Journal of Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

Journal of Pharmacology and Toxicology 1 (4): 362-368, 2006
ISSN 1816-496X

Molecular Modelling Analysis of the Metabolism of Adefovir Dipivoxil

Fazlul Huq
School of Biomedical Sciences, Faculty of Health Sciences,
The University of Sydney, Australia

Abstract: Adefovir dipivoxil (ADV) is an oral prodrug designed to enhance low intestinal absorption of the anti-viral agent adepovil (PMEA). The pivoxil moieties of ADV are rapidly cleaved to produce PMEA during absorption through the gut wall. After entry into the cell, PMEA is phosphorylated to produce ADMP which is further phosphorylated to form ADDP. The antiviral activity of the drug is based on the capacity of ADDP to preferentially inhibit viral DNA replication with relative sparing of host DNA synthesis. The dose-limiting toxicity of ADV is nephrotoxicity associated with high systemic exposure. Molecular modelling analyses based on molecular mechanics, semi-empirical (PM3) and DFT (at B3LYP/6-31G* level) calculations show that ADV and its three metabolites PMEAA, ADMP and ADDP have large LUMO-HOMO energy differences so that they all would be kinetically inert. Thus, although the molecules have some electron-deficient regions on their surface so that they could potentially react with glutathione and nucleobases in DNA, the high kinetic inertness of the molecules is believed to provide protection against such adverse reactions.

Key words: Adefovir, HBV, antiviral agent, molecular modelling, DFT

Introduction

Adefovir dipivoxil [ADV, bis(pivaloyloxymethyl)-9-(2-phosphonoylmethoxyethyl)adenine] is an oral prodrug designed to enhance low intestinal absorption of the anti-viral agent adepovil [PMEA, 9-[2-(phosphonomethoxy)ethyl]adenine] which is a reverse transcriptase inhibitor (Starret *et al*., 1994). ADV has shown to be effective against hepatitis B virus (HBV) in both HBV e antigen-positive and HBV e antigen-negative patients (Marcellin *et al*., 2003, Hadziyannis *et al*., 2003). Chronic hepatitis B remains a global public health problem despite the availability of an effective vaccine (Raney *et al*., 2003). The World Health Organization estimates that approximately 400 million people worldwide are chronically infected with HBV of which at least 30% will develop cirrhosis and/or hepatocellular carcinoma (Ray *et al*., 2004). Also, HBV recurrence and de novo HBV are frequent events in liver transplantation recipients (Barcena *et al*., 2005).

The pivoxil moieties of ADV that serve to enhance its cellular permeability (Srinivas *et al*., 1993) are rapidly cleaved, both chemically and by esterase activity, to produce PMEA during absorption through the gut wall (Naesens *et al*., 1996). After entry into the cell, PMEA is phosphorylated to produce adefovir monophosphate (ADMP) by adenylate kinase 2 (Robbins *et al*., 1995). ADMP is further phosphorylated to form adefovil diphosphate (ADDP). The antiviral activity of PMEA is based on the capacity of ADDP to preferentially inhibit viral DNA replication with relative sparing of host DNA synthesis (Balzarini *et al*., 1991; De Clercq, 1993).

The dose-limiting toxicity of ADV is nephrotoxicity associated with high systemic exposure (Fisher *et al*., 1999). In this study, molecular modelling analyses have been carried out using the program Spartan '04 (Spartan 2004) to investigate the relative stability of ADV and its metabolites with the aim of providing a better understanding on their relative toxicity.

Computational Methods

The geometries of ADV and its metabolites have been optimized based on molecular mechanics, (Fig. 1) semi-empirical and DFT calculations, using the molecular modelling program Spartan '04. 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

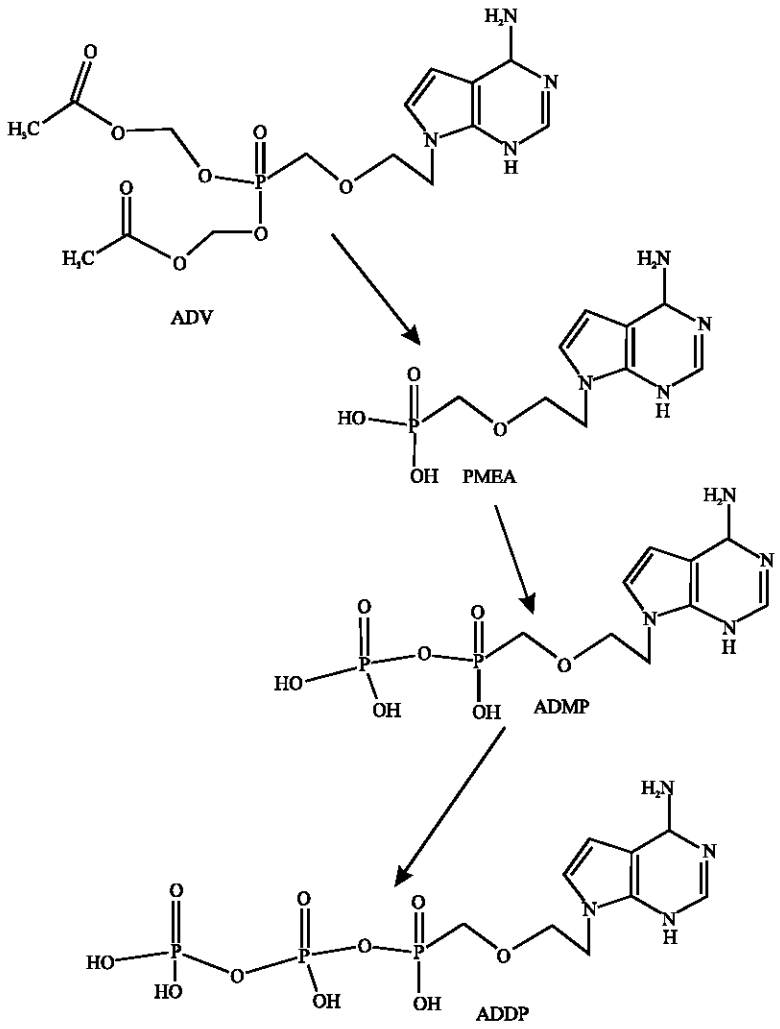

Fig. 1: Metabolic pathways for ADV (Ray *et al*., 2004)

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.

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, energies of HOMO and LUMO as per both PM3 and DFT calculations for ADV and its metabolites PMEA, ADMP and ADDP. Figure 2-5 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 the optimized structures of ADV and its metabolites PMEA, ADMP and ADDP.

The calculated dipole moments from DFT calculations of ADV and its metabolites PMEA, ADMP and ADDP are, respectively 6.9, 1.5, 4.7 and 5.3.

In the case of ADV, PMEA, ADMP and ADDP, the electrostatic potential is found to be more negative around the various oxygen and nitrogen centers, indicating that the positions may be subject to electrophilic attack.

In the case of PMEA, ADMP and ADDP both the HOMOs with high electron density and the LUMOs are found close to the non-hydrogen atoms of the six-membered heterocyclic ring. The overlap of HOMO with high electron density and region of negative electrostatic potential close to sulfur, gives further support to the idea that the position may be subject to electrophilic attack.

The molecular surfaces of ADV, PMEA, ADMP and ADDV, are found to have electron-deficient (blue), neutral (green) and negative (yellow and red) regions indicating that the compounds may be subject to nucleophilic, hydrophobic and electrophilic attacks. Within the cell, the nucleophilic attack may be that due to glutathione and nucleobases in DNA. Reaction with glutathione will induce cellular toxicity by compromising the antioxidant status of the cell whereas that with nucleobases in DNA will cause DNA damage. However, as stated earlier, since ADV and all its metabolites are expected to be kinetically inert, the rate of such adverse reactions may be low.

Table 1: Calculated thermodynamic and other parameters of ADV and its metabolites

Molecule	Calculation type	Total energy (kcal mol^{-1}/ atomic unit*)	Heat of formation (kcal mol^{-1})	Enthalpy (kcal mol^{-1} K^{-1})	Entropy (cal mol^{-1} K^{-1})	Free energy (kcal mol^{-1})
ADV	PM3		-350.24	1484.06	976.30	1192.97
	DFT	-1998.37		1486.97	975.06	
PMEA	PM3		-174.01	624.90	603.60	444.93
	DFT	-1228.15		625.67	602.43	446.15
ADMP	PM3		-388.58	774.35	855.32	519.34
	DFT	-1795.87		775.39	854.23	520.82
ADDP	PM3		-603.57	774.35	855.32	519.34
	DFT	-2363.59		775.41	854.38	520.80

Molecule	Calculation type	Area ($Å^2$)	Volume ($Å^2$)	Dipole moment (debye)	HOMO (eV)	LUMO (eV)	LUMO-HOMO (eV)
ADV	PM3	527.69	481.59	6.9	-8.91	-0.54	
	DFT	522.19	480.67	5.2	-5.90	-0.50	5.40
PMEA	PM3	276.94	236.80	0.70	-8.88	-0.51	8.37
	DFT	272.73	233.71	1.5	-6.00	-0.57	5.43
ADMP	PM3	337.40	283.36	1.7	-8.87	-0.50	8.37
	DFT	324.45	278.00	4.7	-6.10	-0.67	5.43
ADDP	PM3	397.71	329.92	3.5	-8.88	-0.51	8.37
	DFT	374.83	323.15	5.3	-5.86	-0.42	5.44

* in atomic units from DFT calculations

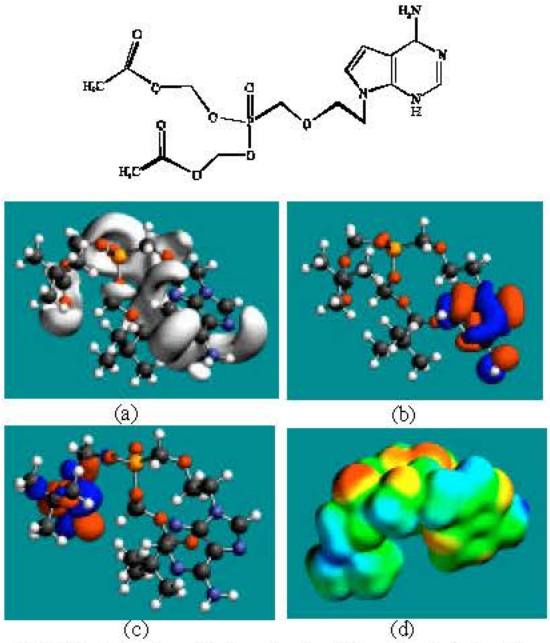

Fig. 2: Structure of ADV giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular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

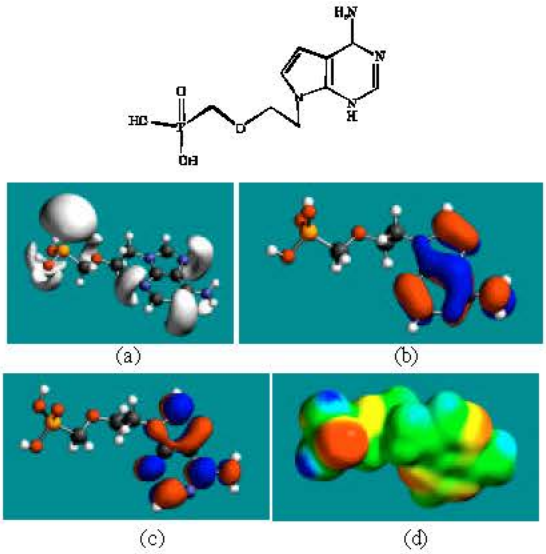

Fig. 3: Structure of PMEA giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular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

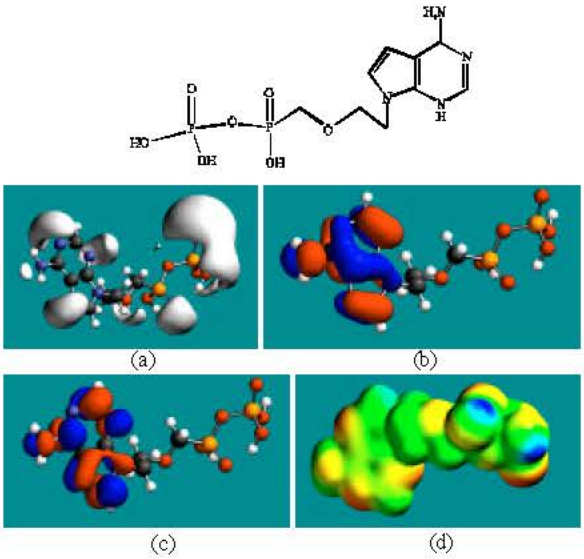

Fig. 4: Structure of ADMP giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral)

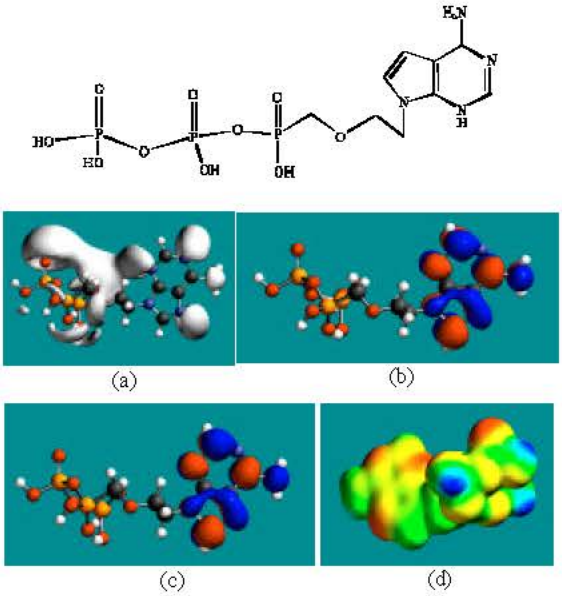

Fig. 5:Structure of ADDP giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral)

When surface area and volume of ADV, PMEA, ADMP and ADDV are compared, it is found that the values for the pharmacologically active molecule ADDP are distinctly different from those of other compounds (Table 1) so that ADV, PMEA and ADMP may not be substrate for the key receptor.

Conclusion

Molecular modelling analyses based on semi-empirical and DFT calculations show that ADV and all its metabolites have large LUMO-HOMO energy differences so that they would be kinetically inert. This means that although all the compounds have some electron-deficient regions on the molecular surface so that they could react with glutathione and nucleobases in DNA, in actual fact the rate of such adverse reactions may not be significant because of the high kinetic inertness of the molecules.

Acknowledgements

Fazlul Huq is grateful to the School of Biomedical Sciences, The University of Sydney for the time release from teaching.

References

Balzarini, J., Z. Hao, P. Herdewijn, D.G. Johns and E. De Clercq, 1991. Intracellular metabolism and mechanism of anti-retrovirus action of 9-(2-phosphonylmethoxyethyl)adenine, a potent anti-human immunodeficiency virus compound. Proc. Natl. Acad. Sci. USA., 88: 1499-1503.

Barcena, R., S. Del Campo, G. Moraleda, T. Cassanovas and M. Prieto *et al*., 2005. Study on the efficacy and safety of adefovir dipivoxil treatment in post-liver transplant patients with Hepatitis B Virus infection and Lamivudine-resistant Hepatitis B Virus. Trnsplantation Proc., 37: 3960-3962.

De Clercq E., 1993. Broad-spectrum anti-DNA and anti-retrovirus activity of phosphonylmethoxyalkylpurines and pyrimidines. Biochem. Pharmacol., 42: 963-972.

Fisher, E., C. Brosgart, D. Cohn, K. Chaloner, C. Pulling, B. Scmetter, B. Alston and W. El-Sadr, 1999. CPCRA 039 Team: Safety of adefovir dipivoxil (ADV) and incidence of proximal renal tubular disorders (PRTD) in a placebo-controlled trial in patients with advanced HIV disease (Abstract 678). Presented at the 6th Conference on Retrovirus and Opportunistic Infections, Chicago, IL, 1999.

Hadziyannis, S.J., N.C. Tassopoulos, E.J. Heathcote, T.T. Chang and G. Kitis *et al*., 2003. Adefovir dipivoxil: A review of its use in chronic hepatitis B e antigen-negative chronic hepatitis B. N. Eng. J. Med., 348: 800-807.

Mansuri, M.M., 1994. Synthesis, oral bioavailability determination and *in vitro* evaluation of prodrugs of the antiviral agent 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA). J. Med. Chem., 37: 1857-1864.

Marcellin, P., T.T. Chang, S.G. Lim, M.J. Tong, W. Sievert and M.L. Shiffman *et al*., 2003. Adefovir dipivoxil for treatment of hepatitis B antigen-positive chronic hepatitis B. N. Engl. J. Med., 348: 808-816.

Naesens, L., J. Balzarini, N. Bischofberger and E. De Clercq, 1996. Antiretroviral activity and pharmacokinetics in mice of oral bis(pivaloyloxymethyl)-9-(2-phosphonylmethoxyethyl)adenine, the bis(pivaloyloxymethyl) ester prodrug of 9-(2-phosphonylmethoxyethyl)adenine. Antimicrob. Agents Chemother., 39: 22-28.

Ray, A.S., J.E. Vela, L. Olson and A. Fridland, 2004. Effective metabolism and long intracellular half life of the anti-Hepatitis B agent adefovir in hepatic cells. Biochem. Pharmacol., 68: 1825-1831.

Robbins, B.L., J. Greenhaw, M.C. Connelly and A. Fridland, 1995. Metabolic pathways for activation of the antiviral agent 9-(2-phosphonylmethoxyethyl)adenine in human lymphoid cells. Antimicrob. Agents Chemother., 39: 2304-2308.

Spartan '04, Wavefunction, Inc. Irvine, CA, USA, 2004.

Srinivas, R.V., B.L. Robbins, M.C. Connelly, Y.F. Gong, N. Bischofberger and A. Fridland, 1993. Antiretroviral activity and pharmacokinetics in mice of oral bis(pivaloyloxymethyl)-9-(2-phosphonoylmethoxyethyl)adenine, the bis(pivaloyloxymethyl) ester prodrug of 9-(2-phosphonylmethoxyethyl)adenine. Antimicrob. Agents Chemother., 40: 22-28.

Starret, J.E., Jr., D.R. Tortolani, J. Russell, M.J.M. Hitchcock, V. Witherock, J.C. Martin, A.K. Raney, R.K. Hamatake and Z. Hong, 2003. Agents in clinical development for the treatment of chronic hepatitis B. Expert Opinion on Invetigational Drugs, 12: 1281-1295.