

ACETAMINOPHEN-INDUCED HEPATOTOXICITY

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ABSTRACT:

The analgesic acetaminophen causes a potentially fatal, hepatic centrilobular necrosis when taken in overdose. The initial phases of toxicity were described in Dr. Gillette's laboratory in the 1970s. These findings indicated that acetaminophen was metabolically activated by cytochrome P450 enzymes to a reactive metabolite that depleted glutathione (GSH) and covalently bound to protein. It was shown that depletion of GSH prevented the toxicity. This finding led to the development of the currently used antidote *N*-acetylcysteine. The reactive metabolite was subsequently identified to be *N*-acetyl-*p*-benzoquinone imine (NAPQI). Although covalent binding has been shown to be an excellent correlate of toxicity, a number of other events have been shown to occur and are likely important in the initiation and repair of toxicity. Recent data have shown that nitrated tyrosine residues as well as acetaminophen adducts occur in the necrotic cells following toxic doses of acetaminophen. Nitrotyrosine was postulated to be mediated by peroxynitrite, a reactive nitrogen species formed by the very rapid reaction of superoxide and nitric oxide (NO). Peroxynitrite is normally detoxified by GSH, which is depleted in acetaminophen toxicity. NO synthesis (serum nitrate plus nitrite) was dramatically increased following acetaminophen. In inducible nitric oxide syn-

thase (iNOS) knockout mice, acetaminophen did not increase NO synthesis or tyrosine nitration; however, histological evidence indicated no difference in toxicity. Acetaminophen did not cause hepatic lipid peroxidation in wild-type mice but did cause lipid peroxidation in iNOS knockout mice. These data suggest that NO may play a role in controlling lipid peroxidation and that reactive nitrogen/oxygen species may be important in toxicity. The source of the superoxide has not been identified, but our recent finding that NADPH oxidase knockout mice were equally sensitive to acetaminophen and had equal nitration of tyrosine suggests that the superoxide is not from the activation of Kupffer cells. It was postulated that NAPQI-mediated mitochondrial injury may be the source of the superoxide. In addition, the significance of cytokines and chemokines in the development of toxicity and repair processes has been demonstrated by several recent studies. IL-1 β is increased early in acetaminophen toxicity and may be important in iNOS induction. Other cytokines, such as IL-10, macrophage inhibitory protein-2 (MIP-2), and monocyte chemoattractant protein-1 (MCP-1), appear to be involved in hepatocyte repair and the regulation of proinflammatory cytokines.

Overdoses of the analgesic and antipyretic acetaminophen represent one of the most common pharmaceutical product poisonings in the United States today (Litovitz et al., 2002). Although considered safe at therapeutic doses, in overdose, acetaminophen produces a centrilobular hepatic necrosis that can be fatal (Prescott, 1980). Whereas the initial biochemical and metabolic events that occur in the early stages of toxicity have been well described, the precise mechanisms of hepatocyte death are poorly understood. Necrosis is recognized as the mode of cell death and apoptosis has been ruled out (Lawson et al., 1999; Gujral et al., 2002).

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¹ Abbreviations used are: NAPQI, *N*-acetyl-*p*-benzoquinone imine; GSH, glutathione; IL, interleukin; TNF- α , tumor necrosis factor- α ; NO, nitric oxide; iNOS, inducible nitric oxide synthase; IFN- γ , interferon- γ ; MIF, macrophage migration inhibitory factor; MPT, mitochondrial permeability transition.

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Several recent excellent reviews on acetaminophen toxicity have been recently published (Bessemers and Vermeulen, 2001; Nelson et al., 2002). The following manuscript will review the role of covalent binding in toxicity as well as other factors recently identified that contribute to the toxicity. These factors include oxidative stress, nitrotyrosine formation, inflammatory cytokines, and the possible importance of mitochondrial permeability transition.

Metabolic Activation of Acetaminophen

Work from Dr. Gillette's laboratory firmly established the importance of metabolism in acetaminophen toxicity. It was shown that acetaminophen is metabolically activated by cytochrome P450 to form a reactive metabolite that covalently binds to protein (Mitchell et al., 1973). The reactive metabolite was found to be *N*-acetyl-*p*-benzoquinone imine (NAPQI¹), which is formed by a direct two-electron oxidation (Dahlin et al., 1984). More recently, the cytochromes 2E1, 1A2, 3A4, and 2A6 (Patten et al., 1993; Thummel et al., 1993; Chen et al., 1998) have been reported to oxidize acetaminophen to the reactive metabolite. Also, Dr. Gillette's laboratory showed that NAPQI is detoxified by glutathione (GSH) to form an acetaminophen-GSH conjugate. After a toxic dose of acetaminophen, total hepatic GSH is depleted by as much as 90%, and as a result, the metabolite

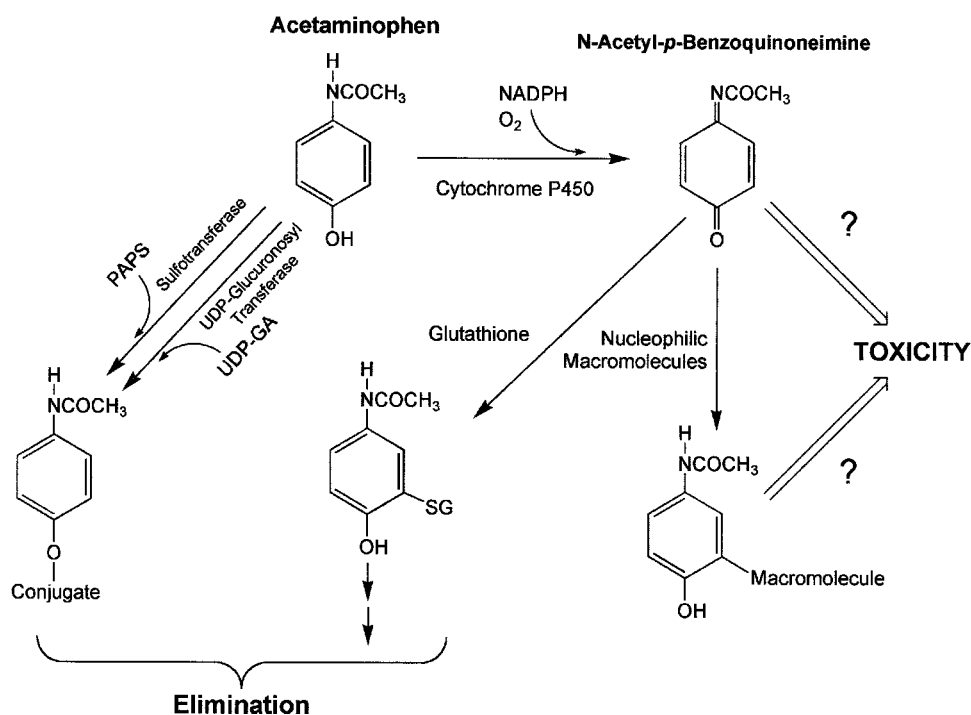


FIG. 1. Schematic representation depicting the role of metabolism in acetaminophen toxicity.

covalently binds to cysteine groups on protein, forming acetaminophen-protein adducts (Mitchell et al., 1973). This mechanism is shown in Fig. 1.

To better understand the role of specific adducts in acetaminophen toxicity, our laboratory and Cohen's laboratory (Bartolone et al., 1988, 1989; Cohen and Khairallah, 1997) developed immunochemical assays that were very specific for acetaminophen covalently bound to cysteine groups on protein (Roberts et al., 1991). Using this antiserum, we reported the relationship between acetaminophen covalent binding and toxicity in mice in a competitive ELISA to quantify the adducts (Pumford et al., 1989, 1990a,b). Western blot assays were used to determine the molecular nature of the acetaminophen-protein adducts (Bartolone et al., 1988; Pumford et al., 1990a,b) and immunohistochemical analyses were used to determine the specific cells that contained the adducts (Bartolone et al., 1989; Roberts et al., 1991). These studies have been reviewed (Hinson et al., 1995; Pumford et al., 1997; Cohen and Khairallah, 1997).

Western blot assays for acetaminophen protein adducts have also been used to study toxicity in human overdose patients. In these studies, adducts have been detected in the blood of human overdose patients with severe toxicity (hepatic transaminases above 5000 IU/l) (James et al., 2001). Using high performance liquid chromatography with electrochemical detection, we recently reported the presence of adducts in the blood samples of overdose patients with mild to moderate toxicity, suggesting that the newer method may be a more sensitive analytical tool than Western blot for the detection of adducts in human sera (Muldrew et al., 2002). This assay may assist in the diagnosis of patients with acute liver failure of indeterminate etiology for which acetaminophen is suspect.

Biochemical Mechanisms of Toxicity

Events that produce hepatocellular death following the formation of acetaminophen protein adducts are poorly understood. One possible mechanism of cell death is that covalent binding to critical cellular proteins results in subsequent loss of activity or function and eventual

cell death and lysis. Primary cellular targets have been postulated to be mitochondrial proteins, with resulting loss of energy production, as well as proteins involved in cellular ion control (Nelson, 1990). Tirmenstein and Nelson (1989) and Tsokos-Kuhn et al. (1988) reported alterations of plasma membrane ATPase activity following toxic doses of acetaminophen.

A number of proteins bound to acetaminophen have been isolated and identified. A review of the individual proteins that were isolated and identified by individual analysis has been published (Cohen and Khairallah, 1997; Pumford et al., 1997). Subsequently, Qui et al. (1998) used matrix-assisted laser desorption ionization mass spectrometry to identify 20 additional proteins containing covalently bound acetaminophen. Table 1 shows the proteins that have been identified to form adducts with acetaminophen. The proteins identified by our laboratory (Pumford et al., 1997) included a 100-kDa cytosolic protein determined by sequence analysis to be the enzyme, *N*-10-formyltetrahydrofolate dehydrogenase. This enzyme is involved in 1-carbon metabolism and oxidizes formaldehyde to carbon dioxide. Studies in mice reported that the activity of this enzyme was decreased by 20% at 2 h after toxic doses of acetaminophen (Pumford et al., 1997). A second protein, a 50-kDa mitochondrial protein, was isolated and determined to be glutamate dehydrogenase (Halmes et al., 1996). This enzyme reversibly metabolizes glutamate to α -ketoglutarate and ammonia. The activity of this enzyme was also decreased approximately 25% at 2 h. Based on the enzyme inhibition data for these two enzymes, it appeared that covalent binding with acetaminophen resulted in only partial inhibition of enzyme activities under toxic conditions in mice. Although it is plausible that partial inhibition of a large number of enzymes may contribute to cell death, the data generated questions relative to the validity of the hypothesis that covalent binding to critical proteins is the only mechanism of acetaminophen toxicity.

Loss of mitochondrial or nuclear ion balance has also been suggested to be a toxic mechanism involved in acetaminophen-mediated cell death since either of these losses can lead to increases in cytosolic

TABLE 1
Acetaminophen-protein adducts

Mass	Fraction	Protein
<i>kDa</i>		
Proteins identified by isolation and sequence analysis ^a		
44	Microsomes	Glutamine synthetase
50	Mitochondria	Glutamate dehydrogenase
54	Mitochondria	Aldehyde dehydrogenase
55–58	Cytosol	Selenium (acetaminophen) binding protein
74–75	Nucleus	Lamin-A
100	Cytosol	N-10 Formyl THF dehydrogenase
Proteins identified by mass spectrometry ^b		
22	Cytosol, mitochondria	Glutathione peroxidase
28	Mitochondria	House keeping protein
29	Cytosol, microsomes	Thioether S-methyltransferase
16	Cytosol	Aryl sulfotransferase
32	Not known	Pyrophosphatase
29	Cytoskeleton	Tropomyosin 5
22	Cytosol, mitochondria	GSH peroxidase
52	Cytosol	Selenium binding protein
52	Cytosol	Acetaminophen binding protein
28	Cytosol	Proteasome subunit C8
45	Cytosol	Methionine adenosyl transferase
46	Ribosomes	Protein synthesis initiation factor 4A
56	Mitochondria	Aldehyde dehydrogenase
54	Cytosol	Aldehyde dehydrogenase
59	Mitochondria	ATP synthetase α -subunit
29	Cytosol	Carbonic anhydrase III
35	Peroxisomes	Urate oxidase
36	Mitochondria, peroxisome	2,4-Dienoyl-CoA reductase
22	Cytosol (macrophages)	Osteoblast-specific factor 3
23	Cytosol, mitochondria	Glutathione transferase π
40	Cytosol	Sorbitol dehydrogenase precursor
32	Cytosol	Glycine N-methyltransferase
32	Cytosol	3-Hydroxyanthranilate 3,4-dioxygenase

THF, tetrahydrofuran.

^a Cohen et al. (1997).

^b Qiu et al. (1998).

Ca²⁺ concentrations, mitochondrial Ca²⁺ cycling, activation of proteases and endonucleases, and DNA strand breaks (Nelson, 1990; Ray et al., 1993; Salas and Corcoran, 1997). The effect of the addition of NAPQI on isolated mitochondria has been reported (Weis et al., 1992) and inhibition of mitochondrial respiration has been investigated as an important mechanism in acetaminophen toxicity (Donnelly et al., 1994).

Early Research on Oxidative Stress

Oxidative stress is another mechanism that has been postulated to be important in the development of acetaminophen toxicity. Thus, increased formation of superoxide would lead to hydrogen peroxide and peroxidation reactions by Fenton-type mechanisms. It has been shown that NAPQI reacts very rapidly with GSH ($k_1 = 3.2 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ at pH 7.0) (Coles et al., 1988), and there are a number of potential mechanisms that have been suggested to play a role. Under conditions of NAPQI formation following toxic acetaminophen doses, GSH concentrations may be very low in the centrilobular cells, and the major peroxide detoxification enzyme, GSH peroxidase, which functions very inefficiently under conditions of GSH depletion (Nakamura et al., 1974), is expected to be inhibited. In addition, during formation of NAPQI by cytochrome P450, the superoxide anion is formed, with dismutation leading to hydrogen peroxide formation (Dai and Cederbaum, 1995). Also, others have suggested that peroxidation of acetaminophen to the semiquinone free radical would lead to redox cycling between the acetaminophen and the semiquinone. This mechanism may lead to increased superoxide and toxicity (de Vries,

1981). However, we found that the semiquinone reacted rapidly to form polymers and no evidence for reaction of oxygen was observed (Potter et al., 1989).

A significant amount of evidence has pointed to the potential involvement of oxidative stress in acetaminophen toxicity. Nakae et al. (1990) reported that administration of encapsulated superoxide dismutase decreased the toxicity of acetaminophen in the rat. Moreover, the iron chelator, deferoxamine, has been shown to decrease toxicity in rats (Sakaida et al., 1995). Our laboratory showed that deferoxamine caused a delay in the rate of development of acetaminophen toxicity in mice, but after 24 h, the relative amount of toxicity was not affected (Schnellmann et al., 1999). These data suggest that an iron-catalyzed Haber-Weiss reaction may also play a role in the development of oxidant stress and injury.

Role of Kupffer Cells

Several laboratories have studied the role of macrophage activation (Laskin et al., 1995) in acetaminophen toxicity. Kupffer cells are the phagocytic macrophages of the liver. When activated, Kupffer cells release numerous signaling molecules, including hydrolytic enzymes, eicosanoids, nitric oxide, and superoxide. Kupffer cells may also release a number of inflammatory cytokines, including IL-1, IL-6, and TNF- α (Laskin et al., 1995), and multiple cytokines are released in acetaminophen toxicity (Blazka et al., 1995; Hogaboam et al., 1999a, 2000; Bourdi et al., 2002a,b). Laskin et al. (1995) examined the role of Kupffer cells in acetaminophen hepatotoxicity by pretreating rats with compounds that suppress Kupffer cell function (gadolinium

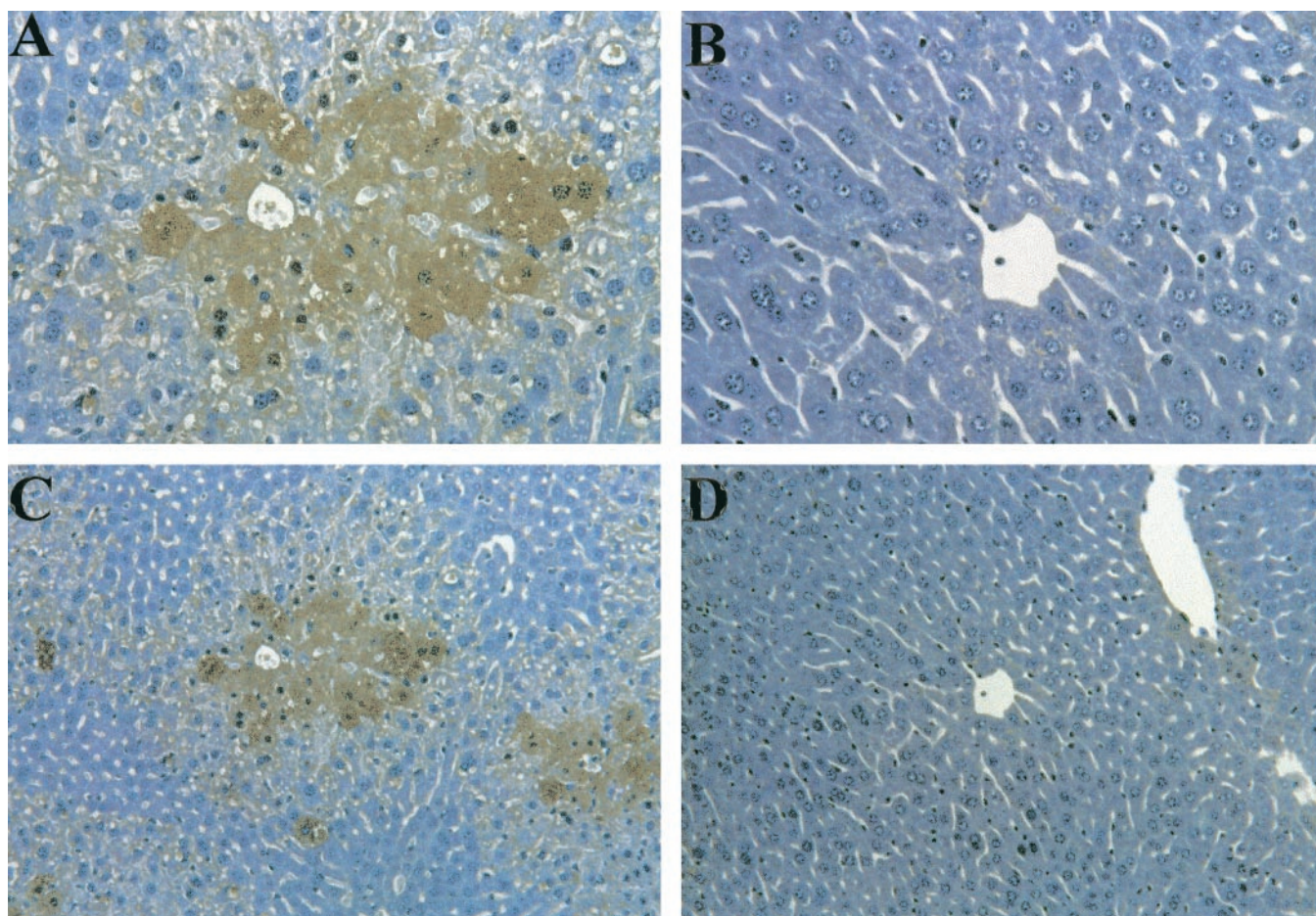


FIG. 2. Immunohistochemical detection of nitrotyrosine in an acetaminophen-treated mouse.

A, mouse treated with 300 mg/kg acetaminophen and sacrificed at 4 h after acetaminophen (40 \times). The brown stain represents nitrotyrosine. B, mouse treated with saline and sacrificed at 4 h and stained for nitrotyrosine. (40 \times). C, mouse treated with 300 mg/kg acetaminophen and sacrificed at 4 h after acetaminophen (20 \times). D, mouse treated with saline and sacrificed at 4 h and stained for nitrotyrosine (20 \times).

chloride and dextran sulfate). These investigators reported that rats pretreated with these compounds were less sensitive to the toxic effects of acetaminophen. Similar effects were reported in the mouse (Blazka et al., 1995). Goldin et al. (1996) showed that treatment of mice with liposomes containing dichloromethylene diphosphonate to knock out the Kupffer cells also decreased acetaminophen toxicity. Similarly, we reported that pretreatment of mice with gadolinium chloride or dextran sulfate decreased the toxic effects of acetaminophen (Michael et al., 1999). These studies suggested a critical role for Kupffer cells in the development of acetaminophen hepatotoxicity; however, recent work by Ju et al. (2002) came to a different conclusion. They pretreated mice with gadolinium chloride and found that the number of Kupffer cells in the liver was only partially decreased. Consistent with previous reports, they showed decreased acetaminophen toxicity in the pretreated mice. However, treatment of mice with dichloromethylene diphosphonate completely depleted the liver of Kupffer cells, but toxicity was increased. These data raise questions relative to the importance of Kupffer cells in acetaminophen toxicity.

Nitrotyrosine and Acetaminophen Toxicity

We recently showed that nitrotyrosine occurs in the centrilobular cells of the liver of acetaminophen-treated mice (Hinson et al., 1998). Immunohistochemical analysis of liver of acetaminophen-treated mice indicated that nitration occurred in the same cells that contained acetaminophen adducts and developed necrosis (Fig. 2) (Hinson et al.,

1998). We postulated that nitrotyrosine was formed by a reaction of peroxynitrite with tyrosine. Nitration of tyrosine has been shown to be an excellent biomarker of peroxynitrite formation (Kaur and Halliwell, 1994). It is formed by a rapid reaction between nitric oxide and superoxide, and we found that NO synthesis (serum levels of nitrate plus nitrite) was increased in acetaminophen toxicity (Hinson et al., 1998). Also, others reported the induction of hepatic iNOS in the acetaminophen-treated rat (Gardner et al., 1998). NO and superoxide react to produce peroxynitrite (ONOO⁻) at a rate near the diffusion-controlled limit ($7 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$). Peroxynitrite is a species that not only leads to the nitration of tyrosine but is also a potent oxidant that can attack a wide range of biological targets (Pryor and Squadrito, 1995), and under conditions of reduced cellular oxidant scavenging capability, peroxynitrite is highly toxic (Beckman and Koppenol, 1996). The anion is relatively stable, but the acid rearranges to nitrate with a half-life of 1.9 s at pH 7.4; the pK is 7.5 (Beckman and Koppenol, 1996). Oxidation reactions can involve one or two-electron processes. Oxidation of lipids (Rubbo, 1998), proteins, or DNA bases (Pryor and Squadrito, 1995) has been reported. Thus, peroxynitrite is an excellent candidate for a toxic species. Moreover, it is normally detoxified by GSH/GSH peroxidase, and GSH is depleted in acetaminophen toxicity (Radi et al., 1991). GSH peroxidase is a key enzyme in this defense mechanism (Sies et al., 1997). Thus, a normal detoxification mechanism for peroxynitrite is impaired. Also, even though acetaminophen itself will detoxify peroxynitrite (Whiteman et

al., 1996), the drug is metabolized rapidly in the mouse and concentrations are low at the time when nitration is observed. However, mechanisms other than peroxynitrite have been recently reported to lead to nitrotyrosine. Thus, myeloperoxidase has been shown to oxidize the nitrite ion to the NO_2 radical, and this is a nitrating species (Davis et al., 2001). Since myeloperoxidase is present in neutrophils and neutrophil recruitment is a late event in acetaminophen toxicity, this mechanism may not be important (Lawson et al., 2000). Also, nitrite may be oxidized by heme or free metals, leading to the NO_2 radical (Thomas et al., 2002).

Nitration of Tyrosine versus Oxidative Stress

The role of tyrosine nitration in acetaminophen toxicity was investigated using inhibitors of nitric oxide synthetase and mice that were genetically deficient in iNOS (Michael et al., 2001). A comparison of the time course for development of acetaminophen toxicity in iNOS knockout mice and wild-type mice indicated no difference in the histological development of toxicity between the two groups of mice. Serum levels of nitrate plus nitrite (a measure of NO synthesis) were increased in the wild-type mice but not in the iNOS knockout mice, and analysis of liver homogenates for tyrosine nitration indicated significant levels in the wild-type mice and a decreased amount in the knockout mice, possibly as a result of endothelial NOS. It was concluded that the major murine enzyme important in tyrosine nitration under normal wild-type conditions was iNOS. Interestingly, these experiments showed that acetaminophen caused a 3-fold increase in lipid peroxidation (oxidative stress) in the iNOS knockout mice but no increase in the wild-type mice. Thus, in the wild-type mice, toxicity was accompanied by tyrosine nitration, whereas in the iNOS knockout mice, toxicity was accompanied by oxidative stress. In a subsequent study (Hinson et al., 2002), we determined the effect of NOS inhibitors on acetaminophen toxicity in mice. The iNOS inhibitor aminoguanidine did not significantly alter acetaminophen toxicity, but it greatly decreased tyrosine nitration and increased lipid peroxidation. Thus, the iNOS inhibitor gave information similar to that obtained in the iNOS knockout mice. These data were interpreted to indicate that excess levels of superoxide were formed in acetaminophen toxicity. When NO is present, as in the wild-type mice, the superoxide preferentially reacts to form peroxynitrite, which nitrates proteins. In the absence of NO, superoxide leads to lipid peroxidation (Fig. 3). These data indicate the importance of NO in the disposition of superoxide, leading to oxidative stress.

Cytokines and Other Inflammatory Mediators in Acetaminophen Toxicity

Several laboratories have reported that inflammatory cytokines are increased in acetaminophen toxicity. Blazka reported the up-regulation of $\text{TNF-}\alpha$ and $\text{IL-1}\alpha$ in the acetaminophen-treated mouse (Blazka et al., 1995, 1996). In addition, he showed that selective immunoneutralization of either $\text{TNF-}\alpha$ or $\text{IL-1}\alpha$ partially decreased toxicity for a period of time. In vitro studies by Kuo showed that $\text{IL-1}\beta$ caused a dose-dependent up-regulation of NO in rat hepatocytes exposed to acetaminophen (Kuo et al., 1997). Consistent with these data, we recently found that $\text{IL-1}\beta$ and NO up-regulation in the acetaminophen-treated mouse are temporally related (James et al., 2003b). Although others have confirmed the up-regulation of $\text{TNF-}\alpha$ in acetaminophen toxicity (Simpson et al., 2000), its role in the mediation of toxicity is somewhat controversial. $\text{TNF-}\alpha$ knockout mice were not protected from toxicity (Boess et al., 1998), and a recent report showed that immunoneutralization of $\text{TNF-}\alpha$ had no effect on toxicity (Simpson et al., 2000). Studies in IL-10 knockout mice suggest that

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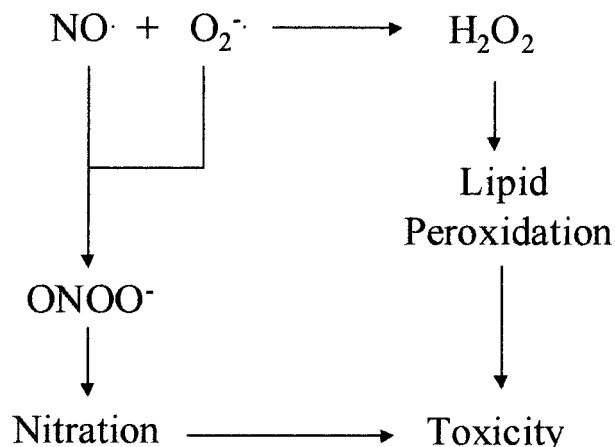


FIG. 3. Schematic representation depicting the role of oxidative stress in acetaminophen toxicity.

IL-10 is protective in APAP toxicity by controlling NO and iNOS formation (Bourdi et al., 2002a). In addition, the increased susceptibility of IL-10 knockout mice to acetaminophen was associated with increased levels of $\text{TNF-}\alpha$ and $\text{IFN-}\gamma$. Thus, it may be that proinflammatory cytokines contribute to the toxicity, and that they are regulated by anti-inflammatory cytokines, such as IL-10 and others.

Macrophage migration inhibitory factor (MIF) is a recently described protein that has characteristics of a cytokine, a hormone, and an enzyme. It may promote the up-regulation of other pro-inflammatory cytokines, adhesion molecules, matrix metalloproteinase-2 expression, NO release, and cyclooxygenase-2 and has counter-regulatory effects on endogenous glucocorticoids (Baugh and Bucala, 2002). Pohl and colleagues reported that MIF was up-regulated in acetaminophen toxicity (Bourdi et al., 2002b). Moreover, MIF knockout mice were less susceptible to acetaminophen and had decreased $\text{IFN-}\gamma$ production and increased heat shock protein expression (Bourdi et al., 2002b). Heat shock proteins are considered to be a protective mechanism against various physiologic and environmental stressors (Bourdi et al., 2002b). In addition, cyclooxygenase-2 has been reported to be hepatoprotective in acetaminophen, possibly due to up-regulation of heat shock proteins (Reilly et al., 2001).

Various chemokines have also been reported to be up-regulated in acetaminophen toxicity. Chemokines were initially recognized for their role in leukocyte recruitment. However, Lawson et al. (2000) showed that neutrophils followed the development of toxicity and that administration of antibodies to $\beta 2$ integrins had no effect on toxicity. Chemokine up-regulation in acetaminophen toxicity appears to be hepatoprotective. Hogaboam showed that macrophage inhibitory protein-2 (MIP-2) and other related chemokines promoted hepatocyte regeneration in acetaminophen-treated mice (Hogaboam et al., 1999a). In additional studies, viral vector delivery of MIP-2 to acetaminophen-treated mice was protective against acetaminophen toxicity (Hogaboam et al., 1999b). Studies in mice deficient in CCR2 , the primary receptor for the chemokine MCP-1 (monocyte chemoattractant protein-1), showed these mice to have increased toxicity to acetaminophen and increased formation of $\text{TNF-}\alpha$ and $\text{IFN-}\gamma$ (Hogaboam et al., 2000). Cumulatively, these studies suggest that cytokines play complex roles in the toxicity and that alterations in the equilibrium of pro- and anti-inflammatory cytokine formation contribute to

the toxicity. Chemokines appear to facilitate hepatocyte regeneration following toxicity, likely by increasing the nuclear translocation of growth-regulatory transcription factors.

Superoxide Formation and Mitochondrial Dysfunction in Acetaminophen Toxicity

Superoxide may be formed via a number of mechanisms including formation from cytochrome P450 (Puntarulo and Cederbaum, 1996) and other enzymes. We evaluated the importance of activation of Kupffer cells, macrophages, or neutrophils (the so-called respiratory burst) in acetaminophen toxicity. This sudden excess utilization of oxygen by activated phagocytes is a result of increased activity of the enzyme, NADPH-oxidase. The result is release of superoxide anion at the outer surface of the plasma membrane (Baggiolini and Wymann, 1990). We utilized mice genetically deficient in Gp91 phox, a critical subunit of NADPH oxidase (James et al., 2003a), and found that these mice and wild-type mice had comparable toxicity to acetaminophen. Also, both knockout mice and wild-type mice had the same degree of tyrosine nitration in the liver. An analysis of mitochondrial glutathione disulfide was found to be comparable in the two groups (James et al., 2003a). These data suggest that the increased superoxide in acetaminophen toxicity is not from activated macrophages.

Available data suggest that mitochondrial dysfunction may be an important mechanism in acetaminophen-induced hepatotoxicity. It is known that mitochondrial permeability transition (MPT) occurs with formation of superoxide, and this may be the source of superoxide leading to peroxynitrite and tyrosine nitration. MPT represents an abrupt increase in the permeability of the inner mitochondrial membrane to small molecular weight solutes. Oxidants such as peroxides and peroxynitrite, Ca^{2+} , and P_i promote the onset of MPT, whereas Mg^{2+} , ADP, low pH, and high membrane potential oppose onset. Associated with the permeability change is membrane depolarization, uncoupling of oxidative phosphorylation, release of intramitochondrial ions and metabolic intermediates, and mitochondrial swelling. MPT mechanisms have been reviewed by Lemasters et al. (1998). In the late 1980s it was shown that cyclosporin A specifically blocked the onset of MPT in a saturable manner, implying a protein channel or pore. It has been subsequently recognized that this pore transports both anionic and cationic solutes of mass less than 1500 Da and is the previously identified multiple conductance channel. Of particular note is the fact that oxidation of vicinal thiols in the pore promote an open conductance state (Lemasters et al., 1998). Moldeus and Orrenius' laboratory reported that addition of NAPQI to isolated rat liver mitochondria caused a decrease in synthesis of ATP and an increase in release of sequestered Ca^{2+} . This release was blocked by cyclosporin A (Weis et al., 1992). These data are consistent with the hypothesis that NAPQI causes MPT, as has been reported about other quinones (Palmeira and Wallace, 1997). This is presumably a result of NAPQI-mediated oxidation of the vicinal thiols at the MPT pore. NAPQI is known to be both an oxidizing agent and an arylating agent, and Tirmenstein and Nelson (1989) have reported that acetaminophen leads to oxidation of protein thiols. Also, McLean and coworkers have reported that inhibitors of MPT decrease acetaminophen toxicity in rat liver slices and in vivo (Beales and McLean, 1996). Lastly, Grewal reported that late addition of *N*-acetylcysteine to freshly isolated mouse liver hepatocytes had no effect on the toxicity; however, dithiothreitol decreased the further development of toxicity (Grewal and Racz, 1993). Also, in similar experiments with isolated hamster hepatocytes, the late addition of dithiothreitol to cells washed free of acetaminophen prevented the further decrease in cell viability and appeared to reverse morphological changes (Tee et al., 1986). Dithiothreitol is known to reduce the vicinal thiols at the MPT pore and

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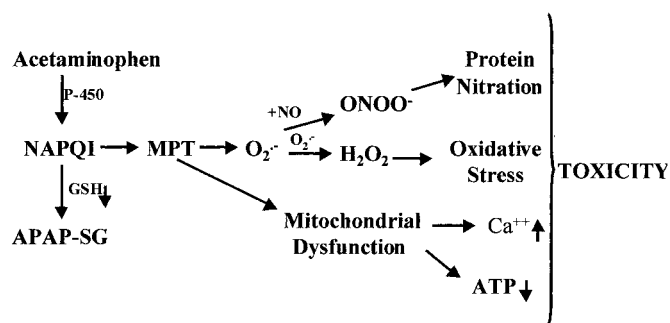


FIG. 4. Schematic representation depicting the role of mitochondrial permeability transition in acetaminophen toxicity.

prevent the further progression of toxicity (Palmeira and Wallace, 1997). These data are consistent with a mechanism whereby acetaminophen toxicity is by NAPQI-mediated oxidation of thiol groups in mitochondria leading to MPT. The toxicity is mediated by mitochondrial dysfunction resulting in production of reactive oxygen/nitrogen species (Fig. 4).

Summary

Dr. Gillette and researchers in his laboratory made fundamental contributions to our understanding of the pathogenesis of drug and chemical toxicity. With the analgesic acetaminophen it was shown that the hepatic necrosis was not a result of the parent compound. Metabolism by cytochrome P450 enzymes to a reactive metabolite was necessary for the production of toxicity. After therapeutic doses of acetaminophen, the reactive metabolite was efficiently detoxified by conjugation with GSH. However, after toxic doses, GSH was depleted by the conjugation reaction and the metabolite covalently bound to protein. Covalent binding correlated with development of toxicity. Treatment with cysteine to increase GSH detoxification was shown to be an effective antidote against toxicity. The latter finding led to the development of *N*-acetylcysteine as the current clinical antidote. Since these initial findings, many investigators have added additional details relative to the concept of how acetaminophen is toxic, and some of these are discussed in this review. Despite these recent studies, the concept of metabolic activation as the critical initiating event in the development of toxicity has remained. Moreover, metabolic activation has proven to be a general mechanism relevant for the development of toxicity for many drugs and chemicals. Dr. Gillette reviewed his major contributions to science in the *Annual Review of Pharmacology and Toxicology* (Gillette, 2000) and in *Drug Metabolism Reviews* (Gillette, 1995).

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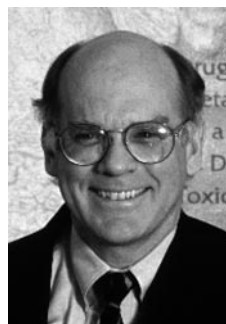


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