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DEVELOPMENT OF AN ANIMAL MODEL FOR DRUG-INDUCED IDIOSYNCRATIC REACTIONS

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DEVELOPMENT OF AN ANIMAL MODEL FOR DRUG-INDUCED IDIOSYNCRATIC REACTIONS

By

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ABSTRACT

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Current hypotheses of individual susceptibility to idiosyncratic reactions include drug metabolism polymorphism and drug allergy. Although some types of idiosyncratic responses can be explained by these hypotheses, evidence is usually poor and cannot be applied to all responses. Chlorpromazine (CPZ) is an antipsychotic drug well documented for idiosyncratic reactions, such as hepatic cholestasis and the neuroleptic malignant syndrome. The clinical manifestations of these responses appear to have an associated inflammatory component. Accordingly, we tested the hypothesis that lipopolysaccharide (LPS), a potent inflammagen, could predispose animals to susceptibility to reactions resembling CPZ idiosyncrasy. In the rat, LPS/CPZ treatment resulted in changes in serum activities for diagnostic endpoints consistent with hepatic cholestasis and necrosis, as well as myonecrosis. Our findings suggest that underlying inflammation may provide an alternative explanation for some idiosyncratic events reported for humans.

This thesis is dedicated to the memory of Jean M. Hornung.

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LIST OF ABBREVIATIONS

ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANA	Antinuclear antibodies
ANOVA	Analysis of variance
ARDS	Acute respiratory distress syndrome
ARF	Acute renal failure
AST	Aspartate aminotransferase
BUN	Blood urea nitrogen
CK	Creatine Kinase
CPZ	Chlorpromazine
CYP	Cytochrome P450
DIC	Disseminated intravascular coagulation
EPS	Extrapyramidal side effects
Fc	Fragment crystallizable
GGT	gamma-glutamyltransferase
IL-1	Interleukin-1
LBP	Lipopolysaccharide binding protein
LPS	Lipopolysaccharide
LSD	Least significant difference
MAPK	Mitogen activated protein kinase
MH	Malignant hyperthermia
MOPSO	[3-(n-morpholino)-2-hydroxypropanesulfonic acid]
NAT	N-acetyl transferase
NF-κB	Nuclear factor kappa B
NK	Natural killer cells
NMS	Neuroleptic malignant syndrome
PMN	Polymorphonuclear leukocyte
SAL	Saline
SEM	Standard error of the mean
SLE	Systemic lupus erythematosis
TFA	Trifluoroacetyl
TLR	Toll-like receptor
TNF-α	Tumor necrosis factor alpha

CHAPTER 1

GENERAL INTRODUCTION

The tragedy of adverse drug reactions gained worldwide attention in the early 1960's with reports of phocomelia associated with the maternal use of thalidomide for the treatment of morning sickness. As a result, thalidomide was promptly removed from the market to prevent further cases. Forty years later, idiosyncratic drug reactions leading to increases in patient mortality continue to result in the removal of drugs from the market. Recently, Duract® (bromfenac sodium), a nonsteroidal anti-inflammatory analgesic indicated for the short term management of acute pain, and Rezulin® (troglitazone), an anti-hyperglycemic agent indicated for the treatment of type II diabetes, joined the list of drugs voluntarily withdrawn from the market due to idiosyncratic liver toxicity and associated mortality.

Idiosyncratic drug reactions pose a problem to patients, clinicians and the pharmaceutical industry. These reactions are unpredictable and sometimes fatal. Because idiosyncratic reactions are not predicted by most clinical trials or current animal testing in the pharmaceutical industry, millions of dollars are spent developing and marketing new drugs without the full extent of the problem being realized until after the drug has been on the market for a period of time. Hence, predictive animal models are sorely needed to help elucidate the pathogenesis of these reactions.

The purpose of this introduction is to provide a general background on idiosyncratic reactions and susceptibility and to illustrate drug idiosyncrasy with the antipsychotic drug, chlorpromazine, as an example. In addition, inflammation will be discussed as a component and possible determinant of susceptibility to drug idiosyncrasy seen in humans. Accordingly, drug idiosyncrasy as it relates to other types and classes of adverse drug reactions will be addressed in Section I. In addition, current hypotheses

surrounding drug idiosyncrasy will be highlighted. In Section II, the therapeutic agent chlorpromazine will be used as an example of a drug that exhibits multiple idiosyncratic reactions in people. Lastly, Section III will provide a discussion of inflammation, especially that resulting from lipopolysaccharide, as a determinant of susceptibility to chemical toxicity, and a hypothesis for its potential involvement in idiosyncratic events.

I. Adverse drug reactions

Adverse drug reactions have been divided into two classes: type A and type B. Type A reactions occur as a result of exaggerated therapeutic effects, diverse pharmacologic and toxicologic actions, or known chemical properties of a drug. A large fraction of adverse drug reactions are type A. These are usually predictable and dose-dependent. Type B reactions are aberrant effects that are not expected from the known, dose-related pharmacological or toxicological actions of a drug. These reactions occur in a small fraction of the population, and are unpredictable and seemingly unrelated to the timecourse of drug administration or dose. Because of their aberrant nature, and the lack of animal models with which to predict idiosyncratic responses, an emphasis will be placed on the discussion of type B reactions and current hypotheses regarding their pathogenesis.

I. A. Type A

I. A. 1. Mechanism of action

Type A reactions result from increased tissue concentrations of a drug or its toxic metabolites. Drug delivery and pharmacokinetics are important variables contributing to

these reactions. Routes of drug administration as well as the vehicles utilized to control its release are factors that can affect the quantity of drug delivered. Once in the body, individual variation in gender, age, body weight, genetic makeup, disease and environmental influences may affect absorption, distribution, metabolism and the elimination of a drug.

I. A. 2. Classification by mechanism

Type A reactions can be separated into three distinct classes by mechanism. These include (1) pharmacological responses, (2) drug intolerance and (3) drug interaction.

A type A adverse pharmacological event is an inherent effect of the drug that is directly related to dose. These events include drug overdose and unavoidable side effects. Drug overdose can occur as a result of improper drug use, or noncompliance. However, drug overdose can also occur as a consequence of a pharmacokinetic imbalance whereby tissue concentrations of a drug or its metabolites accumulate during typical dosing schemes. Side effects of drug use, on the other hand, are predictable but sometimes unavoidable. For example, symptoms such as anxiety, loss of sleep and increased restlessness are common side effects that typically accompany the use of the decongestant, pseudoephedrine.

Intolerance happens when a pharmacologic effect is produced in an individual by an unusually small dose, such that the usual dose tends to induce an overreaction. For example, flurazepam is a benzodiazepine derivative indicated for insomnia or general difficulty in falling asleep. Because of drug intolerance in some people, therapeutic doses

of flurazepam have resulted in excessive CNS depression (Roth and Roehrs, 1991). Drug intolerance can be remedied by adjusting drug dosage.

Unlike drug overdose and drug intolerance, drug interaction is a pharmacological response that is caused by two or more drugs. An example of drug interaction is the pharmacokinetic interactions of macrolide antibacterials and azole antifungal agents with cisapride. Cisapride is indicated for the treatment of a number of gastrointestinal disorders, particularly gastro-esophageal reflux disease in adults and children. Elimination of cisapride is dependent on its metabolism by cytochrome P450 (CYP) 3A4. The macrolide antibacterials clarithromycin, erythromycin and troleandomycin are inhibitors of CYP3A4. Similarly, azole antifungal agents such as fluconazole, itraconazole and ketoconazole are CYP3A4 inhibitors. The concomitant use of these agents with cisapride increases its plasma concentration (Michalets and Williams, 2000). The result of this abnormal accumulation of cisapride is an adverse cardiac event known as torsade de pointes arrhythmia (Piquette, 1999).

I. B. Type B (idiosyncratic)

I. B. 1. Drug idiosyncrasy

Type B reactions are idiosyncratic drug reactions. As previously mentioned, idiosyncratic reactions are uncharacteristic responses of a patient to a drug which do not normally occur with its administration. These responses are clinically manifested in a small percentage (<5%) of the population, are not predicted based on the timecourse of administration and are not apparently dose-related.

I. B. 2. Types of idiosyncratic reactions

Drug idiosyncrasy affects many organ systems including cells of the blood, skin, liver and muscle. Immune hypersensitivity has been attributed to the pathogenesis of all of these responses. The immune system is involved in idiosyncratic skin reactions (e.g. toxic epidermal necrolysis and erythematosis multiforme) (Friedmann et al., 1994), drug-induced lupus (Price and Venables, 1995) and many blood dyscrasias such as hemolytic anemia and agranulocytosis. Evidence for hypersensitivity in idiosyncratic liver disease and other idiosyncratic reactions, however, is circumstantial. As a result, alternative hypotheses have arisen regarding these responses.

I. B. 3. Hypotheses about pathogenesis

I. B. 3. i. Immune hypersensitivity

Immune hypersensitivity is an allergic phenomenon caused by an altered reactiveness of a patient to a drug. Immune hypersensitivity reactions are divided into four classes, designated types I-IV; type I, or anaphylactic reactions, are mediated by IgE antibodies and mast cells; type II, or cytolytic reactions, occur when IgM or IgG antibodies bind to antigen on the surface of cells and activate the complement cascade; type III, or immune complex reactions, are caused by an accummulation of IgM or IgG antibodies in the circulation or in tissue and activation of the complement cascade; type IV, or delayed type hypersensitivity, is mediated by T-cells and macrophages. Drug allergy is characterized by a type II immune hypersensitivity.

Many idiosyncratic reactions include an inflammatory component and occur after patients have been on maintenance therapy with a drug for a period of time, suggesting

that a period of allergic sensitization may be required (Zimmerman, 1981). 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 (Werther and Korelitz, 1957; Hollister, 1957; Shay and Siplet, 1958). For only a few drugs (e.g., halothane) (Kenna et al., 1988; Pohl et al., 1991) have circulating antibodies to drug haptens been demonstrated.

The hapten hypothesis of immune hypersensitivity is as follows: Drugs are bioactivated to reactive metabolites that form drug-protein adducts (haptens) in the liver. The hapten is internalized and processed by Kupffer cells, which then present antigens in association with major histocompatibility complex II to naïve T cells. These hapten-specific T cells then undergo clonal expansion and circulate throughout the body. Local memory T helper cells are reactivated when the liver forms the hapten again upon reexposure to the drug, and they release cytokines (primarily IL-2), thereby eliciting a reaction by direct activation of cytotoxic T cells, natural killer cells (NK) and macrophages. Cytotoxic T cells target antigen-bearing major histocompatability complex class I cells (e.g., hepatocytes) for lysis by tumor necrosis factor-β (lymphotoxin). NK cells and macrophages have Fc (fragment crystallizable) receptors that recognize the Fc portion of IgG on IgG-coated target cells. NK cells, like cytotoxic T cells, can lyse target

cells through the release of cytolytic proteases. Macrophages, on the other hand, phagocytose targeted cells. In addition, B cells produce the antibodies against the hapten-carrying liver cells. Taken together, this gives rise to the clinical manifestations of hepatotoxicity (Furst et al., 1997).

Patients experiencing fulminant halothane hepatitis frequently exhibit rash, arthralgia, eosinophilia, and a variety of autoantibodies, which are characteristic of an immune-mediated drug reaction (Eliasson and Kenna, 1996). Halothane is metabolized in the liver by oxidative and reductive cytochrome P-450-mediated pathways. The reactive metabolite of halothane, trifluoroacetylchloride, covalently binds to hepatocellular proteins and lipids to form trifluoroacetyl (TFA) adducts (Gut, 1998). At least eight distinct trifluoroacetyl adducts have been identified (Eliasson and Kenna, 1996). Sera from patients with severe halothane hepatitis have been shown to contain IgG antibodies that react with these adducts (Kenna et al., 1988). These antibodies are not detectable in the sera from individuals exposed to halothane but who did not sustain liver damage (Kenna et al., 1984). Furthermore, these antibodies have been shown to mediate lymphocyte-dependent killing *in vitro* of hepatocytes bearing drug haptens (Vergani et al., 1980).

Animal models have been developed in the rat, rabbit and guinea pig to study halothane hepatitis. In rats, mild liver necrosis can be produced with phenobarbital pretreatment and hypoxia. These conditions provoke generation of toxic species through a metabolic reductive pathway (McLain et al., 1979). However, these conditions are quite stringent and less likely to be factors in halothane idiosyncrasy. The guinea pig model, on the other hand, offers many similarities to that of human halothane hepatitis. For

example, the guinea pig develops pathologic lesions of the liver closely resembling those seen in human halothane hepatitis when exposed to halothane in the same manner as humans in a clinical situation (Lind et al., 1989). TFA-protein adducts have been identified in guinea pig Kupffer cells, suggesting a role for Kupffer cells as resident antigen presenting cells of the liver (Furst et al., 1997). Additionally, the guinea pig develops an immune response characterized by the formation of antibodies that recognize TFA-protein adducts (Siadat-Pajouh et al., 1987). However, an increased titre of circulating anti-TFA antibody does not correlate with halothane hepatitis following halothane exposure (Hastings et al., 1995). Hence, the role of drug haptens and antibodies produced against them in the pathogenesis of immune hypersensitivity-associated hepatotoxic outcomes remains controversial.

I. B. 3. ii. Genetic Predisposition

Genetic predispositions leading to the polymorphic expression of metabolic enzymes has been hypothesized to either decrease metabolism or increase drug bioactivation, resulting in an accumulation of the drug or bioactive products. These bioactive products are either inherently cytotoxic (Figure 1.1) or contribute to hapten formation (Figure 1.2). Accordingly, genetic polymorphisms in drug metabolizing enzymes (Poolsup et al., 2000) result in rapid and slow metabolizers and thereby may govern susceptibility to intoxication. For example, polymorphisms in N-acetyl transferase (NAT) have been associated with idiosyncratic isoniazid hepatoxicity (Grant et al., 1997). The major pathway of isoniazid metabolism is acetylation. Individuals are classified as rapid or slow acetylators according to the rate at which isoniazid is acetylated by liver

Figure 1.1. Direct cytotoxicity of drugs and their bioactive metabolic products due to polymorphic expression (E*) of metabolic enzymes (E). Differences in isoform expression (E, E*) may result in nontoxic or toxic metabolites, thereby governing susceptibility to toxic responses. Images in this thesis are presented in color.

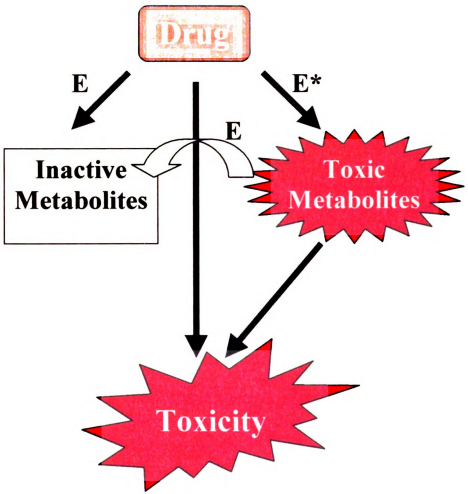
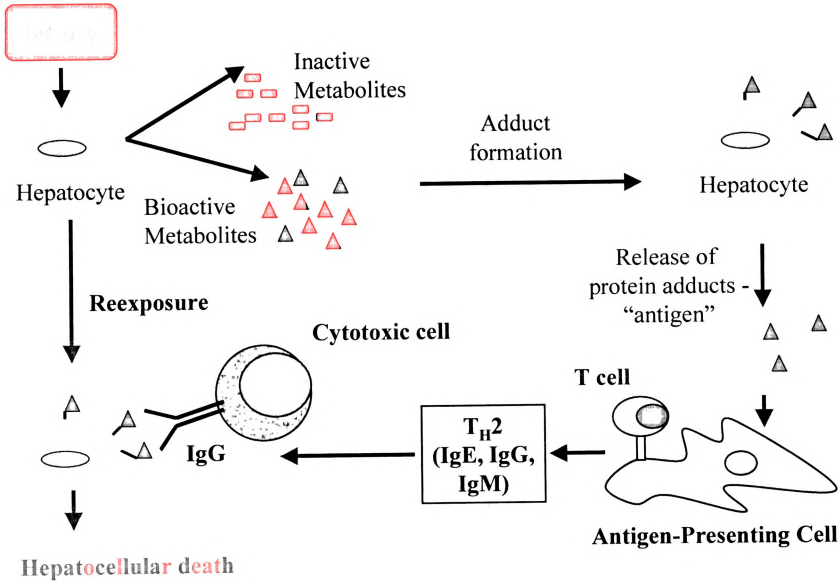

Drug
E
E*
E
Inactive Metabolites
Toxic Metabolites
Toxicity

Figure 1.2. Hapten hypothesis of drug hypersensitivity. Drugs are bioactivated to reactive metabolites that form drug-protein adducts (haptens) in the liver. The hapten is internalized and processed by Kupffer cells that then present antigens in association with major histocompatibility complex II to naïve T cells. These hapten-specific T cells then undergo clonal expansion and circulate throughout the body. Local memory T helper cells are reactivated when the liver forms the hapten again upon reexposure to the drug and release cytokines, thereby eliciting the reaction by direct activation of cytotoxic T cells, natural killer cells and macrophages.


Inactive
Metabolites
Hepatocyte
Bioactive
Metabolites
Adduct
formation
Hepatocyte
Release of
protein adducts -
"antigen"
Reexposure
Cytotoxic cell
T cell
IgG
T_H2
(IgE, IgG,
IgM)
Antigen-Presenting Cell
Hepatocellular death

NAT. Early studies of isoniazid hepatoxicity revealed that patients with the rapid acetylator phenotype hydrolyzed more isoniazid to isonicotinic acid and free hydrazine moiety than slow acetylators (Mitchell et al., 1976). The hydrazine moiety liberated from isoniazid is primarily acetylhydrazine, and studies in animals showed that this metabolite was converted to a potent acylating agent that produced liver necrosis. However, later studies of single-drug regimens of isoniazid have shown that neither slow nor rapid acetylation had any causal influence on isoniazid-induced hepatitis (Gronhagen-Riska et al., 1978; Gurumurthy et al., 1984). Thus, polymorphic expression of metabolic enzymes, including NAT, remains controversial.

Such genetic variability accounts for marked differences in the metabolism of a broad spectrum of drugs and may explain some adverse responses (e.g., drug interactions and drug intolerance). There is insufficient evidence, however, to support polymorphisms in drug metabolizing enzymes as a susceptibility factor in the vast majority of idiosyncratic responses.

I. B. 3. iii. "Two-hit" model

Because hypotheses surrounding drug idiosyncrasy such as drug allergy and metabolic polymorphisms have not been fully supported in the existing literature, Zimmerman (1997) proposed that overt hepatic injury was likely a result of "two hits." The two "hits" may be a combination of an inherent property of the offending drug leading to direct cytotoxicity and the development of a host immune hypersensitivity, as suggested by Zimmerman. Alternatively, these hits might present as a direct effect of the drug in combination with one or more factors that enhance susceptibility to a toxicant, such as underlying disease or inflammatory states.

II. Chlorpromazine

Many drugs have been reported to result in idiosyncratic responses. One such class of drugs is the phenothiazines. CPZ is an aliphatic phenothiazine (Figure 1.3) used primarily in the treatment of psychotic disorders. Its use has been associated with a variety of idiosyncratic responses including agranulocytosis, drug-induced lupus, cholestasis, neuroleptic malignant syndrome and rhabdomyolysis. Because CPZ has been available for clinical use for nearly half a century and is currently available through commercial sources for experimental use, the literature is rich with information regarding its involvement in such reactions. Hence, CPZ is an appropriate "model" agent for drug idiosyncrasy.

II. A. Pharmacologic Actions

CPZ is prescribed clinically for the management of psychotic disorders, nausea and vomiting. CPZ is an aliphatic phenothiazine. Phenothiazines are thought to elicit their antipsychotic and antiemetic effects via dopamine receptor antagonism in the mesolimbic and medullary chemoreceptor trigger zone areas of the brain, respectively.

II. B. Dose-Related Side Effects

Extrapyramidal side effects (EPS's) are involuntary movement disorders that accompany the use of "typical" antipsychotics. EPS's are dose-related, and a significant reduction in risk can be achieved by initiating and maintaining antipsychotic doses in the

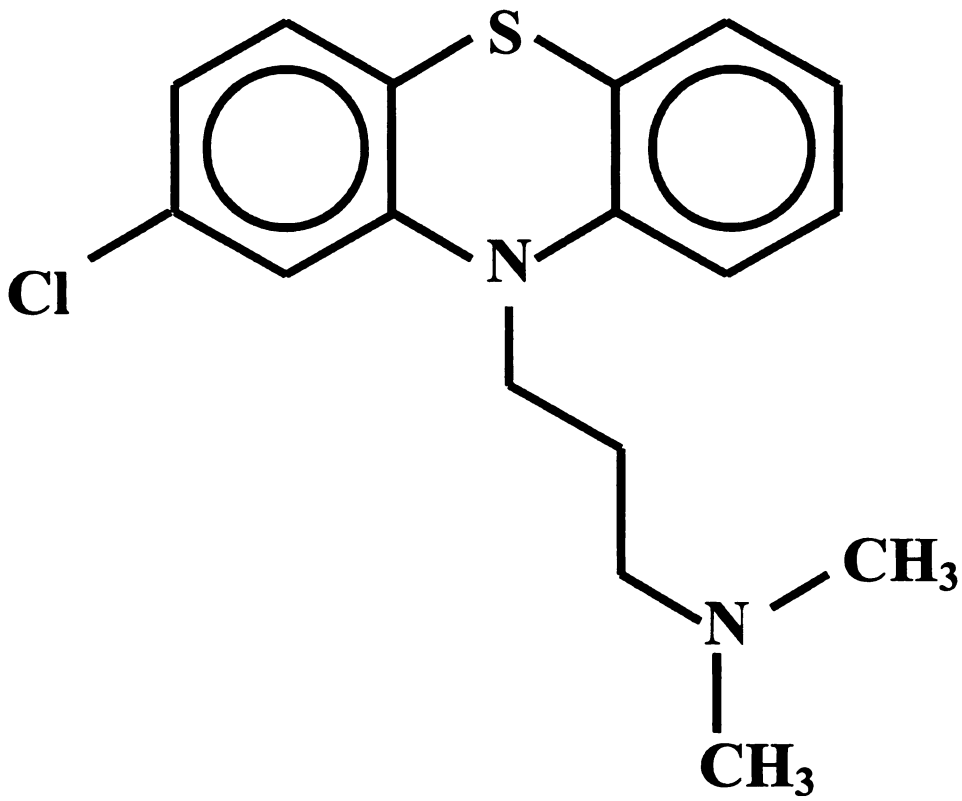


Figure 1.3. Structure of Chlorpromazine (10-[3-dimethylaminopropyl]-2-chlorphenothiazine).

low therapeutic range (Fleischhacker et al., 1990; Tonda and Guthrie, 1994). CPZ is a "typical" antipsychotic drug that produces a broad range of EPS's. These include akathisia (an uncomfortable sense that one must keep moving), acute dystonia (an exaggerated posturing of head, neck or jaw), Parkinsonism (postural instability, stooped posture, shuffling, festinating gate, rigidity, tremor, and akinesia), and tardive dyskinesia (irreversible, involuntary movements frequently involving the facial, buccal, and masticatory muscles) (Ebadi et al., 1990).

The dopaminergic-cholinergic balance hypothesis is the foremost theory of antipsychotic-induced EPS's. An equilibrium of dopaminergic (inhibitory) and cholinergic (excitatory) neuronal activity in the corpus striatum is required for normal motor functioning. The interneuronal dopaminergic blocking action of antipsychotics leads to an absolute decrease of dopamine. It is this relative increase in interneuronal acetylcholine that results in signs and symptoms of EPS's (Marsden and Jenner, 1980).

The serious nature of extrapyramidal side effects seen with "typical" antipsychotics led to the development of "atypical" agents with less frequent EPS's. It has been suggested that this improved profile results from more potent $5HT_{2a}$ receptor antagonism and from weaker dopamine D_2 receptor antagonism (Schotte et al., 1996).

II. C. Idiosyncratic Reactions

In addition to dose-related EPS's, the non-dose-related idiosyncratic (type B) reactions are a confounding factor in CPZ therapy. Idiosyncratic reactions to chlorpromazine include agranulocytosis, drug-induced lupus, hepatic cholestasis, neuroleptic malignant syndrome, and rhabdomyolysis. These reactions are unpredictable

and occur in a small percentage (<5%) of the population using this agent. The following summaries will address the different types of reactions, the estimated incidence, the clinical presentation, and proposed pathogenesis.

II. C. 1. Agranulocytosis

Agranulocytosis is an idiosyncratic event resulting in a marked decrease in circulating granular white blood cells. The incidence of agranulocytosis during treatment with phenothiazines has been estimated at 1/250,000 with short term treatment (Litvak and Kaelbling, 1971). Agranulocytosis is thought to occur by one of two mechanisms. Immune-mediated agranulocytosis is produced by "immunogenic" drugs, and characterized by the early appearance of antibodies to polymorphonuclear neutrophils (PMNs). Agranulocytosis due to direct action of the drug is characterized by morphologic aplasia of marrow and is more likely to occur if the affected host has a concomitant defect in marrow cellular proliferation (Pisciotta, 1990). In vitro studies utilizing human bone marrow cells from CPZ-sensitive individuals showed a distinct suppression of the proliferative response (Pisciotta, 1990). Furthermore, marrow smears from CPZ-sensitive individuals showed distinguishable differences in cell-cycle events as compared to smears obtained from non-sensitive patients (Pisciotta, 1990). These studies lend support to the latter mechanism of action for chlorpromazine-induced agranulocytosis (Kendra et al., 1993).

II. C. 2. Drug-induced lupus

Systemic lupus erythematosis (SLE) is an autoimmune disease characterized by an array of autoantibodies, especially antinuclear antibodies (ANA). Several drugs, including procainamide, hydralazine, CPZ and isoniazid can produce a syndrome resembling SLE, (Price and Venables, 1995). The prevalence of autoantibodies in neuroleptic-treated patients has been reported to be 70 percent (Canoso et al., 1990). Between 10 and 20 percent of ANA-positive individuals develop lupus-like symptoms. Indeed, antinuclear antibodies have been detected in patients taking CPZ (Quismorio et al., 1975; Alarcon-Segovia et al., 1973). However, up to one-half of all patients taking CPZ develop ANAs, but clinical lupus develops in less than 1% (Steen and Ramsey-Goldman, 1988). Patients with drug-induced lupus present with fewer concurrent clinical features than those with idiopathic SLE, and there is a notable paucity of more severe manifestations such as renal or neuropsychiatric disease (Price and Venables, 1995). Otherwise, it is impossible to distinguish drug-induced lupus from idiopathic lupus, except for the association with drugs known to cause lupus and resolution of the syndrome when that drug is discontinued (Uetrecht and Woosley, 1981).

II. C. 3. Hepatic Cholestasis

Intrahepatic cholestasis is the arrest in bile flow resulting from hepatocanalicular or hepatocellular lesions or bile duct injury (Zimmerman, Lewis, 1987). Hepatocanalicular lesions are associated with a portal inflammatory infiltrate. Phenothiazine-induced jaundice is classified as a form of cholestatic hepatocanalicular hepatotoxicity (Regal et al., 1987). The estimated prevalence of jaundice with

chlorpromazine is 1-2%. The onset of jaundice usually occurs during the first one to four weeks of therapy (Regal et al., 1987).

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 alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities indicative of parenchymal cell damage. In many of these, histopathologic findings from liver biopsies consist of modest hepatocellular injury with a portal inflammatory infiltrate that includes lymphoid cells, PMNs, and eosinophils (Gold et al., 1955; Hollister, 1957; Gebhart et al., 1958; Ishak and Irey, 1972). Eosinophils are seen in 25-50% of these cases. Cholangiolitis, or obstruction of the bile ductules, occurs in approximately 25% of these cases (Larrey and Erlinger, 1988). Other reports describe lesions with different zonal distributions and inflammatory characteristics. For example, Ishak and Irey (1972) and Read and coworkers (1961) described biopsy specimens as having hepatocytes "drop out" of a necrotic focus that was infiltrated with PMNs. These biopsies were performed at two weeks and three months after the onset of CPZ-associated jaundice, respectively. This suggests that the type of lesion observed may be unrelated to the time the biopsy was taken during the course of hepatic idiosyncrasy.

Current models for CPZ-induced hepatic cholestasis focus on the direct interaction of CPZ or its metabolites with hepatic cells and their ability to secrete bile acids. CPZ is an amphiphilic cationic detergent that dissolves readily into the lipid bilayer of cell membranes (Boyer, 1978). Ultrastructural data in the isolated, perfused rat liver suggest that CPZ (5.0×10^{-4}M) is directly cytotoxic, exerting its cholestatic effects by

rapidly disturbing the membrane architecture of the sinusoidal surface and interacting with the bile secretory apparatus (Hruban et al., 1978). However, CPZ, when administered at therapeutic doses, is not likely to reach plasma concentrations greater than 3.0×10^{-7}M.

Other models have been designed to verify Zimmerman's "two-hit" hypothesis of direct cytotoxicity combined with the hapten hypothesis of immune hypersensitivity. Mullock et al. (1983) tested the hypothesis that an immune response to CPZ would exacerbate the hepatic injury to CPZ. Hepatic injury occurred in a reproducible fashion in animals treated with CPZ alone; however, there was no correlation between the titre of antibodies in individual animals and the degree of liver damage observed. This suggests that factors other than allergic hypersensitivity may increase susceptibility to overt liver injury from CPZ.

II. C. 4. Neuroleptic Malignant Syndrome

Neuroleptic malignant syndrome (NMS) is a serious adverse side effect associated with typical and atypical antipsychotics. The onset of NMS is not specific to any age group, and there does not appear to be a dose-response relationship (Caroff, 1980). Its estimated prevalence ranges between 0.04% to 0.4% of patients treated with neuroleptics (Addonizio et al., 1987).

Four diagnostic criteria predominate the typical presentation of NMS: hyperthermia, muscular rigidity, autonomic dysfunction (hypertension, tachycardia, tachypnea, and diaphoresis) and an altered conscious state (delirium, mutism, stupor, or coma) (Balzan, 1998; Levenson, 1985). Abnormal laboratory findings such as increases

in creatine kinase (CK) and lactate dehydrogenase as well as leukocytosis may be part of the clinical picture. In addition, thrombocytopenia (Ray, 1997), decreased serum iron concentrations (Lee, 1998; Rosebush, Mazurek, 1991), abnormal liver aminotransferase activities (Cao and Katz, 1999), and electrolyte imbalance (Elizalde-Sciavolino et al., 1998; Park et al., 1987) have been described.

A number of risk factors are associated with NMS. These factors include catatonia (White and Robins, 1991), Parkinson's disease (Ueda et al., 1999), abrupt neuroleptic withdrawal (Amore and Zazzeri, 1995), heat stress (Sterner, 1990), large dosage or rapid neuroleptic dose titration (Gelenberg et al., 1988) and multiple neuroleptic or concurrent lithium therapy (Joseph and Thomas, 1991).

The pathogenesis of NMS is poorly understood. However, current hypotheses focus on hyperthermia as an important event in the development of NMS. For example, Tanii and coworkers (1996) developed a rabbit model based on heat stress, the neuroleptic haloperidol, and atropine sulfate. Administration of haloperidol (1 mg/kg) and atropine (0.4 mg/kg), and exposure to high ambient temperature (35 ^{o}C) resulted in hyperthermia, muscular rigidity and elevated serum CK. This suggested that these parameters could produce an NMS-like condition. However, this condition did not arise as a result of haloperidol administration alone. Atropine sulfate, a muscarinic receptor antagonist, competes with acetylcholine for receptor binding. Physostigmine, an inhibitor of acetylcholinesterase, increases acetylcholine levels thereby reversing the effects of atropine. Physostigmine completely suppressed elevations in body temperature and CK, suggesting that the anticholinergic activity of atropine sulfate was responsible for the potentiation of these responses; not haloperidol.

Pharmacogenetic disorders in muscle such as mutations in the ryanodine receptor, which mediates calcium release during excitation-contraction, result in malignant hyperthermia (MH) from anesthetic triggering agents (McCarthy et al., 2000) such as halothane. It has been hypothesized that the neuroleptic malignant syndrome (NMS), like MH, results from preexisting defects in skeletal muscle metabolism that are unmasked or provoked by neuroleptic exposure (Keck et al., 1995). However, current clinical (Miyatake et al., 1996), as well as animal (Keck et al., 1990) studies do not support this hypothesis.

II. C. 5. Rhabdomyolysis

Rhabdomyolysis is a breakdown in skeletal muscle resulting from disease, trauma or toxic insult. Injury which damages the integrity of the sarcolemma of skeletal muscle can result in the release of potentially toxic components from muscle into the circulation (Knochel, 1993). Chlorpromazine-induced rhabdomyolysis has been reported to occur in patients receiving intramuscular injections (O'Connor, 1980) of the drug and in patients experiencing heat stress (Ellis, 1977) or increased physical exertion (Koizumi et al., 1996). The incidence of chlorpromazine-induced rhabdomyolysis has not been examined; however, there are relatively few reported cases in the literature.

Diagnostic indicators for rhabdomyolysis include elevations in serum CK (at least five times normal values), myoglobinuria, electrolyte imbalance (hyperkalemia, hypocalcemia, and hyerphosphatemia), and release of other muscle enzymes such as lactate dehydrogenase, aldolase, and aminotransferases (Poels and Gabreels, 1993). Complications that may arise from rhabdomyolysis include myoglobinuric renal failure

(Allsop and Twigley, 1987b), hyperkalemia and cardiac arrest (Chan-Tack, 1999) and disseminated intravascular coagulation (DIC) (Taniguchi et al., 1997). Histologic analysis of muscle biopsies reveals necrosis of scattered but numerous muscle fibers, with varying degrees of phagocytosis and regeneration (Ghatak et al., 1973). Subnecrotic changes include Z disc streaming (extension of discs in an irregular line across the long axis of the muscle) and mild inflammation.

III. Inflammation

Idiosyncratic responses to chlorpromazine, such as hepatic cholestasis, NMS and rhabdomyolysis, have an apparent association with inflammation. For example, in cases of hepatic cholestasis, liver histopathology provides evidence for the association of inflammatory cells with various lesions. In addition, patient prodromes (discussed in Chapter 2) suggest an underlying endotoxemia (discussed later in this section). Likewise, risk factors predisposing individuals to NMS and/or rhabdomyolysis include factors known to induce endotoxemia. Because inflammation may play a critical role as a susceptibility factor to these reactions, a discussion of its pathology and LPS as an etiologic agent is presented.

III. A. Acute inflammation

The cardinal features of inflammation were classically defined by the Roman writer Cornelius Celsus as "*rubor et tumor cum calore et dolore*" (redness and swelling with heat and pain). These features result from the cellular and biochemical processes involved; vasodilation and increased blood flow accounts for *rubor*; *calore* is associated

with increased blood flow; *tumor* results from fluid accumulation. Hence, acute inflammation is an early response to injury or infection characterized by dilation and leaking of vessels (Mitchell and Cotran, 1997). These events lead to the recruitment, infiltration and activation of inflammatory cells such as neutrophils.

III. A. 1. Vascular changes

One of the earliest events in inflammation is local vasodilation and increased blood flow. The result of these changes is a penetrable, or leaky, endothelium that allows increased exudate accumulation in the interstitium. Vascular leak may occur as a result of (1) endothelial cell contraction through the release of vasoactive amines such as histamine and serotonin, (2) junctional retraction caused by cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1), (3) direct endothelial cell injury or (4) leukocyte-dependent endothelial injury caused by the release of proteases, reactive oxygen species, and other chemical mediators from leukocytes (Mitchell and Cotran, 1997).

III. A. 2. Leukocytic events

Neutrophils migrate to the site of injury or infection to help clear invading pathogens and degrade damaged or necrotic tissue. With increased vascular permeability and slowing of blood flow, leukocytes marginate to the vessel walls where they roll across activated endothelium. The leukocytes form loose and transient adhesions with the endothelium through adhesion molecules. Once a leukocyte adheres to the endothelium it can begin to migrate between endothelial cells (diapedesis) to the extravascular space.

Leukocytes then travel along a chemical gradient (chemotaxis) to the site of injury, where activated leukocytes can phagocytize cellular debris and release proteolytic enzymes via degranulation. Neutrophils have the potential to prolong inflammation and contribute to tissue damage through the uncontrolled release of proteases, reactive oxygen species and other chemical mediators (Mitchell and Cotran, 1997).

III. A. 3. Soluble mediators

Vascular changes and leukocytic events do not occur independently. Chemical mediators, derived from plasma and/or locally inflamed tissue, may contribute to many of the events seen in inflammation. For example, the cytokines TNF-α and Il-1 are produced by macrophages and monocytes during inflammation. *In vivo* and *ex vivo* studies show that TNF-pretreated neutrophils have depressed migratory responses, suggesting that TNF-α may modulate neutrophil chemotaxis (Schlieffenbaum et al, 1998). TNF-α has also been shown to prime and activate neutophils for superoxide release (Jersmann et al, 1998). Il-1 has effects similar to those of TNF-α, with the exception of neutrophil chemotactic activity. Once neutrophils become activated, they play an important role in host defense through the generation of superoxide and release of proteolytic enzymes. Other chemical mediators involved in vascular and leukocytic events include complement, leukotrienes, prostaglandins, and platelet-activating factor (Mitchell and Cotran, 1997).

III. B. LPS: an etiologic agent for inflammation

III. B. 1. Structure

LPS is a major component of the cell wall of gram-negative bacteria (Raetz et al., 1988). LPS, along with other bacterial macromolecules, is termed "endotoxin" (Hitchcock et al., 1986). It comprises two polysaccharide units, the O-antigen and the core, as well as an amphipathic moiety known as "lipid A." Many of the pathophysiologic effects of LPS can be attributed to the lipid A moiety (Nowotny, 1987).

III. B. 2. Means of exposure

Gram-negative bacterial infection is a common mode of LPS exposure. LPS is embedded within the cell walls of gram-negative bacteria. It can be liberated during cell division and cell death. Hence, antibiotic therapy can contribute to increased LPS exposure (Rotimi et al., 2000).

In humans, a mixture of bacteria colonizes the intestine contributes to metabolism. The gastrointestinal mucosa acts as an impenetrable barrier to the release, or passage, of bacterial flora. However, injury to the mucosal lining may serve as a means by which bacteria or LPS can escape, or translocate, into the systemic circulation. Bacteria that have been associated most frequently with translocation include *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus epidermis,* and Enterococci (Wells et al., 1988). LPS has been proposed to escape the gastrointestinal tract via the portal vein and the lymphatics. A normal function of the liver is to detoxify LPS; however, this function is bypassed when LPS escapes through the lymphatics (Gans and Matsumoto, 1974).

Translocation of bacteria and endotoxin can occur because of direct or indirect injury to the mucosa, or as a result of a disease of the mucosa. Injury with sloughing of the mucosa can occur with irradiation (Hill et al., 1997), inhibitors of cell replication such as cyclophosphamide, or the direct effect of xenobiotic agents. Indirect injury to the mucosa can occur with heat stress (Gathiram et al., 1987), intestinal ischemia (Cuevas and Fine, 1972), hemorrhagic shock (Rush et al., 1989; Baker et al., 1988) and medical procedures involving intestinal manipulation (Lau et al., 2000). Diseases that cause ulcerations of the intestine include intestinal obstruction, Crohn's disease (Ambrose et al., 1984), and ulcerative colitis. Predisposing factors for translocation include use of antibiotics causing alteration of the intestinal flora, changes in the intestinal flora induced by diet, and strenuous exercise leading to gastrointestinal distress.

III. B. 3. Pathophysiologic effects

As a result of clinical and experimental evidence, high-dose LPS exposure has been linked to the pathogenesis of circulatory shock (Ohlsson et al., 1990), disseminated intravascular coagulation (DIC)(Emerson, Jr. et al., 1987), acute respiratory distress syndrome (ARDS)(Martin and Silverman, 1992) and multiple organ failure (Baue, 1993). LPS exposure results in hypotension and decreased cardiac output, hence contributing to circulatory shock (Mozes et al., 1991). Additionally, it directly activates intrinsic and extrinsic pathways of coagulation resulting in DIC. LPS also causes pulmonary vascular hyperresponsiveness (Salzer and McCall, 1990), and might thereby enhance pulmonary hypertension during septic ARDS (Jardin et al., 1979). Multiple organ failure is altered organ function in the setting of sepsis, septic shock, or systemic inflammatory response

syndrome (Johnson and Mayers, 2001). It occurs when either the host's inflammatory or antiinflammatory response to injury (or both) are excessive (Bone, 1996).

III. B. 4. Cellular effects

Once in the circulation, LPS binds to serum factors such as LPS-binding protein (LBP) or endogenous serum proteins such as HDL. LPS:LBP complexes are recognized by cellular CD14 receptors on monocytes, macrophages and leukocytes, or endothelial cells and fibroblasts through a soluble form of CD14. CD14 facilitates LPS interaction with toll-like receptors (TLR), in particular TLR4. TLR4 is responsible for LPS signalling which includes nuclear factor κB (NF-κB) and mitogen activated protein kinase (MAPK) cascades and production of proinflammatory cytokines, such as TNF-α, IL-1 and IL-6.

III. C. Inflammation as a determinant of susceptibility

Exposure to inflammagens is episodic and commonplace, emerging from events such as disease, infection, and GI disturbance (e.g., due to alcohol, altered diet, surgery, etc.) (Roth et al., 1997). As noted above, LPS in large doses leads to sepsis-like changes including DIC, circulatory shock and multiple organ failure (Hewett and Roth, 1993). Small doses of LPS, on the other hand, induce 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 xenobiotic agents. For example, LPS exposure increases liver damage produced by hepatotoxicants 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 inflammagens might provoke untoward responses to drugs that present clinically as idiosyncratic reactions.

IV. Conclusion

There are two types of adverse drug reactions, type A and type B. Most adverse drug reactions are type A. Type B reactions, on the other hand, occur in a small percentage of the population, are not dose-related and lack a predictable timecourse for onset. There are several hypotheses regarding the pathogenesis of these idiosyncratic reactions, but animal models substantiate few. Many classes of drugs result in idiosyncratic responses. The drug chlorpromazine is an antipsychotic therapeutic agent that causes multiple idiosyncratic events in humans. Inflammation has an apparent association with some of these reactions. LPS, a potent inflammagen, has been demonstrated to invoke susceptibility to xenobiotic toxicity. The primary focus of this thesis will be to examine in an animal model the role of an underlying inflammatory state as a susceptibility factor to idiosyncratic responses to CPZ.

V. Hypothesis

Modest endotoxemia predisposes rats to the clinical manifestations of CPZ–induced idiosyncratic reactions seen in people. Hence, the endotoxemic rat cotreated with CPZ is an animal model for chlorpromazine-induced idiosyncratic reactions in people.

VI. Research Goals

1. The primary objective of this research is to identify whether underlying inflammation can predispose rodents to susceptibility to hepatic cholestasis resembling that which occurs idiosyncratically in chlorpromazine-treated people.
2. The second objective of this research is to identify whether underlying inflammation augmented serum CK levels in animals treated with chlorpromazine. Elevated serum CK is an enzymatic marker identified in rhabdomyolysis and NMS that occur as idiosyncratic reactions to CPZ.

CHAPTER 2

UNDERLYING ENDOTOXEMIA AUGMENTS TOXIC RESPONSES TO CHLORPROMAZINE: IS THERE A RELATIONSHIP TO DRUG IDIOSYNCRASY?

2. A. Introduction

CPZ is an antipsychotic drug well documented for a variety of idiosyncratic reactions including hepatic cholestasis and the neuroleptic malignant syndrome (NMS). Case reports of the clinical manifestations of CPZ drug-idiosyncrasy in humans provided evidence for underlying inflammation as an associated component (Table 2.1) For example, patients presenting to clinicians with CPZ-associated jaundice experienced prodromal symptoms of fever, abdominal pain, and gastrointestinal distress in the form of diarrhea and vomiting. Each of these conditions has been associated with endotoxemia. Also, surgical intervention, which leads to endotoxemia (Lau et al., 2000), appeared to provoke or prolong the course of CPZ jaundice. Taken together, these observations suggest a role for inflammation in CPZ drug idiosyncrasy.

To evaluate a role for underlying inflammation in CPZ-induced idiosyncratic reactions, LPS was administered to rats shortly before CPZ to invoke an inflammatory response. Hepatic injury associated with this cotreatment was assessed via serum activities of markers of hepatic cholestasis and necrosis and corresponding liver histopathology. Elevations in serum CK activities were also assessed and compared with diagnostic indicators of rhabdomyolysis and NMS, two idiosyncratic reactions associated with CPZ therapy.

TABLE 2.1

Underlying inflammation suggested by patient prodromes

Study	Prodrome[a]	Cases with at least 1 prodrome	Total cases
Moradpour et al., 1994	F	1	1
Johnson et al., 1979	None	0	1
Russell et al., 1973	None	0	1
Ishak and Irey, 1972	AP (5), D (5), F (12), V (10)	21	36
Levine et al., 1966	AP, V	1	1
Read et al., 1961	D (1)	1	4
Werther and Korelitz, 1957	AP (12), D (1), F (15), V (5)	20	22
Hollister, 1957	AP (11), F (13)	15	17
Hodges and La Zerte, 1955	AP, D, F	1	1
Loftus et al., 1955	F (2), V (1)	2	4
Lomas et al., 1955	AP (2), F (5)	6	11
Van Ommen and Brown, 1955	AP (1), F (2), V (1)	2	3
Gold et al., 1955	AP (2), F (3), V (1)	3	3
Pelner and Waldman, 1955	V	1	1
Movitt et al., 1955	D (2), F (2), V (1)	2	3
McHardy et al., 1955	AP	1	1
Redeker and Balfour, 1955	AP (1), F (1), V (1)	1	2
Lemire and Mitchell, 1954	None	0	2
	Total:	78	114

[a]Evidence for risk of inflammation presented as prodromal symptoms of abdominal pain (AP), diarrhea (D), fever (F), and/or vomiting (V). Number of patients with a specific prodrome are in parentheses.

2. B. Materials and methods

2. B. 1. Materials

Lipopolysaccharide (*Escherichia coli*, serotype 128:B12, lot number 48H4097), Chlorpromazine-hydrochloride, Myoglobin (from horse skeletal muscle) and Kits 59, 58, 245, and 419, for the respective determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyltransferase (GGT) activities were purchased from Sigma Chemical Company. The ALT and AST assays are based on the ability of an aminotransferase to catalyze the transfer of an amino group from an amino acid to an α-ketoacid to yield either pyruvate or oxaloacetate, respectively. Pyruvate is reduced to lactate in the presence of lactate dehydrogenase with simultaneous oxidation of nicotinamide adenine dinucleotide. Likewise, oxaloacetate is reduced to malate in the presence of malate dehydrogenase with simultaneous oxidation of nicotinamide adenine dinucleotide. Aminotransferase activity is proportional to a decrease in absorbance at 340nm. Alkaline phosphatase hydrolyzes p-nitrophenyl phosphate to p-nitrophenol and inorganic phosphate at alkaline pH. The rate of p-nitrophenol formed is measured as an increase in absorbance at 405nm. Gamma-glutamyltransferase catalyzes the transfer of a glutamyl group from L-γ-glutamyl-3-carboxy-4-nitroanilide to glycylglycine to form 5-amino-2-nitrobenzoate that absorbs at 405nm. Kits 450-A and 552-A for the respective measurement of bile acid and bilirubin concentrations were also purchased from Sigma Chemical Company. Bile acids were determined by the oxidation of bile acids to 3-oxo-bile acids that leads to an equimolar quantity of reduced nicotinamide adenine dinucleotide and subsequent oxidation with concommitant reduction of nitro blue tetrazolium to formazan. The increased absorbance

of formazan at 530nm is directly proportional to bile acid concentration in samples. Bilirubin in the presence of a surfactant reacts with diazotized sulfanilic acid to yield a colored complex that absorbs at 540 nm. Bilirubin concentrations are calculated from a set of standards. Kits 67 and 555A for the respective determination of blood urea nitrogen (BUN) and creatinine were also purchased from Sigma Chemical Company. Blood urea nitrogen concentrations are determined by the conversion of blood urea to ammonia and carbon dioxide in the presence of urease. Subsequent reactivity of ammonia and 2-oxoglutarate in the presence of reduced nicotinamide adenine dinucleotide and glutamate dehydrogenase yields glutamate and nicotinamide adenine dinucleotide. Urease activity is proportional to decreases in absorbance at 340nm. Creatinine concentration is determined by the reaction of creatinine with picric acid to form a yellow-orange complex under alkaline conditions. Acidification of the sample destroys the color contribution of creatinine. Hence, the difference in color intensity is proportional to the concentration of creatinine in the sample. Kit 47 for the determination of creatine kinase (CK) was purchased from Sigma Chemical Company. CK activity is determined by a series of reactions in which creatine phosphate and adenosine diphosphate in the presence of CK yields creatine and adenosine triphosphate. Hexokinase subsequently converts adenosine triphosphate and glucose to adenosine diphosphate and glucose-6-phosphate. Lastly, glucose-6-phosphate and nicotinamide adenine dinucleotide in the presence of glucose-6-phosphate dehydrogenase yields 6-phosphogluconate and reduced nicotinamide adenine dinucleotide. The increase in absorbance at 340nm correlates with creatine kinase activity. All immunocytochemical reagents, except rabbit anti-rat neutrophil immunoglobulin, were purchased from Vector Laboratories (Burlingame, CA). 10%

Neutral Buffered Formalin was purchased from Surgipath Medical Industries, Inc. (Richmond, IL). Rectal temperatures were measured using a Tele-Thermometer from Yellow Springs Instrument Company (Yellow Springs, OH).

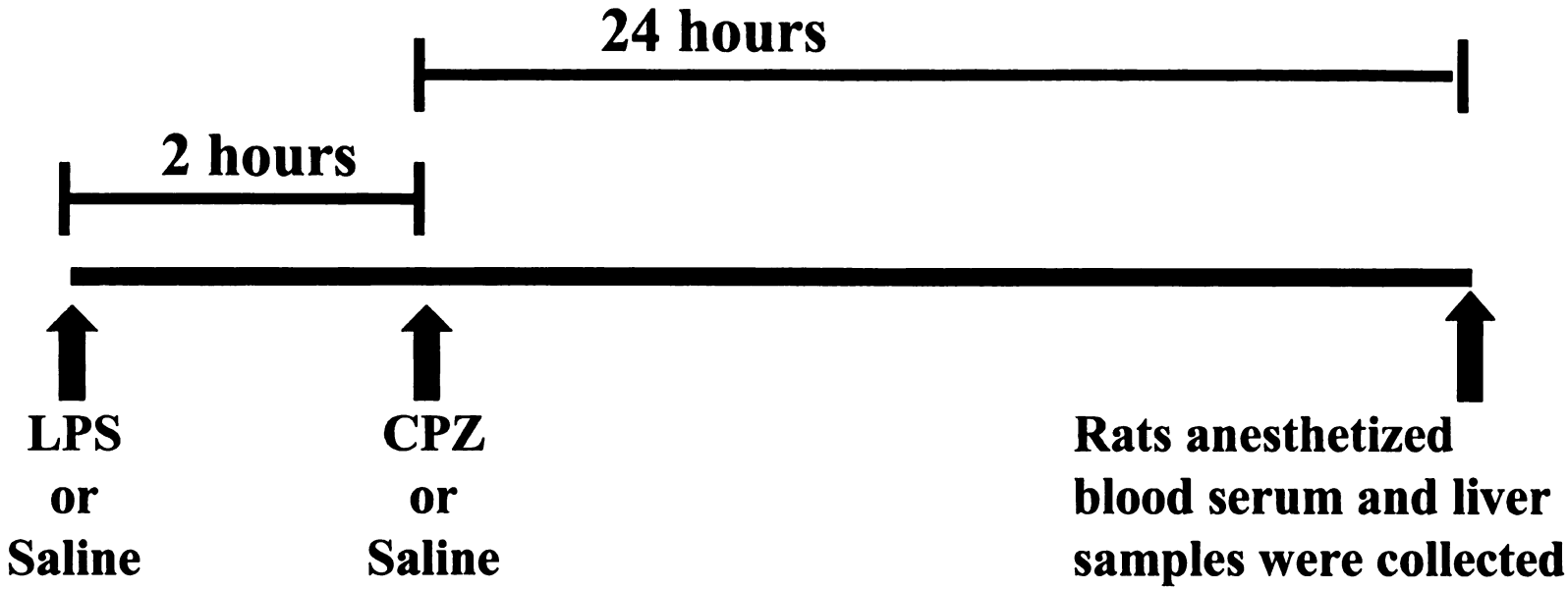
2. B. 2. Animals

Male, Sprague-Dawley rats (CD-Crl:CD-(SD)BR VAF/Plus; Charles River, Portage, MI) weighing 200 – 250 g were used in all studies. Animals were maintained on a 12-hr light and 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*. All animal procedures were approved by the All University Committee on Animal Use and Care.

2. B. 3. Treatment protocol

Animals were fasted 24 hours before the administration of LPS. 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 7.4×10^6 EU/kg, or with an equal volume (equivalent to 0.1% of the body weight) of sterile saline (SAL) vehicle. Two hours after the administration of LPS, CPZ-HCL (70mg/kg) diluted in SAL or an equal volume (equivalent to 0.1% of the body weight) of SAL was injected intraperitoneally. Twenty-four hours after CPZ administration, rats were anesthetized with sodium pentobarbital (50 mg/kg, ip), and a laparotomy was performed (Figure 2.1). Blood was collected from the dorsal aorta, dispensed into a 12 x 75 mm test tube and allowed to clot. Serum was separated by centrifugation and aliquoted for storage at 4-8°C and -20°C until analyzed.

Figure 2.1. Protocol for animals treated with LPS/CPZ. Animals are fasted 24 hours prior to the administration of a noninjurious, low-dose of LPS (7.4 x 10^6 EU/kg) followed 2 hours later by the administration of a nonhepatotoxic dose of CPZ (70mg/kg). Twenty-four hours after CPZ treatment, rats were anesthetized and blood serum and liver samples were collected.


24 hours
2 hours
LPS
or
Saline
CPZ
or
Saline
Rats anesthetized
blood serum and liver
samples were collected

2. B. 4. Serum biomarkers of hepatic injury

To assess the effects of underlying inflammation on CPZ-induced idiosyncratic hepatic injury, serum biomarkers of hepatic cholestasis and necrosis were evaluated. Serum bile acids and total bilirubin, as well as ALP and GGT, were used as markers of hepatic cholestasis and were measured spectrophotometrically using Sigma kits 450-A, 552-A, 245 and 419, respectively. Serum ALT and AST were used as markers of hepatic parenchymal cell injury and were determined spectrophotometrically using Sigma kits 59 and 58, respectively.

2. B. 5. Histologic scoring

To quantify histopathologic hepatic injury, slides of liver sections were coded and evaluated without knowledge of treatment. Three categories of injury (subserosal necrosis, midzonal necrosis and hypereosinophilic hepatocytes) were defined and scored 1 through 5 based on severity.

Subserosal injury was defined as well demarcated lesions, immediately subcapsular, comprised of 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 - 15% of the total circumference, 4 = coagulative necrosis of 15-25% of the total circumference, 5 = coagulative necrosis extending into the lobules and affecting >25% of the total circumference.

Midzonal 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. Liver sections were scored as follows: 1 = no midzonal injury, 2 = 1 – 3 necrotic foci throughout a liver section, 3 = 4 – 6 necrotic foci, 4 = 7 – 9 necrotic foci, 5 = >9 necrotic foci.

Hypereosinophilic hepatocytes were characterized as scattered single hepatocytes or groups of hepatocytes with slightly eosinophilic, cytoplasmic staining and with nuclei slightly smaller than normal and more darkly stained, but not overtly pyknotic. The sinusoidal architecture remained intact. Liver sections were scored as the average of five 20X fields. Each field was scored as follows: 1 = no hypereosinophilic hepatocytes, 2 = scattered single hypereosinophilic cells, 3 = small clusters of hypereosinophilic cells, 4 = large clusters of hypereosinophilic cells without bridging lobules, 5 = clustering of hypereosinophilic cells with bridging of lobules.

2. B. 6. Hepatic neutrophil accumulation

Neutrophils (PMNs) were visualized in liver sections using a previously described immunohistochemical technique (Pearson et al., 1995). In brief, sections were fixed, paraffin embedded and sectioned at 6 μm. 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, 400x, interior random fields (i.e. non-subserosal) using light microscopy.

In addition to PMN accumulation in the liver, PMNs were quantified in the kidney and recorded by distribution in the papilla, medulla, cortex and glomeruli.

2. B. 7. Serum CK activity and isoenzymes

Serum CK activity was measured spectrophotometrically (Sigma kit no.45-1). CK isoforms were separated by applying 5 μl of sample to a 1% agarose gel (8mm thick) at 0 – 5°C with constant voltage (80V) in 0.05M MOPSO buffer [3-(n-morpholino)-2-hydroxypropanesulfonic acid] for 2 hours. The gel was treated with a developing agent (Sigma kit no. 715-AM) for 30 minutes in the dark at 37°C. After 30 minutes, the gel was incubated for an additional 3 hours at room temperature in the dark. The gel was then analyzed by visual inspection for CK-MM (origin), CK-MB and CK-BB (Blum HE et al., 1981).

2. B. 8. Urinalysis

Rats were housed after drug treatment for 24 hours in a metabolism chamber for urine collection. Urine was refrigerated at 4 – 8°C until it was analyzed for myoglobin qualitatively using Multistix 10 SG (Bayer Corp, Elkhart, IN)) reagent strips. Urine testing positive for "blood" on the Multistix test strip was further separated in Ultrafree-MC centrifugal filter devices with a 30,000 MW cutoff. The fraction containing the myoglobin component (< 30,000 MW cutoff) was analyzed using a Multistix reagent strip and by spectrophotometric scanning (300-500nm) for the presence of myoglobin.

2. B. 9. Assessment of nephrotoxicity

Serum was separated from blood by centrifugation and aliquoted for storage at 4-8°C. Serum BUN and creatinine concentrations were used as markers of renal dysfunction and were measured spectrophotometrically using Sigma kits 67 and 555A, respectively.

2. B. 10. Thermoregulatory dysfunction

24 hours after the administration of CPZ, animals were restrained and the rectal temperature of each rat was recorded with a rectal probe (Yellow Springs Instruments, Yellow Springs, OH).

2. B. 11. Complete blood counts

Total and differential blood counts were assessed for leukocytes and platelets. Total white blood cells and platelets were determined on a blood analyzer (Serono Baker Instruments, Allentown, PA), while differential counts were performed on blood smears stained with buffered modified differential Wright-Giemsa stain, to obtain the fraction of neutrophils, lymphocytes and monocytes in each blood sample.

2. B. 12. Neurobehavioral test

A neurobehavioral screening battery (Moser V.C., 1999) that included both observational assessments and manipulative tests was performed on animals 24 hours after CPZ administration. Each observation involved a graded and descriptive response.

A. *Observational Assessments.*

1. Spontaneous activity / arousal. Each rat was observed in an open field (empty cage) for 2 minutes. The response was graded as follows: 1 = Clearly active (walking and running, slight excitement), 2 = Moderate (walking, alert), 3 = Somewhat low (some exploratory movements), 4 = Low (sluggish), 5 = No body movement (stupor, coma).

2. Gait and posture. Each rat was observed in an open field. Scores were assessed as follows: 1 = No abnormality, 2 = Slight abnormality, 3 = Moderate abnormality, 4 = Severe abnormality. In addition to scoring, abnormalities of gait were further described: ataxia (staggering), splayed hindlimbs or feet, walking on toes of hind feet, or forelimbs drag. Postural abnormalities were described as positions of the back, the belly, and the saggital plane.

3. Involuntary motor movements. Each rat was observed in the open field. Tremors, fasiculations (brief spontaneous contractions of a few muscles), clonic (convulsions) and tonic (continuous contraction) movements were ranked according to severity: 1 = None, 2 = Slight, 3 = Moderate, 4 = Severe.

4. Clinical signs. Each rat was observed in the open field for lacrimation (1 = None, 2 = Clear, 3 = Pigmented), changes in hair coat (1 = Normal, 2 = Piloerection), and muscle tone (1 = Normal, 2 = Front limbs or hindlimbs, 3 = Whole body).

B. *Manipulative tests.*

1. Neurological reflexes / reactions. The extensor thrust reflex was used to evaluate the motor/sensory components of the spinal response to pressure on the

footpads. Forceps were gently pressed into the plantar surface of the animal's two hindfeet. The stimulus was withdrawn either immediately following the response or after 30 seconds. The response was graded as 1= present or 2= absent.

2. Postural reactions. The "righting" reaction was tested to measure coordination and balance. Each animal was held in a supine position and then released. Time to "right" was graded as 1 = Normal (< 1 second), 2 = Slow (> 1 second, < 5 seconds), 3 = Very slow (>5 seconds, < 15 seconds), 4 = Did not right.

3. Sensory responses. In an open field each rat was tested for flexor reflex, proprioceptive positioning, and auditory response.

a. Flexor reflex. The presence (grade = 1) or absence (grade = 2) of a retraction of the hindlimb in response to a sharp pinch of the toes.

b. Proprioceptive positioning. The presence (1 = normal, 2 = slow) or absence (grade = 3) of response to flexing the hindlimb so that the dorsal surface of the paw is down on the surface.

c. Auditory response. The magnitude of response to a sound stimulus (finger snap) 5 cm behind each rat. The response was measured as: 1 = obvious reaction (normal), 2 = slight or sluggish reaction, 3 =no reaction (abnormal).

2. B. 14. Data analysis

Serum biomarkers of hepatic cholestasis and necrosis, kidney function, neutrophil accumulation in the liver and kidney, temperatures, blood cell counts and creatine kinase activity were analyzed by a two-way analysis of variance (ANOVA), and individual comparisons were performed using Fisher's least significant difference (LSD) test. When

variances were not homogeneous, data were transformed appropriately before analysis. Results are presented as mean $\pm$ standard error of the mean (SEM). The criterion for statistical significance in all studies was $p<0.05$.

Histologic and neurobehavioral scores were analyzed using the Kruskal-Wallis nonparametric test. Individual comparisons between pairs of treatment groups were performed using Dunn's test. Data are presented as the median with respective 25 and 75^{th} percentile values. The criterion for statistical significance in all studies was $p<0.05$.

2. C. Results

2. C. 1. Markers of hepatic cholestasis and necrosis

LPS and CPZ were administered to rats at noninjurious doses as determined in preliminary experiments. Markers of hepatic cholestasis included serum ALP (Figure 2.2A) and GGT (Figure 2.2B) activities and serum bilirubin (Figure 2.2C) and bile acid (Figure 2.2D) concentrations. 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 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 (Figure 2.3A) and AST (Figure 2.3B) activity. These were not statistically elevated in animals exposed to either LPS or CPZ by themselves. In contrast, treatment with LPS/CPZ resulted in significant increases.

Figure 2.2. Cholestatic liver injury in rats exposed to LPS/CPZ. Animals received either LPS (7.4 x 10^6 EU/kg) or saline followed 2 hours later by treatment with either CPZ (70 mg/kg) or saline. They were killed 24 hours after CPZ exposure, and serum activities or concentrations of (A) alkaline phosphatase (ALP) *n*=(13, 13, 23, 22), (B) gamma glutamyltransferase (GGT) *n*=(12, 12, 18, 17), (C) bilirubin *n*=(4, 4, 6, 5) and (D) bile acids *n*=(4, 4, 6, 5) were determined. The number of animals per treated group are denoted as *n*=(#SAL/SAL, #LPS/SAL, #SAL/CPZ, #LPS/CPZ).

[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 ($p < 0.05$).

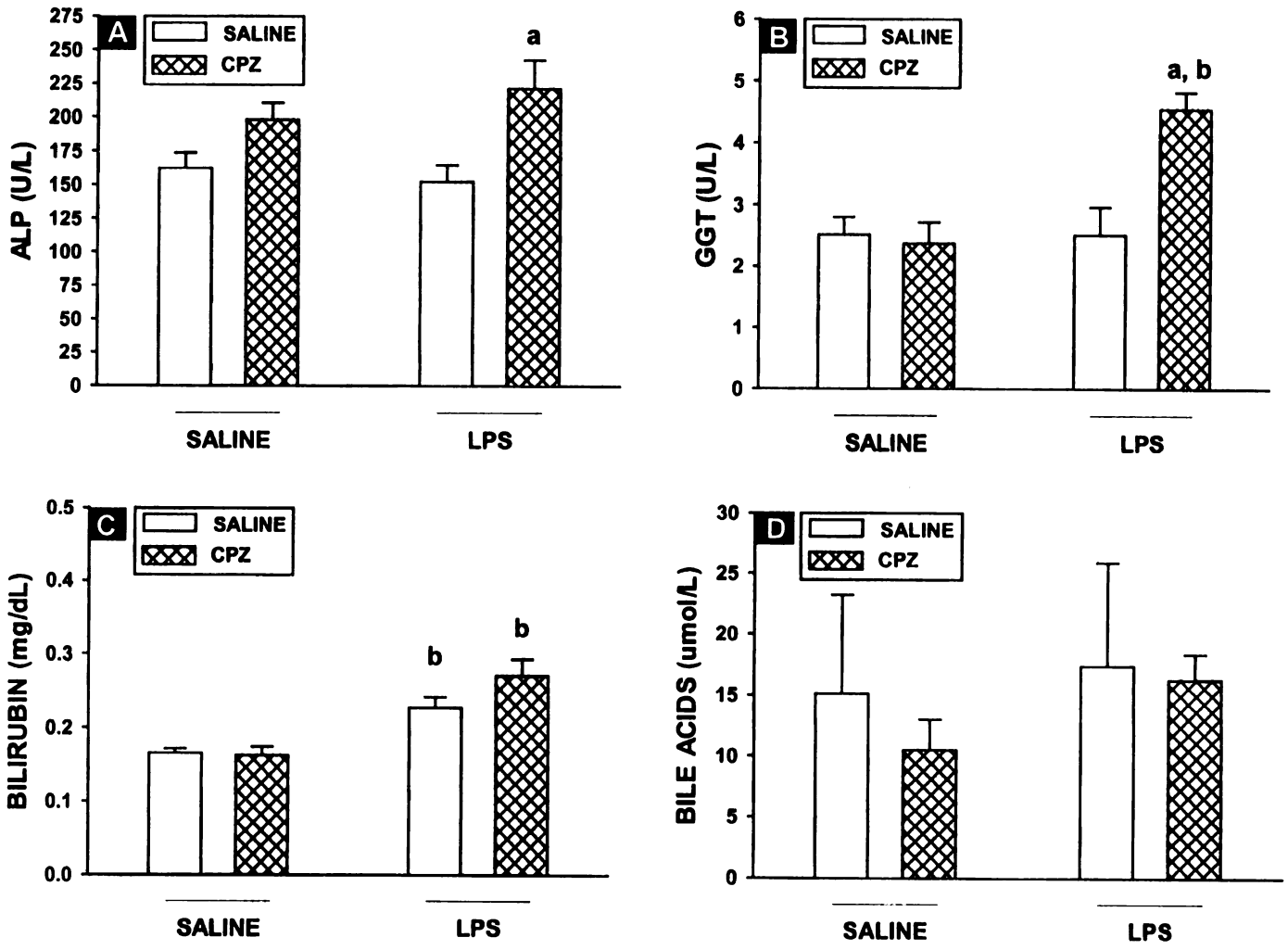
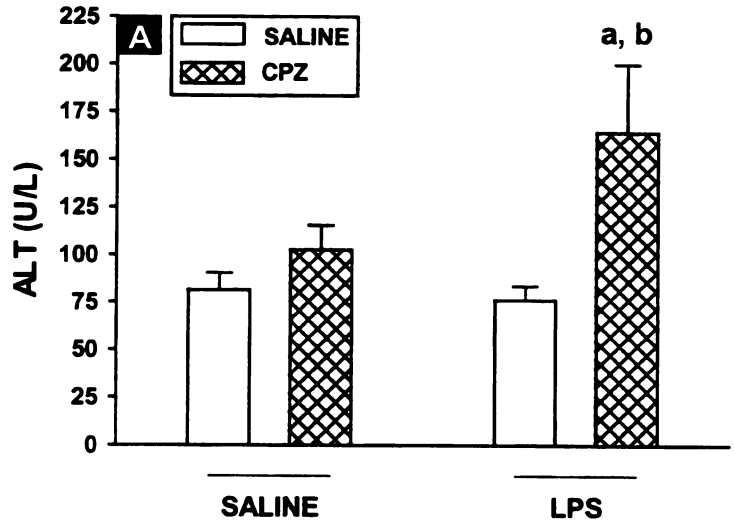
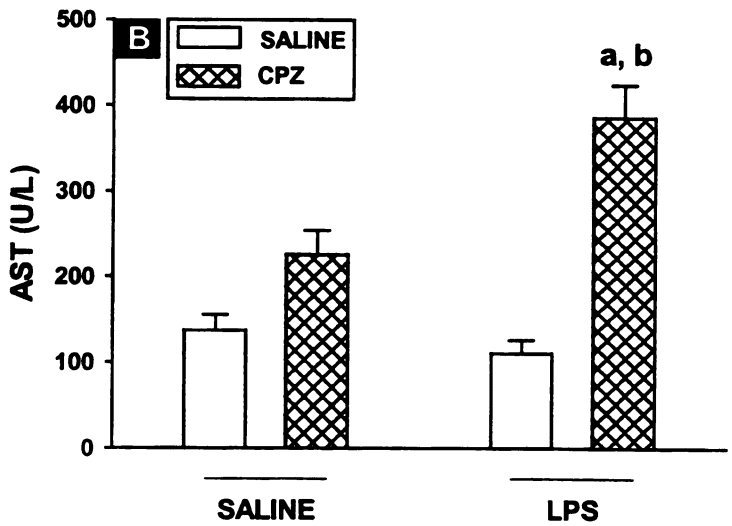

A
SALINE
CPZ
ALP (U/L)
275
250
225
200
175
150
125
100
75
50
25
0
a
SALINE
LPS
B
SALINE
CPZ
GGT (U/L)
6
5
4
3
2
1
0
a, b
SALINE
LPS
C
SALINE
CPZ
BILIRUBIN (mg/dL)
0.5
0.4
0.3
0.2
0.1
0.0
b
b
SALINE
LPS
D
SALINE
CPZ
BILE ACIDS (umol/L)
30
25
20
15
10
5
0
SALINE
LPS

Figure 2.3. Liver injury in rats exposed to LPS/CPZ. Animals received either LPS (7.4 x 10^6 EU/kg) or saline followed 2 hours later by treatment with either CPZ (70 mg/kg) or saline. They were killed 24 hours after CPZ exposure, and serum activities of (A) alanine aminotransferase (ALT) *n*=(10, 10, 17, 17) and (B) aspartate aminotransferase (AST) *n*=(10, 10, 17, 17) were determined. The number of animals per treated group are denoted as *n*=(#SAL/SAL, #LPS/SAL, #SAL/CPZ, #LPS/CPZ).

[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 ($p < 0.05$).


A
SALINE
CPZ
ALT (U/L)
225
200
175
150
125
100
75
50
25
0
a, b
SALINE
LPS


B
SALINE
CPZ
AST (U/L)
500
400
300
200
100
0
a, b
SALINE
LPS

2. C. 2. Histopathology of the liver

In liver sections, two locations of lesions (internal and subserosal) were identified, defined and graded based on severity (Table 2.2). Only livers from animals treated with LPS/CPZ had significantly greater histologic 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 (Figure 2.4). The foci were either subserosal or occurred as midzonal (Figure 2.5) lesions distributed throughout the interior of liver sections.

2. C. 3. Hepatic neutrophil accumulation

Immunohistochemical staining revealed few PMNs scattered throughout the liver sections of animals treated with vehicle or CPZ (Figures 2.5 and 2.6). By contrast, significant neutrophil accumulation was evident in the sinusoids of livers from animals treated with LPS or with LPS/CPZ.

2. C. 4. Serum CK activity

Pronounced increases in serum CK activity characterize phenothiazine idiosyncratic reactions in people (Koizumi et al., 1996; Surmont et al., 1984; Ebadi et al., 1990; Pelonero et al., 1998). CK activity (Figure 2.7) was normal in serum of rats treated with LPS or CPZ alone. However, LPS/CPZ cotreatment markedly increased serum CK activity. 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.

TABLE 2.2

Scoring of Liver Sections of Rats Treated with LPS/CPZ

	Midzonal Necrosis	Subserosal Necrosis	Hypereosinophilic Hepatocytes
SAL/SAL	1.00 (1.00 – 1.00)	1.00 (1.00 – 1.00)	1.40 (1.23 – 1.55)
SAL/CPZ	1.00 (1.00 – 1.38)	1.50 (1.00 – 2.13)	1.40 (1.30 – 1.75)
LPS/SAL	1.00 (1.00 – 1.50)	1.00 (1.00 – 1.00)	1.30 (1.13 – 1.88)
LPS/CPZ	3.50 (1.38 – 4.25) [b]	2.00 (1.50 – 3.63) [a]	1.60 (1.48 – 2.20)

Liver sections from animals treated with LPS and CPZ were evaluated as described under Material and Methods. Three categories of injury (subserosal necrosis, midzonal necrosis and hypereosinophilic hepatocytes) were defined and scored 1 through 5 based on severity. Subserosal necrosis was graded as follows: 1 = no injury or scattered hypereosinophilic hepatocytes, 2 = coagulative necrosis of <5% of the total section circumference, 3 = coagulative necrosis of 5 - 15% of the total circumference, 4 = coagulative necrosis of 15-25% of the total circumference, 5 = coagulative necrosis extending into the lobules and affecting >25% of the total circumference. Midzonal necrosis was graded as follows: 1 = no midzonal injury, 2 = 1 – 3 necrotic foci throughout a liver section, 3 = 4 – 6 necrotic foci, 4 = 7 – 9 necrotic foci, 5 = >9 necrotic foci. Hypereosinophilic hepatocytes were graded as follows: 1 = no hypereosinophilic hepatocytes, 2 = scattered single hypereosinophilic cells, 3 = small clusters of hypereosinophilic cells , 4 = large clusters of hypereosinophilic cells without bridging lobules, 5 = clustering of hypereosinophilic cells with bridging of lobules. N = 7-13. Values are expressed as the median with 25 to 75th quartile distribution in parentheses.

[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 ($p < 0.05$).

Figure 2.4. Midzonal (M) and subserosal (S) necrotic foci in liver sections from rats exposed to LPS and/or CPZ. Liver sections were stained with hematoxylin and eosin. Liver sections represent treatment with vehicle (A), LPS alone (B), CPZ alone (C), and LPS/CPZ (D). Images depict the median score (Table 2.2) for the given treatment.

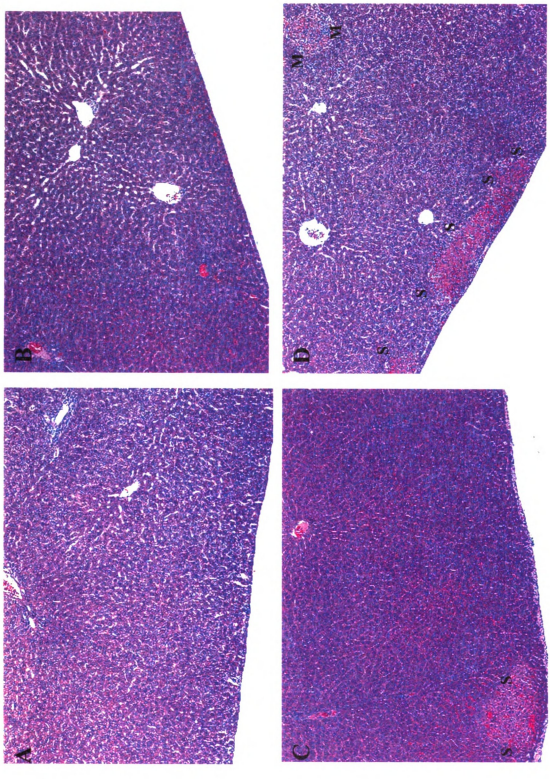
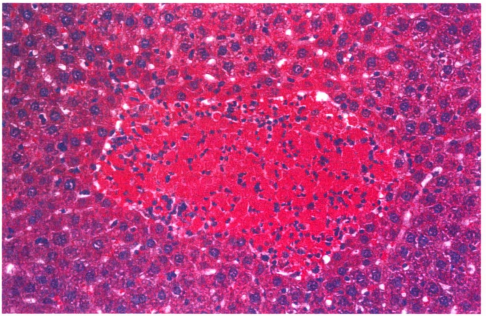

B
D
M
M
S
S
S
S
S
A
C
S
S

Figure 2.5. Midzonal lesion in the liver of rats exposed to LPS/CPZ. Liver sections were stained with hematoxylin and eosin (A) or stained immunohistochemically for neutrophils (B). Midzonal foci are characterized by neutrophil infiltration accompanied by pale, eosinophilic parenchymal cells with indistinct cytoplasmic borders and pyknotic and/or karyolytic nuclei.



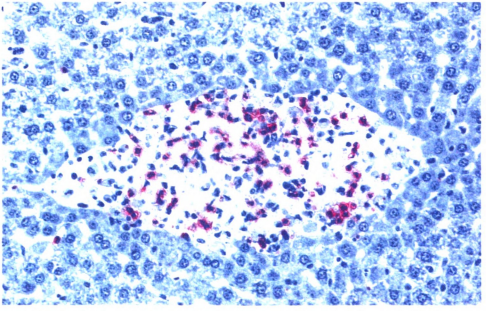


Figure 2.6. Accumulation of neutrophils in the liver of rats exposed to LPS/CPZ. Animals received either LPS (7.4 x 10^6 EU/kg) or saline followed 2 hours later by treatment with either CPZ (70 mg/kg) or saline. They were killed 24 hours after CPZ exposure. N = (8, 6, 12, 13) where n=(#SAL/SAL, #LPS/SAL, #SAL/CPZ, #LPS/CPZ).

[b] Significantly different from respective value in the absence of LPS ($p < 0.05$).

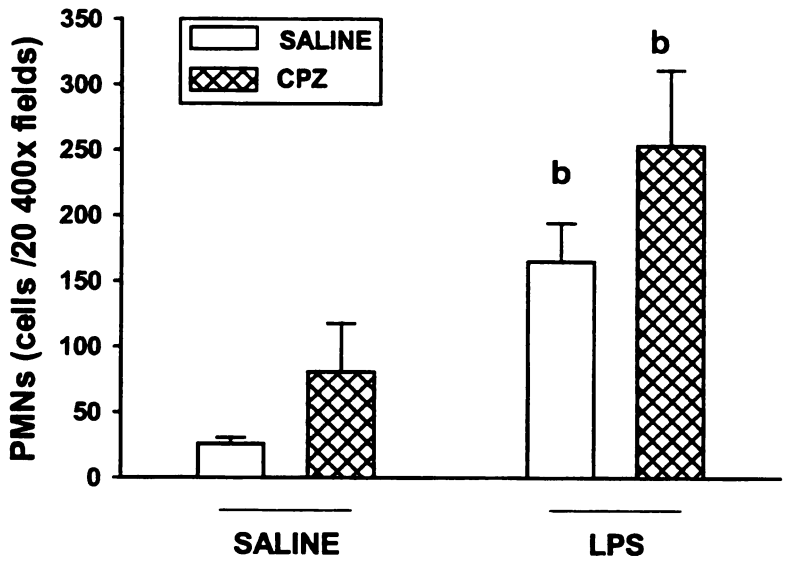
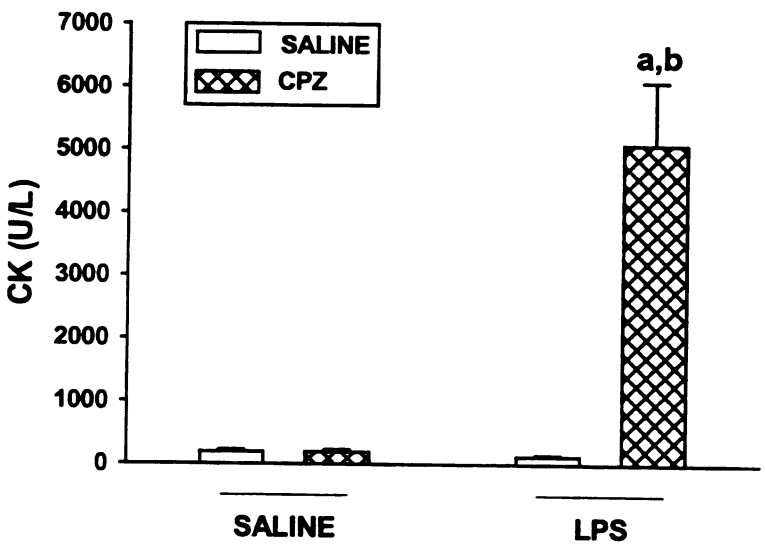

PMNs (cells /20 400x fields)
350
300
250
200
150
100
50
0
SALINE
CPZ
b
b
SALINE
LPS

Figure 2.7. Serum creatine kinase activity in rats exposed to LPS/CPZ. Animals received either LPS (7.4 x 10^6 EU/kg) or saline followed 2 hours later by treatment with either CPZ (70 mg/kg) or saline. They were killed 24 hours after CPZ exposure and serum activity of creatine kinase (CK) was assayed. N = (8, 6, 12, 13) where n=(#SAL/SAL, #LPS/SAL, #SAL/CPZ, #LPS/CPZ).

[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 ($p < 0.05$).


7000
6000
5000
4000
3000
2000
1000
0
CK (U/L)
SALINE
CPZ
a,b
SALINE
LPS

2. C. 5. Assessment of myoglobinuria

The presence of myoglobin in the urine is indicative of myonecrosis and, hence, has been associated with rhabdomyolysis (Schulze, 1982). Qualitative urinalysis using a Multistix test strip (Bayer, Elkhart, IN) was negative for myoglobin for all treatment groups.

2. C. 6. Effect of LPS/CPZ treatment on kidney injury

Acute renal failure is a complication of severe rhabdomyolysis. Serum BUN and creatinine are common markers of renal function. Rats treated with LPS or CPZ alone had modestly, yet significantly greater serum BUN than rats treated with vehicle (Figure 2.8). BUN concentrations in the serum of rats treated with LPS/CPZ were significantly greater than those in rats treated with either LPS or CPZ alone (Figure 2.8A). Serum creatinine was unaffected by any of these treatments (Figure 2.8B).

2. C. 7. Effect of LPS/CPZ on neutrophil accumulation in the kidney

Immunohistochemical staining revealed modest, yet significant, increases in PMNs scattered throughout the papilla, medulla, and cortex in kidney sections of animals treated with CPZ or LPS alone (Table 2.3). Neutrophil accumulation in these regions was greater in kidney sections of animals treated with LPS/CPZ.

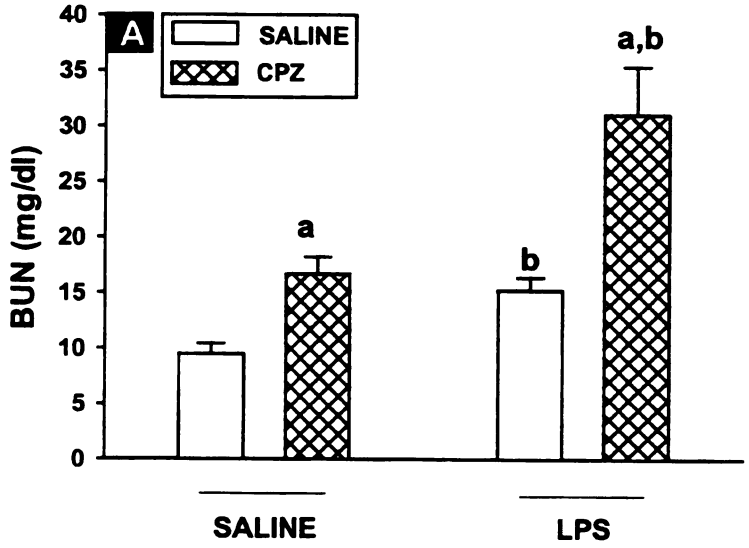
2. C. 8. Effect of LPS/ CPZ treatment on body temperature

Hyperthermia is a hallmark clinical manifestation of NMS in people. The rectal temperature of rats treated with LPS alone was normal at 26 hours after its

Figure 2.8. Kidney function in rats exposed to LPS/CPZ. Animals received either LPS (7.4×10^6 EU/kg) or saline followed 2 hours later by treatment with either CPZ (70 mg/kg) or saline. They were killed 24 hours after CPZ exposure, and serum activities of (A) blood urea nitrogen (BUN) n=(12, 12, 19, 19) and (B) creatinine n=(6, 6, 10, 8) were determined. The number of animals per treated group are denoted as n=(#SAL/SAL, #LPS/SAL, #SAL/CPZ, #LPS/CPZ).

[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 ($p < 0.05$).


A
SALINE
CPZ
BUN (mg/dl)
40
35
30
25
20
15
10
5
0
a
b
a,b
SALINE
LPS

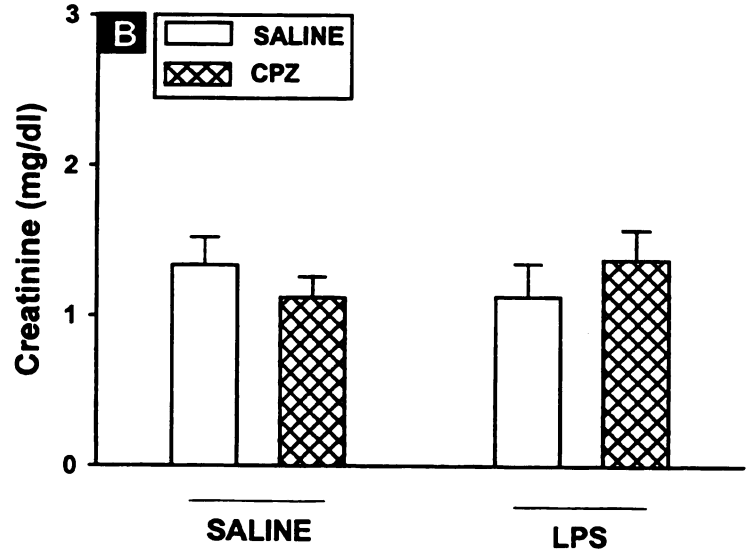

B
SALINE
CPZ
Creatinine (mg/dl)
3
2
1
0
SALINE
LPS

TABLE 2.3

Regional Distribution of Neutrophils in the Kidney

	SALINE		LPS	
	SALINE	**CPZ**	**SALINE**	**CPZ**
Papilla	2.1 ± 1.0	6.3 ± 1.1[a]	10.0 ± 2.0[b]	24.9 ± 7.6[a,b]
Medulla	6.7 ± 3.3	13.3 ± 2.6[a]	34.5 ± 4.0[b]	59.7 ± 7.2[a]
Cortex	44.4 ± 18.6	77.2 ± 10.1[a]	201.9 ± 19.9[b]	361.0 ± 32.9[a,b]
PMNs / 100 Glomeruli	15.8 ± 3.5	33.3 ± 7.1	61.0 ± 7.8[b]	115.5 ± 19.2[a,b]

Rats were treated with nontoxic doses of LPS and/or CPZ as described in Methods. Total neutrophil counts were assessed for each region of the kidney unless otherwise noted. N = (8, 6, 12, 13) where n=(#SAL/SAL, #LPS/SAL, #SAL/CPZ, #LPS/CPZ).

Data are presented as the mean ± the standard error of the mean (SEM).

[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 ($p < 0.05$).

administration. Treatment with CPZ, with and without LPS, resulted in a significant decrease in body temperature 24 hours after its administration (Table 2.4).

2. D. 9. Effect of LPS/CPZ on hematological parameters

Common laboratory diagnostics that are altered during the course of NMS include hematologic parameters such as leukocyte and platelet numbers. The total blood leukocyte concentration was reduced in all animals treated with CPZ. Additionally, CPZ treatment resulted in the decrease of lymphocyte populations. Treatment with LPS did not affect total leukocyte numbers, however it did result in an increase in PMNs. Thrombocytopenia was evident in all animals treated with LPS (Table 2.4).

2. C. 10. Treatment effects on neurobehavioral battery

Mental status changes and muscular rigidity are characteristics of the clinical presentation of NMS. Rats treated with LPS alone experienced tremors, modestly decreased muscle tone and delayed auditory responsiveness. Likewise, rats treated with CPZ alone experienced tremors, decreased muscle tone and delayed auditory responsiveness. Moreover, these rats were sluggish with splayed limbs, and had obvious signs of lacrimation and piloerection. LPS/CPZ treatment rendered animals without movement, splayed limbs, obvious signs of chromodacryorrhea and piloerection, decreased reflex reactions, delayed "righting" response and decreased proprioceptive and auditory responsiveness (Table 2.5).

TABLE 2.4

Changes in Temperature and Blood Cells in Animals Treated with LPS/CPZ

	SALINE		LPS	
	SALINE	**CPZ**	**SALINE**	**CPZ**
Body Temperature (°C)	37.6 ± 0.2	30.1 ± 0.3[a]	37.8 ± 0.8	28.2 ± 0.6[a]
Platelets ($x10^5$ cells/μl)	12.5 ± 1.3	13.0 ± 0.5	4.4 ± 1.0[b]	5.2 ± 1.5[b]
WBCs ($x10^3$ cells/μl)	7.4 ± 0.2	3.3 ± 1.0[a]	10.0 ± 0.3	4.9 ± 0.6[a]
Neutrophils ($x10^3$ cells/μl)	0.6 ± 0.0	1.3 ± 0.3	5.3 ± 0.5[b]	3.3 ± 0.4[b]
Lymphocytes ($x10^3$ cells/μl)	6.6 ± 0.2	1.8 ± 0.3[a]	4.5 ± 0.5	1.4 ± 0.4[a]
Monocytes ($x10^3$ cells/μl)	0.3 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.0

Rats were treated with nontoxic doses of LPS and/or CPZ as described in Methods. Rectal temperature was recorded 24 hours after CPZ injection N = (4, 4, 6, 7) where n=(#SAL/SAL, #LPS/SAL, #SAL/CPZ, #LPS/CPZ). Blood (1.5 ml) was collected from rats into potassium EDTA 24 hours after CPZ administration. Total and differential white blood cell counts were assessed. N = (6, 6, 9, 10). Data are presented as the mean ± standard error of the mean (SEM).

[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 ($p < 0.05$).

TABLE 2.5

Neurobehavioral Scores for Observational and Manipulative Assessments

	SALINE		LPS	
	SALINE	**CPZ**	**SALINE**	**CPZ**
Observational Assessments				
Spontaneous activity	1.0 (1.0 – 2.0)	4.0[a] (3.5 – 5.0)	2.0 (1.0 – 3.0)	5.0[a] (4.0 – 5.0)
Gait and posture	1.0 (1.0 – 1.0)	3.0[a] (3.0 – 4.0)	2.0 (1.0 – 2.0)	4.0[a] (3.0 – 4.0)
Involuntary movements	1.0 (1.0 – 1.0)	1.0[a] (1.0 – 2.0)	1.0[b] (1.0 – 2.0)	1.0 (1.0 – 2.0)
Lacrimation	1.0 (1.0 – 1.0)	2.0[a] (2.0 – 3.0)	1.0 (1.0 – 1.0)	3.0[a] (2.0 – 3.0)
Hair coat	1.0 (1.0 – 1.0)	1.0[a] (1.0 – 2.0)	1.0 (1.0 – 1.0)	1.0[a] (1.0 – 1.75)
Muscle tone	1.0 (1.0 – 1.0)	1.5[a] (1.0 – 3.0)	1.0[b] (1.0 – 2.0)	1.0 (1.0 – 2.0)
Manipulative Tests				
Reflexes and reactions	1.0 (1.0 – 1.0)	1.0 (1.0 – 1.0)	1.0 (1.0 – 1.0)	1.0[a] (1.0 – 1.75)
Postural Reactions	1.0 (1.0 – 1.0)	1.0 (1.0 – 1.0)	1.0 (1.0 – 1.0)	1.0[a] (1.0 – 3.0)
Flexor reflex	1.0 (1.0 – 1.0)	1.0 (1.0 – 1.0)	1.0 (1.0 – 1.0)	1.0 (1.0 – 1.0)
Proprioceptive positioning	1.0 (1.0 – 1.0)	1.0 (1.0 – 1.0)	1.0 (1.0 – 1.0)	2.0[a] (1.0 – 2.0)
Auditory response	1.0 (1.0 – 1.0)	2.0[a] (1.0 – 2.5)	1.0[b] (1.0 – 2.0)	2.0[a] (2.0 – 3.0)

Rats were treated with LPS and/or CPZ as described in Methods. Neurobehavioral scores were recorded 24 hours after CPZ injection. N = (12, 13, 25, 24) where n=(#SAL/SAL, #LPS/SAL, #SAL/CPZ, #LPS/CPZ). Data are presented as the median with 25 to 75th quartile distribution in parentheses.

[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 ($p < 0.05$).

2. E. Discussion

Much attention has focused on metabolic polymorphism and allergic hypersensitivity as causes for drug-induced liver injury. However, the clinical course and pathologic picture of idiosyncratic responses for many drugs call into question critical roles for these. 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; Mayeux, 1997). 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 (Figure 2.5) and the appearance in the plasma of cytokines such as tumor necrosis factor-alpha (TNF-α) (Barton et al., 2000) but no overt liver injury (Figures 2.1 and 2.2). 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. In many of

these, histopathologic findings consist of modest hepatocellular injury with a portal inflammatory infiltrate that includes lymphoid cells, PMNs, and eosinophils (Gold et al., 1955; Hollister, 1957; Gebhart et al., 1958; Ishak and Irey, 1972). However, other reports describe lesions with different zonal distributions and inflammatory characteristics. For example, Ishak and Irey (1972) and Read et al. (1961) described biopsy specimens as having hepatocytes "drop out" of a necrotic focus that was infiltrated with PMNs. These specimens were taken at two weeks and three months after the onset of CPZ-associated jaundice, respectively. This suggests that the type of lesion observed may be unrelated to the time the biopsy was taken during the course of hepatic idiosyncrasy.

The results of the present study suggest that an underlying endotoxemia renders animals susceptible to an increase in serum markers consistent with 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; Johnson et al., 1979; Moradpour et al., 1994) and bilirubin (Ishak and Irey, 1972; Johnson et al., 1979). 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 biliary bile acid pool to the peripheral blood.

In the present study, an elevation in serum enzymes (ALT and AST) and histopathologic findings indicative of hepatocellular necrosis were observed in LPS/CPZ treated rats. Moderate elevation of ALT and AST is not an uncommon finding in the differential diagnosis of drug-induced jaundice (Larrey and Erlinger, 1988; Zimmerman,

1968), 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 (Figures 2.3A-D).

Histologic 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 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, 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 activity. Indeed, serum CK activity in the thousands of units per liter has been reported (Allsop and Twigley, 1987a; Baker and Chengappa, 1995; Pelonero et al., 1998; 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 (Pearlman et al., 1988; 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 exhibited CPZ-related pharmacologic effects by becoming flaccid, sedate and hypothermic. Neurobehavioral screening methods such as a functional observational battery that assesses the neurobehavioral and functional integrity of the rat were utilized to identify changes in neuromuscular (eg., muscle rigidity) and excitability (eg., catatonia) behavior. The neurobehavioral screen is just one of three tests, which also includes neurophysiological and neurochemical measurements, to measure chemically induced changes in the function of the nervous system. Because there is difficulty in distinguishing between the neurobehavioral and pharmacologic effects of chlorpromazine, the assessment of behavior in this model served more as a means of monitoring the well-being of the animals subjected to the various treatment groups than actually providing a statement on neurotoxicity.

Because increases in CK have also been associated with idiosyncratic phenothiazine-induced rhabdomyolysis, urine was analyzed for myoglobin. Myoglobin was not detected by urinalysis, suggesting an absence of rhabdomyolysis. However, a negative test for urinary heme pigments should not exclude a diagnosis of rhabdomyolysis (Gabow et al., 1982). Hence, another biochemical manifestation that presents as a complication of rhabdomyolysis was examined. Acute renal failure (ARF) has been cited to occur in approximately 33% of rhabdomyolytic patients (Gabow et al., 1982) including patients using phenothiazines. The pathogenesis of ARF may be either

prerenal, renal or postrenal. The BUN to creatinine ratio is a diagnostic value useful in predicting the pathogenesis of ARF. The BUN to creatinine ratio has been reported to decrease in rhabdomyolytic patients with ARF as compared to those without ARF (Hamilton et al., 1972). However, others have reported no change in this ratio in either group of patients, even though there was a significant increase in creatinine concentrations (Gabow et al., 1982). In our studies, creatinine concentrations remained unchanged across treatment groups, and BUN concentrations were elevated in all animals treated with LPS or CPZ. The ratio of BUN to creatinine in the LPS/CPZ group is approximately 25:1. In addition, there were increased numbers of neutrophils distributed throughout the kidney in proportions similar to those seen in the liver with each treatment group. The biochemical data, in the absence of other necessary chemical markers, are merely suggestive of a prerenal azotemia (BUN:Creatinine $\geq$ 20:1) in the LPS/CPZ treated group. It could be speculated from observational data and clinical findings in the liver that the source of the prerenal azotemia may be hepatorenal syndrome. Hepatorenal syndrome simply refers to the appearance of renal failure in patients with severe liver disease, in whom there are no intrinsic morphologic or functional cause for the renal failure.

Because the source of serum CK was not known, we determined its isoforms 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). In humans, the elevated serum CK activity has been associated primarily with the MM-isoform (Pearlman et al., 1988; Meltzer et al., 1996). 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 hours before CPZ to produce an underlying inflammation. This LPS dosing regimen allowed for the expression and release of TNF into the plasma and for neutrophil accumulation in liver before exposure to CPZ (Pearson et al., 1995; Barton et al., 1999; Barton et al., 2000). Although this cotreatment regimen caused liver injury, other ones may reduce it. For example, CPZ administered before and up to 30 minutes after a larger dose of LPS inhibited TNF alpha synthesis and decreased lethality and hepatotoxicity (Gadina et al., 1991; Ghezzi et al., 1996; Jansen et al., 1998). Moreover, CPZ dampened neutrophil functions *in vitro*, including chemotaxis and superoxide generation (Bertini et al., 1991). These findings contrast with ours of enhanced injury with LPS coexposure. Hence, the temporal relationship between administration of LPS and exposure to CPZ and the dosages of these agents may be important in determining the qualitative nature of 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 and colleagues (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 following elevated CK activity, despite continued drug treatment. This prompted the authors to suggest that "state-dependent vulnerability factors or exogenous factors not yet identified may be of importance" (Meltzer et al., 1996). One of these factors might be endotoxin. Support for this idea came from a careful review of 15 published reports describing 110 cases of idiosyncratic reactions to phenothiazines. In 71% of these cases, patients had prodromal signs or conditions consistent with mild endotoxemia (Table 2.1). Interestingly, over 40 years ago the medical department of the manufacturer of chlorpromazine described fever and abdominal distress, ie, 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 lend credence to the idea that underlying inflammation might precipitate certain idiosyncratic reactions.

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.

CHAPTER 3

SUMMARY AND CONCLUSIONS

3. A. Historical Summary

In the mid to late 1950's case reports of idiosyncratic hepatic cholestasis associated with CPZ use were first published. The mechanism of action speculated in these reports was that of drug allergy (Gold et al., 1955; Hollister, 1957). The delay of two to three weeks to the onset of jaundice, along with occasionally noted eosinophilic reactions provided the primary evidence for this hypothesis (Van Ommen and Brown, 1955). To this day, allergic hypersensitivity remains the most widely accepted theory of CPZ-associated jaundice. Nevertheless, the hypothesis has not been substantiated through model development and has even been disregarded by some on the weight of clinical evidence to the contrary (Van Ommen and Brown, 1955; Ishak and Irey, 1972).

3. B. Evidence for Underlying Inflammation

A review of case reports of CPZ-associated jaundice provides evidence for an alternative hypothesis regarding factors that may precipitate this drug idiosyncrasy. In the following excerpt of a case provided by Movitt et al. (1955), prodromal symptoms suggestive of an underlying endotoxemia are presented:

> *Case 3* (Letterman Army Hospital, San Francisco). A 42-year-old white woman was admitted into another hospital on November 9, 1954, because of fever and pruritis. From October 23 through 29 she received 125 mg. of chlorpromazine daily for a psychiatric disorder. On October 30 the dose was increased to 250 mg. daily, and on the following morning the patient suddenly developed fever of 103°F., along with nausea, vomiting, and generalized muscular aching. She voluntarily discontinued chlorpromazine on that same day after having taken a total dose of 1.075 gm. On admission to another institution the patient was found to be acutely ill and febrile, but physical examination was not remarkable. Nausea and vomiting subsided, but low-grade fever persisted, and diarrhea became troublesome....
>
> On transfer to Letterman Hospital on November 9, the physical examination was not remarkable. There were no skin lesions, jaundice, hepatomegaly, or abdominal tenderness. Intense pruritis and mild diarrhea were the only

> symptoms....On November 14 mild scleral icterus appeared....The icterus at first rapidly deepened, but about November 20 both jaundice and pruritis began to subside....

Underlying inflammation provoked by endotoxin has been associated with clinical signs of chills, nausea and/or vomiting, fever (Kantor et al., 1983) and diarrhea (Rylander, 1999), signs that are clearly evident two weeks prior to the onset of jaundice in this patient. In yet another case presented by Hodges and LaZerte (1955) similar prodromal symptoms were described, however, surgical intervention appeared to incite an increased jaundice in the patient:

> A 67-year-old white housewife was seized with acute epigastric pain, fever, and chills on Sept. 10, 1954, and was admitted to the hospital. The pain was more severe and different than any she had experienced previously, and it remained localized in the epigastrum and right upper abdomen. Her temperature rapidly reached 104 F....
>
> The significant findings of the physical examination upon admission were mild icterus, temperature of 104 F, and tenderness in the epigastrum and right subcostal areas....
>
> A provisional diagnosis of progressing obstructive jaundice was made, and exploratory laparotomy was done on Sept. 18. At operation the biliary tract was entirely normal. The common bile duct was not dilated. The liver appeared normal, as did the spleen, pancreas, gallbladder, stomach, duodenum, and general peritoneal surfaces.... Immediately postoperatively she received one intramuscular injection of 10 mg. of chlorpromazine for nausea. At this time it was learned that 25 mg. of chlorpromazine had been administered orally twice daily by another physician from Aug. 11 to Sept. 1, for the relief of itching dermatitis. Her medication during the preceding months included phthalylsulfathiazole (Cremothalidine), ½ oz. (15cc.) four times a day, given for diarrhea from Aug. 4 to Aug. 16....
>
> The immediate postoperative course was in no way unusual.... Except for a transient decline in the serum bilirubin level three days postoperatively, there was a progressive increase in jaundice....

Likewise, Van Ommen and Brown (1955) report a similar case in a 64 year old woman, for whom CPZ was prescribed in doses of 25 mg three times daily for the treatment of an anxiety tension state:

> Surgical consultation was obtained....An operative cholangiogram confirmed the absence of dilatation of the common duct, but the passage of dye into the duodenum was delayed. A liver biopsy was done, and on microscopic examination marked bile stasis with phagocytosis of bile pigment by Kupffer cells was demonstrated. The hepatic cells appeared to be normal. Following surgery there was temporary bleeding from the wound, and the patient received two blood transfusions in the immediately post-operative period....The jaundice became progressively more severe.... In the succeeding four weeks, the jaundice gradually subsided....

Gastrointestinal distress and abdominal surgery with manipulation of the GI tract, as mentioned previously, have been implicated in promoting the translocation of endotoxin from the GI tract to detectable levels in the systemic circulation. A plausible connection appears to exist in these cases between an underlying inflammatory state and the onset and/or exacerbation of CPZ jaundice.

3. C. Summary

In this thesis, studies were conducted in a rodent model of inflammation to test the hypothesis that an underlying inflammatory state provoked susceptibility to idiosyncratic reactions seen in humans. Serum markers of hepatic cholestasis and necrosis, as well as histopathologic changes identified in liver sections from animals treated with LPS/CPZ were consistent with clinical and pathologic changes noted in human CPZ-associated jaundice. Additionally, serum creatine kinase activity was augmented in animals treated with LPS/CPZ. This elevation in serum CK activity was similar to that seen in humans; however, human clinical diagnostic features of rhabdomyolysis such as myoglobinuria and a predominant CK-MM isoenzyme profile were not consistent. Likewise, many features of the neuroleptic malignant syndrome such as hyperthermia, muscle rigidity, mental status changes and leukocytosis were not

reproduced. Instead, rats given CPZ exhibited pharmacologic effects of the drug such as sedation and hypothermia. Taken together, these results suggest that underlying inflammation may be a critical factor in precipitating some but not all drug-induced idiosyncratic events.

3. D. Other Hypotheses

Although current animal models have not generally confirmed the involvement of direct cytotoxicity, allergic hypersensitivity or genetic polymorphisms in drug idiosyncrasy, these mechanisms cannot be entirely dismissed. Zimmerman's hypothesis of "two-hits" may indeed have merit. By combining unique aspects from each of the aforementioned hypotheses, in addition to an underlying inflammation, alternative hypotheses can be formulated.

One hypothesis relates immune hypersensitivity and inflammation. Immune hypersensitivity in hepatic drug idiosyncrasy typically follows a timecourse of delayed onset with the occasional histopathologic appearance of eosinophils. Additionally, it is suspected that this period of time is required to mount an immune response to drug haptens. In the model proposed by the current thesis, an underlying inflammatory state augments the hepatotoxicity of CPZ; however, it is an acute model that does not account for a clinically relevant dosing scheme, and the histopathologic picture is completely devoid of eosinophils. Because many cases of human CPZ-associated jaundice occur within several weeks of initiation of drug therapy, and eosinophils and other inflammatory cell types are often a part of the histopathology, subchronic administration of CPZ coupled with an acute episode of underlying endotoxemia may predispose

animals to susceptibility to CPZ hepatic cholestasis as seen in a population of human patients in which eosinophils are a part of the liver histology.

In order to test this hypothesis, rats might be injected with a therapeutic dose of CPZ once daily for two weeks. On the last day, a small dose of LPS would be administered two hours before the scheduled dose of CPZ. Animals would be killed 12, 24, 36 and 48 hours after the last dose of CPZ. Sera would be collected and tested for an IgG fraction and markers of hepatic cholestasis and necrosis. Liver sections would be collected and processed for histopathology including neutrophil and eosinophil staining. It could be speculated that sera may include an Ig component and that the histopathology of liver sections from animals treated in this manner may include a transient, eosinophilic involvement in addition to hepatic necrosis and cholestasis.

Another hypothesis could relate genetic polymorphisms and inflammation to drug idiosyncrasy. Genetic polymorphisms of cytokine promoters and cellular receptors critical to pro-inflammatory responses have been identified in humans, therefore underlying inflammation coupled with a genetic polymorphism of one or more of these may predispose rats to idiosyncratic responses. For example, polymorphisms have been identified in toll-like receptors (Qureshi et al., 1999), CD14 receptors (Hubacek et al., 2000), TNF promoter regions (Yee et al., 2000) and endogenous reactive oxygen species inhibitors (Grant and Bell, 2000). Polymorphisms in genes encoding for these proteins may predispose people to idiosyncratic responses by heightening responses to inflammagens such as LPS. This hypothesis could be tested using rodents with known polymorphic expression of these receptors or promoter regions and a protocol similar to that described within this thesis.

Lastly, structural and/or mechanistic similarities may exist among drugs known to produce idiosyncratic reactions in multiple organ systems. Analysis of structure activity relationships between phenothiazines and other drugs and drug classes known to precipitate similar idiosyncratic events may reveal a common underlying mechanism. For example, chlorpromazine, the drug used in the current thesis, has broad receptor (dopamine, histamine, muscarine, serotonin) binding specificities, many of which are shared by other antipsychotic drug classes with differing affinity. There is no apparent correlation, however, between binding specificity or affinity and the onset of idiosyncratic hepatic cholestasis or NMS with these drugs. Although most of the antipsychotics have been reported to cause idiosyncratic hepatic cholestasis, so have other drugs such as antimalarials and chemotherapeutics that do not share the same pharmacologic receptor binding profiles.

It has been assumed that the many, apparently unrelated effects of drugs are caused by several unrelated biochemical actions (Weiss et al., 1982). However, this may not be true. In fact, the initial event that leads to these varied actions may be the same. This line of reasoning has lead to the suggestion that calmodulin, a ubiquitous protein directly linked with numerous calcium dependent processes, may be a common component in these initial events, and that the unrelated biochemical effects of these drugs may be explained by binding to, and inhibiting, calmodulin (Weiss et al., 1982). Indeeed, CPZ and other phenothiazines are well known calmodulin antagonists (Weiss et al., 1982). Likewise, the antimalarials chloroquin, mefloquin and cyclosporin A competitively bind to calmodulin, thereby adversely affecting parasitic growth (Scheibel et al., 1987). Unlike phenothiazines and antimalarials, chemotherapeutic drugs do not

interact directly with calmodulin. However, the *in vitro* cytotoxic effects of chemotherapeutics, such as the anthracycline, doxorubicin (adriamycin), on resistant leukemia cells are dependent on inhibition of calmodulin by antagonists like the phenothiazines (Ganapathi et al., 1984). Hence, modulation of calmodulin activity may be an important initial event in pharmacologic effects of these drugs and may also play a role in adverse responses.

3. E. Future Studies

Extension of the current hypothesis to other drugs and drug classes linked with human idiosyncrasy will be an important step in validating this model. Like phenothiazines, antiandrogens, antidepressants and antimalarials have been associated with idiosyncratic hepatic injury. Flutamide and cyproterone are antiandrogens that have been linked with idiosyncratic hepatic injury. Clinical (Gomez et al., 1992; Rosman et al., 1993) and experimental evidence suggests a role for inflammation in flutamide hepatotoxicity (Srinivasan et al., 1997). Likewise, the anticonvulsant amitriptyline (Larrey et al., 1988) and the antimalarial trimethoprim (Stevenson et al., 1978) have been clinically linked with hepatic cholestasis and evidence for an underlying inflammatory condition. Examination of biochemical and histopathologic parameters of hepatic injury to these drugs during an underlying endotoxemia may further establish a role for endotoxin as a determinant of susceptibility to hepatic idiosyncratic drug reactions.

REFERENCE LIST

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Addonizio G, Susman VL, Roth SD (1987) Neuroleptic malignant syndrome: review and analysis of 115 cases. Biol Psychiatry 22:1004-1020.

Alarcon-Segovia D, Fishbein E, Cetina JA, Raya RJ, Barrera E (1973) Antigenic specificity of chlorpromazine-induced antinuclear antibodies. Clin Exp Immunol 15:543-548.

Allsop P, Twigley AJ (1987a) The neuroleptic malignant syndrome. Case report with a review of the literature. Anaesthesia 42:49-53.

Allsop P, Twigley AJ (1987b) The neuroleptic malignant syndrome. Case report with a review of the literature. Anaesthesia 42:49-53.

Ambrose NS, Johnson M, Burdon DW, Keighley MR (1984) Incidence of pathogenic bacteria from mesenteric lymph nodes and ileal serosa during Crohn's disease surgery. Br J Surg 71:623-625.

Amore M, Zazzeri N (1995) Neuroleptic malignant syndrome after neuroleptic discontinuation. Prog Neuropsychopharmacol Biol Psychiatry 19:1323-1334.

Baker JW, Deitch EA, Li M, Berg RD, Specian RD (1988) Hemorrhagic shock induces bacterial translocation from the gut. J Trauma 28:896-906.

Baker RW, Chengappa KN (1995) Further study of neuroleptic malignant syndrome [letter]. Am J Psychiatry 152:1831

Balzan MV (1998) The neuroleptic malignant syndrome: a logical approach to the patient with temperature and rigidity. Postgrad Med J 74:72-76.

Barton CC, Barton EX, Ganey PE, Kunkel SL, Roth RA (2000) Bacterial lipopolysacchardie enhances aflatoxin B1 hepatotoxicity in rats by a mechanism that depends on tumor necrosis factor-alpha. Hepatology

Barton CC, Ganey PE, Roth RA (1999) Development of liver injury in rats treated with aflatoxin B_1 and endotoxin, and protection by neutrophil depletion. Toxicological Sciences 48 (Suppl.):55

Baue AE (1993) The multiple organ or system failure syndrome. In: Pathophysiology of shock, sepsis, and organ failure (Schlag G, Redl H eds), pp 1004-1018. Berlin: Springer-Verlag.

Bertini R, Wang JM, Mengozzi M, Willems J, Joniau M, Van Damme J, Ghezzi P (1991) Effects of chlorpromazine on PMN-mediated activities in vivo and in vitro. Immunology 72:138-143.

Blum HE, Weber B, Deus B, Gerok W (1981) The mitochondrial creatine kinase isoenzyme from human heart muscle. In: Creatine kinase isoenzymes : pathophysiology and clincial application (Hermann Lang ed), pp 19Berlin: Springer-Verlag.

Bone RC (1996) Immunologic dissonance: a continuing evolution to our understanding of the Systemic Inflammatory Response Syndrome (SIRS) and the Multiple Organ Dysfunction Syndrome (MODS). Ann Intern Med 125:680-687.

Boyer JL (1978) Mechanisms of chlorpromazine cholestasis: hypersensitivity or toxic metabolite? [editorial]. Gastroenterology 74:1331-1333.

Canoso RT, de Oliveira RM, Nixon RA (1990) Neuroleptic-associated autoantibodies. A prevalence study. Biol Psychiatry 27:863-870.

Cao L, Katz RH (1999) Acute hypernatremia and neuroleptic malignant syndrome in Parkinson disease. Am J Med Sci 318:67-68.

Caroff SN (1980) The neuroleptic malignant syndrome. J Clin Psychiatry 41:79-83.

Chan-Tack KM (1999) Neuroleptic malignant syndrome due to promethazine. South Med J 92:1017-1018.

Cuevas P, Fine J (1972) Route of absorption of endotoxin from the intestine in nonseptic shock. Journal of the Reticuloendothelial Society 11:535-538.

Ebadi M, Pfeiffer RF, Murrin LC (1990) Pathogenesis and treatment of neuroleptic malignant syndrome. Gen Pharmacol 21:367-386.

Eliasson E, Kenna JG (1996) Cytochrome P450 2E1 is a cell surface autoantigen in halothane hepatitis. Mol Pharmacol 50:573-582.

Elizalde-Sciavolino C, Racco A, Proscia-Lieto T, Kleiner M (1998) Severe hyponatremia, neuroleptic malignant syndrome, rhabdomyolysis and acute renal failure: a case report. Mt Sinai J Med 65:284-288.

Ellis FP (1977) Heat illness. II. Pathogenesis. Trans R Soc Trop Med Hyg 70:412-418.

Emerson TE, Jr., Fournel MA, Leach WJ, Redens TB (1987) Protection against disseminated intravascular coagulation and death by antithrombin III in the *Escherichia coli* endotoxemic rat. Circ.Shock 21:1-13.

Fleischhacker WW, Roth SD, Kane JM (1990) The pharmacologic treatment of neuroleptic-induced akathisia. J Clin Psychopharmacol 10:12-21.

Friedmann PS, Strickland I, Pirmohamed M, Park BK (1994) Investigation of mechanisms in toxic epidermal necrolysis induced by carbamazepine. Arch Dermatol 130:598-604.

Furst SM, Luedke D, Gandolfi AJ (1997) Kupffer cells from halothane-exposed guinea pigs carry trifluoroacetylated protein adducts. Toxicology 120:119-132.

Furst SM, Luedke D, Gaw HH, Reich R, Gandolfi AJ (1997) Demonstration of a cellular immune response in halothane-exposed guinea pigs. Toxicol Appl Pharmacol 143:245-255.

Gabow PA, Kaehny WD, Kelleher SP (1982) The spectrum of rhabdomyolysis. Medicine (Baltimore) 61:141-152.

Gadina M, Bertini R, Mengozzi M, Zandalasini M, Mantovani A, Ghezzi P (1991) Protective effect of chlorpromazine on endotoxin toxicity and TNF production in glucocorticoid-sensitive and glucocorticoid-resistant models of endotoxic shock. J Exp Med 173:1305-1310.

Ganapathi R, Grabowski D, Turinic R, Valenzuela R (1984) Correlation between potency of calmodulin inhibitors and effects on cellular levels and cytotoxic activity of doxorubicin (adriamycin) in resistant P388 mouse leukemia cells. Eur J Cancer Clin Oncol 20:799-806.

Gans H, Matsumoto K (1974) The escape of endotoxin from the intestine. Surg.Gynecol.Obstet. 139:395-402.

Gathiram P, Gaffin SL, Brock-Utne JG, Wells MT (1987) Time course of endotoxemia and cardiovascular changes in heat-stressed primates. Aviat Space Environ Med 58:1071-1074.

Gebhart WF, Van Ommen RA, McCormack LJ, Brown CH (1958) Chlorpromazine jaundice. Arch.Intern.Med. 101:1085-1093.

Gelenberg AJ, Bellinghausen B, Wojcik JD, Falk WE, Sachs GS (1988) A prospective survey of neuroleptic malignant syndrome in a short-term psychiatric hospital. Am J Psychiatry 145:517-518.

Ghatak NR, Erenberg G, Hirano A, Golden GS (1973) Idiopathic rhabdomyolysis in children. J Neurol Sci 20:253-268.

Ghezzi P, Garattini S, Mennini T, Bertini R, Delgado HR, Benigni F, Sacco S, Skorupska M, Mengozzi M, Latini R, Kurosaki M, Lombet A, Fradin A, Bonnet J, Rolland Y, Brion JD (1996) Mechanism of inhibition of tumor necrosis factor production by chlorpromazine and its derivatives in mice. Eur J Pharmacol 317:369-376.

Gold H, Rosenberg F, Campbell W (1955) Chlorpromazine (Thorazine) hepatitis: Report of three cases. Annals of Internal Medicine 43:745-757.

Gomez J-L, Dupont A, Cusan L, Tremblay M, Suburu R, Lemay M, Labrie B (1992) Incidence of liver toxicity associated with the use of flutamide in prostate cancer patients. The American Journal of Medicine 92:465-470.

Grant DJ, Bell DA (2000) Bilirubin UDP-glucuronosyltransferase 1A1 gene polymorphisms: Susceptibility to oxidative damage and cancer? Mol Carcinog 29:198-204.

Grant DM, Hughes NC, Janezic SA, Goodfellow GH, Chen HJ, Gaedigk A, Yu VL, Grewal R (1997) Human acetyltransferase polymorphisms. Mutat Res 376:61-70.

Gronhagen-Riska C, Hellstrom PE, Froseth B (1978) Predisposing factors in hepatitis induced by isoniazid-rifampin treatment of tuberculosis. Am Rev Respir Dis 118:461-466.

Gurumurthy P, Krishnamurthy MS, Nazareth O, Parthasarathy R, Sarma GR, Somasundaram PR, Tripathy SP, Ellard GA (1984) Lack of relationship between hepatic toxicity and acetylator phenotype in three thousand South Indian patients during treatment with isoniazid for tuberculosis. Am Rev Respir Dis 129:58-61.

Gut J (1998) Molecular basis of halothane hepatitis. Arch Toxicol Suppl 20:3-17.

Hamilton RW, Gardner LB, Penn AS, Goldberg M (1972) Acute tubular necrosis caused by exercise-induced myoglobinuria. Ann Intern Med 77:77-82.

Hastings KL, Thomas C, Brown AP, Gandolfi AJ (1995) Trifluoroacetylation potentiates the humoral immune response to halothane in the guinea pig. Immunopharmacol Immunotoxicol 17:201-213.

Hewett JA, Roth RA (1993) Hepatic and extrahepatic pathobiology of bacterial lipopolysaccharides. Pharmacol Rev 45:382-411.

Hill GR, Crawford JM, Cooke KR, Brinson YS, Pan L, Ferrara JL (1997) Total body irradiation and acute graft-versus-host disease: the role of gastrointestinal damage and inflammatory cytokines. Blood 90:3204-3213.

Hitchcock PJ, Leive L, Makela PH, Rietschel ET, Strittmatter W, Morrison DC (1986) Lipopolysaccharide nomenclature--past, present, and future. J Bacteriol 166:699-705.

Hodges HH, La Zerte GB (1955) Jaundice and agranulocytosis with fatality following chlorpromazine therapy. JAMA 158:114-116.

Hollister LE (1957) Allergy to chlorpromazine manifested by jaundice. Am J Med 23:870

Hruban Z, Tavoloni N, Reed JS, Boyer JL (1978) Ultrastructural changes during cholestasis induced by chlorpromazine in the isolated perfused rat liver. Virchows Arch B Cell Pathol 26:289-305.

Hubacek JA, Stuber F, Frohlich D, Book M, Wetegrove S, Rothe G, Schmitz G (2000) The common functional C(-159)T polymorphism within the promoter region of

the lipopolysaccharide receptor CD14 is not associated with sepsis development or mortality. Genes Immun 1:405-407.

Ishak KG, Irey NS (1972) Hepatic injury associated with the phenothiazines. Clinicopathologic and follow-up study of 36 patients. Arch Pathol 93:283-304.

Jansen MJ, Hendriks T, Knapen MF, van Kempen LC, van der Meer JW, Goris RJ (1998) Chlorpromazine down-regulates tumor necrosis factor-alpha and attenuates experimental multiple organ dysfunction syndrome in mice [see comments]. Crit Care Med 26:1244-1250.

Jardin F, Gurdjian F, Delille F, Margairaz A (1979) Pulmonary hypertension in the adult respiratory distress syndrome (ARDS) [letter]. Intensive Care Med 5:155-156.

Jersmann HP, Rathjen DA, Ferrante A (1998) Enhancement of lipopolysaccharide-induced neutrophil oxygen radical production by tumor necrosis factor alpha. Infect Immun 66(4):1744-1747.

Johnson D, Mayers I (2001) Multiple organ dysfunction syndrome: a narrative review. Canadian Journal of Anesthesia 502-509.

Johnson EI, Lanford RE, Solomon K (1979) Chlorpromazine, eosinophilia, and hepatotoxicity. Va Med 106:683-684.

Joseph JK, Thomas K (1991) Lithium toxicity--a risk factor for neuroleptic malignant syndrome. J Assoc Physicians India 39:572-573.

Jung K, Neumann R, Cobet G, Nugel E, Egger E (1979) Creatine kinase isoenzyme BB in serum of healthy adults and children. Clin Chim Acta 91:165-168.

Jung K, Neumann R, Lun A, Pohle R, Nugel E (1980) Increased creatine kinase BB activity in rat plasma induced by hypoxia. Enzyme 25:60-63.

Kantor RJ, Carson LA, Graham DR, Petersen NJ, Favero MS (1983) Outbreak of pyrogenic reactions at a dialysis center. Association with infusion of heparinized saline solution. Am J Med 74:449-456.

Keck PEJ, Caroff SN, McElroy SL (1995) Neuroleptic malignant syndrome and malignant hyperthermia: end of a controversy? J Neuropsychiatry Clin Neurosci 7:135-144.

Keck PEJ, Seeler DC, Pope HGJ, McElroy SL (1990) Porcine stress syndrome: an animal model for the neuroleptic malignant syndrome? Biol Psychiatry 28:58-62.

Kendra JR, Rugman FP, Flaherty TA, Myers A, Horsfield N, Barton A, Russell L (1993) First use of G-CSF in chlorpromazine-induced agranulocytosis: a report of two cases. Postgrad Med J 69:885-887.

Kenna JG, Neuberger J, Williams R (1984) An enzyme-linked immunosorbent assay for detection of antibodies against halothane-altered hepatocyte antigens. J Immunol Methods 75:3-14.

Kenna JG, Neuberger J, Williams R (1988) Evidence for expression in human liver of halothane-induced neoantigens recognized by antibodies in sera from patients with halothane hepatitis. Hepatology 8:1635-1641.

Knochel JP (1993) Mechanisms of rhabdomyolysis. Curr Opin Rheumatol 5:725-731.

Koizumi T, Nomura H, Kobayashi T, Hayano T, Kubo K, Sekiguchi M (1996) Fatal rhabdomyolysis during mountaineering. J Sports Med Phys Fitness 36:72-74.

Larrey D, Amouyal G, Pessayre D, Degott C, Danne O, Machayekhi JP, Feldmann G, Benhamou JP (1988) Amitriptyline-induced prolonged cholestasis. Gastroenterology 94:200-203.

Larrey D, Erlinger S (1988) Drug-induced cholestasis. Baillieres Clin Gastroenterol 2:423-452.

Lau LL, Halliday MI, Lee B, Hannon RJ, Gardiner KR, Soong CV (2000) Intestinal manipulation during elective aortic aneurysm surgery leads to portal endotoxaemia and mucosal barrier dysfunction. Eur J Vasc Endovasc Surg 19:619-624.

Lee JW (1998) Serum iron in catatonia and neuroleptic malignant syndrome. Biol Psychiatry 44:499-507.

Lemire RE, Mitchell RA (1954) Regurgitation type jaundice associated with chlorpromazine ("THORAZINE, " SKF 2601-A) administration. Laboratory and Clinical Medicine 44:825

Levenson JL (1985) Neuroleptic malignant syndrome. Am J Psychiatry 142:1137-1145.

Levine RA, Briggs GW, Lowell DA (1966) Chronic chlorpromazine cholangiolitic hepatitis. Gastroenterology 50:665-670.

Lind RC, Gandolfi AJ, Hall PD (1989) The role of oxidative biotransformation of halothane in the guinea pig model of halothane-associated hepatotoxicity. Anesthesiology 70:649-653.

Litvak R, Kaelbling R (1971) Agranulocytosis, leukopenia, and psychotropic drugs. Arch Gen Psychiatry 24:265-267.

Loftus LR, Huizenga KA, Stauffer MH, Rome H.P. (1955) Jaundice caused by chlorpromazine (Thorazine). JAMA 157:1286-1288.

Lomas J, Boardman RH, Markowe M (1955) Complications of chlorpromazine therapy in 800 mental-hospital patients. Lancet 1:1144-1147.

Marsden CD, Jenner P (1980) The pathophysiology of extrapyramidal side-effects of neuroleptic drugs. Psychol Med 10:55-72.

Martin MA, Silverman HJ (1992) Gram-negative sepsis and the adult respiratory distress syndrome. Clin.Infect.Dis. 14(6):1213-1228.

Mayeux PR (1997) Pathobiology of lipopolysaccharide. J Toxicol Environ Health 51:415-435.

McCarthy TV, Quane KA, Lynch PJ (2000) Ryanodine receptor mutations in malignant hyperthermia and central core disease. Hum Mutat 15:410-417.

McHardy G, McHardy R, Canale S (1955) Chlorpromazine (THORAZINE) hepatitis. Gastroenterology 29:184-188.

McLain GE, Sipes IG, Brown BR (1979) An animal model of halothane hepatotoxicity: roles of enzyme induction and hypoxia. Anesthesiology 51:321-326.

Meltzer HY, Cola PA, Parsa M (1996) Marked elevations of serum creatine kinase activity associated with antipsychotic drug treatment. Neuropsychopharmacology 15:395-405.

Michalets EL, Williams CR (2000) Drug interactions with cisapride: clinical implications [In Process Citation]. Clin Pharmacokinet 39:49-75.

Mitchell R.N., Cotran RS (1997) Acute and Chronic Inflammation. In: Basic Pathology (Kumar V, Robbins S, Cotran RS eds), pp 25Philadelphia: W.B. Saunders Co.

Mitchell JR, Zimmerman HJ, Ishak KG, Thorgeirsson UP, Timbrell JA, Snodgrass WR, Nelson SD (1976) Isoniazid liver injury: clinical spectrum, pathology, and probable pathogenesis. Ann Intern Med 84:181-192.

Miyatake R, Iwahashi K, Matsushita M, Nakamura K, Suwaki H (1996) No association between the neuroleptic malignant syndrome and mutations in the RYR1 gene associated malignant hyperthermia. J Neurol Sci 143:161-165.

Moradpour D, Altorfer J, Flury R, Greminger P, Meyenberger C, Jost R, Schmid M (1994) Chlorpromazine-induced vanishing bile duct syndrome leading to biliary cirrhosis. Hepatology 20:1437-1441.

Moser V.C. (1999) Neurobehavioral Screening in Rodents. In: Current Protocols in Toxicology (Maines M.D., Costa L.G., Reed DJ, Sassa S., Sipes I.G. eds), pp 11.2.1New York: John Wiley & Sons, Inc.

Movitt ER, Meyer MA, Snell AM, Goldman MJ, Gibson JR, Gibson J.R., Sullivan BH, Webster JG, Stone RB (1955) Jaundice associated with the administration of chlorpromazine, SKF-2601-A (THORAZINE). Gastroenterology 28:901-913.

Mozes T, Zijlstra FJ, Heiligers JPC, Tak CJAM, Ben-Efraim S, Bonta IL, Saxena PR (1991) Sequential release of tumour necrosis factor, platelet activating factor and eicosanoids during endotoxin shock in anaesthetized pigs; protective effects of indomethacin. Br.J.Pharmac. 104:691-699.

Mullock BM, Hall DE, Shaw LJ, Hinton RH (1983) Immune responses to chlorpromazine in rats. Detection and relation to hepatotoxicity. Biochem Pharmacol 32:2733-2738.

Neuschwander-Tetri BA, Isley WL, Oki JC, Ramrakhiani S, Quiason SG, Phillips NJ, Brunt EM (1998) Troglitazone-induced hepatic failure leading to liver transplantation. A case report [see comments]. Ann Intern Med 129:38-41.

Nowotny A (1987) Review of the molecular requirements of endotoxic actions. Rev Infect Dis 9 Suppl 5:S503-11:S503-S511

O'Connor M (1980) Muscle necrosis induced by intramuscular chlorpromazine. Med J Aust 1:36

Ohlsson K, Bjork P, Bergenfeldt M, Hageman R, Thompson RC (1990) Interleukin-1 receptor antagonist reduces mortality from endotoxin shock. Nature 348:550-552.

Park RH, Jones H, O'Donnell JG, Griffen JS (1987) The neuroleptic malignant syndrome. Scott Med J 32:153

Pearlman C, Wheadon D, Epstein S (1988) Creatine kinase elevation after neuroleptic treatment. Am J Psychiatry 145:1018-1019.

Pearson JM, Schultze AE, Jean PA, Roth RA (1995) Platelet participation in liver injury from gram-negative bacterial lipopolysaccharide in the rat. Shock 4:178-186.

Pelner L, Waldman S (1955) Chlorpromazine-induced jaundice. Postgrad Med 18:349-353.

Pelonero AL, Levenson JL, Pandurangi AK (1998) Neuroleptic malignant syndrome: a review [see comments]. Psychiatr Serv 49:1163-1172.

Piquette RK (1999) Torsade de pointes induced by cisapride/clarithromycin interaction. Ann Pharmacother 33:22-26.

Pisciotta AV (1990) Drug-induced agranulocytosis. Peripheral destruction of polymorphonuclear leukocytes and their marrow precursors. Blood Rev 4:226-237.

Poels PJ, Gabreels FJ (1993) Rhabdomyolysis: a review of the literature. Clin Neurol Neurosurg 95:175-192.

Pohl LR, Thomassen D, Pumford NR, Butler LE, Satoh H, Ferrans VJ, Perrone A, Martin BM, Martin JL (1991) Hapten carrier conjugates associated with halothane hepatitis. Adv Exp Med Biol 283:111-120.

Poolsup N, Li WP, Knight TL (2000) Pharmacogenetics and psychopharmacotherapy. J Clin Pharm Ther 25:197-220.

Price EJ, Venables PJ (1995) Drug-induced lupus. Drug Saf 12:283-290.

Quismorio FP, Bjarnason DF, Kiely WF, Dubois EL, Friou GJ (1975) Antinuclear antibodies in chronic psychotic patients treated with chlorpromazine. Am J Psychiatry 132:1204-1206.

Qureshi ST, Gros P, Malo D (1999) The Lps locus: genetic regulation of host responses to bacterial lipopolysaccharide. Inflamm Res 48:613-620.

Raetz CRH, Brozek KA, Clementz T, Coleman JD, Galloway SM, Golenbock DT, Hampton RY (1988) Gram-negative endotoxin: A biologically active lipid. Cold Spring Harbor Symposia on Quantitative Biol. LIII:973-982.

Ray JG (1997) Neuroleptic malignant syndrome associated with severe thrombocytopenia. J Intern Med 241:245-247.

Read AE, Harrison CV, Sherlock S (1961) Chronic chlorpromazine jaundice. AJMA 31:249-258.

Redeker AG, Balfour DC (1955) Jaundice associated with chlorpromazine (THORAZINE) therapy. Gastroenterology 29:882-888.

Regal RE, Billi JE, Glazer HM (1987) Phenothiazine-induced cholestatic jaundice. Clin Pharm 6:787-794.

Rosebush PI, Mazurek MF (1991) Serum iron and neuroleptic malignant syndrome [see comments]. Lancet 338:149-151.

Rosman AS, Frissora-Rodeo C, Marshall AT, Reiter BP, Paronetto F (1993) Cholestatic hepatitis following flutamide. Digest.Dis.Sci. 38(9):1756-1759.

Roth RA, Harkema JR, Pestka JP, Ganey PE (1997) Is exposure to bacterial endotoxin a determinant of susceptibility to intoxication from xenobiotic agents? Toxicol Appl Pharmacol 147:300-311.

Roth T, Roehrs TA (1991) A review of the safety profiles of benzodiazepine hypnotics. J Clin Psychiatry 52 Suppl:38-41.

Rotimi VO, Verghese TL, Al-Sweih N, Khodakhast FB, Ahmed K (2000) Influence of five antianaerobic antibiotics on endotoxin liberation by gram-negative anaerobes. J Chemother 12:40-47.

Rush BF, Redan JA, Flanagan JJ, Heneghan JB, Hsieh J, Murphy TF, Smith S, Machiedo GW (1989) Does the bacteremia observed in hemorrhagic shock have clinical significance? A study in germ-free animals. Ann Surg 210:342-345.

Russell RI, Allan JG, Patrick R (1973) Active chronic hepatitis after chlorpromazine ingestion. Br Med J 1:655-656.

Rylander R (1999) Health effects among workers in sewage treatment plants. Occup Environ Med 56:354-357.

Salzer WL, McCall CE (1990) Primed stimulation of isolated perfused rabbit lung by endotoxin and platelet activating factor induces enhanced production of thromboxane and lung injury. J Clin Invest 85:1135-1143.

Scheibel LW, Colombani PM, Hess AD, Aikawa M, Atkinson CT, Milhous WK (1987) Calcium and calmodulin antagonists inhibit human malaria parasites (Plasmodium falciparum): implications for drug design. Proc Natl Acad Sci U S A 84:7310-7314.

Schlieffenbaum B, Fehr J, Odermatt B, Sperb R (1998) Inhibition of leukocyte emigration induced during the systemic inflammatory reaction in vivo is not due to IL-8. J Immunol 161(7):3631-3638.

Schotte A, Janssen PF, Gommeren W, Luyten WH, Van Gompel P, Lesage AS, De Loore K, Leysen JE (1996) Risperidone compared with new and reference antipsychotic drugs: in vitro and in vivo receptor binding. Psychopharmacology (Berl) 124:57-73.

Schulze VEJ (1982) Rhabdomyolysis as a cause of acute renal failure. Postgrad Med 72:145-148.

Shay H, Siplet H (1958) Relationship of chemical structure of chlorpromazine to its liver-sensitizing action. Gastroenterology 35:16-24.

Siadat-Pajouh M, Hubbard AK, Roth TP, Gandolfi AJ (1987) Generation of halothane-induced immune response in a guinea pig model of halothane hepatitis. Anesth Analg 66:1209-1214.

Sneed RA, Grimes SD, Schultze AE, Brown AP, Ganey PE (1997) Bacterial endotoxin enhances the hepatotoxicty of allyl alcohol. Toxicol.Appl.Pharmacol. 144:77-87.

Srinivasan R, Buchweitz JP, Ganey PE (1997) Alteration by flutamide of neutrophil response to stimulation. Implications for tissue injury. Biochem.Pharmacol. 53:1179-1185.

Steen VD, Ramsey-Goldman R (1988) Phenothiazine-induced systemic lupus erythematosus with superior vena cava syndrome: case report and review of the literature. Arthritis Rheum 31:923-926.

Sterner S (1990) Summer heat illnesses. Conditions that range from mild to fatal [see comments]. Postgrad Med 87:67-70, 73.

Stevenson DK, Christie DL, Haas JE (1978) Hepatic injury in a child caused by trimethoprim-sulfamethoxazole. Pediatrics 61:864-866.

Surmont DW, Colardyn F, De Reuck J (1984) Fatal complications of neuroleptic drugs. A clinico-pathological study of three cases. Acta Neurol Belg 84:75-83.

Taniguchi N, Tanii H, Nishikawa T, Miyamae Y, Shinozaki K, Inoue Y, Morihara T, Nakano Y, Nishimura T, Takeda M (1997) Classification system of complications in neuroleptic malignant syndrome. Methods Find Exp Clin Pharmacol 19:193-199.

Tanii H, Taniguchi N, Niigawa H, Hosono T, Ikura Y, Sakamoto S, Kudo T, Nishimura T, Takeda M (1996) Development of an animal model for neuroleptic malignant syndrome: heat-exposed rabbits with haloperidol and atropine administration exhibit increased muscle activity, hyperthermia, and high serum creatine phosphokinase level. Brain Res 743:263-270.

Tonda ME, Guthrie SK (1994) Treatment of acute neuroleptic-induced movement disorders. Pharmacotherapy 14:543-560.

Ueda M, Hamamoto M, Nagayama H, Otsubo K, Nito C, Miyazaki T, Terashi A, Katayama Y (1999) Susceptibility to neuroleptic malignant syndrome in Parkinson's disease. Neurology 52:777-781.

Uetrecht JP, Woosley RL (1981) Acetylator phenotype and lupus erythematosus. Clin Pharmacokinet 6:118-134.

Van Ommen RA, Brown CH (1955) Obstructive-type jaundice due to chlorpromazine (THORAZINE). JAMA 157:321-325.

Vergani D, Mieli-Vergani G, Alberti A, Neuberger J, Eddleston AL, Davis M, Williams R (1980) Antibodies to the surface of halothane-altered rabbit hepatocytes in patients with severe halothane-associated hepatitis. N Engl J Med 303:66-71.

Weiss B, Prozialeck WC, Wallace TL (1982) Interaction of drugs with calmodulin. Biochemical, pharmacological and clinical implications. Biochem Pharmacol 31:2217-2226.

Wells CL, Maddaus MA, Simmons RL (1988) Proposed mechanisms for the translocation of intestinal bacteria. Rev Infect Dis 10:958-979.

Werther JL, Korelitz BI (1957) Chlorpromazine Jaundice: Analysis of Twenty-two Cases. AJMA 22:351-366.

White DA, Robins AH (1991) Catatonia: harbinger of the neuroleptic malignant syndrome [see comments]. Br J Psychiatry 158:419-421.

Worman HJ, Ip JH, Winters SL, Tepper DC, Gomes AJ (1992) Hypersensitivity reaction associated with acute hepatic dysfunction following a single intravenous dose of procainamide. J Intern Med 232:361-363.

Yee LJ, Tang J, Herrera J, Kaslow RA, van Leeuwen DJ (2000) Tumor necrosis factor gene polymorphisms in patients with cirrhosis from chronic hepatitis C virus infection. Genes Immun 1:386-390.

Yee SB, Kinser S, Hill DA, Barton CC, Hotchkiss JA, Harkema JR, Ganey PE, Roth RA (2000) Synergistic Hepatotoxicity from Coexposure to Bacterial Endotoxin and the Pyrrolizidine Alkaloid Monocrotaline. Toxicol.Appl.Pharmacol. 166:173-185.

Zimmerman HJ (1968) The differential diagnosis of jaundice. Med Clin North Am 52:1417-1444.

Zimmerman HJ (1981) Drug-induced liver disease: an overview. Semin Liver Dis 1:93-103.

Zimmerman HJ (1997) Effects of Chlorpromazine on the Liver. In: Comprehensive Toxicology (Sipes G, McQueen CA, Gandolfi AJ eds), pp 345Oxford: Elsevier Science Ltd.

Zimmerman HJ, Lewis JH (1987) Drug-induced cholestasis. Med Toxicol 2:112-160.