PharmGKB summary: pathways of acetaminophen metabolism at the therapeutic versus toxic doses

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Introduction

Acetaminophen [*N*-acetyl-*p*-aminophenol (*APAP*) or paracetamol] is widely used for its analgesic and antipyretic properties in many over-the-counter formulations in both adults and children [1,2]. APAP can be synthesized in the body through O-dealkylation of the prodrug phenacetin, a pain-killer that was withdrawn from the market because of nephrotoxicity and carcinogenesis [3]. At the most usual therapeutic adult dose of 1–2 g/day, oral APAP is indicated for fever and for the relief of mild to moderate acute pain [2]. Administration of acetaminophen through the intravenous route has become increasingly widespread, and it has been used as a safe and effective antipyretic and analgesic agent [4]. The maximum recommended therapeutic dose of APAP is 4 g/day in adults and 50–75 mg/kg/day in children. Consumption of a single dose greater than 7 g in an adult and 150 mg/kg in a child is considered potentially toxic to the liver and kidneys because of the highly active metabolite, *N*-acetyl-*p*-benzoquinone imine (NAPQI) [5]. Acetaminophen overdose is one of the most common drug-related toxicities reported to poison centers. APAP is the main cause of acute liver failure in the USA [2,5]. To reduce the risk for hepatotoxicity, the Food and Drug Administration requires that manufacturers limit the amount of acetaminophen in a pill to 325 mg, and that all the formulations containing the drug have a black box warning for potential liver damage [6]. The Food and Drug Administration has also recommended that the healthcare professionals avoid prescribing and dispensing products containing more than 325 mg of APAP per dose [7].

Pharmacokinetics

Acetaminophen has a high oral bioavailability (88%); it is well absorbed and reaches the peak blood concentration within 90 min after ingestion [5]. APAP is not widely bound to plasma proteins and has a plasma half-life of 1.5–2.5 h at the recommended doses [8]. However, after an overdose, metabolism is impaired and the half-life is prolonged to 4–8 h and is directly related to the extent of liver injury [5].

Metabolism

The liver and, to a lesser extent, the kidney and intestine are the major organs implicated in the metabolism of acetaminophen [9]. After a therapeutic dose, APAP is mostly converted to pharmacologically inactive glucuronide (APAP-gluc, 52–57% of urinary metabolites) and sulfate (APAP sulfate, 30–44%) conjugates, with a minor fraction being oxidized to a reactive metabolite NAPQI (5–10%; Fig. 1). Less than 5% of APAP is excreted unchanged [10]. NAPQI is highly reactive and is primarily responsible for acetaminophen-induced hepatotoxicity. Detoxification of NAPQI occurs through its binding to the sulfhydryl group of glutathione (GSH) to form APAP-GSH, which is ultimately excreted in the urine as cysteine and mercapturic acid conjugates (APAP-cys) [5,9]. Acetaminophen disposition involves complex interorgan transport of metabolites between the liver, kidney, and intestine, through bile and the blood stream, to be ultimately eliminated in urine [9]. From the liver, most of the glucuronide and sulfate metabolites get transported to the kidneys through the blood stream, whereas some APAP-gluc appears in the bile with subsequent transport through the intestines into the blood. The kidney is the main site of the disposition of APAP sulfate, either through direct excretion or through further biotransformation followed by renal excretion. Although most of the NAPQI is formed in the liver, the kidney also metabolizes APAP to the toxic metabolite and releases the cysteine conjugate of APAP into the bile and blood for further elimination in urine [9].

At supratherapeutic doses of APAP (more than 4 g/day), the sulfation pathway becomes saturated, whereas glucuronidation and oxidation increase, and a smaller amount of APAP is excreted unchanged. After a highly toxic dose of APAP, glucuronidation gets saturated as well, and higher proportions of the drug are eliminated

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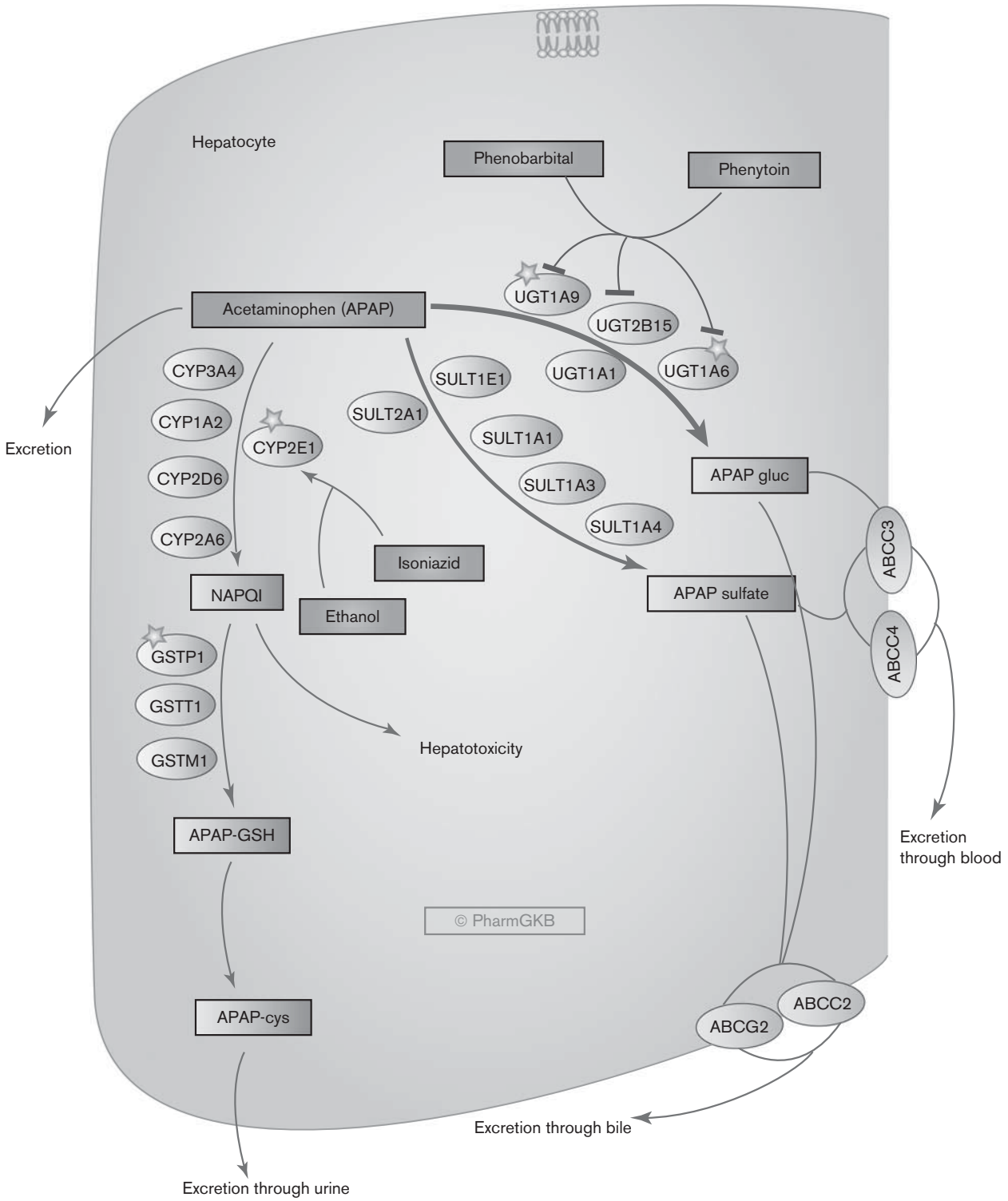
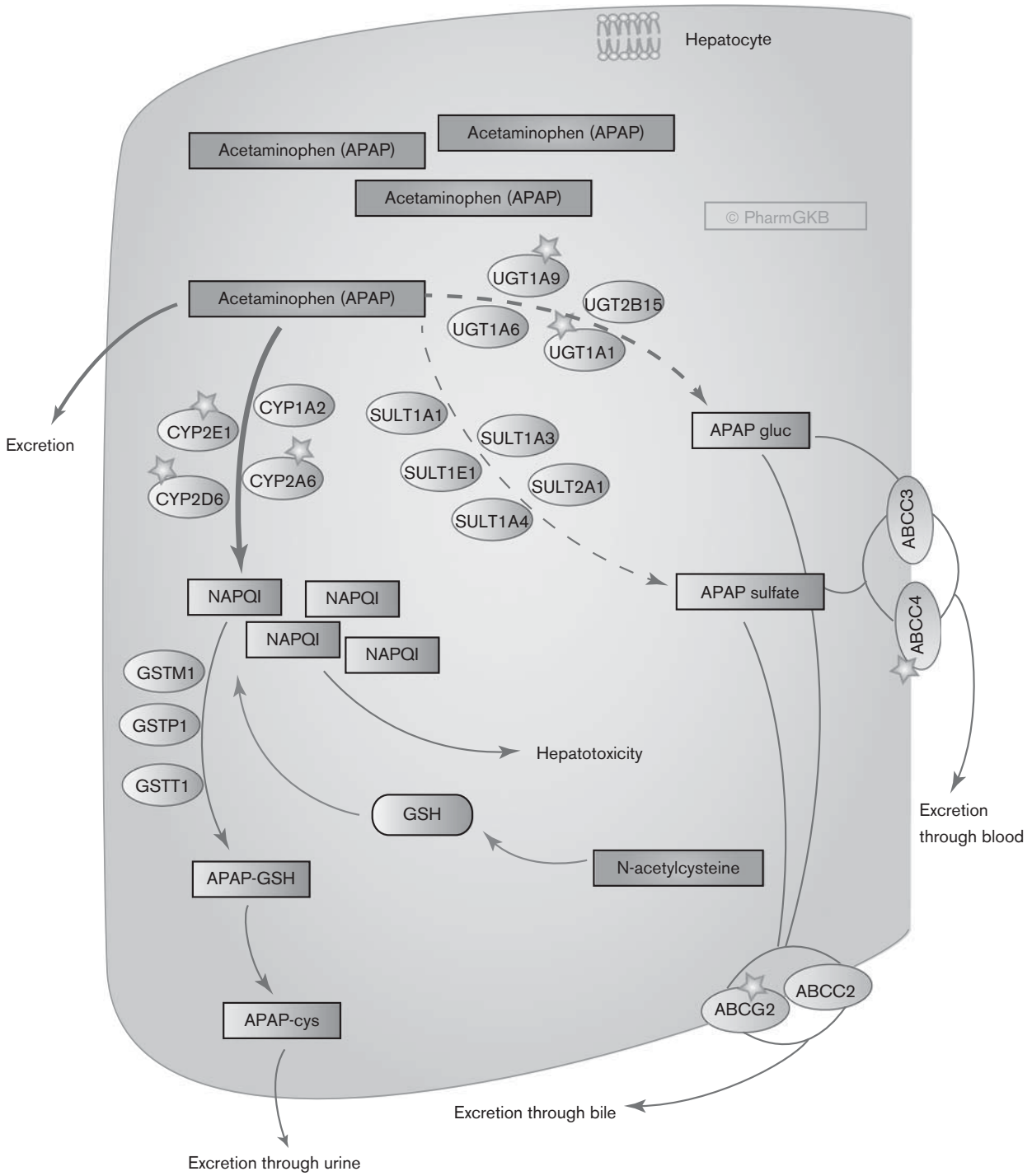
Fig. 1



Metabolism and transport of acetaminophen in the liver at therapeutic doses. Glucuronidation is the main pathway of acetaminophen metabolism, followed by sulfation and a minor contribution from the oxidation route. Oxidation by CYP isozymes yields a reactive metabolite NAPQI that is detoxified by the glutathione pathway. Phenobarbital and phenytoin inhibit acetaminophen glucuronidation, whereas ethanol and isoniazid potentiate acetaminophen oxidation. Enzymes playing a major role in the corresponding pathway are denoted with a star. APAP, acetaminophen; APAP-cys, acetaminophen cysteine; APAP-gluc, acetaminophen glucuronide; CYP, cytochrome P450; NAPQI, *N*-acetyl-*p*-benzoquinone imine. A fully interactive version is available online at *http://www.pharmgkb.org/pathway/PA165986279*.

Fig. 2



Metabolism and transport of acetaminophen in the liver at highly toxic doses. After ingestion of highly toxic doses of acetaminophen, glucuronidation and sulfation pathways get saturated and a higher portion of the drug gets oxidized and excreted unchanged. Excess NAPQI depletes glutathione stores causing liver injury. Administration of NAC provides an exogenous source of glutathione that will neutralize NAPQI and prevent further hepatotoxicity. Enzymes playing a major role in the corresponding pathway are denoted with a star. APAP, acetaminophen; APAP-cys, acetaminophen cysteine; APAP-gluc, acetaminophen glucuronide; NAC, N-acetylcysteine; NAPQI, *N*-acetyl-*p*-benzoquinone imine. A fully interactive version is available online at *http://www.pharmgkb.org/pathway/PA166117881*.

unchanged (~10%) and get oxidized to NAPQI (>15%; Fig. 2). Excess NAPQI eventually depletes GSH stores and starts to form protein adducts through binding to cysteine groups on cellular proteins. NAPQI primarily targets mitochondrial proteins and ion channels, leading to the loss of energy production, ion misbalance, and cell death [5,9,11]. Following animal studies, *N*-acetylcysteine (NAC) was shown to be an effective antidote for acetaminophen overdose in humans [12]. NAC replenishes GSH stores, scavenges reactive oxygen species in the mitochondria, and enhances the sulfation metabolic pathway (Fig. 2). If administered within 8–10 h after an acute overdose, NAC reduces the risk for hepatotoxicity to less than 5%. Overall, NAC prevents liver damage, renal failure, and death, and it is the treatment of choice for APAP poisoning [5,9,10,13]. Extremely high doses of APAP result in severe liver damage accompanied by markedly diminished glucuronidation and sulfation capacities [10]. In patients with fatal centrilobular hepatic necrosis, plasma and urinary levels of the glucuronide metabolite are barely detectable [14].

Glucuronidation of acetaminophen is catalyzed by UDP-glucuronosyl transferases (UGT). UGTs make the APAP molecule more water soluble by transferring the glucuronosyl group from UDP-glucuronic acid [5,8]. Studies in human liver microsomes and cultured hepatocytes have indicated that UGT1A1, UGT1A6, UGT1A9, and UGT2B15 are involved in APAP glucuronidation [15–18]. UGT1A6 is important at low APAP concentrations [16], whereas UGT1A9 and UGT1A1 contribute the most at toxic doses, with UGT1A9 catalyzing within a broad range of pharmacologically relevant APAP concentrations [16, 17]. Genetic polymorphisms in UGTs have been reported to affect APAP metabolism in healthy individuals [19–21] and in a disease state [22–25], as well as after a specific diet [26] (discussed below).

A family of cytosolic enzymes, called sulfotransferases (SULT), carries out sulfation of acetaminophen. SULTs transfer a sulfo group from a substrate 3′-phosphoadenosine-5′-phosphosulfate to APAP, making it more polar and prone to elimination [8]. Using human platelet homogenates as a model for xenobiotic metabolism in the liver, SULT1A1 and SULT1A3/4 were first shown to catalyze APAP sulfation [27]. Human *SULT1A3* and *SULT1A4* genes are very closely related and code for identical SULT proteins [28]. In addition to SULT1A1 and 1A3/4, sulfation of APAP in the human fetal liver is carried out by SULT1E1 and SULT2A1 [29]. This study showed that, in the fetal liver, SULT1A3/4 plays the major role in APAP sulfation; in postnatal development, however, APAP is predominantly sulfated by SULT1A1 and SULT2A1, whereas SULT1A3/4 activity diminishes [29].

Cytochrome P450 (CYP) enzymes catalyze the oxidation of acetaminophen to the reactive metabolite NAPQI [8,9]. The exact contribution of particular CYP isoforms to APAP bioactivation varies and depends on the concentration of the drug. In human liver microsomes, CYP2E1 and CYP1A2 were first reported to convert high doses of APAP to NAPQI [30]. Later studies, combining purified human proteins or human liver microsomes with specific inhibitors confirmed the role of CYP2E1 in bioactivation of toxic levels of APAP, and also reported involvement of CYP2A6 [31,32]. Studies with healthy human volunteers pretreated with the CYP2E1 inhibitor, disulfiram, further confirmed the role of CYP2E1 in APAP oxidation [33]. Using human liver microsomes and human recombinant CYP2D6, this enzyme has been reported to oxidize only very high, toxic doses of APAP, when plasma the APAP concentration reaches 2 mmol/l [34,35]. The role of CYP3A4 in APAP metabolism is controversial, with findings ranging from no significant contribution to it playing the primary role in APAP oxidation [33,35–37]. Studies with human recombinant CYP enzymes and experiments with human CYP3A4 expressed in a hepatoma cell line suggest a major involvement of CYP3A4 in APAP oxidation [35,37]. Conversely, incubation of human liver microsomes with the CYP3A4 inhibitor, troleandomycin, and therapeutic doses of APAP reduced NAPQI formation by 10%; at toxic doses, APAP oxidation was reduced only by 5% [32,36]. In-vivo human studies have further indicated that the contribution of CYP3A4 to acetaminophen oxidative metabolism is negligible. Healthy volunteers pretreated with the CYP3A4 inducer, rifampin, showed insignificant changes in APAP plasma clearance or NAPQI formation [33]. Taken together, human in-vitro and in-vivo studies suggest that CYP3A4 plays a minor role in the bioactivation of low-dose APAP. In addition to CYP450 isoforms, other enzymes might contribute to acetaminophen oxidation. In-vitro experiments have shown the formation of the reactive metabolites NAPQI and *N*-acetyl-*p*-benzosemiquinone imine by prostaglandin H_2 synthases (PTGS) [38,39]. This additional pathway is suggested to be secondary and to be found in tissues with lower CYP activity, such as the kidneys [9,40]. It should be noted, however, that these observations were made using animal microsomes and thus their relevance to acetaminophen metabolism in humans still needs to be investigated.

Acetaminophen metabolism may change under conditions that affect GSH stores. Obesity, liver steatosis, starvation, and fasting lead to GSH depletion and can be considered as risk factors for acetaminophen-induced hepatotoxicity [41,42]. Prolonged fasting results in redirection of acetaminophen metabolism from glucuronidation to the oxidation pathway. Under conditions of fasting, hepatic metabolism is shunted toward gluconeogenesis, making fewer glucose precursors available for glucuronidation. Increased oxidation of acetaminophen after starvation is also due to induction of CYP450 isoforms that start to convert more APAP to the toxic metabolite NAPQI [43]. Fasting was reported to enhance acetaminophen hepatotoxicity after an overdose and after repeated, low doses of the drug [43,44].

Conjugation of NAPQI to GSH occurs through both a spontaneous process and an enzymatic reaction catalyzed by glutathione-*S*-transferases (GSTs) [45]. A non-enzymatic reaction yields a GSH conjugate, 3-(glutathione-S-yl)-acetaminophen (APAP-GSH); a reduction product, free APAP; and an oxidation product, glutathione disulfide. The GST reaction yields APAP-GSH and free APAP. The human cytosolic GST family comprises seven distinct classes of enzymes with numerous genetic variants within each class [46]. Human in-vitro studies with isolated liver and placenta GSTs have shown that GSTP1 is the most effective catalyst of NAPQI conjugation with GSH, followed by GSTT1 and GSTM1 [45]. In the NAPQI reduction reaction, the most efficient human transferase is GSTT1, followed by GSTM1 and GSTP1. Elevated plasma GST has been correlated with acetaminophen-induced hepatotoxicity and is proposed as a sensitive and early biomarker of acute liver damage [47,48]. Unlike alanine and aspartate aminotransferases, GSTs are quickly and robustly released from centrilobular and periportal hepatocytes after APAP overdose. As early as 4 h after APAP poisoning, patients exhibit abnormal plasma GST levels that remain elevated 12 h after ingestion of the drug [48]. Intravenous administration of NAC results in a significant reduction in plasma GST levels beginning at 4 h after the treatment [47,48]. If NAC is not provided within 8 h after APAP intoxication, plasma GST levels will keep increasing, and at 40–50 h, they will be correlated with the time at which major liver damage occurs [48].

In addition to the prevailing pathways of acetaminophen metabolism – glucuronidation, sulfation, and oxidation – acetaminophen might undergo deacetylation. Animal studies have shown that deacetylation of APAP by the liver enzyme *N*-deacetylase yields a minor metabolite *p*-aminophenol [49]. *p*-Aminophenol has been reported to cause nephrotoxicity in rodent models [50,51]; however, its clinical relevance in relation to acetaminophen metabolism by humans is still to be determined. In the brain and the spinal cord, *p*-aminophenol is conjugated with arachidonic acid by the fatty acid amide hydrolase enzyme to form an active metabolite, *N*-arachidonoylphenolamine [49]. In animal studies, *N*-arachidonoylphenolamine was shown to be a potent agonist at the TRPV1 receptor that mediates proinflammatory and painful stimuli [49,52].

Transport

Disposition and elimination of acetaminophen depend on its transport through different cell types. Unlike the parent drug, movement of acetaminophen metabolites requires transporters. Interaction of acetaminophen with common drug carriers has been addressed in the context of two superfamilies of transporters, solute carrier transporters (SLC) and ATP-binding cassette (ABC) transporters [53–56]. ABC transporters mediate efflux of substrates from cells, whereas SLC transporters are responsible for uptake of substrates into cells [57,58]. Excretion of APAP-gluc and sulfate into the bile involves ABCC2 and ABCG2 carriers found in the canalicular membrane of hepatocytes. Movement of APAP-gluc into the blood depends on the ABCC3 transporter, whereas the sulfate metabolite relies on ABCC3 and ABCC4, both located on the sinusoidal side of liver cells [8]. In addition, ABCB1, ABCC1, and ABCC5 transporters might be involved in acetaminophen excretion in humans, as evident from changes in their expression after toxic acetaminophen ingestion [53]. Livers of patients who overdosed on acetaminophen showed upregulation of *ABCC1* and *ABCC4* mRNAs and elevated protein levels of ABCB1, ABCG2, ABCC4, and ABCC5. Increased expression of efflux transporters might be an adaptive change to stop accumulation of toxic metabolites in cells and to prevent additional liver damage. Consistent with this hypothesis is increased hepatocyte proliferation and colocalization of upregulated transporters in the regions of rapidly replicating liver cells [53]. These adaptive responses to toxic levels of acetaminophen result in acquired resistance to a repetitive insult on the liver. This phenomenon is reminiscent of autoprotection observed in experimental animals, where initial exposure to subtoxic doses of acetaminophen protects rodents from subsequent lethal doses of the drug [59,60]. Individual case reports have suggested that humans can develop tolerance to repeated and high doses of acetaminophen without any liver injury [61,62]. Although the mechanism of such resistance to hepatotoxicity from acetaminophen overdose was not fully elucidated in these patients, autoprotection through upregulation of efflux transporters might be responsible for the development of tolerance to chronic and lethal doses of this drug.

SLC transporters comprise two gene superfamilies, the *SLC22A* superfamily, which contains organic cation transporters (OCTs) and organic anion transporters (OATs), and the *SLCO* superfamily, which includes organic anion transporting polypeptides. Organic anion transporting polypeptides mostly transport large, hydrophobic organic anions, whereas OATs transport small and hydrophilic molecules; OCTs mediate cation movement [57]. Using stable cell lines expressing human transporters, interaction of acetaminophen with human OATs (hOATs) and human OCTs (hOCTs) was assessed [63]. Acetaminophen inhibited organic anion uptake mediated by hOAT1 (SLC22A6), hOAT2 (SLC22A7), hOAT3 (SLC22A8), and hOAT4 (SLC22A9). OCT1 (SLC22A1) and OCT2 (SLC22A2) did not mediate the uptake of acetaminophen, but could be inhibited by it, suggesting that acetaminophen could potentially interfere with removal of other drugs relying on these transporters [63]. With regard to the OATP family, in-vitro assays demonstrated that acetaminophen did not interact with OATP1B1 (SLCO1B1) or OATP1B3 (SLCO1B3) transporters [55].

Drug–drug interactions

Numerous drugs have been reported to interact with acetaminophen, leading to exacerbation of its toxicity

[18,64–68]. Several case reports suggested that epileptic patients on long-term anticonvulsant therapy exhibited increased acetaminophen-induced hepatotoxicity [68–71]. In most cases, chronic use of phenytoin or phenobarbital enhanced clinical features of toxicity after acetaminophen overdose [68–70]. It was suggested that epileptic patients exhibit lower bioavailability of acetaminophen because of increased first-pass metabolism of the drug [69]. In-vitro studies with human hepatocytes showed that phenytoin and phenobarbital inhibit acetaminophen glucuronidation, suggesting that other pathways of the drug metabolism, like oxidation to toxic NAPQI, may be potentiated [17,18]. Each drug alone or in combination directly blocked UGT1A6, UGT1A9, and UGT2B15 when coincubated with acetaminophen. Treatment of hepatocytes with phenytoin or phenobarbital increased acetaminophen-induced toxicity in these cells [17,18]. However, controlled studies with human participants showed that coadministration with anticonvulsants increases acetaminophen glucuronidation, suggesting a protective role of anticonvulsant therapy in APAP-induced toxicity [72,73]. Compared with the healthy controls ($n = 20$), epileptic patients on chronic phenytoin therapy ($n = 6$) exhibited a significant elevation in the glucuronide metabolites of APAP, whereas mercapturic acid, sulfate, and cysteine metabolites were reduced [73]. Similarly, patients on long-term therapy with various anticonvulsants ($n = 15$) had a significantly lower urinary recovery of the sulfate conjugate and unchanged drug but a higher recovery of glucuronide metabolites of APAP relative to the healthy participants ($n = 12$) [72]. In light of the contradictory evidence for an acetaminophen–anticonvulsant drug interaction from case reports and in-vitro studies, on the one hand, and small human studies, on the other hand, the safety of coadministration of these drugs should be further investigated. To address this question, a large-scale, controlled human study with patients on chronic anticonvulsant therapy receiving different doses of acetaminophen is warranted.

Many agents, including ethanol and isoniazid, induce CYP450 isozymes during their metabolism [74,75]. The antituberculosis drug isoniazid induces CYP2E1, which is crucial for acetaminophen metabolism through an oxidation pathway. Coadministration of isoniazid with acetaminophen was reported to increase acetaminophen oxidation, promote GSH depletion and NAPQI formation, and ultimately lead to increased hepatotoxicity [65, 66,76]. CYP2E1 is also markedly upregulated by ethanol, and acetaminophen hepatotoxicity in alcoholics is well documented [64]. Low to moderate doses of acetaminophen combined with heavy consumption of alcohol interact to result in an abnormal liver enzyme profile, jaundice, and coagulopathy. Taken together, individuals receiving isoniazid therapy or consuming excessive amounts of alcohol should take particular care when considering acetaminophen to avoid hepatotoxicity due to CYP2E1 induction.

Pharmacodynamics

There is no consensus on the mechanism of action of acetaminophen, with the eicosanoid, endocannabinoid, serotonergic, and nitric oxide pathways implicated in the drug's analgesic effect [1,77]. The main mechanism of action of APAP is linked to its inhibitory effect on the synthesis of prostaglandins (PGs) [77]. PGs are lipids derived from the arachidonic acid pathway that act as mediators of inflammation, fever, and pain [78]. PGs are synthesized upon oxidation of arachidonic acid by PTGS enzymes that possess both cyclooxygenase (COX) and peroxidase functions. The more constitutively expressed PTGS1 and the more readily inducible PTGS2 (by cytokines and growth factors particularly) are commonly referred to as cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), respectively [78]. Both traditional NSAIDs and those designed purposefully to selectively inhibit COX-2 block only the COX activity of the enzymes [79]. However, acetaminophen inhibits both COX isoforms by acting on the peroxide site and reducing the amount of the PTGS oxidized form required for arachidonic acid conversion [2,80,81]. Acetaminophen is often preferred to other NSAIDs as it is thought to be less likely to cause enteropathy. However, this may reflect no more than its relative potency as a PG inhibitor: common therapeutic doses of 1–2 g/day reduce PG formation by ~50% in comparison with the more complete suppression by other traditional NSAIDs like ibuprofen [82]. Acetaminophen readily crosses the blood–brain barrier, and the central nervous system is considered to be the primary site of action of the drug [1]. The central nervous system is characterized by low peroxide tone and thus provides an optimal environment for APAP action [1,78]. Unlike NSAIDs, acetaminophen has only a mild anti-inflammatory effect because of its ability to inhibit PG synthesis only in the presence of low levels of arachidonic acid and peroxides. Thus, it is efficient in suppressing the mild inflammation evoked by extraction of teeth but has little activity in reducing the severe chronic inflammation associated with rheumatoid arthritis or gout [2]. In contrast to NSAIDs, acetaminophen blocks other peroxidase enzymes, such as myeloperoxidase, the inhibition of which results in reduced levels of halogenating oxidants associated with various inflammatory conditions.

Pharmacometabolomics

Pharmacometabolomics, also known as pharmacometabonomics, identifies nongenetic, environmental factors (e.g. age, sex, diet, gut microbiome, disease subtype, concurrent medications) that determine the metabolic state of a patient and affect the overall drug response [83, 84]. Analysis of the patients' biological fluids by mass spectrometry or nuclear magnetic resonance (NMR) spectroscopy helps identify predrug metabolomic signatures that can predict the postdrug exposure effects and provides a molecular basis for variability in drug response. Metabolomics captures complex aspects of human biology, reflective of individual genomics and environmental exposures, and, together with direct

pharmacogenomic approaches, brings closer the prospect of precision medicine [84,85].

The first pharmacometabolomic studies on acetaminophen aimed to identify the biomarkers of drug-induced liver injury [86,87]. Using NMR-based analysis and mathematical models, drug metabolism and toxicity were predicted after rats were treated with a single, toxic dose of acetaminophen [86]. On the basis of the predrug urine metabolome, the mole ratio of acetaminophen glucuronide to parent drug was predicted, whereas the predrug urine metabolites strongly associated with the extent of acetaminophen-induced liver injury. A higher predrug urinary level of the compound taurine was associated with a lower degree of hepatotoxicity, whereas a higher predrug level of trimethylamine-*N*-oxide and betaine was associated with a greater severity of liver damage. This study demonstrated that metabolomic analysis before drug exposure could shed light on the degree of drug-induced hepatotoxicity, a common side effect of acetaminophen [86]. In a follow-up human study, a pharmacometabolomic approach has been used to identify individuals susceptible to acetaminophen-induced liver injury [87]. Human participants were dosed with acetaminophen for a week, followed by urine and serum collection for metabolomic analysis. Urinary metabolomics after acetaminophen treatment distinguished individuals susceptible to mild acetaminophen-induced hepatotoxicity from those who were not. Unlike the study in rats, the human study could not differentiate individuals prone to liver injury based on the predrug urinary metabolome [87]. NMR spectroscopy is further used to identify *p*-cresol as a predose urinary biomarker of acetaminophen metabolism [88]. Human participants with a high predose level of *p*-cresol had low postdose urinary ratios of acetaminophen sulfate to acetaminophen glucuronide. Bacterially derived *p*-cresol competes for sulfation with phenolic drugs, including acetaminophen; therefore, individuals with high levels of *p*-cresol will have a less efficient capacity to metabolize acetaminophen through the sulfation pathway. Most importantly, competition for limited sulfur pools will affect other pathways, such as GSH production. Elevated excretion of *p*-cresol sulfate was accompanied by reduced production of *N*-acetylcysteinyl conjugates of acetaminophen, suggesting an impaired ability to detoxify APAP reactive metabolites. Thus, individuals with a gut microbiome high in *p*-cresol-producing bacteria and ingesting a diet low in sulfur-containing amino acids may be more prone to acetaminophen toxicity, whereas those exposed to the same doses of the drug but having a low *p*-cresol content in the gut may not experience the same adverse reactions to acetaminophen [88].

Pharmacogenomics

Genetic polymorphisms in the drug metabolizing enzymes may be an important factor in the differential therapeutic and toxic responses in humans. Whereas polymorphisms in *UGT*, *CYP*, *SULT*, and *GST* genes are well established [22,23,89–92] and might affect the response to acetaminophen, only polymorphisms in *UGT* genes have been widely studied in relation to APAP pharmacokinetics in humans [20,23,26,93].

Numerous studies have investigated the effect of genetic variation in *UGT* genes on acetaminophen glucuronidation because of the key role of this pathway in acetaminophen metabolism. *UGT1A6* and *UGT1A9* are the main UGT isoforms responsible for acetaminophen glucuronidation in humans (Fig. 1, see the 'Metabolism' section). In-vitro studies with HEK cells stably transfected with various UGT1A6 amino acid variants have indicated that the UGT1A6**2* genotype had a 60% higher glucuronidation activity than the *UGT1A6*1* variant [21]. A repetition of two nucleotides (TA) in the promoter region of the *UGT1A1* gene results in the mutated sequence, referred to as *UGT1A1*28* [94], and leads to a reduction in UGT enzyme activity [95]. However, when assessed in healthy individuals or β-thalassemia patients, selected for the *UGT1A1* genotype, the *UGT1A1*28* variant had no effect on acetaminophen glucuronidation [23,93]. This suggests that enzymatic activity of other UGTs involved in APAP metabolism – UGT1A9, UGT1A6, and UGT2B15 – might compensate for the deficiency in UGT1A1 function. Using liver microsomes from human liver bank samples, three linked single-nucleotide polymorphisms rs10929303, rs1042640, and rs8330 in the UGT1A-3′UTR region were found to be associated with acetaminophen glucuronidation [20]. Of the three single-nucleotide polymorphisms, rs8330 is consistently associated with glucuronidation of acetaminophen at various concentrations of the drug. This suggests that rs8330 could serve as a biomarker of acetaminophen glucuronidation at a wide range of therapeutic and toxic doses of the drug. Moreover, rs8330 demonstrated a lower risk for hepatotoxicity due to acetaminophen glucuronidation in patients with acute liver failure. Investigations into other genotypes – that is, *UGT1A1*28*, *UGT1A6*2*, *UGT1A9* (rs6714486 and rs45625337), and *UGT2B15*2* – did not yield any associations [20]. Finally, the *UGT1A6* and *UGT2B15* genotypes were compared in terms of their contribution to acetaminophen glucuronidation [26]. After a single therapeutic dose of acetaminophen, APAP glucuronidation was significantly influenced by the *UGT2B15*2* polymorphism and very modestly influenced by the *UGT1A6*2* genotype. For *UGT2B15*, the percentage of APAP glucuronide metabolite and the ratio of APAP-gluc to free APAP is diminished, whereas that of APAP sulfate increased across genotypes from **1/*1* to **2/*2* [26].

Several studies have examined the effect of genetic polymorphisms on acetaminophen metabolism under pathological conditions. Metabolism of acetaminophen is affected in patients with Gilbert's syndrome, a chronic unconjugated hyperbilirubinemia [25,96]. The underlying cause of this disorder is a polymorphism in the promoter region of the UGT isoform 1A1 gene

(*UGT1A1*28*) that increases the length of the promoter [97]. This compromises the UGT enzyme activity and therefore leads to increased serum levels of unconjugated bilirubin. Patients with Gilbert's syndrome might be more susceptible to acetaminophen-induced hepatotoxicity because of increased availability of the free drug for the oxidation pathway of metabolism [25,96]. Although contradictory results have been published for acetaminophen glucuronidation, a subgroup of patients with Gilbert's syndrome show a reduction in excretion of APAP glucuronide and a concomitant increase in the elimination of the CYP450 APAP metabolites [22,25,96, 98,99]. A study with a few β-thalassemia/HbE patients aimed at elucidating the effect of a combination of *UGT1A6*2* and *UGT1A1*28* polymorphisms on acetaminophen pharmacokinetics [23]. As compared with wild-type β-thalassemia/HbE patients, patients with the heterozygous *UGT1A6*2* without *UGT1A1*28* genotype exhibited a reduction in the area under the curve of the free drug and of APAP glucuronide, which might be due to the *UGT1A6*2* polymorphism. The same group of patients exhibited elevated alanine aminotransferase but reduced APAP glucuronide levels, suggesting that the *UGT1A6*2* polymorphism is a modifier of acetaminophen glucuronidation in patients with abnormal liver function. β-Thalassemia/HbE patients with both *UGT1A1*28* and *UGT1A6*2* polymorphisms have not demonstrated a significant difference in the pharmacokinetics of acetaminophen [23].

Despite a major role of CYP450 enzymes in acetaminophen-induced toxicity, very few studies have attempted to address the relationship between *CYP* gene polymorphisms and APAP metabolism [90,100]. In a small cohort study, a nonsignificant association between *CYP2E1* promoter *RsaI* restriction fragment length polymorphism and a shorter half-life and elimination rate of acetaminophen has been reported [90]. In the acute liver failure study, genotype frequency differences were evaluated between patients who intentionally consumed a single overdose of acetaminophen and those who unintentionally consumed high doses of the drug over a long period of time. Thus, it should be noted that, although both groups were exposed to the same total amount of acetaminophen, the daily dose in the unintentional group was lower than that normally causing liver failure, and there might have been adaptive changes over time. The carriers of the *CYP3A5* rs776746 A allele were overrepresented in the intentionally overdosed group and were more predisposed to acetaminophen-induced hepatotoxicity than individuals with the G allele, that rendered CYP3A5 enzyme inactive due to aberrant gene splicing [100]. The *CYP3A5* rs776746 A allele polymorphism is associated with increased formation of NAPQI; however, the involvement of CYP3A5 in acetaminophen oxidation has not been reported, and if it occurs, it might be due to a big overlap in substrate selectivity between CYP3A enzymes. Associations with polymorphisms in genes encoding UGT1A1, UGT1A6, UGT1A9, UGT2B15, and SULT1A1 were not detected in the same patient populations [100].

Genetic variability in *SULT* and *GST* genes are not well established, and only a few studies have been conducted in relation to *GST* polymorphisms and acetaminophen detoxification [91,101,102]. In a study investigating associations between polymorphisms in the glutathione-*S*-transferase genes *GSTT1*, *GSTM1*, *GSTP1* and an increased risk for acetaminophen poisoning, prothrombin time was used as a marker of survival in poisoned patients [91]. A borderline association between a high prothrombin time, as an index of a good prognosis, and *GSTT1* homozygous deletion was established, indicating that patients with this polymorphism are more likely to survive after NAC treatment for APAP poisoning. The frequency of the *GSTP1* homozygous variant (Val/Val) was lower in APAP poisoned patients than in healthy individuals, suggesting that this genotype may reduce the risk of being poisoned. However, the *GSTP1* genotype was not associated with prothrombin time, which might have been because of a small sample size in this group ($n = 5$) [91]. A couple of studies address the relationship between prenatal and infant acetaminophen exposure, *GST* polymorphisms in mothers and children, and the risk of developing asthma later in life [101,102]. First, numerous studies reported an association between acetaminophen use during pregnancy and an increased risk for wheezing and later asthma development in infancy and/or childhood [102–105]. It was suggested that this association is related to the maternal polymorphisms in APAP detoxification mechanisms, namely in *GST* genes. Indeed, an increased risk for wheezing is associated with the presence of the *GSTM1* and *GSTT1* genotypes, respectively, in mothers exposed to acetaminophen [101]. Moreover, these risks are further potentiated if both the APAP-consuming mother and her child exhibit the *GSTM1* polymorphism [101]. In a different study, the *GSTP1* polymorphism was found to modify the risk for wheezing in children of age 5 years and was common only among the carriers of the *GSTP1* minor allele [102]. Taken together, these studies demonstrate an interaction of prenatal acetaminophen use and the *GST* genotype of the mother, and in some cases of the child, with airway disease in children.

Finally, two studies reported that genetic variability in the CD44 antigen might predispose patients to acetaminophen-induced liver injury at supratherapeutic doses [106] or to acute liver failure after drug overdose [100]. Evaluation of two independent cohorts of patients, who received 4 g/day APAP for 1–2 weeks, revealed an association between the *CD44 rs1467558* polymorphism and elevated serum alanine aminotransferase levels, a biomarker of hepatocellular injury [106]. Similarly, the same polymorphism was associated with unintentional

acetaminophen-induced acute liver failure [100]. These are the first reports demonstrating that a polymorphism in an immune response gene may predispose an individual to increased acetaminophen-induced hepatotoxicity. However, considering a multitude of physiological and pathological roles of CD44 [107,108], the mechanism of CD44-driven increase in susceptibility to APAP toxicity may be multifactorial and requires further investigation to determine.

Interestingly, polymorphisms in genes encoding acetaminophen-metabolizing enzymes might be responsible for the marked ethnic and racial differences in APAP metabolism and toxicity [109–113]. In comparison with Whites, Hong Kong Chinese were reported to have more rapid absorption, a longer half-life, and a lower clearance of acetaminophen, and exhibited an increased capacity for sulfation but lower capacity for glucuronidation and oxidation of the drug [109,110,114]. Individuals of African descent were shown to have a greater clearance of acetaminophen relative to Caucasians [111]. In terms of hepatotoxicity, metabolic activation of acetaminophen is much lower in Africans than in Caucasians [112], and the rate of acetaminophen-induced hepatotoxicity is low in Asian populations as compared with patients from Western countries [113]. It should be noted, however, that further studies are required to determine whether these associated polymorphisms account for the ethnic differences in acetaminophen pharmacokinetics.

Conclusion

To date, our understanding of the role of genetic polymorphisms in acetaminophen metabolism and toxicity is quite limited and has been primarily studied for *UGT* genes. Considering a high contribution of sulfation in acetaminophen metabolism, the importance of oxidation in APAP toxicity, and the importance of GSH in APAP detoxification, more studies are needed to establish the relationship between polymorphisms in *SULT*, *GST*, and *CYP* genes and interindividual variability in response to acetaminophen. Finally, clinically relevant biomarkers of acetaminophen-induced toxicity are yet to be determined.

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Conflicts of interest

R.B.A. and T.E.K. are stockholders in Personalis Inc. G.A.F. is the McNeil Professor of Translational Medicine and Therapeutics. For the remaining authors there are no conflicts of interest.

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