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Molecular Modelling Analysis of the Metabolism of Troglitazone

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Abstract: In this study, molecular modelling analyses based on molecular mechanics, semi-empirical (PM3) and DFT (at B3LYP/6-31G* level) have been carried out for troglitazone (TGZ) and its metabolites with the aim of obtaining information on their relative toxicity. The results of the analyses show that TGZ and its metabolites have LUMO-HOMO energy differences ranging from 4.1 to 4.9 eV from DFT calculations except TGZQ which has much lower value of 2.69 eV. The values suggest although TGZ and most of its metabolites would be kinetically inert, the metabolite TGZQ would be highly labile. The molecular surfaces of TGZ and its metabolites are found to abound in neutral (green) and electron-rich (red and yellow) regions so that the compounds may undergo lyophilic and electrophilic attacks. TGZ and all its metabolites also possess some electron-deficient regions so that they may be subject to nucleophilic attacks by glutathione and nucleobases as well. However, the rates of such adverse reactions are expected to be low for TGZ and its metabolites except in the case of the highly labile metabolite TGZQ.

Key words: Troglitazone, insulin resistance, type II diabetes mellitus, hepatotoxicity, molecular modelling

INTRODUCTION

Type II diabetes mellitus is a complex disease characterized by insulin resistance resulting from defects in insulin secretion and insulin action (Salitel and Olefsky, 1996). It affects up to 8% of the adult population in developed countries (Kirchheiner *et al*., 2005). Metabolic abnormalities of the disease include hyperinsulinaemia, hyperglycaemia and hyper dislipidaemia (i.e., low levels of high-density lipoprotein or HDL) (Robinson *et al*., 2004; Hollenback *et al*., 1984). A defect in insulin action at the adipocyte has been identified in the pathogenesis of type II diabetes mellitus (Taratani *et al*., 1996). The increase in lipolysis results in excessive increase in the delivery of plasma non-esterified fatty acid (NEFA) and also of peripheral glucose disposal. The glycerol released acts as a substrate for hepatic gluconeogenesis (Christiansen *et al*., 2000). All of these effects serve to aggravate hepatic hyperglycaemia (Robinson *et al*., 2004).

Troglitazone (TGZ; CI-991; (±)-5-(4-(6-hydroxy-2,5,7,8-tetramethylchroman-2-ylmethoxy) benzyl)-thiazolidine-2,4-di-one) was the first marketed thiazolidinedione used for the treatment of type II diabetes mellitus. It acts as a partial agonist for peroxisome proliferator-activated receptor-γ and thus alters gene expression of key proteins involved in glucose metabolism, resulting in an increase of insulin sensitivity in skeletal muscle, liver and adipose tissues (Lehmann *et al*., 1995; Salitel and Olefsky, 1996). TGZ offered significant clinical benefits to many diabetic patients; however it was withdrawn from the m arket in 2000 because of idiosyncratic hepatotoxicity (Enokizono *et al*., 2006). The exact mechanism of hepatotoxicity due to TGZ remains unclear although

a number of mechanisms including metabolic activation (Gan *et al*., 2005), direct mitochondrial injury and cholestasis have been proposed (Pessayre and Larrey, 1988; Funk *et al*., 2001).

The metabolism of TGZ in human subjects and experimental animal species primarily involves sulfation to produce the major metabolite troglitazone sulfate (TGZS), glucuoronidation to produce the metabolite troglitazone glucuronide (TGZG) and oxidative chroman ring opening to produce the quinone metabolite denoted as TGZQ. TGZ has also been reported to form a number of conjugates with glutathione (Kassahun *et al*., 2001; He *et al*., 2004). Figure 1 shows the metabolic pathways of TGZ in humans. The major metabolite TGZS is believed to be the cause of cholestasis induced by TGZ (Enokizono *et al*., 2006). It has been suggested that reactive metabolites of TGZ bind covalently with proteins and cellular nucleophiles such as glutathione (GSH) and cysteine (He *et al*., 2004).

In this study, molecular modelling analyses have been carried out using the program Spartan '02 (Spartan, 2002) to provide information on the relative toxicity of TGZ and its metabolites TGZS, TGZG and TGZQ. No calculation was done for conjugates of TGZ with glutathione. The study was carried out in the Discipline of Biomedical Science, The University of Sydney during February to April 2007. 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 (Huq, 2006a, b).

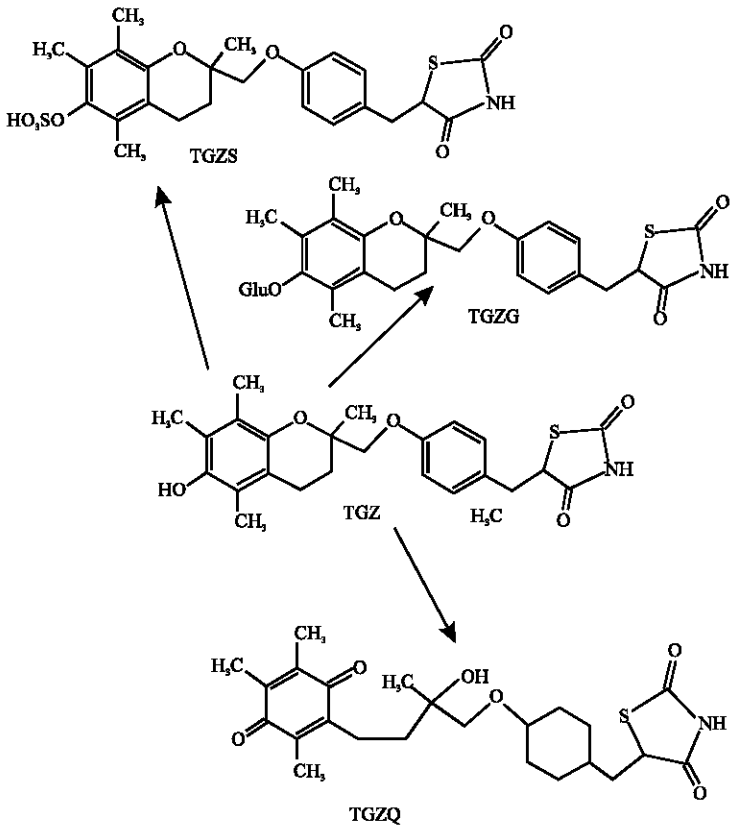

Fig. 1: TGZ metabolites in humans (He *et al*., 2004)

MATERIALS AND METHODS

Only molecular modelling calculations were carried out in this study.

Computational Methods

The geometries of TGZ and its metabolites TGZS, TGZG and TGZQ 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 (Huq and Alsheri, 2006)

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 (Highest Occupied Molcular Orbital) and LUMO (Lowest Unoccupied Molecular Orbital) as per both PM3 and DFT (Density Functional Theory) calculations for TGZ and its metabolites TGZS, TGZG and TGZQ. 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 © 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 optimised structures of TGZ and its metabolites TGZS, TGZG and TGZQ.

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

Molecule	Calculation type	Total energy (kcal mol^{-1} /atomic unit*)	Heat of formation (kcal mol^{-1}) K^{-1})	Enthalpy (kcal mol^{-1} K^{-1})	Entropy (cal $molK^{-1}$	Free energy (kcal mol^{-1})	Solvation energy (kcal mol^{-1})
TGZ	PM3	-178.30	-163.74	314.62	196.46	256.05	-14.56
	DFT	-1759.91		315.91	195.73	257.58	-13.23
TGZS	PM3	-244.99	-220.99	318.93	216.81	254.29	-23.56
	DFT	-2308.48		320.06	215.56	255.82	-21.69
TGZG	PM3	-434.65	-404.19	416.94	250.67	342.20	-30.46
	DFT	-2444.68		418.01	249.38	343.69	-28.51
TGZQ	PM3	-223.32	-190.43	316.70	207.20	254.92	-32.89
	DFT	-1835.12		318.43	206.03	257.03	-30.09

Molecule	Calculation type	Area ($Å^2$)	Volume ($Å^3$)	Dipole moment (debye)	HOMO (eV)	LUMO (eV)	LUMO-HOMO TGZ (eV)
	PM3	449.88	436.62	1.7	-8.45	-1.05	7.40
	DFT	453.47	438.20	2.4	-5.13	-1.03	4.10
TGZS	PM3	496.58	471.47	5.4	-8.67	-0.97	7.70
	DFT	495.18	472.09	3.9	-5.89	-1.00	4.89
TGZG	PM3	592.22	576.82	6.4	-8.96	-0.97	7.99
	DFT	593.99	577.24	6.0	-5.66	-0.96	4.70
TGZQ	PM3	472.07	450.64	6.2	-9.17	-1.55	7.62
	DFT	473.64	452.17	4.6	-6.01	-3.33	2.68

*: In atomic units from DFT calculations

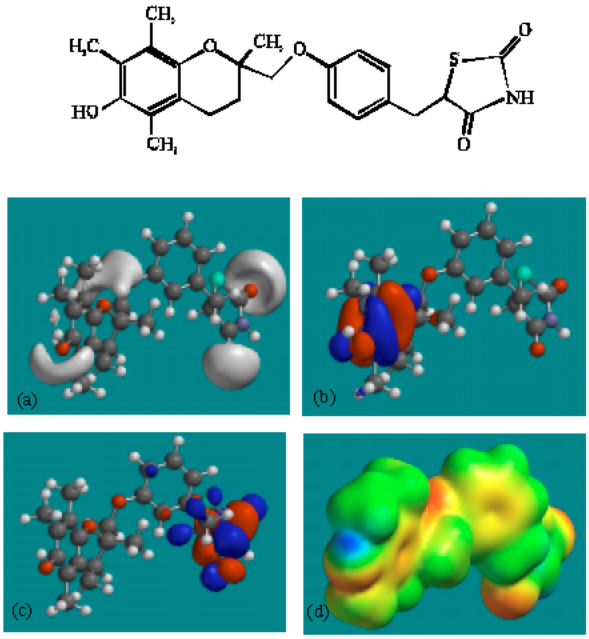

Fig. 2: Structure of TGZ giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density), (c) the LUMOs (where blue indicates LUMOs) and (d) density of electrostatic potential on the molecular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

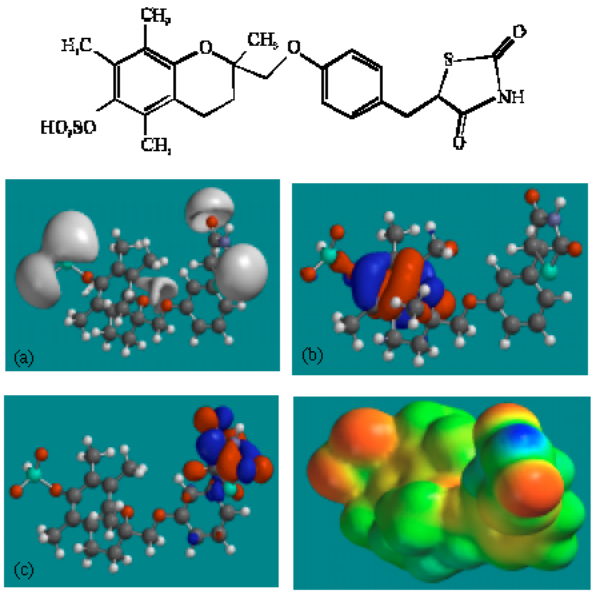

Fig. 3: Structure of TGZS giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density), (c) the LUMOs (where blue indicates LUMOs) and (d) density of electrostatic potential on the molecular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

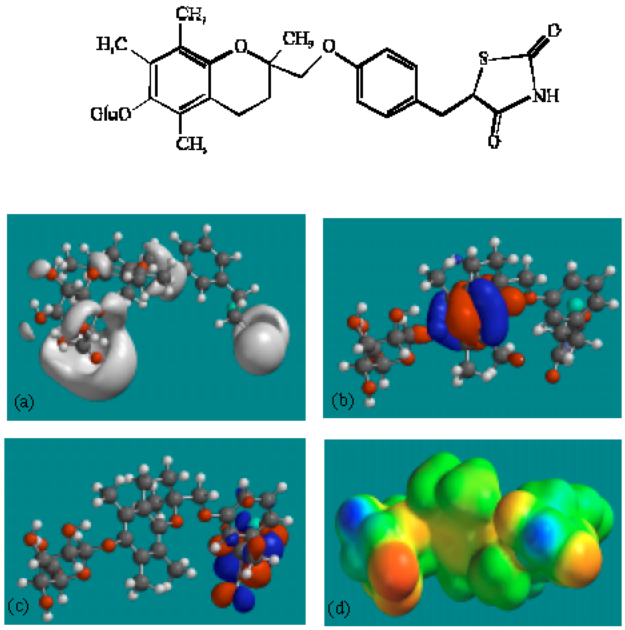

Fig. 4: Structure of TGZG giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicatesLUMOs) and (d) density of electrostatic potential on the molecular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

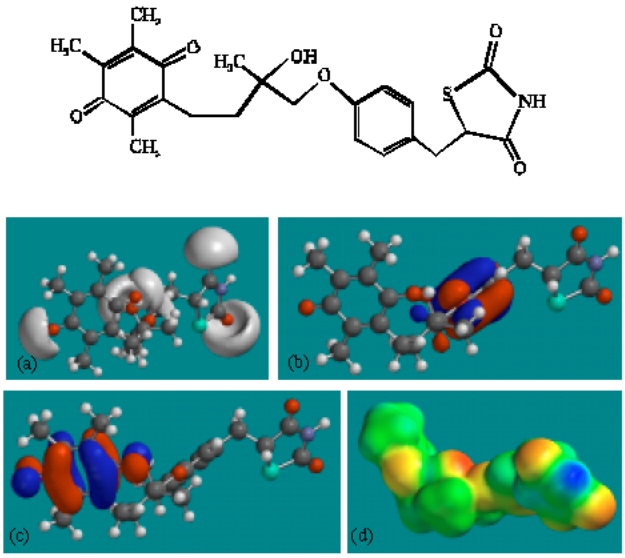

Fig. 5: Structure of TGZQ giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density), (c) the LUMOs (where blue indicates LUMOs) and (d) density of electrostatic potential on the molecular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

The LUMO-HOMO energy differences for TGZ and its metabolites are found to range from 2.68 to 4.89 eV from DFT calculations, indicating the compounds would vary significantly in their kinetic lability. The metabolite TGZQ which has the LUMO-HOMO energy difference of 2.68 eV is expected to be highly labile whereas the metabolite TGZS having the LUMO-HOMO energy difference of 4.89 eV is expected to be fairly inert.

In the case of TGZ and its metabolites TGZS, TGZG and TGZQ, the electrostatic potential is found to be more negative around nitrogen and oxygen atoms, indicating that the positions may be subject to electrophilic attack.

In the case of TGZ, TGZS and TGZG, the HOMOs with high electron density are found to be located on the non-hydrogen atoms of the fused substituted phenyl ring whereas the LUMOs are found to be located on the non-hydrogen atoms of the thiazolidine ring. The separation between the HUMOs with high electron density and the LUMOs may indicate the requirement of electron tunnelling for the activation of the molecules.

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 surfaces of TGZ and its metabolites are found to abound in neutral (green) and electron-rich (red and yellow) regions so that the compounds may undergo lyophilic and electrophilic attacks. TGZS is found to abound most in electron-rich regions so that it may be most subject to electrophilic attacks. The molecular surface of none of the compounds is found to abound in electron-deficient (blue) regions so that the compounds may not react readily with cellular nucleophiles such as glutathione and nucleobases in DNA. This means that none of the compounds may cause significant oxidative stress associated with glutathione depletion or DNA damage associated with oxidation of nucleobases. However, the possibility of such reactions cannot be totally ignored especially in the case of the highly reactive metabolite TGZQ which has one electron-deficient region on the molecular surface.

The solvation energy values of TGZ and its metabolites from PM3 calculations are found to range from -14.6 to -32.9 kcal mol^{-1} with TGZ having the lowest value, indicating that all the metabolites of TGZ would be more soluble in water than the parent drug.

CONCLUSIONS

Troglitazone (TGZ) was the first marketed thiazolidinedione used for the treatment of type II diabetes mellitus. Although TGZ offered significant clinical benefits to many diabetic patients, it was withdrawn from the market in 2000 because of idiosyncratic hepatotoxicity. Molecular modelling analyses based on semi-empirical and DFT calculations show that among TGZ and its metabolites, only the metabolite TGZQ has much lower LUMO-HOMO energy difference. The high kinetic lability and the presence of an electron-deficient region on the molecular surface of TGZQ mean that the rates of its reactions with glutathione and nucleobases in DNA would be appreciable so that the metabolite may induce cellular toxicity and damage to DNA.

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