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Instructors:
GARRY R. BUETTNER, Ph.D.
LARRY W. OBERLEY, Ph.D.

with guest lectures from:
Drs. Freya Q . Schafer, Douglas R. Spitz, and Frederick E. Domann

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Acetaminophen Hepatotoxicity: Underlying Mechanisms of Toxicity and the Protective Role of N-acetylcysteine

By

Joshua S. Eastvold

University of Iowa Roy J. and Lucille A. Carver College of Medicine
Anatomy and Cell Biology

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Abbreviations

APAP	*N*-acetyl-*para*-aminophenol, acetaminophen, paracetamol
$AscH^-$	ascorbate
GSH	glutathione
GSSG	glutathione disulfide
GST	glutathione S-transferase
HRP	horseradish peroxidase
MPT	mitochondrial permeability transition
NAC	*N*-acetylcysteine
NAPQI	*N*-acetyl-*p*-benzoquinone imine
NAPSQI	*N*-acetyl-*p*-benzosemiquinone imine radical
ROS	reactive oxygen species
RNS	reactive nitrogen species

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Abstract

Acetaminophen is a widely used analgesic-antipyretic. While it is considerably safe at low doses, it can cause centrilobular hepatic necrosis if taken in high doses. This toxicity is in part mediated by the metabolic activation of acetaminophen to yield reactive metabolites and subsequent depletion of glutathione stores. With the cell's major antioxidant defense system hindered, covalent modification of vital proteins and oxidative damage ensue. This review will discuss the metabolic pathways of acetaminophen, and the intermediates believed to be involved in its toxicity. Further, the role of N-acetylcysteine (NAC) in the treatment of acetaminophen-induced hepatotoxicity will be discussed.

Introduction

Since its clinical introduction in 1950, acetaminophen (*N*-acetyl-*para*-aminophenol; APAP; paracetamol) has become a widely used analgesic-antipyretic. Although remarkably safe at therapeutic doses, excessive amounts of APAP cause centrilobular hepatic necrosis and acute renal tubular necrosis [1], which is the leading cause of acute liver failure in the United States [2].

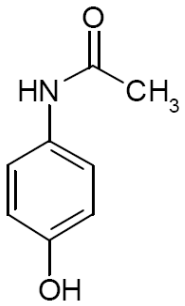
Toxicity is thought to occur *via* two stages: (i) metabolic phase, and (ii) and oxidative phase [11]. The metabolic phase consists of cytochrome P450-mediated oxidation of APAP forming reactive intermediates, such as *N*-acetyl-*p*-benzoquinone imine (NAPQI) [6]. Since NAPQI is detoxified by the glutathione (GSH)-glutathione transferase (GST) system, cellular stores of GSH become depleted resulting in impaired detoxification of peroxides and peroxynitrite, and covalent modification of vital thiol-containing proteins [11].

The susceptibility to oxidative damage (oxidative phase) is thought to cause mitochondrial permeability transition (MPT), which is an increase in permeability of the inner membrane. This membrane perturbation leads to uncoupling of oxidative phosphorylation, ion dysregulation, and superoxide production, which culminate in cell death [11].

Due to the prevalence of APAP-induced acute liver failure, understanding the mechanisms of hepatotoxicity are of utmost clinical importance. This review addresses the metabolic pathways involved in both the normal detoxification, and when these pathways have gone awry resulting in toxicity. Further, the role of N-acetylcysteine (NAC) in the treatment of APAP-induced hepatotoxicity will be discussed.

Structure and Properties

Acetaminophen is white, odorless crystalline powder with a bitter taste.[†] Its structure and physical properties are depicted in **Figure 1.** APAP is derived from the interaction of p-aminophenol and an aqueous solution of acetic anhydride. It is soluble in organic solvents such as methanol and ethanol, but insoluble in water and ether. Maximal UV absorbance is at 245 nm.



MW = 151.2
$C_8H_9NO_2$
Melting point = 169-170° C
pK_A = 9.5
Specific gravity = 1.293

Fig. 1 Structure and physical properties of acetaminophen.

Redox Chemistry

Acetaminophen can participate in many redox reactions, but only those deemed biologically relevant will be discussed here. Acetaminophen can undergo a one-electron oxidation to yield the APAP phenoxyl radical (*N*-acetyl-*p*-benzosemiquinone imine; NAPSQI) and a direct two-electron oxidation to produce *N*-acetyl-*p*-benzoquinone imine (NAPQI) **Figure 2** [6]. The two-electron oxidation pathway is thought to be the major pathway *in vivo*, generating the highly reactive intermediate NAPQI.

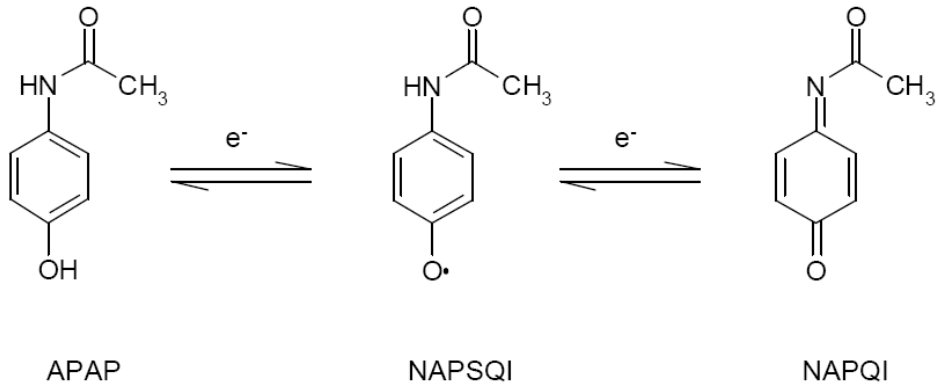


Fig. 2 Oxidation of APAP occurs either through a one-electron or two-electron process.

[†] http://www.pharmweb.net/pwmirror/pwy/paracetamol/pharmwebpicm.html. Visited 2-25-05.

The biological importance of NAPSQI formation is much debated, but several groups have shown that it is formed as an intermediate in the one-electron peroxidase-dependent oxidation of APAP [7, 8]. In the absence of reactive compounds, NAPSQI dismutes rapidly by second-order kinetics with a rate constant of 2.2×10^9 M^{-1} s^{-1} [8]. In the presence of glutathione (GSH) or ascorbate ($AscH^-$), NAPSQI is reduced to form the glutathionyl radical or ascorbate radical, respectively, and regenerate APAP **(Figure 3)** [6]. In the absence of reducing agents (*e.g.*, GSH), two molecules of NAPSQI can polymerize to form APAP dimers (**Figure 4)** [7].

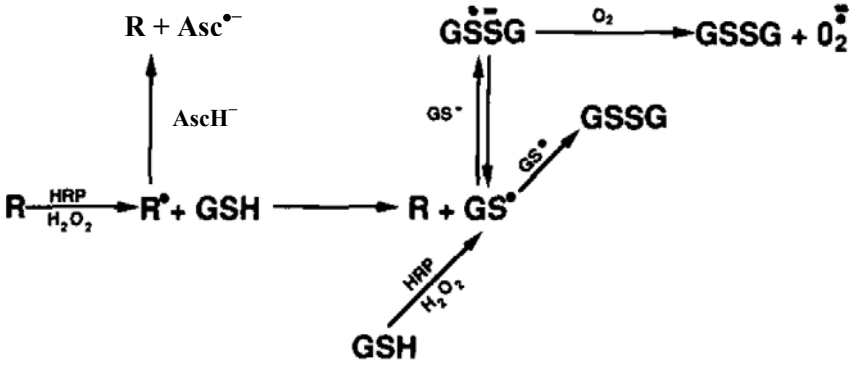


Fig. 3 Scheme for the reactions of ascorbate and glutathione with NAPSQ1. $AscH^-$ (ascorbate), $Asc^{\bullet-}$ (ascorbate radical), $GS^{\bullet}$ (glutathionyl radical), GSH (glutathione), GSSG (glutathione disulfide), HRP (horseradish peroxidase), R (APAP), $R^{\bullet}$ (NAPSQ1). Adapted from [6].

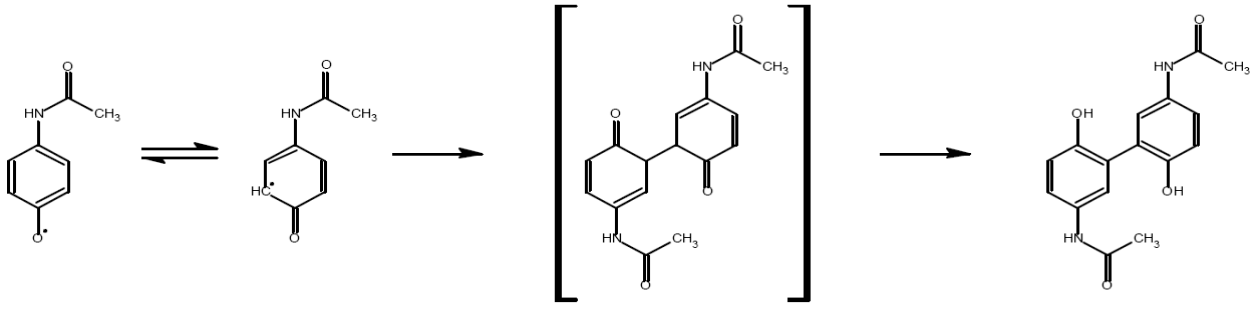


Fig. 4 The polymerization of NAPSQ1 to form APAP dimers. Adapted from [7].

Biochemistry

Acetaminophen exhibits its antipyretic-analgesic activity *via* inhibiting the cyclooxygenase activity of prostaglandin-endoperoxide synthase, which prevents the formation of prostaglandins responsible for inducing fever and pain sensation [13].

At therapeutic doses, 90 percent of APAP is metabolized in the liver *via* the phase II biotransformation enzymes (*e.g.,* sulfotransferase and UDP-glucuronosyl transferase) to yield non-toxic water-soluble compounds that are excreted in the urine (**Figure 5)** [3]. Of the remaining APAP, half is excreted unchanged in the urine and bile. The remaining APAP is oxidized *via* the hepatic cytochrome P450 (2E1, 1A2, 3A4, and 2A6 subfamilies) system to yield the highly reactive, electrophilic intermediate NAPQI [4].

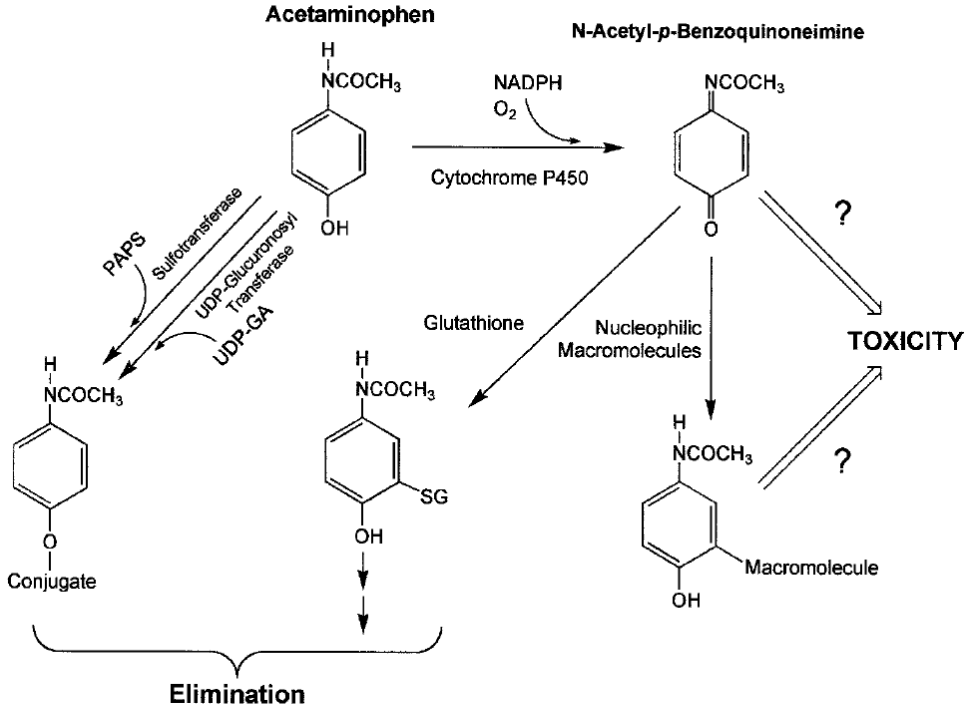


Fig. 5 Metabolic pathways involved in detoxification and hepatotoxicity. Adapted from [11].

Under normal conditions, NAPQI is rapidly conjugated with glutathione (GSH) to form nontoxic cysteine and mercaptate compounds that are renally excreted [5].

With toxic amounts of APAP, the detoxification pathways become saturated and cellular stores of GSH become depleted [5]. This leads to the accumulation of NAPQI, which can react with cellular constituents and induce cell damage. Of importance, NAPQI covalently binds proteins as an APAP-cysteine adduct, presumably rendering them inactive [9]. NAPQI can also oxidize protein thiol groups to disulfides, forming intra- and interprotein crosslinks and GSH-protein mixed disulfides. These APAP adducts correlate to hepatotoxicity and localize to the centrilobular

necrotic areas. A multitude of host protein adducts have been identified and are summarized in **Table 1** [10]. This has been characterized as the metabolic phase (or phase 1) of APAP-induced hepatotoxicity. It has been shown that repletion of GSH prevents toxicity [9], and this is the basis of N-acetylcysteine (NAC) treatment (refer to later section).

Mass kDa	Fraction	Protein
16	Cytosol	Aryl sulfotransferase[b]
22	Cytosol, mitochondria	Glutathione peroxidase[b]
22	Cytosol, mitochondria	GSH peroxidase[b]
22	Cytosol (macrophages)	Osteoblast-specific factor 3[b]
23	Cytosol, mitochondria	Glutathione transferase π[b]
28	Mitochondria	House keeping protein[b]
28	Cytosol	Proteasome subunit C8[b]
29	Cytosol	Carbonic anhydrase III[b]
29	Cytosol, microsomes	Thioether *S*-methyltransferase[b]
29	Cytoskeleton	Tropomyosin 5[b]
32	Not known	Pyrophosphatase[b]
32	Cytosol	Glycine *N*-methyltransferase[b]
32	Cytosol	3-Hydroxyanthranilate 3,4-dioxygenase[b]
35	Peroxisomes	Urate oxidase[b]
36	Mitochondria, peroxisomes	2,4-Dienoyl-CoA reductase[b]
40	Cytosol	Sorbitol dehydrogenase precursor[b]
44	Microsomes	Glutamine synthetase[a]
45	Cytosol	Methionine adenosyl transferase[b]
46	Ribosomes	Protein synthesis initiation factor 4A[b]
50	Mitochondria	Glutamate dehydrogenase[a]
52	Cytosol	Selenium (acetaminophen) binding protein[a,b]
54	Mitochondria	Aldehyde dehydrogenase[a,b]
56	Mitochondria	Aldehyde dehydrogenase
59	Mitochondria	ATP synthetase α-subunit[b]
74	Nucleus	Lamin-A[a]
100	Cytosol	N-10 formyltetrahydrofolate dehydrogenase[a]

Table 1 The APAP-adducts formed during hepatotoxicity. [a]Proteins identified by isolation and sequence analysis. [b]Proteins idenitified by mass spectrometry. Adapted from [11].

The depletion of GSH that occurs during the metabolic phase is a critical event for the progression to the oxidative phase, whereby mitochondrial permeability transition (MPT) and cell death ensue. This second stage is less well characterized, and thus will not be discussed in great

detail. Briefly, the data support the hypothesis proposed by James and Hinson (**Figure 6**) [11]. They propose depleted GSH stores predispose the cell to oxidative stress, since GSH is important in the detoxification of peroxides and peroxynitrite. Increased levels of hydrogen peroxide may initiate oxidative damage *via* Fenton chemistry. One such damaging event is the oxidation of critical vicinal thiol groups at the MPT pore, which leads to an increase in the inner mitochondrial membrane permeability. This leads to uncoupling of oxidative phosphorylation and the release of superoxide, which is a lethal event for the cell.

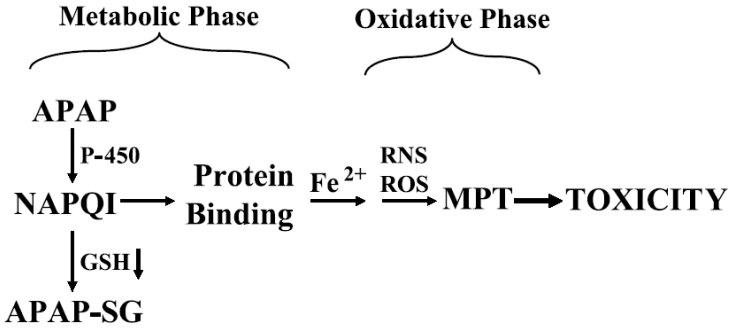


Fig. 6 The proposed two-staged mechanism of APAP hepatotoxicity. MPT (mitochondrial transition permeability), ROS (reactive oxygen species), RNS (reactive nitrogen species).

N-acetylcysteine

N-acetylcysteine is the treatment of choice for patients suffering from APAP toxicity. It functions as an antidote *via* providing cysteine substrate for the replenishment of intracellular GSH stores, thus increasing the ability of the cell to detoxify NAPQI [14]. The administration of GSH is not feasible because it is not taken up by cells, whereas nearly 70% of NAC is metabolized by hepatocytes [15]. NAC also exhibits antioxidant properties, which may limit secondary APAP-induced tissue damage [17].

The administration of NAC is indicated if any of the following conditions is satisfied: (i) the serum APAP concentration is above the "possible hepatic toxicity" line according to the modified Rumack-Matthew nomogram, which relates the time of ingestion to the serum APAP level (ii) presence of liver tenderness, (iii) elevation of aminotransferases, (iv) serum APAP concentrations

greater than 20 mg/ml, and (v) susceptible to hepatoxocity (i.e., chronic alcohol use, fasting, or use of P450-inducing drugs) [16].

If toxicity is suspected, NAC (Mucomyst®) is given PO (by mouth) or by gastric tube as a 140 mg/kg loading dose followed by 17 doses of 70 mg/kg every 4 hours. The initial dose can be given with activated charcoal with no impairment of its efficacy. If the patient cannot tolerate oral NAC (e.g., intractable vomiting) or if the patient has fulminant hepatic failure, NAC can be given intravenously (IV). The initial dose is 140 mg/kg IV over 1 hour, followed by 70 mg/kg every 4 hours for 48 hours [16].

The outcome of APAP is extremely good (no deaths reported) if NAC is given within 10 hrs of overdose, regardless of the initial serum APAP level. Thus, mortality usually results from a delay in seeking medical attention, in recognizing the overdose, or in administering NAC (i.e., > 10 hrs) [16].

Summary

Acetaminophen is a widely used analgesic-antipyretic that can cause centrilobular hepatic necrosis if taken in high doses. This toxicity occurs in two phases: (i) the metabolic activation of acetaminophen to yield reactive metabolites (e.g., NAPQI) and subsequent depletion of glutathione stores (known as the metabolic phase), which progresses to (ii) the oxidative phase, whereby oxidative damage culminates in cell death. The progression into the potentially lethal "oxidative" phase can be prevented if N-acetylcysteine is promptly administered, largely due to its ability to increase intracellular stores of glutathione.

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