Available online at www.sciencedirect.com
SCIENCE DIRECT
European Journal of Pharmacology 542 (2006) 129–135
www.elsevier.com/locate/ejphar

Gomisin A improves scopolamine-induced memory impairment in mice

Dong Hyun Kim [a], Tran Manh Hung [b], Ki Hwan Bae [b], Ji Wook Jung [c], Seungjoo Lee [a], Byung Hoon Yoon [a], Jae Hoon Cheong [d], Kwang Ho Ko [e], Jong Hoon Ryu [a,*]

[a] Department of Oriental Pharmaceutical Science, College of Pharmacy, Kyung Hee University, #1 Hoeki-dong, Dongdeamoon-ku, Seoul 130-701, South Korea
[b] College of Pharmacy, Chungnam National University, Taejon 305-764, South Korea
[c] Department of Herbal Medicinal Resource, College of Health and Welfare, Daegu Haany University, Gyeongsan 712-715, South Korea
[d] Department of Pharmacy, Sahmyook University, Nowon-goo, Seoul 139-742, South Korea
[e] Department of Pharmacology, College of Pharmacy, Seoul National University, San 56-1, Shillim-Dong, Kwanak-Gu, Seoul 151-742, South Korea

Received 22 March 2006; received in revised form 6 June 2006; accepted 12 June 2006
Available online 15 June 2006

Abstract

Gomisin A is a component of the fruits of *Schizandra chinesis* which are widely used as a tonic in traditional Chinese medicine. In the present study, we assessed the effect of gomisin A on the learning and memory impairments induced by scopolamine. The cognition-enhancing effect of gomisin A was investigated using a passive avoidance test, the Y-maze test, and the Morris water maze test in mice. Drug-induced amnesia was induced by treating animals with scopolamine (1 mg/kg, i.p.). Gomisin A (5 mg/kg, p.o.) administration significantly reversed scopolamine-induced cognitive impairments in mice by the passive avoidance test and the Y-maze test ($P<0.05$), and also improved escape latency in the Morris water maze test at 5 mg/kg ($P<0.05$). Moreover, in an *in vitro* study, gomisin A was found to inhibit acetylcholinesterase activity in a dose-dependent manner (IC_{50} value; 15.5 μM). These results suggest that gomisin A may be a useful cognitive impairment treatment, and its beneficial effects are mediated, in part, via enhancing the cholinergic nervous system.

Keywords: Gomisin A; Memory; Passive avoidance test; Y-maze test; Morris water maze; Acetylcholinesterase

1. Introduction

Dementia has several etiologies, such as, Lewy body disease, Pick's disease, cerebrovascular disease, and Alzheimer's disease, the last of which has been reported to be the most common cause (Evans et al., 1989; Nordberg, 1996). Alzheimer's disease has been estimated to account for 50%–60% of dementia cases in persons over 65 years of age (Francis et al., 1999). Alzheimer's disease is a progressive neurodegenerative disease and a major and increasing public health concern. The characteristic pathological features of the central nervous system (CNS) in Alzheimer's disease are senile plaque, neurofibrillary tangle formation, aberrant oxidative and inflammatory processes, and neurotransmitter disturbances. Moreover, cholinergic deficit is a consistent neuropathological occurrence that is associated with memory loss, and has been correlated with the severity of Alzheimer's disease (Bierer et al., 1995; Collerton, 1986; Giacobini, 1990; Read, 1987). The restoration of cholinergic function remains a rational target for development programs targeting the treatment of the symptoms of Alzheimer's disease. Moreover, the prolonging of the availability of acetylcholine released into the neuronal synaptic cleft has been used as a means of enhancing cholinergic function in Alzheimer's disease. This prolongation may be achieved by inhibiting acetylcholine hydrolysis by acetylcholinesterase by administering acetylcholinesterase inhibitors.

The fruits of *Schizandra chinesis* (Fructus Schizandrae) have been traditionally used in Korea, Japan and China to treat coughs, mouth dryness, spontaneous sweating, dysentery, and insomnia (Zhu, 1998), and it has been reported that Fructus Schizandrae has an ameliorating effect on cycloheximide-induced amnesia in rats (Hsieh et al., 2001). In addition, the beneficial effect of Fructus Schizandrae on cycloheximide-induced amnesia was found to be amplified by treatment with serotonergic 5-HT_2 receptor antagonists, but to be attenuated by

* Corresponding author. Tel.: +82 2 961 9230; fax: +82 2 966 3885.
E-mail address: jhryu63@khu.ac.kr (J.H. Ryu).

0014-2999/$ - see front matter © 2006 Published by Elsevier B.V.
doi:10.1016/j.ejphar.2006.06.015

serotonergic 5-HT_{1A} receptor agonists and cholinergic receptor antagonists. However, the active principals in Fructus Schizandrae have not been identified. Here, we reported that Gomisin A, a lignan purified from the dried fruits of *S. chinesis*, has an ameliorating effect on scopolamine-induced learning and memory impairments in mice. Although gomisin A has been reported to protect the liver from hepatotoxicity induced by acetaminophen and to have an anti-inflammatory effect (Ohkura et al., 1990; Yamada et al., 1993), no reports have been issued on the anti-amnestic effects of gomisin A.

2. Materials and methods

2.1. Animals

Male ICR mice, weighing 25–30 g, were purchased from the Orient Co., Ltd, a branch of Charles River Laboratories (Seoul). Animals were housed 5 or 6 per cage, allowed access to water and food *ad libitum*, and maintained in a constant temperature (23±1 °C) and humidity (60±10%) environment under a 12-h light/dark cycle (light on 07.30–19.30 h). Animal treatment and maintenance were carried out in accordance with the Principle of Laboratory Animal Care (NIH publication No. 85-23, revised 1985) and the Animal Care and Use Guidelines of Kyung Hee University, Korea.

2.2. Materials

Tacrine (9-amino-1, 2, 3, 4-tetrahydroacridine hydrochloride), (−) scopolamine hydrobromide, acetylthiocholine iodide and DTNB (5, 5′-dithiobis [2-nitrobenzoic acid]) were purchased from the Sigma Chemical Co. (USA). All other materials were obtained from normal commercial sources and were of the highest grade available.

2.3. Isolation and identification of gomisin A

The dried fruits of *Schizandra chinensis* (20 kg) were refluxed with hot methanol twice and concentrated in vacuum, and a residue (4.5 kg) was obtained. The methanol extract was suspended in H_2O and partitioned successively with hexane, ethylacetate and butanol, to give hexane (512 g), ethylacetate (554 g) and butanol (886 g) soluble fractions, respectively. The hexane fraction was subjected to a silica gel column chromatography (12×25 cm) and eluted with a stepwise gradient of hexane : acetone to yield seven fractions (H1–H7) on the basis of their thin layer chromatography (TLC) behaviors. Fraction H5 (36 g) was chromatographed on a silica gel column (5×30 cm) eluted with hexane:acetone (10:1) to give ten subfractions (H5.1–H5.10). Repeated chromatography subfraction H5.8 on a silica gel column using hexane:ethylacetate (5:1), a crystal (2.5 g) was obtained by crystallization from the collected subfraction H5.8.5.

The crystal compound was isolated as colorless needles, mp. 80–85 °C. The fast atom bombardment mass spectrometry (FABMS) gave [M^+] at *m/z* 416 corresponding to the molecular formula $C_{23}H_{28}O_7$. The UV spectrum showed absorption maximum at 218, 281 and 290 nm, and the IR spectrum exhibited vibration bands due to hydroxyl group (3470 cm^{-1}) and aromatic rings (1618, 1590 cm^{-1}), indicating that this compound is a dibenzocyclooctadiene lignan (Ikeya et al., 1979). The ^1H NMR spectrum displayed the presence of a secondary methyl δ 0.81 (3H, d, J=7.5 Hz, H-18), which was shielded by the aromatic ring and a tertiary methyl δ 1.24 (3H, s, H-17) attached to a carbon carrying a hydroxyl group. Two benzylic methylene groups appeared at δ 2.33 (1H, dd, J=8.1, 14.1 Hz, H-9) and 2.57 (1H, dd, J=1.5, 14.1 Hz, H-9′), δ 2.34 (1H, d, J=13.5 Hz, H-6) and 2.67 (1H, d, J=13.5 Hz, H-6') were characteristic signals of lignan structures (Ikeya et al., 1980). The presence of a methylenedioxyl moiety δ 5.96 (2H, s), four methoxyls on the aromatic ring δ 3.51–3.90 (O–CH_3), two aromatic protons δ 6.47 (1H, s, H-11) and δ 6.62 (1H, s, H-4) were also observed. The dibenzocyclooctadiene skeleton was further confirmed by the ^{13}C NMR spectrum which disclosed highly resolved carbon signals comprising two methylenes δ 33.7 (C-9) and δ 40.5 (C-6), one methylenedioxyl (δ 100.7), six oxygenated aromatic quaternary carbons δ 152.1 (C-1), δ 140.6 (C-2), δ 151.9 (C-3), δ 147.2 (C-12), δ 134.5 (C-13), and δ 141.1 (C-14). On the other hand, four methoxyls δ 55.9, δ 59.6, δ 60.5, and δ 61.0, two methyls δ 15.8 (C-18) and δ 30.1 (C-17), and one oxygenated quaternary carbon (δ 71.6 ppm) suggested that the hydroxyl group was located at C-7. In addition, a secondary methyl δ 40.2, two quaternary carbons δ 121.7 (C-15) and δ 124.0 (C-16), and two aromatic carbons δ 110.2 (C-4) and δ 105.8 (C-11) were also observed in agreement with the literature. From the above spectroscopic data and the published evidences (Ikeya et al., 1979), the structure of this compound could be assigned as gomisin A (Fig. 1).

2.4. Passive avoidance task

Passive avoidance task was carried out in identical illuminated and non-illuminated boxes (Gemini Avoidance System, San Diego, USA). The illuminated compartment (20×20×20 cm) contained a 100 W bulb, and the floor of non-illuminated compartment (20×20×20 cm) was composed of 2 mm stainless steel rods spaced 1 cm apart. These compartments were separated

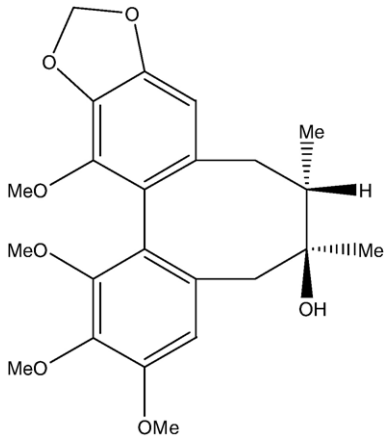

Fig. 1. Structure of gomisin A.