Underlying Endotoxemia Augments Toxic Responses to Chlorpromazine: Is There a Relationship to Drug Idiosyncrasy?

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ABSTRACT

Idiosyncratic reactions occur in a small fraction (typically <5%) of the population taking therapeutic drugs. Chlorpromazine (CPZ) is a phenothiazine, antipsychotic drug that has caused several idiosyncratic responses during its therapeutic use. Clinical evidence suggests that conditions associated with inflammation are risk factors for the appearance of these responses. Accordingly, we tested the hypothesis that an inflammatory stimulus, bacterial lipopolysaccharide (LPS), renders animals susceptible to CPZ-induced idiosyncratic reactions seen in humans. Male Sprague-Dawley rats (200–250 g) were fasted for 24 h. A small dose of LPS (7.4 × 10^6 EU/kg from Escherichia coli) or its vehicle (saline) was administered by tail vein 2 h before an intraperitoneal injection of CPZ (70 mg/kg) or its vehicle (saline). Cholestasis and hepatocellular necrosis were evaluated as increased concentrations of serum bile acids and bilirubin and increased activities of alkaline phosphatase, γ-glutamyltransferase, alanine aminotransferase, and aspartate aminotransferase. With the exception of bile acids, these serum markers were elevated in animals treated with LPS/CPZ. Histopathological lesions in liver sections were consistent with these findings. Elevated serum creatine kinase activity, which is associated with human idiosyncratic responses to phenothiazines, was also found in animals treated with LPS/CPZ, but not with either LPS or CPZ alone. These results raise the possibility that concurrent, modest inflammation may underlie susceptibility of individuals to certain idiosyncratic reactions and may form the basis for an animal model with which to understand and predict drug idiosyncrasy.

Drug idiosyncrasy is an untoward biological response to a therapeutic agent occurring in a small percentage of individuals. Idiosyncratic responses appear to occur independently of dose and have an inconsistent temporal relationship to the course of drug administration (Hollister, 1957). Although drug metabolism polymorphisms and drug allergy are widely presumed to underlie idiosyncratic responses, convincing evidence to support these as causes is lacking for the majority of drugs. Reproducing such responses in animals has been met with little success. Inasmuch as drug idiosyncrasy results in human suffering and considerable cost to pharmaceutical companies, animal models that are able to predict such responses in people before a drug is marketed could have great benefit.

Among the many drugs that have caused idiosyncratic reactions in people are aliphatic phenothiazines. For example, chlorpromazine (CPZ) (Thorazine, 10-[3-dimethylaminopropyl]-2-chlorophenothiazine) is a tricyclic antidepressant that has been used as a sedative and antiemetic and for the management of psychotic disorders. Two types of adverse reactions result from phenothiazine usage. First, extrapyramidal side effects such as pseudoparkinsonism, dystonic reactions, and akathisia are common, dose-related side effects that likely result from the blockade of dopamine receptors and action at cholinergic receptors (Holloman and Marder, 1997). Second, the clinical use of CPZ has resulted in idiosyncratic reactions that include hepatic cholestasis (Regal et al., 1987), neuroleptic malignant syndrome (NMS) (Wong, 1996), and increased serum creatine kinase activity suggestive of rhabdomyolysis (Koizumi et al., 1996). As with other idiosyncratic drug reactions, mechanisms of action remain unclear.

Animal models (Ros et al., 1979; Mullock et al., 1983) and in vitro studies (Abernathy et al., 1977; Hruban et al., 1978; Tavoloni et al., 1979) have indicated that CPZ is intrinsically toxic to the liver. However, the toxicity is dose-related and reproducible, unlike the hepatic injury seen clinically in humans, for which a small percentage of the population consuming this drug is susceptible.

Zimmerman (1997) proposed that overt hepatic injury resulting from CPZ was likely a result of “two hits”, in the sense that the intrinsic toxicity of CPZ, coupled with another fac-

ABBREVIATIONS: CPZ, chlorpromazine; NMS, neuroleptic malignant syndrome; LPS, lipopolysaccharide; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, γ-glutamyltransferase; PMN, polymorphonuclear leukocyte; CK, creatine kinase; TNF-α, tumor necrosis factor-α.
tor, might precipitate hepatic disease. In an animal model, Mullock et al. (1983) tested the hypothesis that an immune response to CPZ would exacerbate its hepatotoxicity. Hepatic injury occurred in a reproducible manner in animals treated with CPZ; however, there was no correlation between the titer of antibodies in individual animals and the degree of liver damage observed. This suggests that factors other than allergic hypersensitivity may increase susceptibility to liver injury.

It has been hypothesized that inflammation is a determinant of susceptibility to the toxic effects of xenobiologic agents (Roth et al., 1997). Exposure to agents that cause inflammation is episodic and commonplace, emerging from infection and other events. For example, bacterial endotoxin (lipopolysaccharide (LPS)) is a potent inflammmagen that can translocate from the intestine into the systemic circulation during disturbances of the gastrointestinal tract or other stresses. Mild endotoxia has been associated with events such as liver or intestinal disease, gastrointestinal distress from alcohol consumption, altered diet, surgery, and other factors (for review, see Roth et al., 1997).

When administered to rodents in large doses, LPS leads to sepsis-like changes, including fever, disseminated intravascular coagulation, circulatory shock, and multiple organ failure (Hewett and Roth, 1993). Exposure to smaller amounts, on the other hand, induces noninjurious and modest inflammatory changes in experimental animals and people. Although noninjurious by themselves, such small doses of LPS can enhance the toxic response to xenobiologic agents. For example, LPS exposure increases liver damage produced by hepatotoxins such as monocrotaline (Yee et al., 2000), aflatoxin B$_1$ (Barton et al., 2000), allyl alcohol (Sneed et al., 1997), and others (Roth et al., 1997). Similarly, an inflammatory state induced by the presence of circulating endotoxin or other inflammmagens might provoke untoward responses to drugs.

CPZ-induced cholestasis is associated with a marked hepatic infiltration of inflammatory cells such as neutrophils and lymphocytes (Moradpour et al., 1994). Additionally, CPZ-induced idiosyncratic reactions have been reported after acute gastrointestinal distress or prolonged physical stress (Lo et al., 1989; Lowy et al., 1995; Wong, 1996). Each of the latter has been associated with endotoxia (Roth et al., 1997), raising the possibility that underlying inflammation may precipitate idiosyncratic reactions. Accordingly, we hypothesized that CPZ idiosyncratic reactions may be reproduced in animals with concurrent, modest inflammation. As an initial test of this hypothesis, we determined whether cotreatment of rats with CPZ and a small dose of LPS would result in toxic responses mimicking manifestations of human idiosyncratic reactions to this class of drugs.

**Materials and Methods**

**Animals.** Male Sprague-Dawley rats (CD-Crl:CD-(SD)BR VAF/Plus; Charles River, Portage, MI) weighing 200 to 250 g were used. Animals were maintained on a 12-h light/dark cycle under conditions of controlled temperature and humidity for 1 week. Food (Rodent Chow; Teklad, Madison, WI) and tap water were provided ad libitum.

**Treatment Protocol.** Animals were fasted for 24 h before the administration of LPS (Escherichia coli, serotype 012:B12, 1.8 $\times$ 10$^6$ EU/kg; Sigma Chemical, St. Louis, MO). They were then allowed food ad libitum. Water was provided ad libitum at all times. Rats were treated intravenously with LPS at a dose of 7.4 $\times$ 10$^6$ EU/kg, or with an equal volume of sterile saline vehicle (Abbott Diagnostics, Abbott Park, IL). This dose of LPS alone does not produce overt liver injury. Two hours after the administration of LPS, CPZ-HCl (70 mg/kg) (Sigma Chemical) diluted in sterile saline or an equal volume of sterile saline was injected intraperitoneally. Twenty-four hours after CPZ administration, rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and a laparotomy was performed. Blood was collected from the abdominal aorta into a sterile 5-ml syringe. Blood was dispensed into a 12 $\times$ 75-mm test tube and allowed to clot. Serum was separated by centrifugation and aliquoted for storage at 4–8°C or −20°C until analyzed.

**Assessment of Hepatotoxicity.** Hepatic necrosis and cholestatic injury were assessed by measuring the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and $\gamma$-glutamyltransferase (GGT), and the concentrations of bile acids and bilirubin (kits 59-UV, 58-UV, 245-UV, 419-UV, 450-A, and 552-A, respectively; Sigma Chemical). Liver sections were fixed in formalin, embedded in paraffin, cut at 6 $\mu$m, stained with hematoxylin and eosin, and evaluated for liver injury.

**Histological Scoring.** Slides of liver sections were coded and evaluated without knowledge of treatment. Two categories of injury (suberosal and internal) were defined based on location within the liver sections and scored on a scale of 1 to 5 based on severity.

Suberosal injury was defined as well demarcated lesions, immediately subcapsular, comprising groups of hypereosinophilic hepatocytes with indistinct cell borders and with nuclear pyknosis and/or karyolysis and neutrophilic infiltration. These lesions were scored as follows: 1, no injury or scattered hypereosinophilic hepatocytes; 2, coagulative necrosis of <5% of the total section circumference; 3, coagulative necrosis of 5 to 15% of the total circumference; 4, coagulative necrosis of 16 to 25% of the total circumference; and 5, coagulative necrosis extending into the lobules and affecting >25% of the total circumference.

Internal lobar injury was defined as well demarcated, midzonal lesions comprising markedly hypereosinophilic hepatocytes lacking cell border definition and with nuclear pyknosis and/or karyolysis, neutrophilic infiltration, and loss of sinusoidal architecture. Microscopically, these lesions comprised between 5 and 15% of a 200 $\times$ field. Liver sections were scored as follows: 1, no midzonal injury; 2, one to three necrotic foci per liver section; 3, four to six necrotic foci; 4, seven to nine necrotic foci; and 5, more than nine necrotic foci.

**Quantification of Hepatic Polymorphonuclear Leukocyte (PMN) Accumulation.** PMNs were visualized in liver sections by using a previously described immunohistochemical technique (Pearson et al., 1995). In brief, sections were fixed in formalin, paraffin-embedded, and sectioned at 6 $\mu$m. Paraffin was removed from the tissue sections with xylene. PMNs within the liver tissue were stained using a primary rabbit anti-rat PMN immunoglobulin followed by biotinylated goat anti-rabbit IgG, avidin-conjugated alkaline phosphatase, and Vector Red substrate. Hepatic PMNs were quantified in 20, 400 $\times$ random fields in the interior of each section by using light microscopy. PMNs accumulating in the subcapsular space were not quantified due to clustering of immunohistochemically stained cells and the associated difficulty in enumerating these cells.

**Assessment of Creatine Kinase (CK) Activity.** Serum CK activity was measured spectrophotometrically (kit 45-1; Sigma Chemical). CK isozymes were separated by applying 5 $\mu$L of serum with hematoxylin and eosin, and evaluated for liver injury.
were discerned by their increased cathodal migration from the origin, respectively (Blum et al., 1981).

Statistical Analysis. Results are presented as means ± S.E.M. Data were analyzed by a two-way analysis of variance, and individual comparisons were performed using Fisher’s least significant difference test. When variances were not homogeneous, data were log-transformed before analysis. Nonparametric data are presented as the median with 25th and 75th quartile values. Nonparametric data were analyzed by a Kruskal-Wallis analysis of variance on ranks with individual comparisons performed using Dunn’s test. The criterion for statistical significance in all studies was \( p < 0.05 \).

Results

Serum Markers of Hepatic Cholestasis and Necrosis. LPS and CPZ were administered to rats at doses that were nontoxic by themselves in preliminary experiments. Markers of hepatic cholestasis included serum ALP (Fig. 1A) and GGT (Fig. 1B) activities and serum bilirubin (Fig. 1C) and bile acid (Fig. 1D) concentrations. At 24 h, the activities of ALP and GGT were unaffected by either LPS or CPZ treatment; however, cotreatment with LPS and CPZ resulted in significant increases. LPS treatment resulted in a modest yet statistically significant increase in serum bilirubin that was unaffected by CPZ treatment. Bile acid concentrations were not altered by any of the treatments. Markers of hepatocellular injury included serum ALT (Fig. 2A) and AST (Fig. 2B) activity. These were not elevated in animals exposed to either LPS or CPZ by themselves. In contrast, treatment with LPS/CPZ resulted in significant increases. Results of a limited study (\( n = 3 \)) indicated that markers of hepatocellular injury were significantly elevated by 16 h in LPS/CPZ-treated animals (data not shown).

To explore the relationship of the toxic response to drug dose, rats were given various nontoxic doses of CPZ in conjunction with a noninjurious dose of LPS. The hepatotoxic response depended on CPZ dose, with a threshold between 25 and 50 mg of CPZ/kg for markers of biliary injury (Fig. 3A) and hepatic parenchymal cell damage (Fig. 3, B and C).

Histopathology of Liver. In liver sections, two locations of lesions (internal and subserosal) were identified, defined, and graded based on severity (Table 1). Only livers from animals treated with LPS/CPZ had significantly greater histological scores than control animals. These livers were characterized by foci marked by neutrophil infiltration accompanied by pale, eosinophilic parenchymal cells with indistinct cytoplasmic borders and pyknotic and/or karyolytic nuclei (Fig. 4). The foci were either subserosal or occurred as midzonal lesions distributed throughout the interior of liver sections.

Fig. 1. Markers of cholestatic liver injury in rats exposed to LPS/CPZ. Animals received either LPS (7.4 \( \times 10^6 \) EU/kg) or saline followed 2 h later by treatment with either CPZ (70 mg/kg) or saline. They were killed 24 h after CPZ exposure, and serum activities of ALP (A) and GGT (B), and concentrations of bilirubin (C) and bile acids (D) were determined; \( n = 4 \) to 23. a, significantly different from respective value in the absence of CPZ (\( p < 0.05 \)); b, significantly different from respective value in the absence of LPS (\( p < 0.05 \)).
Markers of hepatocellular injury in rats exposed to LPS/CPZ. Animals received either LPS (7.4 × 10^7 EU/kg) or saline followed 2 h later by treatment with either CPZ (70 mg/kg) or saline. They were killed 24 h after CPZ exposure, and serum activities of ALT (A) and AST (B) were determined; n = 10 to 17. a, significantly different from respective value in the absence of CPZ (p < 0.05); b, significantly different from respective value in the absence of LPS (p < 0.05).

**Fig. 2.** Markers of hepatocellular injury in rats exposed to LPS/CPZ. Animals received either LPS (7.4 × 10^7 EU/kg) or saline followed 2 h later by treatment with either CPZ (70 mg/kg) or saline. They were killed 24 h after CPZ exposure, and serum activities of ALT (A) and AST (B) were determined; n = 10 to 17. a, significantly different from respective value in the absence of CPZ (p < 0.05); b, significantly different from respective value in the absence of LPS (p < 0.05).

**Neutrophil Accumulation in Liver.** Immunohistochemical staining revealed few PMNs scattered throughout the liver sections of animals treated with vehicle or CPZ (Fig. 5). In contrast, significant neutrophil accumulation was evident in the sinusoids of livers from animals treated with LPS or with LPS/CPZ.

**Serum Creatine Kinase.** Pronounced increases in serum CK activity characterize phenothiazine idiosyncratic reactions in people (Surrourn et al., 1984; Ebadi et al., 1990; Koizumi et al., 1996). CK activity (Fig. 6) was normal in serum of rats treated with LPS or CPZ alone. However, LPS/CPZ cotreatment markedly increased serum CK activity. This response depended on CPZ dose, demonstrating a threshold between 25 and 50 mg of CPZ/kg (Fig. 3). Results of a limited study (n = 3) indicated that serum CK activity was significantly elevated by 16 h after LPS/CPZ treatment (data not shown). CK exists in several isoforms that can be differentiated by gel electrophoresis. Visual analysis of the electrophoretic banding pattern of CK isoenzymes revealed that CK in serum of LPS/CPZ-cotreated rats was predominantly the BB isoform (data not shown).

In addition to increased CK activity, a common clinical manifestation of rhabdomyolysis is myoglobinuria (Schulze, 1982). Qualitative urinalysis by using a Multistix test strip (Bayer, Elkhart, IN) was negative for myoglobin for all treatment groups (data not shown).

**Discussion**

There are two popular hypotheses regarding what factors render an individual susceptible to an idiosyncratic adverse drug response. One of these is that genetic polymorphisms in drug-metabolizing enzymes result in rapid and slow metabolizers and thereby governs susceptibility to intoxication (Poolsup et al., 2000). There is insufficient evidence, however, to support polymorphisms in drug-metabolizing enzymes as a susceptibility factor in the majority of idiosyncratic responses. Drug allergy has also been invoked as a potential explanation for drug idiosyncrasy. However, evidence for allergy as a basis for all but a few idiosyncratic drug reactions is weak. Indeed, idiosyncratic responses sometimes occur with the onset of drug therapy (Worman et al., 1992) or may happen after months of successful maintenance therapy (Neuschwander-Tetri et al., 1998) (i.e., periods longer than typically required for allergic sensitization). Moreover, there have been cases in which maintenance drug therapy has been reinstituted without incident after an idiosyncratic reaction to the drug has subsided, rendering allergy as a cause of the reaction unlikely (Hollister, 1957; Werther and Korelitz, 1957). For only a few drugs (e.g., halothane) (Kenna et al., 1988; Pohl et al., 1991) have circulating antibodies to drug hapten been demonstrated, but whether these cause idiosyncratic responses remains controversial. Thus, causes of drug idiosyncrasy remain largely unknown.

Some reports of CPZ idiosyncrasy suggest an association with an underlying inflammatory state (Ishak and Irey, 1972), which may be a determinant of sensitivity to chemical toxicity (Roth et al., 1997). Accordingly, we hypothesized that clinical manifestations of human CPZ idiosyncrasy could be reproduced in experimental animals treated with this drug in the presence of concurrent inflammation.

Endotoxin activates pathways that lead to inflammatory events in the liver (Hewett and Roth, 1993). In the present study, a nonhepatotoxic dose of LPS was used to induce a modest inflammatory response. This dose leads to a mild, yet significant accumulation of inflammatory cells (Fig. 6) and the appearance in the plasma of cytokines such as tumor necrosis factor-α (TNF-α) (Barton et al., 2000) but no overt liver injury (Figs. 1 and 2). Exposure to this dose of LPS converted an otherwise nontoxic dose of CPZ into one that was hepatotoxic. This suggests that concurrent, modest inflammation renders rats sensitive to hepatotoxic effects of CPZ. This result provides an alternative hypothesis regarding factors that provoke CPZ-induced idiosyncratic responses.

Hepatic cholestasis is a common manifestation of drug-induced liver injury (Larrey and Erlinger, 1988). Human clinical findings of phenothiazine idiosyncratic hepatic injury generally include elevations of serum markers of cholestasis and modest increases in ALT and AST activities indicative of parenchymal cell damage. The results of the present study suggest that an underlying endotoxemia renders animals susceptible to hepatic cholestasis from CPZ. Exposure of
animals to LPS/CPZ resulted in a significant release into serum of ALP, GGT, and bilirubin. In humans with phenothiazine-associated jaundice, a wide range of values has been reported for ALP (Ishak and Irey, 1972; Moradpour et al., 1994) and bilirubin (Ishak and Irey, 1972). Bile acids were not significantly elevated in the present study. At first glance, this seems contrary to phenothiazine-induced cholestasis in humans. However, because this is an acute model there may not have been ample time for a disturbance in bile acid metabolism to result in a shift of the bile acid pool to the peripheral blood.

In the present study, an elevation in serum enzymes (ALT and AST) and histopathological findings indicative of hepatocellular necrosis occurred in LPS/CPZ-treated rats. Moderate elevation of ALT and AST is not an uncommon finding in the differential diagnosis of drug-induced jaundice (Zimmerman, 1968; Larrey and Erlinger, 1988), and it occurs in CPZ idiosyncrasy in people (Ishak and Irey, 1972). Indeed, there was an association between increased ALT and AST activities in serum and treatment-related hepatocellular necrosis observed morphologically.

Histological examination revealed mild to no parenchymal cell alterations in liver sections of animals treated with vehicle or LPS alone. The liver sections of animals treated with CPZ had infrequent subserosal necrotic foci that increased markedly in frequency and size with concurrent administration of LPS. In addition to subserosal lesions, animals treated with LPS/CPZ had interior midzonal lesions similar to those seen in the livers of animals treated with large, hepatotoxic doses of LPS (Hewett and Roth, 1993). Neutrophils were associated with both the midzonal and subserosal lesions. Although phenothiazine idiosyncrasy in people is associated with lesions of variable morphology (Ishak and Irey, 1972), the coagulative necrosis we observed was strikingly similar to lesions described by Ishak and Irey (1972) in patients who suffered from CPZ idiosyncrasy.

One of the most pronounced changes that characterize phenothiazine idiosyncrasy in humans is elevated serum CK

### TABLE 1
Lesions in livers of rats treated with LPS/CPZ
Liver sections from animals treated with LPS and CPZ were evaluated as described under Materials and Methods. See text for detailed description of lesions. n = 7 to 13.

<table>
<thead>
<tr>
<th>Lesion</th>
<th>SAL/SAL</th>
<th>SAL/CPZ</th>
<th>LPS/SAL</th>
<th>LPS/CPZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midzonal Necrosis</td>
<td>1.00 (1.00–1.00)</td>
<td>1.00 (1.00–1.38)</td>
<td>1.00 (1.00–1.50)</td>
<td>3.50 (1.38–4.25)*</td>
</tr>
<tr>
<td>Subserosal Necrosis</td>
<td>1.00 (1.00–1.00)</td>
<td>1.50 (1.00–2.13)</td>
<td>1.00 (1.00–1.00)</td>
<td>2.00 (1.50–3.63)*</td>
</tr>
</tbody>
</table>

*Significantly different from respective value in the absence of LPS (p < 0.05).

Significantly different from respective value in the absence of CPZ (p < 0.05).

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![Fig. 3. Dose dependence of LPS/CPZ hepatotoxicity. Animals received a nontoxic dose of LPS (7.4 × 10^6 EU/kg) followed 2 h later by treatment with a nontoxic dose of CPZ (10, 25, 50, or 70 mg/kg). They were killed 24 h after CPZ exposure, and serum activities of ALP (A), ALT (B), AST (C), and CK (D) were determined; n = 3. •, significantly different from 10 mg/kg CPZ cotreatment (p < 0.05).](image)
Fig. 4. Liver lesions in rats exposed to LPS and/or CPZ. Liver sections were stained with hematoxylin and eosin. Treatment with vehicle (A) or with LPS alone (B) yielded no obvious hepatocellular lesions. In livers of rats treated with CPZ alone (C), occasional subserosal lesions were evident but were much less frequent and pronounced than those in livers of rats given LPS/CPZ (D). The latter livers also contained numerous midzonal lesions in the section interiors. Images are from livers exhibiting the median score (Table 1) for the various treatments. S, subserosal lesion; M, interior, midzonal lesion.

Fig. 5. Neutrophil accumulation in livers of rats exposed to LPS/CPZ. Animals received either LPS (7.4 × 10⁶ EU/kg) or saline followed 2 h later by treatment with either CPZ (70 mg/kg) or saline. They were killed 24 h after CPZ exposure. n = 6 to 13. b, significantly different from respective value in the absence of LPS (p < 0.05).

Fig. 6. Serum creatine kinase activity in rats exposed to LPS/CPZ. Animals receiving either LPS (7.4 × 10⁶ EU/kg) or saline followed 2 h later by treatment with either CPZ (70 mg/kg) or saline. They were killed 24 h after CPZ exposure, and serum activity of CK was assayed. n = 6 to 13. a, significantly different from respective value in the absence of CPZ (p < 0.05); b, significantly different from respective value in the absence of LPS (p < 0.05).
activity. Indeed, serum CK activity in the thousands of units per liter has been reported frequently (Allsop and Twigley, 1987; Ebadi et al., 1990). In our study, neither LPS nor CPZ alone altered serum CK activity, but a large increase occurred in rats cotreated with these agents. Thus, LPS/CPZ coadministration in rats resembled human phenothiazine idiosyncrasy in this regard. In patients taking phenothiazines, increased serum CK sometimes occurs in association with NMS and rhabdomyolysis as manifestations of idiosyncrasy. However, pronounced elevations of CK have also occurred idiosyncratically in the absence of either of these conditions (Meltzer et al., 1996). In humans, the elevated serum CK activity has been associated primarily with the MM isoform (Meltzer et al., 1996). In the present study, animals did not meet criteria consistent with NMS (Ebadi et al., 1990) such as muscle rigidity, catatonia, or hyperthermia. Instead, the animals became sedate and hypothermic. Additionally, myoglobin was not detected by urinalysis, suggesting an absence of rhabdomyolysis. Because the source of serum CK was not known, we determined its isoforms by using agarose gel electrophoresis. This resulted in the identification of the CK-BB isoform. CK-BB occurs in brain and other tissues and is the predominant isoform in normal rat plasma (Jung et al., 1980), in contrast to its near absence in human plasma (Jung et al., 1979). Thus, like phenothiazine idiosyncrasy in people, LPS/CPZ-treated rats had a pronounced increase in serum CK activity; however, the source of the enzyme in rats and humans may be different.

In the present study, LPS was administered 2 h before CPZ to produce an underlying inflammation. This LPS dosing regimen allowed for the expression and release of TNF-α into the plasma and for neutrophil accumulation in liver before exposure to CPZ (Pearson et al., 1995; Barton et al., 1999, 2000). Although this cotreatment regimen caused liver injury, other ones may reduce it. For example, CPZ administered before and up to 30 min after a larger dose of LPS inhibited TNF-α synthesis and decreased lethality and hepatotoxicity (Gadina et al., 1991; Ghezzi et al., 1996; Jansen et al., 1998). Moreover, CPZ dampens neutrophil functions in vitro, including chemotaxis and superoxide generation (Bertini et al., 1991). Hence, the temporal relationship between administration of LPS and exposure to CPZ may be important in determining the toxic outcome.

The time to onset of an idiosyncratic event during maintenance drug therapy is unpredictable. Likewise, the occurrence of conditions (see above) that cause endotoxemia or exposure to other inflammagens also varies within and among people. Thus, the variable and unpredictable nature of drug idiosyncrasy is consistent with the variable nature of episodes of modest endotoxemia that people experience (Roth et al., 1997).

Although our studies in rats suggest the possibility that modest inflammation may play a precipitating role in certain drug idiosyncrasies, this has not been explored in people. Meltzer et al. (1996) observed that some patients with elevated plasma CK activity during antipsychotic drug treatment showed no recurrence after rechallenge with the drug, whereas others did. Moreover, some people experienced a normalization of values after elevated CK activity, despite continued drug treatment. This prompted Meltzer et al. (1996) to suggest that “state-dependent vulnerability factors or exogenous factors not yet identified may be of importance”. One of these factors might be endotoxin. We reviewed 15 published reports describing 110 cases of idiosyncratic reactions to phenothiazines. In 70% of these cases, patients had prodromal signs or conditions that occur during mild endotoxemia (i.e., diarrhea, abdominal discomfort, fever, and/or vomiting). Interestingly, more than 40 years ago the medical department of the manufacturer of CPZ described fever and abdominal distress (i.e., signs associated with endotoxemia) as characteristic of events preceding idiosyncratic jaundice from this drug (Loftus et al., 1955). Although it is impossible to assign cause and effect from an analysis of case reports, these observations are consistent with the idea that underlying inflammation might precipitate certain idiosyncratic reactions. Further study will be required to establish a relationship between concurrent inflammation and idiosyncratic responses in people.

Molecular and biochemical mechanisms responsible for the ability of LPS to render rats sensitive to CPZ toxicity were not explored in this study. The capacity of LPS to activate inflammatory cells to release proinflammatory mediators is likely to be involved. Previous studies of the interaction of LPS with other xenobiotic agents have uncovered neutrophils, TNF-α, and cyclooxygenase products as critical mediators of liver injury (Barton et al., 1999, 2000; Ganey et al., 2001). These mediators precipitate secondary events such as cellular generation of reactive oxygen species and release from cells of toxic proteases that might cause overt injury to a parenchymal cell homeostatically altered by CPZ exposure. Interestingly, inflammatory mediators that are critical to enhancement of toxicity by LPS exposure differ for different xenobiotic agents (Barton et al., 2000; Ganey et al., 2001). Inflammatory cytokines produced during LPS exposure also influence the expression of xenobiotic-metabolizing enzymes in the liver (Yoshida et al., 1982). Accordingly, it is possible that LPS affects the metabolism of CPZ, and that this contributes to toxicity. Additional exploration is needed to uncover mechanisms by which LPS interacts with CPZ.

In conclusion, this study demonstrated that characteristics of human phenothiazine idiosyncrasy could be reproduced in rats cotreated with CPZ and a small dose of LPS that causes a modest inflammatory response. This result raises the possibility that modest, concurrent inflammation may be a critical factor in precipitating idiosyncratic responses to some drugs. If this proves upon further study to be true, it may provide a basis for creation of animal models to predict drug idiosyncrasy in humans and for studying underlying mechanisms.

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References


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