Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Ethnopharmacology 100 (2005) 216–220
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Short communication

Monoamine oxidase B (MAO-B) inhibition by active principles from *Uncaria rhynchophylla*

Wen-Chi Hou [a], Rong-Dih Lin [b], Cheng-Tang Chen [a], Mei-Hsien Lee [a,*]

[a] *Graduate Institute of Pharmacognosy, Taipei Medical University, 250 Wu-Hsing Street, Taipei 110, Taiwan*
[b] *Department of Internal Medicine, Taipei Municipal Ho-Ping Hospital, Taipei 100, Taiwan*

Received 22 July 2004; received in revised form 18 January 2005; accepted 26 March 2005
Available online 10 May 2005

Abstract

Attenuation of monoamine oxidase B (MAO-B) activity may provide protection against oxidative neurodegeneration. For this reason, inhibition of MAO-B activity is used as part of the treatment of Parkinson's and Alzheimer's patients. The hook of *Uncaria rhynchophylla* (Miq.) Jacks. (Rubiaceae) is a traditional Chinese herbal drug that is generally used to treat convulsive disorders. In this study, the fractionation and purification of *Uncaria rhynchophylla* extracts using a bioguided assay isolated two known compounds, (+)-catechin and (−)-epicatechin. The compounds inhibited MAO-B, as measured by an assay of rat brain MAO-B separated by electrophoresis on a 7.5% native polyacrylamide gel. The IC_{50} values of (+)-catechin and (−)-epicatechin were 88.6 and 58.9 μM, respectively, and inhibition occurred in a dose-dependent manner, as measured by the fluorescence method. The Lineweaver–Burk plot revealed K_i values for (+)-catechin and (−)-epicatechin of 74 and 21 μM, respectively. This suggests that these two compounds, isolated here for the first time from *Uncaria rhynchophylla*, might be able to protect against neurodegeneration in vitro, and, therefore, the molecular mechanism deserves further study. This finding may also increase interest in the health benefits of *Uncaria rhynchophylla*.

Keywords: *Uncaria rhynchophylla*; Monoamine oxidase B; (+)-Catechin; (−)-Epicatechin

1. Introduction

The hook of *Uncaria rhynchophylla* (Miq.) Jacks. (Rubiaceae) is a traditional Chinese herbal drug that is described as having the ability to remove heat, check hyperfunction of the liver, and relieve dizziness, tremor, and convulsion (Hsieh et al., 1999). The herb is also used to treat headache, dizziness resulting from hypertension (Kuramochi et al., 1994), and convulsive disorders, such as epilepsy. *Uncaria rhynchophylla* has also been found to inhibit tumor necrosis factor-α (TNF-α) and nitric oxide production in BV-2 mouse microglial cells in vitro. The neuroprotective effects of *Uncaria rhynchophylla* following transient global ischemia have also been evaluated (Suk et al., 2002). In previous studies, a number of alkaloids from the genus *Uncaria* have been reported to act as antihypertensive principles (Chang et al., 1979; Aisaka et al., 1985; Masumiya et al., 1999; Watanabe et al., 2003).

Monoamine oxidase (MAO), a flavin-containing enzyme, is widely distributed in both the central and peripheral nervous systems (Waldmeier, 1987; Muller et al., 1993) and plays a central role in the control of substrate availability and activity. MAO catalyzes the oxidation of a variety of amine-containing neurotransmitters to yield the corresponding aldehyde, hydrogen peroxide (H_2O_2), and ammonia (O'Brien et al., 1993). MAO exists in two forms, MAO-A and MAO-B, which are distinguished on the basis of different pharmacological and biochemical characteristics. MAO is a key enzyme in catecholamine metabolism, and increased catecholamine metabolism seen in aging has been extensively studied. The regulation of MAO activity may alleviate symptoms and slow the progression of neurodegenerative disorders. In humans,

* Corresponding author. Tel.: +886 2 2736 1661x6151.
E-mail address: lmh@tmu.edu.tw (M.-H. Lee).

0378-8741/$ – see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2005.03.017

MAO-B activity increases with age (Fowler et al., 2002) and is especially elevated in certain neurodegenerative diseases (Castagnoli and Murugesan, 2004; Dewey, 2004; Girgin Sagin et al., 2004; Nagatsu, 2004). Therefore, inhibition of MAO-B activity may improve the quality of life of the elderly.

In a previous paper, we reported the inhibitory effect of 27 Chinese herbal medicines on MAO-B derived from rat brain homogenates (Lin et al., 2003). None of the medicines had previously been evaluated for MAO-B inhibition. *Uncaria rhynchophylla* exhibits inhibition similar to these medicines. In this study, MAO-B activity was used to guide the isolation of any bioactive compounds present in *Uncaria rhynchophylla*. The active constituents were isolated, their structures elucidated, and their MAO-B inhibitory effects evaluated individually.

2. Materials and methods

2.1. Material

The hooks of *Uncaria rhynchophylla* were purchased from the Chinese drug market in Taipei, Taiwan, and identified by Dr. H.C. Chang, Bureau of Food and Drug Analysis, Department of Health Executive Yuan, Taiwan, Republic of China. A reference specimen (no. M-10) was deposited in the Graduate Institute of Pharmacognosy, Taipei Medical University, Taiwan.

2.2. Extraction and isolation

The dried hooks (2 kg) were extracted three times with methanol at room temperature for one week. The solutions were combined and evaporated under reduced pressure to yield a total extract. The extract was fractionated with *n*-hexane, ethyl acetate and *n*-butanol. Fractions determined to be active by means of a bioguided assay were passed through a Diaion HP-20 column and eluted with a gradient solvent system of methanol (MeOH) in water (H_2O). After monitoring the resulting fractions for bioactivity, the active fractions were combined, passed through an octadecylsilane (ODS) column, and eluted with a MeOH–H_2O solvent system.

2.3. HPLC analysis

The combined active fractions were passed through a reverse-phase high-performance liquid chromatograph (HPLC) at a rate of 1.0 ml/min. The column was a Licrospher RP-18, 4.6 mm × 250 mm (Merck, Darmstadt, Germany), and the solvent system was MeOH:H_2O (20:80) that was degassed prior to use. An LC-10A pump (Shimadzu Corporation, Chromatographic Instruments Division, Kyoto, Japan) was connected to an SPD-6A ultraviolet spectrophotometric detector set at UV 280 nm.

2.4. Identification of isolated compounds

The structures of the purified compounds were identified by direct comparisons of their retention times, melting points and spectral data (^{1}H-NMR and ^{13}C-NMR) with data found in the literature.

2.5. Animals

Wistar rats (300–350 g) were purchased from the National Laboratory Animal Breeding and Research Center, Taiwan. The rats were maintained under a 12 h light–dark cycle. Water and pelletized feed were supplied ad libitum. Animal studies followed the guidelines of protocols approved by the National Science Council. The protein content of rat brain homogenates was estimated using bovine serum albumin (BSA) as the standard and showed a linear regression relationship ($y = 0.9370x + 0.3235$, $r^2 = 0.9926$) within the range of 62.5–500 μg/ml of BSA.

2.6. Enzyme preparation

The MOA-B was prepared from the brains of Wistar rats, as described previously (Endo et al., 1994).

2.7. Polyacrylamide gel electrophoresis (PAGE)

The identified compounds were mixed with rat brain homogenates (protein content 6.86 mg/ml) in 50 mM phosphate buffer (pH 7.5) overnight and then electrophoresed on native 7.5% polyacrylamide gels (Lee et al., 2002).

2.8. Fluoroscopy of MAO-B inhibition

The MAO-B inhibition was measured with a fluorescence microplate reader (FLA-2000 Reader, Fujifilm, Japan) with the filter set for excitation at 473 ± 10 nm and emission at 580 ± 10 nm (Lin et al., 2003), using benzylamine as the substrate. The compound L-deprenyl (an MAO inhibitor) was used as a positive control.

2.9. Kinetic analysis of MAO-B inhibition

The 50% inhibitory concentration (IC_{50}) values for the test samples and for various concentrations of benzylamine (0.5, 1, 2, 4, 8 and 16 mM) were determined. The apparent inhibition constants (K_i) for the isolated compounds were calculated using Lineweaver–Burk plots.

3. Results

The 40% methanol eluate from the Diaion HP-20 column showed good activity, as did the subsequent 10% methanol fraction from the ODS column. Purification and analysis

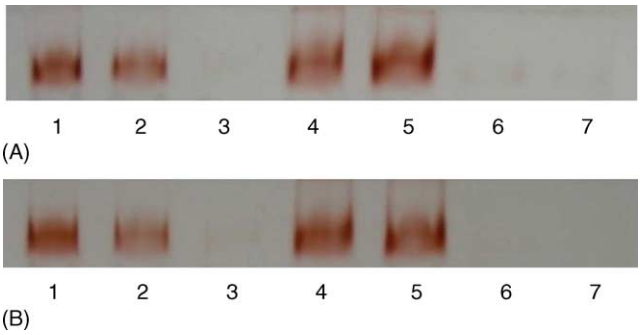

Fig. 1. The MAO-B activity inhibitory effects of isolated principles of *Uncaria rhynchophylla* on 7.5% native polyacrylamide gels, (A) for (+)-catechin; (B) for (−)-epicatechin. Lane 1, control; lane 2, clorgyline (20 μM, MAO-A inhibitor) added; lane 3, L-deprenyl (20 μM, MAO-B inhibitor) added; lane 4, 13.8 μM sample added; lane 5, 27.6 μM sample added; lane 6, 41.4 μM sample added; lane 7, 55.2 μM sample added.

of this fraction by reverse-phase HPLC identified two major compounds, identified from the literature as (+)-catechin (Zhang et al., 1990) and (−)-epicatechin (Bae et al., 1994). The yields of (+)-catechin and (−)-epicatechin were 0.0441 and 0.0378%, respectively.

Analysis of the MAO-B inhibitory activity showed that (+)-catechin (Fig. 1A) and (−)-epicatechin (Fig. 1B) inhibited MAO-B, when compared to L-deprenyl. Both compounds, at concentrations of 41.4 and 55.2 μM, inhibited MAO-B activity (Fig. 1). Furthermore, these two isolated compounds exhibited dose-dependent MAO-B inhibitory activity during fluorospectrophotometry: the IC_{50} values of

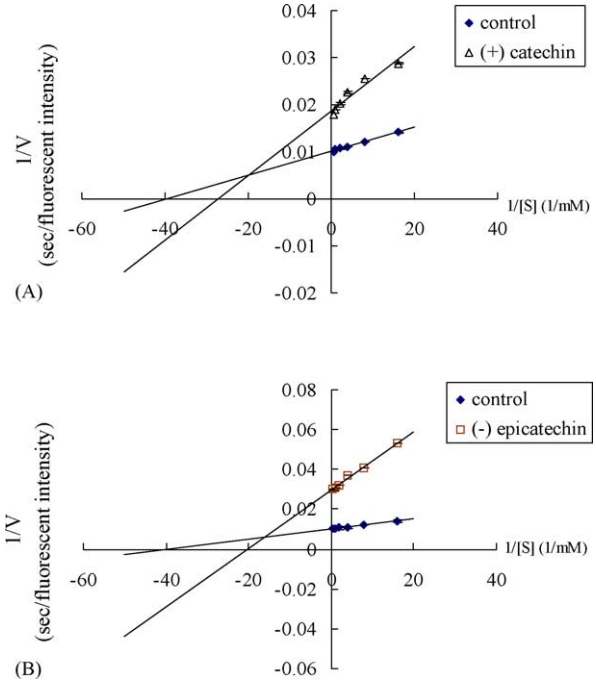

Fig. 2. . Kinetic analysis of (+)-catechin and (−)-epicatechin (IC_{50} values) on MAO-B by Lineweaver–Burk plots.

(+)-catechin and (−)-epicatechin were 88.6 and 58.9 μM, respectively. The Lineweaver–Burk plots for both IC_{50} concentrations, using various concentrations of the benzylamine substrate, indicated that (+)-catechin and (−)-epicatechin, acted as mixed-type inhibitors with respect to the substrate benzylamine and had K_i values against this substrate of 74 and 21 μM, respectively (Fig. 2A and B, respectively). The IC_{50} value of the positive control, L-deprenyl (an MAO-B inhibitor), was 0.31 μM and the K_i value was 0.002 μM. L-Deprenyl also showed the mixed-type mode of inhibition (Lin et al., 2003).

4. Discussion

Uncaria rhynchophylla has been reported to contain various alkaloids including rhynchophylline, corynoxeine, corynantheine and hirsutine, among others (Yamanaka et al., 1983; Ozaki, 1989). These compounds show antihypertensive, neuroprotective and vasodilative effects (Horie et al., 1992; Kuramochi et al., 1994; Gonzalez-Polo et al., 2003; Spencer, 2003) when total extracts of *Uncaria rhynchophylla* are used.

MAO-B enzymes from bovine, rat and human species all have very similar amino acid sequences (Abell and Kwan, 2001); therefore, rat brain homogenates were used to detect MAO-B inhibition in this study. MAO-B prefers hydrophobic substrates such as phenylethylamine and benzylamine and provides protection by catalyzing the oxidation of xenobiotics derived from dietary or environmental sources (Tieu et al., 2003). The generation of hydrogen peroxide (H_2O_2) by MAOs is also considered to be a cytotoxic factor involved in oxidative stress and nigral cell degeneration in Parkinson's disease. Therefore, pretreatment of animals with MAO-B inhibitors (e.g., deprenyl and pargyline) has been described as protective against neurotoxicity (Sziraki et al., 1994).

The original crude extract of *Uncaria rhynchophylla* showed an IC_{50} concentration of 30 μg/ml (Lin et al., 2003). When the concentration of the present extract of *Uncaria rhynchophylla* was 100 μg/ml, the MAO-B inhibitory activity was 85.2%, as well as the IC_{50} concentrations of (+)-catechin and (−)-epicatechin were 25.7 μg/ml (88.6 μM) and 17.1 μg/ml (58.9 μM) in the present study. The yields of (+)-catechin and (−)-epicatechin were 0.0441 and 0.0378%, respectively. In this study, the isolated constituents showed MAO-B inhibitory effects, but the yields were lower. The other eluates from the ethyl acetate fraction also exhibited MAO-B inhibition, but the activity was less than that seen with the isolated fractions. Further investigation of these eluates may yield additional active compounds contained in *Uncaria rhynchophylla*.

(+)-Catechin and (−)-epicatechin are abundant in green tea. Catechin and its derivatives are thought to contribute to the beneficial effects ascribed to tea, such as the effective scavenging of reactive oxygen species seen in vitro (Higdon

and Frei, 2003). They may also function indirectly as antioxidants through their effects on transcription factors and enzyme activity. (+)-Catechin was also reported to inhibit MAO-B activity in C6 astrocyte cells; the IC_{50} value was less than 1 μM (Mazzio et al., 1998).

In the present study, we isolated the active compounds by following their inhibitory effect on MAO-B in rat brain homogenates in vitro. This is the first report to isolate and identify the active compounds from *Uncaria rhynchophylla* as polyphenols, and to show that they exhibit MAO-B inhibition. Therefore, in addition to the known alkaloids contained in *Uncaria rhynchophylla*, which are usually thought to exert an effect on various neurological symptoms, the polyphenols (+)-catechin and (−)-epicatechin are also present and active in *Uncaria rhynchophylla*. They have been shown in vitro to have the potential to protect against neurodegeneration; in addition, they have antioxidant activity. Therefore, their molecular mechanism of action deserves scrutiny. These findings may increase interest in the health benefits of *Uncaria rhynchophylla* and lead to the inclusion of *Uncaria rhynchophylla* in dietary supplements and functional foods.

Acknowledgements

This study was supported by a grant from the Taipei Medical University (Grant No. TMC89-Y05-A118). The authors also thank the Juridical Person of Yen's Foundation, Taiwan.

References

Abell, C.W., Kwan, S.W., 2001. Molecular characterization of monoamine oxidases A and B. Progress in Nucleic Acid Research and Molecular Biology 65, 129–156.

Aisaka, K., Hattori, Y., Kihara, T., Ishihara, T., Endo, K., Hikino, H., 1985. Hypotensive action of 3 alpha-dihydrocadambine, an indole alkaloid glycoside of *Uncaria* hooks. Planta Medica, 424–427.

Bae, Y., Burger, J., Steynberg, J., Ferreira, D., Hemingway, R., 1994. Flavan and procyanidin glycosides from the bark of blackjack oak. Phytochemistry 35, 473–478.

Castagnoli, K., Murugesan, T., 2004. Tobacco leaf, smoke and smoking, MAO inhibitors Parkinson's disease and neuroprotection; are there links? Neurotoxicology 25, 279–291.

Chang, C.C., Tung, L.H., Chen, R.R., Chiueh, C.C., 1979. A study on the antihypertensive action of uncarine A, an alkaloid of *Uncaria formosana* used in Chinese herb medicine. Taiwan Yi Xue Hui Za Zhi 78, 61–69.

Dewey Jr., R.B., 2004. Management of motor complications in Parkinson's disease. Neurology 62, S3–S7.

Endo, Y., Hayashi, H., Sato, T., Maruno, M., Ohta, T., Nozoe, S., 1994. Confluentic acid and 2′-*O*-methylperlatolic acid, monoamine oxidase B inhibitors in a Brazilian plant *Himatanthus sucuuba*. Chemical and Pharmaceutical Bulletin 42, 1198–1201.

Fowler, J.S., Logan, J., Volkow, N.D., Wang, G.J., MacGregor, R.R., Ding, Y.S., 2002. Monoamine oxidase: radiotracer development and human studies. Methods 27, 263–277.

Girgin Sagin, F., Sozmen, E.Y., Ersoz, B., Mentes, G., 2004. Link between monoamine oxidase and nitric oxide. Neurotoxicology 25, 91–99.

Gonzalez-Polo, R.A., Soler, G., Alonso, J.C., Rodriguez-Martin, A., Fuentes, J.M., 2003. MPP(+) causes inhibition of cellular energy supply in cerebellar granule cells. Neurotoxicology 24, 219–225.

Higdon, J.V., Frei, B., 2003. Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions. Critical Reviews in Food Science and Nutrition 43, 89–143.

Horie, S., Yano, S., Aimi, N., Sakai, S., Watanabe, K., 1992. Effects of hirsutine, an antihypertensive indole alkaloid from *Uncaria rhynchophylla*, on intracellular calcium in rat thoracic aorta. Life Sciences 50, 491–498.

Hsieh, C.L., Chen, M.F., Li, T.C., Li, S.C., Tang, N.Y., Hsieh, C.T., Pon, C.Z., Lin, J.G., 1999. Anticonvulsant effect of *Uncaria rhynchophylla* (Miq.) Jack. in rats with kainic acid-induced epileptic seizure. American Journal of Chinese Medicine 27, 257–264.

Kuramochi, T., Chu, J., Suga, T., 1994. Gou-teng (from *Uncaria rhynchophylla* Miquel)-induced endothelium-dependent and -independent relaxations in the isolated rat aorta. Life Sciences 54, 2061–2069.

Lee, M.-H., Chuang, M.T., Hou, W.-C., 2002. Activity staining of plasma amine oxidase after polyacrylamide gel electrophoresis and its application to natural inhibitor screening. Electrophoresis 23, 2369–2372.

Lin, R.-D., Hou, W.-C., Yen, K.Y., Lee, M.-H., 2003. Inhibition of monoamine oxidase B (MAO-B) by Chinese herbal medicines. Phytomedicine 10, 650–656.

Masumiya, H., Saitoh, T., Tanaka, Y., Horie, S., Aimi, N., Takayama, H., Tanaka, H., Shigenobu, K., 1999. Effects of hirsutine and dihydrocorynantheine on the action potentials of sino-atrial node, atrium and ventricle. Life Sciences 65, 2333–2341.

Mazzio, E.A., Harris, N., Soliman, K.F., 1998. Food constituents attenuate monoamine oxidase activity and peroxide levels in C6 astrocyte cells. Planta Medica 64, 603–606.

Muller, T., Kuhn, W., Przuntek, H., 1993. Therapy with central active catechol-*O*-methyltransferase (COMT)-inhibitors: is addition of monoamine oxidase (MAO)-inhibitors necessary to slow progress of neurodegenerative disorders? Journal of Neural Transmission General Section 92, 187–195.

Nagatsu, T., 2004. Progress in monoamine oxidase (MAO) research in relation to genetic engineering. Neurotoxicology 25, 11–20.

O'Brien, E.M., Kiely, K.A., Tipton, K.F., 1993. A discontinuous luminometric assay for monoamine oxidase. Biochemical Pharmacology 46, 1301–1306.

Ozaki, Y., 1989. Pharmacological studies of indole alkaloids obtained from domestic plants, *Uncaria rhynchophylla* Miq. and *Amsonia elliptica* Roem. et Schult. Nippon Yakurigaku Zasshi—Folia Pharmacologica Japonica 94, 17–26.

Spencer, J.P., 2003. Metabolism of tea flavonoids in the gastrointestinal tract. Journal of Nutrition 133, 3255S–3261S.

Suk, K., Kim, S.Y., Leem, K., Kim, Y.O., Park, S.Y., Hur, J., Baek, J., Lee, K.J., Zheng, H.Z., Kim, H., 2002. Neuroprotection by methanol extract of *Uncaria rhynchophylla* against global cerebral ischemia in rats. Life Sciences 70, 2467–2480.

Sziraki, I., Kardos, V., Patthy, M., Patfalusi, M., Gaal, J., Solti, M., Kollar, E., Singer, J., 1994. Amphetamine-metabolites of deprenyl involved in protection against neurotoxicity induced by MPTP and 2′-methyl-MPTP. Journal of Neural Transmission 41, 207–219.

Tieu, K., Perier, C., Caspersen, C., Teismann, P., Wu, D.C., Yan, S.D., Naini, A., Vila, M., Jackson-Lewis, V., Ramasamy, R., Przedborski, S., 2003. D-Beta-hydroxybutyrate rescues mitochondrial respiration and mitigates features of Parkinson disease. Journal of Clinical Investigation 112, 892–901.

Waldmeier, P.C., 1987. Amine oxidases and their endogenous substrates (with special reference to monoamine oxidase and the brain). Journal of Neural Transplantation Supplement 23, 55–72.

Watanabe, H., Zhao, Q., Matsumoto, K., Tohda, M., Murakami, Y., Zhang, S.H., Kang, T.H., Mahakunakorn, P., Maruyama, Y., Sakak-

ibara, I., Aimi, N., Takayama, H., 2003. Pharmacological evidence for antidementia effect of Choto-san (Gouteng-san), a traditional Kampo medicine. Pharmacology, Biochemistry and Behavior 75, 635–643.

Yamanaka, E., Kimizuka, Y., Aimi, N., Sakai, S., Haginiwa, J., 1983. Studies of plants containing indole alkaloids. IX. Quantitative analysis of tertiary alkaloids in various parts of *Uncaria rhynchophylla* Miq. Yakugaku Zasshi. Journal of the Pharmaceutical Society of Japan 103, 1028–1033.

Zhang, X., Ling, L., Dai, R., 1990. Constituents of the seed coat of *Arachis hypogaea* L. China Journal of Chinese Materia Medica 15, 356–358.