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Molecular Modelling Analysis of the Metabolism of Aspirin

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In this study, molecular modelling analyses using molecular mechanics, semi-empirical and DFT calculations have been carried out to investigate the relative stability of aspirin and its metabolites. The results support the idea that the metabolites of aspirin, especially salicyluric acid, can be more toxic than the parent compound because of higher reactivity. It may cause glutathione depletion, thus compromising the antioxidant status of the cell.

Key Words: Aspirin, Salicylic acid, Salicyluric acid, Toxicity, Molecular modelling.

INTRODUCTION

Acetyl salicylic acid, commonly known as aspirin, is one of the most widely used minor analgesics[1]. Aspirin and its metabolite salicylic acid may also act as scavengers of reactive oxygen species, such as hydroxyl radical (*OH)[2]. There are numerous studies demonstrating that aspirin modifies the history of atherosclerosis[3]. However, the drug is often an important cause of poisoning in humans that results from both overdoses and therapeutic use. In children, majority of the deaths due to aspirin poisoning are from therapeutic overdose. Aspirin is also known to cause gastric injury and delay ulcer healing[4]. Whereas low doses of aspirin are effective in the prophylaxis of myocardial infarction, high doses are necessary for the prevention of stroke[5]. Salicylic acid, the *in vivo* metabolite of aspirin, inhibits the β-oxidation of short-chain fatty acids.

The toxic effects of aspirin are considered to be partly biochemical and partly physiological with no clear target organ[1]. When used repeatedly at therapeutic doses, aspirin may accumulate in the patient eventually reaching concentration levels that are toxic[1]. This is because both metabolism of aspirin and its elimination can reach saturation. In humans, aspirin is mostly hydrolyzed by esterases to salicylic acid which is the main metabolite of the drug. Salicylic acid is further metabolized by conjugation with glucoronic acid or glycine. Some salicylic acid is also hydrolyzed to 2,5-dihydroxy benzoic acid (known as gentisic acid) and 2,3-dihydroxy benzoic acid[2]. Fig. 1 gives a schematic representation of metabolism of aspirin.

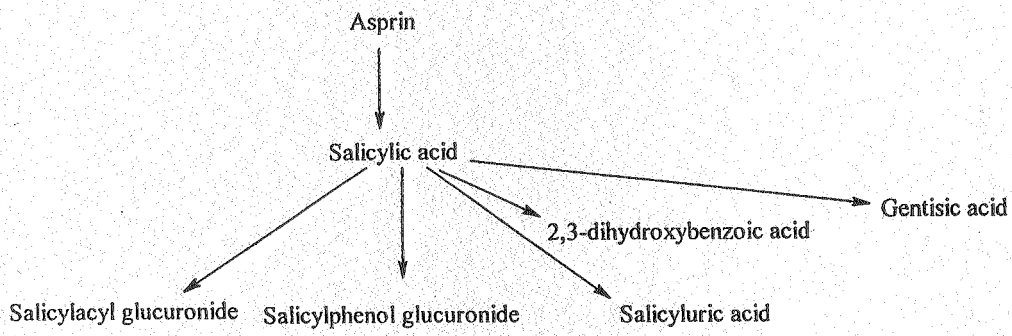

Fig. 1. Schematic representation of metabolism of aspirin

Since these conjugation steps are saturable, relative rate of elimination is reduced as the dose is increased. Table-1 shows that the half-life of aspirin in the body increases significantly even with a small increase in the number of tablets.

TABLE-1
DOSE EFFECT ON THE DISPOSITION OF ASPIRIN AND ITS TOXICITY
(Based on M.J. Ellenhorn and D.G. Barceloux, Medical Toxicology, Elsevier Science Publishing, 1988)

Number of tablets (dose)	$T_{1/2}$* (h)	$V_D^{\#}$ (L kg^{-1})	Blood level (μg mL^{-1})	Symptoms of toxicity
1 (300 mg)	3	0.10	43	None
2 (600 mg)	4	0.15	57	None
3 (900 mg)	5	0.17	76	None
12 (3.6 g)	6	0.20	25	Tinnitus
30 (9 g)	20	0.25	51.4	Hyperventilation, respiratory alkalosis, metabolic acidosis, fever
70 (21 g)	> 20	0.30	1000	Coma, convulsions, respiratory failure, renal failure
100 (30 g)	> 20	0.33	1299	Death

*$T_{1/2}$: plasma half-life; # v_D: volume of distribution.

Aspirin toxicity causes a mixed acid-base disorder characterized by respiratory alkalosis and metabolic acidosis as described below[6]. Aspirin interferes with the function of the electron transport chain in the mitochondria, leading to uncoupling of ATP production that results into decreased production of ATP, increased utilization of oxygen and increased production of carbon dioxide. This causes the patient to hyperventilate. Salicylate also directly influences the control of breathing. Hyperventilation results in an increase in the pH of the blood known as respiratory alkalosis. The body corrects for this by eliminating sodium bicarbonate into the urine and the pH drops. However, in children and after severe overdoses in adults, the pH may fall so much that the patient may enter metabolic acidosis that may cause a change in the distribution of the salicylate. Salicylate, the main metabolite of aspirin, is a weak acid so that as the pH is lowered, the proportion of its un-ionized form will increase. As a result, distribution into tissues (particularly the brain) will increase. Overall, the patient will experience a lack

of ATP in the critical organs such as the brain, heart and the temperature rises since energy not used in the production of ATP is dissipated as heat. The excretion of bicarbonate that occurs in response to the increase in blood pH results in loss of Na^+ and water and the rise in body temperature that causes sweating. As a result, the patient becomes dehydrated. As the pH of the urine decreases, the salicylate and its metabolites are less readily excreted so that the drug is not eliminated from the body, thus worsening the situation. In short, the toxicity of aspirin is due the biochemical effects of low levels of ATP and acidosis.

Aspirin causes necrosis at high concentrations but does not induce cell cycle arrest[7]. In this study, molecular modelling analyses have been carried out using the programs HyperChem 7.0[8] and Spartan '02[9] to investigate the relative stability of aspirin and its metabolites in order to obtain knowledge on their biodistribution and elimination.

Computational Methods

The geometries of aspirin, salicylic acid, salicylacyl glucuronide, salcylphenol glucuronide, salicyluric acid, gentisic acid and 2,3-dihydroxybenzoic acid have been optimized based on molecular mechanics, semi-empirical and DFT calculations. Molecular mechanics calculations were carried out using MM+ force field. Semi-empirical calculations were carried out using the routine PM3. DFT calculations were carried at B3LYP/6-31G* level. For the optimized structures, single point calculations were carried out to give heat of formation, enthalpy, entropy, free energy, dipole moment and solvation energy, HOMO and LUMO.

RESULTS AND DISCUSSION

Table-2 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 aspirin, salicylic acid, salicylacyl glucuronide, salicylphenol glucuronide, salicyluric acid, gentisic acid and 2,3-dihydroxybenzoic acid. Figs. 2, 3, 4, 5, 6, 7 and 8 give the optimized structures of aspirin, salicylic acid, salicylacyl glucuronide, salicylphenolic glucuronide, salicyluric acid, gentisic acid and 2,3-dihydroxybenzoic acid as per PM3 calculations using the program HyperChem 7.0. The structures also give 2D contours of total electrostatic potential. The solvation energies of aspirin, salicylic acid, 2,3-dihydroxybenzoic acid, gentisic acid, salicyluric acid, salicylalyl glucuronide and salicylphenolic glucuronide from PM3 calculations in kcal mol^{-1} are respectively –11.56, –15.79, –17.43, –19.22, –27.27, –48.29 and –51.88, respectively indicating that the metabolites of aspirin are more soluble in water than the parent drug and hence can be more easily excreted. It may be noted that although the metabolite salicyluric acid has high solvation energy, its dipole moment is low. The results point to the complexity of the process of dissolution in water where interactions such as hydrogen bonding, ionization and resonance stabilization may play significant roles. The same conclusion can be drawn from DFT calculations

TABLE-2
CALCULATED THERMODYNAMIC AND OTHER PARAMETERS FOR ASPIRIN AND ITS METABOLITES

Molecule	Calculation type	Total energy (kcal mol^{-1})	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})	Free energy (kcal mol^{-1})	Dipole moment (debye)	HOMO (eV)	LUMO (eV)	LUMO-HOMO (eV)
spirin	PM3	–156.53	–144.97	106.49	107.06	–11.56	74.57	6.53	–10.36	–0.96	9.40
	DFT	–648.69	–	106.13	107.92	–6.65	73.96	6.14	–7.43	–1.68	5.75
alicylic acid	PM3	–128.98	–113.19	81.65	87.69	–15.79	55.50	4.17	–9.58	–0.76	8.82
	DFT	–496.06	–	79.65	84.15	–14.34	54.56	4.30	–6.42	–1.59	4.83
,3-dihydroxy-enzoic acid	PM3	–170.22	–152.79	85.42	97.67	–17.43	56.30	6.50	–9.55	–0.71	8.84
	DFT	–571.25	–	83.71	95.46	–15.14	55.25	7.50	–6.39	–1.21	5.18
entisic acid	PM3	–173.05	–153.83	103.98	83.52	–19.22	79.08	4.91	–9.39	–0.72	8.67
	DFT	–571.28	–	83.58	95.48	–18.02	55.12	5.17	–6.16	–1.33	4.83
alicyluric acid	PM3	–175.57	–148.51	149.58	92.01	–27.07	122.15	4.70	–9.35	–0.63	8.72
	DFT	–703.88	–	114.29	115.73	–21.23	79.79	1.84	–4.98	–1.73	3.25
alicylalyl ucuronide	PM3	–486.97	–438.69	196.19	154.53	–48.29	150.12	5.75	–9.92	–0.92	9.00
	DFT	–1369.37	–	182.22	152.34	–21.02	92.22	6.38	–7.20	–2.10	5.10
alicylphenolic ucuronide	PM3	–491.00	–439.12	196.34	157.30	–51.88	149.44	5.32	–10.49	–1.30	9.19
	DFT	–1369.37	–	190.03	156.44	–20.53	91.78	6.42	–7.15	–2.18	4.97

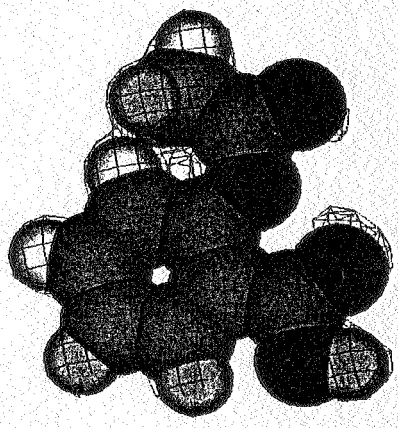

Fig. 2. Structure of aspirin giving 2D contours of total electrostatic potential

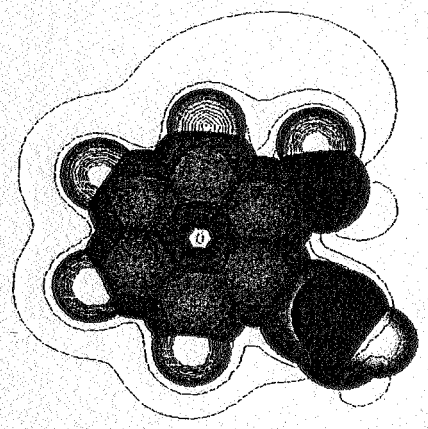

Fig. 3. Structure of salicylic acid giving 2D contours of total electrostatic potential

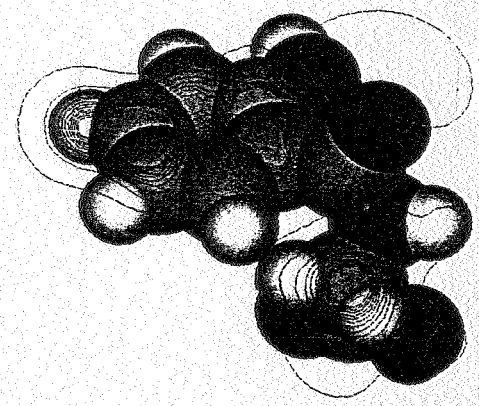

Fig. 4. Structure of salicyluric acid giving 2D contours of total electrostatic potential

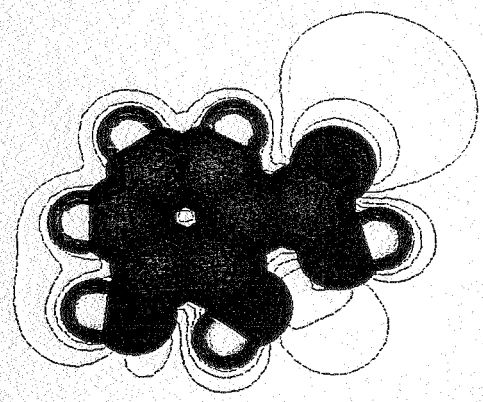

Fig. 5. Structure of 2,3-dihydroxybenzoic acid giving 2D contours of total electrostatic potential

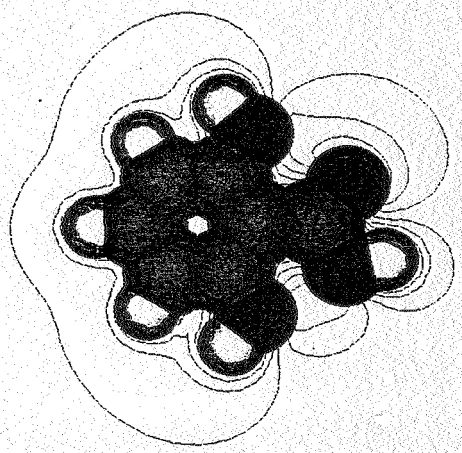

Fig. 6. Structure of 2,5-dihydroxybenzoic acid giving 2D contours of total electrostatic potential

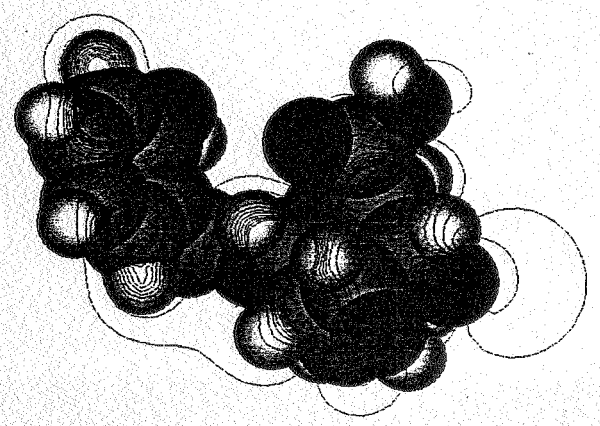

Fig. 7. Structure of salicylalyl glucuronide giving 2D contours of total electrostatic potential

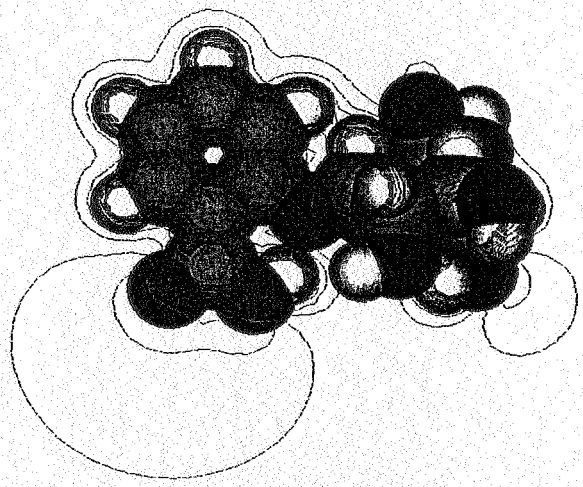

Fig. 8. Structure of salicylphenol glucuride giving 2D contours of total electrostatic potential

as well. It should be noted that in the body aspirin is quickly hydrolyzed to salicylic acid. The other point to note is that all the metabolites of aspirin have smaller LUMO-HOMO differences than aspirin indicating that the metabolites would be more reactive kinetically than aspirin. This may mean that the toxicity of aspirin may be associated more with the metabolites than the parent compound. However, for metabolite salicylic acid the difference between HOMO and LUMO energies is only slightly smaller than that for the parent compound, meaning that salicylic acid is only marginally more reactive than aspirin. The terminal metabolite salicyluric acid is found to have the lowest LUMO-HOMO energy difference from DFT calculations, meaning that the compound would be much more reactive than aspirin itself or its other metabolites. Thus, salicyluric acid is expected to react more readily with biomolecules. However, the solvation energy of salicyluric acid is much greater than that of aspirin (–21.23 kcal mol^{-1} for the former and –6.65 kcal mol^{-1} for the latter as per DFT calculations), meaning that salicyluric acid can be more easily eliminated from the body than aspirin. The contours of total electrostatic potential show the concentration of negative charges around the carbonyl oxygen in all the compounds so that the positions may be subject to electrophilic attack. When the density of electrostatic potential on the surface of salicyluric acid is considered, it is found that there are electron-rich and electron-deficient regions on its surface so that the compound may be subject to both electrophilic and nucleophilic attacks. The latter means that the compound may react readily with cellular antioxidant glutathione and nucleobases in DNA, thus causing cellular toxicity.

Conclusion

Aspirin is one of the most widely used minor analgesics. In humans, aspirin is mostly hydrolyzed by esterases to salicylic acid which is the main metabolite of the drug. Salicylic acid is further metabolized by conjugation with glucoronic acid or glycine. Some salicylic acid is also hydrolyzed to 2,5-dihydroxybenzoic acid (known as gentisic acid) and 2,3-dihydroxybenzoic acid. Molecular model-

ling analyses support the idea that metabolites of aspirin especially salicyluric acid can be more toxic than the parent compound because of higher reactivity. The metabolite may react to cause glutathione depletion, thus compromising antioxidant status of the cell.

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