



Journal of
**Pharmacology and
Toxicology**

ISSN 1816-496X



Academic
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A Molecular Modelling Analysis of Toxicity of Fosamax and Risedronate

Fazlul Huq

Discipline of Biomedical Science, School of Medical Sciences, Faculty of Medicine,
Cumberland Campus, C42, The University of Sydney, Lidcombe, NSW, Australia

Abstract: Fosamax (FSM) and risedronate (RDT) are a second and a third generation aminobisphosphonate respectively that are approved for the prevention and treatment of osteoporosis in post-menopausal women and elderly men. Molecular modelling analyses based on molecular mechanics, semi-empirical (PM3) and DFT (at B3LYP/6-31G* level) calculations show that RDT has a much larger LUMO-HOMO energy difference than FMX so that it would be more inert kinetically than FMX. The molecular surface of FSM is found to abound more in electron-rich red and yellow regions than that of RDT so that FSM may be subject to electrophilic attack. This means that FSM can compete better than RDT, with phospholipids for the binding sites on the surface of the mucus gel layer, thus causing a much greater reduction in the protective hydrophobic barrier. The lower gastric irritating action of FSM and RDT at low pH may be explained as being due to partial neutralization of surface charge on the molecules as a result of association with readily available hydrogen ions. Also at low pH, hydrogen ion may displace sodium ion from FSM producing the acid form of the molecule that is found to have much lower negative charge on its molecular surface.

Key words: Osteoporosis, post-menopausal women, fosamax, alendronate sodium, risedronate, molecular modelling

INTRODUCTION

Osteoporosis may be defined as a disorder of low bone mass, Bone Mineral Density (BMD) and deterioration of bone quality due to increased bone turnover, leading to defective skeletal function (WHO, 1994). It is a major health problem worldwide affecting at least 30% of post-menopausal women. It has been estimated that one in two women over the age of 50 and one in eight men over the same age will have an osteoporosis-related fracture in their lifetime (Riggs and Melton, 1992). Such fractures in post-menopausal women and elderly men can cause substantial morbidity and mortality and seriously compromise the quality of life.

Bisphosphonates are widely used in the treatment of various diseases of bone mineral metabolism (Russell and Rogers, 1999). Alendronate sodium (Fosamax; FMX) is a second generation aminobisphosphonate approved for the prevention and treatment of osteoporosis in post-menopausal women and elderly men. It causes sustained increase in BMD, reduces bone turnover rate and the risk of vertebral, hip, wrist and other fractures. In clinical trials, FMX has been shown to reduce fracture risk by 50%. FMX binds to hydroxyapatite and inhibits bone resorption. It is poorly absorbed from gastrointestinal tract and its absorption is further reduced if taken with any substance that could bind to the drug. Hence strict restrictions on food and the use of other medications are necessary to prevent a decrease in efficacy of the drug. Bisphosphonic acid ligand possesses strong chelating properties capable of binding with Ca(II), Mg(II) and Fe(II), in a bidentate manner and its affinity for calcium can be increased if hydroxyl group is attached to the geminal carbon atom (Jung *et al.*, 1973). It has recently been reported that FMX can cause damage in the gastric mucosa and worsen the gastric ulcerogenic

response to indomethacin. Risedronate (RDT) is a third-generation bisphosphonate with a nitrogen atom as a part of a pyridine ring (Fig. 2). Like FMX, RDT also has mucosal irritative and healing impairment effects in the stomach. However, these adverse effects are less pronounced for RDT than FMX, so that RDT may be considered to be safer than FMX. The mechanisms by which FMX, RDT and other bisphosphonates irritate the gastric mucosa remain unknown. Proposed hypotheses include cytotoxic action of adenosine triphosphate (ATP), disruption of cellular permeability barrier and interference with mevalonate pathway necessary for lipid synthesis (Lichtenberger *et al.*, 2000; Luckman *et al.*, 1998; Tarnawski and Halter, 1995). It has also been suggested that N-bisphosphonates cause damage in the stomach through topical irritant action on the stomach (Kanatsu *et al.*, 2004). Osteonecrosis of the jaws is also a recently described side effect of bisphosphonate therapy. Patients with multiple myeloma and metastatic carcinoma to the skeleton who are receiving i.v. nitrogen-containing bisphosphonates are a great risk for osteonecrosis of the jaws (Woo *et al.*, 2006).

The bisphosphonates because of molecular similarities can compete with phospholipids for the binding sites on the surface of the mucus gel layer and in so doing can reduce the protective hydrophobic barrier. It is however found that gastric irritating action of RDT and FMX is less pronounced at pH 4.0 than at pH 7.0 (Kanatsu *et al.*, 2004). Further study would be required to determine the exact mechanism underlying the gastric irritating action of bisphosphonates, taking into account into consideration the pH dependency and structure-activity relationship. It is also known that the gastric ulcer healing is further delayed in presence of NSAIDs that are frequently administered along with bisphosphonates in patients with arthritis or osteoporosis. Again, the mechanism remains unknown although it may be noted that wound healing involves multiple phases including synthesis of extracellular matrix materials, angiogenic response and epithelial migration over newly granulated tissue, ultimately resulting in tissue rebuilding (Tarnawski and Halter, 1995).

In this study, molecular modelling analysis has been carried out using the program Spartan '04 (Spartan, 2004) to obtain information on the reactivity of FMX and RDT and distribution of charge on their molecular surfaces with the aim of providing a better understanding of their toxicity. It is proposed that the greater gastric irritating action of FMX than RDT is associated with greater accumulation of negative charge on its molecular surface.

COMPUTATIONAL METHODS

The geometries of FMX have been optimised based on molecular mechanics, 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 optimised 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.

Table 1: Calculated thermodynamic and other parameters of FMX and RDT

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)
Fosamax	PM3		-434.10	130.78	146.76	87.02
	DFT	-1586.16		134.27	137.57	93.25
RDT	PM3		-350.89	130.03	140.05	88.27
	DFT	-1462.31		133.48	130.52	94.57

Molecule	Calculation type	Area (Å ²)	Volume (Å ³)	Dipole moment (debye)	HOMO (eV)	LUMO (eV)	LUMO-HOMO (eV)
Fosamax	PM3	253.70	204.00	9.6	-9.13	-1.21	7.89
	DFT	241.35	200.55	7.6	-6.17	-1.75	4.32
RDT	PM3	258.56	220.33	2.1	-9.97	-0.20	9.77
	DFT	244.98	216.39	2.5	-7.09	-0.83	6.26*

In atomic units from DFT calculations

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 FMX and RDT. Figures 1 and 2 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 optimised structures of FMX and RDT.

The LUMO-HOMO energy differences for FSM and RDT from DFT calculations are found to be 4.32 and 6.26 eV, respectively, indicating that RDT would be more inert kinetically than FSM. In the case of FSM, the HOMOs with high electron density are found to be centred mostly on the non-hydrogen atoms of the aminopropyl moiety whereas the LUMOs are found to be centred on the sodium. In the case of RDT, both the HOMOs with high electron density and the LUMOS are found to be centred on the non-hydrogen atoms of the pyridine ring.

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

The overlap of HOMO with high electron density and region of negative electrostatic potential at some positions, gives further support to the idea that the positions may be subject to electrophilic attack.

The molecular surface of FSM is found to abound more in electron-rich (red and yellow) regions than that of RDT so that FSM is more likely to interact with biomolecules electrically (being subject to electrohilic attack) than RDT. This means that FSM can compete better than RDT, with phospholipids for the binding sites on the surface of the mucus gel layer, thus causing a much greater reduction in the protective hydrophobic barrier. The lower gastric irritating action of FSM and RDT at low pH may be explained as being due to partial neutralization of surface charge on FSM as a result of association with readily available hydrogen ions. Another reason for low toxicity of FSM at low pH, may be due to the displacement of sodium ion by hydrogen ion resulting into acid form of FSM (FSMA). The surface of FSMA unlike that of FSM does not abound in electron-rich regions. Also at low pH, hydrogen ion may displace sodium ion from FSM producing the acid form of the molecule that is found to have much lower negative charge on its molecular surface (Fig. 3).

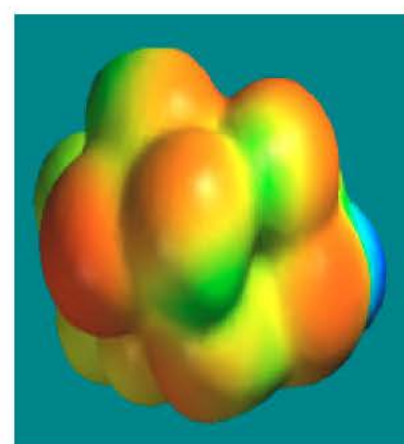
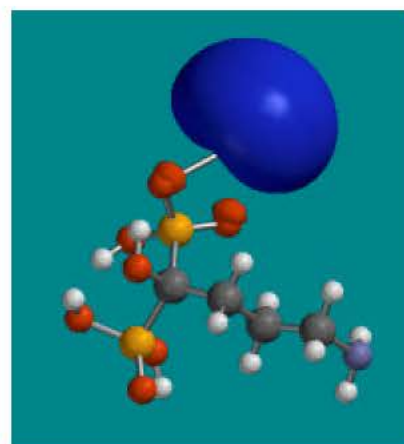
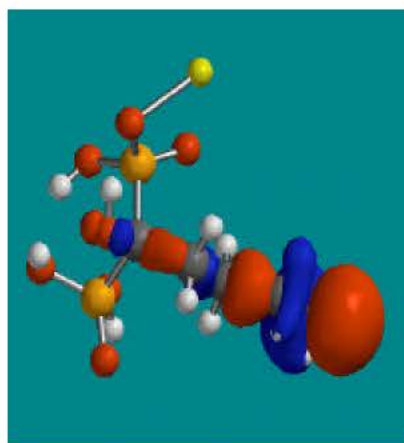
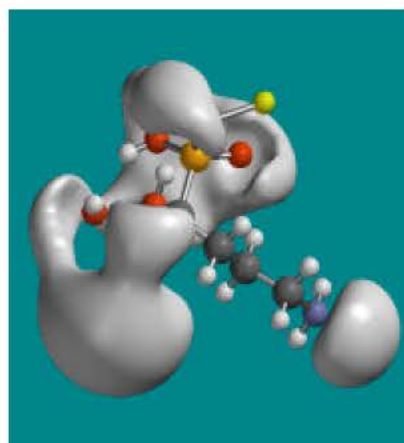
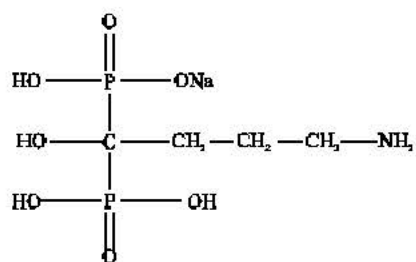
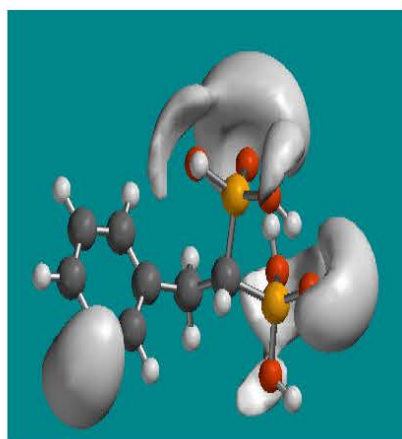
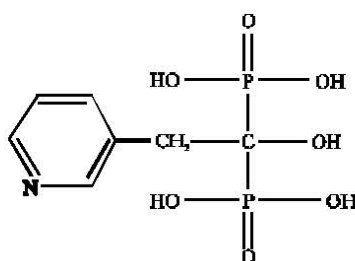
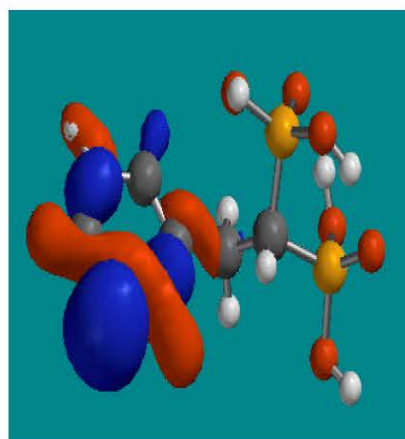


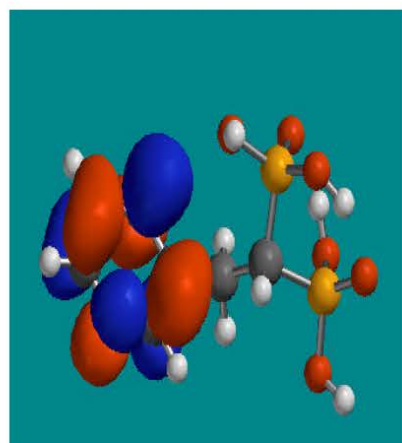
Fig. 1: Structure of FMX giving in, (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density), (c) the LUMOs (where blue indicates LUMOs) and in, (d) density of electrostatic potential on the molecular surface (where red indicates negative, blue indicates positive and green indicates neutral)



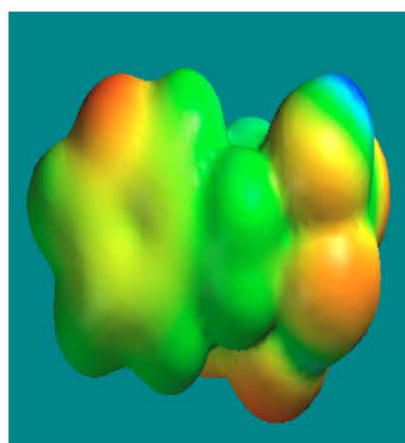
(a)



(b)



(c)



(d)

Fig. 2: Structure of RDT 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)

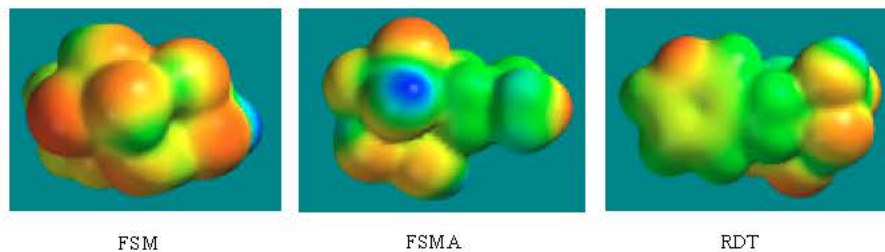


Fig. 3: Density of electrostatic potential on the molecular surfaces (where red indicates negative, blue indicates positive and green indicates neutral) of FSM, FSMA and RDT

CONCLUSION

Molecular modelling analyses based on semi-empirical and DFT calculations show that RDT has larger LUMO-HOMO energy difference than FSM so that RDT would be more kinetically inert. The molecular surface of FSM is found to abound more in electron-rich red and yellow regions than that of RDT so that FSM may be more subject to electrophilic attack than RDT. This means that FSM can compete better than RDT, with phospholipids for the binding sites on the surface of the mucus gel layer, thus causing a much greater reduction in the protective hydrophobic barrier. The lower gastric irritating action of FSM and RDT at low pH may be explained as being due to partial neutralization of surface charge on the molecules as a result of association with readily available hydrogen ions.

ACKNOWLEDGMENTS

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

ABBREVIATIONS

FMX: Alendronate sodium; Fosamax
RDT: Risedronate
DFT: Density functional theory
LUMO: Lowest unoccupied molecular orbital
HOMO: Highest occupied molecular orbital

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