Hepatoprotective Lignan Compound from Schizandrae Fructus on Tacrine-induced Cytotoxicity in Hep G2 Cells

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In the course of search for hepatoprotective agents from oriental traditional medicines, one compound was isolated from the MeOH extract of the fruits of *Schizandra chinensis*, and its structure was determined as gomisin J (1). The evaluation for hepatoprotective activity of isolated compound 1 on drug-induced cytotoxicity was conducted, and compound 1 showed protective effect with an EC_{50}value of 86.0 ± 5.3 μM against tacrine-induced cytotoxicity in Hep G2 cells. One of the well-known hepatoprotective agents, silybin, used as a positive control, and the hepatoprotective effect of compound 1 is similar with that of silybin (EC_{50} value = 90.5 ± 9.8 μM).

Key words : Schizandrae Fructus, Lignan, Gomisin J, Hepatoprotective, Tacrine, Hep G2

Introduction

In the searching for the hepatoprotective agents from the natural sources, employing the relevantmodels of human liver toxicosis are used in order to identify agents with therapeutic potential. Tacrine (1,2,3,4-tetrahydro-9-aminoacridine hydrochloride) is a non-competitive acetylcholinesterase inhibitor that is one of drugs approved for the treatment of Alzheimer's disease. However, prolonged use of tacrine has been found to cause dose-dependent peripheral side effects and hepatotoxicity in about 30-50% of the patients, which seriously limits its clinical use[1]. As part of the search for natural products with protective effects on tacrine-induced cytotoxicity have been conducted in recent years[2-4].In the present study, human liver-derived cell (Hep G2) was employed for the screening of hepatoprotective agents against tacrine-induced cytotoxicity, since the cell line retains many cellular functions, and is known to be comparable with rat primary hepatocytes in tacrine-induced cytotoxicity[5].

Schizandrae Fructus, the fruits of *Schizandra chinensis* Baillon (Schizandraceae), has been used for the treatment of cough, asthma, spermatorrhea, diarrhea, and insomnia in Chinese traditional medicine[6]. Recently, lignan compounds have been isolated from the *S. chinensis* and showed to have antioxidant activity[7], glutamate-induced neurotoxicity effects[8], and inhibitory activity on NFAT transcription[9].

As a part of our continuing research to find novel hepatoprotective active compounds from natural sources, we studied on the MeOH extract of Schizandrae Fructus, and this paper describes the isolationand structure determination of active principle, as well as the hepatoprotective effect against tacrine-induced cytotoxicity in Hep G2 cells.

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Material and Methods

1. Plant material

The dried fruits of *S. chinensis* were purchased from the University Oriental Drugstore, Iksan, Korea in June 2005. The voucher specimen (No. WP05-295) was deposited at the Herbarium of the College of Pharmacy, Wonkwang University (Korea).

2. General instrumental equipment

NMR spectra were recorded in $CDCl_3$ using a JEOL Eclipse-500 MHz spectrometer (500 MHz for ^1H, 125 MHz for ^{13}C), and chemical shifts are quoted versus tetramethylsilane, ESI-MS spectra were measured on a Quattro LC-MS (Micromass). Column chromatography was performed on silica gel 60 (70-230 mesh, Merck) and YMC-GEL ODS-A (S-75 ㎛, YMC). In TLC silica gel 60 F254 plate (Merck) were used. Spots were detected under UV light or after spraying with 10% H_2SO_4 reagent, followed by heating.

3. Isolation and identification of compound 1

The dried fruits of *S. chinensis*(600 g) were extracted twice with MeOH (2 L) under the ultrasonic condition for 3 h. The MeOH extract (113 g) was suspended in H_2O and partitioned successively with n-hexane, CH_2Cl_2, EtOAc, and BuOH. The CH_2Cl_2-soluble extract (5.0 g) was subjected to column chromatography on silica gel, which was using hexane-EtOAc (2:1) to give five fractions (Fr. 1, 95.8 mg; Fr. 2, 182.3 mg; Fr. 3, 635.4 mg; Fr. 4, 806.6 mg; Fr. 5, 841.7 mg). The fraction 1 was subjected to column chromatography on YMC gel (75%, MeOH in H_2O) gave compound 1 (11.0 mg).

Compound 1 Colorless powder; (-)-ESI-MS m/z 387 [M-H]-, ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 6.62 (2H, s, H-4, 11), 3.92 (3H, s, OCH_3-2), 3.91 (3H, s, OCH_3-13), 3.51 (6H, s, OCH_3-1, 14), 2.54 (1H, dd, J = 7.3, 13.7 Hz, H-6b), 2.45 (1H, dd, J = 1.8, 13.7 Hz, H-6a), 2.23 (1H, dd, J = 9.2, 12.8 Hz, H-9b), 2.01 (1H, d, J = 12.8 Hz, H-9a), 1.88 (1H, m, H-7), 1.79 (1H, m, H-8), 0.96 (3H, d, J = 7.3 Hz, CH_3-17), 0.72 (3H, d, J = 7.3 Hz, CH_3-18); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 150.4 (C-1), 137.7 (C-2), 147.6 (C-3), 113.2 (C-4), 134.9 (C-5), 38.9 (C-6), 33.8 (C-7), 41.0 (C-8), 35.3 (C-9), 140.3 (C-10), 110.1 (C-11), 148.8 (C-12), 137.4 (C-13), 150.3 (C-14), 121.5 (C-15), 122.5 (C-16), 21.8 (C-17), 12.6 (C-18), 60.1 (OCH_3-1, 14), 61.1 (OCH_3-2, 13).

4. Assay for hepatoprotective activity on tacrine-induced cytotoxicity in Hep G2 cells

Tacrine-induced cytotoxicity assay was conducted using a minor modification of the method described by Song *et al.*[10]. Briefly, human hepatoma Hep G2 cells from the American Type Culture Collection were maintained at 2 × 10^5cells/well in complete medium consisting of RPMI supplemented with 10% heat-inactivated FBS, penicillin G (100 IU/ml), and streptomycin (100 mg/ml), and then incubated at 37 °C in a humidified atmosphere containing 5% CO_2 and 95% air. Cytotoxicity was assessed by MTT assay by incubating cells for 2-h in the corresponding medium in the presence of 1.1 mM tacrine. Compound 1 was tested at three different concentrations (10, 40, and 80 μM) in triplicate. The EC_{50} value for hepatoprotective effect (defined as percentage viability versus the respective control) were calculated by linear regression using mean values, and are expressed as means S.D. of three independent experiments. Silybin was used as a positive control.

Results and Discussion

In the present study aiming at the searching for the hepatoprotective compounds from the oriental medicines, the isolation and identification of hepatoprotective component of Schizandrae Fructus was performed. Repeated column chromatography of the MeOH extract of Schizandrae Fructus on silica gel and YMC-gel ODS-A let to the isolation of compound 1. The chemical structure of 1 was elucidated by various spectroscopic methods as gomisin J[11], by comparing data with those previously reported (Fig. 1). Compound 1 was tested for its hepatoprotective activity in our in vitro assay system. Compound 1 showed the protective effect in a concentration-dependent manner against tacrine-induced cytotoxicity in human liver-derived Hep G2 cells with EC_{50} value of 86.0± 5.3 μM (Fig. 2). When the EC_{50} value of compound 1 compared to that of silybin (EC_{50} = 90.5 ± 9.8 μM), which used as a positive controls, it is revealed that the hepatoprotective effect of compound 1 was similar with that of silybin.

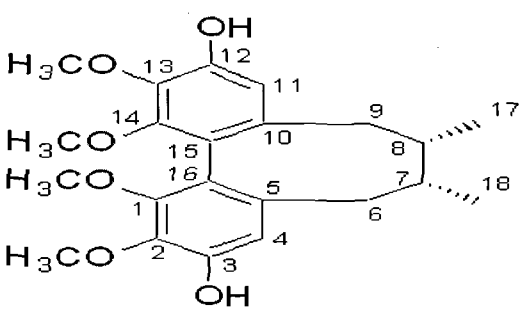

Fig. 1. Chemical structure of compound 1.

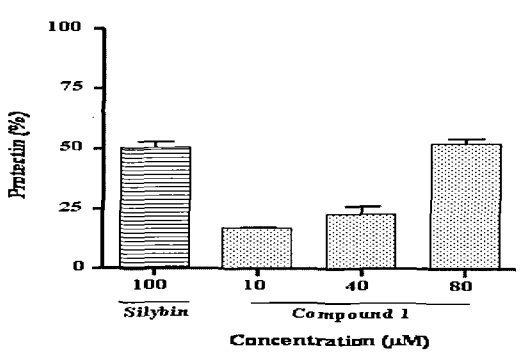

Fig. 2. Hepatoprotective effect of compound 1 on tacrine-induced cytotoxicity in Hep G2 cells. Cytotoxicity was assessed after incubating for 2-h with 1.1 mM of tacrine in RPMI medium. The values shown represent the means ±S.D. of three experiments. Silybin was used as a positive control.

Some reports have appeared hepatoprotective effect of *S. chinensis*, Hikino *et al.*[12] examined the antihepatotoxic activities of twenty-two pure compounds by tetrachloride (CCl_4)- and galactosamine (GalN)-induced cytotoxicity in primary cultured rat hepatocytes as model systems. As a result, wuweizisu C and schisantherin D exhibited prominent protective actions in CCl_4-treated cultures, and deoxygomisin A, gomisin N, wuweizisu C, gomisin C and schisantherin D were effective in

preventing GalN-induced cell damage. Although the tacrine- and CCl_4- induced hepatoprotective effect of schisandrin B had been reported[13,14], the hepatoprotective effect of compound 1 have not, to the best of our knowledge, been reported previously, which suggests that this compound be further evaluated as potential hepatoprotective agents.

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References

1. Watkins, P.B., Zimmermann, H.J., Knapp, M.J., Gracon, S.I., Lewis, K.W. Hepatotoxic effects of tacrine administration in patients with Alzheimer's disease. J. Am. Med. Assoc. 271:992-998, 1994.
2. An, R.B., Oh, H., Kim, Y.C. Phenolic constituents of Galla Rhois with hepatoprotective effects on tacrine- and nitrofurantoin-induced cytotoxicity in Hep G2 cells. Biol. Pharm. Bull. 28:2155-2157, 2005.
3. An, R.B., Sohn, D.H., Kim, Y.C. Hepatoprotective compounds of the roots of Cudrania tricuspidata on tacrine-induced cytotoxicity in Hep G2 cells. Biol. Pharm. Bull. 29:838-840, 2006.
4. Oh, H., Kim, J.S., Song, E.K., Cho, H., Kim, D.H., Park, S.E., Lee, H.S., Kim, Y.C. Sesquiterpenes with hepatoprotective activity from Cnidium monnieri on tacrine-induced cytotoxicity in Hep G2 cells. Planta Med. 68:748-749, 2002.
5. Halliwell, B. Free radicals, antioxidants and human disease: curiosity, cause or consequence? Lancet, 344:721-724, 1994.
6. Zhu, Y.P. Chinese Materia Medica. Amsterdam, The Netherlands, Harwood Academic Publishers. p 653, 1998.
7. Choi, Y.W., Takamatsu, S., Khan, S.I., Srinivas, P.V., Ferreira, D., Zhao, J., Khan, I.A. Schisandrene, a dibenzocyclooctadiene lignan from Schisandra chinensis: structure-antioxidant activity relationships of dibenzocyclooctadiene lignans. J. Nat. Prod. 69:356-359, 2006.
8. Kim, S.R., Lee, M.K., Koo, K.A., Kim, S.H., Sung, S.H., Lee, N.G., Markelonis, G.J., Oh, T.H., Yang, J.H., Kim, Y.C. Dibenzocyclooctadiene lignans from Schisandra chinensisprotect primary cultures of rat cortical cells from glutamate-induced toxicity. J. Neurosci. Res. 76:397-405, 2004.
9. Lee, I.S., Lee, H.K., Dat, N.T., Lee, M.S., Kim, J.W., Na, D.S., Kim, Y.H. Lignans with inhibitory activity against NFAT transcription from Schisandra chinensis. Planta Med. 69:6364, 2003.
10. Song, E.K., Cho, H., Kim, J.S., Kim, N.Y., An, N.H., Kim, J.A., Lee, S.H., Kim, Y.C. Diarylheptanoids with free radical scavenging and hepatoprotective activity in vitro from Curcuma longa. Planta Med. 67:876-877, 2001.
11. Seo, S.M., Lee, H.J., Park, Y., Lee, M.K., Park, J.I., Paik, K.H. Lignans from the fruits of Schizandra chinensis and their inhibitory effects on dopamine content in PC12 cells. Nat. Prod. Sci. 10:104-108, 2004.
12. Hikino, H., Kiso, Y., Taguchi, H., Ikeya, Y. Antihepatotoxic actions of lignoids from Schizandra chinensis fruits. Planta Med. 50:213-218, 1984.
13. Ip, S.P., Poon, M.K., Che, C.T., Ng, K.H., Kong, Y.C., Ko, K.M. Schisandrin B protects against carbon tetrachloride toxicity by enhancing the mitochondrial glutathione redox status in mouse liver. Free Radical Bio. Med. 21:709-712, 1996.
14. Pan, S.Y., Han, Y.F., Carlier, P.R., Pang, Y.P., Mak, D.H.F., Lam, B.Y.H., Ko, K.M., Schisandrin B protects against tacrine- and bis(7)-tacrine-induced hepatotoxicity and enhances cognitive function in mice. Planta Med. 68:217-220, 2002.