Does cytochrome P450 liver isoenzyme induction increase the risk of liver toxicity after paracetamol overdose?

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Abstract: Paracetamol (acetaminophen, N-acetyl-p-aminophenol, 4-hydroxyacetanilide) is the most common cause of acute liver failure in developed countries. There are a number of factors which potentially impact on the risk of an individual developing hepatotoxicity following an acute paracetamol overdose. These include the dose of paracetamol ingested, time to presentation, decreased liver glutathione, and induction of cytochrome P450 (CYP) isoenzymes responsible for the metabolism of paracetamol to its toxic metabolite N-acetyl-p-benzoquinoneimine (NAPQI). In this paper, we review the currently published literature to determine whether induction of relevant CYP isoenzymes is a risk factor for hepatotoxicity in patients with acute paracetamol overdose. Animal and human in vitro studies have shown that the CYP isoenzyme responsible for the majority of human biotransformation of paracetamol to NAPQI is CYP2E1 at both therapeutic and toxic doses of paracetamol. Current UK treatment guidelines suggest that patients who use a number of drugs therapeutically should be treated as “high-risk” after paracetamol overdose. However, based on our review of the available literature, it appears that the only drugs for which there is evidence of the potential for an increased risk of hepatotoxicity associated with paracetamol overdose are phenobarbital, primidone, isoniazid, and perhaps St John’s wort. There is no evidence that other drugs often quoted as increasing risk, such as carbamazepine, phenytoin, primidone, rifampicin, rifabutin, efavirenz, or nevirapine, should be considered risk factors for hepatotoxicity in patients presenting with acute paracetamol overdose.

Keywords: paracetamol, overdose, hepatotoxicity, isoenzymes

Background
Paracetamol (acetaminophen, N-acetyl-p-aminophenol, 4-hydroxyacetanilide) overdose causing fatal hepatotoxicity was first reported in humans in 1966.1,2 Since then, paracetamol poisoning has become the most common cause of acute liver failure in many developed countries/regions, including the UK, US, Canada, Australia, and Scandinavia.3–7 Paracetamol poisoning may result from either an acute overdose (where the drug is ingested at a single point in time, usually within one hour), or as a staggered supratherapeutic ingestion over a longer period. This article will focus on single-ingestion paracetamol poisoning.

A number of factors are important in determining the risk of toxicity after paracetamol poisoning, and these influence the decision to administer antidotal treatment with N-acetylcysteine.8–13 The plasma paracetamol concentration plotted against time after ingestion is compared with a “treatment line” to determine the need for antidotal therapy. There are a number of different treatment lines used around
the world. In addition, in the UK, there are currently two treatment lines, ie, one for those deemed to be at usual risk of paracetamol-related hepatotoxicity, and a lower line used for those deemed to be at “high” risk of paracetamol-related hepatotoxicity. Common factors used to determine risk of paracetamol-related hepatotoxicity include depletion of liver glutathione (eg, malnutrition, eating disorders) and induction of liver cytochrome P450 (CYP) 2E1 (eg, chronic ethanol use, phenytoin, or other enzyme-inducing drugs). In the UK, the process of determining which treatment line to base treatment on following a single-time point paracetamol overdose is known as “risk stratification”. This relies on both laboratory measurement of paracetamol concentration and assessment of the patient history to determine the interval after ingestion, which appears to be reasonably reliable in this patient population. In addition to determining the need for treatment, clinicians need to consider the potential for adverse reactions to the antidote.

Mechanism of paracetamol-related hepatotoxicity

The parent paracetamol molecule is not in itself associated with toxicity. At therapeutic doses, paracetamol is predominantly metabolized by hepatic sulfation and glucuronidation, with less than 5%–10% being metabolized by the hepatic CYP system (predominantly CYP2E1 and CYP3A4) to N-acetyl-p-benzoquinoneimine (NAPQI), a highly reactive intermediate metabolite responsible for paracetamol-related hepatotoxicity. At therapeutic doses of paracetamol, this metabolite is effectively detoxified by reduced glutathione into cysteine and mercapturic conjugates which are renally excreted. Following a supratherapeutic dose or overdose of paracetamol, glucuronidation/sulfation conjugation pathways are saturated, and therefore a greater proportion of paracetamol is metabolized to NAPQI. Intrahepatic glutathione stores may be insufficient to detoxify the greater NAPQI, and when glutathione concentrations fall to approximately 30% of normal, NAPQI covalently binds to hepatic cells. This is associated with irreversible hepatocellular necrosis which, in severe cases, results in liver transplant or death.

Factors reported to be associated with increased risk

There are a number of factors which potentially impact on the risk of an individual developing hepatotoxicity following an acute paracetamol overdose. These can be broadly divided into the following categories: dose of paracetamol ingested; time from ingestion to treatment with N-acetylcysteine; increased metabolism of paracetamol to NAPQI due to induction of the relevant CYP isoenzymes; and reduction in liver glutathione stores. In this paper, we will review the evidence for increased risk of hepatotoxicity following paracetamol overdose due to CYP isoenzyme induction unrelated to ethanol.

Evidence for induction of CYP isoenzyme system

As discussed in the introduction, the toxic metabolite of paracetamol is NAPQI, and this is formed by oxidative metabolism via the CYP system. This system is subdivided into multiple isoenzyme groups with different substrate specificity. Animal studies in the 1970s demonstrated that paracetamol is metabolized by the CYP system. More recent investigations have centered on which isoenzyme(s) metabolize paracetamol, and the CYP1A2, CYP2A6, CYP2D6, CYP2E1, and CYP3A4 isoenzymes have all been investigated. The published literature is largely based on in vitro and animal models, with very few human studies in vivo. We will now summarize the available literature on the relative importance of these isoenzymes for paracetamol metabolism.
CYP1A2
An in vitro study of IgG-mediated inhibition of CYP1A2 and CYP2E1 suggested that CYP1A2 contributed 30%–56% of metabolism, compared with the 30%–78% performed by CYP2E1.38 Unlike CYP2A6 and CYP2E1, inhibition of CYP1A2 and CYP2A4 in another in vitro study failed to reduce the production of NAPQI.44 A mouse model subsequently suggested that, unlike CYP2E1, CYP1A2 activity increased after higher doses of paracetamol.40 Double knockout mice for both CYP1A2 and CYP2E1 were protected against paracetamol-induced hepatotoxicity and glutathione depletion after massive overdose. However, the significance of these findings in animal studies is unclear, because human volunteer studies suggest that CYP1A2 inducers do not increase production of NAPQI after therapeutic doses of paracetamol.41

CYP2A6
CYP2A6 seems to oxidize paracetamol preferentially to 3-OH-acetaminophen, which is a nontoxic metabolite in a 3:1 ratio to NAPQI.35 Inhibition of human CYP2A6 in vitro reduces the amount of NAPQI produced.44 Therefore, from a toxicological perspective, it is unlikely that CYP2A6 plays an important role in paracetamol-related hepatotoxicity.

CYP2D6
In vitro, human CYP2D6 is one-third as efficient as CYP2E1, with a 4%–22% contribution to paracetamol metabolism, especially at higher paracetamol doses.42 This observation deserves to be investigated further because, of all the isoenzymes, CYP2D6 is known to have substantial interindividual variation.45

CYP3A4
The evidence for the degree of CYP3A4 involvement in paracetamol metabolism in humans remains mixed. An in vitro study suggested that human CYP3A4 contributes 1%–20% of total metabolism, especially at lower doses; despite being a substrate, paracetamol also appeared to inhibit its activity.39 However, a subsequent in vitro study showed that CYP3A4 inhibition did not affect NAPQI formation.44 A more recent in vitro study using human recombinant cytochromes suggested that bioactivation of paracetamol at both therapeutic 50 µM and toxic 1 mM concentrations is largely due to CYP3A4, ahead of CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP2E1.43 This study has been criticized for the lack of cytochrome b5 in the model, which may have resulted in suppression of CYP2E1 activity.46 Mice humanized for CYP3A4 show enhanced paracetamol-induced hepatotoxicity following overdose.47 However, two separate human volunteer studies using therapeutic paracetamol doses failed to show any enhancement of NAPQI formation following CYP3A4 induction with either rifampicin or carbamazepine.36,48 Overall, it appears that the contribution of CYP3A4 to paracetamol metabolism and NAPQI formation in humans is likely to be limited.

CYP2E1
CYP2E1 is widely considered to be the main isoenzyme responsible for NAPQI formation. Human CYP2E1 has been shown in vitro to contribute to 30%–78% of paracetamol metabolism.38 In vitro inhibition of CYP2E1 also significantly reduces the amount of NAPQI produced.44 Compared with CYP2A6, CYP2E1-mediated oxidative metabolism produces NAPQI preferentially (as opposed to 3-OH-acetaminophen, which is a nontoxic metabolite produced by CYP2A6), and CYP2E1 is the more efficient isoenzyme at toxic doses of paracetamol.35 Studies in mice show that CYP2E1 is equally efficient for NAPQI production at both low and higher doses of paracetamol,40 CYP2E1-null mice experience less hepatotoxicity compared with wild-type mice for comparable doses of paracetamol,49 and double knockout mice for CYP2E1 and CYP1A2 are protected against both hepatotoxicity and glutathione depletion despite massive paracetamol overdose.37 In human volunteers given a therapeutic paracetamol dose, CYP2E1 suppression with disulfiram pretreatment resulted in significantly less NAPQI production.36

Evidence for increased hepatotoxicity via altered paracetamol metabolism
There are a number of pharmacological agents that induce the CYP isoenzymes involved in paracetamol metabolism. These include carbamazepine, phenobarbital, phenytoin, primidone, rifampicin, rifabutin, efavirenz, nevirapine, and St John’s wort.13 Currently in the UK, therapeutic use of these medications is considered a “high-risk” factor for paracetamol-related hepatotoxicity, and patients are treated using the 100 line rather than the 200 line. We will now review the evidence on whether therapeutic use of and/or concurrent exposure to these drugs is actually likely to put patients at higher risk of paracetamol-related hepatotoxicity.

Phenobarbital
Co-administration of phenobarbital and paracetamol has been linked with enhanced toxicity in vitro, in animal models, and in humans, although this toxicity may be the result of suppressed
glucuronidation rather than CYP induction.\textsuperscript{18,21,23,50–59} Paracetamol-poisoned rats and mice show enhanced hepatotoxicity when pretreated with phenobarbital, in association with either normal or increased recovery of cysteine and mercapturic conjugates.\textsuperscript{17,21,54} In rats, phenobarbital appears to accelerate the onset of hepatotoxicity (24 versus 72 hours) rather than worsen the overall degree of hepatonecrosis.\textsuperscript{54} In another rat model, coadministration of phenobarbital 150 mg/kg one hour before paracetamol 400 mg/kg suppressed glucuronidation, and this correlated with hepatotoxicity; the speed at which this interaction occurred suggests the increased hepatotoxicity was not a result of CYP induction.\textsuperscript{56} Furthermore, in vitro studies in human hepatocyte cultures show that phenobarbital only induces CYP2B6 and CYP3A4 but not the more relevant CYP2E1.\textsuperscript{52,53,60,61} In vitro studies in human hepatocyte cultures also show that coadministration (but not pretreatment) of phenobarbital suppresses glucuronidation, with reduced uridine 5′-diphosphate-glucuronosyltransferase, in particular the UGT1A6, UGT1A9, and UGT2B19 isoenzymes, and this correlates with toxicity.\textsuperscript{50,51} A single, controlled, crossover, volunteer study reported that coadministration of subtoxic phenobarbital and paracetamol doses resulted in increased recovery of mercapturic and cysteine conjugates, indicating increased production of NAPQI.\textsuperscript{21} These reports suggest that phenobarbital suppresses paracetamol glucuronidation, resulting in a larger burden of paracetamol diverted to mercapturic conjugates.\textsuperscript{17,21,54} In rats, phenobarbital appears to accelerate NAPQI production, and that this effect is without relevant CYP induction. However, there is little clinical evidence for enhanced hepatotoxicity after paracetamol overdose in phenobarbital-treated patients. Published case reports involve unreliable histories. One reported a patient with an undisclosed ingestion of phenobarbital preceding an overdose of 11 g paracetamol, whilst another patient had ingested therapeutic doses on a long-term basis before the onset of hepatotoxicity, and the case description does not exclude the possibility of undisclosed repeated supratherapeutic paracetamol ingestion.\textsuperscript{58,62} There are no case series or controlled clinical trials looking at the effect of phenobarbital treatment alone prior to paracetamol overdose or of phenobarbital-paracetamol coingestion. In a retrospective study of patients with fulminant hepatic failure, 18 paracetamol-poisoned patients on antiepileptic therapy (five receiving phenobarbital) were reported to have significantly “worse” hepatotoxicity compared with an historical unmatched series of patients not receiving antiepileptic drugs. However, the antiepileptic medications cannot be clearly implicated, because these patients presented more than 8 hours after paracetamol ingestion and were not treated with N-acetylcysteine.\textsuperscript{59}

In summary, whilst there are limited data in patients with paracetamol poisoning, from in vitro, animal, and human volunteer studies, it appears that phenobarbital predominantly increases the risk of paracetamol poisoning by reduction of glucuronidation and resultant increased metabolism to NAPQI, rather than by CYP isoenzyme induction. Potentially, it is not just patients who are taking phenobarbital therapeutically who are at risk but also patients who coingest phenobarbital with paracetamol in overdose.

Phenytoin

As well as CYP3A4 induction, phenytoin may also affect paracetamol glucuronidation. In vitro, phenytoin induces human CYP3A4 up to two-fold, but reduces paracetamol glucuronidation by 50%.\textsuperscript{50,51,61} However, in vivo, phenytoin-treated volunteers receiving a therapeutic dose of paracetamol have increased urinary glucuronide conjugates compared with controls, alongside either reduced or unchanged mercapturic and cysteine conjugates, which suggests phenytoin should not enhance paracetamol-induced hepatotoxicity by a reduction in glucuronidation.\textsuperscript{58,63} Although there are anecdotal reports of more severe hepatotoxicity, these are not reliable, because they describe phenytoin-treated patients with substantial paracetamol overdoses over 12 g and either delayed or unreliable times of ingestion. Suchin et al reported a phenytoin-treated patient ingesting an overdose of paracetamol 25 g, who presented with established hepatotoxicity; the reported plasma paracetamol concentration and clinical course suggests an undisclosed delayed presentation.\textsuperscript{64,65} Minton et al reported on another phenytoin-treated patient who developed hepatotoxicity following paracetamol poisoning. In this case, N-acetylcysteine was withheld because the timed plasma paracetamol concentration was below the 200 line, but the patient would have qualified for therapy based on the 150 line.\textsuperscript{57} McClements et al reported on another phenytoin-treated patient who presented over 24 hours after ingestion of 45 g of paracetamol and died after treatment with cimetidine (instead of N-acetylcysteine).\textsuperscript{66} The study that is most often quoted to support antiepileptic medication as a risk factor for paracetamol toxicity is an uncontrolled study of 18 patients on antiepileptic medications (seven on phenytoin, either as monotherapy or part of combination therapy) who had significantly worse hepatotoxicity compared with patients not on antiepileptic treatment.\textsuperscript{59} However, the patients presented late (median time to presentation was 14 hours), eight did not have any detectable serum paracetamol, 15 did not receive N-acetylcysteine, and the comparison was with a historical series of unmatched control patients. Therefore, we feel it
is not possible to determine that the hepatotoxicity was due to the antiepileptic drugs rather than other factors. Overall, insufficient data exist to determine whether paracetamol-induced hepatotoxicity might be more common or more severe in patients normally receiving phenytoin.

### Primidone

Primidone is metabolized into phenobarbital in vivo. In a small limited study, the pharmacokinetics of 1000 mg oral and intravenous paracetamol in six epileptic patients on long-term treatment with various combinations of primidone, phenobarbital, phenytoin, and carbamazepine was compared with six nonepileptic controls. The number treated with primidone was not documented. Following the oral paracetamol dose, reduced bioavailability but without altered urinary excretion was noted in the patients on antiepileptics, leading the authors to conclude that antiepileptic medications enhanced hepatic first-pass metabolism of paracetamol. The effect of primidone alone cannot be discerned from this study. There are no specific studies addressing the interaction between paracetamol and primidone alone. However, we would anticipate that because primidone is a prodrug of phenobarbital, the risks would be similar to phenobarbital, whereby it increases risk of paracetamol-related hepatotoxicity by reducing glucuronidation, and therefore results in an increase in metabolism of paracetamol to NAPQI.

### Carbamazepine

Carbamazepine induces human CYP3A4 in vitro and in vivo. There are no data to suggest that carbamazepine induces CYP2E1 or alters glucuronidation/sulfation of paracetamol. In a controlled human volunteer study, the urinary excretion of paracetamol metabolites was no different between carbamazepine-treated epileptics and carbamazepine-naive healthy controls, demonstrating that NAPQI production is not affected by carbamazepine. No convincing data exist to examine the impact of normal therapeutic use of carbamazepine on risk of hepatotoxicity after paracetamol overdose.

### Rifampicin

In vitro, rifampicin is a human CYP3A4 and CYP2D6 inducer. When administered to mice humanized for both the pregnane X receptor and CYP3A4, rifampicin is associated with worsened paracetamol-induced hepatotoxicity. However, in a human volunteer study, pretreatment with rifampicin enhanced CYP3A4 activity without increasing the production of NAPQI following a subtherapeutic 500 mg paracetamol dose. There are no data to suggest that rifampicin induces CYP2E1 or alters glucuronidation/sulfation of paracetamol. Furthermore, there are no published cases associating rifampicin alone with worsened paracetamol-induced hepatotoxicity.

### Rifabutin, efavirenz, nevirapine

These agents are also CYP3A4 inducers but there are no in vitro, animal, or human studies to demonstrate that therapeutic use of these drugs is associated with increased formation of NAPQI. There are no data to suggest that these drugs induce CYP2E1 or alter glucuronidation/sulfation of paracetamol.

### St John’s wort

In human volunteer studies, St John’s wort has been shown to induce significant CYP3A4 activity. There is also a report of CYP2E1 induction by St John’s wort in human volunteers. However, there are no reports of an interaction between St John’s wort and paracetamol, either therapeutically or following overdose.

### Isoniazid

Isoniazid, a hepatotoxic drug in its own right, ligand-stabilizes CYP2E1, which means binding initially inhibits CYP2E1 before inducing it. This process is reflected in controlled human volunteer studies that show initial NAPQI formation is reduced before being enhanced. In one study of 10 slow acetylators (patients with slower isoniazid metabolism due to N-acetyltransferase phenotype) pretreated with isoniazid for 7 days, a 500 mg paracetamol dose resulted in 49%–63% less NAPQI formation during isoniazid treatment compared with 3 days after cessation of isoniazid. In another study, concurrent isoniazid and paracetamol doses resulted in 57% less NAPQI formation, but a paracetamol dose 12 hours after the isoniazid dose resulted in significantly more NAPQI formation in fast acetylators compared with slow acetylators. There are no data to suggest that isoniazid alters the glucuronidation/sulfation of paracetamol. On the basis of the currently available literature, it is not possible to be conclusive as to the potential for isoniazid to increase the risk of hepatotoxicity following paracetamol overdose. We would advise that, given the potential for therapeutic isoniazid use to induce CYP2E1, those taking isoniazid who present with paracetamol overdose should be treated as “high-risk”. It is unclear as to whether ingestion of isoniazid in a nonchronic user could theoretically reduce the risk of paracetamol-related hepatotoxicity by inhibiting paracetamol metabolism via...
CYP2E1 to NAPQI, meaning that this individual would then be “normal-risk” rather than “high-risk”. However, because there is no conclusive evidence to be able to determine the time at which CYP2E1 induction occurs relative to the time of isoniazid ingestion, we feel that to reduce confusion and potential harm to the individual, concurrent or recent single ingestion of isoniazid in relation to paracetamol should be considered a “high-risk” factor for paracetamol-related hepatotoxicity.

Conclusion

The current literature suggests that the CYP isoenzyme responsible for the majority of human biotransformation of paracetamol to NAPQI is CYP2E1 at both therapeutic and toxic doses of paracetamol. The contribution of other isoenzymes, such as CYP1A2, CYP2D6, and particularly CYP3A4 at toxic doses, is unclear, but appears to be minimal.

Current UK guidelines suggest that patients who use a number of drugs therapeutically should be treated as “high-risk” after paracetamol overdose. However, based on our review of the available literature, it appears that the only drugs for which there is the potential for an increased risk are phenobarbital, primidone, isoniazid, and perhaps St John’s wort. For phenobarbital, and its precursor primidone, the overriding clinical concern is the induction of CYP2E1 which is the major isoenzyme responsible for the metabolism of paracetamol to NAPQI. For isoniazid ingestion, we feel that to reduce confusion and potential harm to the individual, concurrent or recent single ingestion of isoniazid in relation to paracetamol should be considered a “high-risk” factor for paracetamol-related hepatotoxicity.

Disclosure

The authors report no conflicts of interest in this work.

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