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Molecular Modelling Analysis of the Metabolism of Naltrexone

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Abstract: Naltrexone (NTX) is a potent opioid antagonist that has been used in the treatment of alcohol dependence and opioid addiction. In humans, it has a plasma half-life of 2-14 h and volume distribution of 15 L kg⁻¹. NTX therapy is associated with a number of gastrointestinal adverse effects including abdominal pain, nausea and vomiting, thus limiting its clinical utility. A major disappointment has been the poor patient compliance with the therapy. Reasons for non-adherence include poor motivation, cognitive impairment and the adverse effects of the drug. Molecular modelling analyses based on molecular mechanics, semi-empirical (PM3) and DFT (at B3LYP/6-31G* level) calculations show that NTX, NTXOL and HMNTXOL have high LUMO-HOMO energy differences so that they would be kinetically inert. The presence of electron-deficient regions on the molecular surface indicates that the compounds can react with cellular glutathione, thus causing glutathione depletion and hence oxidative stress. Comparable surface area and volume for NTX and NTXOL indicates that the two compounds can be substrates for the same binding sites in opioid receptors.

Key words: Naltrexone, opioid antagonist, alcohol dependence, molecular modelling

Introduction

Naltrexone (NTX, N-cyclopropylmethylnoroxymorphone) is a potent opioid antagonist (for μ -, κ - and δ -receptors) that is widely used in the treatment of alcohol dependence and opioid addiction (Volpicelli *et al.*, 1992; Takemori and Portgese, 1992; Veebey and Mule, 1975). In laboratory setting, NTX has been found to decrease craving for alcohol in both social (Davidson *et al.*, 1996) and heavy drinkers (Davidson *et al.*, 1999). A major disappointment of NTX has been the poor patient compliance with the therapy (Pillali *et al.*, 2004). Reasons for non-adherence include poor motivation, cognitive impairment and the adverse effects such as abdominal pain, nausea and vomiting, that limit its clinical utility (Kranzler *et al.*, 2000). (Dunbar *et al.*, 2006). The ability of alcohol to disrupt behavioural control and an individual's ability to recognize that he/she has an illness requiring treatment may also be a reason for non-compliance (Dunbar *et al.*, 2006).

The drug has a high extraction ratio in the liver and is subjected to first pass metabolism (Meyer *et al.*, 1984; Bertolotti *et al.*, 1997). Its bioavailability after oral administration ranges from 5 to 40%. Although neurochemical mechanisms underlying the attenuation of ethanol intake by opioid receptor antagonists have not been clearly defined, interaction with dopaminergic neurotransmission system seems to play an important role (Lee *et al.*, 2005). The major metabolite of NTX is 6- β -naltrexol (NTXOL) in humans and several other animal species but not in rodents (Misra *et al.*, 1976; Meyer *et al.*, 1984). It is formed by rapid reduction of NTX. Following oral administration of 100 mg of NTX in humans, plasma concentration of NTX was found to range from 2 to 20 $\mu\text{g L}^{-1}$ while that

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of NTXOL ranged from 75 to 200 $\mu\text{g L}^{-1}$ (Meyer *et al.*, 1984) so that the plasma level of NTXOL is approximately 2- to 3-fold greater than that of NTX (Meyer *et al.*, 1984). NTXOL is also an opioid receptor antagonist although it is about hundred times less potent than NTX at the μ -receptor (Chatterjee and Inturrisi, 1975). Another metabolite of NTX is 2-hydroxy-3-O-methylnaltrexol (HMNTXOL) (Wall and Perez-Reyes, 1984). All the three compounds can form glucuronides.

In this study, molecular modelling analyses have been carried out using the program Spartan (2002) to investigate the relative stability of NTX and its metabolites with the aim of providing a better understanding on their relative toxicity.

Computational Methods

The geometries of NTX and its metabolites have been optimised based on molecular mechanics (Fig. 1), semi-empirical and DFT calculations, using the molecular modelling program Spartan '02. 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

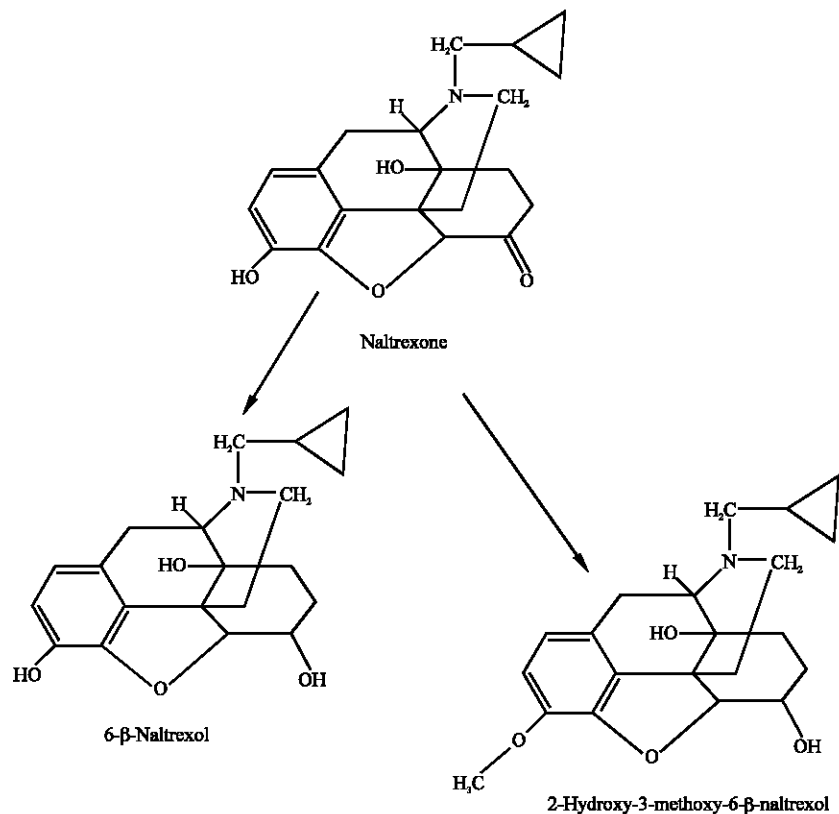


Fig. 1: Metabolic pathways for naltrexone (Ramenskaya *et al.*, 2005)

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.

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 NTX and its metabolite NTXOL and HMNTXOL. Figure 2-4 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 surface charges (where red indicates negative, blue indicates positive and green indicates neutral) in (d) as applied to the optimised structures of NTX, NTXOL and HMNTXOL.

The calculated solvation energies of NTX, NTXOL and HMNTXOL from PM3 calculations in kcal mol⁻¹ are, respectively -13.96, -13.95 and -13.81 and their dipole moments from DFT calculations are 7.0, 5.4 and 5.0, respectively. The values suggest that NTX, NTXOL and HMNTXOL would have similar solubility in water. NTX, NTXOL and HMNTXOL are also found to have similar LUMO-HOMO energy differences, indicating that the compounds would have similar kinetic lability.

In the case of NTX, NTXOL and HMNTXOL the electrostatic potential is found to be more negative around various oxygen atoms such as hydroxyl, carbonyl and ethereal oxygen atoms, indicating that the positions may be subject to electrophilic attacks.

In the case of NTX, NTXOL and HMNTXOL both HOMOs with high electron density and the LUMOs are found centred mostly on the non-hydrogen atoms of the phenyl ring and the ethereal oxygen atom.

The overlap or close proximity of positions of HOMOs with high electron density and those of negative electrostatic potential give further support to the idea that the positions may be subject to electrophilic attacks.

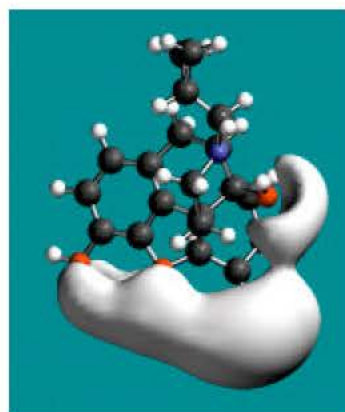
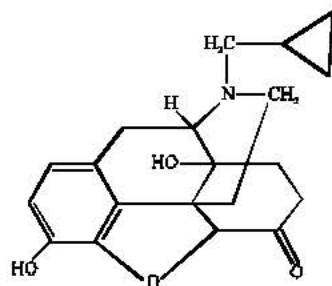
When the electron densities of electrostatic potential on molecular surfaces of NTX, NTXOL and HMNTXOL (Fig. 2d-4d) are considered, it can be seen that all the three compounds have red negative, blue positive and green neutral regions, indicating that the compounds may be subject to electrophilic, nucleophilic and hydrophobic interactions. Nucleophilic attack means that the compounds may react

Table 1: Calculated thermodynamic and other parameters of naltrexone and its metabolites NTXOL and HMNTXOL

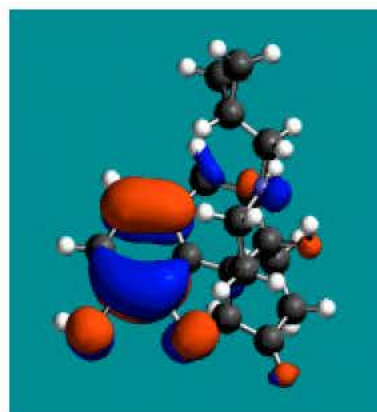
Molecule	Calculation type	Total energy (kcal mol ⁻¹ / atomic unit*)	Heat of formation (kcal mol ⁻¹)	Enthalpy (kcal mol ⁻¹ K ⁻¹)	Entropy (cal mol ⁻¹ K ⁻¹)	Solvation energy (kcal mol ⁻¹ K ⁻¹)
NTX	PM3	-79.49	-65.53	263.25	140.06	-13.96
	DFT	-1131.51		264.63	139.59	-15.25
NTXOL	PM3	-92.97	-79.03	278.10	141.63	-13.95
	DFT	-1132.71		278.99	140.71	-15.22
HMNTXOL	PM3	-71.19	-57.38	312.43	153.30	-13.81
	DFT	-1211.30		313.53	152.21	-15.13

Molecule	Calculation type	Free energy (kcal mol ⁻¹)	Area (Å ²)	Volume (Å ³)	Dipole moment (debye)	HOMO (eV)	LUMO (eV)	LUMO-HOMO (eV)
NTX	PM3	221.50	328.78	335.42	6.6	-9.00	-0.12	8.88
	DFT	223.03	330.84	335.85	7.0	-5.71	-0.27	5.44
NTXOL	PM3	235.85	335.05	340.28	5.3	-8.91	-0.01	8.90
	DFT	237.06	337.30	340.75	5.4	-5.55	0.07	5.62
HMNTXOL	PM3	266.73	365.11	375.35	5.3	-8.90	0.05	8.95
	DFT	268.17	365.11	375.35	5.0	-5.56	0.06	5.62

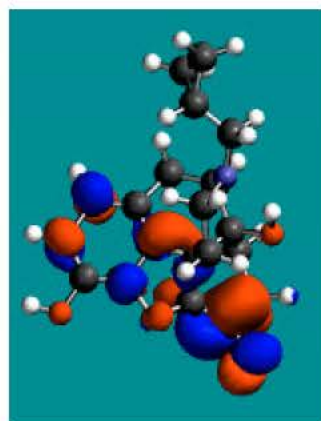
* In atomic units from DFT calculations



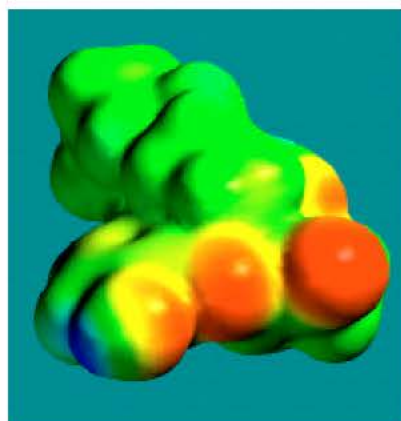
(a)



(b)

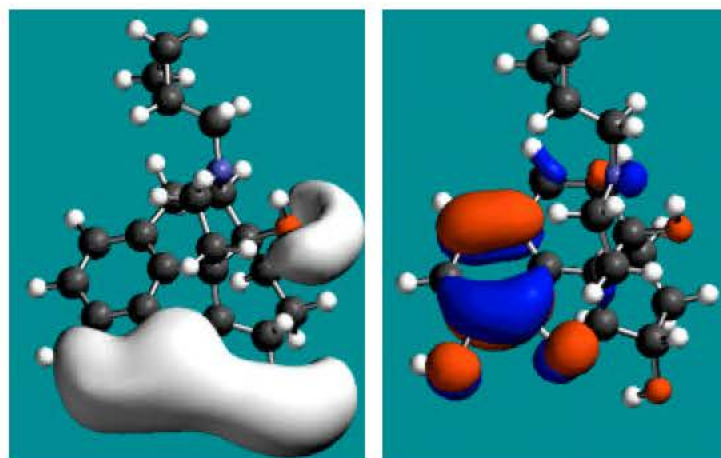
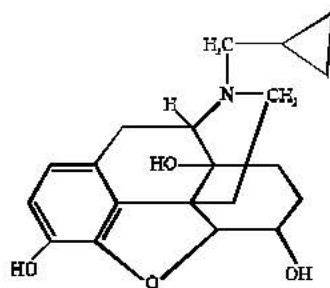


(c)



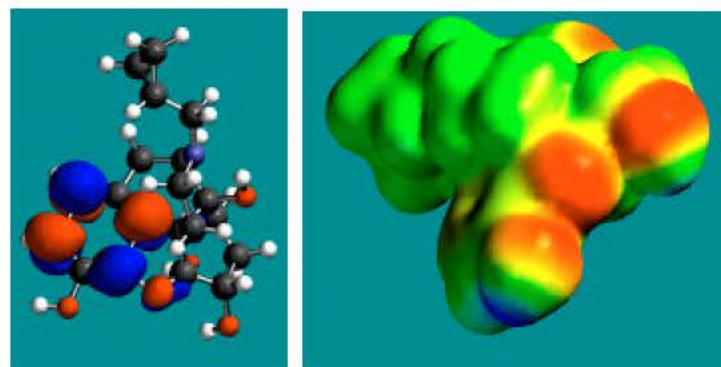
(d)

Fig. 2: Structure of NTX 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. 3: Structure of NTXOL 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)

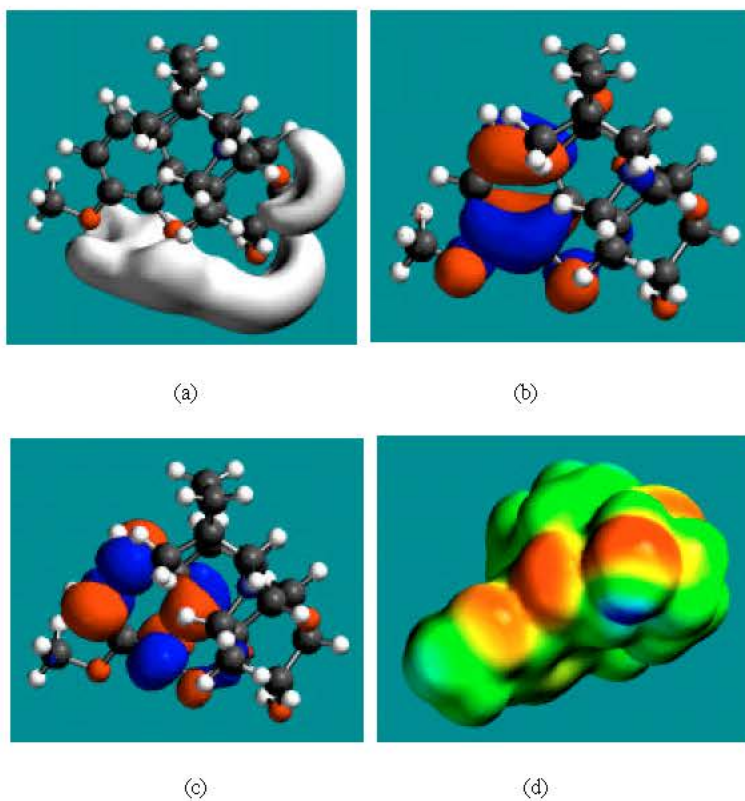
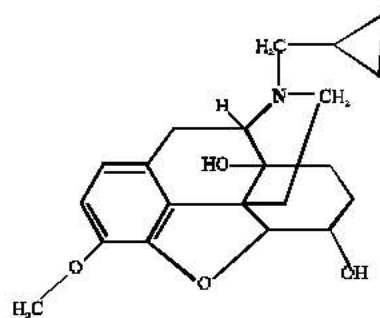


Fig. 4: Structure of HMTXOL giving in: (a) the electrostatic potential (greyish envelope denotes negative electrostatic potential), (b) the HOMOs, (where red indicates HOMOs with high electron density) (c) the LUMOs (where blue indicates LUMOs) and in (d) density of electrostatic potential on the molecular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

with cellular glutathione, thus causing glutathione depletion and hence compromising the anti-oxidant status of the cell thereby inducing cellular toxicity. When the surface areas (330.84 \AA^2 for NTX, 337.30 \AA^2 for NTXOL and 365.11 \AA^2 for HMNTXOL from DFT calculations) and volumes (335.85 \AA^3 for NTX, 340.75 \AA^3 for NTXOL and 375.35 \AA^3 for HMNTXOL from DFT calculations) are considered, it is found that the values for HMNTXOL are distinctly different from those of NTX and NTXOL which are found to be similar so that NTX and NTXOL (but HMNTXOL) can be substrates for the same binding sites.

Conclusion

Molecular modelling analyses based on molecular mechanics, semi-empirical and DFT calculations show that NTX, NTXOL and HMNTXOL can all bind to cellular glutathione, thus causing its depletion and hence producing oxidative stress. However the compounds are found to be kinetically inert so that the rates of such reactions are expected to be low. Comparable surface areas and volumes for NTX and NTXOL indicate that both the compounds can be substrates for the same binding sites in opioid receptors.

Abbreviations

NTX	Naltrexone (N-cyclopropylmethylnoroxymorphone)
NTXOL	6- β -Naltrexol
HMNTXOL	2-Hydroxy-3-O-methylnaltrexol
DFT	Density functional theory
LUMO	Lowest unoccupied molecular orbital
HOMO	Highest occupied molecular orbital

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