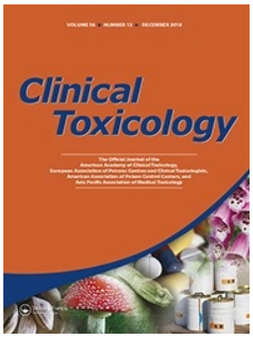

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REVIEW

Metabolic and mitochondrial treatments for severe paracetamol poisoning: a systematic review

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ABSTRACT

Background: Paracetamol (acetaminophen) remains a leading cause of poisoning in Europe, North America, and Australia. For over four decades, acetylcysteine has been the antidote of choice. However, despite the use of acetylcysteine, some patients who ingest very large doses of paracetamol or who reach hospital late in the course of their poisoning, develop acute liver failure. Some will develop metabolic acidosis indicating mitochondrial toxicity.

Objective: We review the experimental and clinical data reported with the use of cimetidine, fomepizole, and calmangafodipir in the treatment of paracetamol toxicity to determine if these treatments alone or in combination with acetylcysteine might be of benefit.

Methods: We searched Ovid Medline 1946–2020, Embase 1947–2020, Scopus 2004–2020, Cochrane Databases of Systematic Reviews (CDSR), Cochrane Central Register of Controlled Trials (CENTRAL), and clinicaltrials.gov 1997–2020 for records including the concepts of paracetamol poisoning and cimetidine, fomepizole, calmangafodipir, and acetylcysteine. We included basic science studies in animals and all available study types in humans. We reviewed the reference lists of included articles to search for references missed in the original search. We registered the protocol in PROSPERO.

Results: We completed all search strategies on 20 August 2019, 27 January 2020, and 15 June 2020. These produced 6,826 citations. We identified and deleted 2,843 duplicate resulting in a total of 3,856 unique citations. After applying inclusion and exclusion criteria, 89 studies remained. The largest numbers of studies described the past use of cimetidine, and the more recent use of fomepizole.

Cimetidine: There is good animal evidence that cimetidine blocks CYP 2E1 with the potential to inhibit the toxic metabolism of paracetamol. Early case reports were inconclusive regarding the benefit to humans in paracetamol poisoning. Two comparative trials found no benefit of cimetidine in paracetamol poisoning, but few patients had severe poisoning.

Fomepizole: There is good animal evidence that fomepizole blocks CYP 2E1 with the potential to inhibit the toxic metabolism of paracetamol. There are no comparative trials of fomepizole for acute paracetamol poisoning. Case reports are inconclusive due to multiple other interventions including the use of acetylcysteine in all cases. The benefit of fomepizole as adjunct treatment has not been demonstrated.

Calmangafodipir: Calmangafodipir, a drug mimicking superoxide dismutase, has emerged as a potential treatment for severe paracetamol toxicity because the formation of superoxide free radicals appears to explain part of the mitochondrial toxicity of extremely large paracetamol overdoses. Calmangafodipir has reached Phase I/II trial of safety in humans with acute paracetamol overdose. Planning for a Phase III study of efficacy is currently underway.

Conclusions: The vast majority of patients with acute paracetamol overdose enjoy excellent outcomes with acetylcysteine alone. Although cimetidine and fomepizole inhibit CYP 2E1 in animals, there is insufficient evidence to recommend their use either as a primary treatment or adjunct therapy in paracetamol poisoning. Calmangafodipir remains investigational.

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Paracetamol; CYP 2E1; cimetidine; fomepizole; mitochondria; calmangafodipir

Background

What's past is prologue
William Shakespeare, *The Tempest*, Act 2, Scene I.

Paracetamol remains a leading pharmaceutical cause of poisoning in overdose throughout Europe, North America, and Australia. Within a decade of its introduction into human use as an antipyretic and analgesic in the 1950s [1], its propensity for hepatic toxicity became apparent [2–4]. Within another decade, acetylcysteine emerged as the antidote of choice for acute paracetamol poisoning [5], and it has remained the antidote of choice for more than four decades.

While the majority of patients with paracetamol overdose recover from their paracetamol poisonings treated with acetylcysteine, some very large paracetamol overdoses exceed the detoxifying capacity of acetylcysteine used in contemporary infusion protocols. These very large overdoses tend to produce hepatic injury, acute liver failure, and mitochondrial dysfunction.

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Since the earliest research, we learned that cytochrome P450 enzymes (later identified as CYP 2E1, CYP 1A2, and CYP 3A4) oxidize paracetamol and produce its characteristic liver injury [6]. In addition to the detoxifying action of endogenous glutathione and exogenous acetylcysteine, piperonyl butoxide – an inhibitor of metabolism by hepatic microsomes – reduced hepatic injury in rats [7]. In a later study, genetic knock-out mice expressing neither CYP 2E1 nor CYP 1A2 consistently survived with no elevation of ALT activity despite paracetamol doses twice the 100% lethal dose in wild-type mice [8]. Together these data suggest the possibility of adding a metabolic inhibitor as a potential treatment for paracetamol poisonings. Later research on the mechanisms of mitochondrial dysfunction in severe paracetamol poisoning has opened a new frontier in the treatment of severe paracetamol overdose. Three drugs represent the past, present, and likely future of metabolic or mitochondrial treatment (in addition to acetylcysteine) in cases of severe paracetamol poisoning.

Objectives

We review the experimental data as well as the human experience with cimetidine, fomepizole, and calmangafodipir. We look back at past research and experience to apply these lessons to the present and to suggest the way forward into the future care of patients with severe paracetamol overdose.

Methods

We undertook a systematic review in adherence to PRISMA guidelines [9] concerning drugs approved human drugs with clinical or experimental evidence of use as an adjunctive treatment for severe paracetamol poisoning. A medical librarian (LHY) searched the literature for records including the concepts of paracetamol poisoning and cimetidine, fomepizole, calmangafodipir, and acetylcysteine. The librarian created search strategies using a combination of keywords and controlled vocabulary in Ovid Medline 1946–present, Embase 1947–present, Scopus 2004–present, Cochrane Databases of Systematic Reviews (CDSR), Cochrane Central Register of Controlled Trials (CENTRAL), and clinicaltrials.gov 1997–present. All search strategies were completed 19 August 2019 and yielded 6,699 results. We deleted 2,843 duplicate records after using published de-duplication processes described resulting in a total of 3,856 unique citations included in the project library [10]. Updated literature searches completed on 27 January 2020 and 15 June 2020 identified an additional 129 unique citations for analysis. Fully reproducible search strategies for each database can be found in the appendix.

From the primary searches, we reviewed the titles and abstracts of articles for further full-text review. We included all available human study designs (case reports, case series, retrospective cohort studies, and prospective comparative trials), animal experiments, and *in vitro* experimental data. We set an *a priori* focus on cimetidine, fomepizole, and calmangafodipir, but we included any sources describing or mentioning the use of a drug approved for human use for any indication in the setting of paracetamol toxicity. We excluded studies of botanical products. We sought to summarize the evidence surrounding the use of adjunctive medication (in addition to acetylcysteine) aimed at inhibiting the oxidation of paracetamol by cytochrome P450 enzymes (particularly CYP 2E1) or treating mitochondrial dysfunction in patients with severe paracetamol toxicity. After applying these inclusion and exclusion criteria, we analyzed the 89 remaining records. We classified the quality of evidence using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) system of rating quality of evidence and grading strength of recommendations [11].

Results

Cimetidine

Cimetidine, the first approved histamine-2 receptor antagonist, is associated with a large number of drug–drug interactions mediated through various hepatic enzymes, including CYP 2E1 [12,13]. This led to research into whether cimetidine would be effective in mitigating paracetamol poisoning.

In vitro studies

In a series of experiments, Mitchell et al. [14] compared cimetidine and ranitidine in murine hepatic microsomes bathed in paracetamol 1 mM (151 mg/L) and 10 mM (1510 mg/L) with cimetidine concentrations between 0.1 and 1 mM. In the first experiment, cimetidine, but not ranitidine, showed a dose-dependent reduction in cysteinyl-paracetamol formation with 56% reduction at 1 mM of cimetidine. This effect persisted in the second (paracetamol 10 mM) experiment with a 50% reduction in cysteinyl-paracetamol formation at 1 mM of cimetidine.

Dalhoff and Poulsen [15] incubated rat hepatocytes in 60 μg/mL (0.24 mM) of cimetidine for 30 min before incubating them in paracetamol 5 mM (755 mg/L). They found a 38% decrease in the formation of glutathionyl-paracetamol in cimetidine treated cells.

Hazai et al. [16] studied several inhibitors of different CYP enzymes in cultured human hepatic microsomes incubated in paracetamol at 50 μM or 250 μM. Microsomes exposed to paracetamol produced NAPQI in proportion to their CYP 2E1 activity (measured by 6-hydroxylation of chlorzoxazone). Inhibitors of CYP 1A2 (a-naphthoflavone) and CYP 3A4 (troleandomycin) had no effect on NAPQI formation. A comparison of four CYP 2E1 inhibitors (each at 100 μM) found that cimetidine had no apparent effect on NAPQI formation, but fomepizole, disulfiram, and diethyl-dithiocarbamate reduced NAPQI formation by 65, 93, and 77%, respectively. The concentrations for 50% inhibition of CYP 2E1 (IC_{50}) were 50 μM for fomepizole and 8 μM for disulfiram. This study suggests

fomepizole may be effective but that cimetidine may be ineffective.

These conflicting results may stem from higher cimetidine and paracetamol concentrations in the first two studies and lower concentrations studied by Hazai et al.

Animal studies

Thirteen studies have investigated the use of cimetidine in experimental models of paracetamol poisoning.

In the first of these Mitchell et al. [17] compared cimetidine 120 m/kg or acetylcysteine 1000 mg/kg in one of two schedules: 1 h before plus 6 h after paracetamol 500 mg/kg or 4 h plus 10 h after paracetamol. Pretreatment with either cimetidine or acetylcysteine resulted in 100% survival compared to 43% of the control group receiving only paracetamol. Cimetidine appeared slightly inferior to acetylcysteine when started at 4 h after paracetamol. Cimetidine and acetylcysteine similarly reduced AST and ALT activities by 80–90% in pretreatment and 50–60% in post-treatment. No rats received both treatments in combination.

Rudd et al. [18,19] studied cimetidine 200 mg/kg given 30 min before paracetamol 500 mg/kg in mice plus cimetidine 100 mg/kg given hourly for 7 h. Comparison groups received either cimetidine or paracetamol only. The median AST activity was 121 U/L in the treatment group compared to 996 U/L in the paracetamol only group.

In another study, mice received cimetidine 100 mg or acetylcysteine 600 mg/kg 1 h after paracetamol 1,200 mg/kg [20]. Seven-day survival was 100% in the acetylcysteine group, 68% in the cimetidine group, and 24% in the control group.

Peterson et al. [21] gave mice cimetidine 100 mg/kg 1 h before and 1 h after paracetamol 350 mg/kg. They found that cimetidine prevented a rise in ALT activity and tissue necrosis seen on microscopy.

Miners et al. [22] gave mice cimetidine 50 mg/kg or 100 mg/kg 30 min before and again 1 h after paracetamol 200 mg/kg. Both doses of cimetidine blunted the rise in ALT activity assessed at 7 h after paracetamol from ALT 1960 ± 291 U/L to 225 ± 42 U/L and 192 ± 30 U/L, respectively, but cimetidine had little effect on the proportions of oxidative and conjugated paracetamol metabolites recovered in the urine over 24 h.

Mitchell et al. [14] pretreated rats with 3-methycholanthrene (intended to increase oxidative drug metabolism) and gave them cimetidine 150 mg/kg 30 min before paracetamol 500 mg/kg. Cimetidine reduced glutathione depletion with a minimum of 13.9 ± 1.3% of glutathione remaining after paracetamol alone versus 30.3 ± 1.9% remaining after cimetidine. Cimetidine also reduced the covalent binding of paracetamol to hepatic proteins from 552 ± 23.8 nmol/g of protein to 170 ± 31.6 nmol/g of protein.

The same author group later studied varying doses and combinations of cimetidine and acetylcysteine in rats [23]. They found that the combination of cimetidine 50 mg/kg plus acetylcysteine 400 mg/kg resulted in greater survival (89%), smaller increases in AST and ALT activities, and slower depletion of glutathione than either agent alone (67% after cimetidine 75 mg/kg, 56% after acetylcysteine 400 mg/kg.

Murase et al. [24] administered cimetidine 200 mg/kg to rats pretreated with 3-methylcholantrene 72 h before paracetamol 800 mg/kg. The rats received two doses of cimetidine 1 h before and 1 h after the paracetamol. Cimetidine treatment resulted in a slight elevation in mean AST and ALT activities (171 U/L and 22 U/L) compared to 5,760 U/L and 1,862 U/L after paracetamol alone, reduced glutathione depletion and prevented necrosis on microscopy. Ranitidine and famotidine had little or no effect on transaminase activities.

Another rat study by Okuno et al. [25] corroborated the effect of cimetidine on rats poisoned with paracetamol and again found that cimetidine blunted the increases in AST and ALT activities and the decrease in glutathione following paracetamol.

Al-Mustafa et al. [26] studied transaminase activities and survival to 24 h in mice dosed with paracetamol 400 mg/kg by gavage. Mice received either acetylcysteine 400 mg/kg 2 h after paracetamol dosing, or acetylcysteine 400 mg/kg plus cimetidine 150 mg/kg 2 h after paracetamol dosing, or cimetidine 150 mg/kg 2 h post-paracetamol dosing, or cimetidine 150 mg/kg at 2 h and 6 h post-paracetamol dosing. The administration of acetylcysteine alone resulted in a survival rate of 72.7% (compared to 37.5% in control animals), whereas the administration of acetylcysteine and a single dose of cimetidine resulted in a survival rate of 100%. The administration of either one or two doses of cimetidine produced a survival rate of 85.7 and 93.3%, respectively. Although the administration of acetylcysteine significantly ($p < 0.05$) decreased both ALT and AST activities compared to control, co-administration of acetylcysteine, and one dose of cimetidine resulted in no increase in AST or ALT activities.

Sajedianfard and colleagues [27,28] performed two sets of experiments with mice and rabbits. Both experiments used cimetidine alone at different dosing intervals after paracetamol. The first article described mice receiving cimetidine 12.5 mg/kg at 0, 1, 2, 4, or 8 h after paracetamol 3 mg/kg [27]. There was no consistent effect on mortality, activities of AST and ALT, or histopathology. The experiment used much lower cimetidine doses than any previous study without explanation for the selected dose. The reported paracetamol dose was two orders of magnitude lower than all other murine studies. The report lacked details of the timing of outcome measurements, so assessment of the study is impossible.

Their second study in rabbits included nine different treatment groups of cimetidine 40 mg/kg in single or divided doses after paracetamol 3.24 g/kg [28]. Outcomes included ALT, AST, and arginase activities and total bilirubin concentration at 12 and 36 h after paracetamol. The single doses of cimetidine at 2 or 4 h appeared to have better measures of all outcomes than did cimetidine at 0 h. The reason for this is unclear.

Zira et al. [29] compared cimetidine 50 mg/kg and acetylcysteine 150 mg/kg in rabbits given paracetamol 2 g/kg. The outcomes were AST and ALT activities at 24 h. The AST and ALT activities in the control group were 405.2 ± 88.3 U/L and 1487.3 ± 21.6 U/L respectively. Cimetidine treatment alone

resulted in AST and ALT activities of 119.2 ± 15.8 U/L and 190.8 ± 15.7 U/L respectively. Acetylcysteine produced the lowest AST and AST activities (79.2 ± 6.7 U/L and 122.8 ± 7.2 U/L), which were significantly lower than in the cimetidine group and similar to the unpoisoned control group.

Taken together, these animal studies generally showed that cimetidine reduced hepatotoxicity caused by paracetamol. Acetylcysteine was superior to cimetidine in the three studies comparing the two treatments [17,26,29]. The only study including a treatment arm with both acetylcysteine and cimetidine found that the combination was superior to either treatment alone [26].

Human experience

Nine review articles on paracetamol poisoning have mentioned the possibility of the beneficial effect of cimetidine based upon early animal studies [13,30–37]. Most cited little or no human evidence. Among these, a review by Kaufenberg and Shephard [36] did include human data, but seven of their sources involved therapeutic doses of paracetamol (650–1,000 mg) [14,38–43]. All of these showed no demonstrable change in pharmacokinetics or metabolism of paracetamol, but all were quite far from the severe paracetamol overdoses of interest to the present discussion.

Case reports and case series in paracetamol overdose. Seven case reports or case series mentioned cimetidine either in adjunctive use or use preceding the paracetamol overdose [20,44–49]. Jackson et al. [20] described a case of an 18-year-old woman with two different paracetamol overdoses (reportedly 10 g each) approximately 1 month apart. The first overdose included over 1,200 mg of cimetidine and "small amounts" of flurazepam and methaprilene/scopolamine while the second included only paracetamol. Her apparent plasma half-life was 4.4 h after the first overdose and 3.3 h after the second overdose. The authors did not comment on the possible role of the antimuscarinic co-ingestant in the first overdose.

Smith et al. [44] reported a case of a 20-year-old woman with a paracetamol concentration of 459 μmol/L (69.3 mg/L) 11 h after a reported 10 g paracetamol overdose. Following a loading dose of 150 mg/kg of acetylcysteine, she had an urticarial rash and sensation of fullness in the throat. She responded well to adrenaline, hydrocortisone, and promethazine, but receive no further acetylcysteine. In lieu of acetylcysteine, she received a single dose of 40 mg/kg of cimetidine, and recovered uneventfully with no abnormal hepatic enzyme activities. With a dose of acetycysteine sufficient to neutralize any NAPQI formed, the role of cimetidine is unclear.

Kaysen et al. [45] described five alcoholic patients with hepatic and renal injury after taking "therapeutic" doses of paracetamol. One of these, a 41-year-old woman who reported taking 20 tablets of paracetamol/codeine with an estimated total of 19.5 g of paracetamol within 3 d while continuing to drink 0.5 L of whiskey per day. Her AST and ALT activities on arrival were 23,000 U/L and 939 U/L, respectively. She died on the eighth hospital day after a complicated course. Some features of her case make it less clear that paracetamol alone caused her demise, and the role of cimetidine is impossible to assess.

A 5-year case series by Monteagudo and Folb [46] summarized 91 cases paracetamol poisoning. Out of the 91 patients, 10 received cimetidine. There were no details about the cimetidine dose, frequency, and duration nor about the severity of these paracetamol poisonings. We cannot tell whether poisoning severity, clinician preference, or other factors motivated the addition of cimetidine. The contribution of cimetidine to patient outcomes is impossible to assess.

Three case reports plausibly suggested the benefit of cimetidine in addition to acetylcysteine in patients with severe paracetamol poisonings. Kadri et al. [47] described a patient chronically taking cimetidine 400 mg nightly before she overdosed on 50 tablets of paracetamol with a plasma paracetamol concentration of 7,400 μmol/L (1,117 mg/L) and arterial pH of 7.20 on arrival about 8 to 10 h later. She received IV acetylcysteine and bicarbonate infusion. She recovered with no ALT above 30 U/L and no prothrombin ratio above 1.2. This suggested a possible contribution of the cimetidine to her recovery despite an extremely large paracetamol overdose.

Rolband and Marcuard [48] described a single case with a 4-h paracetamol concentration of 410 mg/L (2715 μmol/L). In addition to oral acetylcysteine (140 mg/kg once then 70 mg/kg every 4 h), she received IV cimetidine 600 mg once then 80 mg/h constant infusion for 4 d. Her highest AST activity was 83 U/L, and she recovered uneventfully. This case also suggested the benefit of cimetidine in a high-risk paracetamol overdose.

Block et al. [49] described a 24-year-old woman who arrived to the hospital with hypothermia (19 °C), hypotension 50 mmHg/30 mmHg, a paracetamol concentration of 943 μmol/L (142 mg/L) h, and a pH of 7.13 about 18 h after an overdose of dextropropoxyphene-paracetamol and paracetamol. In addition to IV acetylcysteine and rewarming, she received cimetidine (dose, frequency, and route unstated). Her ALT activity 4 d after admission was 27 U/L with a normal bilirubin and prothrombin time. Although hypotension, hypothermia, or co-ingestion of dextropropoxyphene could plausibly have affected either absorption or metabolism of paracetamol, it is possible that cimetidine contributed to her recovery.

Comparative trials in overdose. Only two comparative trials attempted to assess the effect of the addition of cimetidine to IV acetylcysteine in patients with acute paracetamol overdoses [50,51]. First, Burkhart et al. [50] undertook a 2-year study of patients referred by telephone to Rocky Mountain Poison and Drug Center with paracetamol concentrations above the 150 line and starting acetylcysteine at least 8 h after an overdose. They attempted to allocate patients to acetylcysteine (oral or intravenous) in even-numbered months and acetylcysteine plus cimetidine 300 mg IV every 6 h in odd-numbered months. The acetylcysteine only group had 66 patients while the acetylcysteine plus cimetidine

group had 41 patients. The authors neither commented on this imbalance nor revealed whether they analyzed patients by intent-to-treat or as treated. Otherwise, the two groups were similar at enrollment. Patients receiving acetylcysteine had a slightly longer time to paracetamol concentration, slightly longer time to first acetylcysteine dose, and slightly lower paracetamol concentrations, but none of these comparisons reached statistical significance. Outcomes including peak values of AST, ALT, and bilirubin as well as proportions of patients with AST $>$ 100 U/L or AST $>$ 1,000 U/L were similar between groups. No patient died from paracetamol toxicity, and no patient developed encephalopathy. They concluded that the addition of cimetidine in these patients had no effect on hepatotoxicity. They speculated that larger or more frequent doses of cimetidine might be helpful.

After the Burkhart study, clinical interest in cimetidine appeared to collapse. Two decades later, Ebrahimi et al. [51] randomized patients with acute paracetamol overdoses with paracetamol concentrations above the treatment line to the three-bag IV acetylcysteine protocol plus IV cimetidine 300 mg every 6 h or acetylcysteine alone. Their primary outcome measures were AST and ALT activities at 4, 12, 24, and 48 h after admission. The groups had similar characteristics on arrival. The authors reported mean time from overdose to arrival of about 9 h in both groups with a similar mean concentration of paracetamol, and similar mean AST and ALT activities at arrival. Several flaws in the presentation and data analysis make this study more difficult to interpret. Although the authors used parametric testing (Student's *t*-test) for obviously non-parametric data, the two groups appeared to maintain similar characteristics at each time interval. Paracetamol was undetectable in most patients at 24 h and in all patients by 48 h. The authors did not report any occurrences of acute liver failure. They concluded that the addition of cimetidine 300 mg every 6 h ha no effect on outcomes and echoed speculation by Burkhart et al. [50] 20 years earlier that larger cimetidine doses might be effective.

A common limitation shared by the Burkhart et al. [50] and Ebrahimi et al. [51] studies is that both mainly enrolled patients who were above but near the 150 line with few, if any, high-risk patients. Thus, we would expect these patients to have good outcomes with acetylcysteine alone. If cimetidine were to have any benefit in some patients with paracetamol overdose, perhaps higher-risk patients (higher paracetamol concentrations on arrival, late presenting patients with unmetabolized paracetamol, etc.) would be those who might derive benefit.

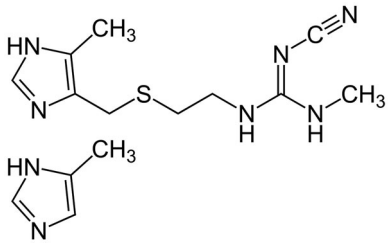

Figure 1. Shared chemical structure of cimetidine (top) and fomepizole (bottom).

Fomepizole

Fomepizole (Figure 1) is an approved antidote for poisoning by ethylene glycol or methanol [52,53]. Fomepizole is a potent inhibitor of alcohol dehydrogenase with a minimal effective concentration of 10 μM in monkeys [54] and humans [52,53,55]. Fomepizole also inhibits CYP 2E1 at a slightly higher concentration (IC_{50} = 50 μM) [16]. This effect led to animal research and some human clinical experience with fomepizole for acute paracetamol poisoning.

In addition to inhibiting CYP 2E1, fomepizole directly inhibits the c-jun N-terminal kinase (JNK) pathway of mitochondrial dysfunction [56,57].

In vitro studies

Three *in vitro* studies provide evidence of the mechanisms by which fomepizole may reduce paracetamol toxicity. First, Dai and Cederbaum [57] undertook a series of experiments using a human hepatoma cell line (MVh2E1-9) genetically engineered to express high levels of CYP 2E1 and a second cell line (MV-5) engineered to express no CYP 2E1. They bathed the cell cultures with L-buthionine sulfoximime (inhibitor of glutathione synthesis) and paracetamol. They assessed cell injury by measuring leakage of lactate dehydrogenase (LDH) in the cell media and by microscopy. They first demonstrated dose-dependent paracetamol toxicity in the MVh2E1-9 cells but not the MV-5 cells. Then they found that the addition of fomepizole at 0.5 mM and 2.0 mM prevented LDH leakage from the MVh2E1-9 cells.

Hazai et al. [16] studied the effects of several inhibitors of different CYP enzymes in cultured human hepatic microsomes from five human donors. Microsomes exposed to paracetamol produced NAPQI in proportion to their CYP 2E1 activity (measured by 6-hydroxylation of chlorzoxazone). Inhibitors of CYP 1A2 (a-naphthoflavone) and CYP 3A4 (troleandomycin) had no effect on NAPQI formation. Inhibition of CYP 2E1 with diethyldithiocarbamate reduced NAPQI formation by 77% compared to control. A comparison of four inhibitors of CYP 2E1 found that disulfiram, diethyl-dithiocarbamate, fomepizole, and diallyl sulfide (in order of effectiveness) all reduced NAPQI formation relative to control. Cimetidine had no apparent effect on NAPQI formation in this experiment. The concentrations for 50% inhibition of CYP 2E1 were 50 μM for fomepizole and 8 μM for disulfiram. This study suggests fomepizole may be effective but that cimetidine may be ineffective.

In 2018, Akakpo et al. [56] studied fomepizole in cultures of human hepatocytes bathed in 10 mM (1512 mg/L) paracetamol with or without 2 mM (164 mg/L) fomepizole. After 24 h, they measured ALT activities in the cell media and examined the cells by microscopy. They found that paracetamol treated cells released 40% of cellular ALT into the medium while cells in treated with paracetamol plus fomepizole, fomepizole alone, or control all released less than 10% of cellular ALT.

Animal studies

A study in rats by Burk et al. [58] investigated the effects of fomepizole as a CYP enzyme inhibitor. Rats in all three

groups received paracetamol 750 mg/kg with or without fomepizole 100 mg/kg given 30 min before followed by 50 mg/kg 8 h after paracetamol [58]. Pretreatment included isoniazid for 10 d, phenobarbital for 4 d, or no pretreatment. Isoniazid and phenobarbital each amplified the paracetamol toxicity measured by ALT at 24 and 48 h. ALT activities (mean ± SD in mU/mL) at 24 h were 969 ± 760 (no inducer), 4,450 ± 1,880 (phenobarbital), and 8,300 ± 4,100 (isoniazid). Fomepizole prevented ALT elevation in the three pretreatment groups (46 ± 13, 64 ± 12, and 54 ± 19, respectively). Fomepizole also reduced glutathione depletion.

In 1994, Brennan et al. [59] gave rats 2,000 mg/kg of paracetamol followed 4 h or 8 h later by fomepizole 50 mg/kg, fomepizole 400 mg/kg, or no antidote. A fourth group received no paracetamol or fomepizole. They sacrificed the animals at 28 h for measurement of AST and ALT and for the microscopic examination of the livers. Rats receiving fomepizole 400 mg or 50 mg at 4 h had lower AST and ALT activities ($p < 0.01$ and $p < 0.05$, respectively for the two doses) than control animals and also had nearly normal liver microscopy. Although mean AST and ALT activities were lower when fomepizole 50 mg/kg and fomepizole 400 mg/kg were administered 8 h after paracetamol, these results were not statistically significant from those in double-control rats.

Küçükardali et al. [60] studied six groups of ten rats each. Groups 1 through 5 all received 2000 mg/kg paracetamol by gavage, while group 6 was the control group. Antidotal treatment in groups 2 through 5 included: group 2) acetylcysteine 140 mg/kg by gavage then 70 mg/kg every 4 h for 7 doses; group 3) fomepizole 50 mg/kg; group 4) fomepizole 200 mg/kg; and group 5) fomepizole 200 mg/kg and acetylcysteine as in group 2. The measured serum AST and ALT activity and graded hepatic necrosis on microscopy using the same grading system as did Brennan et al. [59]. All antidotal combinations reduced ($p < 0.001$) AST and ALT activities compared to group 1 and reduced the degree of necrosis. However, higher activities of ALT were found in group 5 than in group 2 ($p < 0.05$), and higher activities of AST were found in group 5 than in group 3 ($p < 0.01$). Combining fomepizole and acetylcysteine offered no additional benefit over acetylcysteine alone.

Akakpo et al. [56] studied fomepizole 50 mg/kg versus saline vehicle in mice treated with 300 mg/kg of paracetamol. At 6 h after an overdose, mice treated with fomepizole had essentially no elevation in plasma ALT activity and no hepatic necrosis seen on microscopy. By comparison, the control mice had marked elevation of ALT and characteristic hepatic necrosis. They also measured plasma concentrations of paracetamol, its glucuronide and sulfate conjugates, and its oxidative metabolites. Fomepizole had no effect on concentrations of paracetamol and paracetamol conjugates, but fomepizole treatment resulted in much lower concentrations of cysteinyl, acetylcysteinyl, and glutothionyl metabolites.

Akakpo et al. [61] followed this with a post-treatment study of rats given paracetamol 300 mg/kg or 600 mg/kg. Ninety minutes after paracetamol, the animals received fomepizole 50 mg/kg or 200 mg/kg, acetylcysteine 500 mg/kg, or a combination of fomepizole and acetylcysteine. After the lower dose of paracetamol (300 mg/kg), post-treatment with fomepizole was almost as effective as acetylcysteine in preventing elevated ALT activity at 6 and 24 h. The combination of either dose of fomepizole with acetylcysteine was better than fomepizole alone but not better than acetylcysteine alone. The protection of fomepizole alone was lost when it was administered 3 h after paracetamol. Six hours after paracetamol, rats treated with fomepizole 50 mg/kg had twice as much glutathione per g of liver tissue, one-seventh the ratio of glutathione disulfide to total glutathione (GSSG/GSH), and one-half the concentration of cysteinyl paracetamol adducts in comparison to rats receiving no antidote. After the higher dose of paracetamol (600 mg/kg), post-treatment with fomepizole resulted in the lowest mean ALT activity (less than 200 U/L compared to just over 400 U/L after acetylcysteine, and approximately 4600 U/L with no antidote.

Together these *in vitro* and animal studies confirmed the central role of CYP 2E1 in paracetamol toxicity and demonstrated fomepizole's ability to prevent or treat paracetamol toxicity in these experimental models. The favorable effect of fomepizole was consistent among the studies. Only one of the studies compared the combination of fomepizole and acetylcysteine to either agent alone [61].

Human experience

The published human experience comprises seven case reports (three as abstracts), three small case series (one as an abstract), and one human volunteer study in supratherapeutic paracetamol exposure. The case reports and case series together describe 17 patients with one patient shared between an abstract and later publication. The human volunteer study enrolled six subjects in a crossover control study.

A 2013 case report by Zell-Kanter et al. [62] described a patient found obtunded with agonal respirations after an apparent suicide attempt. She underwent immediate intubation in the ED, where her initial arterial pH was 6.9 with lactate of 22 mEq/L. Her initial paracetamol concentration was 1,141 mg/L (7,548 μmol/L), and a repeat concentration was 1,193 mg/L (7,892 μmol/L) several hours later. The authors highlighted this case as "the highest reported acetaminophen level in a patient successfully treated with only intravenous N-acetylcysteine along with supportive care" [62]. Although the case explicitly mentioned an empiric dose of fomepizole early in the case due to concern for possible toxic alcohol poisoning, the authors made no further comment regarding fomepizole.

In a 2016 commentary about this case report, Yip and Heard [63] suggested that inhibition of CYP 2E1 by fomepizole may have contributed to the favorable outcome and that some patients with unusually large paracetamol ingestions might benefit from the addition of fomepizole.

A 2015 case report by Villano et al. [64] described a 28-year-old man who developed altered mental status 16 h after police arrest and confinement in jail. He was unresponsive, tachycardic, tachypneic, and acidotic (arterial pH 6.97). These findings prompted empiric treatment with fomepizole (dose and time not stated) and consideration for hemodialysis. His

initial paracetamol concentration was 616 mg/L (4,075 μmol/L). He then received IV acetylcysteine for 45 h until paracetamol was undetectable. His highest observed AST activity was 67 U/L. He recovered without hemodialysis. The authors made no comment about the role of fomepizole.

Four similar case reports described patients treated with fomepizole, acetylcysteine, and hemodialysis. A 2017 abstract by Appleqvist and Lindeman [65] described a young man with a paracetamol concentration of 687 mg/L (4,545 μmol/L) and lactate of 11 mmol/L about 2.5 h after ingestion of about 100 g of paracetamol. Activated charcoal 50 g was given, and treatment with acetylcysteine was started at 4 h after ingestion. The paracetamol concentration at 6 h was 475 mg/L. Out of concern for the risk of complications, he received fomepizole 15 mg/kg (from 5.5 h) and underwent dialysis. The second acetylcysteine dose was also doubled (25 mg/kg/h) at 7 h with the initiation of continuous venovenous hemodiafiltration (CVVHDF) (effluent flow 48 mL/kg/h) to compensate for extracorporeal elimination of acetylcysteine. The third acetylcysteine dose remained at this level until 16 h when it was lowered (12.5 mg/kg/h). Acetylcysteine and CVVHDF were terminated at 40 h. The ALT activity was minimally elevated to 70 U/L at 8.5 h and was normal at 60 h. Kidney function was unaffected.

A 2019 case report by Kiernan et al. [66] described a 68-year-old woman who was unconscious (GCS 3) after reported ingestion of 208 tablets of paracetamol 500 mg/diphenhydramine 25 mg). On arrival at the ED she had a GCS of 3 without spontaneous respiration and was intubated and ventilated. The patient subsequently became hypotensive requiring five IV push doses of epinephrine (total 100 μg), followed by dopamine (10 μg/kg/min increased to 15 μg/kg/min), and then a norepinephrine infusion (10 μg/min). Her paracetamol concentration was 1,017 mg/L (6,728 μmol/L) with a lactate concentration of 7.6 μmol/L. The initial AST activity was 21 IU/L (10–34 IU/L) and ALT activity was 99 IU/L (8–37 IU/L). A drug screen of abuse detected only methadone, and serum liquid chromatography/mass spectroscopy detected caffeine, dihydrocodeine/hydrocodol, lidocaine, monoethylglycinexylidide, and diphenhydramine. She received IV sodium bicarbonate (for prolonged QRS interval), acetylcysteine, and fomepizole 15 mg/kg and underwent immediate hemodialysis. During hemodialysis, the rate of acetylcysteine administration was doubled to 200 mg/kg and then tripled to 300 mg/kg, and a subsequent dose of fomepizole10 mg/kg was administered due to concern that both antidotes were being removed by hemodialysis. No further transaminase activities were reported but she was discharged from a psychiatric hospital 8 d later.

A 2020 case report by Woolum et al. [67] described a 55-year-old man with unresponsiveness and a paracetamol concentration of 883 mg/L (5,841 μmol/L), a venous pH of 7.08, and a lactate concentration of 17.5 mmol/L. He received IV acetylcysteine, a continuous infusion of sodium bicarbonate due to a prolonged QRS duration of 114 ms and concomitant acidotic state, and intravenous fomepizole 15 mg/kg due to the patient's anion gap metabolic acidosis. Within 3 h of hospital admission, he underwent a 3-h course of conventional hemodialysis. Due to the severity of his paracetamol ingestion (subsequently confirmed by the patient as 65 g), the acetylcysteine infusion rate was increased from 6.25 mg/kg/h to 18.75 mg/kg/h for the duration of treatment. He received no fomepizole after the initial first dose due to improving pH and lack of detection of toxic alcohols. Peak AST/ALT activities on day 2 were 1791 U/L and 743 U/L respectively.

A 2020 abstract by Colon-Hidalgo et al. [68] an 18-year-old woman who arrived in the ED 10 h after ingestion of approximately 46 g of paracetamol. Her initial paracetamol concentration was >250 mg/L (>1,655 μmol/L). She received oral and IV acetylcysteine (doses not specified). Her repeat paracetamol concentration 4 h later was still >250 mg/L, which prompted the addition of fomepizole 15 mg/kg and 4 h of hemodialysis followed by 10 mg/kg fomepizole and 12 h of continuous venovenous hemodialysis (CVVHD). Her paracetamol concentration declined to 137 mg/L (906 μmol/L) after HD and was undetectable 18 h later. Her peak transaminase activities were AST 3,820 IU/L, ALT 4,346 IU/L with an INR of 2.4. She survived and was discharged to a psychiatric facility 6 d after admission.

All 4 patients recovered after combined treatment including hemodialysis intensified acetylcysteine infusions (doubling or tripling the infusion rates or combining IV and oral acetylcysteine), and one or two doses of fomepizole. Since paracetamol is dialyzable, and since the guidelines from the Extracorporeal Treatments in Poisoning (EXTRIP) Workgroup recommend hemodialysis in at least two of these cases [69], the relative contribution of fomepizole is unclear.

Rampon et al. [70] reported on 6 patients who received intravenous acetylcysteine (30 mg/mL at 150 mg/kg load followed by 12.5 mg/kg/h) and fomepizole (15 mg/kg once followed by 10 mg/kg every 12 h) as an adjunct therapy and 1 patient also received hemodialysis. A 41-year-old woman presented after ingesting temazepam and lorazepam and had a paracetamol concentration of 140 mg/L (927 μmol/L) and a salicylate concentration of 45 mg/dL 4 h after ingestion. Due to persistently high paracetamol concentrations and "concern for co-ingestants including salicylates", she underwent one session of hemodialysis. The peak ALT activity was 79 U/L and AST activity was 51 U/L 62 h after ingestion. A 42-year-old woman had a paracetamol concentration of 135 mg/L at 4 h and received IV acetylcysteine. As the paracetamol concentration rose to 251 mg/L (1,582 μmol/L) at 6 h, she received fomepizole 15 mg/kg. She had no increases in ALT or AST activity. A 9-year-old boy with a paracetamol concentration of 281 mg/L (1,860 μmol/L) at 4 h received IV acetylcysteine. His repeat paracetamol concentration was still 239 mg/L (1,582 μmol/L) at 6 h, and he received fomepizole. His ALT activity did not rise. A 15-year-old girl had a 2 h paracetamol concentration of 236 mg/L (1,562 μmol/L) and started on acetylcysteine. As the paracetamol concentration rose to 311 mg/L (2,058 μmol/L) at 8.5 h, the dose of acetylcysteine was increased to 18.75 mg/kg/h and fomepizole was administered. The girl developed no biochemical evidence of liver damage. A 42-year-old woman was admitted after ingesting ibuprofen, loperamide, and paracetamol. She had a 5 h paracetamol concentration of 201 mg/L and received IV

acetylcysteine. Due to elevated paracetamol concentrations and concern that co-ingestants might delay the absorption of paracetamol and prolong elimination, fomepizole was also given. The ALT activity remained normal. A 15-year-old girl had a 4 h paracetamol concentration of 361 mg/L (2,390 μmol/L) and received IV acetylcysteine. Fomepizole was added at 5.5 h. She did not develop hepatic damage. A graph of their serial paracetamol concentrations illustrated that a paracetamol half-life of approximately 4 h in 5 of the 6 patients.

Shah and Beuhler [71] recently described a 33-year-old man who presented to the emergency department with abdominal pain after taking 50 paracetamol 500 mg tablets over the preceding 2 d. He was tachycardic and tachypneic. The paracetamol concentration was 337 mg/L and his AST and ALT activities were respectively 137 IU/L and 194 IU/L; arterial pH 7.24 (3 h after admission); and lactate 4.1 mmol/L. He had a history of chronic ethanol use, but the ethanol concentration was not detectable on arrival. IV acetylcysteine was started 9 h after arrival, with 150 mg/kg given over 1 h, 12.5 mg/kg/h for 4 h, and then 6.25 mg/kg/h for 9 h, after which the rate was switched to 15 mg/kg/h. He received fomepizole 15 mg/kg IV 11 h after arrival as an adjunct for the expected severe paracetamol toxicity. Peak transaminase activities occurred 2 d after admission, with an AST activity of 198 IU/L and an ALT activity of 301 IU/L. Acetylcysteine was discontinued 3 d after admission, and he was discharged.

Shah et al. [72] have also reported in abstract the clinical course of 3 patients poisoned with paracetamol who received acetylcysteine and fomepizole IV (doses and timing of antidotes not stated). The abstract included few clinical details, but all patients recovered. A 62-year-old woman was admitted after an acute overdose of paracetamol. Her paracetamol concentration approximately 5 h after ingestion was 1,014 mg/L (6,708 μmol/L) with normal transaminase activities at 5 h post-ingestion but no other biochemical data. A 56-year-old woman with acute on chronic ingestion of paracetamol had an initial paracetamol concentration of 298 mg/L (1,971 μmol/L) with AST and ALT activities of 245 U/L and 326 U/L respectively. No other biochemical data were given. A 58-year-old woman was admitted at an unknown time after the acute ingestion of paracetamol. The paracetamol concentration was 22.4 mg/L (148 μmol/L), the ALT activity was 3,246 IU/L, and the AST activity was 5,971 IU/L. No other biochemical data were provided. Insufficient details are given to draw any conclusions on the benefit of either antidote.

The same author group described a case series of 2 patients in a letter to the editor [73]. One patient was the second patient in the abstract with no new details presented. The other patient was a 44-year-old woman with a history of alcohol and polysubstance use who had altered mental status with lactate of 14.3 mmol/L and an arterial pH of 7.276. Paracetamol concentration was 108.1 mg/L (716 μmol/L) 13 h after arrival after arrival with AST and ALT activities of 1,516 IU/L and 1,190 IU/L, respectively. She then received oral acetylcysteine 140 mg/kg once and 70 mg/kg every 4 h beginning 19 h after arrival. The poison center recommended fomepizole 15 mg/kg. By day 3, her INR rose to 6.7 with AST and ALT activities of >5,000 IU/L and 8,372 IU/L, respectively. She then received IV acetycysteine 150 mg/kg load followed by an infusion of 15 mg/kg/h. She survived to hospital discharge.

Kang et al. [74] undertook a double-blinded, placebo-controlled trial of IV fomepizole in human volunteers with a supratherapeutic oral paracetamol dose of 80 mg/kg. Subjects received fomepizole 15 mg/kg IV just before the oral paracetamol and 10 mg/kg 12 h later. The measured paracetamol, conjugates, and metabolites in plasma and urine over 24 h. Peak paracetamol concentrations averaged approximately 121 mg/L (800 μmol/L) at 1 h with 4-h paracetamol concentrations of approximately 68 mg/L (450 μmol/L) in both treatments. Their principal finding was fomepizole reduced the fraction recovered in the urine as oxidative metabolites decreased from 4.48–0.51% of the ingested dose.

The single controlled trial of fomepizole in supratherapeutic paracetamol exposure [74] is consistent with the *in vitro* and animal data that fomepizole inhibits oxidation of paracetamol by CYP 2E1. The combined administration of acetylcysteine and fomepizole and occasionally the use of hemodialysis in these case reports [62,64–73] limit conclusions from these reports. The benefit of fomepizole as adjunct treatment is unclear.

Calmangafodipir

Severe metabolic acidosis following very large paracetamol overdoses reflects mitochondrial injury. NAPQI production exceeding the detoxifying capacity of available glutathione reacts with sulfhydryl groups to form both cytosolic and mitochondrial protein adducts. Mitochondrial adducts appear to produce superoxide and peroxynitrite free radicals. These reactive oxygen species produce further oxidative damage to the mitochondrion and proteins [75,76]. These molecules begin a cascade leading to mitogen-activated protein kinase (MAP kinase) phosphorylation in the cytosol. This further leads to phosphorylation of c-jun N-terminal kinase (JNK) and its movement to the outer membrane of the mitochondrion. JNK disrupts the electron transport chain and promotes the opening of the mitochondrial permeability transition pore (MPTP), which depolarizes the mitochondrial outer membrane [75,77]. The loss of the outer mitochondrial membrane integrity leads to the release of intermembrane proteins into the cytosol, which is largely responsible for the cellular death in paracetamol-mediated liver injury [78].

Manganese superoxide dismutase (MnSOD) is a mitochondrial metalloprotein that scavenges superoxide free radicals [79]. Its principal role is to catalyze the conversion of superoxide (O_2^-) to oxygen (O_2) and hydrogen peroxide (H_2O_2).

Several compounds mimic the role of MnSOD in animals. Of these, only the calmangafodipir has reached a human trial in paracetamol poisoning. Calmangafodipir or $Ca_4Mn(DPDP)_5$ is a modification of mangafodipir (MnDPDP). Both share a structure with a phosphorylated dimer of pyridoxine

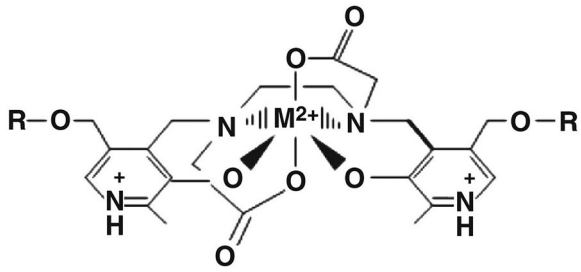

Figure 2. Shared chemical structure of mangafodipir (manganese dipyridoxyl diphosphate or MnDPDP), manganese pyridoxyl ethylenediamine (MnPLED), and calmangafodipir (tetracalcium monomanganese penta(dipyridoxyl diphosphate or Ca4Mn(DPDP)5)). Source: Reference [80], https://doi.org/10.1016/j.tranon.2017.04.012. Used under Creative Commons license CC BY-NC-ND.

surrounding a divalent cation (Figure 2). Mangafodipir has manganese in the metal-binding site, and calmangafodipir has either calcium or manganese in the metal-binding site with a ratio of 4 Ca^{+2} to 1 Mn^{+2}. Mangafodipir previously had FDA and EMA approval as an MRI contrast agent from 1997 to 2012 [81,82].

In vitro studies

In a cell culture of HuH7 human hepatoma cells, mangafodipir 500 mM protected cells from oxidative stress by the addition of xanthine oxidase or hydrogen peroxide and increased the proportion of surviving cells from 20–21% to 86–88%. In both the animal and cell culture experiments, mangafodipir was somewhat more effective than MnTBAP [83].

Animal studies

Animal experiments illustrate that MnSOD plays a critical role in defending the mitochondria from oxidative stress of paracetamol toxicity. Mice bred for heterozygous MnSOD deficiency (SOD±) exhibit more severe paracetamol toxicity than do wild type (SOD+/+) mice [84].

Toxic doses of paracetamol gradually inhibit MnSOD by nitration of the enzyme. Agarwal et al. found that mice injected with paracetamol 100 mg/kg, 200 mg/kg, or 300 mg/kg showed a dose-dependent decrease in MnSOD activity over time [85]. Mice receiving the two higher doses had an over 50% decrease in MnSOD activity at 1 h coinciding with nitration of tyrosine residues on MnSOD. MnSOD activity remained low at 2 h before returning to baseline at 4 h and surpassing baseline at 6 h after paracetamol 300 mg/kg.

Ferret et al. [86] found that manganese III tetrakis (5, 20, 15, 20 benzoic acid) (MnTBAP) 10 mg/kg or 20 mg/kg in mice poisoned with paracetamol 500 mg/kg or 1,000 mg/kg. Compared to acetylcysteine, MnTBAP produced dose-dependent improvement in survival to 24 h and lower AST concentrations.

Mangafodipir (manganese dipyridoxyl diphosphate or MnDPDP) 10 mg/kg given to mice 2 h before 500 mg/kg paracetamol resulted in AST and LDH activities that were one-eighth and one-fourth, respectively, of those seen in the paracetamol poisoned mice with no antidote [83]. Further, survival in mice given mangafodipir 6 h after 1000 mg/kg paracetamol improved survival to 24 h to 67% from 17% in control mice.

Additionally, mice administered the MnSOD mimetic (2-(2,2,6,6-Tetramethylpiperidin-1-oxyl-4-ylamino)-2-oxoethyl)triphenylphosphonium chloride (MitoTEMPO) experienced less liver damage than untreated mice in paracetamol overdose when administered 1.5 h or 3 h after paracetamol [87,88]. These findings indicate that MnSOD therapy might prevent paracetamol-mediated hepatic injury in humans.

Dear et al. [89] studied calmangafodipir in mice treated with paracetamol 300 mg/kg followed by acetylcysteine 300 mg/kg or calmangafodipir 10 mg/kg or both at different time intervals up to 6 h after paracetamol 300 mg/kg. Acetylcysteine was effective when given at 1 h after paracetamol but not at 2.5 h. However, calmangafodipir prevented ALT elevation and hepatic necrosis even when given at 6 h after paracetamol.

Human experience

Calmangafodipir and mangafodipir share a property of mimicking manganese superoxide dismutase (MnSOD). Because the formation of superoxide free radicals appears to explain part of the mitochondrial toxicity of extremely large paracetamol overdoses, calmangafodipir has emerged as a potential treatment for severe paracetamol toxicity. Calmangafodipir and mangafodipir have human phase 2/phase 3 data in treating peripheral neuropathy caused by oxidative stress from platinum-based chemotherapy [90,91].

A human study of calmangafodipir has progressed to a phase 1/phase 2 study of safety and tolerability in humans with acute paracetamol overdose treated with the Scotland and Newcastle Antiemetic Protocol (SNAP) [92]. This industry-funded study recruited 24 patients with an acute paracetamol overdose meeting treatment criteria in the UK. Three sequential cohorts of 8 patients included 6 patients receiving IV calmangafodipir once after the loading infusion of acetylcysteine (100 mg/kg in 200 mL fluid over 2 h) and 2 control patients receiving only acetylcysteine. The three treatment doses of calmangafodipir were 2 μmol/kg, 5 μmol/kg, and 10 μmol/kg (6 calmangafodipir patients and 2 control patients in each cohort). These doses matched those used in the PLIANT trial [91]. The authors assessed patients for liver injury by laboratory measurements of ALT and INR as well as several experimental biomarkers. The authors concluded that calmangafodipir is safe and tolerable for patients with acute paracetamol overdose.

Several aspects of the study limit any inferences about the efficacy of calmangafodipir. First, the size and design of the study were not intended to study efficacy. Its goal was to explore the range of well-tolerated doses to lay the foundation for future study. Second, the study enrolled patients with low risk of hepatotoxicity. The study included patients meeting the UK treatment threshold of 100 mg/L (660 μmol/L) at 4 h. Just over half of the patients would not have received acetylcysteine in other countries using the 150 line as the threshold for treatment. Further, only 2 patients had initial paracetamol concentrations above the high-risk 300 line. Seven patients presented more than 8 h after the

overdose, and only one of them presented more than 16 h after the overdose. One would forecast that most of the study patients would have good outcomes with IV acetylcysteine alone. A phase 3 study is likely to be forthcoming.

Other medications

Several other medications with approved human uses have been investigated for effect in paracetamol poisoning. Metformin has five *in vitro* and murinestudies indicating the effect of metformin in reducing JNK activation and possibly inhibiting the oxidation of paracetamol [93–97]. Three rat studies suggest that disulfiram inhibits CYP 2E1 and prevents oxidation of paracetamol to NAPQI [16,80,98]. Several other animal studies have explored effects of methylthioninium chloride (methylene blue) [99], carvedilol [100,101], diltiazem [102,103], amlodipine [104], nifedipine [103], verapamil [103], lisinopril [104], enalapril [105], allopurinol [104], trifluoperazine [103] and gemfibrozil [106] in murine models of paracetamol poisoning. One human volunteer study found that pretreatment with disulfiram before a single dose of 500 mg paracetamol reduced urinary recovery of glutathionyl paracetamol by two-thirds from 9.11% to 2.80% of the ingested dose [107]. Our search revealed no human case reports, case series, or comparative trials of any of these medications in paracetamol poisoning.

Discussion

Cimetidine generally appeared effective in reducing hepatotoxicity in animal and cellular models. Its effect appeared to be synergistic with acetylcysteine. Human studies were inadequate to conclude the benefit of severe paracetamol poisoning. The majority of human reports involved therapeutic (non-toxic) doses of paracetamol and some case reports of low quality. The two comparative human trials generally enrolled patients with paracetamol concentrations near the 150 line with a low risk of severe hepatotoxicity. From these, we cannot determine the likelihood of a beneficial effect of cimetidine in patients with more severe paracetamol poisonings.

As with cimetidine, the animal and cellular models for fomepizole are encouraging and indicate actions mitigating both the oxidative metabolism and mitochondrial toxicity. Human case reports and small case series vary in quality, but all are poor, and many contain insufficient information making judgment difficult. As all patients received acetylcysteine, often in the increased dose and for prolonged periods, and several underwent hemodialysis, the additional effect of fomepizole is impossible to assess. The single human volunteer study of a supratherapeutic dose of paracetamol shows that fomepizole alone prevents oxidative metabolism of paracetamol.

Calmangafodipir and its analog mangafodipir have some safety data in humans. However, the sole study to date enrolling humans with paracetamol overdose included patients just above the 100 line with few, if any, patients presenting at high risk of mitochondrial toxicity. All patients in the POP-1 trial likely would have enjoyed the same good outcomes with acetylcysteine alone. Future research should focus on patients with very high paracetamol concentrations and those with metabolic acidosis.

It is conceivable that some patients meeting the EXTRIP guidelines for hemodialysis could avoid hemodialysis with the addition of either fomepizole or calmangafodipir to acetycysteine. A few case reports suggest this possibility with fomepizole, but human experience with calmangafodipir in severe paracetamol poisoning is lacking.

It remains unknown how the addition of any of these treatments would compare to increasing the delivery of acetylcysteine. Pharmacokinetic modeling suggests that higher acetylcysteine infusion rates are likely necessary for very large paracetamol overdoses with concentrations above the 300 line of the paracetamol treatment nomogram [108].

These metabolic and mitochondrial treatments are unlikely to benefit the majority of patients with paracetamol overdoses who will have good outcomes with acetylcysteine. This is particularly true in the UK, where treatment now starts at the 100 line. We suggest considering additional treatment only for patients with at least one characteristic of severe poisoning. We suggest that may include any of the following: a paracetamol concentration above the 300 line, metabolic acidosis, positive King's College criteria, or late presentation with evident liver injury and measurable paracetamol.

Conclusions

For cimetidine, the published human data are of low quality and appear to conflict with animal data. At this time we cannot recommend cimetidine in the setting of severe paracetamol poisoning. If an effective dose of cimetidine exists in humans with paracetamol poisoning, it remains unknown.

For fomepizole, there are no comparative trials of fomepizole for acute paracetamol poisoning. The currently available information comprises low quality, uncontrolled human observations which are inconclusive due to multiple other interventions including the use of acetylcysteine, often in the increased dose and for prolonged periods, in all cases. The benefit of fomepizole as adjunct treatment has not been clearly demonstrated.

Calmangafodipir is a potential therapy that remains experimental, so no recommendation is possible at this time. We look forward to future research in its effects on severe paracetamol poisoning.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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