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Butyrylcholinesterase: K variant, plasma activity, molecular forms and rivastigmine treatment in Alzheimer's disease in a Southern Brazilian population

G.F. Bono [a,*], D.P. Simão-Silva [a], M.S. Batistela [a], N.D. Josviak [a], P.F.R. Dias [a], G.A. Nascimento [a], R.L.R. Souza [a], M.R. Piovezan [b], R.K.M. Souza [c], L. Furtado-Alle [a]

[a] Department of Genetics, Federal University of Paraná, Curitiba, Brazil
[b] Clinical Hospital of the Federal University of Paraná, Curitiba, Brazil
[c] Curitiba Neurology Institute, Curitiba, Brazil

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disorder in which there is a decline of cholinergic function. The symptomatic AD treatment involves the use of ChEIs (cholinesterase inhibitors) as rivastigimine, a dual inhibitor. The human butyrylcholinesterase (BChE) is an enzyme that has specific roles in cholinergic neurotransmission and it has been associated with AD. In the serum, BChE is found in four main molecular forms: G1 (monomer); G1-ALB (monomer linked to albumin); G2 (dimer); and G4 (tetramer). The interaction between the products of *BCHE* gene and *CHE2* locus results in *CHE2 C5+* and *CHE2 C5-* phenotypes. *CHE2 C5+* phenotype and BChE-K are factors that influence on BChE activity. This work aimed to verify the proportions of BChE molecular forms, total and relative activity in 139 AD patients and 139 elderly controls, taking into account *K* variant, *CHE2* locus, rivastigmine treatment and clinical dementia rating (CDR) of AD patients. Phenotypic frequencies of *CHE2 C5+* and frequency of the carriers of the *K* allele were similar between groups. Total BChE activity in plasma was significantly lower in AD patients than in elderly controls. Furthermore, we found that reduction on plasma BChE activity is associated directly with AD progression in AD patients and that rivastigmine treatment has a stronger effect on BChE activity within the CDR2 group. The reduction in BChE activity did not occur proportionally in all molecular forms. Multiple regression analysis results confirmed that AD acts as the main factor in plasma BChE activity reduction and that severe stages are related with an even greater reduction. These findings suggest that the reduction of total plasma BChE and relative BChE molecular forms activity in AD patients is probably associated with a feedback mechanism and provides a future perspective of using this enzyme as a possible plasmatic secondary marker for AD.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder and the most common cause of dementia. It is characterized pathologically by the presence of amyloid plaques and neurofibrillary tangles in the brain besides decline of cholinergic function. The cholinergic deficits in AD are strongly correlated with cognitive impairment (Roberson and Harrell, 1997) which led to the formulation of the "cholinergic hypothesis" (Davies and Maloney, 1976). This hypothesis states that the inability to transmit neurologic impulses across brain synapses is the cause of cognitive, global, and behavioral dysfunctions associated with dementia (Ladner and Lee, 1998).

Cholinesterases (ChE) are a family of enzymes that catalyze the hydrolysis of acetylcholine (ACh) into choline and acetic acid, an essential process for the restoration of the cholinergic neuron. There are two cholinesterase types: acetylcholinesterase (AChE; EC 3.1.1.7) and butyrylcholinesterase (BChE; EC 3.1.1.8). Both enzymes participate in cholinergic neutrotransmission by hydrolyzing acetylcholine in the central and peripheral nervous systems (Pohanka, 2011).

Based on cholinergic deficits in AD, cholinesterase inhibitors (ChEIs) are the first-line drugs in the symptomatic treatment of AD by inhibiting cholinesterase and thus resulting in increased synaptic levels of acetylcholine neurotransmitter. Currently the most prescribed ChEIs are donepezil, galantamine and rivastigmine. These drugs are commonly used to treat patients with mild-to-moderate AD (Qaseem et al., 2008) although Ferris et al.'s (2013) *post-hoc* analysis reinforces that rivastigmine may be an effective therapy in the treatment of severe AD. Individual ChEIs differ from each other with respect to their pharmacologic properties. Donepezil and galantamine

* Corresponding author. P.O. Box 19071, 81531-980; Curitiba, PR, Brazil. Tel.: +55 41 33611554; fax: +55 41 32662042.
E-mail address: gleysefb@gmail.com (G.F. Bono).

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are short-acting reversible competitive inhibitors, whereas rivastigmine is actively metabolized by cholinesterase, thus making it an intermediate-acting or 'pseudo-irreversible' inhibitor with an intermediate duration of action (Jann et al., 2002). Although the primary target of these agents is acetylcholinesterase (donepezil and galantamine), rivastigmine shows equal affinity for both AChE and BChE enzymes (Jann et al., 2002). These agents do not stop disease progression, but clinical studies have shown that they temporarily stabilize cognitive impairment and help to maintain global function, often delaying the need for patient placement in nursing homes by several months (Michaelis, 2003).

Human butyrylcholinesterase (BChE; EC 3.1.1.8) is the secondary acetylcholine (ACh)-hydrolyzing enzyme encoded by the *BCHE* gene (3q26.1-q26.2) (Arpagaus et al., 1990) and it is found in the neurofibrillary tangles and amyloid plaques of AD (Gómez-Ramos and Morán, 1997), which suggests that it functions as a potential modulator in the process. BChE is synthesized in the liver and distributed to several parts of the organism, including brain (Wescoe et al., 1947). The butyrylcholinesterase activity is increased in elderly brain (60–90 years) (Perry et al., 1978) and in AD patients' brain, mainly in the hippocampus and temporal cortex, suggesting a relationship with the loss of episodic memory in AD (Arendt et al., 1992; Perry et al., 1978) and cognitive decline in dementias (Perry et al., 2003), respectively. Acetylcholinesterase (AChE) activity, however, is reduced in AD brain and there was no correlation between age and AChE activity (Perry et al., 1978). Studies suggest that BChE may participate in the transformation of beta-amyloid (Aβ) from an initially benign form to an eventually malignant form associated with neuritic tissue degeneration and clinical dementia (Darvesh et al., 2011; Guillozet et al., 1997). However, other researchers found that BChE attenuates amyloid fibril formation and its presence in amyloid plaques implies that this enzyme incorporates into Aβ fibrils at a late phase of their formation (Diamant et al., 2006; Podoly et al., 2010).

In the serum, BChE is found in four molecular forms: G1 (monomer); G1-ALB (monomer linked to albumin); G2 (dimer) and the most common form, G4 (tetramer) (Masson, 1979). The interaction between BChE tetramer and a protein encoded by *CHE2* locus (2q33-35) results in a complex named C5 band, identified in electrophoresis only in 10.3% of a Southern Brazilian population (Chautard-Freire-Maia et al., 1991). The *CHE2 C5+* and *CHE2 C5−* phenotypes correspond to the presence and absence of the band, respectively (Harris et al., 1962). *CHE2 C5+* individuals have BChE activity approximately 30% higher than *CHE2 C5−* individuals (Chautard-Freire-Maia et al., 1991). Over 65 genetic variants were described for the *BCHE* gene (Souza et al., 2005), and the *K* variant (BChE-K; 1615A; rs1803274) is the most studied as a risk factor for AD and it was primarily associated with 33% reduction of BChE molecules in plasma (Rubinstein et al., 1978).

Considering the debate about the change in BChE expression and hydrolytic activity and the role on AD neuropathology, this work aims to verify the proportions of BChE molecular forms (G1, G1-ALB, G2 and G4), total and relative activities in AD patients and elderly controls, taking into account K variant and *CHE2* locus. Moreover, this study has the purpose to assess the total and molecular forms activity of butyrylcholinesterase in AD patients treated and not treated with rivastigmine at different stages of the disease.

2. Materials and methods

2.1. Sample

Blood samples were collected from 139 patients with Alzheimer's disease from the Clinical Hospital of the Federal University of Paraná (HC-UFPR) and Curitiba Neurology Institute (INC); and 139 elderly controls cognitively healthy, both groups constituted mainly of euro-Brazilian from Southern Brazil. The present work was designed as a case–control study and both groups were paired for sex, age and years of schooling. Other forms of dementia have been excluded in the patient group with AD. All subjects read, accepted and signed the term of informed consent. This study was previously approved by the ethical committee from HC-UFPR under registration 1192.117.11.08. The AD patients were diagnosed according to the NIA-AA (National Institute on Aging and Alzheimer's Association) criteria for probable AD (McKhann et al., 2011). Clinical Dementia Rating (CDR) originally developed by Hughes et al. (1982) and adapted by Morris (1993) was used to classify the degree of dementia in mild (CDR1), moderate (CDR2) and severe (CDR3). The mini-mental state examination (MMSE) is a screening test that was used to detect cognitive impairment in patients and controls. It was originally published by Folstein et al. (1975) and the Portuguese version used in the present study was developed by Bertolucci et al. (1994). The control group was screened by neuropsychological tests and submitted to an evaluation of impairment of daily activities, and then selected according to reference values for the corresponding age group.

2.2. Laboratory analysis

The samples were coded and processed to obtain the plasma. Plasma BChE activity was measured using propionylthiocholine as substrate at 25 °C as the protocol of Dietz et al. (1972) modified by Evans and Wroe (1978). *CHE2* locus phenotypes were identified by acid agar gel electrophoresis (pH: 6.50) (Van Ros and Vervoot, 1973). The detection of BChE bands in plasma was made according to Boberg et al. (2010). Relative Intensity (RI) of each band was measured using KODAK 1D Image Analysis Software produced by KODAK, and the relative activity (RA) of each band (G1, G1-ALB, G2 and G4) was the result of multiplication of total BChE plasma activity by the RI of each band detected in the polyacrylamide gel. DNA extraction was performed by a salting-out method (Lahiri and Nurnberger, 1991) and then diluted to a final concentration of 20 ng/μL. Genotyping of *K* variant (rs1803274) was obtained by TaqMan SNP Genotyping Assay produced by Life Technologies according to Simão-Silva et al. (2013).

2.3. Statistical analysis

Allele and genotype frequencies of the *K* variant and phenotype frequencies of the *CHE2* locus were obtained by direct counting and compared between groups with χ^2 test with assistance of the Clump program (Clump Sham and Curtis, 1995). Kolmogorov–Smirnov test was used to test for normality of variables distribution. The comparisons between means were performed by t-test (parametric variables) or by Mann–Whitney test (non- parametric variables). Multiple regression analysis was performed to evaluate the independent effect of variables. A 5% level of significance was adopted for all the statistical analyses performed.

3. Results

3.1. Sample characterization

Patients and elderly control characteristics are shown in Table 1. Women–men ratios were similar in patient and control group ($\chi^2 = 2.53$, $p = 0.11$). The patient group was a compound of 52 men and 87 women. In the rivastigmine treated patient group ($n = 37$), all individuals treated with this drug were included. In the no-rivastigmine treated group ($n = 99$), all patients who were not taking this drug, while under treatment with another type of ChEI or memantine, were included. The CDR groups analyzed were

Table 1
Means ± standard deviations (S.D.) for age, years of schooling and Mini Mental State Examination (MMSE) scores in patients and controls and *p* values for comparisons between means.

	Patients n = 139	Controls n = 139	p^2
	Mean ± S.D.	Mean ± S.D.	
Age[1]	72.16 ± 9.54	71.17 ± 8.08	0.37
Years of schooling	5.99 ± 5.08	7.13 ± 4.9	0.06
MMSE	14.33 ± 8.29	27.09 ± 2.39	0.00

[1] Patient's age was determined according to the age of onset of AD symptoms (n = 116).
[2] *t*-test for parametric variable; Mann–Whitney U test for non-parametric variable.

age-homogeneous (CDR1 = 71.2 ± 8.21; CDR2 = 72.3 ± 11.23; CDR3 = 72.2 ± 9.02).

3.2. Case–control study: AD patients × elderly controls

Table 2 shows that mean total BChE activity in plasma was significantly higher in elderly controls (6.45 KU/L ± 1.49) than in patients with AD (5.52 KU/L ± 1.54; t = 5.17, $p = 4.49 \times 10^{-7}$) as the relative mean activity of G4, G1-ALB and G1 bands. Mean total BChE activity and relative activity of G4 and G1 BChE bands remained significantly lower in patients than in controls even when only no-rivastigmine treated patients were considered (p = 0.0016; p = 0.0056; p = 0.0095, respectively). The reduction in BChE activity did not occur proportionally in all molecular forms, since the dimer showed no significant difference in activity in patients and controls.

Frequencies of loco *CHE2* phenotypes and of carriers of the *K* allele were similar between patients and controls ($\chi^2 = 0.46$, p = 0.50 and $\chi^2 = 0.63$, p = 0.43, respectively). The genotype distributions were in Hardy–Weinberg equilibrium.

Since mean total BChE activity was significantly higher in the control group than in the AD patient group, a multiple regression analysis was conducted considering AD presence, sex, *CHE2* phenotype, *K* variant presence and rivastigmine treatment as independent variables and total BChE activity as the dependent variable. These results confirmed that AD and *K* variant are independent factors for decreasing total BChE activity in plasma ($\beta = 0.29 \pm 0.06$, $p = 2.43 \times 10^{-6}$; $\beta = 0.19 \pm 0.06$, p = 0.0018, respectively).

Whereas significant difference was found between the means of molecular forms activity in patients and controls, it was conducted another regression analysis considering each molecular form as a dependent factor. Thus, about G1 and G1-ALB, both AD and *K* variant act as independent factors in the decrease of activity. On the other hand, considering G4 as the dependent variable, only AD is a significant factor in the decrease of tetramer activity ($\beta = 0.28 \pm 0.06$, $p = 7.09 \times 10^{-6}$). Regarding the dimer, only *K* variant acts in the decrease of this molecular form activity ($\beta = -0.17 \pm 0.06$, p = 0.0053).

Table 2
Means ± standard deviations (S.D.) and *p* value of total BChE activity (ATV) and relative activity of BChE bands (G4, G2, G1-ALB and G1) in patients and controls.

	Patients n = 139	Controls n = 139	*p* (*t*-test)
	Mean (KU/L) ± S.D.	Mean (KU/L) ± S.D.	
ATV	5.52 ± 1.54	6.45 ± 1.49	4.49×10^{-7}
G4	3.24 ± 0.93	3.78 ± 1.06	9.17×10^{-6}
G2	0.77 ± 0.36	0.84 ± 0.54	0.1100
G1 – ALB	0.73 ± 0.38	0.87 ± 0.39	0.0028
G1	0.78 ± 0.45	0.97 ± 0.49	0.0008

Table 3
Means ± standard deviations (S.D.) of MMSE scores, total BChE activity (ATV) (KU/L) and relative activity of BChE bands (G4, G2, G1-ALB and G1) (KU/L) within each CDR patient group.

	CDR1 n = 48	CDR2 n = 47	CDR3 n = 41
	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
MMSE	21.23 ± 4.79	15.11 ± 5.64	5.37 ± 5.30
ATV	5.88 ± 1.39	5.54 ± 1.63	5.11 ± 1.56
G4	3.39 ± 0.93	3.33 ± 1.02	2.94 ± 0.77
G2	0.79 ± 0.32	0.74 ± 0.40	0.79 ± 0.38
G1-ALB	0.77 ± 0.35	0.73 ± 0.41	0.70 ± 0.38
G1	0.93 ± 0.47	0.74 ± 0.41	0.67 ± 0.46

3.3. Patients study: rivastigmine treated × no-rivastigmine treated and AD progression

Table 3 shows means of MMSE score, total BChE activity and relative activities of BChE bands (G4, G2, G1-ALB and G1) in each CDR group. The means of MMSE score were significantly different between CDR1 and CDR2 ($t = 5.71$; $p = 1.37 \times 10^{-7}$); CDR1 and CDR3 ($t = 14.83$; $p = 1.5 \times 10^{-25}$) and CDR2 and CDR3 ($t = 8.31$; $p = 1.23 \times 10^{-12}$). Means of ATV were significantly different only between CDR1 and CDR3 (t = 2.47; p = 0.015) and the same was true for means of relative activity of G4 (t = 2.47; p = 0.016) and G1 (t = 2.61; p = 0.011).

Mean total BChE activity and mean relative activity of G4, G2 and G1-ALB BChE bands were significantly higher in the no-rivastigmine treated patients than in the rivastigmine-treated patients. The means of MMSE scores were not different between rivastigmine-treated and no-rivastigmine treated patients (Table 4).

There were no significant differences in total BChE activity and mean MMSE scores between rivastigmine-treated and no-rivastigmine treated patients in CDR1 and CDR3 groups. However, in the CDR2 group, the mean total BChE activity was significantly lower in the rivastigmine-treated patients (4.28 KU/L ± 1.19) than no-rivastigmine treated patients (6.07 KU/L ± 1.50; t = 3.98, p = 0.0003) (Table 5) as the relative activities of all BChE molecular forms (Table 6).

Since mean total BChE activity was different among CDRs groups and between rivastigmine-treated and no-rivastigmine treated patients, a multiple regression analysis was conducted considering CDR, sex and rivastigmine treatment as independent variables and total BChE activity as the dependent variable. This analysis confirmed that CDR acts as an independent factor in the decrease of total plasma BChE activity ($\beta = 0.21 \pm 0.09$, p = 0.0259). Considering G4 and G1 as dependent variables, similar results were obtained.

Table 4
Means ± standard deviations (S.D.) of total BChE activity (ATV), relative activity of BChE bands (G4, G2, G1-ALB and G1) and MMSE score in rivastigmine-treated patients (RVG) and no-rivastigmine treated patients (No-RVG).

	RVG n = 37*	NO-RVG n = 99*	*p* (*t*-test)
	Mean (KU/L) ± S.D.	Mean (KU/L) ± S.D.	
MMSE	14.27 ± 8.18	14.47 ± 8.34	0.90
ATV	4.76 ± 1.52	5.84 ± 1.45	0.0002
G4	2.82 ± 0.85	3.41 ± 0.92	0.0009
G2	0.64 ± 0.30	0.82 ± 0.37	0.0067
G1 – ALB	0.59 ± 0.35	0.79 ± 0.38	0.0054
G1	0.72 ± 0.54	0.82 ± 0.42	0.2600

* In the "Patients study", the total number of patients was 136 because there was no information on the treatment for all 139.

Table 5
Means ± standard deviations (S.D.) of MMSE and total BChE activity (ATV) in rivastigmine-treated patients (RVG) and no-rivastigmine treated patients (No-RVG) within each CDR group.

	CDR1 n = 48*		CDR2 n = 47*		CDR3 n = 41*	
	ATV (KU/L)	MMSE	ATV (KU/L)	MMSE	ATV (KU/L)	MMSE
RVG	5.51 ± 1.36	22.5 ± 4.97	4.28 ± 1.19	15.71 ± 3.89	4.65 ± 1.88	5.75 ± 5.59
No-RVG	6.00 ± 1.41	20.92 ± 4.82	6.07 ± 1.50	14.85 ± 6.28	5.39 ± 1.39	5.18 ± 5.24
p (*t*-test)	0.3352	0.3650	0.0003	0.6356	0.1768	0.7625

* In the "Patients study", the total number of patients was 136 because there was no information on the treatment for all 139.

Table 6
Means ± standard deviations (S.D.) of relative activity of the BChE bands (G4, G2, G1-ALB AND G1) in rivastigmine-treated patients (RVG) and no-rivastigmine treated patients (No-RVG) within the CDR2 group. All BChE bands activities are presented in KU/L.

	CDR2 n = 47		
	RVG n = 14	No-RVG n = 33	*p* (*t*-test)
G4	2.82 ± 0.88	3.55 ± 1.01	0.0235
G2	0.46 ± 0.27	0.86 ± 0.38	0.0009
G1- ALB	0.46 ± 0.21	0.84 ± 0.43	0.0023
G1	0.55 ± 0.28	0.83 ± 0.43	0.0323

4. Discussion

The evidence of the *BCHE* knockout mouse has an essentially normal phenotype (Li et al., 2000; Mesulam et al., 2002) and that numerous human BChE mutations, many that silence the enzyme with minimal physiological effect, suggest that the enzyme may be redundant. However, according to Johnson and Moore's (2012) review, structurally and functionally, BChE is neither a vestigial nor a degenerate AChE, but a unique enzyme with detoxification and synaptic efficiency. Phylogenetic analysis of BChE and AChE expression indicates that these two enzymes have emerged from a common precursor whose function was to hydrolyze acetylcholine. Therefore, the *ACHE* and *BCHE* genes arose by gene duplication after the emergence of cholinergic systems (Chatonnet and Lockridge, 1989; Hall and Spierer, 1986; Pritchard et al., 1994; Toutant, 1989). Thus, *BCHE* appears to be a good example of a gene that has survived by subfunctionalization, the proposal in which two genes, original and duplicate, split the functions of the original gene between them (Johnson and Moore, 2012).

It is noteworthy that BChE is found in the central and peripheral nervous systems and it is the major ACh hydrolyzing enzyme in plasma (Lampón et al., 2012) which is virtually free of AChE (Brimijoin and Hammond, 1988). BChE levels in the body exceed those of AChE in all tissues except muscle and brain (Li et al., 2000). The human body contains ten times more molecules of BChE than AChE (Manoharan et al., 2007). In the Alzheimer's disease, acetylcholinesterase is lost up to 85% in specific brain regions, whereas butyrylcholinesterase levels, chiefly the G1 form, rise with disease progression (Arendt et al., 1992; Perry et al., 1978).

Our results showed that there is a reduction in total plasma BChE activity in AD patients when compared to elderly controls (Table 2). Furthermore, we also found that there is a reduction in the total plasma BChE activity with the AD progression as well as in the MMSE scores in AD patients. The decrease in total BChE activity was particularly significant between mild (CDR1) and severe AD stage (CDR3) patient groups (Table 3). Interestingly, the regression analysis results showed that AD is the main factor that influences the BChE activity in the case–control study, and the stage of disease (CDR) is the main factor that acts further reducing the plasma activity of this enzyme among patients. This fact highlights that Alzheimer's disease is the first element that alters the homeostasis of the peripheral BChE activity while the evolution of the disease is the second one that exacerbates this decline in patients.

These results may be explained by BChE kinetic response to concentrations of ACh. It is known that BChE is less efficient in ACh hydrolysis at low concentrations but highly efficient at higher ones, a situation in which AChE becomes substrate inhibited (Silver, 1974). Considering that AD is characterized by a cholinergic neuron loss and a progressive decline in acetylcholine (ACh) levels, the peripheral higher BChE activity becomes unnecessary in this condition, remaining at lower levels by a feedback mechanism. This hypothesis is supported by the fact that the activity of this enzyme is reduced peripherally with disease progression while high levels are progressively found in the brain with AD. According to Giacobini et al. (2002), the absolute levels of BChE activity in plasma and CSF (cerebrospinal fluid) were not significantly associated. This suggests that the BChE activity and its changes measured in CSF of AD patients does not have its origin from plasma, but most likely from the brain, presumably as a consequence of the higher BChE activity found in the brain of AD patients.

Additionally, we found that the decrease in the activity of BChE in patients was not homogeneously distributed to all molecular forms. We observed a significant reduction in G4, G1 and G1-ALB relative activities, but this did not occur with the G2 band (Table 2). Similarly, we found that this reduction was also not homogeneous when considering disease progression in patients. In this case, there were no significant reductions in the relative activity of G2 and G1-ALB between CDR1 and CDR3 patient groups (Table 3). These results differ from those of Boberg et al. (2010) and Silva et al. (2012) who reported a homogeneous reduction of all molecular forms, in a case–control study with obese and in a physical exercise intervention study with obese adolescents, respectively. Considering the different results found in the present study, it is possible that AD interferes in the regulation of proportionality between the relative activities of BChE molecular forms, which may further affect the role of this enzyme in the peripheral cholinergic system in AD progression.

Whereby BChE activity appears to be involved in the transformation of Aβ plaques from a benign diffuse state to the compact malignant form (Guillozet et al., 1997), non-selective inhibition may therefore help to slow down the formation of these plaques in AD patients' brain (Giacobini, 2000). Evidence suggests that inhibition of both AChE and BChE by rivastigmine may be beneficial in treating the cognitive decline of AD (Giacobini et al., 2002) and might have the potential to provide the greatest long-term benefits (Darreh-Shori and Soininen, 2010).

Activities of AChE in CSF and of BChE in plasma and CSF are stronglyinhibited by rivastigmine, and this inhibition is associated with improved cognitive performance (Giacobini et al., 2002). Similarly, the Darreh-Shori et al. (2002) study verified that rivastigmine causes persistent inhibition of AChE and BChE in CSF (by 36% for AChE and 45% for BChE) as well as plasma (27% for AChE and 33% for BChE) in eleven patients with mild AD. We found that patients

treated with rivastigmine have total BChE and G4, G2 and G1-ALB activities significantly lower compared with patients not treated with this inhibitor (Table 4), confirming the action of this drug on the plasma BChE activity.

We found a significant reduction in plasma BChE activity in the CDR2 group rivastigmine treated patients when compared with no-rivastigmine treated ones (Table 5). Similar results occurred with the relative activity of all the molecular forms of BChE in the CDR2 group (Table 6). These findings suggest that a stronger rivastigmine effect is observed in patients with moderate AD than in mild and severe stage patients. The peripheral BChE inhibition was not correlated with changes of performance in speed/attention- and memory-related tasks (Giacobini et al., 2002) but the common side effects (nausea, gastrointestinal upset, and diarrhea) observed due to ChEIs acting in the peripheral nervous system are most notable for rivastigmine (Bullock et al., 2006; Casey et al., 2010). Also, our results show that the side effects may be intensified in the CDR2 patient group just because the rivastigmine peripheral action seems to be more intense than in CDR1 and CDR3 groups. The reduction in BChE activity observed in the moderate phase is a relevant clinical finding, whereas the cholinergic signaling is involved in peripheral homeostasis through activation of the parasympathetic system and mediating both neuromuscular and inflammatory responses (Ofek and Soreq, 2013).

The BChE-K (K variant) is characterized by the substitution A539T in the tetramerization domain of the enzyme. Primarily, it was associated with 33% reduction of BChE molecules in plasma (Rubinstein et al., 1978) and with a 30% decreased capacity of hydrolyzing butyrylthiocholine (Bartels et al., 1992). However, Altamirano et al. (2000) reported that BChE-K demonstrates no apparent differences from wild-type BChE and this mutation does not affect hydrolytic activity neither tetramer formation. According to Podoly et al. (2009) BChE-K is inherently unstable and shows impaired quaternary organization when compared with the wild-type, resulting in reduced hydrolytic activity and predicting prolonged acetylcholine maintenance and protection from AD. However, other findings demonstrated that K variant was considerably less effective in attenuating the accumulation of Aβ fibrils than BChE wild-type (Diamant et al., 2006). Thus, this variant may pose either a risk or a protective factor in AD (Podoly et al., 2009). This study found no association between the K variant and AD, similar to the study of Simão-Silva et al. (2013) in a southeastern Brazilian population. The debate of the BChE function on AD neuropathology was accompanied by several studies that aimed to verify association between BChE-K and Alzheimer's disease. Some of them found the BCHE-K was associated with AD (Lehmann et al., 1997; McIlroy et al., 2000; Raygani et al., 2004; Tilley et al., 1999; Wiebusch et al., 1999) and others found no association (Alvarez-Arcaya et al., 2000; Ki et al., 1999; Singleton et al., 1998). Bizzarro et al. (2010), in turn, suggest a protective effect of K variant, since the authors found it in a lower frequency in AD patients when compared to healthy controls and to fronto-temporal dementia (FTD) patients. In contrast to Podoly et al. (2009), in the present study it was verified that the K variant acts by reducing G2, G1 and G1-ALB relative activities and not the tetramer (G4), which suffered greater influence of AD itself.

In conclusion, we demonstrated that there is lower total plasma BChE activity in AD patients than in elderly controls and that the reduction on plasma BChE activity is associated directly with AD progression. Thus, this work shows evidence that AD acts as a main factor in lowering plasma BChE activity and that severe stages are related with an even greater reduction. Another interesting finding was the fact that rivastigmine showed a stronger effect in reducing peripheral BChE activity in patients with moderate AD, which are, therefore, more likely to suffer side effects. Furthermore, we observed that the reduction of relative activity of BChE molecular forms was not homogeneous. The failure to maintain proportionality between molecular forms of BChE in AD may suggest that the disease interferes in the homeostasis regulation and may further affect the role of this enzyme in the peripheral cholinergic system. We propose that plasma BChE activity may be used as a secondary marker in AD, and that periodic measurement of peripheral BChE activity may be useful for evaluating AD progression, especially as this is an assessment that lacks side effects.

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