Journal of Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

Journal of Pharmacology and Toxicology 2 (2): 142-150, 2007
ISSN 1816-496X

Molecular Modelling Analysis of the Metabolism of Paracetamol

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Abstract: Paracetamol is probably the most versatile and widely used analgesic and antipyretic drug all over the world and is also one of the commonest means of committing suicide. In humans, high doses of paracetamol can cause hepatotoxicity and sometimes nephrotoxicity. The drug is metabolized by different hepatic pathways to produce metabolites paracetamol sulfate, paracetamol glucuronide, NAPQI, PLCC and PNALCC. of these paracetamol sulfate and paracetamol glucuronide form the largest proportion. The metabolic activation of aspirin is associated with the formation of NAPQI which is highly toxic but is normally detoxified by reaction with glutathione. Molecular modelling analyses based on molecular mechanics, semi-empirical (PM3) and DFT (at B3LYP/6-31G* level) show that among paracetamol and its metabolites, NAPQI has the highest kinetic lability, lower solubility in water and possibly lower thermodynamic stability.

Key words: Paracetamol, paracetamol sulfate, glutathione, necrosis, molecular modelling

INTRODUCTION

Paracetamol (*N*-acetyl-p-aminophenol, 4-hydroxyacetanilide, also called acetaminophen) is probably the most versatile and widely used analgesic and antipyretic drug all over the world (Rocha *et al*., 2005) and is also one of the commonest means of committing suicide. When taken at the therapeutic dose, around 500 mg, the drug is safe being remarkably free from adverse effects, but when taken in overdose (15 to 20 tablets) it causes centrilobular hepatitic necrosis (Devalia *et al*., 1982; James *et al*., 1975). Overdose is in often a common problem in paracetamol use eg repeated self medication with paracetamol tablets possibly along with cold medications which may also contain the drug has lead to fatal overdosage. In normal doses, paracetamol is well absorbed and reaches peak level in blood with 1 h. In healthy adults, elimination half life is about 2 h (Prescott, 1981).

The metabolism of paracetamol is well established and has been studied extensively by NMR spectroscopy (Bales *et al*., 1984). The drug is metabolized by different hepatic pathways but mainly by conjugation with sulfate and glucuronic acid to produce 4-acetamidophenol sulfate and 4-acetamidophenol glucuronide respectively. Only a minor portion is metabolized by oxidation (catalysed by microsomal mono-oxygenases), producing *N*-acetyl-p-benzoquinoneimine (NAPQI). NAPQI, an alkylating agent of thiol groups, is more toxic and normally detoxified by reaction with glutathione (GSH). Alternatively, NAPQI is detoxified by reduction to paracetamol with the formation of GSSG. Thus, the major metabolites of paracetamol at the therapeutic dose level are: paracetamol sulfate, paracetamol glucuronide, paracetamol L-cysteinyl conjugate [more exactly 3-(L-cysteinyl)-4-acetamidophenol] (PLCC) and paracetamol N-acetyl-L-cysteinyl conjugate [more exactly 3-(N-acetyl-L-cysteinyl)-4-acetamidophenol] (PNALCC) (Nicholls *et al*., 1997). Of these, as stated earlier, paracetamol sulfate and paracetamol glucuronide form the largest proportion. Other minor

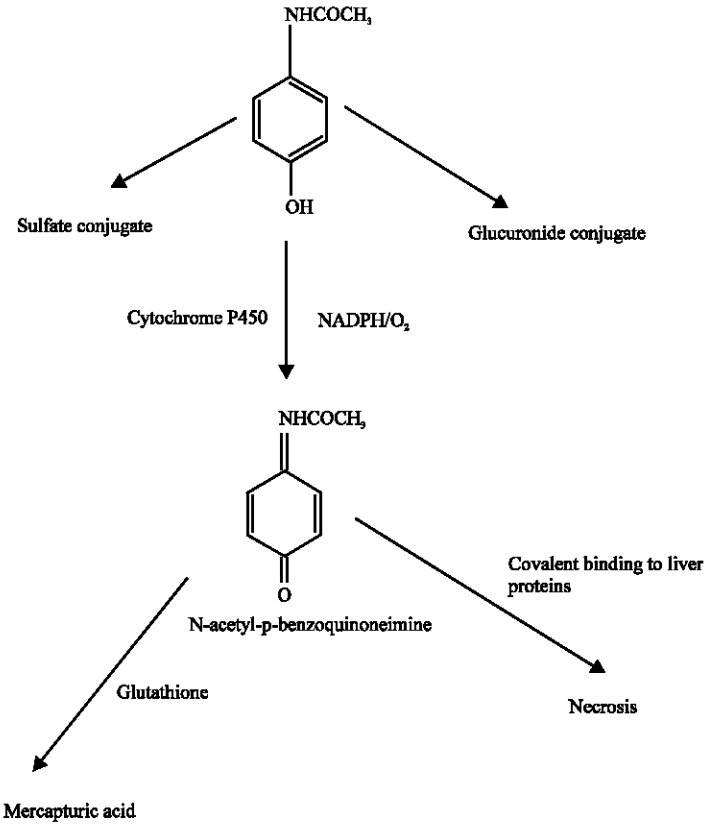


Fig. 1: Schematic representation of hepatitic bioactivation and detoxification of paracetamol (Based on Thomas, 1993)

metabolites may also be observed, usually from drug overdose (Duggin, 1993). Figure 1 gives schematic representation of the hepatitic bioactivation and detoxification of paracetamol. When paracetamol is taken in toxic doses, it causes depletion of heaptitic glutathione thus compromising the antioxidant status of cells (Thomas, 1993). Toxicity due to paracetamol in humans occurs via binding of paracetamol metabolites, as evidenced by 3-(cystein-S-yl) paracetamol adducts whose level in serum increase in proportion to the degree of paracetamol-induced hepatitic damage (Hinson *et al*., 1990). Sometimes renal failure occurs due to paracetamol poisoning in the absence of hepatitic necrosis (Thomas, 1993).

The toxicity of paracetamol is enhanced by factors that cause depletion of glutathione, increase the formation of NAPQ or reduce the antioxidant capacity of liver (Thomas, 1993). For example, a low protein die or pre-treatment with diethyl maleate cause glutathione depletion and enhance toxicity, as do hepatitic P450 inducing agents, including alcohol, phenobarbitone and 3-methylcholanthrene (McLean and Day, 1975). Conversely toxicity due to paracetamol is reduced by glutathione receptors such as cysteine and butylated hydroxyanisol, hepatitic enzyme inhibitors such as piperonyl butoxide (Mitchell *et al*., 1973), antioxidants and inhibitors of lipid peroxidases (Mansuy *et al*., 1986). Currently accepted treatment of paracetamol overdose uses N-acetylcysteine given either orally or intravenously. Provided the antidote is given within 10-12 h of the overdose fatal liver damage may be avoided.

In this study, molecular modelling analyses have been carried out using the programs HyperChem 7.0 (HyperChem, 2002) and Spartan '02 (Spartan, 2002) to obtain information on the relative stability of paracetamol and its metabolites leading a better understanding of the toxicity of the drug.

COMPUTATION METHODS

The geometries of paracetamol and its metabolites paracetamol sulfate, paracetamol glucuronide, NAPQI, PLCC and PNALCC have been optimized based on molecular mechanics, semi-empirical and DFT calculations, using the molecular modelling programs HyperChem 7.0 (2002) and Spartan (2002). No calculations were done for the glucuronides of the compounds. Molecular mechanics calculations were carried out using MM+ force field. Semi-empirical calculations were carried out using the routine PM3. DFT calculations were carried using the program Spartan '02 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 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 minimized the chances of the structures being trapped in local minima rather than reaching global minima. 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, structures corresponding to global minimum or close to that were reached in most cases. Although RMS gradient of 0.001 may not be sufficiently small for vibrational analysis, it is believed to be so for calculations associated with electronic energy levels. For the optimized structures, single point calculations were carried to give heat of formation, enthalpy, entropy, free energy, surface area, volume, dipole moment and solvation energy, HOMO and LUMO. The work was carried out in the School of Biomedical Sciences, The University of Sydney during the period June 2005 to February 2006.

RESULTS AND DISCUSSION

Table 1 gives the total energy, heat of formation as per PM3 calculation, enthalpy, entropy, free energy, dipole moment, energies of HOMO and LUMO as per both PM3 and DFT calculations for paracetamol and its metabolites: paracetamol sulfate, paracetamol glucuronide,

Table 1: Calculated thermodynamic and other parameters for paracetamol 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})
Paracetamol	PM3	-76.83	-65.19	106.44	98.33
	DFT	-515.49		106.54	100.45
Paracetamol	PM3	-214.60	-200.73	133.86	126.72
sulfate	DFT	-1140.45		133.67	126.78
Paracetamol	PM3	-329.41	-308.39	202.70	159.27
glucuronide	DFT	-1200.28		202.73	159.22
NAPQI	PM3	-33.81	-25.33	91.13	100.43
	DFT	-514.23		91.00	100.10
PLCC	PM3	-163.54	-141.00	167.08	145.21
	DFT	-1236.23		167.12	145.02
PNALCC	PM3	-202.82	-178.77	192.14	156.69
	DFT	-1388.85		192.45	156.34
Mercapturic	PM3	-95.16	-76.99	183.24	140.13
acid	DFT	-1259.26		183.12	140.23

Table 1: Continue

Molecule	Calculation type	Solvation energy (kcal mol^{-1} K^{-1})	Free energy (kcal mol^{-1})	Dipole moment (debye)	HOMO (eV)	LUMO (eV)	LUMO-HOMO (eV)
Paracetamol	PM3	-11.63	77.12	3.15	-8.88	-0.08	8.80
	DFT	-9.99	76.60	4.04	-5.84	-0.42	5.42
Paracetamol	PM3	-13.87	96.08	3.38	-8.81	-0.43	8.38
sulfate	DFT	-18.43	95.89	5.52	-5.67	-0.78	4.89
Paracetamol	PM3	-21.02	161.22	3.01	-9.10	-0.20	8.90
glucuronide	DFT	-16.10	155.28	3.36	-6.07	-0.70	5.37
NAPQI	PM3	-8.49	61.18	2.78	-10.38	-1.72	8.66
	DFT	-6.88	61.15	2.75	-7.04	-3.44	3.60
PLCC	PM3	-22.55	123.78	7.00	-9.21	-0.88	8.33
	DFT	-20.79	123.90	8.55	-6.06	-1.04	5.02
PNALCC	PM3	-24.05	145.42	5.91	-9.00	-0.75	8.25
	DFT	-18.37	145.87	6.65	-5.83	-0.79	5.04
Mercapturic	PM3	-18.17	141.46	4.22	-9.06	-1.08	7.98
acid	DFT	-17.56	141.33	7.28	-6.41	-1.79	4.63

* in atomic unit from DFT calculations

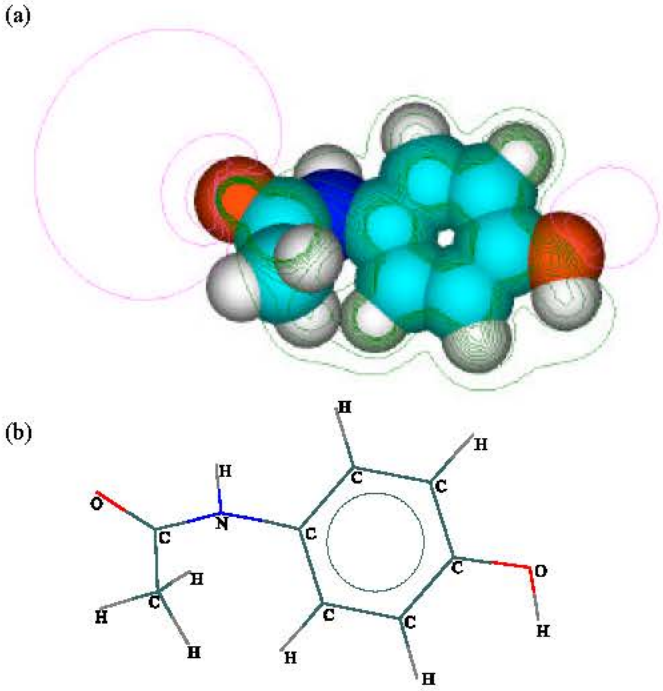
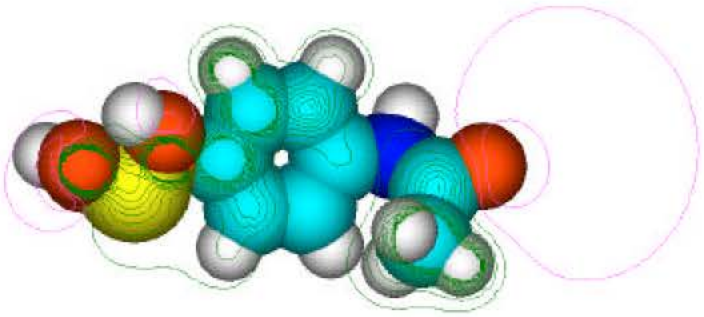


Fig. 2: Structure of paracetamol giving 2D contours of total electrostatic potential in (a) and atoms labelling in (b)

NAPQI, PLCC and paracetamol PNALCC. Figure 2-8 give the optimized structures of paracetamol, paracetamol sulfate and paracetamol glucuronide, NAPQI, PLCC and PNALCC as per PM3 calculations using the program HyperChem 7.0. Among all the metabolites and the parent compound, NAPQI has also the lowest solvation energy, meaning it would be least soluble in water. When the LUMO-HOMO energy differences are compared, it is found that NAPQI has the smallest difference (3.60 eV from DFT calculations for NAPQI as against values of 4.6 to 5.4 eV for the other compounds), indicating that NAPQI would be much more labile kinetically. NAPQI is also found to least negative heat of formation which may

(a)



(a)

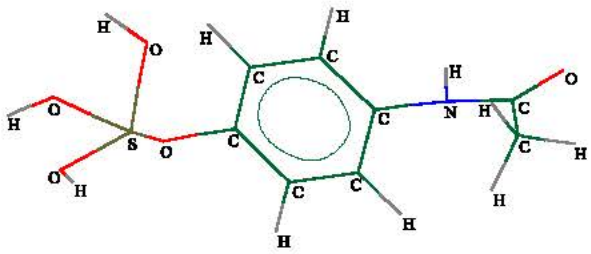
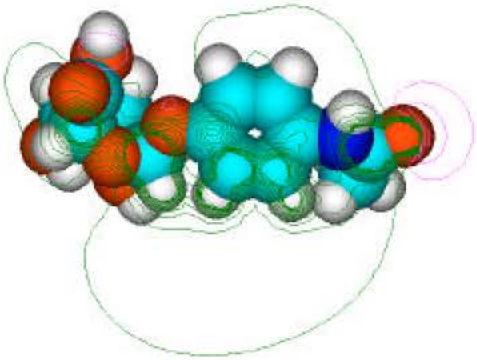


Fig. 3: Structure of paracetamol sulfate giving 2D contours of total electrostatic potential in (a) and atoms labelling in (b)

(a)



(b)

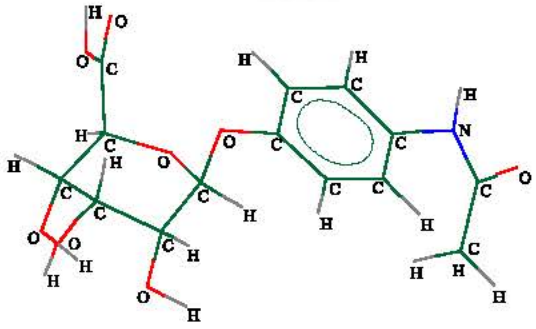


Fig. 4: Structure of paracetamol glucuronide conjugate giving 2D contours of total electrostatic potential in (a) and atoms labelling in (b)

(a)

(b)

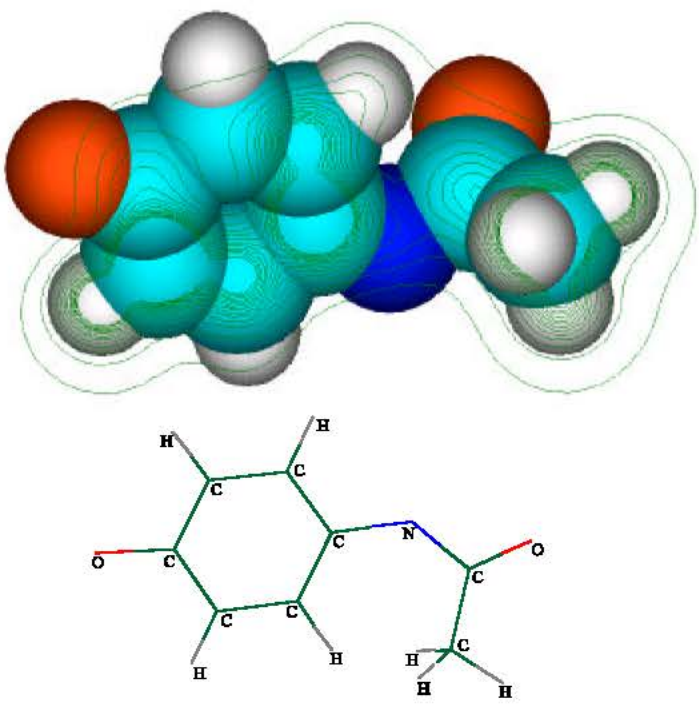


Fig. 5: Structure of NAPQI giving 2D contours of total electrostatic potential in (a) and atoms labelling in (b)

(a)

(b)

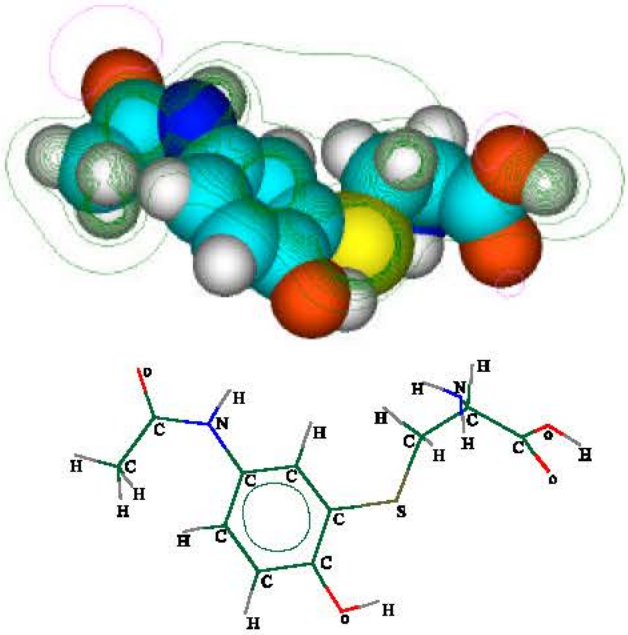


Fig. 6: Structure of PLCC giving 2D contours of total electrostatic potential in (a) and atoms labelling in (b)

(a)

(b)

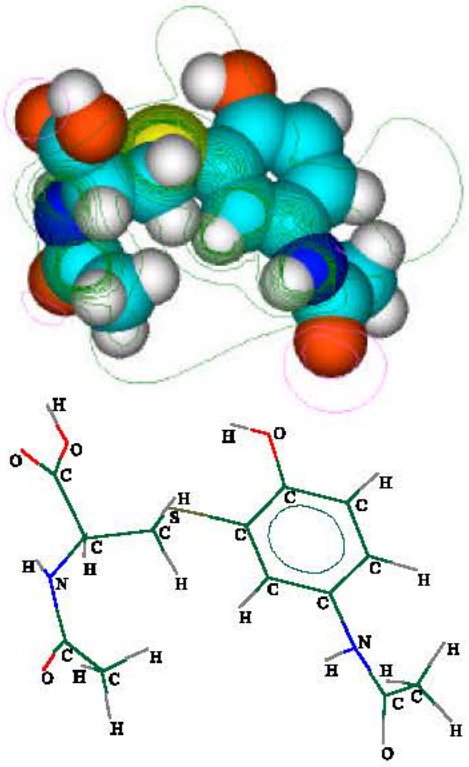


Fig. 7: Structure of PNALCC giving 2D contours of total electrostatic potential in (a) and atoms labelling in (b)

(a)

(b)

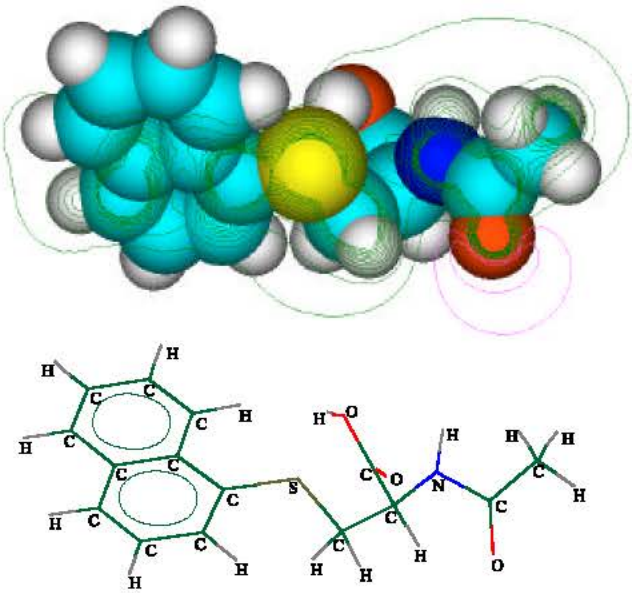
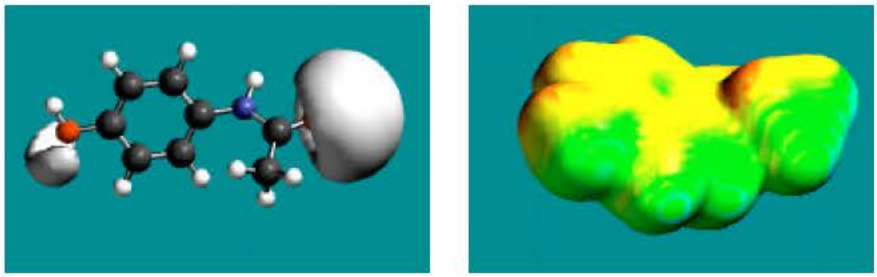
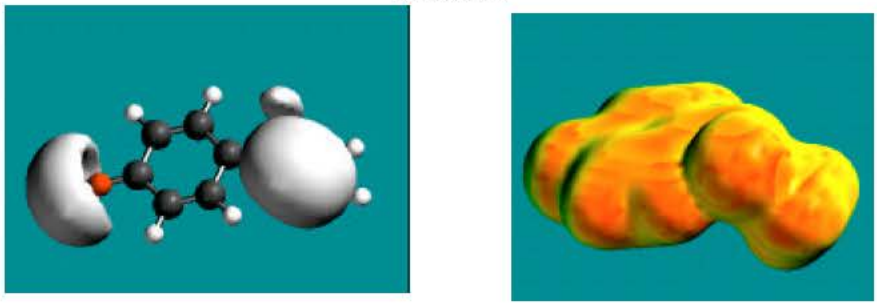


Fig. 8: Structure of mercapturic acid giving 2D contours of total electrostatic potential in (a) and atoms labelling in (b)



Paracetamol



NAPQI

Fig. 9: Electrostatic potential (where the grey envelopes denote regions of negative electrostatic potential) and distribution of surface charges (where green means neutral, red means highly negative and blue means positive) as applied to optimized structures of paracetamol and its most toxic metabolite NAPQI

mean that NAPQI would be least stable thermodynamically. As stated earlier, NAPQI is a reactive electrophile which is an alkylating agent of the thiol groups (Rocha *et al*., 2005; Dahlin *et al*., 1984). It causes depletion of glutathione and it also binds covalently to a variety of hepatic cell proteins (Cohen *et al*., 1997), increasing susceptibility of liver to oxidative stress. It may be noted that free radicals are formed after intoxication with paracetamol (Lores *et al*., 1995) that cause a reduction in the content of hepatitic ascorbic acid (Rocha *et al*., 2005). Thus it has been suggested that the administration of ascorbic acid should be considered for the treatment of acute paracetamol intoxication. It can be seen that NAPQI has the highest heat of formation and the lowest LUMO-HOMO separation (as per DFT calculations) meaning that the metabolite has the lowest thermodynamic and kinetic stability. Electrostatic potential contours show that both carbonyl oxygen atoms are strong electrophilic sites in NAPQI.

As expected, the terminal metabolites paracetamol sulfate, paracetamol glucuronide and PLALCC all are found to have high solvation energy, in agreement with their high solubility in water.

Figure 9 gives the electrostatic potential (where the grey envelopes denote regions of negative electrostatic potential) and distribution of surface charges (where green means neutral, red means highly negative and blue means positive) as applied to optimized structures of paracetamol and its most toxic metabolite NAPQI. It can be seen that the visible surface of NAPQI has a greater negative charge than that of paracetamol even though the solvation energy of paracetamol is greater than that of NAPQI. It was noted earlier that NAPQI is highly electrophilic in nature which means that there should be positively charged regions on the other side of NAPQI (which is in fact found on the other side of the molecule; not shown) or electrophilic attack may be preceded by electronic excitation of NAPQI.

CONCLUSIONS

Paracetamol is probably the most versatile and widely used analgesic and antipyretic drug all over the world and is also one of the commonest means of committing suicide. In humans, high doses of paracetamol can cause hepatotoxicity and sometimes nephrotoxicity. The drug is metabolized by different hepatic pathways to produce paracetamol sulfate, paracetamol glucuronide, NAPQI, PLCC and PNALCC. The metabolic activation of aspirin is associated with the formation of NAPQI which is highly toxic but is normally detoxified by reaction with glutathione. Molecular modelling analyses show that the high toxicity of NAPQI can be explained in terms of its higher kinetic lability, lower solubility in water and possibly lower thermodynamic stability.

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