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Inhibitory Effect of Corynoline Isolated from the Aerial Parts of *Corydalis incisa* on the Acetylcholinesterase

Dae Keun Kim

College of Pharmacy, Woosuk University, Samrye 565-701, Korea

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In the course of screening Korean natural products for acetylcholinesterase (AChE) inhibitory activity, it was found that a methanolic extract of the aerial parts of *Corydalis incisa* (Papaveraceae) showed significant inhibitory effects on AChE. Corynoline isolated from this plant inhibited AChE activity in a dose-dependent manner, and the IC_{50} value of corynoline was 30.6 μM. The AChE inhibitory activity of corynoline was reversible and noncompetitive.

Key words: *Corydalis incisa*, Papaveraceae, Acetylcholinesterase inhibitor, Corynoline

INTRODUCTION

Alzheimer's disease (AD) is characterized by the presence of excessive amounts of neuritic plaques containing amyloid β protein and loss of cholinergic markers in the brain (Selkoe *et al.*, 1994; Park *et al.*, 1996). In AD patients deficit of cholinergic functions in the brain results in memory impairments. An important therapeutic strategy for activating central cholinergic functions has been the use of inhibitors of AChE, the enzyme responsible for the metabolic hydrolysis of acetylcholine (Bartus *et al.*, 1982; Perry, 1986; Bartus, 2000). Some AChE inhibitors like physostgmine or tacrine are known to have limitations such as short half-life or side-effects like hepatotoxicity, and alkylpyridinium polymers, dehydroevodiamine and carbamate type AChE inhibitors have been reported, but because of bioavailability problems and possible side-effects, there still is great interest in finding better AChE inhibitors (Park *et al.*, 1996; Rhee *et al.*, 2001).

Searching for AChE inhibitors, natural resources such as medicinal or natural plants were screened for anti-AChE activity. It was found that a total methanolic extract of the aerial parts of *Corydalis incisa* (Papaveraceae) showed significant inhibition towards AChE. The anti-AChE activity of the methanolic extract was found to be mainly concentrated in the crude alkaloid fraction. As a result, we isolated corynoline from the crude alkaloid fraction.

Correspondence to: Dae Keun Kim, College of Pharmacy, Woosuk University, Samrye 565-701
E-mail: ckkim@woosuk.ac.kr

This paper deals with the isolation of corynoline from *Corydalis incisa* and the inhibitory effect of corynoline on AChE.

MATERIALS AND METHODS

General procedure

^{1}H- and ^{13}C-NMR spectra were determined on a JEOL JMN-EX 400 spectrometer. TLC was carried out on Merck precoated silica gel F_{254} plates and silica gel for column chromatography was Kiesel gel 60 (230-400 mesh, Merck). All other chemicals and solvents were analyticlal grade and used without further purification. Acetylthiocholine iodide (ATCh), 5,5-dithiobis-2-nitrobenzoic acid (DTNB) and AChE (Type V-S, used for comparing with the prepared enzyme from the mouse brain) were purchased from Sigma Chemical Co..

Plant materials

The aerial parts of *C. incisa* were collected in May 2001 at Sunchang, Chonbuk, Korea. A voucher specimen is deposited in the herbarium of college of pharmacy, Woosuk University (WSU-01-019).

Extraction and isolation

Corynoline was isolated from the dried aerial parts of *C. incisa* as reported previously (Kim *et al.*, 2000). Its structure was determined by physicochemical and spectral data (Fig. 1).

Corynoline: mp 217-218°C; $[\alpha]_D^{25}$ +110 (MeOH, c 0.1); ^{1}H-NMR and ^{13}C-NMR data were in good agreement with

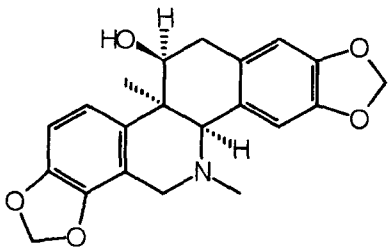

Fig. 1. Structure of corynoline

those reported in the literature (Kim *et al.*, 2000).

Acetylcholinesterase inhibition assay

The AChE assay was measured by the modified method of Ellman *et al.* using acetylthiocholine iodide as a substrate (Ellman *et al.*, 1961). For the enzyme source, mouse brain was homogenized with 5 volumes of a homogenation buffer [10 mM Tris-HCl (pH 7.2), containing 1 M NaCl, 50 mM $MgCl_2$, and 1% triton X-100] (Rieger *et al.*, 1980), then centrifuged at 10,000 g for 30 min. The resulting supernatant was used as an enzyme source. All extraction steps were carried out at 4°C. Protein concentration was determined using the BCA kit (biocinchoninic acid, Sigma Co., USA) with bovine serum albumin (BSA) as the protein standard. The rates of hydrolysis by AChE were monitored spectrophotomatically using a 96-well microtiter plate format. Each extract (10 μl) was mixed with an enzyme solution (10 μl) and incubated at 37°C for 30 min. Absorbance at 450 nm was read immediately after adding an Ellmans reaction mixture [70 μl, 0.5 mM acetylthiocholine, 1 mM 5, 5-dithil-bis-(2-nitrobenzoic acid)] in a 50 mM sodium phosphate buffer (pH 8.0) to the above reaction mixture. Reading was repeated for 10 min at 2 min intervals to verify that the reaction occurred linearly. Blank reaction was measured by substituting saline for the enzyme (Chung *et al.*, 2001; Park *et al.*, 1996).

RESULTS AND DISCUSSION

In the course of our search for AChE inhibitors from natural products, it was observed that the methanol extract of the aerial parts of *C. incisa* showed potent inhibition (IC_{50} = 31 μg/ml) of AChE prepared from the mouse brain. Using several chromatographic techniques, corynoline was isolated as an active constituent from the crude alkaloid fraction.

Corynoline inhibited AChE activity in a dose-dependent manner (Fig. 2). The concentration of this compound required for IC_{50} determined to be 30.6 μM, while the IC_{50} value of a positive control, berberine (Hwang *et al.*, 1996), was 3.6 μM. The inhibition mechanism of corynoline was more studed *in vitro*. Inhibition of AChE by corynoline was

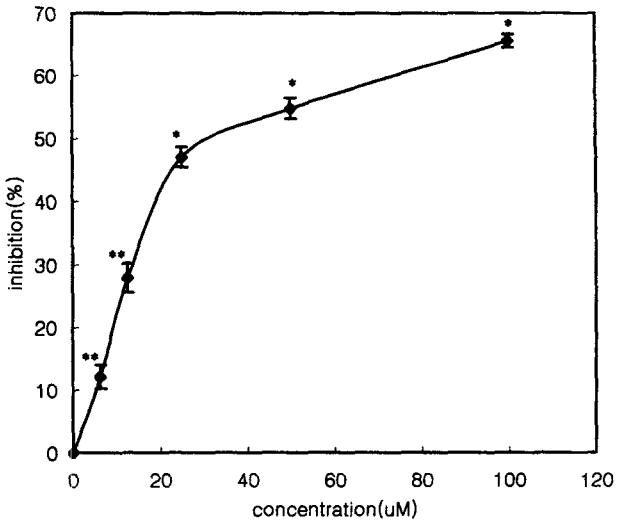

Fig. 2. The inhibitory activity of corynoline on AChE. Differs significantly from the control, effective *p < 0.05, **p < 0.01

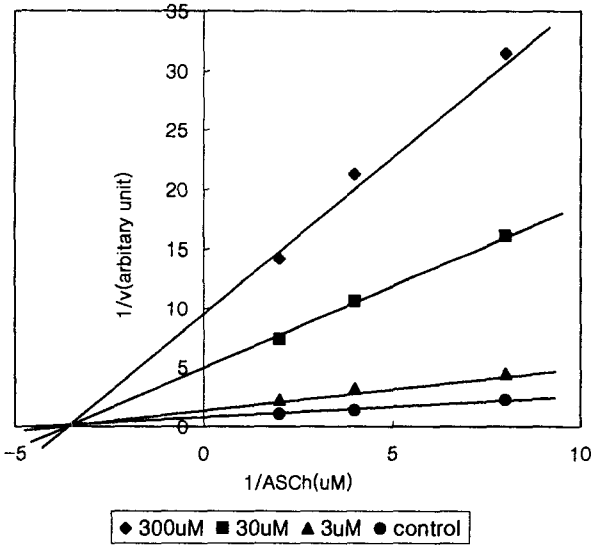

Fig. 3. Lineweaver-Burk plot of 1/v vs. 1/ASCh in the presence or absence of corynoline

independent of incubation time (up to 60 min, data not shown). This result suggests that corynoline inhibited AChE reversibly. The kinetic analysis of AChE inhibition of corynoline is shown in Fig. 3. The K_m and V_{max} values were calculated from the Lineweaver-Burk plot. The V_{max} value of AChE as plotted against [ATCh] was decreased significantly by the addition of corynoline. However, the K_m value was not changed. These results indicate that corynoline inhibited AChE in a noncompetitive manner.

In this study, we have shown that corynoline isolated from *C. incisa* inhibits AChE activity. This is less effective than that of tacrine derivatives. However, this compound

was purified from a natural medicinal plant. And this low molecular material could easily reach the site of action following oral administration, since the molecule could cross the blood-brain barrier, the tight junction controlling the transport of material into the brain (Broadwell *et al.*, 1993) In conclusion, the present study indicated that the methanolic extract of *C. incisa*, and its isolated isoquinoline alkaloid, corynoline may be useful for the treatment of AD. But further study on the anti-ammestic activity of corynoline is needed.

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