Screening of Various Phenolic Acids and Flavonoid Derivatives for their Anticholinesterase Potential

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Alzheimer's disease (AD), the most common form of dementia, is a neurodegenerative disease characterized by progressive cognitive deterioration together with declining activities of daily living and neuropsychiatric symptoms or behavioural changes. The oldest, on which most currently available drug therapies are based, is known as the "cholinergic hypothesis" and suggests that AD begins as a deficiency in the production of the neurotransmitter acetylcholine. Therefore, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitors have gained a great popularity for the treatment of AD. In this study, we screened *in vitro* inhibitory activities of a number of phenolic acids (chlorogenic, caffeic, gallic, and quinic acids) as well as of various flavonoid derivatives (genistein, biochanin A, naringin, apigenin, quercetin, luteolin-7-*O*-rutinoside, kaempferol-3-*O*-galactoside, diosmin, silibinin, and silymarin) against AChE and BChE at 1 mg/ml concentration using a microplate-reader assay based on the Ellman method. Among them, only quercetin showed a substantial inhibition (76.2%) against AChE, while genistein (65.7%), luteolin-7-*O*-rutinoside (54.9%), and silibinin (51.4%) exerted a moderate inhibition on BChE.

Key words: Phenolic Acid, Flavonoid, Alzheimer's Disease, Acetyl-/Butyrylcholinesterase

Introduction

Xanthones, as a special type of flavonoids, have been shown to be the potential inhibitors of acetylcholinesterase (AChE) (Brühlmann *et al.*, 2004), which prompted us to screen a number of flavonoids and some other phenolic compounds for finding new AChE and butyrylcholinesterase (BChE) inhibitors of natural origin. Hence, our target was to investigate possible *in vitro* inhibitory potentials of a number of phenolic compounds including chlorogenic, caffeic, gallic, and quinic acids along with a range of flavonoid derivatives such as apigenin, quercetin, genistein, biochanin A, luteolin-7-*O*-rutinoside, kaempferol-3-*O*-galactoside, diosmin, naringin, silibinin, and silymarin against AChE and BChE at a concentration of 1 mg/ml using ELISA based on the Ellman method.

Materials and Methods

Test compounds

The phenolic compounds tested were obtained from the respective manufacturers as follows: chlorogenic acid (Sigma; C3878), caffeic acid (Dr. Th. Schuchardt & Co.; 822029), gallic acid (Sigma; G7384), quinic acid (ChromaDex; ASB-D0017175-001), genistein (Sigma; G6776), biochanin A (Sigma; D2016), diosmin (Sigma; D3525), naringin (Koch-Light Laboratories Ltd.; 4161h), apigenin (Serva; 13700), quercetin (Serva; 34120), silibinin (Sigma; S0417), and silymarin (Sigma; S0292). On the other hand, luteolin-7-*O*-rutinoside was previously isolated from *Gonocytisus angulatus* (L.) Spach. (Fabaceae) (Tosun and Akyüz, 1997), while kaempferol-3-*O*-galactoside was obtained from *Calluna vulgaris* L. (Ericaceae) as described elsewhere (Orhan *et al.*, 2007a).

Determination of AChE and BChE inhibitory activities

The modified spectrophotometric method of Ellman and all the used enzymes and reagents were employed as described in details in our previous publications (Ellman *et al.*, 1961; Orhan and Şener, 2003; Orhan *et al.*, 2004, 2007b).

Statistical analysis of data

Data obtained from *in vitro* experiments were expressed as mean standard error (± SEM). Sta-

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tistical differences between the treatments and the control were evaluated by ANOVA test. $P < 0.05$ was considered to be significant [$*P < 0.05$; $**P < 0.01$; $***P < 0.001$].

Results and Discussion

In the current study, *in vitro* inhibitory activities of four phenolic acids (chlorogenic, caffeic, gallic, and quinic acids) as well as of various flavonoid derivatives (apigenin, quercetin, genistein, biochanin A, luteolin-7-*O*-rutinoside, kaempferol-3-*O*-galactoside, diosmin, naringin, silibinin, and silymarin) were tested against AChE and BChE at 1 mg/ml using a microplate-reader assay based on the Ellman method. Quercetin was found to be the most effective one towards AChE with 76.2% inhibition, whereas genistein had the highest inhibition (65.7%) against BChE, followed by luteolin-7-*O*-rutinoside and silibinin (54.9% and 51.4%, respectively) (Table I). The rest of the flavonoid derivatives and phenolic acids were either inactive or insignificantly active. The compounds exerted a better inhibition on BChE rather than on AChE.

Up to date, quite a lot of studies have reported affirmative effects of phenolics in neurodegenerative diseases depending upon their antioxidative properties (Behl and Moosmann, 2002; Ramassamy, 2006). However, there has been a small number of data on AChE and BChE inhibitory activities of phenolic compounds. For instance, Wilson and Quan (1958) screened the inhibition of AChE by a number of (hydroxyphenyl)-trimethylammonium derivatives, their dimethyl carbamates, and other related compounds and concluded that it was evident that the binding contribution of a phenolic hydroxy group arises from hydrogen bonding to a group in the enzyme. This group can be located so as to satisfy both the requirements for a hydrogen bond with a hydroxy group of the best phenol and for a covalent link with the carbonyl carbon atom of the best dimethyl carbamate. The atom of the enzyme which forms a covalent bond with the carbonyl carbon atom of the carbamates is the basic group of the esteratic site.

More recently, a few research also reported AChE inhibitory effects of non-nitrogenous compounds, which have led to the conclusion about their binding properties since a positively charged moiety (the nitrogen-containing part), which played the main role in bonding with the anionic aspartate residue of the enzyme, can be no longer responsible for this inhibition (Houghton and Howes, 2005). In 2004, there was one article pointing out to the AChE inhibition of non-nitrogenous compounds, which sparkled seven xanthone-type compounds being highly active against the enzyme by a convenient microtiter plate assay (Brühlmann *et al.*, 2004). Furthermore, four among these xanthones had already been described as monoamine oxidase (MAO) inhibitors, making them dual AChE/MAO inhibitors of great interest. Besides, the effect of tea polyphenols (TP) on cognitive and anticholinesterase activities was examined in scopolamine-treated mice by Kim *et al.* (2004) and TP exhibited a dramatic inhibitory effect on AChE. The authors suggested that TP might be functional in the treatment of Alzheimer's disease

Table I. AChE and BChE inhibitory activities of phenolic compounds (1 mg/ml).

Compound	Inhibition (%)	
	AChE	BChE
Chlorogenic acid	–[a]	30.8 ± 0.81[b,***]
Caffeic acid	–	–
Gallic acid	15.7 ± 1.02***	48.8 ± 0.88***
Quercetin	76.2 ± 0.99**	46.8 ± 1.35***
Quinic acid	–	–
Apigenin	–	–
Genistein	–	65.7 ± 1.24***
Biochanin A	–	–
Luteolin-7-*O*-rutinoside	24.7 ± 0.34***	54.9 ± 0.98***
Kaempferol-3-*O*-galactoside	–	–
Naringin	–	13.7 ± 0.56***
Diosmin	–	–
Silibinin	–	51.4 ± 1.05***
Silymarin	–	43.2 ± 0.78***
Galanthamine	99.8 ± 0.31	80.3 ± 1.14

[a] No inhibition.
[b] Values are expressed as means ± SEM ($n = 3$); $P > 0.05$; $*P < 0.05$; $**P < 0.01$; $***P < 0001$.

(AD). Another study performed on the methanol extract of *Citrus junos* had a significant inhibitory effect on AChE *in vitro*, in which the active component was identified as naringenin, a major flavonone derivative, after sequential fractionations (Heo *et al.*, 2004). Naringin, the major flavonoid glycoside in grapefruit which is metabolized to the flavonone naringenin in humans, was studied herein for its anticholinesterase effect and showed no inhibition against both enzymes at all. The reason why naringenin was active while naringin was completely inactive could be due to the structural differences and also to their interactions with the active sites of the enzymes. In our study, two isoflavones, genistein and biochanin A, displayed a contrast effect on BChE. As seen in Table I, while biochanin A was inactive, genistein had a good inhibition on BChE with 65.7%. This may again be brought about by the structural difference as biochanin A contains a methoxy instead of a hydroxy group in the C ring, and might diminish the activity.

On the other hand, quercetin was reported to have a good anti-BChE activity and a weak inhibition towards insect AChE and no effect on human AChE (Sivori *et al.*, 1999; Zhang, 2006; Zhang *et al.*, 2006), whereas it showed the highest inhibitory activity against AChE (76.2%) and moderate activity against BChE (46.8%) in our experiment. Taking flavonoids into consideration from the point of AChE inhibition view, there has been also one interesting work by Ji and Zhang (2006). In that report, the structural requirements for flavonoids as inhibitors of AChE and BChE were examined, and it was stated that the catechol moiety on ring B has positive effects on the enzyme-inhibiting activities of quercetin contributing to its binding to the enzyme. However, the difference observed between results of our study and the previous data might result from the source of the enzyme.

Because of the unclear pathogenesis of AD, there have been several hypothesis associated with the disease such as amyloid-β peptide-containing plaque formation, excess metal ions, oxidative stress, and reduced acetylcholine levels. As proposed by Zhang *et al.* (2006), finding more than one approach with multifunction for AD treatment draws attention to researchers. On the other hand, plant phenolics are well-known for their potent antioxidant effects and in addition to this property, the phenolic compounds like quercetin in this study also possessing an anticholinesterase effect might be considered to be of therapeutic potential. Obviously, the active compounds, quercetin in particular, deserve further molecular docking and structure-activity relationship studies.

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