.K., Kim, T.H. and Han, B.H.: Antithrombotic activities of saponins from *Ilex pubescens, Arch. Pharm. Res.* **10,** 115 (1987).
10. Hamberg, M., Svensson, J. and Samuelsson, E.: Prostaglandin endoperoxides, A new concept concerning the mode action and release of prostaglandins, *Proc. Nat. Acad. Sci. USA,* **71,** 3824 (1974).
11. Tanaka, S., Kuwai, Y. and Tabata, M.: Isolation of monoamine oxidase inhibitor from *Glycyrrhiza uralensis* roots and structure-activity relationship, *Planta Medica,* **53,** 5 (1987).
12. Yamazaki, M., Satoh, Y., Maebayashi, Y. and Horie, Y.: Monoamine oxidase inhibitors from fungus, *Emericella navahoensis, Chem. Pharm. Bull.* **36,** 670 (1988).
13. Von Korff, R.W.: Monoamine oxidase: Structure, function and altered functions (Signer, T.P., Von Korff, R.W. and Murphy, D.L., eds.), p.1, Academic press, New York (1979).
14. Russell, S.M., Davey, J. and Mayer, R.L.: The vectorial orientation of human monoamine oxidase in the mitochondrial outer membrane, *Biochem. J.* **181,** 7 (1979).
15. Abell, C.W.: Monoamine oxidase A and B from human liver and brain, *Methods in Enzym.* **142,** 638 (1987).
16. Paech, C., Salach, J.I. and Singer, T.P.: Suicide inactivation of monoamine oxidase by transphenylcyclopropylamine. In Singer, T.P., Von Korff, R.W. and Murphy, D.L. (eds): Monoamine oxidase: Structure, function and al-

tered functions, Academic Press, New York (1979).
17. Maycock, A.L., Abeles, R.H. and Salach J.I.: The structure of the covalent adduct formed by interaction of 3-dimethylamino-1-propyne and the flavin of mitochondrial amine oxidase, *Biochem.* **15**, 114 (1976).
18. Kenney, W.C., Nagy, J. and Salach, J.I.: Structure of the covalent phenylhydrazine adduct of monoamine oxidase. In Singer, T.P., Von Korff, R.W. and Murphy, D.L. (eds): Monoamine oxidase: Structure, function and altered functions, Academic Press, New York (1979).