ORIGINAL PAPER

QUANTUM CHEMISTRY IN EVALUATION OF ANTAGONISM IN PLATELET AGGREGATION BETWEEN ADRENALINE AND CLOPIDOGREL

EMILIA AMZOIU[1], MANUEL AMZOIU[1], RODICA SIRBU[2], GABRIELA STANCIU[3*], MATI ERAND[4], ROXANA SANDULOVICI[4]

Manuscript received: 03.12.2017; Accepted paper: 22.02.2018; Published online: 30.03.2018.

Abstract. *Drug-platelet agregation receptor interaction of pro and antiaggregants is only possible for a certain distribution of electron density in the molecule that generates electron-rich regions. Molecular electrostatic potential can be used to assess the cuantomolecular lipophilicity, because it characterizes the polarity of a certain region on the van der Waals surface of the molecule. Energy difference between the levels HOMO and LUMO is explaining the stability of the molecule, a low value indicating that the molecule is highly reactive, the most stable molecule is adrenaline. The tendency of donation - acceptance of electrons is described by electronegativity. Greater electronegativity is presented by thiol active metabolite of clopidogrel explaining bigger reactivity of this substance. In case of antagonism between proaggregant adrenaline and antiaggregant clopidogrel it was found that there is not an interaction between the two molecules but only competition for receptor.*

Keywords*: clopidogrel, adrenaline, electron density, proaggregant, antiaggregant*

1. INTRODUCTION

In a fundamental physicochemical approach to platelet aggregation phenomena based on the Dereaghin-Landau Verwey Overbeek theory, the stability of blood and the lack of platelet aggregation is the consequence of their negative electrical charge and the rejection between the double electrical layers formed at the membranes. Classical DLVO forces alone are not sufficient to accurately predict aggregation behavior. More recently, theory was extended to include ionic, steric, elastic [1] and osmotic forces [2-3] in so called extended DLVO theory (XDLVO). In this physico-chemical context all negative charged molecules which adsorb at membranes have to present antiagregant activity and all positively charged are potentially proaggregant. This is more or less true in vitro but, in vivo , there are only a few antiaggregant drugs, essential treatment of cardiovascular and neurological stroke being based on aspirine and clopidogrel.

[1] University of Medicine and Pharmacy of Craiova, Faculty of Pharmacy, 200349 Craiova, Romania. E-mail: emanro2002@yahoo.com.
[2] Ovidius University of Constanta, Faculty of Pharmacy, Department of Physical Chemistry, 900527 Constanta, Romania. E-mail: sirbu_27@yahoo.com.
[3] Ovidius University of Constanta, Department of Chemistry and Chemical Engineering, 900527 Constanta, Romania. * Corresponding author: gstanciu@univ-ovidius.ro.
[4] Titu Maiorescu University, Faculty of Pharmacy, Department of Pharmaceutical Technology, 040317 Bucharest, Romania. E-mail: roxana.sandulovici@yahoo.com.

Intrinsic platelet aggregation is very low and is not directly measurable. It is much elevated before and immediately after vascular accidents, involving an increased risk of relapse or transient vascular accident. As a result, antiaggregation treatment is mandatory in all cases, but the treatment with a drug or combination of antiaggregation drugs is one of long duration. Instead of the effect on intrinsic aggregation, it is evaluated the effect of antiaggregant treatments on aggregation induced by a proaggregate, usually adrenaline. The challenge of antiaggregant therapy is the variability of clinical effect and frequent "resistance" to treatment [4] that implies an increased risk of recurrent atherothrombotic events [5].

Present paper is a part of a large program for physico-chemical approach of blood cell aggregation of the Carol Davila and Craiova universities of medicine and pharmacy and particularly the interaction between the standard proaggregant - adrenaline and a major antiaggregant - clopidogrel (Fig. 1). Beyond scientific interest, the problem may also have immediate clinical implications.

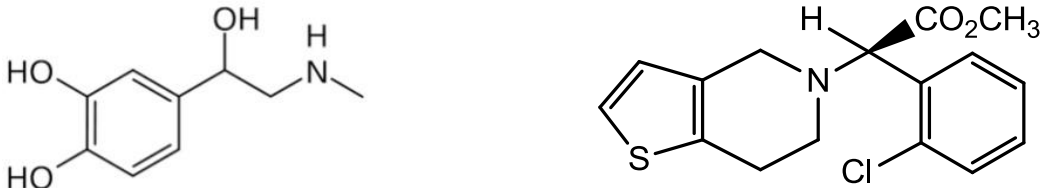


Figure 1. The adrenaline and clopidogrel formulas

After oral administration, clopidogrel is extensively biotransformed to at least eight metabolites (Fig. 2) [6]. It is accepted that clopidogrel itself has no antiaggregant properties [7], and that its therapeutic effect is dues to its active metabolite: 2-{1-1{1-(2-clorofenil)-2-metoxi-2-oxoetil}-4-sulfanil-3-piperidiniliden acid acetic [6] obtained from intermediate 2-oxoclopidogrel (Fig. 2), which is not active *in vitro* [8].

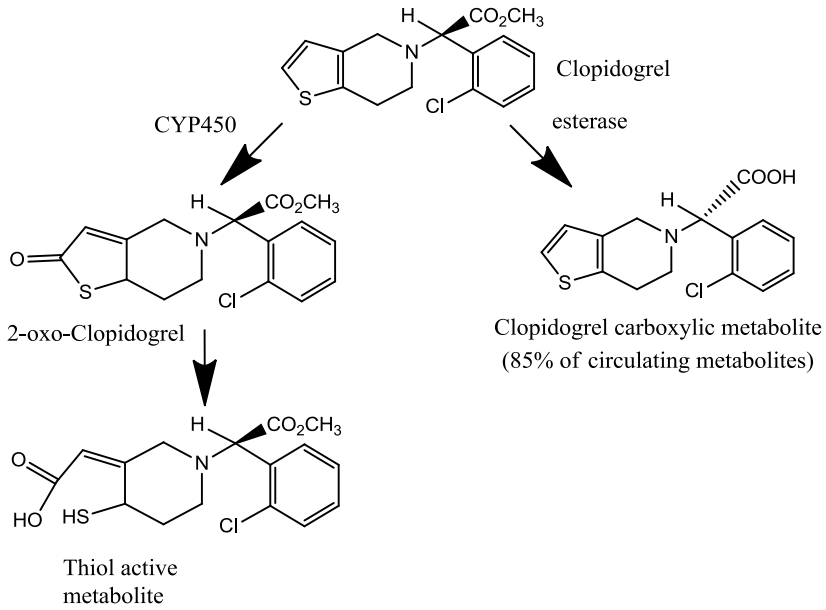
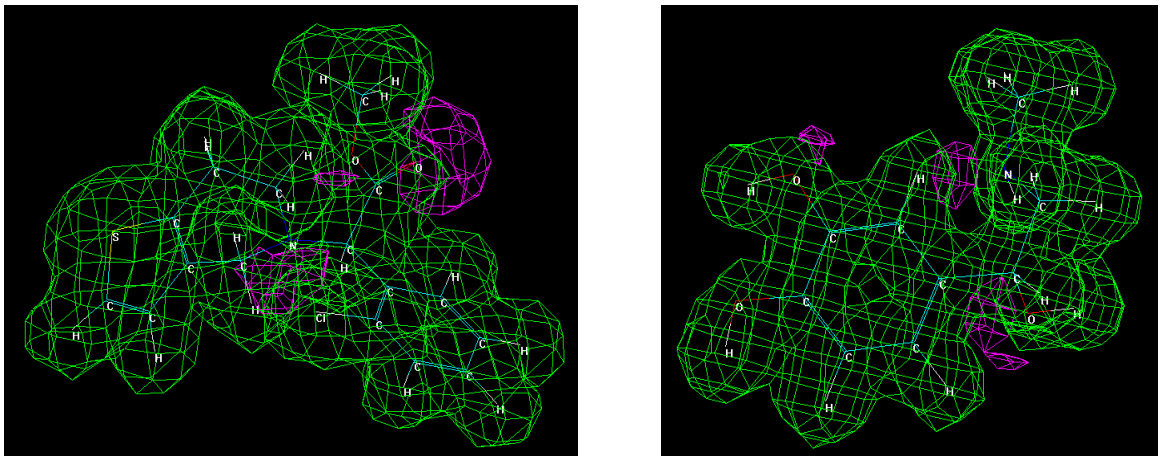


Figure 2. Biotransformation of clopidogrel into metabolites

2. CALCULATING MOLECULAR PROPERTIES

Molecular geometries were modeled and optimized with RHF method (Restricted Hartree-Fock) in PM3 semiempirical approximation using the program HyperChem [9]. These geometries were used in empirical calculations of molecular orbitals [10], obtaining data regarding the electronic structure of the substance under investigation (molecular electronic levels, electron population, dipole moment, net electric charges on atoms, partitions energy by type of chemical bonds or interactions, orders of bond or free valence).

In the case of the two molecules studied, based on the molecular property called electrostatic potential was obtained three-dimensional electron density map, the different values of electrostatic potential is marked by color (Fig. 3). Molecular electrostatic potential can be used to assess the cuantomolecular lipophilicity, because it characterizes the polarity of a certain region on the van der Waals surface of the molecule. Regions with high values of potential (positive areas colored in green) will strongly attract water molecules and regions with low values (negative areas colored in purple) will not attract water, being considered hydrophobic.



a **b**

Figure 3. The surface of molecular electrostatic potential of clopidogrel (a) and adrenaline (b).

The interactions between chemical substances and biological receptors, interactions that give rise to appropriate biological responses, generally takes place by electron transfer in both directions: ligand (OC-MO) => receptor and ligand (UN-MO) <= receptor (Fig. 4).

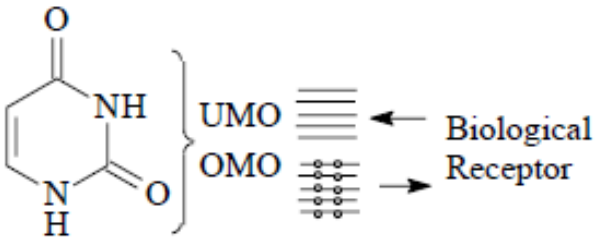
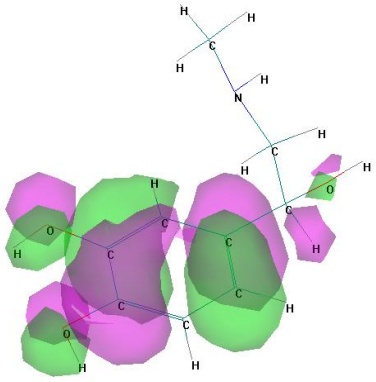
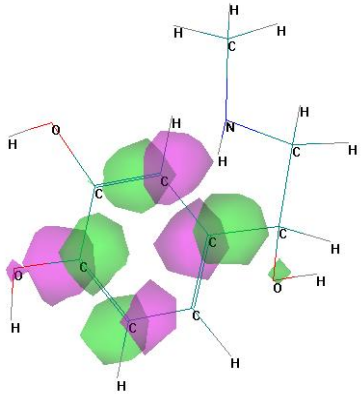


Figure 4. The ligand – receptor interaction by electron transfer [11].

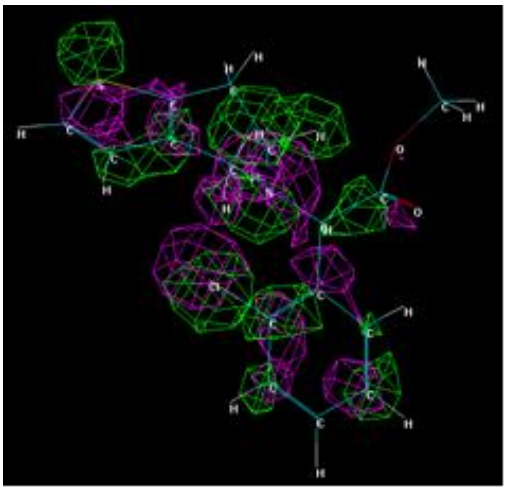
Therefore frontier orbitals are very important parameters to describe the interaction ligand (drug) - biological receptor and for molecules studied (Fig. 5) shows the following energy values.



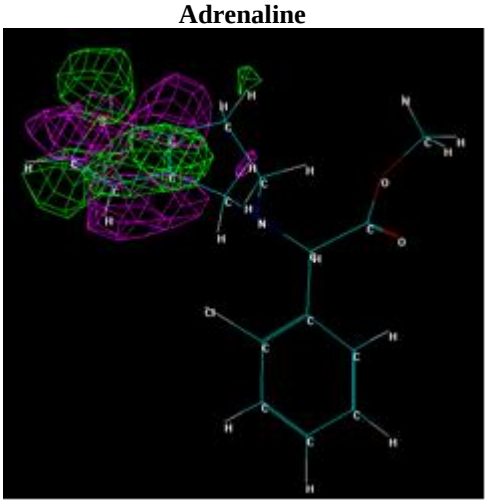
E_{HOMO} = - 8.6748 eV
Adrenaline



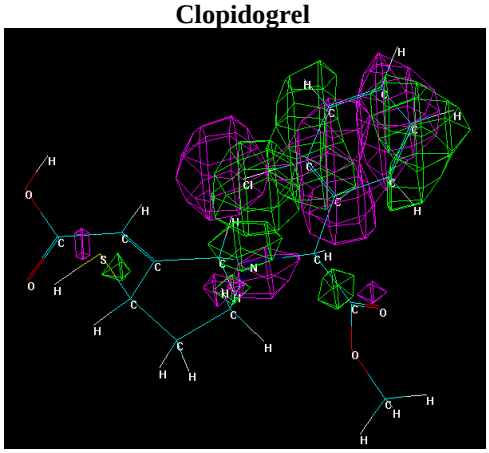
E_{LUMO} = 0.4042 eV
Adrenaline



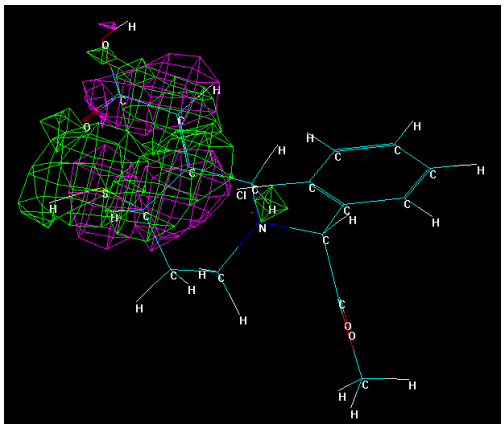
E_{HOMO} = - 9.0177 eV
Clopidogrel



E_{LUMO} = - 0.2824 eV
Clopidogrel



E_{HOMO} = - 9.1695 eV
Thiol



E_{LUMO} = - 0.6466 eV
Thiol

Figure 5. Frontier molecular orbitals.

The green color (Fig. 5) indicates a positive polarity and the color purple - the negative polarity. The molecular areas study for the two types of molecular orbitals show their contribution to the formation of atomic orbitals. Descriptor value E_{HOMO} allow an appreciation of the donor properties of a molecule or its oxidation trends. Instead, the descriptor value E_{LUMO} allow the assessment of acceptor properties of a molecule or its reduction trends.

Molecules which have maximum E_{HOMO} are most susceptible to electrophilic attack, and for which E_{LUMO} has the maximum value are more susceptible to nucleophilic attack. Energy difference between the levels HOMO and LUMO ($E = E_{LUMO} - E_{HOMO}$) is also an important molecular descriptor from chemically point of view explaining the stability of the molecule, a low value indicating that the molecule is highly reactive. From the values shown in Table 1, the most stable molecule adrenaline.

Table 1. The values of molecular descriptors.

Substance	***ΔE [eV]***	***λ [nm]***	***η [%]***
Adrenaline	9.079	4.1353	4.5584
Clopidogrel	8.7353	4.6500	4.3676
Thiol	8.5229	4.9080	4.2614

Sum and difference of energies of frontier molecular orbitals are related to the chemical reactivity of molecules by quantum descriptors of global reactivity, absolute electronegativity (λ) and absolute hardness (η) [12-13]:

$$\lambda = -\frac{E_{LUMO} + E_{HOMO}}{2} \qquad \eta = \frac{E_{LUMO} - E_{HOMO}}{2}$$

The tendency of donation - acceptance of electrons is described by electronegativity λ. Rigidity (hardness) is a quantity which describes a system opposition to atomic or molecular electron density variation in the system. Greater electronegativity is presented by thiol active metabolite of clopidogrel explaining bigger reactivity of this substance.

Another parameter derived from molecular quantum chemical calculations is electric dipole moment (μ), reflecting the partial separation of electric charge in the molecule. The molecular descriptor is also a predictor of the chemical reactivity of the molecules. This parameter is a measure of the molecular system polarization. Table 2 shows the values of the dipole moment for molecules studied. As we can see, the greater the dipole moment belongs to thiol active metabolite of clopidogrel, which may explain its increased reactivity, that is its antiplatelet activity.

Table 2. The values of molecular descriptors.

Substance	***M [g/mol]***	***Dipole moment [D]***
Adrenaline	183.208	2.049
Clopidogrel	321.829	2.063
2oxoclopidogrel	337.829	3.354
Thiol	356.098	3.832
Clopidogrel carboxil	307.803	1.980

The existence in the drug molecule of partialy highly negatively charged atoms determine a better drug-receptor interaction. The theoretical study of the electronic density distribution of the S atoms of the molecules studied is presented in Table 3.

Table 3. Atomic charges obtained by Mulliken population analysis [14].

Substance	***Atom***	***Mulliken atomic charge***	***Mulliken atomic population***
Clopidogrel	S	0.020665	15.979335
2oxo clopidogrel	S	-0.109856	16.109856
Thiol	S	-0.143685	16.143685

The presented data show a better participation of the S atom of the thiol metabolite molecule of clopidogrel at the chemical interaction with the biological receptor. Transport of

drugs through cell membranes depends on their solubility in biological membranes and their partition coefficient, which expresses the differential solubility of the substances in aqueous and organic solvents. In Table 4 are presented the values of this parameter theoretically calculated.

Table 4. The values for the partition coefficient

Substance	***log P***
Clopidogrel	3.81
Adrenaline	-0.61

Clopidogrel is more lipophilic (log P > 1) than adrenaline (log P < 1). We can considere adrenaline, more or less amphiphilic (log P = - 0.61, but close to 1, ie solubility in both water and octanol), that have a hydrophobic part (bigger) and one hydrophilic (OH, NH, SH). Given the fact that adrenaline is proaggregant and clopidogrel is an antiaggregant prodrug, interaction between them would have been possible if besides the mechanism of receptor interaction there would be another biophysical mechanism through effects at the level of the double electric layers of the membranes. This has been highlighted [15] the erythrocyte antiaggregant effect of clopidogrel in the absence of activation by hepatic metabolism. Last but not least, it should be taken into account that the effects, at least at the level of the double electric layers, depend on the concentration at the action site [16].

CONCLUSION

Our results didn't indicate a possible direct interaction between clopidogrel or clopidogrel metabolites and adrenaline. An opposite effect on the structure of the double layer of platelet membrane appears following the positive residual charge of adrenaline and residual negative charge of clopidogrel and metabolites. Carboxiclopidogrel is most positively charged but its effect is lower than that of active metabolite. Complementary, biochemical component of antiagregant effect arise from -SH reactive group, which lead to bridges with other -SH groups from the so called receptor.

REFERENCES

[1] Fritz, G. et al., *Langmuir*, **18**, 6381, 2002.
[2] Ortega, V. J. et al., *J. Colloid Interface Sci*. **184**, 259, 1996.
[3] Phenrat, T. et al., *J. Nanopart. Res*., **10**, 795, 2008.
[4] Tantry, U.S. et al., *Expert Opin Pharmacother*, **6**, 2027, 2005.
[5] Matetzky, S. et al., *Circulation*, **109**, 3171, 2004.
[6] Pereillo, J.M. et al., *Drug Metab Dispos*, **30**(11), 1288, 2002.
[7] Plavix, *Summary of product characteristics*, revised 26 July 2013.
[8] Savi, P. et al., *Thromb Haemostasis*, **84**, 891, 2000.
[9] http://www.4shared.com/file/E6zKU0pg/portable_hyperchem_807.html
[10] Amzoiu, E. et al., *Revue Roumaine de Chimie*, **56**(7), 711, 2011.
[11] Amzoiu, E. et al., *Revue Roumaine de Chimie*, **54**(8), 671, 2010.
[12] Obot, I.B. et al., *J. Environ. Chem. Eng*., **10**, 431, 2013.
[13] Janak, J., *Phys. Rev. B*, **18**, 7165, 1978.
[14] Mulliken, R.S., *J. Chem. Phys*., **3**(9), 573, 1935.
[15] Borisova, S. et al., *Farmacia*, **64**(4), 785, 2016.
[16] Vatasescu, A. et al, *Farmacia*, **59**(2), 161, 2011.