academicJournals

Vol. 12(14), pp. 1675-1681, 3 April, 2013
DOI: 10.5897/AJB12.2699
ISSN 1684–5315© 2013 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Inhibition of cholinesterases by phenolic acids detected in beer: A dose-response model approach

D. Szwajgier

Department of Biotechnology, Human Nutrition and Food Commodity Science, University of Natural Sciences in Lublin, Skromna 8, 20-704 Lublin, Poland. E-mail: dszwajgier@hotmail.com. Tel: +48 (81) 462 33 68. Fax +48 (81) 462 33 54.

Accepted 15 March, 2013

This study tested the *in vitro* anti-acetylcholinesterase (AChE) and anti-butyrylcholinesterase (BChE) activities of simple phenolic acids using Ellman's spectrophotometric method. 15 phenolic acids (at levels found in beer) exerted differentiated anticholinesterase activities, with the most efficient being chlorogenic, p-coumaric, p-hydroxybenzoic and caffeic acids. When present at levels similar to those detected in plasma after beer consumption (500 ml), vanillic, caffeic, syringic and p-coumaric acids exhibited anti-BChE activity. Phenolic acids were not interactive in a complex solution at concentrations found in plasma after beer consumption. Combined phenolic acids (p-hydroxyphenylacetic, ferulic, vanillic, caffeic, syringic, sinapic and p-coumaric, at 0.01 mM) showed both anticholinesterase activity and synergy between the individual compounds. Also, synergy was found in the case of selected pairs of phenolic acids (each at 0.01 mM), calculated using the method of expected inhibition and the interactive index of combination. These results support the idea that simple phenolic acids from beer can play a role in neuroprotection, but further studies need to be conducted.

Key words: Acetylcholinesterase, Alzheimer's disease, beer, butyrylcholinesterase, phenolic acids.

INTRODUCTION

Alzheimer's disease (AD) is a progressively developing neurodegenerative disorder of the central nervous system (CNS), mainly occurring in the population aged over 65 years. Significant cholinergic pathway deficits can be observed during the development of AD (Rao et al., 2007), due to the increase in butyrylcholinesterase (BChE) and acetylcholinesterase (AChE) activity (Whitehouse et al., 1981). Accordingly, the only current treatment for AD is based on the application of cholinesterase inhibitors (Figure 1) in order to inhibit AChE and BChE (Gruntzendler and Morris, 2001).

Beer is a complex solution of 600 or more compounds (Kunze, 2010). Earlier studies have indicated that limited beer consumption either results in a decreased risk of dementia or AD (Mukamal et al., 2003) or is not positively connected with a higher risk of neurodegenerative changes in the CNS (Luchsinger et al., 2004). More than half of the research papers published over the last 20 years (out of a total number of approximately 45), and collected in a volume by Collins et al. (2009), prove that low consumption of beer reduces the risk of dementia. On the other hand, a minority of the papers in the same review indicate the risk of neurodegeneration in the CNS after beer consumption due to its ethanol content. Beer components have not been studied in detail with regard to their protective effects towards CNS, although the role of phenolic acids has been previously noted in this context. Ferulic acid (FA) has been a neuroprotectant in the CNS of experimental animals (Cho et al., 2005; Cheng et al., 2008; Yabe et al., 2010). FA, p-coumaric (p-CA), 4-hydroxybenzoic (4-OH-BA) and sinapic (SNA) acids have acted as efficient *in vitro* AChE and BChE inhibitors (Szwajgier and Borowiec, 2012). SNA and FA have participated in the regulation of the ACh-signalling system in experimental

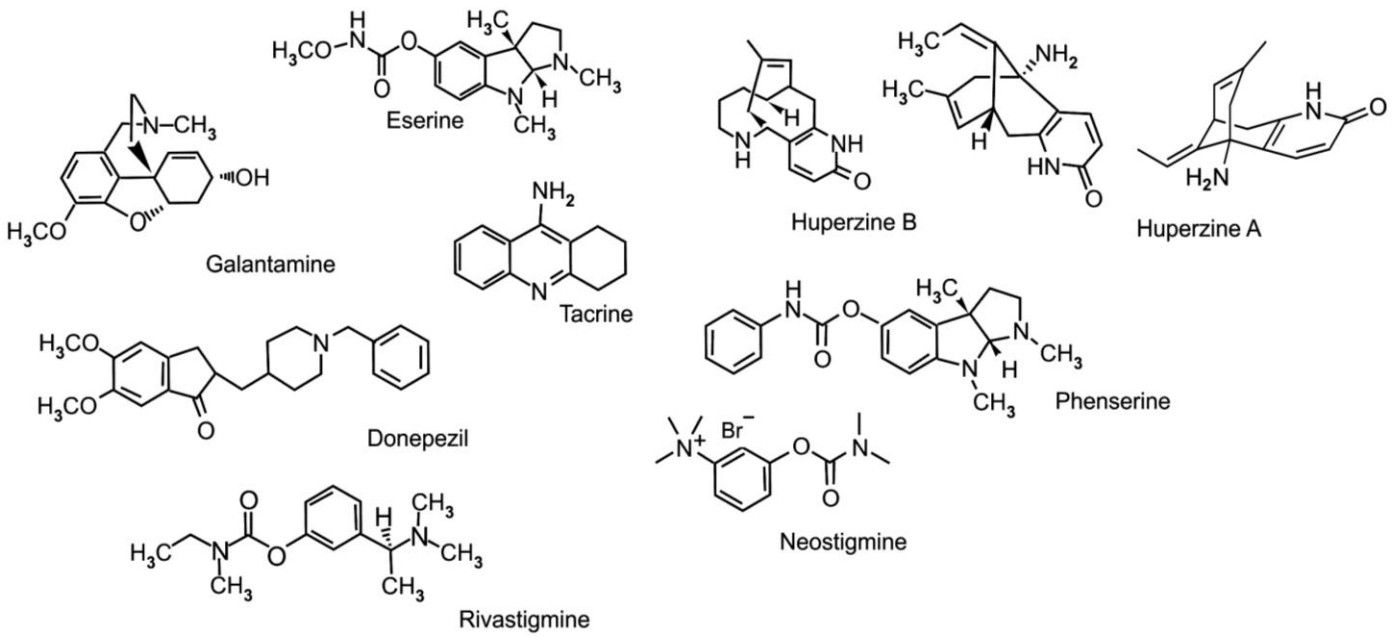

Figure 1. Chemical structures of cholinesterase inhibitors.

animals (Yan et al., 2001; Hsieh et al., 2002; Kim et al., 2004, 2007; Karakida et al., 2007). Amyloid-β plaque deposition has been significantly decreased in the brains of mice fed nordihydroguaiaretic (NDGA) and rosmarinic (RA) acids. Treatment with RA has caused an increase in the content of soluble β-amyloid (Hamaguchi et al., 2009).

A number of phenolic acids have been detected in different types of beer: caffeic (CA), chlorogenic (CHL), cinnamic (CNA), o-coumaric (o-CA), p-CA, FA, gallic (GA), homovanillic (HVA), 3- and 4-hydroxybenzoic (3- and 4-OH-BA), 4-hydroxyphenylacetic (4-OH-PA), protocatechuic (PCA), sinapic (SA), syringic (SRA) and vanillic (VA) acids (Montanari et al., 1999; Gerhäuser et al., 2002; Nardini and Ghiselli, 2004; Nardini et al., 2006; Szwajgier et al., 2007; Szwajgier, 2009). It is of note that phenolic acids are effectively absorbed from beer into plasma after beer consumption. In a study by Bourne et al. (2000), the peak time for urinary excretion of FA after consumption of low-alcohol beer was approximately 8 h, and the mean cumulative amount excreted was 5.8 ± 3.2 mg. The increase in the content of phenolic acids in plasma after drinking a single beer (500 ml) reached 40% of the basal level and amounted to 10 mg of phenolic acids in total (Bourne et al., 2000). The amount of phenolic acids absorbed from a single glass of beer (500 ml) can be considered remarkably high. The levels of CA, FA, VA, 4-OH-PA, p-CA, SA and SRA detected in plasma 30, 60 or 120 min after beer consumption were in the range of 0.4 to 160 ng/ml (Ghiselli et al., 2000; Nardini et al., 2006). Therefore, the aim of the present work was to study the anticholinesterase activity of phenolic acids at the concentrations previously detected in beer as well as in human plasma after beer consumption. The synergy/antagonism or zero interactivity between the individual phenolic acids was estimated in model systems. Inhibitions calculated using the zero interactive response method and the interactive index of combination were compared with experimental ones.

MATERIALS AND METHODS

Reagents

The following reagents were purchased from Sigma-Aldrich (Poznań, Poland): VA (94770), FA (128708), 4-OH-BA (H20059), 4-OH-PA (H50004), CA (C0625), SA (D7927), PCA (37580), CNA (C80857), p-CA (C9008), o-coumaric acid (o-CA, H22809), 3-OH-BA (H20008), SRA (S6881), GA (G7384), CHA (C3878), HVA (H1252), acetylthiocholine (ATChI, 01480), butyrylthiocholine (BTCh, B3128), 5,5'-dithiobis-2-nitrobenzoic acid (DTNB, D8130), eserine (E8375), acetylcholinesterase (AChE, C3389) and butyrylcholinesterase (BChE, C7512). Other reagents were purchased from POCh S.A. (Gliwice, Poland).

Solutions of phenolic compounds

Standard solutions of phenolic acids were prepared daily in a minimal volume of ethanol (98.0% (v/v), followed by dilution to the final concentration in double deionized water. Ethanol content in final solutions was maximum of 4% (v/v). The presence of ethanol in test samples had no influence on the activity of AChE or BChE (results not shown). Two sets of solutions were prepared during this study.

Inhibition of AChE and BChE

Enzyme activities were measured in a 96-well microplate reader (Tecan Sunrise, Grödig, Austria) using a modified spectrophotometric method (Ellman et al., 1961). All reagent solutions were prepared in Tris-HCl buffer (50 mM, pH 8.0). The test solution was

composed of 0.035 ml of ATChI or BTCh (1.5 mM), 0.035 ml of the studied sample, 0.08 ml of Tris-HCl buffer (50 mM, pH 8.0), 0.02 ml of AChE or BChE solution (0.28 U/ml) and 0.175 ml of 0.3 mM DTNB (containing 10 mM NaCl and 2 mM $MgCl_2$). Also, samples containing Tris-HCl buffer instead of the test solutions were run in the same way. The absorbance (405 nm, 22°C) was read after 30 (AChE) or 10 min (BChE). The increase in the absorbance due to the spontaneous hydrolysis of the substrate was monitored using blank samples containing DTNB and ATCh (BTCh) supplemented with Tris-HCl buffer to the final reaction volume. The absorbance originating from the blank sample was subtracted from the absorbance of the test sample. The false-positive effect was verified according to Rhee et al. (2003), with minor modifications. After the addition of ATChI (BTCh), buffer and the enzyme, the sample was left for 30 (AChE) or 10 min (BChE). Then, a test solution was added, followed by direct addition of DTNB and measurement of the absorbance. Each solution of the phenolic acid was analyzed in eight repeats.

The inhibitory activity of test compounds was expressed using eserine equivalents (nM serine), as proposed previously (Szwajgier and Borowiec, 2012). In short, 12 solutions of eserine at 7.1 nM - 7.27 µM were prepared and their anticholinesterase activities were measured as described above. Calibration curves were plotted for each enzyme. In the results section, the inhibitory activity of the test solution was expressed as the equivalent concentration of eserine (mM) required to exert the same inhibitory activity.

Calculation of the expected inhibition of AChE and BChE

The inhibition of AChE and BChE was determined in eight repeats using model solutions of the individual phenolic acids in the range of 0 to 0.1 mM, followed by establishing dose-response curves. The IC_{50} (defined as the concentration of the individual phenolic acid that inhibited 50% of the enzyme activity) was calculated using the dose-response curves. The expected enzyme inhibition was calculated using the dose-response curves of individual phenolic acids at concentrations previously detected in beers (Gerhäuser et al., 2002; Montanari et al., 1999; Nardini et al., 2006; Nardini and Ghiselli, 2004; Szwajgier, 2009). Similarly, the expected enzyme inhibition by individual phenolic acids was calculated at levels similar to their content in plasma using the dose-response curves and the content of phenolic acids in plasma previously reported by other authors (Ghiselli et al., 2000; Nardini et al., 2006).

Determination of the interaction between individual phenolic acids

The expected inhibition of the mixture of phenolic acids was calculated according to Berenbaum (1985) using the protocol described in detail by Savelev et al. (2003). Briefly, the method assumes zero interaction between the individual components of a complex solution. The expected inhibition of AChE (BChE) by a number of phenolic acids in the solution was calculated in order to satisfy both the equation for zero interaction response - $d_1/D_1 + d_2/D_2 + \ldots + d_n/D_n = 1$ - and the dose-response curves of each compound. D_1, D_2,…D_n are the concentrations of individual phenolic acids that are able, singly, to produce the same inhibitory effect as compounds present at concentrations d_1, $d_2 \ldots d_n$ in the complex solution. The inhibition of AChE and BChE in the complex solution was measured using the spectrophotometric method described above. Synergy was assumed if the observed inhibition was significantly ($P < 0.05$) higher than the expected inhibition, and antagonism was observed if a reverse result was obtained.

Synergy/antagonism was also calculated using another method based on the interaction index as described by Savelev et al. (2003). In this method, the observed inhibition of AChE or BChE by a complex solution of phenolic acids was substituted for “y” in each dose-response curve of the individual compounds. The calculated x values (D_1, $D_2 \ldots D_n$) corresponding to y were then read. D values as well as the actual concentrations of phenolic acids in the solution (d_1, $d_2 \ldots d_n$) were used to calculate the interaction index using the equation: $d_1/D_1 + d_2/D_2 + \ldots + d_n/D_n = 1$. Synergy was assumed if the interaction index was significantly lower than 1.0, whereas antagonism was observed if the values of the interaction index significantly exceeded 1.0.

First, a solution containing all free phenolic acids at concentrations similar to their levels in plasma after beer consumption was studied (1000 nM p-OH-PA, 50 nM FA, 40 nM VA, 30 nM CA, 20 nM SRA, 20 nM SA and 10 nM p-CA). Also, the expected and observed inhibitions of both enzymes by the solution containing all free phenolic acids (each at a final concentration 0.01 mM) were tested in eight repeats. Finally, synergy/antagonism/zero interaction was studied using phenolic acids in pairs, each at a final concentration of 0.01 mM. The observed inhibitions of cholinesterases by the test solutions were calculated using experimental calibration curves determined using eserine. Expected as well, additive inhibitions of combinations of phenolic acids were calculated using dose-response curves of the individual compounds.

Statistical analysis

Mean values ± standard deviation (STATISTICA 8.0, StatSoft, Cracow, Poland) were calculated. Tukey’s test ($P \leq 0.05$) was used to denote statistically significant results.

RESULTS

In the present paper, synergy/antagonism/zero interacttion between individual phenolic acids was tested using the expected response method instead of the summation method, because dose-response curves of all the phenollic acids were nonlinear (Figure 2). Most of the phenolic acids exhibited anticholinesterase activities in the presence of AChE and/or BChE. The highest inhi-bitory activity was shown by FA (Table 1). However, this was mainly attributable to the fact that the content of this acid used for calculations was the highest. A number of other phenolic acids, even though used at much lower concentrations than FA, exerted considerable anticho-linesterase activeties. Phenolic acids were unable to in-hibit 50% of AChE or BChE activity in the applied test conditions, with the exception of p-CA (IC_{50} values at 336 and 160 mM calculated for AChE and BChE, respect-tively).

AChE was not inhibited by phenolic acids at concentrations equal to their average levels in plasma after beer consumption (Table 2). The ability to inhibit BChE was observed in the case of VA, CA, SRA and p-CA, with the last showing considerable anti-BChE activity and equal to SRA, despite it having the lowest concentration among all phenolic acids.

The synergy/antagonism/zero interaction of the complex solution of all the phenolic acids at concentrations similar to those present in plasma after beer consumption (p-OH-PA 1000 nM, FA 50 nM, VA 40 nM, CA 30 nM, SRA 20 nM, SA 20 nM, p-CA 10 nM) were calculated. The expected inhibition of AChE (1.5 nM of eserine) was equal to the additive one (1.4 ± 0.2 nM of eserine). No

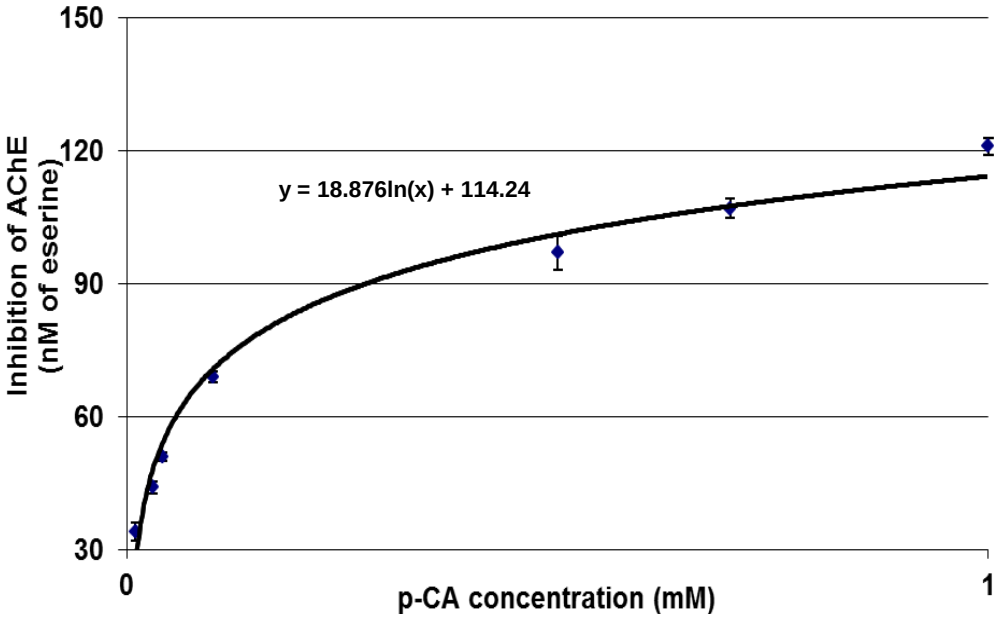

Figure 2. Anti-AChE dose-response curve for p-CA, n = 8.

Table 1. Expected inhibitions of AChE and BChE by HPLC standards of phenolic acids at concentrations equal to their average, total levels in beer (calculated using dose-response curve equations of individual compounds).

Phenolic acid	Total levels detected in beer (µM)[a]	Inhibition (nM of eserine)	
		AChE	BChE
FA	53.5-86.3	39.5-50.1	42.8-53.1
SA	0.4-27.5	0-40.7	0-19.1
PCA	0.5-24.0	-	0-25.2
CNA	17.6	-	-
VA	3.5-12.5	2.3-11.2	5.1-9.1
p-CA	2.2-11.9	0–29.2	6.2-36.0
CA	0.2-11.7[b]	0-7.4	9.6-34.1
p-OH-PA	4.1-5.3	-	-
p-OH-BA	0.2-4.6	0-6.3	7.0-19.5
m-OH-BA	3.6	-	4.9
o-CA	3.5	-	-
SRA	0.2-3.4	-	4.7–8.8
GA	2.9	-	10.4
CHA	0.3-0.7[b]	3.8-13.9	0-6.5
HVA	0.5[b]	-	-

[a]Based on results obtained by Gerhäuser et al. (2002), Montanari et al. (1999), Nardini et al. (2006), Nardini and Ghiselli (2004) and Szwajgier (2009). [b]Free CHA and CA levels were used for the calculations instead of total content because caffeic acid is a product of the alkaline hydrolysis of chlorogenic acid. Only free homovanillic acid was detected by the cited authors and it was this form that was used for the calculations.

experimental inhibition of BChE was observed in this study. However, the results presented in Table 2 show that individual phenolic acids at low concentrations were capable of inhibiting BChE but not AChE.

Further tests were performed using model solutions of phenolic acids at 0.01 mM. First, the synergy/antagonism/zero interaction of all the phenolic acids in a solution were studied (Table 3). The observed inhibitions of AChE and BChE were significantly ($P < 0.05$) higher than the corresponding expected inhibitions calculated from dose-response curves. Also, the interaction indexes for the combination of compounds in the solution were

Table 2. Expected inhibition of BChE by HPLC standards of free phenolic acids at levels equal to their levels in plasma after beer consumption (calculated using dose-response curve equations of individual compounds).

Phenolic acid	Maximal elevation in plasma after beer consumption (nM)[a]	Inhibition (nM of eserine)
p-OH-PA	1051.5	-
FA	56.6	-
VA	35.7	0.6
CA	25.0	0.3
SRA	21.2	0.2
SA	19.2	-
p-CA	9.1	0.2

[a]Based on results obtained by Ghiselli et al. (2000) and Nardini et al. (2006).

Table 3. Anticholinesterase activities of p-OH-PA, FA, VA, CA, SRA, SA and p-CA (each compound at 0.01 mM).

Inhibition ± SD of all phenolic acids in combination (nM of eserine)			
AChE		BChE	
Expected[a]	Observed[b]	Expected[a]	Observed[b]
57.4	91.3±7.9 (0.66±0.13)[c]	107.5	129.0±10.5 (0.74±0.21)[c]

[a]Calculated zero interactive response of the combination; [b]Inhibition obtained experimentally (n = 8); [c]Results in the brackets are the interaction indexes of the combination.

significantly ($P < 0.05$) lower than 1.0, indicating synergy between the individual compounds. Therefore, in the next part of the study, the interactions of pairs of phenolic acids were tested (Table 4). Most of the phenolic acids in pairs showed no interaction, as measured using these two methods. Co-operation (at $P < 0.05$) in the inhibition of AChE was observed for p-CA + FA, FA + CA and CA + VA, as well as for CA + VA and p-CA + CA in the inhibition of BChE. In the case of p-CA + CA (inhibition of BChE) and VA + CA (inhibition of both enzymes), the experimental inhibition was significantly higher than the calculated additive inhibition of the individual phenolic acids. However, no pairwise interaction between these phenolic acids was observed when the interaction index of combination (~1.00) was calculated. On the other hand, interaction indexes of p-CA + SA and VA + SRA were significantly lower than 1.00 (synergy). In some cases, the observed inhibition of the enzyme was significantly higher (p-CA + VA, AChE) or, conversely, lower (p-CA + SA, BChE) than the additive inhibition of the individual compounds.

DISCUSSION

Well-known cholinesterase inhibitors currently in use (Figure 1) are compounds of higher molecular weights (200 to 400 g/mol) than simple phenolic acids. However, some authors have previously isolated and characterized cholinesterase inhibitors of molecular weights similar to or even lower than those of phenolic acids. Anticholinesterase activities of complex solutions (crude extracts from *Salvia lavandulaefolia*) reflected both the synergistic and antagonistic interactions of the components (Perry et al., 2000, 2001). In a study by Savelev et al. (2003), low-molecular weight (136.23 to 220.35 g/mol) terpenoids from *S. lavandulaefolia* (1,8-cineole, camphor, α-pinene, borneol, caryophyllene oxide, linalool and bornyl acetate) were studied for anticholinesterase activities, and the synergy between individual compounds in model solutions was established. 1,8-Cineole was found to be the most effective inhibitor of AChE (IC_{50} 0.06 ± 0.01 mg/ml, M_w = 154.25 g/mol), and a positive interaction between 1,8-cineole and α-pinene and 1,8-cineole and caryophyllene oxide was observed. Also, antagonism between selected *Salvia* constituents (camphor and 1,8-cineole) was noted. The role of anti-AChE compounds has also been reported in the prolongation of the active time of combined compounds in solution (Savelev et al., 2003). In another study, linalool and α-terpineol from different essential oils exhibited anticholinesterase activities (Howes et al., 2003). Wang et al. (2007) isolated a very effective antioxidant and AChE inhibitor, hydroquinone (M_w = 110.11 g/mol), from the rhizome of *Rhodiola rosea*. Miyazawa et al. (1997) estimated the anti-AChE activity of 17 monoterpenoid compounds (hydrocarbons, alcohols and ketones) with a *p*-menthane skeleton (M_w = 134.22-156.27 g/mol). The highest anti-AChE activity was exhibited by (+)-pulegone and α-terpinene. Terpene ketones were more efficient

Table 4. Inhibition of cholinesterases by combinations of phenolic acids (each phenolic acid at 0.01 mM).

Phenolic acid	Interaction index of the combination (AChE)	Inhibition ± SD by combined phenolic acid (nM of eserine)					
		AChE			BChE		
		Expected[d]	Observed[e]	Additive[f]	Expected[d]	Observed[e]	Additive[f]
p-CA+FA	0.94±0.15	27.9	33^{c}±2.4	30.8^{c}± 3.7	38.8	34.2^{bc}±3.1	33.6^{bc}±2.2
p-CA+SA	0.84±0.08	38.3	36.5^{c}±2.5	37.7^{c}±5.1	36.0	33.9^{bc}±1.3	40.0^{c}±6.2
p-CA+p-OH-PA	1.08±0.09	25.6	-	22.8^{b}±2.4	30.0	26.5^{a}±0.6	29.8^{ab}±4.1
p-CA+CA	0.90±0.11	34.4	35.0^{c}±2.1	32.4^{c}±4.8	61.2	60.3^{c}±0.8	64.6^{c}±6.0
p-CA+VA	0.92±0.13	34.9	-	30.7^{c}±4.4	42.3	47.8^{c}±1.3	45.8^{c}±6.1
p-CA+SRA	1.21±0.18	26.9	25.2^{bc}±1.5	29.8^{c}±3.4	50.2	55.3^{c}±3.0	53.6^{c}±4.0
FA+SA	0.94±0.09	17.3	16.8^{ab}±2.5	19.9^{bc}±3.0	11.5	13.1^{a}±2.4	12.8^{a}±6.4
FA+p-OH-PA	1.17±0.11	7.5	-	8.0^{a}±1.3	6.7	7.3^{a}±2.5	6.6^{a}±4.3
FA+CA	0.87±0.20	10.9	14.7^{a}±1.9	12.6^{a}±3.7	32.5	27.1^{a}±3.3	33.4^{bc}±3.2
FA+VA	0.92±0.09	14.5	-	15.9^{ab}±3.3	17.6	-	14.6^{a}±2.0
FA+SRA	1.12±0.03	9.9	6.7^{a}±2.6	11.0^{a}±2.3	29.4	-	27.4^{ab}±6.3
SA+p-OH-PA	0.89±0.14	7.3	6.6^{a}±2.4	7.9^{a}±2.7	7.9	10.7^{a}±2.0	8.0^{a}±2.3
SA+CA	0.95±0.11	18.1	20.6^{b}±2.9	17.5^{ab}±3.1	35.1	36.0^{bc}±2.1	32.8^{bc}±3.2
SA+VA	0.90±0.21	22.8	25.9^{bc}±3.6	19.8^{bc}±4.7	14.4	13.6^{a}±2.7	11.0^{a}±6.3
SA+SRA	1.14±0.19	8.4	7.4^{a}±1.3	9.0^{a}±1.7	26.5	-	23.8^{ab}±6.3
p-OH-PA+CA	1.07±0.06	7.9	8.6^{a}±0.5	9.0^{a}±2.4	24.6	19.0^{a}±1.3	25.6^{ab}±4.1
p-OH-PA+VA	0.89±0.12	10.0	6.7^{a}±1.3	7.9^{a}±2.0	7.8	5.4^{a}±2.2	8.4^{a}±4.2
p-OH-PA+SRA	1.11±0.15	-	-	-	17.8	16.4^{a}±3.2	14.6^{a}±4.2
CA+VA	0.92±0.14	15.8	21.6^{b}±2.6	17.5^{ab}±4.4	36.1	40.3^{c}±2.4	35.6^{c}±1.2
CA+SRA	0.96±0.12	8.0	10.0^{a}±2.0	6.6^{a}±3.4	47.5	47.1^{c}±2.2	48.4^{c}±6.1
VA+SRA	0.92±0.07	9.4	11.7^{a}±2.2	7.9^{a}±3.0	7.1	9.4^{a}±1.8	9.6^{a}±3.2

[d]Calculated zero interactive response of the combinations; [e]Inhibition obtained experimentally (n = 8); [f]The sum of the expected inhibitions of individual phenolic acids calculated from dose-response curve equations. In each column, different superscript letters a, b and c denote significant differences between samples (P > 0.05).

inhibitors than terpene alcohols, which showed an identical inhibitory activity to terpene hydrocarbon compounds. (+)-p-Menth-1-ene and α-terpinene were equally strong as inhibitors as terpene ketones.

In the present study, it was not unequivocally established which derivatives of which acid, cinnamic or benzoic, exerted higher inhibitory activities (FA vs. VA, SA vs. SRA, PCA vs. CA and p-CA vs. p-OH-BA). The presence of -OH and -OCH_3 groups elevated anti-ChE activity, as shown by the fact that CNA exerted no inhibitory activity, while most of its derivatives were active towards at least one of the enzymes. The presence of a -OH group in the para position elevated anti-ChE activity in comparison to a -OH group in the ortho position (p-CA vs. o-CA, p-OH-BA vs. m-OH-BA). The presence of a -OH group in the meta-position (CA) elevated inhibitory activity in comparison to the presence of a -OCH_3 group (FA). However, this was not observed in the case of PCA and VA. It is of note that a single substitution in the phenol ring in the para position (as in p-OH-BA or p-CA) was especially beneficial for inhibitory activity. As can be seen in Figure 1, efficient cholinesterase inhibitors possess hydrophyllic as well as hydrophobic regions. The synergistic and/or antagonistic interactions between individual phenolic acids may depend on the site of action of the compound in the cholinesterase which is determined by hydrophyllic and/or hydrophobic binding of the ligand to the enzyme. If a compound is located in the peripheral site of the enzyme, it can block the entrance of the substrate at the narrow aromatic gorge of the enzyme.

The studies citied in the introduction section have shown that phenolic acid levels in plasma after beer consumption are low. However, it had been previously proven that phenolic acids (FA, VA, p-CA and CNA) were effective neuroprotectants *in vivo* and in neuronal cell cultures at low micromolar concentrations (Schroeter et al., 2000; Kanski et al., 2002; Chang et al., 2009). In this paper, the total molar concentration of any two phenolic acids in the model solution (10 + 10 µM) was approximately 16-fold higher than the total maximum identified levels of these phenolic acids in plasma (~1.22 µM) (Table 2). Therefore, it can be supposed that a zero interaction between two or more phenolic acids in plasma after the consumption of 500 ml of beer should be expected. In conclusion, it can be stated, however, that a more thorough account of the neuroprotective properties of the minor phenolic acids detected in beer is needed, for example CA, p-OH-BA, SA, SRA or GA. Also, expanded *in vivo* experiments on the role of phenolic acids in Alzheimer's disease, especially as cholinesterase inhibitors, are desireable.

ACKNOWLEDGEMENTS

This scientific work was financed by the Ministry of Science and Higher Education of the Republic of Poland (Scientific Grant 2339/B/P01/2010/38) and by resources of Scientific Project POIG 01.01.02-00-061/09.

Abbreviations

ACh, Acetylcholine; **AChE,** acetylcholinesterase; **BChE,** butyrylcholinesterase; **ChAT,** choline acetyltransferase; **VA,** vanillic acid; **FA**, ferulic acid; **FAEE,** ferulic acid ethyl ester; **m-OH-BA,** m-OH-benzoic acid; **p-OH-BA,** p-OH-benzoic acid; **p-OH-PA,** p-OH-phenylacetic acid; **CA,** caffeic acid; **SA,** sinapic acid; **PCA,** protocatechuic acid; **CNA,** cinnamic acid; **p-CA,** p-coumaric acid; **o-CA,** o-coumaric acid; **SRA,** syringic acid; **GA,** gallic acid; **CHA,** chlorogenic acid; **HVA,** homovanillic acid; **ATChI,** acetylthiocholine iodide; **BTCh,** S-butyrylthiocholine chloride; **DTNB,** 5,5'-dithiobis-2-nitrobenzoic acid; **AD,** Alzheimer's disease.

REFERENCES

Berenbaum MC (1985). The expected effect of a combination of agents: the general solution. J. Theor. Biol. 114:122-130.

Bourne L, Paganga G, Baxter D, Hughes P, Rice-Evans C (2000). Absorption of ferulic acid from low-alcohol beer. Free Radic. Res. 32(3):273-280.

Chang Ho C, Kumaran A, Sun Hwang L (2009). Bio-assay guided isolation and identification of anti-Alzheimer active compounds from the root of Angelica sinensis. Food Chem. 114:246-252.

Cheng CY, Su SY, Tang NY, Ho TY, Chiang SY, Hsieh CL (2008). Ferulic acid provides neuroprotection against oxidative stress-related apoptosis after cerebral ischemia/reperfusion injury by inhibiting of ICAM-1 mRNA expression in rats. Brain Res. 13:136-150.

Cho JY, Kim HS, Kim DH, Yan JJ, Suh HW, Song DK (2005). Inhibitory effects of long-term administration of ferulic acid on astrocyte activation induced by intracerebroventricular injection of beta-amyloid peptide (1-42) in mice. Prog. Neuro- Psychoph. 29:901-907.

Collins MA, Neafsey EJ, Mukamal KJ, Gray MO, Parks DA, Das DK, Korthuis RJ (2009). Alcohol in moderation, cardioprotection, and neuroprotection: Epidemiological considerations and mechanistic studies. Alcoholism 33: 206-219.

Ellman GL, Lourtney DK, Andres V, Gmelin G (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7:88-95.

Gerhäuser C, Alt AP, Klimo K, Knauft J, Frank N, Becker H (2002). Isolation and potential cancer chemopreventive activities of phenolic compounds of beer. Phytochem. Rev. 1:369-377.

Ghiselli A, Natella F, Guidi A, Montanari L (2000). Beer increases plasma antioxidant capacity in humans. J. Nutr. Biochem. 11: 76-80.

Gruntzendler J, Morris JC (2001). Cholinesterase inhibitors for Alzheimer's disease. Drugs 61: 41-52.

Hamaguchi T, Ono K., Murase A, Yamada M (2009). Phenolic compounds prevent Alzheimer's pathology through different effects on the amyloid-β aggregation pathway. Am. J. Pathol. 175:2557-2565.

Howes MJR, Perry NSL, Houghton PJ (2003). Plants with traditional uses and activities relevant to the management of Alzheimer's disease and other cognitive disorders. Phytother. Res. 17: 1-18.

Hsieh MT, Tsai FH, Lin YC, Wang WH, Wu CR (2002). Effects of ferulic acid on the impairment of inhibitory avoidance performance in rats. Planta Med. 68:754-756.

Kanski J, Aksenova M, Stoyanova A, Butterfield DA (2002). Ferulic acid antioxidant protection against hydroxyl and peroxyl radical oxidation in synaptosomal and neuronal cell culture systems in vitro: structure-activity studies. J. Nutr. Biochem. 13:273-281.

Karakida F, Ikeya Y, Tsunakawa M, Yamaguchi T, Ikarashi Y, Takeda S, Aburada M (2007). Cerebral protective and cognition-improving effects of sinapic acid in rodents. Biol. Pharm. Bull. 30:514-519.

Kim HS, Cho JY, Kim DH, Yan JJ, Lee HK, Suh HW, Song DK (2004). Inhibitory effects of long-term administration of ferulic acid on microglial activation induced by intracerebroventricular injection of beta-amyloid peptide (1-42) in mice. Biol. Pharm. Bull. 27:120-121.

Kim MJ, Choi SJ, Lim S-T, Kim HK, Heo HJ, Kim E-K, Jun W-J, Cho HY, Kim YJ, Shin DH (2007). Ferulic acid supplementation prevents trimethyltin-induced cognitive deficits in mice. Biosci. Biotechnol. Biochem. 71:1063-1068.

Kunze W (2010). Technology Brewing & Malting. 4th edition, VLB, Berlin. pp. 224-322.

Luchsinger JA, Tang M-X, Siddiqui M, Shea S, Mayeux R (2004). Alcohol intake and risk of dementia. J. Am. Geriatr. Soc. 52:540-546.

Miyazawa M, Watanabe H, Kameoka H (1997). Inhibition of acetylcholinesterase activity by monoterpenoids with a p-menthane skeleton. J. Agric. Food Chem. 45:677-679.

Montanari L, Perretti G, Natella F, Guidi A, Fantozzi P (1999). Organic and phenolic acids in beer. Lebensm. Wiss. Technol. 32:535-539.

Mukamal KJ, Kuller LH, Fitzpatrick AL, Longstreth WT Jr, Mittleman MA, Siscovick DS (2003). Prospective Study of Alcohol Consumption and Risk of Dementia in Older Adults. J. Am. Med. Assoc. 289:1405-1413.

Nardini M, Ghiselli A (2004). Determination of free and bound phenolic acids in beer. Food Chem. 84:137-143.

Nardini M, Natella F, Scaccini C, Ghiselli A (2006). Phenolic acids from beer are absorbed and extensively metabolized in humans. J. Nutr. Biochem. 17:14-22.

Perry NSL, Houghton PJ, Sampson J, Theobald AE, Hart S, Lis-Balchin M, Hoult JRS, Evans P, Jenner P, Milligan S, et al., (2001). In-vitro activity of S. lavandulaefolia (Spanish sage) relevant to treatment of Alzheimer's disease. J. Pharm. Pharmacol. 53:1347-1356.

Perry NSL, Houghton PJ, Theobald A, Jenner P, Perry EK (2000). In-vitro inhibition of human erythrocyte acetylcholinesterase by Salvia lavandulaefolia essential oil and constituent terpenes. J. Pharm. Pharmacol. 52:895-902.

Rao AA, Sridhar GR, Das UN (2007). Elevated butyrylcholinesterase and acetylcholinesterase may predict the development of type 2 diabetes mellitus and Alzheimer's disease. Med. Hypotheses 69:1272-1276.

Rhee IK, van Rijn RM, Verpoorte R (2003). Qualitative determination of false-positive effects in the acetylcholinesterase assay using thin layer chromatography. Phytochem. Anal. 14:127-131.

Savelev S, Okello E, Perry NSL, Wilkins RM, Perry EK (2003). Synergistic and antagonistic interactions of anticholinesterase terpenoids in *Salvia lavandulaefolia* essential oil. Pharmacol. Biochem. Behav. 75:661-668.

Schroeter H, Williams RJ, Matin R, Iversen L, Rice-Evans CA (2000). Phenolic antioxidants attenuate neuronal cell death following uptake of oxidized low-density lipoprotein. Free Radic. Biol. Med. 12 1222-1233.

Szwajgier D (2009). Content of individual phenolic acids in worts and beers and their possible contribution to the antiradical activity of beer. J. Inst. Brew. 115:243-252.

Szwajgier D, Borowiec K (2012). Phenolic acids from malt are efficient acetylcholinesterase and butyrylcholinesterase inhibitors. J. Inst. Brew. 118:40-48.

Szwajgier D, Waśko A, Zapp J, Targoński Z (2007). An attempt to identify the low molecular feruloylated oligosaccharides in beer. J. Inst. Brew. 113:185-195.

Wang H, Zhou G, Gao X, Wang Y, Yao W (2007). Acetylcholinesterase inhibitory-active components of *Rhodiola rosea* L. Food Chem. 105:24-27.

Whitehouse PJ, Price DL, Clark AW, Coyle JT, DeLong MR (1981). Alzheimer's disease: evidence for selective loss of cholinergic neurons in the nucleus basalis. Ann. Neurol. 10:115-126.

Yabe T, Hirahara H, Harada N, Ito N, Nagai T, Sanagi T, Yamada H (2010). Ferulic acid induces neural progenitor cell proliferation in vitro and *in vivo*. Neuroscience 165:515-524.

Yan JJ, Cho JY, Kim HS, Kim KL, Jung JS, Huh SO, Suh HW, Kim YH, Song DK (2001). Protection against β-amyloid peptide toxicity *in vivo* with long-term administration of ferulic acid. Brit. J. Pharmacol. 133:89-96.