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ENVIRONMENTAL MODULATION OF PARACETAMOL TOXICITY

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INTRODUCTION

Paracetamol (acetaminophen) is normally a very safe drug but when taken in substantial overdosage it can cause acute centrilobular hepatic necrosis. Without specific antidotal therapy 8 to 10% of unselected hospital patients with paracetamol overdosage develop severe liver damage with plasma aminotransferase activity exceeding 1000 i.u./l, about 1% die with fulminant hepatic failure and 1% develop acute renal failure requiring dialysis[1]. The incidence of paracetamol overdosage has increased considerably in many countries during the last decade or two. In the U.K. in 1984, 176 deaths were recorded from poisoning with paracetamol alone and 305 with paracetamol plus other drugs, notably d-propoxyphene[2]. There has also been concern that paracetamol might cause hepatic and renal damage in chronic alcoholics even when taken in therapeutic doses[3-6] but the evidence for this is unconvincing. Paracetamol hepatotoxicity has received much attention in recent years and it is used increasingly as a model of liver injury induced by chemical agents.

MECHANISMS OF PARACETAMOL HEPATOTOXICITY

A minor route of paracetamol biotransformation involves metabolic activation by cytochrome P-450 dependent mixed function oxidase to form a reactive arylating metabolite (N-acetyl-p-benzoquinoneimine)[7,8]. This is normally inactivated by preferential conjugation with reduced glutathione through the action of glutathione-S-transferase[9] but large doses deplete hepatic glutathione and paracetamol is covalently bound to hepatic constituents[8,10]. Subsequent irreversible cell damage is probably initiated by the oxidation of SH groups of key enzymes, particularly ATP-dependent plasma membrane Ca^{2+} translocases[11,12].

Paracetamol hepatotoxicity depends primarily on the balance between the rate of formation of N-acetyl-p-benzoquinoneimine and the rate of synthesis of glutathione. It may therefore be modulated by environmental factors which influence the following:

1. The rate of absorption and delivery of paracetamol to the liver.
2. The activity of the isozyme of cytochrome P-450 which is responsible for its metabolic activation.
3. The capacity of the major parallel pathways of elimination by glucuronide and sulphate conjugation.
4. The hepatic content and rate of synthesis of reduced glutathione.

RATE OF ABSORPTION AND DELIVERY TO THE LIVER

The average single acute threshold dose of paracetamol which must be absorbed to produce severe liver damage in man is about 250 mg/Kg but there is considerable individual variation[1]. Little is known of the effects of changes in absorption rate on the hepatotoxicity of paracetamol after single acute or repeated doses. However, toxicity is likely to be reduced if absorption is slowed by food or drugs such as narcotic analgesics which inhibit gastric emptying. Many treatments have been shown to modify paracetamol hepatotoxicity but often the precise mechanisms have not been established. It is possible that effects on absorption and delivery to the liver may contribute in some cases.

THE METABOLIC ACTIVATION OF PARACETAMOL

In their original studies, Mitchell and his colleagues[7,8] showed that experimental paracetamol-induced liver injury and its covalent binding were enhanced by pretreatment with inducers of cytochrome P-450 such as phenobarbitone and 3-methylcholanthrene, and reduced by inhibitors such as piperonyl butoxide and cobaltous chloride. This fundamental relationship between microsomal enzyme activity and toxicity has since been amply confirmed in animals. Other examples include potentiation by acetone[13] and chronic administration of ethanol[14,15], and protection by acute ethanol[16], carbon disulphide[17] and cimetidine[18]. However, in some studies toxicity has not been increased by pretreatment with phenobarbitone[19,20] and it is not possible to extrapolate results from animals to man because of species differences in the substrate specificities of the isozymes of cytochrome P-450 involved in the oxidation of paracetamol. Liver microsomal enzyme activity and the severity of paracetamol toxicity may be modified by age[21], diet and nutritional state[22,23] but these factors may also alter glutathione status.

Environmental factors in man

In man, microsomal enzyme activity could be influenced by environmental factors such as drugs, ethanol, smoking, food additives, and exposure to household, industrial and agricultural chemicals etc, and this probably contributes to the marked individual variation in susceptibility to the hepatotoxicity of paracetamol[1,24]. However, despite much speculation, the role of these factors is uncertain. There have been anecdotal accounts of allegedly more severe liver damage after paracetamol overdosage in patients who had previously been taking drugs which cause microsomal enzyme induction[25,26], and in chronic alcoholics who have been presumed to be in an induced state[3,27]. Unfortunately the variation in susceptibility following overdosage is such that it is never possible to determine whether the outcome in an individual patient was influenced by previous drug therapy or chronic ethanol intake. It has also been claimed that the therapeutic use of paracetamol can cause severe liver damage in chronic alcoholics[3-6] but in many of these patients it was clearly taken in excess or in overdosage[28].

There seems little doubt that chronic alcoholics are more vulnerable to the acute toxic effects of paracetamol in overdose[1], but this is probably due more to impaired syn-

thesis of glutathione[29,30] than to increased metabolic activation as a result of microsomal enzyme induction. Although chronic intake of ethanol[15] and pretreatment with phenobarbitone[7] may enhance the oxidation of paracetamol and potentiate its hepatotoxicity in animals, this has not been demonstrated in man. Indeed, as judged by the fractional urinary recovery of the cysteine and mercapturic acid conjugates, the metabolic activation of paracetamol is not increased in patients who have been induced by chronic use of anticonvulsants and rifampicin, or in regular heavy consumers of ethanol[31]. These findings conflict with those reported in animals and presumably reflect species differences in drug metabolism.

Selective inhibition of the metabolic activation of paracetamol is an obvious approach to the treatment of overdosage but this has not been pursued to any extent. Cimetidine has been considered in this context as an inhibitor of microsomal oxidation. In rats it reduces the urinary excretion of the cysteine and mercapturic acid conjugates and has some protective effect[18] but once again there are species differences since cimetidine has no effect on the metabolic activation of paracetamol in mice[32] or in man[33]. On the other hand, the acute administration of ethanol dramatically reduces the urinary recovery of cysteine and mercapturic acid conjugates both in animals[16] and in man[33]. Many of our patients drink alcohol before taking an overdose of paracetamol and in doing so are probably unwittingly protecting themselves. There is some evidence that liver damage is less severe if alcohol is taken at the same time[34], but on the other hand it certainly does not prevent severe liver damage[35]. In one recent study there was no evidence that previous alcohol consumption worsened the prognosis and simultaneous ingestion of alcohol with paracetamol had no effect on the outcome[36].

Individual variation and extensive metabolic activation

Individual variation in susceptibility to the toxicity of paracetamol is presumably related at least in part to the remarkable individual variation in the extent of its metabolic activation. We recently found a 60-fold range in the fractional urinary recovery of cysteine and mercapturic acid conjugates of the drug in a population survey of healthy young subjects in Scotland, Ghana and Kenya[37]. In contrast, the range in sulphate and glucuronide conjugation varied no more than threefold. In addition there were highly significant ethnic differences in the metabolic activation of paracetamol (Figure). The mean combined recovery of the oxidative metabolites was 9.3% in the Scots but only 5.2% in the Ghanaians and 4.4% in the Kenyans. On this basis the Africans would be more resistant to the hepatotoxicity of paracetamol than the Scots and there are obvious implications for the toxicity of other drugs and chemicals caused by oxidative metabolic activation. The question to be answered is whether these ethnic differences are due to environmental or genetic factors. The Africans used less tobacco and alcohol, but this did not appear to be the explanation. They were probably exposed less to environmental inducing agents and their diet contained less animal fat and protein. A low protein intake may impair drug metabolism[38], but at the same time hepatic glutathione may be reduced[22,39].

Clinical reports on the role of dietary factors in paracetamol toxicity are conflicting. Toxic hepatitis in one patient who had taken excessive doses was attributed to cachexia and a negative nitrogen balance[40] while in another with malnutrition due to severe anorexia nervosa, it was considered noteworthy that the liver was not damaged after an overdose despite treatment with N-acetylcysteine![41]

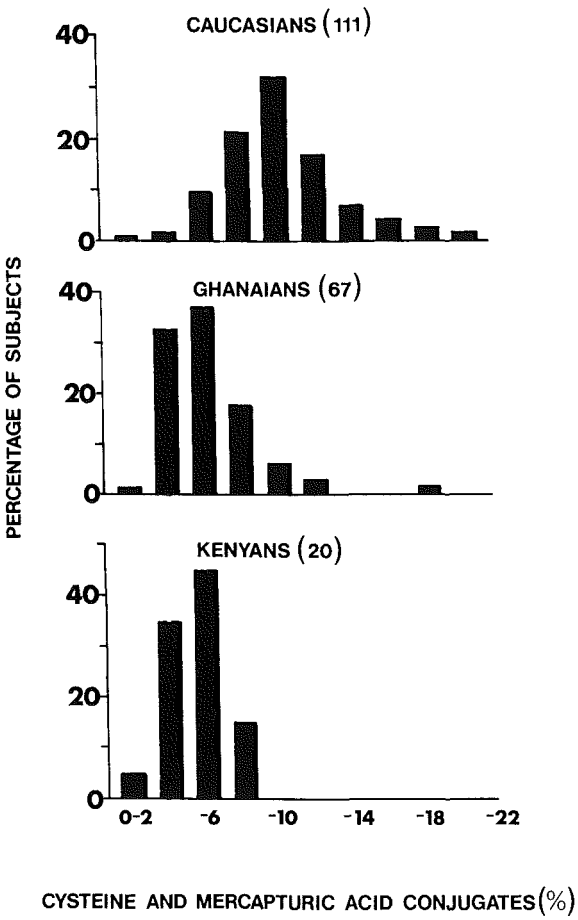

Figure. Frequency distributions of the 24 hour urinary recovery (% of total) of the cysteine and mercapturic acid conjugates of paracetamol following a single oral dose of 1.5 g in healthy subjects in Scotland, Ghana and Kenya. (From reference 37)

The population survey revealed that a small minority of individuals produce abnormally large amounts of cysteine and mercapturic acid conjugates of paracetamol with recoveries in the range of 15-25% (Figure). There was no obvious cause for this in any of the subjects but the implication is that these "extensive activators" would be at considerably

increased risk of paracetamol toxicity. This may be relevant to the alleged occurence of liver damage following therapeutic doses of paracetamol although it seems most unlikely that the threshold dose for toxicity could be reduced to this extent. I recently had under my care a 27 year old chronic alcoholic who developed severe liver damage and renal failure after taking paracetamol in allegedly normal doses over one day during a drinking bout. On admission four days later the plasma paracetamol concentration was 1.1 mg/l, and this almost certainly indicates overdosage. Three weeks after taking the paracetamol, during convalescence in hospital with complete abstinence from ethanol, he was given an oral test dose of 20 mg/Kg of paracetamol and blood and urine samples were collected for 24 hours. At this time liver function tests were normal apart from mild elevation of the plasma γ-glutamyltransferase activity. The plasma paracetamol half life was normal at 2.1 hours, but the cysteine and mercapturic acid conjugates accounted for 21.4% of the total drug and metabolites in the urine (Table).

TABLE

EXTENSIVE METABOLIC ACTIVATION OF PARACETAMOL IN A PATIENT WHO DEVELOPED LIVER DAMAGE AND RENAL FAILURE FOLLOWING ALLEGED BUT PROBABLY EXCESSIVE THERAPEUTIC USE

Percentage 24 hour urinary recovery of paracetamol metabolites after a single oral dose of 20 mg/Kg in the patient and in 12 healthy subjects

	Glucuronide	Sulphate	Cysteine & mercapturate
Healthy subjects	57 $\pm$11	30 $\pm$10	8.2 $\pm$1.1
Patient with liver & renal damage	49.5	29.2	21.4

The patient was studied 3 weeks later during convalescence. Values are means $\pm$S.D.

This very abnormal pattern of metabolism is consistent with increased sensitivity to paracetamol on the basis of extensive metabolic activation. The role of ethanol in this case is unknown but if induction had occurred, it should have largely disappeared after abstinence for 3 weeks.

PARACETAMOL ELIMINATION CAPACITY

The major pathways of paracetamol elimination are glucuronide and sulphate conjugation. Glucuronide conjugation is the dominant route, accounting for about 60% of a therapeutic dose and a larger proportion of an overdose. The plasma paracetamol half life after a therapeutic dose is 1.5 to 2.5 hours and there is relatively little individual vari-

ation: after a hepatotoxic overdose the half life is prolonged from the outset according to the severity of liver damage[1].

It has been claimed that both glucuronide and sulphate conjugation of paracetamol become saturated after overdosage so that a greater proportion of the dose is shunted to the toxic route of metabolism[42,43]. However, in man the capacity for glucuronide conjugation is very large and there is no evidence for saturation of this pathway except in very rare cases of gross intoxication with plasma paracetamol concentrations of about 1000 mg/l[44]. On the other hand, the sulphate conjugation of paracetamol is partially saturated with high therapeutic doses and completely saturated after a large overdose. Availability of inorganic sulphate is also a limiting factor and sulphate conjugation after overdosage can be partly restored by treatment with N-acetylcysteine which presumably provides inorganic sulphate[44]. The plasma concentrations of inorganic sulphate are transiently reduced by therapeutic doses of paracetamol but there is adaptation with elevated concentrations during chronic use[45].

The glucuronide conjugation of paracetamol is marginally increased by microsomal enzyme induction[46] and by oral contraceptives in females[47], and sulphate conjugation may be reduced by dietary deficiencies and agents which compete for this reaction[44]. However, environmental factors are unlikely to greatly influence the toxicity of paracetamol through effects on these routes of elimination.

GLUTATHIONE STATUS AND PROTECTION AGAINST HEPATOTOXICITY

Glutathione performs a vital protective role against oxidative cell injury and its hepatic content and synthetic capacity are important determinants of paracetamol toxicity[8,10]. Experimental liver damage and covalent binding induced by paracetamol are greatly increased if glutathione is depleted by diethyl maleate[10], or its synthesis is inhibited by buthionine sulphoximine[48], and reduced by precursors such as cysteine[10], N-acetylcysteine[48,49] and methionine[22]. In man, the early administration of agents such as cysteamine, N-acetylcysteine and methionine has proved remarkably effective in preventing liver damage, renal failure and death following paracetamol overdosage[1]. Glutathione synthesis is limited by the availability of cysteine, and N-acetylcysteine and methionine are thought to act primarily by facilitating this process after conversion to cysteine[50-53]. The free thiols may also reduce N-acetyl-p-benzoquinoneimine back to paracetamol[51,54].

Glutathione and other thiols may also protect hepatocytes against otherwise lethal injury after covalent binding has occurred. When isolated hamster hepatocytes are incubated with paracetamol for 1½ hours there is no immediate loss of viability, but if the paracetamol is then removed there is subsequent extensive injury. This can be prevented by exposing the cells to dithiothreitol and N-acetylcysteine (but not methionine) after removal of the paracetamol. The events initiated by the covalent binding of paracetamol which lead to cell necrosis can therefore be reversed by thiols, probably through reactiv-

ation of the oxidised SH groups of membrane Ca^{2+} translocase with restoration of intracellular calcium homeostasis[55]. This late protective action probably explains why N-acetylcysteine can prevent liver damage in man even when given 10 to 12 hours after an overdose of paracetamol has been taken[1]. By this time, most of the drug would already have been metabolised.

Environmental factors

In animals, hepatic glutathione is reduced and paracetamol toxicity increased by factors such as fasting and a low protein diet[22,23,56]. An extended fast greatly increases the turnover, hepatic efflux and plasma clearance of glutathione[57], and even the commonly used suspending agents methylcellulose and carboxymethylcellulose can produce species-dependent changes in its hepatic content[56]. Acute and chronic ethanol treatment has variable effects on hepatic glutathione in animals[15,16,58,59]. Recent studies have shown that acute ethanol reduces glutathione synthesis without increasing its consumption[60] while turnover and hepatic efflux are increased after withdrawal from chronic ethanol treatment[61,62]. Glutathione synthesis is impaired in chronic alcoholics[29] and its hepatic content reduced in patients with alcoholic cirrhosis[30]. These findings may account for the increased sensitivity of chronic alcoholics to paracetamol hepatotoxicity. Glutathione synthesis is stimulated following depletion by various agents and there is a greater rate of turnover in young than in older rats. It has been suggested that this increased turnover might explain the decreased susceptibility of young children to liver damage after paracetamol overdose[63].

Some of the many sulphur-containing compounds which protect against paracetamol-induced liver damage probably do so by providing cysteine to maintain glutathione synthesis. For example, L-2-oxothiazolidine-4-carboxylate and its methyl analogue provide particularly efficient intracellular delivery of cysteine[64,65]. Propylthiouracil is a substrate for glutathione-S-transferase and reacts directly with the toxic metabolite of paracetamol[66]: it protects against paracetamol hepatotoxicity even when glutathione is depleted[67]. The liver contains very large amounts of glutathione-S-transferase and it seems unlikely that changes in its activity would have marked effects on paracetamol toxicity. However, the alkylating diuretics ethacrynic and tienilic acids strongly inhibit several of the isozymes of glutathione-S-transferase and might thereby potentiate toxicity[68]. Conversely, glutathione-S-transferase activity is increased, and paracetamol hepatotoxicity decreased by dithiolthiones and the antioxidant butylhydroxyanisole[69]. Other antioxidants including vitamin E[70], catechins[71] and ascorbic acid[72] also have some protective effect but the mechanisms are uncertain.

Glutathione status is clearly of great importance as a modulator of paracetamol hepatotoxicity and it can be influenced by a variety of environmental factors. Apart from the proven effectiveness of agents such as N-acetylcysteine in preventing liver damage after paracetamol overdosage and the probable impairment of glutathione synthesis in chronic alcoholics, the relevance of these factors in man is largely unknown.

SUMMARY

Environmental factors can modulate paracetamol hepatotoxicity through effects on microsomal enzyme activity and glutathione status. In animals, the hepatotoxicity and covalent binding of paracetamol is increased and decreased respectively by agents which stimulate and inhibit its metabolic activation. However, there seem to be important species differences in the specificity of the isozyme(s) of cytochrome P-450 involved in this reaction. In man, the metabolic activation of paracetamol is not increased by chronic treatment with anticonvulsants, rifampicin or heavy ethanol consumption. It is decreased by acute ethanol but not cimetidine.

There are great individual and ethnic differences in the extent to which paracetamol undergoes metabolic activation. A minority of individuals are "extensive activators" who would seem to be at increased risk of toxicity. The role of environmental factors in these individuals is unknown.

Experimental paracetamol hepatotoxicity depends critically on glutathione status, and this may be influenced by drugs, diet and fasting. In man, the increased susceptibility of chronic alcoholics to toxicity may be related to impaired glutathione synthesis. The early administration of glutathione precursors such as N-acetylcysteine effectively prevents liver damage and death after paracetamol overdosage. In addition to facilitating glutathione synthesis, thiols may reverse oxidative damage to critical enzymes after covalent binding has taken place.

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Discussion - Environmental modulation of acetaminophen toxicity.

G. Zbinden

Are there any statistics about a possible higher risk for liver tumors in patients surviving from paracetamol overdosage?

L.F. Prescott

This is a very relevant point that, unfortunately, we cannot answer. It is very difficult to follow up these patients, but some have been followed for months or even years. In these patients, unless there was very severe damage, liver function has always returned to normal and the appearance of the liver on biopsy is also normal. However, a long term risk of hepatic carcinoma is a possibility. The problems of paracetamol overdosage started about twenty years ago, and I think that we have to look for this complication in the coming years.

O. Pelkonen

It has been published that paracetamol can cause cancer in the rat.

L.F. Prescott

Yes, but in these studies a rather unusual strain of rats was used and there were marked sex diffferences. There have been other long term studies with paracetamol in which no increased incidence of tumors was detected.

J. Brodeur

Are you aware of studies where the genetic distribution of glutathione transferase activity has been studied?

L.F. Prescott

I do not know of any studies in man, but as far as the conjugation of the reactive metabolite of paracetamol is concerned, the enzyme is present in very large excess and variation is unlikely to be of importance.

R. Lauwerys

Do you know if patients with chronic active hepatitis, who

probably have low concentrations of glutathione in their liver are more susceptible to the acute toxic effects of paracetamol?

L.F. Prescott

The number of such patients is too low to draw any conclusion. On the other hand, an association between the onset of chronic active hepatitis and paracetamol intake has been sought but not found.

G.J. Mulder

Is it possible to formulate paracetamol with one of the drugs used in the treatment of overdosage, in order to prevent toxicity?

L.F. Prescott

Methionine gives a bad smell and N-acetylcysteine poses some problems of stability. However, an N-acetylmethionine ester of paracetamol, which releases the two agents in the body, has been prepared and is currently being evaluated.

B. Kobusch

There have been several animal studies showing that hepatotoxicity induced by different agents can be reduced by calcium channel blockers. Do you know whether this line of actuation has been tried in man?

L.F. Prescott

This is certainly an area of interest, but I do not know of any studies in man. The timing of the administration of the protective agent in clinical use may be critical.

E.E. Ohnhaus

Can you comment on the possible use of ranitidine in paracetamol intoxication?

L.F. Prescott

A normal dose of ranitidine has no effect on the metabolism of paracetamol in man, as judged by the urinary excretion of the major metabolites. The effects of ranitidine on paracetamol

toxicity in animals are complex and dose-related : ranitidine given at about the same time as a toxic dose of paracetamol can either protect or enhance toxicity, depending on the dose.

S. Erill

Your data on fractional urinary recovery of cysteine and mercapturic acid conjugates of paracetamol in different populations seem most interesting to me. As you mention, this has obvious implications for the toxicity of paracetamol and probably other drugs. For many years, the prevailing view has been that etnic differences in the incidence of side effects are more apparent than real, and now I wonder whether this should be considered critically.

L.F. Prescott

We have identified a group of very extensive metabolic activators. We do not know the exact implications. In clinical pharmacology we tend to be very concerned about poor metabolizers of drugs, but extensive metabolizers may also be at increased risk if metabolites cause toxicity.

R. Lauwerys

A reduced excretion of cysteine and mercapturic acid conjugates is always interpreted as an indication of low production of toxic metabolites, but after small doses of paracetamol it might actually reflect a reduced pool of hepatic glutathione and, therefore, just mean an increased susceptibility.

L.F. Prescott

This is unlikely. If different animal species are ranked in order of susceptibility to paracetamol toxicity, this correlates well with the proportion of the dose which is recoverable in the urine as the cysteine and mercapturic acid conjugates. Furthermore, it is not just the size of the pool of glutathione, which is important, but rather the maximun capacity to synthesize glutathione.

G.J. Mulder

Another factor that should be taken into consideration is that

glutathione conjugates of paracetamol are excreted into the bile. Since there is probably some enterohepatic circulation, diet and other factors may influence the urinary recovery of these conjugates.

L.F. Prescott

Most of the gluthatione-derived conjugates seem to be excreted in the bile as the cysteine conjugate. This is probably reabsorbed and acetylated in the kidney to form the mercapturic acid conjugate. In any event, the overall recovery of metabolites after a single therapeutic dose of paracetamol is about 95% so that the amount lost in the bile must be very small.

P. Juul

There is some discussion concerning the possible nephrotoxicity of the combined use of paracetamol and aspirin for long periods of time. Can you comment on that?

L.F. Prescott

As far as I know, there have been no studies published which specifically point to the development of chronic renal disease in patients who have taken that combination. There have also been very few reports involving paracetamol. The chronic renal toxicity of analgesic abuse is probably related to prostaglandin inhibition and there may be some potentiation of one of these analgesics by the other in studies in experimental animals. I do not think that there is any evidence that this occurs in man.