Archives of Toxicology
https://doi.org/10.1007/s00204-021-03211-z

REVIEW ARTICLE

Comparing *N*-acetylcysteine and 4-methylpyrazole as antidotes for acetaminophen overdose

Jephte Y. Akakpo[1] · Anup Ramachandran[1] · Steven C. Curry[2] · Barry H. Rumack[3] · Hartmut Jaeschke[1]

Received: 9 November 2021 / Accepted: 20 December 2021

Abstract

Acetaminophen (APAP) overdose can cause hepatotoxicity and even liver failure. *N*-acetylcysteine (NAC) is still the only FDA-approved antidote against APAP overdose 40 years after its introduction. The standard oral or intravenous dosing regimen of NAC is highly effective for patients with moderate overdoses who present within 8 h of APAP ingestion. However, for late-presenting patients or after ingestion of very large overdoses, the efficacy of NAC is diminished. Thus, additional antidotes with an extended therapeutic window may be needed for these patients. Fomepizole (4-methylpyrazole), a clinically approved antidote against methanol and ethylene glycol poisoning, recently emerged as a promising candidate. In animal studies, fomepizole effectively prevented APAP-induced liver injury by inhibiting Cyp2E1 when treated early, and by inhibiting c-jun N-terminal kinase (JNK) and oxidant stress when treated after the metabolism phase. In addition, fomepizole treatment, unlike NAC, prevented APAP-induced kidney damage and promoted hepatic regeneration in mice. These mechanisms of protection (inhibition of Cyp2E1 and JNK) and an extended efficacy compared to NAC could be verified in primary human hepatocytes. Furthermore, the formation of oxidative metabolites was eliminated in healthy volunteers using the established treatment protocol for fomepizole in toxic alcohol and ethylene glycol poisoning. These mechanistic findings, together with the excellent safety profile after methanol and ethylene glycol poisoning and after an APAP overdose, suggest that fomepizole may be a promising antidote against APAP overdose that could be useful as adjunct treatment to NAC. Clinical trials to support this hypothesis are warranted.

Keywords Acetaminophen · *N*-Acetylcysteine · 4-Methylpyrazole · Fomepizole · c-Jun N-terminal kinase · Hepatotoxicity

Abbreviations

ADH	Alcohol dehydrogenase
AIF	Apoptosis inducing factor
AKI	Acute kidney injury
ALF	Acute liver failure
APAP	Acetaminophen
ASK1	Apoptosis signal-regulating kinase 1
Cyp2E1	Cytochrome P450 2E1
DMSO	Dimethyl sulfoxide
FDA	Food and Drug Administration (US)
GSH	Reduced glutathione
JNK	C-Jun N-terminal kinase
MAPK	Mitogen activating protein kinase
4MP	4-Methylpyrazole
MKK4	Mitogen-activated protein kinase kinase 4
MPTP	Mitochondrial membrane permeability transition pore
NAPQI	*N*-Acetyl-*p*-benzoquinone imine
Nrf2	Nuclear factor erythroid 2-related factor 2
USNMS	United States National Multicenter Study

✉ Hartmut Jaeschke
hjaeschke@kumc.edu

[1] Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, 3901 Rainbow Blvd, MS 1018, Kansas City, KS 66160, USA

[2] Division of Clinical Data Analytics and Decision Support, and Division of Medical Toxicology and Precision Medicine, Department of Medicine, University of Arizona College of Medicine-Phoenix, Phoenix, AZ, USA

[3] Department of Emergency Medicine and Pediatrics, University of Colorado School of Medicine, Aurora, CO, USA

Introduction

Acetaminophen (APAP), or paracetamol, is a widely available analgesic and anti-pyretic drug on the market since the 1950s (Ohashi and Kohno 2020). It is considered safe

Published online: 03 January 2022

at therapeutic doses (Dart and Bailey 2007; Lavonas et al. 2010). However, because it is present in hundreds of medications, including both prescription drugs and numerous over-the-counter drug formulations, intentional and accidental overdosing is a significant problem. A consequence of acute or chronic overdosing can be severe liver injury and even acute liver failure and death (Fisher and Curry 2019). After the potential for severe liver injury by an APAP overdose was first recognized (Davidson and Eastham 1966), a mouse model of APAP hepatotoxicity was developed by Mitchell and coworkers who subsequently demonstrated the importance of hepatic reduced glutathione (GSH) as defense against a reactive metabolite of APAP (Jollow et al. 1973; Mitchell et al. 1973a, b). This led to the introduction of *N*-acetylcysteine (NAC) as a clinical antidote against APAP poisoning in the 1970s (Rumack and Bateman 2012). Despite this progress, APAP overdosing accounts for 30–50,000 hospitalizations per year and 300–500 annual deaths in the US (Blieden et al. 2014). In most western countries, in general, acute liver failure caused by an APAP overdose is the dominant etiology, including in the UK (57%) and the US (46%) (Bernal and Wendon 2013). Interestingly, these numbers have remained virtually unchanged in the last 20 years despite the availability of NAC.

Thus, there is obviously a need for improvement in therapeutic strategies for APAP-induced acute liver injury. However, due to inherent limitations of NAC treatment, this progress would have to come from additional drugs with alternate modes of action. More recently, 4-methylpyrazole (4MP, fomepizole) has come into focus as a potential adjunct treatment used with NAC in APAP overdose patients (Jaeschke et al. 2020; Mullins et al. 2020; Ramachandran and Jaeschke 2021). This review will compare the mechanisms of action of NAC and 4MP in preclinical studies and in patients and discuss the advantages and problems of each antidote.

Mechanisms of acetaminophen hepatotoxicity

Understanding the mechanisms of APAP-induced liver injury is critical for identification of therapeutic targets and ultimately developing clinically applicable antidotes. The mouse model of APAP toxicity, first described by Mitchell and coworkers (Jollow et al. 1973; Mitchell et al. 1973a, b), proved to be an essential tool that not only offered an initial insight into the mechanism of APAP-induced cell death, but has served as the basis for most relevant mechanistic studies since (Jaeschke et al. 2012, 2019; Ramachandran and Jaeschke 2019) (Fig. 1). Although most of the administered drug is conjugated with glucuronic acid or sulfate by phase II enzymes in hepatocytes and excreted into bile and plasma, less than 10% of a therapeutic APAP dose is oxidized by cytochrome P450 enzymes, especially Cyp2E1, to form the reactive metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI) (McGill and Jaeschke 2013). NAPQI can readily be detoxified by conjugation with GSH, which limits protein adduct formation and prevents toxicity. Because protein adducts are removed by autophagy (Nguyen et al. 2021a; Ni et al. 2016), even chronic use of therapeutic doses of APAP does not cause liver injury (Temple et al. 2006). However, after an APAP overdose, excessive NAPQI formation leads to GSH depletion and extensive protein binding in the cytosol, but also in mitochondria, which is the critical event for the initiation of toxicity (Tirmenstein and Nelson 1989; Xie et al. 2015a).

Protein adducts in mitochondria trigger an initial electron leak at the level of complex III of the electron transport chain, which results in superoxide formation on the outside of the inner mitochondrial membrane and, thus, triggers a mild oxidant stress in the cytosol (Nguyen et al. 2021b). This causes activation of redox-sensitive mitogen activated protein kinases such as ASK1 (apoptosis-signal-regulating kinase 1) (Nakagawa et al. 2008; Xie et al. 2015b), which activate MKK4 (mitogen-activated protein kinase kinase 4) and ultimately leads to phosphorylation of c-jun N-terminal kinase (JNK) (Hanawa et al. 2008; Zhang et al. 2017). P-JNK then translocate to the mitochondria, binds to the anchor protein Sab and triggers further impairment of the electron transport chain through inactivation of Src, a mitochondrial kinase (Hanawa et al. 2008; Win et al. 2011, 2016). These events cause a strong amplification of mitochondrial superoxide formation mainly through complex I and III into the mitochondrial matrix (Nguyen et al. 2021b).

Importantly, the superoxide radical rapidly reacts with nitric oxide and forms the very potent oxidant, peroxynitrite, which is the ultimate oxidant responsible for APAP's toxicity (Cover et al. 2005; Knight et al. 2002; Saito et al. 2010a). This amplified oxidative and nitrosative stress causes the opening of the mitochondrial membrane permeability transition pore (MPTP) leading to collapse of the mitochondrial inner membrane potential and cessation of ATP synthesis (Kon et al. 2004; Masubuchi et al. 2005). In addition, the MPTP opening will cause matrix swelling and rupture of the outer mitochondrial membrane with release of intermembrane proteins such as endonuclease G and apoptosis-inducing factor (AIF), which translocate to the nucleus and cause DNA fragmentation (Bajt et al. 2006, 2008). The widespread mitochondrial dysfunction in the cell together with the nuclear fragmentation leads to necrotic cell death after APAP overdose (Du et al. 2016; Jaeschke et al. 2012; Ramachandran and Jaeschke 2019). Importantly, most of these mechanisms have been verified in human hepatocytes and in APAP overdose patients (McGill et al. 2011, 2012; Xie et al. 2014).

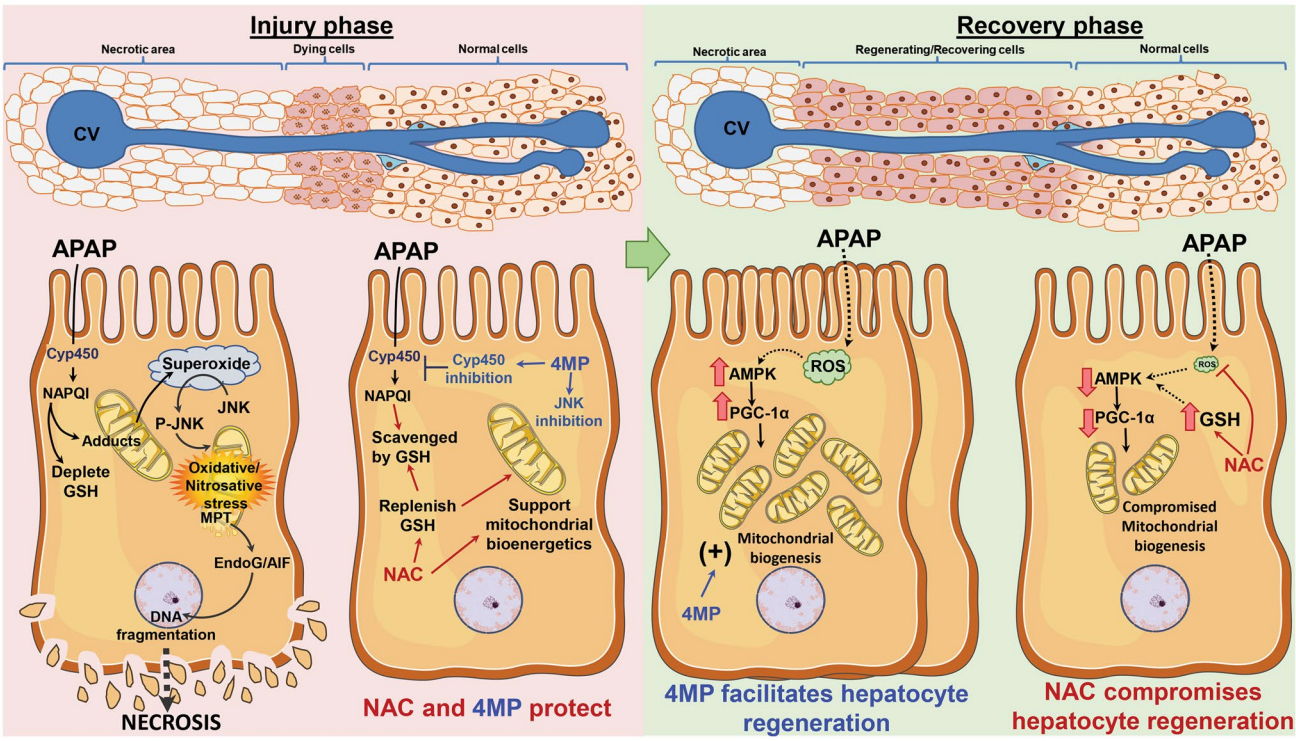

Fig. 1 4MP protects throughout the continuum of APAP pathophysiology while NAC has restricted benefit to the injury phase. APAP pathophysiology can be divided into an injury phase (left) where active hepatocyte cell death produces ongoing centrilobular necrosis, and a recovery phase (right), where recovery and regeneration of surviving cells repopulates areas of necrosis. During the injury phase, enhanced production of the reactive metabolite NAPQI from APAP depletes hepatic GSH stores and subsequently forms mitochondrial protein adducts. This results in superoxide release into the cytosol which activates the MAP kinase, JNK, inducing its translocation to mitochondria. This amplifies mitochondrial oxidative and nitrosative stress, resulting in induction of the mitochondrial permeability transition pore (MPTP) opening and release of mitochondrial intermembrane proteins endonuclease G (EndoG) and apoptosis inducing factor (AIF) into the cytosol with their translocation to the nucleus. This then induces DNA fragmentation which ultimately causes hepatocyte necrosis. Since NAC and 4MP target several of these early mechanisms, both are protective during the injury phase, where 4MP inhibits cytochrome P450 to prevent NAPQI formation and also inhibits JNK to prevent amplification of mitochondrial damage, while NAC replenishes hepatic GSH stores and supports mitochondrial bioenergetics. However, during the recovery phase, when mild reactive oxygen species (ROS) production may enable activation of the AMPK and PGC1α mediated pathways of mitochondrial biogenesis in regenerating hepatocytes, 4MP or NAC treatment has differing outcomes in mice. While 4MP treatment seems to enhance these beneficial responses, NAC compromises mitochondrial biogenesis by modulating intracellular ROS and GSH levels to blunt the APAP-induced AMPK response

Although there are many additional events including ER stress (Uzi et al. 2013), Nrf2 (nuclear factor erythroid 2-related factor 2) activation (Enomoto et al. 2001), mitophagy (Ni et al. 2012) and mitochondrial biogenesis (Du et al. 2017) that can modulate the degree of liver injury after an APAP overdose, the described mechanisms represent the fundamental events responsible for cell death and, thus, serve as targets against APAP toxicity.

N-Acetylcysteine as an antidote for APAP toxicity

Development of *N*-acetylcysteine (NAC) for treatment of APAP toxicity

The pioneering work by Mitchell et al. (1973b) at the National Institutes of Health elucidated the initial mechanism of toxicity of acetaminophen and demonstrated the protective role of glutathione in detoxification. They concluded that the administration of a sulfhydryl nucleophile such as cysteamine given up to 6–8 h after overdosage might protect patients from toxicity (Mitchell et al. 1974). Based on this mechanistic insight, in February of 1974 Elliott Piperno and Daniel Berssenbruegge at McNeil Laboratories began investigation of drugs on the market which

were sulfhydryl nucleophiles in mice and beagle dogs. A conference attended by numerous investigators was held in 1974 at the company to discuss these investigations.

In May of 1974 a letter was published regarding cysteamine for paracetamol overdosage (Prescott et al. 1974). The letter stated, "However, it is likely that other SH-containing compounds such as dimercaprol (B.A.L.), penicillamine and *N*-acetylcysteine would also protect the liver from overdosage. *These drugs are available in hospitals and might be tried as a last resort in patients with severe paracetamol overdosage if cysteamine cannot be obtained*."

In early 1975 the results of the experimental work at McNeil examining five of the seven possible drugs for treatment of overdosage, and comparing NAC and cysteamine, was presented and subsequently published in 1976 (Piperno and Berssenbruegge 1976). Based on this, an abbreviated new drug application was submitted to the U.S. Food and Drug Administration (FDA) in early 1975 to initiate a clinical trial utilizing NAC. Piperno et al (1978) published additional experimental work comparing NAC and methionine. That work had been presented in 1975 and discussed in earlier meetings. Late treatment with methionine demonstrated a decreased survival rate, unlike NAC.

A publication from Koch-Weser and a letter to the FDA questioned the ethics of a placebo randomized controlled trial, although he wrote it would be desirable (Koch-Weser 1976). The FDA then concluded that the United States National Multicenter Study (USNMS) should not be controlled and would rely solely upon a 1971 publication for historical controls during the approval process (Prescott et al. 1971). The USNMS was initiated in September of 1976 after obtaining approval from the FDA. The drug master file holder was Mead-Johnson, Inc. and they had not certified that NAC was pyrogen free and declined to undertake those studies. It was therefore approved only for oral use in the USNMS study. Oral NAC was theoretically more effective due to its first pass effect. However, outside of the US, the intravenous version was available and was preferable due to the taste and smell of oral NAC (Prescott et al. 1977). The USNMS dosage was calculated based on the stoichiometry of NAC in relation to APAP (Rumack 2002). In contrast, the IV dosing regimen utilized initially in Edinburgh was empirically based (Rumack and Bateman 2012).

The first report regarding the use of oral NAC for APAP overdose was published in May of 1977 (Peterson and Rumack 1977). A report of the first 416 patients treated in the USNMS was published in 1978 (Rumack and Peterson 1978). This was followed in 1981 with a report on 662 cases treated in the USNMS which further demonstrated effectiveness (Rumack et al. 1981). Mead-Johnson's NDA 13-601 for use of NAC solution (MUCOMYST) was approved by the FDA for oral use in APAP overdose on January 31, 1985.

The USNMS comprehensive publication in 1988 in the New England Journal of Medicine included 11,195 suspected cases of APAP overdose of which 2540 were treated with oral NAC (Smilkstein et al. 1988). When the study began accepting patients in September of 1976 very few hospitals could quantify plasma APAP levels, so all samples were shipped to the University of Colorado for analyses. Protocol criteria were based on the original "200 line" (200 µg/mL at 4 h post-ingestion) with a 25% safety factor required by the FDA, resulting in the "150 line" for inclusion (150 µg/mL at 4 h post-ingestion) (Rumack and Matthew 1975; Rumack and Peterson 1978; Rumack et al. 1981) (Fig. 2). APAP levels at that time were not completed for several days after collection due to shipping, and patients were treated based on the history.

Of the 2540 cases, 517 of them were eliminated after full treatment with NAC because their APAP levels did not reach inclusion criteria. A later analysis showed that some of those 517 patients developed a transaminase of greater than 1000 IU/L despite treatment (Rumack 2002). Likely this was due to inaccuracies in patient histories and dependence on reported times of ingestion. A comparative study of intravenous and oral NAC comparing a total of 4048 patients, with 2086 in the intravenous group and 1962 in the oral group, was published in 2009 (Yarema et al. 2009). The incidence of hepatotoxicity was 13.95% in the intravenous group and 15.85% in the oral group. The relative risk of hepatotoxicity was lower in the intravenous group when NAC was initiated within 12 h. There was no difference between intravenous and oral groups treated with NAC between 12 to 18 h. The relative risk of hepatotoxicity was lower in the oral group when NAC was initiated after 18 h. This result is likely due to the longer duration of treatment and greater dose intensity of oral administration (1330 mg/kg total dose) compared to intravenous dosing (300 mg/kg total dose) and not due to the route of administration (Rumack and Bateman 2012).

NAC has been used for the past 40 + years but is not always effective with patients who ingest massive amounts of APAP, who are delayed in obtaining treatment, or who potentially possess some genetic variations (Rumack and Bateman 2012; Tortora et al. 2018).

Mechanisms of protection by NAC and its limitations in preclinical models

Based on the initial studies by Mitchell and coworkers demonstrating the importance of hepatic GSH depletion as a prerequisite for extensive protein binding and toxicity (Mitchell et al. 1973b), it was shown that NAC treatment 1 h after APAP effectively promoted detoxification of NAPQI in mice, as indicated by the enhanced formation of APAP-GSH metabolites (Corcoran et al. 1985b), and attenuated protein adduct formation (Corcoran et al. 1985a), which correlated

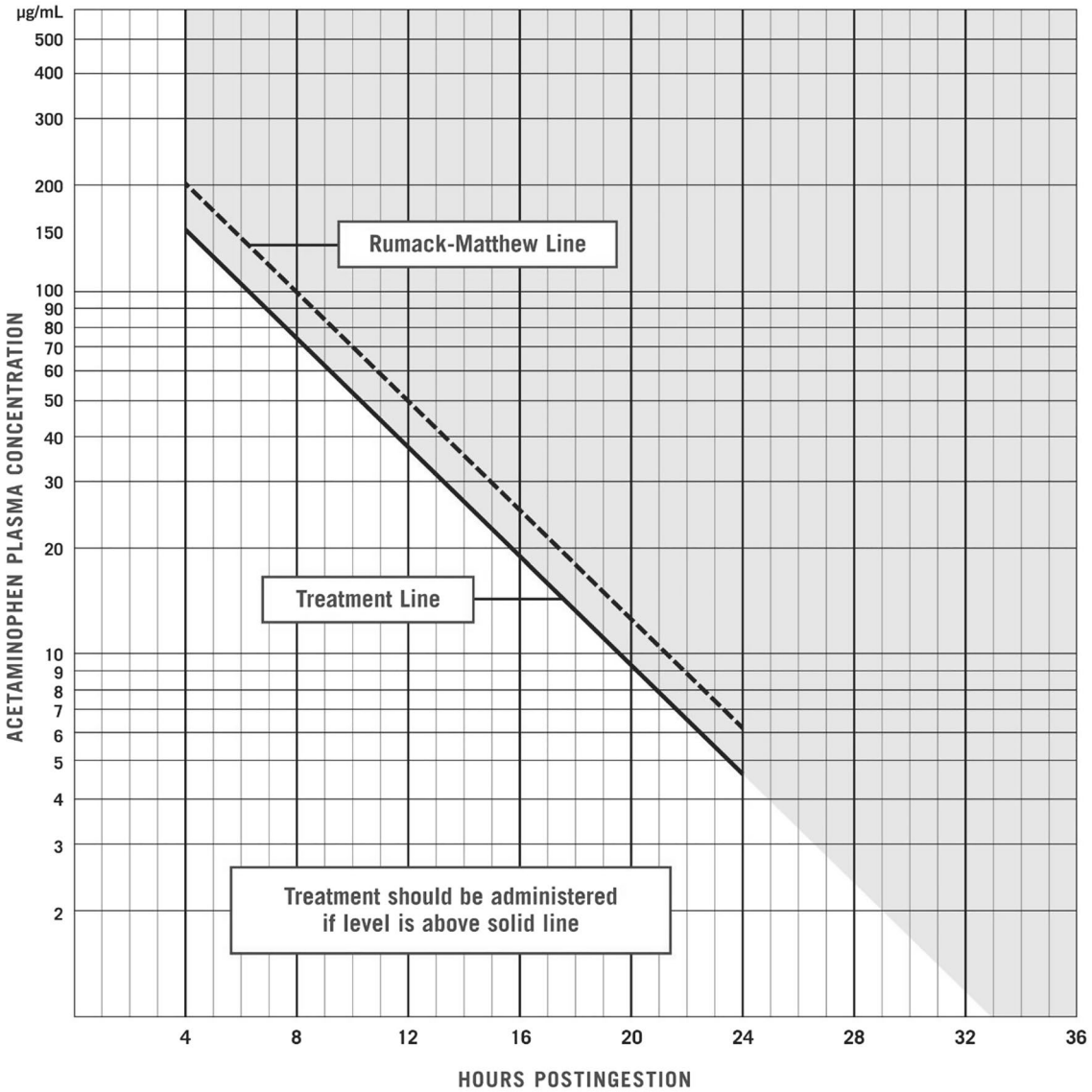

Fig. 2 Rumack–Matthew nomogram. Nomogram with the original 200-line and the 150-line used in the United States National Multicenter Study (USNMS) protocol (Rumack and Matthew 1975; Rumack et al. 1981). Source: Wikimedia Commons

with reduced toxicity. Importantly, Corcoran and Wong (1986) clearly showed that NAC did not directly conjugate with NAPQI, but first required synthesis of GSH, which then detoxified NAPQI (Fig. 1). This mechanism of protection by NAC is highly effective because protein adduct formation is the key initiating event in the toxicity. However, this mechanism requires that treatment with NAC occurs as early as possible during the metabolism phase of APAP toxicity. The duration of APAP metabolism depends on the dose ($\leq$1.5 h after 300 mg/kg or $\leq$6 h after 600 mg/kg in mice) (McGill et al. 2013).

Because GSH can directly interact with and detoxify peroxynitrite (Knight et al. 2002) and is a co-substrate for glutathione peroxidase to reduce hydrogen peroxide (Brigelius-Flohé and Flohé 2020), animal studies showed that delayed treatment with NAC or exogenous GSH can also protect against APAP toxicity without scavenging NAPQI (James et al. 2003; Knight et al. 2002; Saito et al. 2010b). Although this expands the therapeutic window, the caveat is that due to the selective mitochondrial oxidant stress and peroxynitrite formation (Jaeschke 1990; Knight et al. 2001; Saito et al. 2010a), cytosolic GSH synthesis and subsequent transport of GSH into mitochondria is required to be effective. Interestingly, if an excess of NAC is provided, i.e., more than needed for GSH synthesis, NAC can be metabolized to promote formation of Krebs cycle intermediates, which support mitochondrial energy metabolism and ATP formation and, thus, limit cell death (Saito et al. 2010b). Together these

mechanisms allow a therapeutic window for the efficacy of NAC of 0–2.5 h after APAP administration in mice.

Due to the prolonged absorption of large doses of orally administered drug in most patients and the prolonged mechanisms of cell death in human hepatocytes compared to mouse hepatocytes (Xie et al. 2014), the efficacy of NAC is generally considered very high if treatment is started within 8–10 h after the overdose in patients with gradually declining effects afterwards (Smilkstein et al. 1988).

As discussed, NAC is highly effective when administered relatively early after an APAP overdose in animal models and in patients. However, there are also some limitations. In the mouse model, it could be demonstrated that severely delayed and continuous treatment with NAC into the regeneration phase after APAP-induced liver injury significantly delayed recovery (Akakpo et al. 2021; Yang et al. 2009) (Fig. 1). This effect of NAC appears to be caused by the downregulation of key activators of mitochondrial biogenesis leading to reduced cell proliferation and repair of necrotic areas (Akakpo et al. 2021). Although this effect of NAC is very striking in mice after an APAP overdose, the relevance of this detrimental effect has not been thoroughly investigated in humans. Only a single prospective study looked at late NAC treatment in ALF patients and observed less cerebral edema and improved survival with NAC, but the rate of recovery of liver function was not affected by NAC (Keays et al. 1991). Given the variable time of initiation and duration of NAC treatment in patients, a potential effect of prolonged NAC treatment on liver recovery in humans may warrant further assessment.

It is well known that APAP overdose can also cause kidney injury in humans (O'Riordan et al. 2011; Tujios et al. 2015) and in mice (Emeigh Hart et al. 1991), especially after severe overdoses (Akakpo et al. 2020). Like hepatocytes, APAP is metabolized by Cyp2E1 present in kidney tubular cells (Arzuk et al. 2018) to generate NAPQI, which is conjugated with GSH, and once GSH is depleted, binds to proteins (Emeigh Hart et al. 1991; Hart et al. 1994, 1995). Interestingly, inhibition of Cyps in the kidney protects against APAP nephrotoxicity (Akakpo et al. 2020) but treatment with NAC, despite preventing liver injury, had no effect on kidney injury in mice (Slitt et al. 2004). It has been hypothesized that the APAP-Cys metabolite causes depletion of renal GSH levels through interactions with the γ-glutamyl cycle, which enhances the susceptibility to APAP-induced acute kidney injury in mice (Stern et al. 2005a, b).

Whether these findings in mice translate to the human pathophysiology remains unclear. When early presenting patients were treated with NAC, both liver and kidney injury were prevented (Prescott et al. 1979). On the other hand, late-presenting patients can develop both severe liver and kidney injury despite NAC treatment (Davenport and Finn 1988). The potential lack of protection by NAC against APAP-induced kidney injury and its mechanism requires further studies in both mice and in patients.

Clinical efficacy of NAC and adverse events

NAC treatment is currently the only FDA-approved antidote against APAP overdose and represents the standard of care for this indication. In principle there are two dosing regimens in use, an oral and an intravenous treatment protocol. In the US, the approved dosing for oral NAC is a loading dose of 140 mg/kg body weight followed by 70 mg/kg every 4 h for 17 doses. The approved dosing for intravenous NAC is a 21-h course, which includes a loading dose of 150 mg/kg body weight given over 1 h followed by 50 mg/kg given over the next 4 h and 100 mg/kg given over the final 16 h (Fisher and Curry 2019). This is commonly described as the 3-bag method, with each dose mixed in a separate IV bag.

Based on the original oral treatment protocol, NAC treatment is highly effective in preventing liver injury and liver failure when administered within the first 8–10 h after APAP ingestion (Rumack et al. 1981; Smilkstein et al. 1988). Beyond that time, the efficacy gradually declines but is still partially effective when given up to 24 h. Because a 48-h IV protocol (140 mg/kg loading dose and 12 times 70 mg/kg for a total of 980 mg/kg) was as effective as the 72-h oral or the 21-h IV protocol, but more effective for late-presenting patients than the 21-h regimen (Heard et al. 2014; Smilkstein et al. 1991), it was concluded that the higher dose of NAC and longer treatment period, rather than the route of administration, was responsible for the improved outcome. Another comparison between IV treatment and historical oral data of an Australian cohort of patients did not find relevant differences in outcome (hepatotoxicity) between oral and IV protocols (Buckley et al. 1999). However, because of the shorter treatment period of the IV protocol and the higher incidence of vomiting during the oral protocol, which can reduce the availability of NAC, the 21-h IV regimen is preferred by most centers for early-presenting uncomplicated overdoses (Buckley et al. 1999; Heard and Green 2012; Klein-Schwartz and Doyon 2011).

NAC therapy is safe, generally well tolerated, and serious side effects are uncommon. Bebarta et al (2010), in a multicenter comparison, assessed the adverse effects of the oral and the IV NAC treatment regimens. Nausea and vomiting were the most common side effects affecting 23% and 9% of patients after oral and IV NAC treatment, respectively. On the other hand, anaphylactoid reactions were observed in 6% of patients after the IV NAC treatment versus only 2% in patients during the oral regimen (Bebarta et al. 2010). The severity of anaphylactoid reactions correlated with lower APAP and higher histamine serum levels (Daoud et al. 2020; Pakravan et al. 2008), and fewer anaphylactoid side effects were observed in

patients who co-ingested an antihistamine (Daoud et al. 2020). IV NAC results in much higher peak plasma NAC concentrations than oral dosing, also explaining higher rates of anaphylactoid reactions during loading doses, especially when the NAC loading dose is infused relatively rapidly.

Accidental large overdoses of NAC in the treatment of APAP toxicity produce seizures, cerebral edema, and even death. Fortunately, these are rare events, but the tragic consequences speak to the importance of compulsively and accurately delivering NAC at the intended dose (Bailey et al. 2004; Heard and Schaeffer 2011; Srinivasan et al. 2015).

Hepatotoxicity can develop in patients despite early treatment with a standard protocol of NAC, which may be related in most cases to very high overdoses of APAP (Cairney et al. 2016; Doyon and Klein-Schwartz 2009; Marks et al. 2017) and variations in reliability of histories concerning time(s) of ingestion. Under such conditions, more flexible NAC treatment schedules are commonly used. If, at the end of the 21-h NAC infusion regimen, plasma APAP concentrations have not fallen to low or undetectable levels, or if transaminase activities have increased above the normal range, NAC therapy has been continued until there is clear evidence for improvement of liver function in the patient (Dart and Rumack 2007; Fisher and Curry 2019).

Around the world, including the US, many centers are delivering IV NAC using modifications of the original 3-bag protocol for various reasons, which include: decreasing errors and delays in NAC administration, decreasing anaphylactoid reactions, providing for increased NAC dosing in patients with very large APAP ingestions or in those in whom plasma APAP concentrations have not fallen, and may even have risen, after 21 h of NAC infusion; and delivering more NAC to patients whose liver function is worsening, despite 21 h of NAC therapy. It is beyond the scope of this review to describe and compare these variations in NAC dosing, but as an example, some centers load patients with 150 mg NAC per kg body weight over 1 h and then continue infusions at 15 mg/kg/h indefinitely, until plasma APAP levels have fallen, and liver function studies are not worsening, and the patient is clearly improving (Pauley et al. 2015). As another example, Chiew et al. (2017) reported that an increased infusion of NAC during the 21-h treatment period, especially a doubling of the dose from 100 to 200 mg/kg/16 h with the 3rd bag, was associated with reduced hepatotoxicity in patients with massive overdoses (> 40 g of APAP). That NAC is not very effective for patients who present late after overdose, and that current NAC doses may be inadequate for massive overdoses (Hendrickson 2019), a safe and effective adjunct therapy would be desirable.

4-Methylpyrazole as an antidote against APAP toxicity

Development of 4MP as antidote against toxic alcohols and ethylene glycol

The development of 4MP (fomepizole) as an antidote against toxic alcohol and ethylene glycol poisoning is a classic example of translational toxicology where mechanistic basic science investigations discover viable therapeutic targets, which eventually result in the development of clinically approved drugs (McMartin 2010). It was well-established in the 1960s that both methanol and ethylene glycol toxicity were dependent on their metabolism initiated by the enzyme alcohol dehydrogenase (ADH) (McMartin et al. 1975; Wacker et al. 1965). 4MP was recognized as an ADH inhibitor around 1970 in both experimental animals and in humans (Blomstrand and Theorell 1970; Li and Theorell 1969; Theorell et al. 1972). This led to the assessment of 4MP as an antidote in methanol poisoning (Blomstrand et al. 1979; McMartin et al. 1975, 1980) and ethylene glycol poisoning in animals (Clay and Murphy 1977; Mundy et al. 1974) and in humans (Baud et al. 1986–1987; Burns et al. 1997). Due to its high effectiveness as an ADH inhibitor and its favorable safety profile after single and repeated dosing (Jacobsen et al. 1988, 1990), initial clinical studies were performed in France (Baud et al. 1986–1987) and the US (Burns et al. 1997), which paved the way for phase III trials for both indications in the US (Brent et al. 1999, 2001). The favorable results of these trials ultimately led to FDA approval for 4MP (fomepizole) as an antidote against ethylene glycol and methanol poisoning in 1997 and 2000, respectively (McMartin 2010).

Within a few years after approval, fomepizole replaced ethanol as the standard of care for methanol and ethylene poisoning in most countries (McMartin 2010; Mégarbane 2010). The main reason for the success of fomepizole is that compared to ethanol, fomepizole has a higher potency of inhibiting ADH, and drug levels can be more easily controlled, which makes it less labor-intensive to administer. Fomepizole also causes substantially fewer side-effects (McMartin 2010). The very limited side-effects of fomepizole have also been confirmed by retrospective reviews of cases over the years (Lepik et al. 2009; Rasamison et al. 2020). Despite ultimately being a success story, it took 30 years from the discovery of 4MP as an ADH inhibitor to the clinical approval as an antidote. As McMartin (2010) pointed out, there are substantial problems that

have to be solved when trying to bring a compound to the market that was identified in academic preclinical studies. These include finding a source that can generate the drug certified for human use, difficulties in obtaining funding for very applied preclinical investigations and initial clinical safety and proof-of-concept studies, and the challenges of getting multi-center clinical trials organized and financed in the face of limited interest by pharmaceutical companies (due to limited patient numbers and/or lack of patent protection). Nevertheless, in the case of 4MP (fomepizole) the perseverance of a number of basic scientists and clinicians paid off and laid the groundwork for future repurposing of this drug for other indications as will be discussed.

Mechanisms of protection by 4MP and its efficacy in preclinical models of APAP toxicity

4MP has been used sporadically as a P450 inhibitor in various APAP toxicity models in the rat (Burk et al. 1990) and in Cyp-overexpressing HepG2 cells (Dai and Cederbaum 1995). However, more recent in vitro experiments demonstrated that 4MP is a relatively specific Cyp2E1 inhibitor, with an IC_{50} of 50 µM (Hazai et al. 2002). The first clinical use of 4MP in a patient with a massive APAP overdose was reported when due to suspected additional alcohol poisoning the patient was treated with both NAC and 4MP (Zell-Kanter et al. 2013). In a later commentary, Yip and Heard (2016) hypothesized that 4MP may have contributed to the positive outcome in this patient and raised the possibility that 4MP could be an adjunct therapy after a high APAP overdose. Based on this background, 4MP was tested in the clinically relevant mouse model of APAP-induced liver injury. Co-treatment of 4MP with APAP effectively prevented APAP-induced liver injury (Akakpo et al. 2018) (Fig. 1). The fact that 4MP strongly attenuated hepatic GSH depletion and almost completely eliminated the formation of protein adducts and of all oxidative metabolites of APAP, i.e., APAP-GSH, APAP-Cys and APAP-NAC, suggested that 4MP acted as a Cyp2E1 inhibitor (Akakpo et al. 2018). These in vivo data were confirmed with an in vitro assay in liver homogenate using a Cyp2E1/Cyp1A2 substrate (Akakpo et al. 2018). A rough estimate also confirmed an IC_{50} value around 50 µM (Akakpo et al. 2018). However, a P450 inhibitor alone, even if specific for Cyp2E1, would be of limited use and no relevant advantage over NAC when APAP levels are elevated. Thus, it was investigated whether a delayed treatment with 4MP beyond APAP's metabolism phase would still be effective. Indeed, administering 4MP 1.5 h after APAP did not affect protein adducts in mice, but still eliminated APAP-induced liver injury (Akakpo et al. 2019). Mechanistically, this was because 4MP also prevented JNK activation and translocation to the mitochondria, eliminated mitochondrial oxidant stress and dysfunction, and prevented nuclear DNA fragmentation. This suggested that 4MP prevented JNK activation by directly inhibiting JNK (Akakpo et al. 2019), a fact confirmed by modeling experiments indicating that 4MP inhibits JNK enzymes by competing with the binding of ATP (Akakpo et al. 2019) (Fig. 1).

In addition to the efficacy in mouse models, 4MP eliminated APAP-induced cell death in primary human hepatocytes when given simultaneously with APAP, likely through inhibition of Cyp2E1 (Akakpo et al. 2018). However, 4MP was also effective when administered 18 h after APAP (Akakpo et al. 2021). Given the substantially later activation and mitochondrial translocation of JNK in human hepatocytes compared to mice (Xie et al. 2014), the late protective effect was likely caused by inhibition of JNK activation (Akakpo et al. 2021). Interestingly, under these conditions, NAC was only modestly effective (Akakpo et al. 2021). Taken together, 4MP is more effective and can be used at later time points than NAC in preclinical animal models.

A major limitation of late NAC treatment, at least in mice, is the delay in hepatocyte regeneration through inhibition of mitochondrial biogenesis after prolonged NAC administration (Akakpo et al. 2021; Yang et al. 2009). In contrast, delayed 4MP administration, besides still being effective in reducing the late injury, did not show any inhibitory effect on regeneration but seemed to promote mitochondrial biogenesis and recovery (Akakpo et al. 2021) (Fig. 1). At this point, however, this effect is only observed in mice and awaits confirmation in humans.

As discussed, an APAP overdose can also cause acute kidney injury (AKI) in addition to hepatotoxicity, and NAC treatment appears to have limited efficacy in preventing this kidney injury in mice (Slitt et al. 2004). However, 4MP given at the same time as APAP also prevented kidney damage in mice (Akakpo et al. 2020). This effect was caused by inhibition of Cyp2E1 in the kidney as indicated by the reduction of renal oxidative APAP metabolites and protein adducts (Akakpo et al. 2020). Because APAP overdose did not induce JNK activation in the murine kidney, the protective effect was unlikely due to JNK inhibition. However, it remains unclear how far 4MP treatment can be delayed and still be effective against AKI in the mouse model.

Clinical effects of 4MP and potential adverse events

Previous safety studies have shown that single or multiple oral doses of 4MP between 10 and 20 mg/kg are generally well-tolerated without relevant side effects (Jacobsen et al. 1988, 1990). Single oral doses of 50 or 100 mg/kg 4MP caused temporary nausea and dizziness without changes in blood or urine chemistries (Jacobsen et al. 1988). After a loading dose of 15 mg/kg followed by maintenance doses of 5 mg/kg every 12 h, mild, transient

ALT/AST increases were observed in 6 out 15 subjects (Jacobsen et al. 1990). However, this occurred only after 96–144 h of treatment (Jacobsen et al. 1990), a time frame not used in APAP overdose patients. 4MP (fomepizole) has been used clinically as an antidote against methanol and ethylene glycol poisoning for almost 20 years. A recent study assessing adverse effects of 4MP during its clinical use for the last 16 years in France indicated very limited side effects of standard 4MP treatment for methanol or ethylene glycol poisoning in humans (Rasamison et al. 2020). Among more than 500 patients surveyed, only 36 patients (7%) reported mild and transient side effects, with injection site pain/burning (36% of the patients with side effects) and nausea/vomiting (22%) being the most frequently adverse reactions reported (Rasamison et al. 2020).

To translate findings from the mouse model with regards to 4MP-mediated protection against APAP hepatotoxicity to humans, a cross-over clinical study was performed, where human volunteers were given a single oral supratherapeutic dose (80 mg/kg) of APAP alone or with IV infusion of 15 mg/kg 4MP and a second 4MP dose of 10 mg/kg 12 h later (Kang et al. 2020). Drug metabolites of APAP were monitored in plasma and in urine over 24 h. 4MP did not significantly affect plasma APAP, APAP-glucuronide and APAP-sulfate levels but reduced detectable oxidative metabolites (APAP-Cys and APAP-NAC) by > 90% suggesting that 4MP effectively inhibited Cyp2E1 and prevented, to a large degree, NAPQI formation (Kang et al. 2020). No adverse effects of 4MP treatment were noted in this volunteer study.

A number of case studies with a total of more than 25 patients have been reported on the use of 4MP, in addition to treatment with NAC (Chiu et al. 2021; Kiernan et al. 2019; Link et al. 2021; Rampon et al. 2020; Shah et al. 2021). Most cases involved patients with very high overdoses of APAP where there was a risk that standard or even prolonged NAC treatment may be insufficient to prevent acute liver failure (Chiu et al. 2021; Kiernan et al. 2019; Link et al. 2021; Rampon et al. 2020; Shah et al. 2021). In all cases, there was a positive outcome, i.e., moderate or no liver injury and all patients survived, except in one case of a patient with late presentation after a massive APAP overdose who was refractory to NAC and 4MP treatment, and renal replacement therapy (Cuninghame et al. 2021). Although the mostly positive outcome may suggest that 4MP is effective in these cases, there is no direct proof that 4MP was actually the cause of this beneficial effect. In addition, it needs to be kept in mind that positive results are more likely to be reported. However, these case reports indicate that patients with severe overdose can tolerate 4MP. Thus, a randomized controlled trial is clearly warranted to provide evidence for the clinical efficacy of 4MP in APAP overdose patients.

Summary and conclusions

NAC, the only currently approved antidote against APAP overdose, is safe and generally well tolerated. Both standard oral and IV NAC treatment regimens are highly effective in preventing hepatotoxicity when treating patients with moderate overdoses within 8–10 h after APAP ingestion. However, when some patients present late and/or ingest a massive overdose of APAP (> 30–40 g), the standard NAC protocol may be insufficient to prevent severe liver injury or even acute liver failure. Although modifications of the standard protocol may improve the efficacy, there are limitations of how much additional NAC can be infused.

Thus, additional antidotes with different mechanisms of action could be useful as adjunct therapy to NAC. Fomepizole (4MP) was identified in preclinical models to be an effective inhibitor of Cyp2E1, thereby preventing NAPQI formation. Additional studies found 4MP to also be an inhibitor of JNK, which prevents the amplification of the mitochondrial oxidant stress and cell death after an APAP overdose in mice and in human hepatocytes. In contrast NAC, through formation of GSH, attempts to scavenge NAPQI or reactive oxygen species after they have formed. 4MP also demonstrates benefits with late dosing in mice and human hepatocytes through prevention of JNK activation.

Unlike NAC, 4MP prevents kidney injury after high APAP overdoses and promotes hepatic regeneration in mice. As a clinically approved antidote against methanol and ethylene glycol poisoning for two decades, 4MP displays an excellent safety profile. More recently, it has been used successfully in healthy volunteers to prevent reactive metabolite formation after a mild APAP overdose and was well-tolerated in a number of high-risk APAP overdose patients. Thus, based on the current understanding of the mechanism of action and its therapeutic use in mice and in human hepatocytes, 4MP is highly effective in preventing APAP-induced liver and kidney injury and has the potential to extend the therapeutic window of NAC for treatment of APAP overdose. It is reassuring that standard doses of 4MP used in methanol and ethylene glycol poisoning were effective in inhibiting Cyp2E1 in healthy volunteers and were well-tolerated in overdose patients, who generally showed improved outcome.

In summary, the accumulated preclinical and clinical data strongly suggest that 4MP could be an excellent candidate as an adjunct therapeutic to NAC in treating selective APAP overdose patients. Based on the available evidence, a randomized controlled trial to test this hypothesis is warranted.

Acknowledgements Work discussed in the review was funded in part by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Grants R01 DK102142 (HJ) and R01 DK125465 (AR), and National Institute of General Medicine (NIGMS) funded Liver Disease COBRE Grants P20 GM103549 (HJ) and P30 GM118247 (HJ), and grants from McNeil Consumer Healthcare, Inc (HJ). JYA was funded by a Predoctoral Fellowship (F31 DK120194).

Declarations

Conflict of interest Jephte Akakpo, Anup Ramachandran: no disclosures. Barry Rumack, Steven Curry, Hartmut Jaeschke: consultation regarding acetaminophen hepatotoxicity for Johnson and Johnson Consumer Health. Steven Curry, Hartmut Jaeschke: received research grants on mechanism of acetaminophen hepatotoxicity from McNeil Consumer Health, Inc.

References

Akakpo JY, Ramachandran A, Kandel SE, Ni HM, Kumer SC, Rumack BH, Jaeschke H (2018) 4-Methylpyrazole protects against acetaminophen hepatotoxicity in mice and in primary human hepatocytes. Hum Exp Toxicol 37:1310–1322

Akakpo JY, Ramachandran A, Duan L, Schaich MA, Jaeschke MW, Freudenthal BD, Ding WX, Rumack BH, Jaeschke H (2019) Delayed treatment with 4-methylpyrazole protects against acetaminophen hepatotoxicity in mice by inhibition of c-jun N-terminal kinase. Toxicol Sci 170:57–68

Akakpo JY, Ramachandran A, Orhan H, Curry SC, Rumack BH, Jaeschke H (2020) 4-Methylpyrazole protects against acetaminophen-induced acute kidney injury. Toxicol Appl Pharmacol 409:115317

Akakpo JY, Jaeschke MW, Ramachandran A, Curry SC, Rumack BH, Jaeschke H (2021) Delayed administration of *N*-acetylcysteine blunts recovery after an acetaminophen overdose unlike 4-methylpyrazole. Arch Toxicol 95:3377–3391

Arzuk E, Turna B, Sözbilen M, Orhan H (2018) Inter-individual and inter-organ variability in the bioactivation of paracetamol by human liver and kidney tissues. Environ Toxicol Pharmacol 61:8–17

Bailey B, Blais R, Letarte A (2004) Status epilepticus after a massive intravenous *N*-acetylcysteine overdose leading to intracranial hypertension and death. Ann Emerg Med 44:401–406

Bajt ML, Cover C, Lemasters JJ, Jaeschke H (2006) Nuclear translocation of endonuclease G and apoptosis-inducing factor during acetaminophen-induced liver cell injury. Toxicol Sci 94:217–225

Bajt ML, Farhood A, Lemasters JJ, Jaeschke H (2008) Mitochondrial bax translocation accelerates DNA fragmentation and cell necrosis in a murine model of acetaminophen hepatotoxicity. J Pharmacol Exp Ther 324:8–14

Baud FJ, Bismuth C, Garnier R, Galliot M, Astier A, Maistre G, Soffer M (1986–1987) 4-Methylpyrazole may be an alternative to ethanol therapy for ethylene glycol intoxication in man. J Toxicol Clin Toxicol 24:463–483

Bebarta VS, Kao L, Froberg B, Clark RF, Lavonas E, Qi M, Delgado J, McDonagh J, Arnold T, Odujebe O, O'Malley G, Lares C, Aguilera E, Dart R, Heard K, Stanford C, Kokko J, Bogdan G, Mendoza C, Mlynarchek S, Rhyee S, Hoppe J, Haur W, Tan HH, Tran NN, Varney S, Zosel A, Buchanan J, Al-Helial M (2010) A multicenter comparison of the safety of oral versus intravenous acetylcysteine for treatment of acetaminophen overdose. Clin Toxicol (phila) 48:424–430

Bernal W, Wendon J (2013) Acute liver failure. N Engl J Med 369:2525–2534

Blieden M, Paramore LC, Shah D, Ben-Joseph R (2014) A perspective on the epidemiology of acetaminophen exposure and toxicity in the United States. Expert Rev Clin Pharmacol 7:341–348

Blomstrand R, Theorell H (1970) Inhibitory effect on ethanol oxidation in man after administration of 4-methylpyrazole. Life Sci II 9:631–640

Blomstrand R, Ostling-Wintzell H, Löf A, McMartin K, Tolf BR, Hedström KG (1979) Pyrazoles as inhibitors of alcohol oxidation and as important tools in alcohol research: an approach to therapy against methanol poisoning. Proc Natl Acad Sci USA 76:3499–3503

Brent J, McMartin K, Phillips S, Burkhart KK, Donovan JW, Wells M, Kulig K (1999) Fomepizole for the treatment of ethylene glycol poisoning. Methylpyrazole for Toxic Alcohols Study Group. N Engl J Med 340:832–838

Brent J, McMartin K, Phillips S, Aaron C, Kulig K (2001) Methylpyrazole for Toxic Alcohols Study Group. Fomepizole for the treatment of methanol poisoning. N Engl J Med 344:424–429

Brigelius-Flohé R, Flohé L (2020) Regulatory phenomena in the glutathione peroxidase superfamily. Antioxid Redox Signal 33:498–516

Buckley NA, Whyte IM, O'Connell DL, Dawson AH (1999) Oral or intravenous *N*-acetylcysteine: which is the treatment of choice for acetaminophen (paracetamol) poisoning? J Toxicol Clin Toxicol 37:759–767

Burk RF, Hill KE, Hunt RW Jr, Martin AE (1990) Isoniazid potentiation of acetaminophen hepatotoxicity in the rat and 4-methylpyrazole inhibition of it. Res Commun Chem Pathol Pharmacol 69:115–118

Burns MJ, Graudins A, Aaron CK, McMartin K, Brent J (1997) Treatment of methanol poisoning with intravenous 4-methylpyrazole. Ann Emerg Med 30:829–832

Cairney DG, Beckwith HKS, Al-Hourani K, Eddleston M, Bateman DN, Dear JW (2016) Plasma paracetamol concentration at hospital presentation has a dose-dependent relationship with liver injury despite prompt treatment with intravenous acetylcysteine. Clin Toxicol (phila) 54:405–410

Chiew AL, Isbister GK, Kirby KA, Page CB, Chan BSH, Buckley NA (2017) Massive paracetamol overdose: an observational study of the effect of activated charcoal and increased acetylcysteine dose (ATOM-2). Clin Toxicol (phila) 55:1055–1065

Chiu MH, Jaworska N, Li NL, Yarema M (2021) Massive acetaminophen overdose treated successfully with *N*-acetylcysteine, fomepizole, and hemodialysis. Case Rep Crit Care 2021:6695967

Clay KL, Murphy RC (1977) On the metabolic acidosis of ethylene glycol intoxication. Toxicol Appl Pharmacol 39:39–49

Corcoran GB, Wong BK (1986) Role of glutathione in prevention of acetaminophen-induced hepatotoxicity by *N*-acetyl-L-cysteine in vivo: studies with *N*-acetyl-D-cysteine in mice. J Pharmacol Exp Ther 238:54–61

Corcoran GB, Racz WJ, Smith CV, Mitchell JR (1985a) Effects of *N*-acetylcysteine on acetaminophen covalent binding and hepatic necrosis in mice. J Pharmacol Exp Ther 232:864–872

Corcoran GB, Todd EL, Racz WJ, Hughes H, Smith CV, Mitchell JR (1985b) Effects of *N*-acetylcysteine on the disposition and metabolism of acetaminophen in mice. J Pharmacol Exp Ther 232:857–863

Cover C, Mansouri A, Knight TR, Bajt ML, Lemasters JJ, Pessayre D, Jaeschke H (2005) Peroxynitrite-induced mitochondrial and endonuclease-mediated nuclear DNA damage in acetaminophen hepatotoxicity. J Pharmacol Exp Ther 315:879–887

Cuninghame S, Lotfy K, Cameron P (2021) Massive acetaminophen overdose with metabolic acidosis refractory to *N*-acetylcysteine,

fomepizole, and renal replacement therapy. Toxicol Rep 8:804–807
Dai Y, Cederbaum AI (1995) Cytotoxicity of acetaminophen in human cytochrome P4502E1-transfected HepG2 cells. J Pharmacol Exp Ther 273:1497–1505
Daoud A, Dalhoff KP, Christensen MB, Bøgevig S, Petersen TS (2020) Two-bag intravenous *N*-acetylcysteine, antihistamine pretreatment and high plasma paracetamol levels are associated with a lower incidence of anaphylactoid reactions to *N*-acetylcysteine. Clin Toxicol (phila) 58:698–704
Dart RC, Bailey E (2007) Does therapeutic use of acetaminophen cause acute liver failure? Pharmacotherapy 27:1219–1230
Dart RC, Rumack BH (2007) Patient-tailored acetylcysteine administration. Ann Emerg Med 50:280–281
Davenport A, Finn R (1988) Paracetamol (acetaminophen) poisoning resulting in acute renal failure without hepatic coma. Nephron 50:55–56
Davidson DG, Eastham WN (1966) Acute liver necrosis following overdose of paracetamol. Br Med J 2(5512):497–499
Doyon S, Klein-Schwartz W (2009) Hepatotoxicity despite early administration of intravenous *N*-acetylcysteine for acute acetaminophen overdose. Acad Emerg Med 16:34–39
Du K, Ramachandran A, Jaeschke H (2016) Oxidative stress during acetaminophen hepatotoxicity: sources, pathophysiological role and therapeutic potential. Redox Biol 10:148–156
Du K, Ramachandran A, McGill MR, Mansouri A, Asselah T, Farhood A, Woolbright BL, Ding WX, Jaeschke H (2017) Induction of mitochondrial biogenesis protects against acetaminophen hepatotoxicity. Food Chem Toxicol 108(Pt A):339–350
Emeigh Hart SG, Beierschmitt WP, Bartolone JB, Wyand DS, Khairallah EA, Cohen SD (1991) Evidence against deacetylation and for cytochrome P450-mediated activation in acetaminophen-induced nephrotoxicity in the CD-1 mouse. Toxicol Appl Pharmacol 107:1–15
Enomoto A, Itoh K, Nagayoshi E, Haruta J, Kimura T, O'Connor T, Harada T, Yamamoto M (2001) High sensitivity of Nrf2 knockout mice to acetaminophen hepatotoxicity associated with decreased expression of ARE-regulated drug metabolizing enzymes and antioxidant genes. Toxicol Sci 59:169–177
Fisher ES, Curry SC (2019) Evaluation and treatment of acetaminophen toxicity. Adv Pharmacol 85:263–272
Hanawa N, Shinohara M, Saberi B, Gaarde WA, Han D, Kaplowitz N (2008) Role of JNK translocation to mitochondria leading to inhibition of mitochondria bioenergetics in acetaminophen-induced liver injury. J Biol Chem 283:13565–13577
Hart SG, Beierschmitt WP, Wyand DS, Khairallah EA, Cohen SD (1994) Acetaminophen nephrotoxicity in CD-1 mice. I. Evidence of a role for in situ activation in selective covalent binding and toxicity. Toxicol Appl Pharmacol 126:267–275
Hart SG, Cartun RW, Wyand DS, Khairallah EA, Cohen SD (1995) Immunohistochemical localization of acetaminophen in target tissues of the CD-1 mouse: correspondence of covalent binding with toxicity. Fundam Appl Toxicol 24:260–274
Hazai E, Vereczkey L, Monostory K (2002) Reduction of toxic metabolite formation of acetaminophen. Biochem Biophys Res Commun 291:1089–1094
Heard K, Green J (2012) Acetylcysteine therapy for acetaminophen poisoning. Curr Pharm Biotechnol 13:1917–1923
Heard K, Schaeffer TH (2011) Massive acetylcysteine overdose associated with cerebral edema and seizures. Clin Toxicol (phila) 49:423–425
Heard K, Rumack BH, Green JL, Bucher-Bartelson B, Heard S, Bronstein AC, Dart RC (2014) A single-arm clinical trial of a 48-hour intravenous *N*-acetylcysteine protocol for treatment of acetaminophen poisoning. Clin Toxicol (phila) 52:512–518
Hendrickson RG (2019) What is the most appropriate dose of *N*-acetylcysteine after massive acetaminophen overdose? Clin Toxicol (phila) 57:686–691
Jacobsen D, Sebastian CS, Blomstrand R, McMartin KE (1988) 4-Methylpyrazole: a controlled study of safety in healthy human subjects after single, ascending doses. Alcohol Clin Exp Res 12:516–522
Jacobsen D, Sebastian CS, Barron SK, Carriere EW, McMartin KE (1990) Effects of 4-methylpyrazole, methanol/ethylene glycol antidote, in healthy humans. J Emerg Med 8:455–461
Jaeschke H (1990) Glutathione disulfide formation and oxidant stress during acetaminophen-induced hepatotoxicity in mice in vivo: the protective effect of allopurinol. J Pharmacol Exp Ther 255:935–941
Jaeschke H, McGill MR, Ramachandran A (2012) Oxidant stress, mitochondria, and cell death mechanisms in drug-induced liver injury: lessons learned from acetaminophen hepatotoxicity. Drug Metab Rev 44:88–106
Jaeschke H, Ramachandran A, Chao X, Ding WX (2019) Emerging and established modes of cell death during acetaminophen-induced liver injury. Arch Toxicol 93:3491–3502
Jaeschke H, Akakpo JY, Umbaugh DS, Ramachandran A (2020) Novel therapeutic approaches against acetaminophen-induced liver injury and acute liver failure. Toxicol Sci 174:159–167
James LP, McCullough SS, Lamps LW, Hinson JA (2003) Effect of *N*-acetylcysteine on acetaminophen toxicity in mice: relationship to reactive nitrogen and cytokine formation. Toxicol Sci 75:458–467
Jollow DJ, Mitchell JR, Potter WZ, Davis DC, Gillette JR, Brodie BB (1973) Acetaminophen-induced hepatic necrosis. II. Role of covalent binding in vivo. J Pharmacol Exp Ther 187:195–202
Kang AM, Padilla-Jones A, Fisher ES, Akakpo JY, Jaeschke H, Rumack BH, Gerkin RD, Curry SC (2020) The effect of 4-methylpyrazole on oxidative metabolism of acetaminophen in human volunteers. J Med Toxicol 16:169–176
Keays R, Harrison PM, Wendon JA, Forbes A, Gove C, Alexander GJ, Williams R (1991) Intravenous acetylcysteine in paracetamol induced fulminant hepatic failure: a prospective controlled trial. BMJ 303(6809):1026–1029
Kiernan EA, Fritzges JA, Henry KA, Katz KD (2019) A case report of massive acetaminophen poisoning treated with a novel "triple therapy": *N*-acetylcysteine, 4-methylpyrazole, and hemodialysis. Case Rep Emerg Med 2019:9301432
Klein-Schwartz W, Doyon S (2011) Intravenous acetylcysteine for the treatment of acetaminophen overdose. Expert Opin Pharmacother 12:119–130
Knight TR, Kurtz A, Bajt ML, Hinson JA, Jaeschke H (2001) Vascular and hepatocellular peroxynitrite formation during acetaminophen toxicity: role of mitochondrial oxidant stress. Toxicol Sci 62:212–220
Knight TR, Ho YS, Farhood A, Jaeschke H (2002) Peroxynitrite is a critical mediator of acetaminophen hepatotoxicity in murine livers: protection by glutathione. J Pharmacol Exp Ther 303:468–475
Koch-Weser J (1976) Drug therapy. Acetaminophen. N Engl J Med 295(23):1297–1300
Kon K, Kim JS, Jaeschke H, Lemasters JJ (2004) Mitochondrial permeability transition in acetaminophen-induced necrosis and apoptosis of cultured mouse hepatocytes. Hepatology 40:1170–1179
Lavonas EJ, Reynolds KM, Dart RC (2010) Therapeutic acetaminophen is not associated with liver injury in children: a systematic review. Pediatrics 126:e1430–e1444
Lepik KJ, Levy AR, Sobolev BG, Purssell RA, DeWitt CR, Erhardt GD, Kennedy JR, Daws DE, Brignall JL (2009) Adverse drug events associated with the antidotes for methanol and ethylene

glycol poisoning: a comparison of ethanol and fomepizole. Ann Emerg Med 53:439–450
Li TK, Theorell H (1969) Human liver alcohol dehydrogenase: inhibition by pyrazole and pyrazole analogs. Acta Chem Scand 23:892–902
Link SL, Rampon G, Osmon S, Scalzo A, Rumack BH (2021) Fomepizole as an adjunct in acetylcysteine treated acetaminophen overdose patients: a case series. Clin Toxicol (phila). https://doi.org/10.1080/15563650.2021.1996591 (**Online ahead of print**)
Marks DJB, Dargan PI, Archer JRH, Davies CL, Dines AM, Wood DM, Greene SL (2017) Outcomes from massive paracetamol overdose: a retrospective observational study. Br J Clin Pharmacol 83:1263–1272
Masubuchi Y, Suda C, Horie T (2005) Involvement of mitochondrial permeability transition in acetaminophen-induced liver injury in mice. J Hepatol 42:110–116
McGill MR, Jaeschke H (2013) Metabolism and disposition of acetaminophen: recent advances in relation to hepatotoxicity and diagnosis. Pharm Res 30:2174–2187
McGill MR, Yan HM, Ramachandran A, Murray GJ, Rollins DE, Jaeschke H (2011) HepaRG cells: a human model to study mechanisms of acetaminophen hepatotoxicity. Hepatology 53:974–982
McGill MR, Sharpe MR, Williams CD, Taha M, Curry SC, Jaeschke H (2012) The mechanism underlying acetaminophen-induced hepatotoxicity in humans and mice involves mitochondrial damage and nuclear DNA fragmentation. J Clin Investig 122:1574–1583
McGill MR, Lebofsky M, Norris HR, Slawson MH, Bajt ML, Xie Y, Williams CD, Wilkins DG, Rollins DE, Jaeschke H (2013) Plasma and liver acetaminophen-protein adduct levels in mice after acetaminophen treatment: dose-response, mechanisms, and clinical implications. Toxicol Appl Pharmacol 269:240–249
McMartin KE (2010) Antidotes for alcohol and glycol toxicity: translating mechanisms into treatments. Clin Pharmacol Ther 88:400–404
McMartin KE, Makar AB, Martin G, Palese M, Tephly TR (1975) Methanol poisoning. I. The role of formic acid in the development of metabolic acidosis in the monkey and the reversal by 4-methylpyrazole. Biochem Med 13:319–333
McMartin KE, Hedström KG, Tolf BR, Ostling-Wintzell H, Blomstrand R (1980) Studies on the metabolic interactions between 4-methylpyrazole and methanol using the monkey as an animal model. Arch Biochem Biophys 199:606–614
Mégarbane B (2010) Treatment of patients with ethylene glycol or methanol poisoning: focus on fomepizole. Open Access Emerg Med 2:67–75
Mitchell JR, Jollow DJ, Potter WZ, Davis DC, Gillette JR, Brodie BB (1973a) Acetaminophen-induced hepatic necrosis. I. Role of drug metabolism. J Pharmacol Exp Ther 187:185–194
Mitchell JR, Jollow DJ, Potter WZ, Gillette JR, Brodie BB (1973b) Acetaminophen-induced hepatic necrosis. IV. Protective role of glutathione. J Pharmacol Exp Ther 187:211–217
Mitchell JR, Thorgeirsson SS, Potter WZ, Jollow DJ, Keiser H (1974) Acetaminophen-induced hepatic injury: protective role of glutathione in man and rationale for therapy. Clin Pharmacol Ther 16:676–684
Mullins ME, Yeager LH, Freeman WE (2020) Metabolic and mitochondrial treatments for severe paracetamol poisoning: a systematic review. Clin Toxicol (phila) 58:1284–1296
Mundy RL, Hall LM, Teague RS (1974) Pyrazole as an antidote for ethylene glycol poisoning. Toxicol Appl Pharmacol 28:320–322
Nakagawa H, Maeda S, Hikiba Y, Ohmae T, Shibata W, Yanai A, Sakamoto K, Ogura K, Noguchi T, Karin M, Ichijo H, Omata M (2008) Deletion of apoptosis signal-regulating kinase 1 attenuates acetaminophen-induced liver injury by inhibiting c-Jun N-terminal kinase activation. Gastroenterology 135:1311–1321
Nguyen NT, Akakpo JY, Weemhoff JL, Ramachandran A, Ding WX, Jaeschke H (2021a) Impaired protein adduct removal following repeat administration of subtoxic doses of acetaminophen enhances liver injury in fed mice. Arch Toxicol 95:1463–1473
Nguyen NT, Du K, Akakpo JY, Umbaugh DS, Jaeschke H, Ramachandran A (2021b) Mitochondrial protein adduct and superoxide generation are prerequisites for early activation of c-jun N-terminal kinase within the cytosol after an acetaminophen overdose in mice. Toxicol Lett 338:21–31
Ni HM, Bockus A, Boggess N, Jaeschke H, Ding WX (2012) Activation of autophagy protects against acetaminophen-induced hepatotoxicity. Hepatology 55:222–232
Ni HM, McGill MR, Chao X, Du K, Williams JA, Xie Y, Jaeschke H, Ding WX (2016) Removal of acetaminophen protein adducts by autophagy protects against acetaminophen-induced liver injury in mice. J Hepatol 65:354–362
Ohashi N, Kohno T (2020) Analgesic effect of acetaminophen: a review of known and novel mechanisms of action. Front Pharmacol 11:580289
O'Riordan A, Brummell Z, Sizer E, Auzinger G, Heaton N, O'Grady JG, Bernal W, Hendry BM, Wendon JA (2011) Acute kidney injury in patients admitted to a liver intensive therapy unit with paracetamol-induced hepatotoxicity. Nephrol Dial Transplant 26:3501–3508
Pakravan N, Waring WS, Sharma S, Ludlam C, Megson I, Bateman DN (2008) Risk factors and mechanisms of anaphylactoid reactions to acetylcysteine in acetaminophen overdose. Clin Toxicol (phila) 46:697–702
Pauley KA, Sandritter TL, Lowry JA, Algren DA (2015) Evaluation of an alternative intravenous *N*-acetylcysteine regimen in pediatric patients. J Pediatr Pharmacol Ther 20:178–185
Peterson RG, Rumack BH (1977) Treating acute acetaminophen poisoning with acetylcysteine. JAMA 237:2406–2407
Piperno E, Berssenbruegge DA (1976) Reversal of experimental paracetamol toxicosis with *N*-acetylcysteine. Lancet 2(7988):738–739
Piperno E, Mosher AH, Berssenbruegge DA, Winkler JD, Smith RB (1978) Pathophysiology of acetaminophen overdosage toxicity: implications for management. Pediatrics 62(5 Pt 2 Suppl):880–889
Prescott LF, Roscoe P, Wright N, Brown SS (1971) Plasma-paracetamol half-life and hepatic necrosis in patients with paracetamol overdosage. Lancet 1(7698):519–522
Prescott LF, Matthew H, Todd JW (1974) Cysteamine for paracetamol overdose (letter). Lancet 1(7864):998
Prescott LF, Park J, Ballantyne A, Adriaenssens P, Proudfoot AT (1977) Treatment of paracetamol (acetaminophen) poisoning with *N*-acetylcysteine. Lancet 2(8035):432–434
Prescott F, Illingworth RN, Critchley JA, Stewart MJ, Adam RD, Proudfoot AT (1979) Intravenous *N*-acetylcystine: the treatment of choice for paracetamol poisoning. Br Med J 2:1097–1100
Ramachandran A, Jaeschke H (2019) Acetaminophen hepatotoxicity. Semin Liver Dis 39:221–234
Ramachandran A, Jaeschke H (2021) Oxidant stress and acetaminophen hepatotoxicity: mechanism-based drug development. Antioxid Redox Signal 35:718–733
Rampon G, Wartman H, Osmon S, Scalzo A (2020) Use of fomepizole as an adjunct in the treatment of acetaminophen overdose: a case series. Toxicol Commun 4:1–4
Rasamison R, Besson H, Berleur MP, Schicchi A, Mégarbane B (2020) Analysis of fomepizole safety based on a 16-year post-marketing experience in France. Clin Toxicol (phila) 58:742–747
Rumack BH (2002) Acetaminophen hepatotoxicity: the first 35 years. J Toxicol Clin Toxicol 40:3–20

Rumack BH, Bateman DN (2012) Acetaminophen and acetylcysteine dose and duration: past, present and future. Clin Toxicol (phila) 50:91–98

Rumack BH, Matthew H (1975) Acetaminophen poisoning and toxicity. Pediatrics 55:871–876

Rumack BH, Peterson RG (1978) Acetaminophen overdose: incidence, diagnosis, and management in 416 patients. Pediatrics 62(5 Pt 2 Suppl):898–903

Rumack BH, Peterson RC, Koch GG, Amara IA (1981) Acetaminophen overdose. 662 cases with evaluation of oral acetylcysteine treatment. Arch Intern Med 141(3 Spec No):380–385

Saito C, Lemasters JJ, Jaeschke H (2010a) c-Jun N-terminal kinase modulates oxidant stress and peroxynitrite formation independent of inducible nitric oxide synthase in acetaminophen hepatotoxicity. Toxicol Appl Pharmacol 246:8–17

Saito C, Zwingmann C, Jaeschke H (2010b) Novel mechanisms of protection against acetaminophen hepatotoxicity in mice by glutathione and *N*-acetylcysteine. Hepatology 51:246–254

Shah KR, Fox C, Geib AJ, Murphy C, Kopec K, Kerns Ii W, Dulaney A, Beuhler MC (2021) Fomepizole as an adjunctive treatment in severe acetaminophen ingestions: a case series. Clin Toxicol (phila) 59:71–72

Slitt AL, Dominick PK, Roberts JC, Cohen SD (2004) Standard of care may not protect against acetaminophen-induced nephrotoxicity. Basic Clin Pharmacol Toxicol 95:247–248

Smilkstein MJ, Knapp GL, Kulig KW, Rumack BH (1988) Efficacy of oral *N*-acetylcysteine in the treatment of acetaminophen overdose. Analysis of the national multicenter study (1976 to 1985). N Engl J Med 319:1557–1562

Smilkstein MJ, Bronstein AC, Linden C, Augenstein WL, Kulig KW, Rumack BH (1991) Acetaminophen overdose: a 48-hour intravenous *N*-acetylcysteine treatment protocol. Ann Emerg Med 20:1058–1063

Srinivasan V, Corwin D, Verceles AC (2015) An accidental overdose of *N*-acetylcysteine during treatment for acetaminophen toxicity. Clin Toxicol (phila) 53:500

Stern ST, Bruno MK, Hennig GE, Horton RA, Roberts JC, Cohen SD (2005a) Contribution of acetaminophen-cysteine to acetaminophen nephrotoxicity in CD-1 mice: I. Enhancement of acetaminophen nephrotoxicity by acetaminophen-cysteine. Toxicol Appl Pharmacol 202:151–159

Stern ST, Bruno MK, Horton RA, Hill DW, Roberts JC, Cohen SD (2005b) Contribution of acetaminophen-cysteine to acetaminophen nephrotoxicity II. Possible involvement of the gamma-glutamyl cycle. Toxicol Appl Pharmacol 202:160–171

Temple AR, Benson GD, Zinsenheim JR, Schweinle JE (2006) Multicenter, randomized, double-blind, active-controlled, parallel-group trial of the long-term (6–12 months) safety of acetaminophen in adult patients with osteoarthritis. Clin Ther 28:222–235

Theorell H, Chance B, Yonetani T, Oshino N (1972) The combustion of alcohol and its inhibition by 4-methyl-pyrazole in perfused rat livers. Arch Biochem Biophys 151:434–444

Tirmenstein MA, Nelson SD (1989) Subcellular binding and effects on calcium homeostasis produced by acetaminophen and a non-hepatotoxic regioisomer, 3′-hydroxyacetanilide, in mouse liver. J Biol Chem 264:9814–9819

Tortora L, Ruha AM, Ramos KS, Jaeschke H, Rumack BH, Kang AM, Padilla-Jones A, Wilhelms K, Curry SC (2018) Pharmacogenomic analysis of a patient with severe hepatotoxicity and hemolysis after acetaminophen overdose despite early *N*-acetylcysteine therapy (abstract). Clin Toxicol 56:983–984

Tujios SR, Hynan LS, Vazquez MA, Larson AM, Seremba E, Sanders CM, Lee WM, Acute Liver Failure Study Group (2015) Risk factors and outcomes of acute kidney injury in patients with acute liver failure. Clin Gastroenterol Hepatol 13:352–359

Uzi D, Barda L, Scaiewicz V, Mills M, Mueller T, Gonzalez-Rodriguez A, Valverde AM, Iwawaki T, Nahmias Y, Xavier R, Chung RT, Tirosh B, Shibolet O (2013) CHOP is a critical regulator of acetaminophen-induced hepatotoxicity. J Hepatol 59:495–503

Wacker WE, Haynes H, Druyan R, Fisher W, Coleman JE (1965) Treatment of ethylene glycol poisoning with ethyl alcohol. J Am Med Assoc 194:1231–1233

Win S, Than TA, Han D, Petrovic LM, Kaplowitz N (2011) c-Jun N-terminal kinase (JNK)-dependent acute liver injury from acetaminophen or tumor necrosis factor (TNF) requires mitochondrial Sab protein expression in mice. J Biol Chem 286:35071–35078

Win S, Than TA, Min RW, Aghajan M, Kaplowitz N (2016) c-Jun N-terminal kinase mediates mouse liver injury through a novel Sab (SH3BP5)-dependent pathway leading to inactivation of intramitochondrial Src. Hepatology 63:1987–2003

Xie Y, McGill MR, Dorko K, Kumer SC, Schmitt TM, Forster J, Jaeschke H (2014) Mechanisms of acetaminophen-induced cell death in primary human hepatocytes. Toxicol Appl Pharmacol 279:266–274

Xie Y, McGill MR, Du K, Dorko K, Kumer SC, Schmitt TM, Ding WX, Jaeschke H (2015a) Mitochondrial protein adducts formation and mitochondrial dysfunction during *N*-acetyl-*m*-aminophenol (AMAP)-induced hepatotoxicity in primary human hepatocytes. Toxicol Appl Pharmacol 289:213–222

Xie Y, Ramachandran A, Breckenridge DG, Liles JT, Lebofsky M, Farhood A, Jaeschke H (2015b) Inhibitor of apoptosis signal-regulating kinase 1 protects against acetaminophen-induced liver injury. Toxicol Appl Pharmacol 286:1–9

Yang R, Miki K, He X, Killeen ME, Fink MP (2009) Prolonged treatment with *N*-acetylcystine delays liver recovery from acetaminophen hepatotoxicity. Crit Care 13:R55

Yarema MC, Johnson DW, Berlin RJ, Sivilotti ML, Nettel-Aguirre A, Brant RF, Spyker DA, Bailey B, Chalut D, Lee JS, Plint AC, Purssell RA, Rutledge T, Seviour CA, Stiell IG, Thompson M, Tyberg J, Dart RC, Rumack BH (2009) Comparison of the 20-hour intravenous and 72-hour oral acetylcysteine protocols for the treatment of acute acetaminophen poisoning. Ann Emerg Med 54:606–614

Yip L, Heard K (2016) Potential adjunct treatment for high-risk acetaminophen overdose. Clin Toxicol (phila) 54:459

Zell-Kanter M, Coleman P, Whiteley PM, Leikin JB (2013) A gargantuan acetaminophen level in an acidemic patient treated solely with intravenous *N*-acetylcysteine. Am J Ther 20:104–106

Zhang J, Min RWM, Le K, Zhou S, Aghajan M, Than TA, Win S, Kaplowitz N (2017) The role of MAP2 kinases and p38 kinase in acute murine liver injury models. Cell Death Dis 8:e2903