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Molecular Modelling Analysis of the Metabolism of Codeine

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Abstract: Molecular modelling analyses based on molecular mechanics, semi-empirical (PM3) and DFT (at B3LYP/6-31G* level) calculations show that the metabolite codeinone is more kinetically labile than the parent drug codeine and other metabolites and that it can be subject to nucleophilic attack due to the presence of electron-deficient regions on its molecular surface. This means that the metabolite can react with glutathione thus causing glutathione depletion and can also cause oxidation of nucleobases in DNA thus producing DNA damage. Depletion of glutathione induces oxidative stress as it compromises the anti-oxidant status of the cell.

Key words: Codeine, morphine, norcodeine, molecular modelling

Introduction

Codeine (3-methoxymorphine) is a commonly used opinoid (Caraco *et al*., 1997) prescribed for postoperative pain relief and is frequently recommended for paediatric use (Dolhery, 1999). It has a lower incidence of opioid-related side effects that make it popular for younger age groups including neonates (Williams *et al*., 2002) although there are some doubts about the efficacy and reliability of the drug (Williams *et al*., 2001). There are three types of opioid receptors, termed μ (mu), δ (delta) and κ (kappa), which are expressed in the brain and spinal cord (Chen *et al*., 2005). The effects of codeine are generally similar to those of morphine although it is much less potent e.g., codeine at a dose of 120 mg produces the same effect as that of 10 mg morphine. Codeine potentiates the analgesic activity of both aspirin and paracetamol. When administered in the usual doses of 30 or 60 mg, the most common side-effect is constipation. Some patients experience unpleasant feelings whilst others experience the pleasant ones. Codeine and alcohol are the intoxicants involved in the abuse of cough syrup (Smith and Reynard, 1992).

The main metabolic pathway for codeine is via conjugation with glucuronic acid to form codeine-6-glucuronide. Minor metabolic pathways include O-demethylation to form morphine (M) and N-demethylation to form norcodeine. Approximately 10% of the population lack the enzyme responsible for the conversion of codeine into morphine in the liver (Galbraith *et al*., 2001). Norcodeine can undergo O-demethylation to form normorphine. Norcodeine and morphine also conjugate with glucuronic acid to form corresponding glucuronides. The O-demethylation of codeine is catalysed by CYP2D6. Although the mechanism of codeine analgesia is not fully understood, there is convincing evidence from both animal and human studies that it is wholly or partly due to morphine (Williams *et al*., 2002). Animal and adult human studies have shown that there is a significant variability in both pharmacokinetics and pharmacodynamics of codeine. Over fifty genetic variations are known to exist for CYP2D6 that leads to a wide spectrum of metabolic capabilities within populations (Williams *et al*., 2002). Individuals with dysfunctional allelic variants of CYP2D6 are phenotypically described as poor metabolizers and are less sensitive to codeine (Thompson *et al*., 2004). Figure 1 shows the metabolic pathways of codeine in humans.

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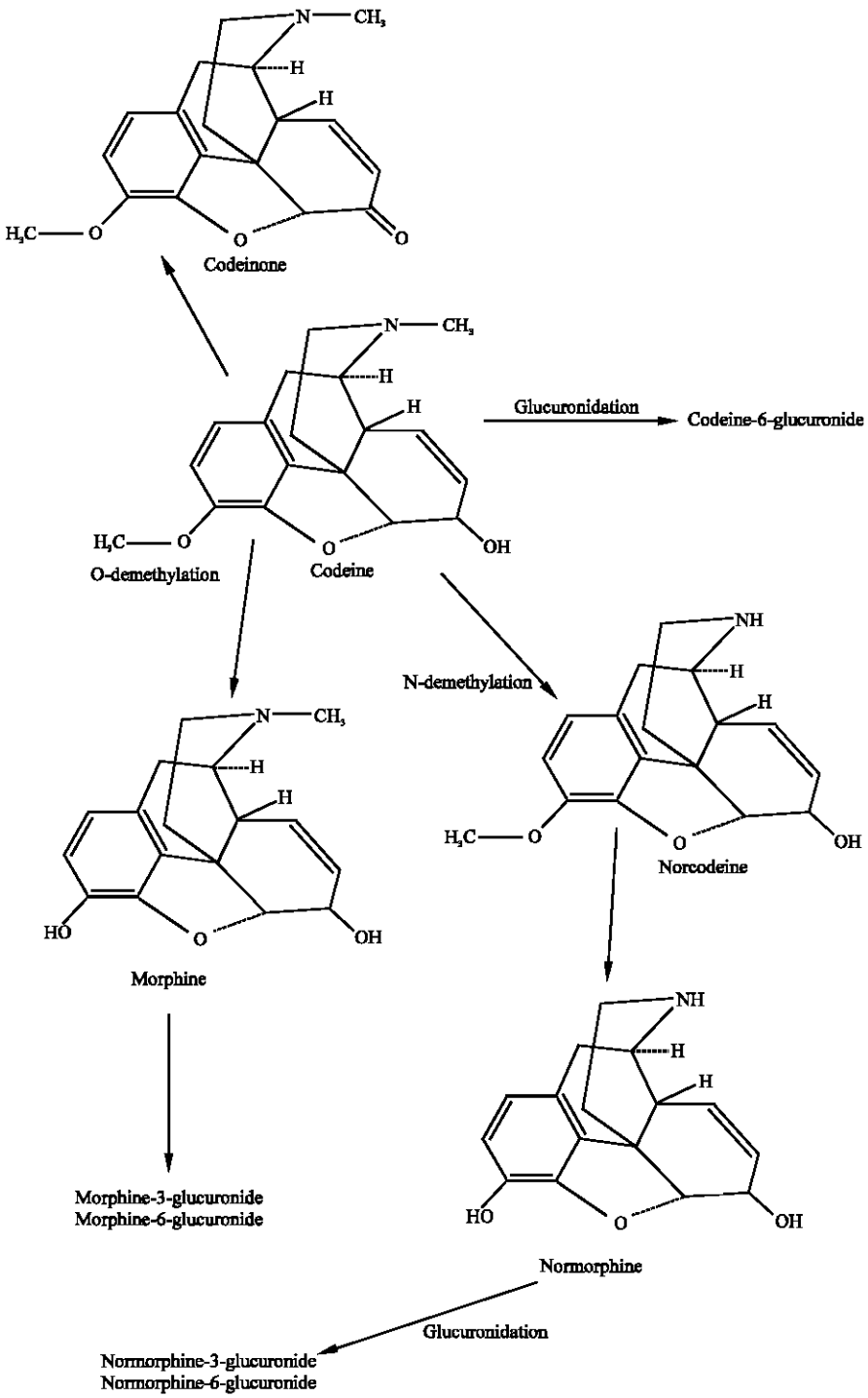


Fig. 1: Metabolic pathways for codeine (Thompson *et al*., 2004)

Although much information is available on the biotransformation of codeine and other opiates, little is known about the toxicity of their metabolites (Jairaj *et al*., 2003). Morphine and codeine are hepatotoxic at high doses in isolated rat hepatocytes (Ellington and Rosen, 1987). Their 6-dehydrogenated metabolites, codeinone and morphinone which have been identified after treatment of guinea pigs with codeine and morphine (Ishida *et al*., 1991, 1998) are likely to be toxic as the metabolites can cause depletion of antioxidant glutathione (Nagamatsu and Hasegawa, 1992). In this study, molecular modelling analyses have been carried out using the programs HyperChem 7.0 (2002) and Spartan '02 (2002) with the aim of providing information on relative toxicity of codeine and its metabolites.

In this study, molecular modelling analyses have been carried out using the programs HyperChem 7.0 (2002) and Spartan '02 (2002) to investigate the relative stability of codeine and its metabolites with the aim of providing information on their toxicity.

Materials and Methods

The geometries of codeine, norcodeine, normorphine, normorphine-3-glucuronide, codenine-6-glucuronide,morphine-3-glucuonide,morphine-6-glucuonide,normorphine-6-glucuonideandcodeinone have been optimised based on molecular mechanics, semi-empirical and DFT calculations, using the molecular modelling programs Spartan '02 and HyperChem 7.0. 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. For the optimised structures, single point calculations were carried to give heat of formation, enthalpy, entropy, free energy, dipole moment and solvation energy, energies of 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 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 sufficiently low for calculations associated with electronic energy levels. The study was carried out in the School of Biomedical Sciences, The University of Sydney during the period November 2005 to March 2006.

Results and Discussion

Table 1 gives the total energy, heat of formation as per PM3 calculation, enthalpy, entropy, free energy, dipole moment, surface area, volume, energies of HOMO and LUMO as per both PM3 and DFT calculations for codeine, norcodeine, normorphine, normorphine-3-glucuronide, codenine-6-glucuronide,morphine-3-glucuonide,morphine-6-glucuonide,normorphine-6-glucuonideandcodeinone.

Figure 2-6 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 surface charges (where red indicates negative, blue indicates positive and green indicates neutral) in (d) as applied to the optimised structures of codeine and four selected metabolites morphine, norcodeine, normorphine and codeinone.

The solvation energy of codeine is reasonably large and that of all the metabolites are even larger so that codeine and all its metabolites can be easily excreted via urine. The metabolite that has solvation energy closest to that of codeine is codeinone formed from the oxidation of hydroxyl group. As expected, norcodeine and morphine formed from codeine due to N- and O-demethylation, respectively have higher solvation energies than codeine. As noted earlier, the major metabolite is codeine-6-glucuronide that is expected to be excreted quite readily.

The heats of formation in kcal mol^{-1} of codeine and codeine-6-glucuronide as per PM3 calculations are, respectively -68.72 and -314.83. The large difference in heats of formation of codeine

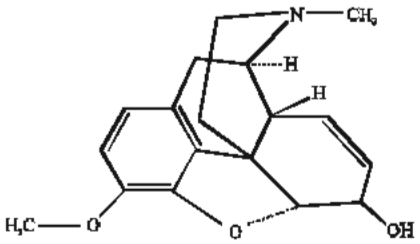
Table 1: Calculated thermodynamic and other parameters for morphine and its metabolites ('DM' stands for dipole moment)

Molecule	Calculation type	Total energy (kcal mol^{-1}/ atomic unit*)	ΔH_f (kcal mol^{-1})	Enthalpy (kcal mol^{-1})	Entropy (cal mol^{-1} K^{-1})	Solvation energy (kcal mol^{-1})	Free energy (kcal mol^{-1})
Codeine	PM3	-77.26	-68.72	236.35	133.15	-8.54	196.66
	DFT	-978.92		238.44	134.86	-9.23	198.25
Norcodeine	PM3	-78.79	-67.19	218.65	125.89	-11.60	181.12
	DFT	-939.61		219.34	126.23	-11.98	181.72
Morphine	PM3	-0.50	14.43	218.17	122.01	-14.94	181.79
	DFT	-939.48		232.46	123.86	-15.40	195.55
Normorphine	PM3	-0.08	17.48	200.21	116.62	-17.40	165.44
	DFT	-901.40		212.44	117.34	-18.80	177.47
Normorphine-3-glucuronide	PM3	-278.33	-253.84	335.87	182.45	-24.48	281.45
	DFT	-1586.18		324.45	178.45	-31.22	271.27
Codeine-6-glucuronide	PM3	-331.62	-314.83	339.11	191.45	-16.79	282.04
	DFT	-1663.70		333.56	188.12	-20.45	277.50
Morphine-3-glucuronide	PM3	-278.33	-253.84	335.87	182.45	-24.48	281.45
	DFT	-1625.48		338.56	183.44	-26.43	283.89
Morphine-6-glucuronide	PM3	-279.63	-255.68	335.37	185.34	-23.95	280.11
	DFT	-1625.49		342.85	187.23	-25.23	287.01
Norcodeine-6-glucuronide	PM3	-327.77	-310.19	321.14	181.21	-17.58	267.12
	DFT	-1624.38		322.87	182.23	-17.98	268.57
Codeinone	PM3	24.24	34.89	220.98	129.16	-9.65	182.47
	DFT	-977.59		222.02	129.87	-10.23	183.32

Molecule	Calculation type	DM (debye)	Surface area (Å^2)	Volume (Å^3)	HOMO (eV)	LUMO (eV)	LUMO-HOMO(eV)
Codeine	PM3	2.08	301.23	302.03	-8.71	0.16	8.87
	DFT	2.47	298.95	301.52	-5.42	0.09	5.51
Norcodeine	PM3	1.91	282.46	282.49	-8.74	0.13	8.87
	DFT	2.02	280.83	282.41	-5.45	0.06	5.51
Morphine	PM3	2.32	275.43	280.43	-8.98	-0.06	8.92
	DFT	1.67	272.83	276.70	-5.73	-1.52	4.21
Normorphine	PM3	1.60	258.88	261.43	-9.00	-0.07	8.93
	DFT	2.15	265.08	266.70	-5.49	0.14	5.63
Normorphine-3-glucuronide	PM3	9.48	412.86	407.12	-9.02	-0.10	8.92
	DFT	5.69	408.69	406.10	-5.82	-0.17	5.65
Codeine-6-glucuronide	PM3	5.36	447.25	442.89	-8.87	-0.00	8.77
	DFT	3.75	433.23	440.24	-5.68	-0.28	5.40
Morphine-3-glucuronide	PM3	9.48	421.58	424.66	-9.32	-0.27	9.05
	DFT	11.19	421.30	425.27	-5.73	-0.3	5.42
Morphine-6-glucuronide	PM3	6.82	431.93	426.98	-9.09	-0.15	8.94
	DFT	8.52	423.65	425.09	-5.67	-0.26	5.41
Norcodeine-6-glucuronide	PM3	4.72	421.60	422.23	-9.05	-0.11	8.94
	DFT	5.18	421.53	422.11	-5.64	-0.20	5.44
Codeinone	PM3	3.11	291.59	296.19	-9.23	-0.37	8.83
	DFT	3.33	293.05	296.78	-5.68	-1.58	4.10

* in atomic units from DFT calculations

and codeine-6-glucurnide suggest that the conversion of codeine to codeine-6-glucuronide may be spontaneous although a firm conclusion can only be drawn when Gibb's energy change for the conversion is calculated taking into consideration all reactants and products. It should however be noted whether a reaction is spontaneous or not may not be a critical factor in biological systems where reactions are often coupled such that the Gibb's free energy for the overall process is negative.



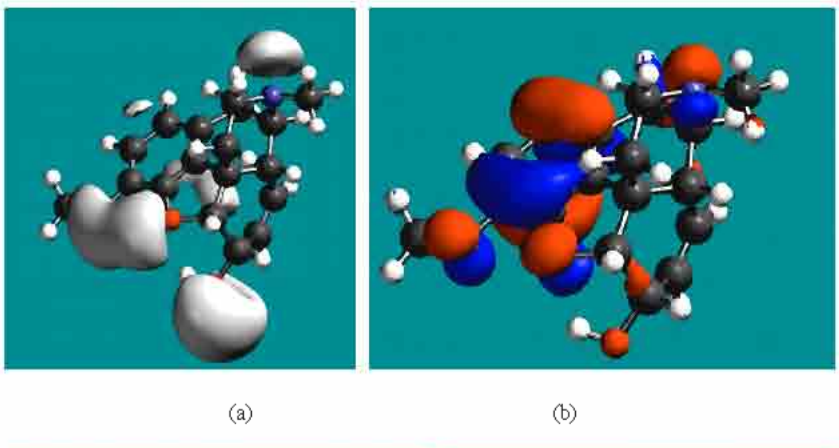
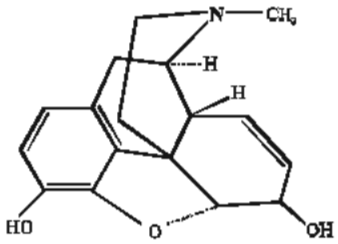


Fig 2 Structure of codeine giving in (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) surface electric charges (where red indicates negative,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



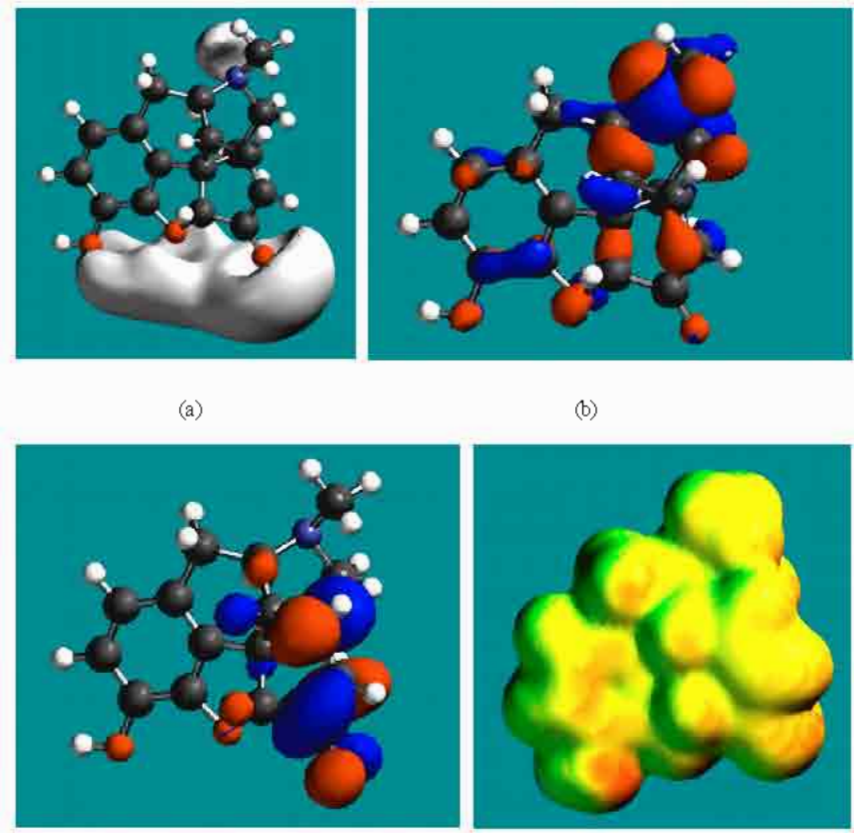
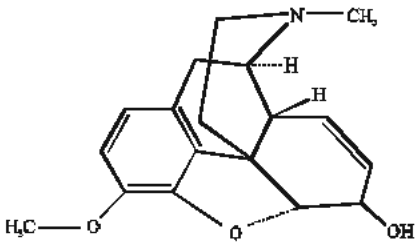


Fig 3 Structure of morphine giving in (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) surface electric charges (where red indicates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



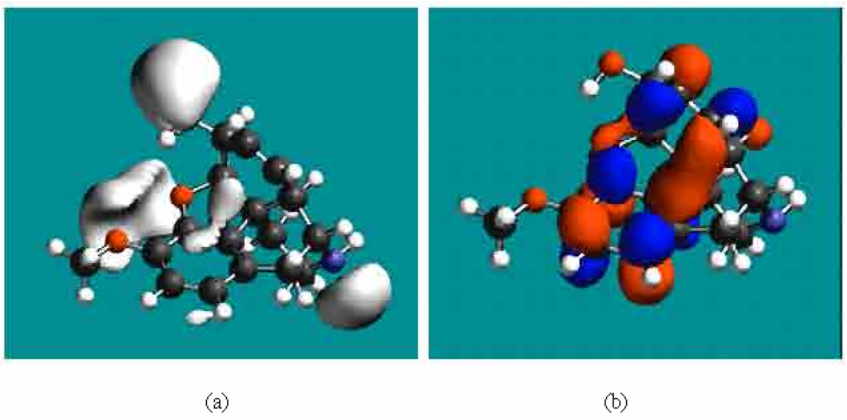
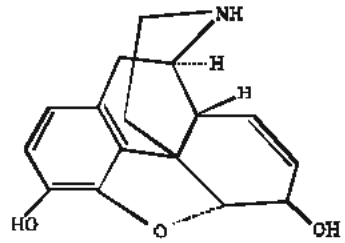
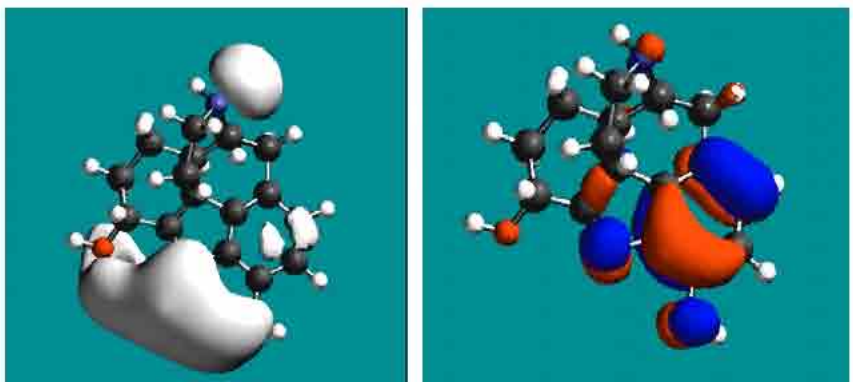
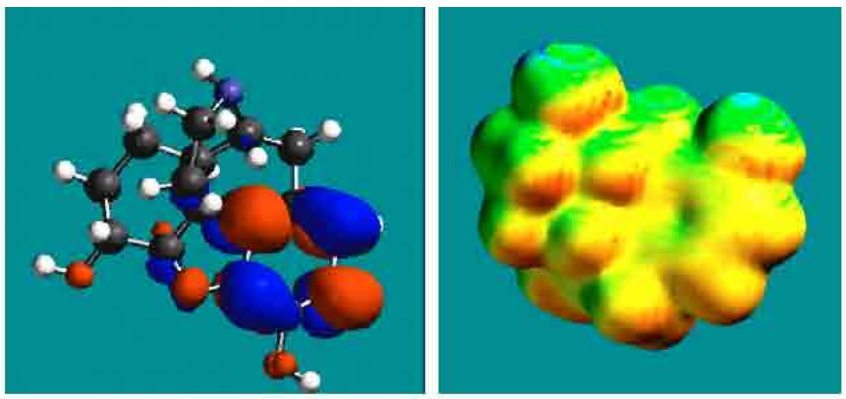


Fig 4 Structure of norcodeine giving in (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) surface electric charges (where red indicates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





(a) (b)



(c) (d)

Fig 5 Structure of normorphine 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) surface electric charges (where red indicates negative, blue indicates positive and green indicates neutral)

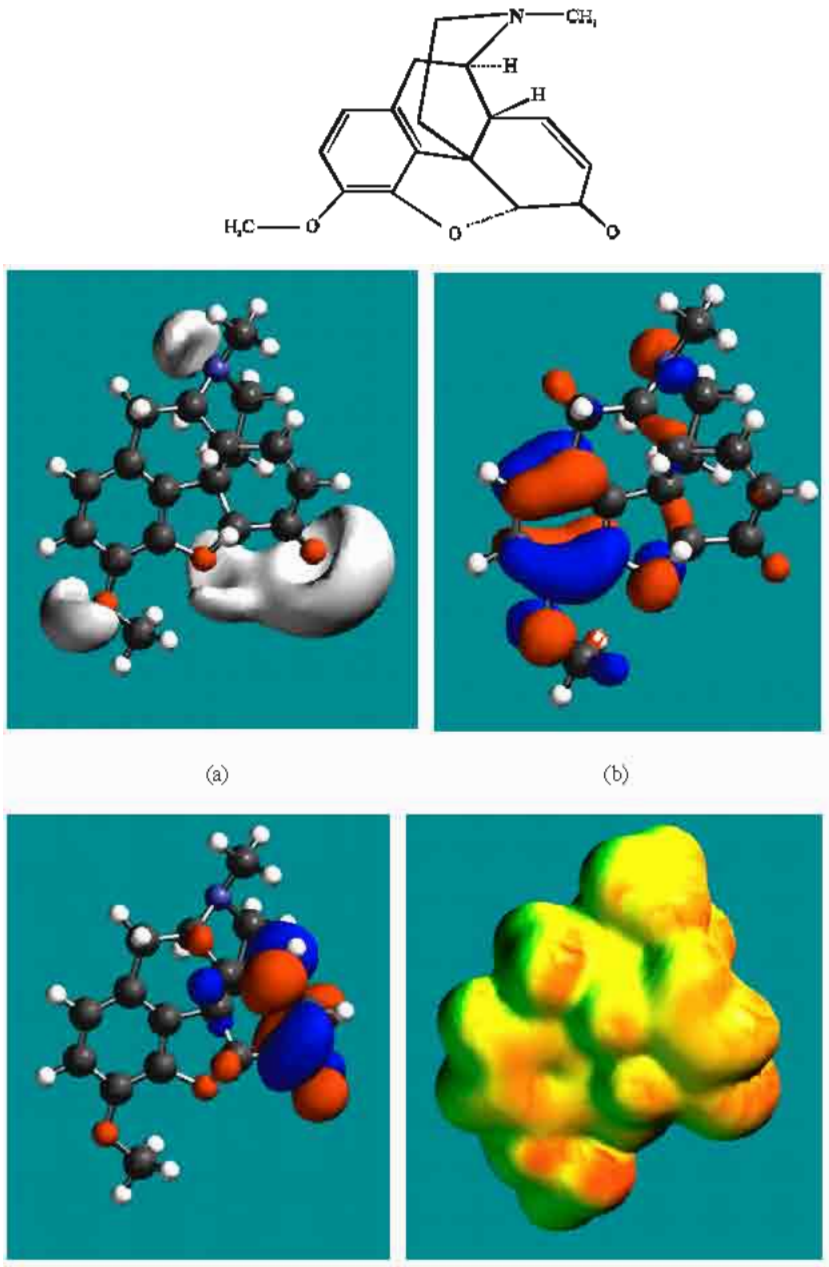


Fig 6 Structure of codeinone giving in (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) surface electric charges (where red indicates negative, blue indicates positive and green indicates neutral)

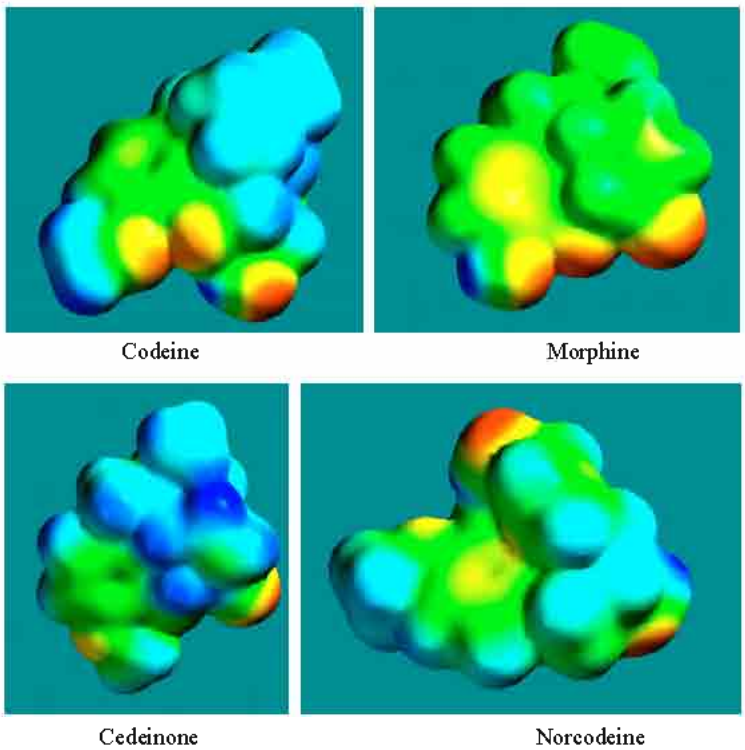


Fig. 7: Density of electrostatic potentials on the surfaces of codeine, morphine, codeinone and norcodeine where red stands for large negative, blue for large positive and green for neutral

The LUMO-HOMO energy differences for codeine and most of its metabolites as per DFT calculations are of the order of 5.5 eV except for codeinone which has a significantly smaller value of 4.1 eV. The smaller LUMO-HOMO energy difference suggests that codeinone would be significantly more kinetically labile (although not extremely labile) than codeine and other metabolites. The higher kinetic lability of codeinone means that it can bind more readily with the cellular antioxidant glutathione, thus causing its depletion and hence compromising the antioxidant status of the cell. This is particularly so as the molecular surface of codeinone has sites that may be more likely to be subject to nucleophilic attack (considered more fully later).

In the case of codeine, morphine, norcodeine, normorphine and codeinone the electrostatic potential is found to be more negative around oxygen and nitrogen centres, indicating that the positions may be subject to electrophilic attack.

In the case of codeine, HOMOs with large electron density are found to be centred on the oxygen atom of the five-membered ring and two carbon atoms of the aromatic ring and in the case of morphine, HOMOs with large electron density are found to be centred on the hydroxy oxygen, the tertiary nitrogen and a number of carbon atoms of the aromatic ring. In the case of norcodeine, HOMOs with large electron density are found to be centred on a number of carbon atoms and the hydroxyl oxygen atoms. In the case of codeinone also, HOMOs with high electron density surround a number of atoms including both oxygen and carbon atoms.

The molecular surfaces of morphine, normorphine and codeinone are found to have greater negative charges than that those of codeine and norcodeine indicating that the morphine, normorphine and codeinone may be subject to electrophilic attack more readily than codeine and norcodeine (Fig. 2-6). However, codeinone may also be subject to nucleophilic attack as evident from the density of electrostatic potential plot given in Fig. 7 where red stands for large negative, blue for large positive

and green for neutral. It can be seen that although codeine and its three selected metabolites morphine, codeinone and norcodeine, have defined regions of negative electrostatic potential (that was considered earlier) the reactive metabolite codeinone has also got more of defined positive electrostatic potential regions where it can undergo nucleophilic attack by electron-rich molecules such as reduced glutathione and nucleobases present in DNA. It is thus suggested that the toxicity of codeine is mediated at least in part via the reaction of codeinone with glutathione and nucleobases.

Conclusions

Molecular modelling analyses show that codeine and its metabolites have moderate to large solvation energy values indicating that the compounds would have moderate or high solubility in water and hence can be easily excreted via urine. The metabolite codeinone has a much smaller LUMO-HOMO energy difference so that the compound would be more kinetically labile. The higher reactivity of codeinone and the presence of localized electron-deficient regions on its molecular surface indicate that it will react more readily with biomolecules including glutathione, thus compromising anti-oxidant status of the cell.

References

Caraco, Y., J. Sheller and A.J. Wood, 1997. Pharmacogenetic determinants of codeine induction by rifampin: The impact on codeine's respiratory, psychomotor and miotic effects. J. Pharmacol. Exp. Ther., 281: 330-336.

Chen, S.-L., E.Y.-K. Huang, L.-H. Chow and P.-L. Tao, 2005. Dextromethorphan differentially affects opioid antinociception in rats. Br. J. Pharmacol., 144: 400-404.

Dolhery, C., 1999. Therapeutic Drugs. 2nd Edn., Churchill Livingstone, Edinburgh, C317.

Ellington, S.P. and G.M. Rosen, 1987. Codeine-mediated hepatotoxicity in isolated rat hepatocytes. Toxicol. Pharmacol., 90: 156-165.

Galbraith, A., S. Bullock and E. Manias, 2001. Fundamentals of Pharmacology. 3rd Edn., Prentice Hall, pp: 363.

HyperCube HyperChem, Release 7 for Window, 7.0 Edn., HyperCube, Edn., 2002.

Ishida, T., M. Yano and S. Toki, 1991. *In vivo* formation of codeinone and morphinone from codeine. Isolation and identification from guinea pig bile. Drug Metabol. Dispos., 19: 895-899.

Ishida, T., M. Yano and S. Toki, 1998. *In vivo* formation of codeinone-glutathione adduct: Isolation and identification of a new metabolite in the bile of codeine-treated guinea pig. J. Anal. Toxicol., 22: 567-572.

Jairaj, M., D.G. Watson, M.H. Grant and G.G. Skellern, 2003. The toxicity of opiates and their metabolites in HepG2 cells. Chem. Biol. Interact., 146: 121-129.

Nagamatsu, K. and A. Hasegawa, 1992. Covalent binding of morphine to isolated rat hepatocytes. Biochem. Pharmacol., 43: 2631-2635.

Smith, C.M. and A.M. Reynard, 1992. Textbook of Pharmacology. Saunders, Philadelphia, USA.

Spartan '02, 2002. Wavefunction, Inc. Irvine, CA, USA, 2002.

Thompson, C.M., H. Wojno, E. Greiner, E.L. May, K.C. Rice and D.E. Selley, 2004. Activation of G-Proteins by morphine and codeine congenes: Insights to the relevance of O- and N-demethylated metabolites at μ- and δ-Opioid receptors. J. Pharmacol. Exp. Ther., 308: 547-554.

Williams, D.G., D.J. Hatch and R.F. Howard, 2001. Codeine phosphate in paediatric medicine. Br. J. Anaes., 86: 413-421.

Williams, D.G., A. Patel and R.F. Howard, 2002. Pharmacogenetics of codeine metabolism in an urban population of children and its implications for analgesic reliability. Br. J. Anaes., 89: 839-845.