MULTIVARIATE STUDY OF ACHEIS MOLECULES
Mapping Pharmacophoric Profile of AChEIs Via PCA

Érica Cristina Moreno Nascimento and João Batista Lopes Martins
Laboratório de Química Computacional, Universidade de Brasília, Campus Universitário Darcy Ribeiro, Brasília, Brazil

Keywords: Alzheimer disease, AChE Inhibitors, DFT, Multivariate analysis, PCA.

Abstract: Alzheimer's disease (AD) is a degenerative dementia. The causes of AD are not well determined, and the most popular strategy for AD treatment is the cholinergic hypothesis, that consists in the use of drugs with an inhibitory effect on acetylcholinesterase (AChE) enzyme, to prevent the decrease on the neurotransmitter (acetylcholine) concentration in synaptic clefts. Structural, electronic and spatial parameters of 10 drugs with known inhibitory effect on AChE (AChEI) were determined. The parameter values were obtained by means of calculations at B3LYP/6-31+G(d,p) level. The multivariate analysis of principal components (PCA) method was applied to 18 parameters to determine the pharmacophoric profile. PCA study was performed to reduce the sample space of properties and get the ones that are major AChEI components.

1 INTRODUCTION

Alzheimer's disease (AD) is the most common type of dementia in population over 60 years old (Pivetta, 2008; Sugimoto, 2002; Sippl, 2001). It is a progressive and degenerative disease (Alcaro, 2002).

In AD patients, the concentration of acetylcholine (ACh) neurotransmitter is markedly reduced in the transmission of neural impulses. This occurs due to the small production of ACh in neurons, causing the cholinergic deficit, where the cognitive function remains severely impaired (Francis, 1999). Based on this information, it was suggested the cholinergic hypothesis. The hypothesis consists of applying drugs that can bring benefits and significant improvement on AD patient cognitive functions. (Camps, 2002; Sugimoto, 2002).

The acetylcholinesterase inhibitors (AChEI) are currently the main strategy for the treatment of AD patients in a cholinergic therapy (Sugimoto, 2002). Some AChE inhibitors act by competing with ACh, others inhibit the OH group acylation of amino-acid residue Ser200, forming a carbamoyl ester, more stable than the acetate and less able to leave the enzyme active site (GORGE) (Alcaro, 2002).

Some drugs act as inhibitors of AChE, shown in Figure 1, among them tacrine (THA), first drug approved by FDA for the AD treatment (Proctor, 2000; Sugimoto, 2002), followed by donepezil (E2020), rivastigmine (RIVA) and galantamine (GALA) (Racchi, 2004). Some have been studied and tested clinically for AD treatment, such as physostigmine (PHYSO), and others are in testing phase and are promising candidates to approval, including huperzine A (HUPE), metrifonate (METRI), dichlorvos (DDVP), phenserine (PHEN) and the tacrine dimer (DIMTHA) (Racchi, 2004; Camps, 2002; Kaur, 2000). These drugs, are indicated for the treatment in mild to moderate stages, when the patient still has independent cognitive activity.

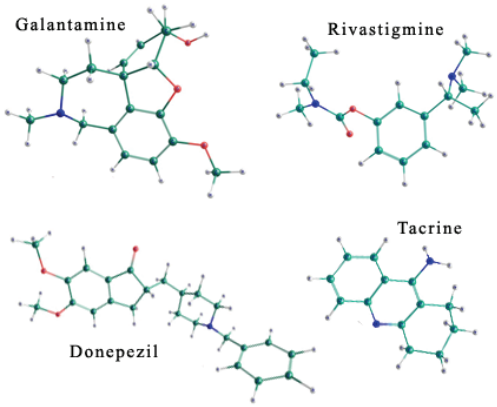

Figure 1: Some AChEI molecules structures.

Biochemically, these AChEI molecules have in common the inhibitory action on the AChE.

Cristina Moreno Nascimento É. and Batista Lopes Martins J. (2010).
MULTIVARIATE STUDY OF ACHEIS MOLECULES - Mapping Pharmacophoric Profile of AChEIs Via PCA.
In *Proceedings of the First International Conference on Bioinformatics*, pages 245-249
DOI: 10.5220/0002745602450249

However, the chemical class (pharmacophoric groups) and structures involved in such molecules have non similar chemical characteristics. Therefore, quantum mechanical calculations to find electronic structure properties were needed to determinate the common and relevant properties that these molecules are sharing.

In this study the structural and electronic properties of acetylcholinesterase enzyme inhibitors were theoretically obtained and related to their activity by multivariate analysis by seeking the principal components (PCA), which were used to establish the pharmacophoric profile of those drugs.

Multivariate analysis is a powerful tool to investigate candidates to AChE inhibitors. PCA was used to determinate significant properties in classical acetylcholinesterase inhibitors (Nascimento, 2008). PCA was also applied to propose promising new candidates, in order to produce potential active AChEI molecules for the DA treatment (de Paula et al., 2009).

This study aims to obtain pharmacophoric profile of AChEI molecules and to contribute to the development of new inhibitors of AChE. We are interested in provide insights for new descriptors of known AChE inhibitor molecules in order to contribute to the understanding how these drugs are correlated to each other using PCA chemometrics method.

2 COMPUTATIONAL DETAILS

Density functional theory (DFT) at B3LYP hybrid functional level was applied in this study. The 6-31+G(d,p) basis set was used. The geometries of target drugs were optimized using internal coordinates. The theoretical calculations were performed using Gaussian03 (Frisch, 2003) program, in order to determine the best electronic and geometrical parameters. OSIRIS (Sander, 2001) program was used to determine the logP and logS parameters. PCA study was carried out using MATLAB® (Rahman, 2009) program.

Pharmacophoric profile of AChEI molecules was acquired using the PCA multivariate statistical method. This method was used to correlate the studied AChEI molecule properties and their inhibitory activity, as well as to reduce the initial set of parameters (electronic and structural properties), generating the most relevant ones. The eighteen parameters used in the PCA analysis were: dipole, HOMO, HOMO-1, LUMO and LUMO+1 energies, heteroatom charge, hydrogen charge of most acid atom, molecular volume, H-H distance, partition coefficient logP, logS, number of hydrogen receptors and donors (H_{recp} e H_{don}), number of aromatic rings, (LUMO-HOMO) GAP, molecular size (largest intramolecular distance), rotation degrees freedom and Polar Surface Area (PSA), using the optimized geometries.

2.1 PCA Method

PCA is a major technique employed in chemometric analysis to study a set of multivariate data (Mizutani, 2004). PCA method has two aims: the decrease of variable sets, and the selection of the best properties linearly independent to represent a system (principal components).

As a general example, we have a chemical experiment performed with ***m*** numbers of molecules resulting in ***n*** numbers of properties. Studies using PCA data processing (**D**) is performed by considering the ***n*** numbers of variables (properties) system on ***m*** numbers of objects (molecules). Then, the generated matrix D (Figure 2) consists of ***m***x***n*** elements. The j-th variable is represented by a column vector and the i-th object is represented by a row vector, also called the response vector, and can be described by a point in n-dimensional space (Anderson, 1984).

$$\mathbf{D} = \begin{bmatrix} d_{11} & d_{12} & d & \cdots & d_{ij} & \cdots & d_{1n} \\ d_{21} & d & d & \cdots & d & \cdots & d \\ \vdots & & & & & & \\ d_{m1} & \cdots & \cdots & \cdots & \cdots & \cdots & d_{mn} \end{bmatrix}$$

Figure 2: ***mxn*** matrix of data set.

The aim of PCA study is to explain the variance and covariance structure of an aleatory vector of variables, using a linear combination of original set yielding the principal components (PCs) (Anderson, 1984).

The PCs can be seen as axes of maximum distribution of objects. We can visualize the layout of objects in these new sets of axes. The layout formed by the projection of these objects in the main components is called the graph of scores. Its coordinates are derived from the product of the data matrix by the matrix of eigenvectors. If the first two or three eigenvectors explain a significant amount of the total variance, a plot of scores are the

coordinates that are accurate in many dimensions of the larger original space (Gnanadesikan, 1997; Marriot, 1974).

PCA study was conducted using the auto-stepping method, since the structural and electronic properties have different dimensions.

3 RESULTS AND DISCUSSIONS

Table 1 shows the most relevant properties of the AChEI optimized geometries at B3LYP/6-31+G(d,p) level. PCA cumulative variance using four principal components, PC1, PC2, PC3 and PC4, are 38.3, 59.2, 73.2 and 83.3%, respectively, which are significant for the whole set.

Table 1: Most relevant properties of AChEI molecules at B3LYP/6-31+G(d,) level.

AChEI	Volume ($Å^3$)	Size (Å)	H-H (Å)	HOMO-1 (eV)	logP	Aromatic rings
DDVP	185	7.849	1.796	-8.57	1.66	0
DIMTHA	606	19.386	1.998	-6.71	3.88	4
E2020	454	12.254	2.342	-6.07	4.14	3
GALA	329	10.29	2.359	-6.03	1.39	2
HUPE	286	9.051	1.625	-6.75	2.60	1
METRI	207	7.068	2.307	-8.30	0.80	0
PHEN	391	14.808	2.491	-6.07	2.99	1
PHYSO	321	12.927	2.319	-6.08	1.94	2
RIVA	312	11.242	2.460	-6.53	2.86	1
THA	236	9.516	1.683	-6.56	3.13	2

To increase accuracy and to determine the most relevant properties a systematic study was carried out for all possible combinations of 18 properties for all 10 drugs.

The variables number was reduced to 6 (described below), nevertheless keeping the sample space of 10 objects (AChE): molecular volume, molecular size, H-H distance, HOMO-1 energy, partition coefficient logP and number of aromatic rings. The criterion for reducing the variables number to six was to improve the cumulative variances percent using combinatorial analysis of the 18 properties of 10 drugs.

The information that describes the drugs as AChEI molecules may be represented by three principal components. Figure 3 depicts PC1xPC2 scores. PC1 (with 63.5% of variance) x PC2 (accounting for 19.1% of variance) x PC3 (9.1% of variance), satisfactorily account for more than 90% of the variance of the entire data set.

Equations 01, 02 and 03 show the calculated PC1, PC2 and PC3 coefficients, respectively. The PC1 was represented essentially by the drug volume and size (structural parameters). For PC2 the most relevant are the H-H distance and HOMO-1 orbital energy (structural and electronic parameters), while PC3 is mainly represented by the H-H distance.

All 6 properties listed are positive in PC1, i.e., the 6 properties contributes in the first principal component. 62% of the variance is represented by the PC1 (Equation 01).

$$PC1(\%) = 24_{Volume} + 22_{Size} + 2_{H\text{-}H} + 14_{HOMO\text{-}1} + 17_{logP} \quad (1)$$

$$PC2(\%) = 0.1_{Volume} + 0.1_{Size} + 78_{H\text{-}H} + 8_{HOMO\text{-}1} - 11_{logP} \quad (2)$$

$$PC1(\%) = 14_{Volume} - 17_{Size} - 3_{H\text{-}H} + 63_{HOMO\text{-}1} + 3_{logP} \quad (3)$$

Figure 3 shows that PC1 tends to cluster the AChEI molecules by the six selected properties. It follows the formation of groups of well-defined AChE molecules, four of the AChEI molecules, already approved by FDA for AD group treatment, form a cluster, GALA, RIVA, PHYSO and PHEN. The distribution along PC1 is satisfactory, since the AChE with similar molecular volume are considerable closer to each other: GALA/ RIVA/ PHYSO/ PHEN and HUPE/ THA. AChEI molecules with smaller molecular volumes are in negative scores region, the AChEI with molecular volumes between 236 and 606 $Å^3$ are in regions of positive scores.

On the other hand, PC2 is dominated by the H-H distance (+0.88224), which separates compounds into two groups according to the distance between the two most acid hydrogens: the first group is found within values of H-H less than 2.0 Å . The second group is within values higher than 2.0 Å distances. There is a clear separation between the groups, Table 1 shows that the E2020 has intermediate values for all properties, except for logP, which has negative coefficient and is the dispersion element of PC2.

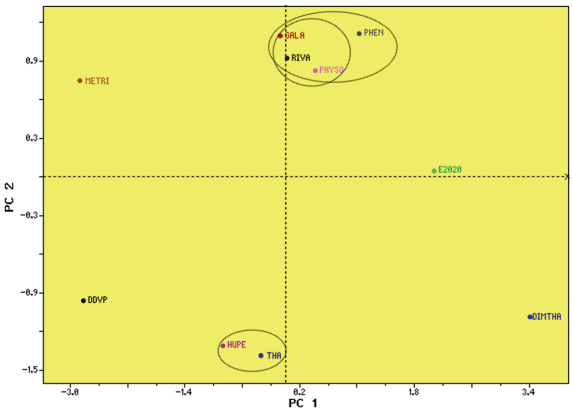

Figure 3: PC1 versus PC3 scores at level B3LYP/6-31+G(d,p).

Figure 4 shows PC1 versus PC3 scores plot. There are two patterns in PC3, i.e., DDVP / METRI and GALA / PHYSO / RIVA. Which can be explained, since PC3 is dominated by the orbital energy of HOMO-1 (+0.79272) (see Equation 3). These molecules have values close to the HOMO-1 and the molecular volume (Table 1).

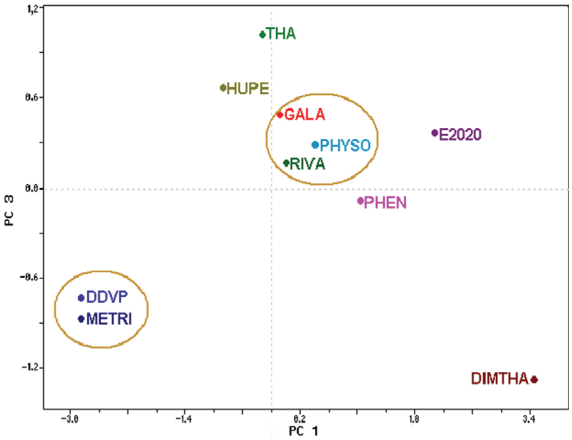

Figure 4: PC1 versus PC3 scores at level B3LYP/6-31+G(d,p).

As can be seen in Figures 3 and 4, the equations generated in PCs indicate that the electronic properties are the most significant in the AChEI molecules study, such as energy of HOMO-1. The structural parameters that also contribute are: molecular volume, size of the drug and H-H distance.

4 CONCLUSIONS

The PCA study showed that electronic property, – HOMO-1 orbital energy, logP, numbers of aromatic ring, and structural parameters - volume, drug size and H-H - are the most significant properties, i.e., the principal components of the AChEI drugs pharmacophforic profile.

Thus, it is estimated that a good candidate to inhibit the acetylcholinesterase enzyme must includes: partition coefficient values between 0.8 and 4.9; logS between -5.0 and -1.5; polar surface area between 30.0 and 60.0 $Å^2$. The torsional degrees number of freedom sufficient to be able to rearrange itself adequately inside the AChE active site is also important. Other desirable features for the AChEI molecules are: preferably aromatic systems or groups that simulate surface electron density of aromatic systems; sufficient amount of hydrogen acceptors and few donors of hydrogen. Furthermore, according to B3LYP/6-31+G(d,p) level results the inhibitor should have: HOMO-1 orbital energy between -8.60 and -6.00 eV; and the distance among the two more acidic hydrogens molecule between 1.600 – 2.500 Å. Together, all these properties participate in the pharmacophoric profile of the studied AChEIs molecules.

ACKNOWLEDGEMENTS

This study is supported by CNPq, and Funpe/UnB. We also acknowledge the computational resource of CENAPAD/SP.

REFERENCES

Alcaro, S., Scipione, L., Ortuso, F., Posca, S., Rispoli, V. & Rotiroti, D. (2002). Molecular modeling and enzymatic studies of the interaction of a choline analogue and acetylcholinesterase. *Bioorganic & Medicinal Chemistry Letters, 12, 2899-2905.*

Anderson, T. W. 1984. *An Introduction to Multivariate Statistical Analysis*. John Wiley & Sons. New York, 2nd edition.

Camps, P. (2002). Cholinergic Drugs in Pharmacotherapy of Alzheimer's Disease. *Mini Reviews in Medicinal Chemistry*, 2, 11-25.

de Paula A.A.N., Martins J.B.L., dos Santos, M.L., Nascente, L. de C., Romeiro, L.A.S., Áreas, T.F.M.A., Vieira, K.S.T., Gambôa, N.F., Castro, N.G., Gargano, R. (2009). New potential AChE inhibitor candidates. *European Journal of Medicinal Chemistry, 44, 3754–3759.*

Francis, P. T. (1999) The cholinergic hypothesis of Alzheimer's disease: a review of progress. *J Neurosurg Psychiatry*, 66, 137-147.

Frisch, M. J., G.W. Trucks, H.B.S., G.E. Scuseria, M.A. Robb, J.R.C., Zakrzewski, V.G., Montgomery, Jr. J.A., Stratmann, R.E., Dapprich, J.C.B.S., Millam, J.M., Daniels, A.D., Strain, K.N.K.M.C., Farkas, O., Tomasi, J., Barone, V., Cammi, M.C.R., Mennucci, B., Pomelli, C., Adamo, C., Clifford, S., Petersson, J.O.G.A., Ayala, P.Y., Cui, Q., Morokuma, K., Rabuck, D.K.M.A.D., Raghavachari, K., Foresman, J.B., Ortiz, J.C.J.V., Stefanov, B.B., Liu, G., Liashenko, A., Komaromi, P.P.I., Gomperts, R., Martin, R.L., Fox D.J., Al-Laham, T.K.M.A., Peng, C.Y., Nanayakkara, A., Gonzalez, C., Challacombe, M., Gill, P.M.W., Johnson, B., Chen, W., Wong, M.W., Andres, J.L., Gonzalez, C., Head-Gordon, M., Replogle E.S. And Pople, J.A. (2003). Gaussian03 (Rev. A11) Pittsburgh, PA, Gaussian Inc.

Gnanadesikan, R. 1997. *Methods for statistical data analysis of multivariate observations*. John Wiley & Sons. New York, 2nd edition.

Kaur, J. & Zhang, M. Q. (2000). Molecular modelling and QSAR of reversible acetylcholinesterase inhibitors. *Current Medicinal Chemistry*, 7, 273-294.

Marriott, F. H. C. 1974. *Interpretation of multiple observations*. Academic Press. London, 1st edition.

Nascimento, É. C. M.; Martins, J. B. L.; Santos, M. L.; Gargano, R. (2008). Theoretical study of classical acetylcholinesterase inhibitors. *Chemical Physics Letters, 458, 285-289*.

Pivetta, M. (2008). Na raiz do Alzheimer. *Pesquisa Fapesp*. 21, 16-21.

Racchi, M., Mazzucchelli, M., Porrello, E., Lanni, C. & Govoni, S. (2004). Acetylcholinesterase inhibitors: novel activities of old molecules. *Pharmacological Research*., 50, 441-451.

Rahman, O. M., J., Foster, A. & Gertler, P. (1994-2009) MATLAB. U.S. Patent Nos. 6,857,118; 6,973,644; 6,993,772; 7,010,364; 7,051,333; 7,051,338; 7,096,154; 7,139,686; 7,165,253; 7,181,745; 7,228,239; 7,231,631; 7,237,237; 7,340,441; 7,353,502; 7,359,805; 7,365,311; 7,369,127; 7,400,997; 7,428,737; 7,454,659; 7,454,746; 7,460,123. USA.

Sander, T. (2001). OSIRIS Property Explorer. Actelion Pharmaceuticals Ltd, Gewerbestrasse 16, 4123 Allschwil, Switzerland.

Sippl, W., Contreras, J. M., Parrot, I., Rival, Y. M. & Wermuth, C. G. (2001). Structure-based 3D QSAR and design of novel acetylcholinesterase inhibitors. *Journal of Computer-Aided Molecular Design*. 15, 395-410.

Sugimoto, H., Ogura, H., Arai, Y., Iimura, Y. & Yamanishi, Y. (2002)**.** Research and development of donepezil hydrochloride, a new type of acetylcholinesterase inhibitor. *Japanese Journal of Pharmacology*, 89, 7-20.