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MAGNOLOL AND HONOKIOL ISOLATED FROM *MAGNOLIA OFFICINALIS* PROTECT RAT HEART MITOCHONDRIA AGAINST LIPID PEROXIDATION

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Abstract—In isolated rat heart mitochondria lipid peroxidation was induced with ADP and ferrous sulfate ($FeSO_4$). Oxygen consumption and malondialdehyde (MDA) production were measured to quantitate lipid peroxidation. The antioxidant effects of magnolol and honokiol purified from *Magnolia officinalis* were 1000 times higher than that of α-tocopherol. The IC_{50} values of magnolol and honokiol for inhibition of oxygen consumption were 8.0×10^{-8} M and 1.0×10^{-7} M, respectively, while that of α-tocopherol was 1.0×10^{-4} M. Magnolol at 0.5 μM inhibited 71.4 ± 9.4% of oxygen consumption and 59.3 ± 4.6% MDA production. At the same concentration, honokiol inhibited 78.1 ± 4.7% of oxygen consumption and 86.9 ± 4.0% of MDA production. Of conjugated diene formation 48.4 ± 4.6% and 53.1 ± 3.4% were inhibited by 0.5 μM magnolol and honokiol, respectively. Also both magnolol and honokiol exhibited free radical scavenging activities as shown by the diphenyl-*p*-picrylhydrazyl assay, but they were less potent than α-tocopherol.

Key words: lipid peroxidation, mitochondria, magnolol, honokiol, *Magnolia officinalis*

Antioxidants are of interest for the treatment of cellular degeneration, drug toxicity, irradiation damage and ischemic/reperfusion injury [1]. All these abnormalities are considered to be associated with lipid peroxidation [2]. Since redox reactions are most prominent in mitochondria, these are constantly susceptible to oxidative stress [3, 4]. The heart is one of the target organs for injury by oxygen free radicals [5, 6], and several studies have demonstrated that lipid peroxidation in cardiac mitochondria plays an important role in the pathogenesis of cardiac dysfunction [7].

Many chemical components in Chinese medicinal herbs have antioxidant activity. For example, Rb_1, a saponin purified from *Panax ginseng*, inhibits lipid peroxidation in liver and cardiac homogenate [8]. Celastrol, a triterpine isolated from *Tripterygium wilfordii*, is 15 times more potent than α-tocopherol in inhibiting lipid peroxidation in rat liver mitochondria [9]. Schisanhenol, a schzandrin isolated from *Fructus schizandrae*, has been shown to protect rat heart mitochondria against Adriamycin®-induced toxicity by scavenging the hydroxyl radicals generated by electron transfer from Adriamycin semiquinolone radicals to hydrogen peroxide [10]. Several phenolic compounds isolated from *Salvia miltiorrhiza* were found to protect liver microsomes, hepatocytes and erythrocytes against peroxidative damage [11].

In this study, we screened six compounds extracted from five Chinese medicinal herbs for their effects on the lipid peroxidation of rat heart mitochondria. These compounds have previously been tested for their effect on platelet aggregation [12–16]. We found that magnolol and honokiol (Scheme 1) isolated from *Magnolia officinalis* were most potent in inhibiting lipid peroxidation in rat heart mitochondria, and their potencies were much higher than that of α-tocopherol, a frequently used antioxidant.

MATERIALS AND METHODS

Mitochondria were prepared from the hearts of male rats as described previously [17]. Sprague–Dawley rats weighing between 200 and 300 g were killed by decapitation and their hearts were quickly excised, opened and washed thoroughly with ice-cold 0.25 M sucrose. The hearts were finely minced with a pair of scissors into 10 vol. of SEH buffer, containing 0.25 M sucrose, 0.5 mM EGTA and 3 mM HEPES (pH 7.4). The suspension of minced heart was treated with Nagarse at a concentration of 0.1 mg/mL. After 15 min incubation at 0° with occasional stirring, the supernatant was discarded and the remaining tissue washed twice with 0.25 M sucrose solution. The dispersed heart tissue was then homogenized with a glass pestle and diluted with sucrose solution to 10 mL/g heart tissue. The homogenates were centrifuged at 400 *g* for 5 min in the JA 20 rotor of a Beckman J2/21 refrigerated high-speed centrifuge. The supernatant was decanted and centrifuged at 700 *g* for 5 min. After recentrifugation of the supernatant at 2500 *g* for 10 min, the pellet was resuspended in a KCl–Tris solution

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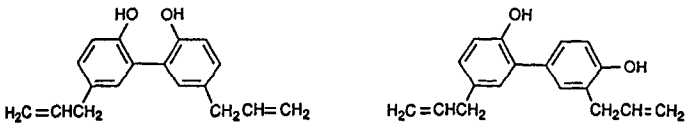

Magnolol Honokiol

Scheme 1.

containing 175 mM KCl and 20 mM Tris–HCl (pH 7.4), and centrifuged again for 10 min at 6000 *g*. The mitochondria-rich, red-brown lower layer was suspended in 0.5 mL of KCl–Tris solution/g heart tissue and stored at 0° for 3 days before it was used for the lipid peroxidation experiment. Freshly prepared mitochondria, like liver and myocardial homogenate [8], were resistant to lipid peroxidation, so that a cold storage period was required. The amount of protein in mitochondria was determined by the Lowry method [18], with bovine serum albumin as a standard.

Lipid peroxidation in rat heart mitochondria was measured according to the method described by Sassa *et al.* [9]. Peroxidation was started by addition of ADP and $FeSO_4$ to mitochondrial suspension. Final concentrations of ADP and $FeSO_4$ were 1 and 0.1 mM, respectively. The amount of oxygen consumed during the 6 min incubation period was monitored with a Clark-type oxygen electrode in a Gilson 5/6 oxygraph (Gilson Medical Electronics, U.S.A.). The total volume of the assay medium in the reaction chamber was 1.6 mL. The amount of oxygen consumed during peroxidation was calculated assuming that the saturated concentration of oxygen at 25° is 258 μM. At the end of the incubation period, 0.3 mL of mitochondrial suspension was mixed with 0.1 mL of 15.2% trichloroacetic acid. MDA* was assayed with a thiobarbituric acid technique and the level was used to represent the amount of lipid peroxides formed during incubation [19]. Tetramethoxypropane was used as an external standard. For estimation of conjugated diene, 0.3 mL of mitochondrial suspension was mixed with 0.7 mL of ethanol and then centrifuged at 3000 *g* for 10 min. The UV absorption of the supernatant at 234 nm was measured with ethanol as a blank [20].

It should be pointed out that MDA measured with the thiobarbituric acid technique is not specific for lipid peroxidation, as aldehyde derivatives from other compounds such as purines and amino acids are known to react. However, all the reacting compounds are the result of free radical damage. Besides, compounds such as sialic acid and glucose may interfere with UV absorption at 234 nm, but in mitochondria suspension, there is no reason to suspect that these compounds are changing in concentration.

Magnolol and honokiol were isolated as described by Fujita *et al.* [21]. They were dissolved in KCl–Tris buffer containing 0.5% (v/v) DMSO. At this concentration DMSO had no effect on lipid peroxidation.

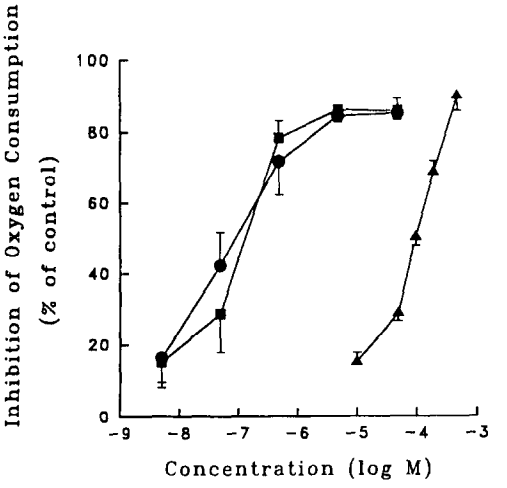

Fig. 1. Dose–response curves for the inhibitory effect of magnolol (●), honokiol (■) and α-tocopherol (▲) on oxygen consumption in rat heart mitochondria. Data points represent means ± SEM of six experiments.

For studying the anti-oxidant effect, a solution of the tested compound was pipetted into the reaction chamber prior to the co-incubation of mitochondria and ADP/Fe^{2+}. Oxygen consumption, MDA production and conjugated diene formation in rat heart mitochondria in the presence of a tested compound were compared with those in the absence of the compound.

The radical scavenging activities of tested compounds were determined from the change in the optical absorbance at 517 nm due to scavenging of the stable free radical of DPPH. According to the method of Blois [22], 10 mL of 100 mM acetate buffer, pH 5.5, 10 mL of ethanol and 5 mL of a 500 nM ethanolic solution of DPPH were first mixed, then 5 mL of an ethanolic solution of a tested compound was added. The change in optical density was monitored with a spectrophotometer (Beckman DU-50).

RESULTS

Figure 1 shows the dose–response curves of the

* Abbreviations: MDA, malondialdehyde; DMSO, dimethyl sulfoxide; DPPH, diphenyl-*p*-picrylhydrazyl.

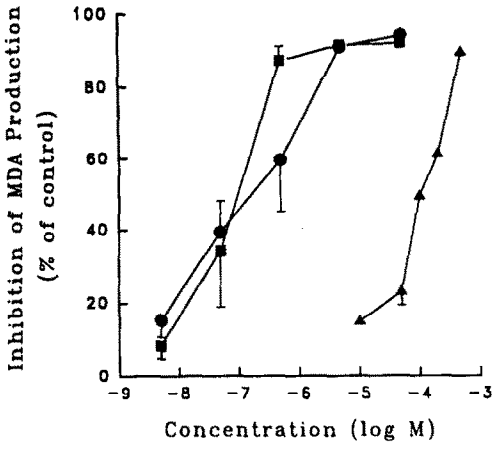

Fig. 2. Dose–response curves for the inhibitory effect of magnolol (●), honokiol (■) and α-tocopherol (▲) on MDA production in rat heart mitochondria. Data points represent means ± SEM of six experiments.

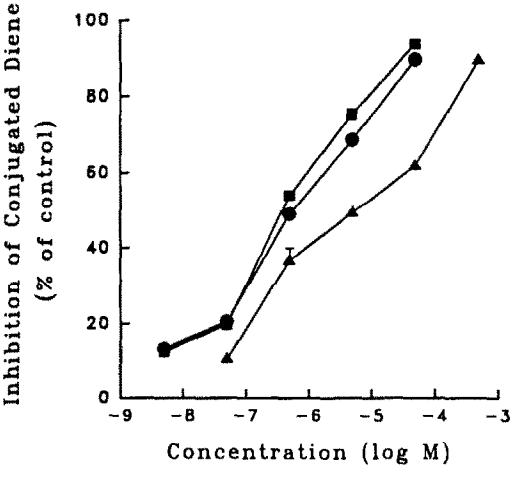

Fig. 3. Dose–response curves for the inhibitory effect of magnolol (●), honokiol (■) and α-tocopherol (▲) on conjugated diene formation in rat heart mitochondria. Data points represent means ± SEM of three experiments.

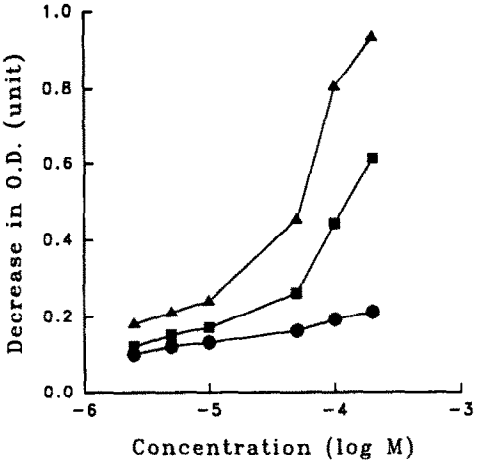

Fig. 4. Dose–response curves for the effect of magnolol (●), honokiol (■) and α-tocopherol (▲) on scavenging of DPPH radicals after incubation for 90 min. Decrease in optical density (O.D., expressed in arbitrary units) at 517 nm of DPPH due to scavenging of its radical was taken as a measure of the radical scavenging activity. Data points represent means ± SEM of three experiments.

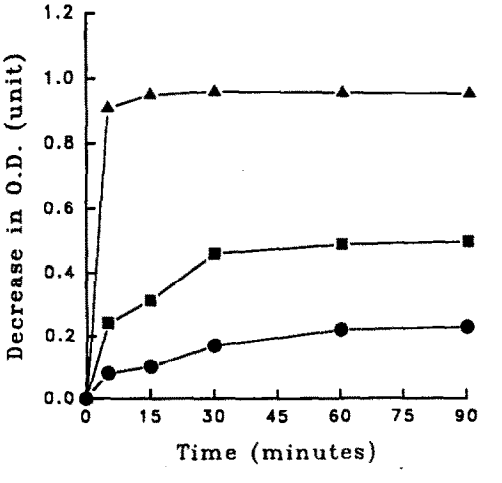

Fig. 5. Time course for the effect of magnolol (●), honokiol (■) and α-tocopherol (▲) on scavenging of DPPH radicals. The concentrations of tested compounds were fixed at 200 μM. Decrease in optical density (O.D.) was expressed in arbitrary units. Data points represent means ± SEM of three experiments.

inhibitory effect of magnolol, honokiol and α-tocopherol on oxygen consumption due to lipid peroxidation in rat heart mitochondria. It can be seen that the inhibitory effects of magnolol and honokiol were stronger than that of α-tocopherol. The IC_{50} values of magnolol, honokiol and α-tocopherol were 8.0×10^{-8}, 1.0×10^{-7} and 1.0×10^{-4} M, respectively.

Figure 2 shows the inhibitory effect of magnolol, honokiol and α-tocopherol on MDA production and Fig. 3 shows the effect on conjugated diene formation. The IC_{50} values of magnolol, honokiol and α-tocopherol on MDA production were 1.1×10^{-7}, 1.0×10^{-7} and 1.1×10^{-4} M, respectively (Fig. 2). Those on conjugated diene formation were 5.2×10^{-7}, 4.9×10^{-7} and 5.1×10^{-6} M, respectively (Fig. 3).

Figure 4 shows the dose–response curve for magnolol, honokiol and α-tocopherol in decolorizing DPPH after incubation for 90 min. Unlike their effect on oxygen consumption, MDA and diene formation, the scavenging effects of magnolol and honokiol were weaker than that of α-tocopherol. Figure 5 shows the time course of their effect on DPPH. α-Tocopherol caused an instant decrease in

Table 1. Inhibitory effects of magnolol and honokiol on oxygen consumption and MDA production in rat heart mitochondria induced by various concentrations of $FeSO_4$

$FeSO_4$ (μM)	Magnolol (50 μM)		Honokiol (50 μM)	
	O_2 consumption	MDA production	O_2 consumption	MDA production
50	86.0 ± 1.2	82.8 ± 2.4	85.7 ± 2.2	82.1 ± 0.6
75	84.3 ± 3.4	78.2 ± 2.1	86.2 ± 0.8	80.3 ± 2.8
100	82.8 ± 2.6	86.3 ± 3.5	90.1 ± 1.8	80.7 ± 2.5
125	89.7 ± 1.8	85.4 ± 0.8	84.6 ± 3.2	78.8 ± 3.6
150	86.6 ± 2.4	80.8 ± 3.0	82.3 ± 2.2	79.0 ± 3.1

Values are expressed as percentage of control; means ± SEM; N = 3.

the absorbance of DPPH while magnolol and honokiol caused a more gradual decrease in absorbance until it reached a plateau after 60 min.

DISCUSSION

Before observing the antioxidant activities of magnolol and honokiol, we tested four other compounds isolated from Chinese medicinal herbs: norathyriol from *Radix gentianae* (Longdan), osthole from *R. angelicae pubescentis* (Duhuo), protopine from *Rhizoma corydalis* (Yanhusuo) and apigenin from *Apium graveolens*. None of these inhibited lipid peroxidation by 50% at concentrations up to 500 μM.

Magnolol and honokiol are two prominent constituents isolated from the bark of *M. officinalis* which is a commonly used Chinese medicinal herb. They have been found to possess pharmacological activities such as central nervous inhibition [23], muscle relaxation [24], antibacteria activity [25], antiplatelet aggregation [12] and prevention of peptic ulcer. However, the antioxidant effects of magnolol and honokiol have not been reported.

Lipid peroxidation is a complex process. In this study, we first monitored oxygen consumption following the induction of lipid peroxidation in cardiac mitochondria by $FeSO_4$. Then, we tested both the intermediate (conjugated diene) and the final (MDA) products of lipid peroxidation. Both magnolol and honokiol were more potent than α-tocopherol in inhibiting oxygen consumption, as well as diene and MDA formation. From the IC_{50} values, the inhibition of oxygen consumption and MDA formation by magnolol and honokiol were found to be around 1000 times higher than by α-tocopherol. For conjugated diene formation, the potencies of magnolol and honokiol were 10 times higher than that of α-tocopherol.

To understand the mechanism of the antioxidant effect of magnolol and honokiol, we monitored the decrease in optical absorbance at 517 nm following the trapping of the unpaired electron of DPPH. A positive DPPH test suggested that magnolol and honokiol were free radical scavengers. As magnolol and honokiol possess two phenolic groups, it is possible that magnolol and honokiol inhibit lipid peroxidation by forming complexes with iron. This possibility was excluded in our supplementary experiment which showed that the inhibitory effects of magnolol and honokiol on oxygen consumption and MDA formation were not influenced by changing the concentration of $FeSO_4$ (Table 1). Therefore, it can be concluded that magnolol and honokiol acted on free radicals and mitochondria rather than on iron.

Unlike their effects on oxygen consumption, conjugated diene and MDA formation, the free radical scavenging activities of magnolol and honokiol shown with the DPPH test were lower than that of α-tocopherol. Besides, α-tocopherol caused an instant decrease in optical absorbance of DPPH while magnolol and honokiol a more gradual one. Disparity between the effects of antioxidants on oxygen consumption, MDA formation and DPPH assay is most likely due to the fact that the DPPH test is a chemical reaction which is not performed by mitochondria. The orientation of the phenolic hydroxyl groups of antioxidants in the mitochondrial membrane and the differential affinity of antioxidants for various types of lipid peroxyl radicals formed in the mitochondrial membrane may both influence their relative potencies.

Recently, it was reported that a slimming regimen including Chinese medicinal herbs induced rapid progressive interstitial renal fibrosis in young women [26]. *M. officinalis* was one of the two Chinese medicinal herbs listed in that regime and magnolol and honokiol were confirmed to be present. Although there was no evidence to support that these two compounds were responsible for the adverse reaction in these patients, the safety record of magnolol and honokiol has to be established before they can be recommended as medicine.

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REFERENCES

1. Halliwell B, Reactive oxygen species in living systems: source, biochemistry and role in human disease. *Am J Med* **91**: 14S–22S, 1991.
2. Sies H, Oxidative stress: from basic research to clinical application. *Am J Med* **91**: 31S–38S, 1991.
3. Wiswedel I, Ulbricht O and Augustin W, Studies of lipid peroxidation in isolated rat heart mitochondria. *Biomed Biochim Acta* **2**: 73–76, 1989.

4. Hruszkewycz A, Evidence for mitochondrial DNA damage by lipid peroxidation. *Biochem Biophys Res Commun* **153**: 191–197, 1988.
5. Ferrari R, Ceconi C, Curello S, Cargnoni A, Alfieri O, Pardini A, Marzollo P and Visioli O, oxygen free radicals and myocardial damage: protective role of thiol-containing agents. *Am J Med* **91**: 95S–121S, 1991.
6. Ambrosio G, Myocardial reperfusion injury: mechanism and management—a review. *Am J Med* **91**: 86S–88S, 1991.
7. Fukuchi T, Kobayashi A, Kaneko M, Ichiyama A and Yamazaki N, Possible involvement of free radicals and antioxidants in the early stages of the development of cardiomyopathy in BIO 14.6 Syrian hamster. *Jpn Heart J* **32**: 655–666, 1991.
8. Deng JW, Guan YY and Kwan CY, Effects of ginseng saponins on lipid peroxidation in liver and cardiac muscle homogenates. *Biochem Arch* **6**: 359–365, 1990.
9. Sassa H, Takaishi Y and Terada H, The triterpene celastrol as a very potent inhibitor of lipid peroxidation in mitochondria. *Biochem Biophys Res Commun* **172**: 890–897, 1990.
10. Lin TJ, Liu GT, Pan Y, Liu Y and Xu GZ, Protection by schisanhenol against adriamycin toxicity in rat mitochondria. *Biochem Pharmacol* **42**: 1805–1810, 1991.
11. Liu GT, Zhang TM, Wang BE and Wang YW, Protective action of seven natural phenolic compounds against peroxidative damage to biomembranes. *Biochem Pharmacol* **43**: 147–152, 1992.
12. Teng CM, Chen CC, Ko FN, Lee LG, Huang TF, Chen YP and Hsu HY, Two antiplatelet agents from *Magnolia officinalis*. *Thromb Res* **50**: 757–765, 1988.
13. Teng CM, Lin CN, Ko FN, Cheng KL and Huang TF, Novel inhibitory actions on platelet thromboxane and inositolphosphate formation by xanthones and their glycosides. *Biochem Pharmacol* **38**: 3791–3795, 1989.
14. Ko FN, Wu TS, Liou MJ, Huang TF and Teng CM, Inhibition of platelet thromboxane formation and phosphoinositides breakdown by osthole from *Angelica pubescens*. *Thromb Hemost* **62**: 996–999, 1989.
15. Ko FN, Wu TS, Lu ST, Wu YC, Huang TF and Teng CM, Antiplatelet effects of protopine isolated from *Corydalis tubers*. *Thromb Res* **56**: 289–298, 1989.
16. Teng CM, Lee TG, Ko FN and Huang TF, Inhibition of platelet aggregation by apigenin from *Apium graveolens*. *Asia Pacific J Pharmacol* **3**: 85–89, 1988.
17. Vercesi A, Reynafarje B and Lehninger AL, Stoichiometry of H^+ ejection and Ca^{2+} uptake coupled to electron transport in rat heart mitochondria. *J Biol Chem* **253**: 6379–6385, 1978.
18. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
19. Dousset N, Negre-Salvayre A, Lopez M, Salvayre R and Douste-Blazy L, Ultraviolet-treated lipoproteins as a model system for the study of the biological effects of lipid peroxides on cultured cell. I. Chemical modifications of ultraviolet-treated low density lipoproteins. *Biochim Biophys Acta* **1045**: 219–223, 1990.
20. Esterbauer H, Striegl G, Puhl H and Rotheneder M, Continuous monitoring of *in vitro* oxidation of human low density lipoproteins. *Free Rad Res Commun* **6**: 67–75, 1989.
21. Fujita M, Itokawa H and Sashida Y, Honokiol, a new phenolic compound isolated from the bark of *Magnolia obovata Thunb*. *Chem Pharm Bull* **20**: 212–213, 1972.
22. Blois MS, Antioxidant determinations by the use of a stable free radical. *Nature* **181**: 1119–1200, 1958.
23. Watanabe K, Watanabe H, Goto Y, Yamaguchi M, Yamamoto N and Hagino K, Pharmacological properties of magnolol and honokiol extracted from *Magnolia officinalis*: central depressant effects. *Planta Med* **49**: 103–108, 1983.
24. Teng CM, Yu SM, Chen CC, Huang YL and Huang TF, EDRF-release and Ca^{2+}-channel blockade by magnolol, an antiplatelet agent isolated from Chinese herb *Magnolia officinalis*, in rat thoracic aorta. *Life Sci* **47**: 1153–1161, 1990.
25. Clark AM, Elferaly FS and Li WS, Antimicrobial activity of phenolic constituents of *Magnolia grandiflora* L. *J Pharm Sci* **70**: 951–952, 1981.
26. Vanherweghem JL, Depierreux M, Tielemans C, Abramowicz D, Dratwa M, Jadoul M, Richard C, Vandervelde D, Verbeelen D, Vanhaelen-Fastre F and Vanhaelen M, Rapid progressive interstitial renal fibrosis in young women: associated with slimming regimen including Chinese herbs. *Lancet* **341**: 387–391, 1993.