Renal excretion of paracetamol | experiment

Paracetamol, known as acetaminophen in the USA, is one of the most commonly used analgesic and antipyretic drugs available over-the-counter. Its common name derives from the full chemical name: para-acetyl-amino-phenol, with the chemical formula $C_8H_9NO_2$ and a molecular weight of 151. 17.

Paracetamol does not have any significant anti-inflammatory action and therefore cannot be accurately described as a non-steroidal anti-inflammatory drug (NSAID), as was once thought. Its mechanism of action is still poorly understood but some studies have suggested that it inhibits a variant of the cyclo-oxygenase enzyme COX-1, which has been designated COX-3 (Swierkosz et al., 2002). Paracetamol acts mainly in the central nervous system and endothelial cells, rather than in platelets and immune cells. Boutaud and colleagues (2002) hypothesised that this may be explained by the high levels of peroxides found in the latter cell types, which inhibit the action of paracetamol. There has been some debate on the subject, with other researchers proposing an inhibitory action against COX-2 (Graham & Scott, 2005). Further research is required to fully elucidate the mechanism of action at the molecular level.

Following oral administration and absorption from the gastrointestinal tract, paracetamol enters the blood and is distributed throughout the body. It is metabolised by enzymes in the hepatocytes of the liver and the majority is converted to inactive metabolites by conjugation with sulphate or glucuronide. This is then filtered out of the blood by the kidneys and into the urine, via active renal tubular secretion. A small portion of paracetamol remains unaltered and passes into the urine via glomerular filtration and

passive absorption (Morris & Levy, 1984). There is also a small proportion of the paracetamol that is metabolised by the cytochrome P450 system, which results in the formation of cysteine or glutathione conjugates and mercapturic acid conjugates. These products of oxidative metabolism are also excreted renally (Andrews et al. 1976).

Paracetamol has a low therapeutic index, so the therapeutic dose is very close to the toxic dose. Toxicity can occur following a single large dose (> 10g) or with chronic lower doses (4-5g/d) and is usually seen as hepatotoxicity, which can result in death within several days (Wikipedia).

Toxicity occurs when the enzymes responsible for catalysing sulphate and glucuronide conjugation become saturated, forcing metabolism to be increasingly dependent upon the cytochrome P450 system. This results in formation of a toxic metabolite, N-acetyl-p-benzo-quinone imine (NAPQI), which is normally mopped up by binding to the sulphydryl group of glutathione to form inactive conjugates and mercapturic acid. Toxicity occurs when the glutathione supply becomes exhausted and NAPQI binds indiscriminately to molecules within the cell, such as membranes, to cause cell damage and death, seen as acute hepatic necrosis.

1. Major pathway for normal metabolism
2. Minor pathway via cytochrome P450 system produces toxic metabolite (NAPQI), shown in red. Normally this is detoxified by binding to glutathione.
3. Toxicity occurs when pathways 1 and 2 are overloaded and NAPQI binds to molecules of the cell, causing damage.

Modified from Rang et al. 1995.

The aim of this experiment is to investigate the renal excretion of paracetamol, by measuring the levels of paracetamol metabolites in human urine over 6 hours following an oral dose of 500mg. The total excretion will be assessed using the spectrophotometric method. From this data the elimination rate constant (K_E) and the half-life ($T_{1/2}$) will be calculated. Qualitative analysis of the various metabolites will be conducted using appropriate chemical identification techniques.

METHOD

A standard stock solution of paracetamol was prepared at 1mg/cm^3 and dilutions were made to give a range of known concentrations. 1 cm^3 of the paracetamol solution was added to 1 cm^3 blank urine and 4 cm^3 4M HCl, and mixed thoroughly. A blank duplicate was also prepared, using water instead of urine. After an hour in a boiling water bath the tubes were cooled and water added, up to 10 cm^3. 1 cm^3 of this hydrolysed urine solution was added to 10 cm^3 of colour forming solution, mixed and allowed to stand for 40 minutes. The absorbance of each solution was measured, using the spectrophotometer, zeroing the instrument using the drug free urine sample in between solutions. This produced the readings for the calibration curve. The collected timed urine samples were then processed in the same way, adding 1 cm^3 water instead of paracetamol solution.

RESULTS AND DISCUSSION

Known concentrations of paracetamol underwent spectrophotometry to measure the absorbance at 620nm. These results were used to produce a

calibration curve (figure 3). The timed urine samples were then analysed following the same protocol and the absorbance at 620nm was used, in conjunction with the calibration curve to ascertain the concentration of paracetamol in the urine. Unfortunately, half of the samples produced absorbances outside the range of the calibration curve. Because this curve is non-linear, extrapolation and dilution cannot be used to accurately deduce the concentration of paracetamol in the urine. For the purposes of this report the concentration for these samples has been declared as ' greater than 800ug/cm $^{3'}$. This is not very satisfactory and further experiments must be done to extend the range of the calibration curve to the maximum absorbancy of the timed samples. The values of K_E and $T_{1/2}$ have been calculated to demonstrate the procedure, but are inaccurate and will need revising once accurate concentrations have been established form the calibration curve.

Table 1:

Timed urine sample	Mean absorbance 620nm	Conc. ug/cm3	Vol. Urine (ml)	Total drug (ug of paracetamol)	Excret mg/h
0	0	0	0	0	0
1 hour	0. 256	192	245	47040	47
2 hours	1. 918	> 800	50	40000	40
3 hours	1. 769	> 800	38	30400	30. 4
4 hours	1. 028	> 800	55	44000	44

5 hours	0. 349	246	135	33210	33. 2
6 hours	0. 255	192	160	30720	30. 7

Table 1 contains the absorbance results of the timed urine samples and the deduced concentration of paracetamol in the urine, as well as the hourly excretion rate. The total amount of paracetamol excreted over the 6 hour period was 225. 3mg, which is 45% of the orally administered dose. Due to problems discussed above, this is an underestimate of the true percentage of dose excreted renally, which has been found to be 55-70% by other studies (Steventon et al., 1996).

When log of the excretion rate (equivalent to total drug excreted per hour) is plotted against time, a linear plot should be achieved, from which K_E can be estimated. This is shown in Figure 4, but is likely to need revising.

The slope of this straight line equates to : K_E/2. 303, which gives a value for K_E of 0. 094. Using the formula: $T_{1/2} = 0. 692/ K_E$, the value of $T_{1/2} = 7. 36$ hours.

This states that it takes the body 7. 36 hours to excrete half of the drug administered. This is longer than the 1-4 hours usually quoted for paracetamol (Rang et al. 1995), and is not surprising given the underestimation of the paracetamol urine concentration. With proper calibration, this would be expected to decrease to nearer the previously found results.

There were no results for the qualitative studies for metabolite composition, but it would be expected that sulphate and glucuronide conjugates would constitute the majority of the sample, with a smaller quantity of unchanged paracetamol, cysteine/glutathione and mercapturic acid metabolites.

These results only represent one individual on one day and replications of this experiment are crucial. Nutritional status, recent alcohol consumption, ethnic background, concurrent drug usage and illness must all be taken into account as factors that may affect paracetamol metabolism and excretion (Riordan & Williams, 2002, Patel & Tang, 1992).

Further analysis of paracetamol excretion

- Hepatotoxicity and drug interactions

Table 2 shows how concurrent use of phenobarbital, an anti-epileptic drug, can increase the severity of liver damage caused by paracetamol administration and its subsequent metabolism.

Table 2: Effect of Phenobarbital on paracetamol induced hepatotoxicity

Treatment Dose of Paracetamol (mg/kg) Severity of liver necrosis

None3751-2+

Phenobarbital 375 2-4+_____

This occurs due to metabolism of phenobarbital by enzymes of the P450 cytochrome system, which results in upregulation of their production. As explained in the introduction (see fig. 2), P450 enzymes also metabolise paracetamol, to form the toxic metabolite NAPQI. This is normally a minor

pathway but as the amount of P450 enzymes available increases, the activity of this pathway also increases. This results in a larger than normal amount of NAPQI, which is mopped up and inactivated by glutathione. Glutathione supplies will eventually run out, which occurs sooner if the person is malnourished. When this happens the toxic metabolite binds to cell components, causing necrosis. To prevent this occurring, such as in cases of overdose, N-acetylcysteine can be given (Routledge et al., 1998), which is required for glutathione synthesis and helps to boost it. This allows a greater amount of the toxic metabolite to be mopped up and reduces cell damage.

- Paracetamol metabolism following hepatotoxicity

Table 3:

Plasma paracetamol

concentrations (ug/cm3)

PatientsPlasma

paracetamol4 hrs after12hrs after

Half life (h)ingestioningestion

_____

no liver damage (18)2. 9 +/= 0. 3163 +/= 20 29. 5 +/= 6

liver damage (23) 7. 2+/= 0. 7 296 +/= 26 124 +/= 22___

Table 3 shows that, in a study, the ability of patients with liver damage to eliminate paracetamol from the blood is much decreased, compared to

healthy people. This is seen by the prolonged half-life and the high levels of paracetamol in the plasma. The plasma level does come down by 12 hrs, which indicates that there is enough functional liver reserve to metabolise some of the drug, but the level is still very high. To ascertain whether it is just conjugation that is affected, or whether all the pathways are affected equally it would be necessary to quantify the levels of different metabolites in the blood and urine. As conjugation is responsible for the majority of metabolism, damage to all systems will still show up as affecting conjugation the most.

In theory reduced clearance of a substance is useful for monitoring the severity of liver damage, but in the case of paracetamol it would be unwise as it could potentiate the hepatotoxic effects and worsen the liver condition. It is also unnecessary as there are already a number of reliable blood tests for liver function and damage.

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