Asian Journal of Chemistry Vol. 20, No. 4 (2008), 3121-3126

Molecular Modelling Analysis of the Metabolism of Oseltamivir

FAZLUL HUQ
Discipline of Biomedical Science, School of Medical Sciences, Faculty of Medicine
Cumberland Campus, C42, The University of Sydney, Lidcombe, NSW, Australia
Fax: (61)(2)93519520; Tel: (61)(2)93519522; E-mail: f.huq@usyd.edu.au

Molecular modelling analyses based on molecular mechanics, semi-empirical (PM3) and DFT (at B3LYP/6-31G* level) calculations show that oseltamivir (OSE) and its metabolite oseltamivir carboxylate (OSA) have large LUMO-HOMO energy differences of 4.7 to 4.6 eV, respectively indicating that the compounds would be moderate inert kinetically. The molecular surfaces of the compounds are found to abound in neutral green and electron-rich red and yellow regions so that they may be subjected to lyophilic and electrophilic attacks. The molecular surfaces of OSE and OSA are also found to possess a small amount of electron-deficient blue regions so that the compounds may be subject to nucleophilic attacks. Nucleophilic attacks may be due to glutathione and nucleobases in DNA as a result of which depletion of glutathione and oxidation of nucleobases in DNA may occur. The former would induce oxidative stress and hence cellular toxicity whereas the latter would cause DNA damage. However, because of kinetic inertness of the molecules and paucity of electron-deficient regions, the rates of such adverse reactions are expected to be low.

Key Words: Influenza, Oseltamivir, Tamiflu, Neuraminidase inhibitor, Molecular modelling.

INTRODUCTION

Influenza (A and/or B) is a highly contagious respiratory disease and presents a significant global health problem that affects more than 10 % of the population every year[1]. Each annual epidemic is associated with substantial morbidity and mortality, with 150,000 hospitalizations and tens of thousands of death occurring in the USA alone[2].

Oseltamivir (Tamiflu, Ro 64-0796, OSE) is the first orally administered neuraminidase (NA) inhibitor[3] approved for use as a therapeutic and prophylactic agent against human influenza virus infection. Neuraminidase enzyme activity is essential in the replication of influenza virus, playing an important role in the elution of newly synthesized virions from infected cells and helping the movement of virus through mucus in the respiratory tract[4]. Oseltamivir is effective in adults, children as well as high-risk populations such as elderly, who are more susceptible to the risk of influenza-associated

complications[5]. OSE is a pro-drug that is rapidly absorbed from gastrointestinal tract and on hydrolysis produces the active metabolite oseltamivir carboxylate (Ro 64-0802, OSA) (Fig. 1) catalyzed by hepatic esterases. OSA is exclusively excreted into urine without further metabolism in both humans and experimental animals. Plasma concentrations of OSA reach a maximum 3-4 h after ingestion and at that time, substantially exceed concentrations of OSE by ≥ 20 fold[3]. The active metabolite OSA interacts with highly conserved residues of influenza NA and is therefore likely to be effective against all strains of influenza virus regardless of antigenic changes. Its apparent resistance to biodegradation and hydrophilicity, OSA is likely to enter receiving water from sewage treatment works and this may be significant in the case of a flu epidemic when stockpiles of Tamiflu are likely to be deployed[6].

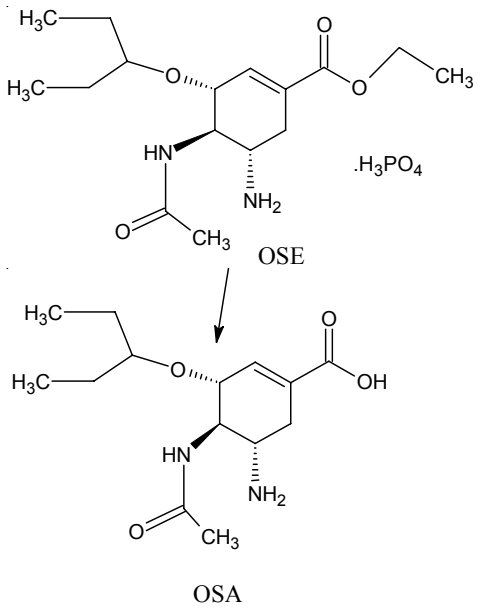

Fig. 1. Hydrolysis of OSE to form the active metabolite OSA

In this study, molecular modelling analyses have been carried out using the program Spartan '02[7] for OSE and its metabolite OSA, with the aim of providing information related to any toxicity caused by the compounds. Previous studies have shown that xenobiotics and their metabolites which are kinetically labile and abound in electron-deficient regions on the molecular surface tend to induce cellular toxicity due to glutathione depletion and cause DNA damage due to oxidation of nucleobases in DNA[8,9] whereas the ones that abound in electron-rich regions can act as antioxidants.

The work was carried out in the Discipline of Biomedical Science, School of Medical Sciences, The University of Sydney during May to August 2007.

COMPUTATIONAL METHOD

The geometries of OSE and its metabolite OSA have been optimized based on molecular mechanics, semi-empirical and DFT calculations, using the molecular modelling program Spartan '02. Molecular mechanics calculations were carried out using MMFF force field. Semi-empirical calculations were carried out using the routine PM3. DFT calculations were carried at B3LYP/6-31G* level. In optimization calculations, a RMS gradient of 0.001 was set as the terminating condition. For the optimized structures, single point calculations were carried out to give heat of formation, enthalpy, entropy, free energy, dipole moment, solvation energy, energies for HOMO and LUMO. The order of calculations: molecular mechanics followed by semi-empirical followed by DFT ensured that the structure was not embedded in a local minimum. To further check whether the global minimum was reached, some calculations were carried out with improvable structures. It was found that when the stated order was followed, structure corresponding to the global minimum or close to that could ultimately be reached in all cases. Although RMS gradient of 0.001 may not be sufficiently low for vibrational analysis, it is believed to be sufficient for calculations associated with electronic energy levels[10].

RESULTS AND DISCUSSION

Table-1 gives the total energy, heat of formation as per PM3 calculation, enthalpy, entropy, free energy, surface area, volume, dipole moment and energies of HOMO and LUMO as per both PM3 and DFT calculations for OSE and its metabolite OSA. Figs. 2-3 give the regions of negative electrostatic potential (greyish-white envelopes) in (a), HOMOs (where red indicates HOMOs with high electron density) in (b), LUMOs in (c) and density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral) in (d) as applied to optimized structures of OSE and OSA, respectively.

TABLE-1
CALCULATED THERMODYNAMIC AND OTHER PARAMETERS OF OSE AND ITS METABOLITE OSA

A	B	C	D	E	F	G	H	I	J	K	L	M	N
OSE	PM3	-188.70	-179.08	285.12	169.56	234.57	-9.61	368.53	337.39	4.5	-9.64	0.07	9.71
	DFT	-1036.98		286.67	168.31	236.51	-9.13	375.43	338.79	5.3	-6.06	-1.36	4.70
OSA	PM3	-193.63	-181.87	249.22	154.64	203.11	-11.76	325.21	298.23	4.1	-9.67	-0.12	9.55
	DFT	-958.35		250.59	153.16	204.95	-11.14	331.60	299.80	4.9	-6.11	-1.52	4.59

A = Molecule; B = Calculation type; C = Total energy (kcal mol^{-1}/atomic unit*); D = Heat of formation (kcal mol^{-1})'; E = Enthalpy (kcal mol^{-1} K^{-1}); F = Entropy (cal mol^{-1} K^{-1}); G = Free energy (kcal mol^{-1}); H = Solvation energy (kcal mol^{-1}); I = Area ($Å^2$); J = Volume ($Å^3$); K = Dipole moment (debye); L = HOMO (eV); M = LUMO (eV); N = LUMO-HOMO (eV).
*In atomic units from DFT calculations.

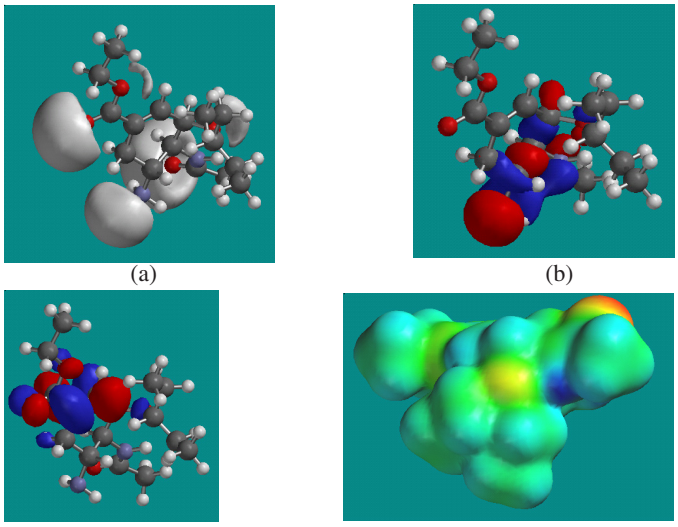

Fig. 2. Structure of OSE giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral)

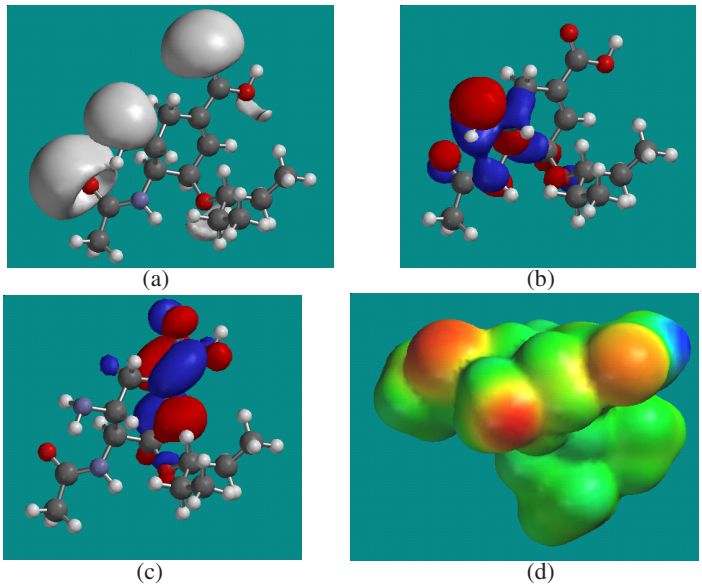

Fig. 3. Structure of OSA giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral)

The LUMO-HOMO energy differences for OSE and OSA from DFT calculations are 4.70 and 4.59 eV, respectively (Table-1), indicating that both the compounds would be moderately inert kinetically.

The solvation energies of OSE and OSA obtained from PM3 calculations are -9.61 and -11.76 kcal mol^{-1}, respectively (Table-1), indicating that the two compounds would be lypophilic. OSE is found to have slightly higher solvation energy than OSA whereas the latter has a larger dipole moment than the former. As observed previously, the results indicate complexity of solution in which such processes as hydrogen bonding and resonance stabilization may be playing key roles[10].

In the case of OSE and OSA, the electrostatic potential is found to be more negative around the various oxygen centres, indicating that the positions may be subject to electrophilic attack.

In the case of OSE, the HOMOs with high electron density are found to be close to the non-hydrogen atoms of the acetamide moiety whereas the LUMOs are found to be located on the non-hydrogen atoms of the ester moiety. In the case of OSA also, the HOMOs with high electron density are found to be located on the non-hydrogen atoms of the acetamide moiety whereas the LUMOs are found close to the non-hydrogen atoms of the carboxyl group.

The molecular surfaces of OSE and OSA are found to abound in neutral green regions so that the compounds may be subjected to lyophilic attacks. The molecular surfaces of both the compounds are also found to possess some negatively charged red and yellow regions (more in the case of OSA) so that the compounds may also be subjected to electrophilic attacks. The molecular surfaces of OSE and OSA are also found to possess some electron-deficient blue regions (more in the case of OSE) so that the compounds may be subject to nucleophilic attacks. Nucleophilic attacks may be due to glutathione and nucleobases in DNA, resulting into glutathione depletion and oxidation of nucleobases in DNA. The former would induce oxidative stress and hence cellular toxicity whereas the latter would cause DNA damage. However, because of the kinetic inertness of the molecules, the rates of such adverse reactions are expected to be low unless speeded up enzymatically.

Conclusion

Influenza (A and/or B) is a highly contagious respiratory disease and presents a significant global health problem that affects more than 10 % of the population every year. Oseltamivir (OSE) is the first orally administered neuraminidase (NA) inhibitor approved for use as a therapeutic and prophylactic agent against human influenza virus infection. Molecular modelling analyses based on molecular mechanics, semi-empirical (PM3) and

DFT (at B3LYP/6-31G* level) calculations show that OSE and its metabolite OSA have moderately large to large LUMO-HOMO energy differences indicating that the compounds would be inert kinetically. The molecular surfaces of OSE and OSA are found to abound in neutral green and electron-rich red and yellow regions so that the compounds may be subject to lyophilic and electrophilic attacks. The presence of electron-rich regions on the molecular surface may render some antioxidant properties to the molecules. OSE and OSA are also found to possess some electron-deficient blue regions so that they may be subject to nucleophilic attacks. However, because of the kinetic inertness of the molecules, the rates of such adverse reactions are expected to be low unless speeded up enzymatically.

ACKNOWLEDGEMENT

The author is grateful to the Discipline of Biomedical Science, School of Medical Sciences, The University of Sydney for substantial time release from teaching.

REFERENCES

1. J.W. Massarella, G.Z. He, A. Dorr, K. Nieforth, P. Ward and A. Brown, *J. Pharmacol.*, **40**, 836 (2000).
2. K.L. Nichol, A. Lind, K.L. Margolis, M. Murdoch, R. McFadden, M. Hauge and M. Drake, *N. Engl. J. Med.*, **333**, 889 (1995).
3. P. Snell, C. Oo, A. Dorr and J. Barrett, *Br. J. Clin. Pharmacol.*, **54**, 372 (2002).
4. M. Von Itzstein, W.Y. Wu, G.B. Kok, M.S. Pegg, J.C. Dyason, B. Jin, T. Van Phan, M.L. Smythe, H.F. White, S.W. Oliver, P. Colman, J. Varghese, D. Ryan, J. Woods, R. Bethell, V. Hotham, J. Cameron and C. Penn, *Nature*, **363**, 418 (1993).
5. R. Robson, A. Buttimore, K. Lynn, M. Brewster and P. Ward, *Nephrol. Dial Transplant*, **21**, 2556 (2006).
6. A.C. Singer, M.A. Nunn, E.A. Gould and A.C. Johnson, *Environ. Health Persp.*, **115**, 102 (2007).
7. Spartan '02, Wavefunction, Inc. Irvine, CA, USA (2002).
8. F. Huq, *Int. J. Pure Appl. Chem.*, **1**, 155 (2006).
9. F. Huq, *J. Pharmacol. Toxicol.*, **1**, 328 (2006).
10. F. Huq, *Int. J. Pure Appl. Chem.*, **1**, 331 (2006).

(*Received*: 5 September 2007; *Accepted*: 19 January 2008) AJC-6224