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Inflammation and Drug Idiosyncrasy—Is There a Connection?

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Abstract

“Drug idiosyncrasy” refers to untoward reactions to drugs that occur in a small fraction of patients and have no obvious relationship to dose or duration of therapy. The liver is a frequent target for toxicity. Much of the conventional thinking about mechanisms of drug idiosyncrasy has centered on hypotheses that the reactions have a metabolic basis involving drug metabolism polymorphisms or that they arise from a specific immune response to the drug or its metabolite(s). However, for very few drugs does convincing evidence exist for either of these mechanisms. The erratic temporal and dose relationships that characterize idiosyncratic drug responses suggest the possibility that some event during the course of therapy renders tissues peculiarly susceptible to toxic effects of the drug. For example, episodes of inflammation are commonplace in people, and results of numerous studies in animals indicate that a modest inflammatory response can enhance tissue sensitivity to a variety of toxic chemicals. These observations have led to the hypothesis that an episode of inflammation during drug therapy could decrease the threshold for drug toxicity and thereby render an individual susceptible to a toxic reaction that would not otherwise occur (i.e., an “idiosyncratic” response). This hypothesis can explain the features of drug idiosyncrasy using fundamental pharmacologic principles, and results of recent animal studies are supportive. Knowledge gaps that need to be filled before the hypothesis should be widely accepted are discussed.

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Idiosyncratic Reactions to Drugs. Adverse drug reactions are an important source of morbidity and mortality in people. For example, a meta-analysis of 39 prospective studies revealed over 2 million cases of hospitalization and more than 100,000 deaths due to adverse drug reactions in the U.S. in 1994 (Lazarou et al., 1998). Idiosyncratic responses to drugs are one type of adverse drug reaction. For purposes of this commentary, “drug idiosyncrasy” is defined as an adverse reaction that does not arise from drug-drug interaction and that meets several criteria. Unlike typical toxic responses to xenobiotic agents, which are dose-related and unfold in a characteristic temporal pattern, idiosyncratic drug reactions (a) occur in a small fraction of people exposed to the drug (usually <5%), (b) are typically unrelated to the drug’s pharmacologic effect, (c) demonstrate no obvious relation to dose and (d) occur with inconsistent temporal patterns in relation to drug exposure (Zimmerman, 1993). Since these reactions occur with low incidence in animals as they do in humans, they are not usually predicted from results of preclinical testing in which relatively small groups of animals are used. Some patients who respond idiosyncratically do so after the first or second administration of the drug, whereas others require weeks or months of therapy. Although idiosyncratic reactions can involve numerous tissues, the liver is commonly a target.

Idiosyncratic drug reactions have obvious importance to human health. The type and severity of the reactions vary with the drug and the affected individual, but some result in permanent disability or death. In addition to having direct effects on human health, these adverse drug reactions have resulted in removal of otherwise efficacious drugs from the market which had the potential to improve human health and reduce suffering. Because of the infrequency of their occurrence and the lack of animal models for preclinical evaluation, idiosyncratic reactions are typically not discovered until a drug is in phase 2 clinical trials or actually on the market and being widely used by people. If animal models existed that could predict some idiosyncratic reactions, these could be used to identify potential problems earlier

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and to direct appropriate preventive actions. The latter might include additional monitoring for specific reactions during clinical trials, research to understand mechanisms that could lead to strategies to prevent, minimize or treat the idiosyncratic reaction, and consideration of related, alternative drug candidates for development.

Many drugs precipitate idiosyncratic toxicities. As an example, antipsychotic drugs such as chlorpromazine and clozapine cause infrequent reactions such as rhabdomyolysis (breakdown of striated muscle) and liver toxicity (Ishak and Irey, 1972; Caroff, 1980; Nankivell et al., 1994; Goldman, 1996). These and other reactions may occur together or independently in a responding patient. The bizarre nature of these reactions is illustrated in a case of a patient treated with clozapine (Meltzer et al., 1996). After several weeks of uneventful maintenance therapy, he developed a pronounced increase in serum creatine kinase activity (suggesting rhabdomyolysis) and cholestatic liver injury, as evidenced by an increase in serum gamma-glutamyl transferase (GGT) activity. When clozapine therapy was discontinued, both of these markers of tissue injury returned to normal. Interestingly, when therapy was reinstated several weeks later, the toxic reaction did not return. This seemingly erratic relationship between onset of an untoward response and drug exposure is typical of idiosyncratic reactions.

An example of a drug that caused life-threatening, idiosyncratic reactions in patients is troglitazone. Mild, reversible hepatotoxicity from this antidiabetic drug appeared in premarketing clinical trials in <2% of patients. However, after the drug appeared on the market in early 1997, several cases of severe, even fatal liver failure emerged (e.g., Fukano et al., 2000; Murphy et al., 2000). These cases forced the withdrawal of the drug from the market in 2000. Patients experiencing liver failure had elevated serum enzymes and bilirubin as well as other clinical signs of hepatocellular necrosis and cholestasis. These signs appeared at widely varying times after onset of drug therapy. Livers from affected patients had necrosis and inflammatory infiltrates, including neutrophil (PMN) accumulation. Because of the idiosyncratic nature of the

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hepatotoxicity (i.e., occurring in a small minority of patients and with no obvious relationship to dose) and the lack of understanding of mechanisms, “it is not currently possible to predict which patients treated with the drug will develop liver injury” (Murphy et al., 2000).

Modes and Mechanisms: Conventional Wisdom. Much of the conventional thinking about mechanisms of drug idiosyncrasy has centered on hypotheses that the reactions have a metabolic basis involving drug metabolism polymorphisms or that they arise from a specific immune response to the drug or its metabolite(s). For a few drugs, evidence exists for these as underlying causes of idiosyncrasy. An example is the proposed importance of acetylation polymorphism in the toxic responses to hydrazines and aromatic amine drugs, such as isoniazid (INH; Stevens et al., 1999; Weber, 1999). INH causes hepatotoxicity in <10% of patients taking the drug. Human polymorphisms in INH acetylation were recognized over 40 years ago, and results of initial epidemiological studies suggested that rapid acetylators of INH were more susceptible to INH hepatotoxicity (reviewed by Weber et al., 1983). Studies in animals, which identified acetylhydrazine as a hepatotoxic metabolite, seemed to support a role for acetylation polymorphism as the determinant of sensitivity to INH hepatotoxicity. However, several subsequent epidemiological studies failed to confirm the initial link between rapid acetylation status and INH hepatotoxicity in humans (Singapore, 1977; Gurumurthy et al., 1984). Accordingly, although INH acetylation is likely to be causally involved in its hepatotoxicity, it appears that other factors are necessary to precipitate an idiosyncratic reaction in people.

Similarly, frequent speculation about a specific immune response (e.g., antigen-antibody reaction) directly damaging liver or other tissues as an explanation for drug idiosyncrasy is without convincing support for all but a few drugs. This hypothesis has become a “default assumption,” typically based on the lack of obvious relationship of the reaction to drug dose and on observations that some patients don’t experience reactions until they have been treated with the drug for a period long enough to develop antibodies. For a few drugs, autoantibodies to

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drug-protein adducts have been detected in serum of patients, but the role of these in causing idiosyncratic reactions remains questionable (Kitteringham et al., 1995). Halothane hepatotoxicity is one of the most widely studied reactions. It is clear that halothane is metabolized to reactive species that bind covalently to liver proteins and that humans generate antibodies to these altered proteins (Njoku et al., 2002); however, many halothane-exposed individuals develop antibodies but do not experience hepatitis. Such findings have raised doubt about whether such autoantibodies have a pathological role in hepatitis induced by halothane and other volatile anesthetics (Njoku et al., 2002). In some patients reacting to certain drugs, the occurrence of antinuclear or antimitochondrial antibodies in plasma has been taken as evidence of immune-mediated hypersensitivity to drugs, but whether these antibodies actually cause tissue injury or arise as a response to cellular destruction caused by other mechanisms is not known. An excellent discussion of issues that are unresolved and sometimes in conflict with a specific immune response as a direct, underlying cause of drug idiosyncrasy appears in a commentary by Uetrecht (Uetrecht, 1999).

For most drugs, claims as to either metabolic polymorphism or allergy being the underlying cause of idiosyncratic hepatotoxicity are largely speculation with incomplete or no support. Returning to troglitazone as an example, there have been speculations about immunological hypersensitivity and metabolic polymorphisms as origins for the severe, hepatotoxic reactions, but the mechanism remains unknown. The liver pathology and laboratory tests in some patients do not support immunological hypersensitivity as a cause of troglitazone-induced liver failure (Fukano et al., 2000), and there is no convincing evidence that metabolism of troglitazone contributes to the pathogenesis *in vivo*. For many drugs, the clinical literature is replete with case reports in which one or both of these mechanisms is invoked as an explanation, but with no supporting evidence. Thus, animal models that could reproduce the idiosyncratic effects of even some drugs or drug classes could increase our understanding;

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however, because [a] dose and temporal relationships have been difficult to define, [b] reactions occur in a small fraction of people, [c] virtually nothing is known about mechanisms and [d] the responses are similarly infrequent in animals, animal models that predict idiosyncratic responses are largely lacking.

Zimmerman (1993) proposed years ago that interplay between intrinsic biological effects of a drug and host vulnerability might precipitate some idiosyncratic reactions. “Host vulnerability” is unlikely to be constant within any individual. Indeed, the erratic temporal and dose relationships that characterize drug idiosyncrasy suggest the possibility that some event during the course of therapy renders tissues particularly susceptible to toxic effects of the drug. If so, then the precipitating event must happen occasionally and irregularly to account for the infrequent and erratic occurrence of idiosyncratic reactions.

Inflammatory infiltrates, including neutrophils and other leukocytes, often characterize liver lesions in patients who suffer idiosyncratic drug reactions (e.g., see Khouri et al., 1987; Murphy et al., 2000; Fukano et al., 2000). This and other characteristics of idiosyncrasy led Uetrecht to propose that necrosis or cell stress imposed by reactive drug metabolites provides a “danger signal” that activates macrophages or other cells to produce cytokines required for an antibody- or T-cell-mediated specific immune responses (Uetrecht, 1999). In this paradigm, the innate immune system (ie, inflammation) is proposed as a necessary factor to precipitate a damaging specific immune response. However, studies in animals (see below) have revealed that mild inflammation can enhance the sensitivity of the liver to chemically induced damage without invoking a specific immune response. These observations raised to us the possibility that at least some idiosyncratic drug reactions may be explained by episodes of modest inflammation that occur during the course of therapy. The balance of this perspectives article will expand on this idea with a short discussion of episodes of inflammation, their ability to enhance hepatotoxic responses and how these observations have led to an alternative

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hypothesis about the origin of idiosyncratic drug responses.

Episodic Exposure to Inflammagens. Inflammatory episodes occur commonly in people and animals. Conditions that are associated with inflammation include arthritis, atherosclerosis, asthma and many other diseases, infection due to bacteria or viruses, specific immune responses to antigens, and exposure to toxins elaborated by microorganisms. Many bacterial and viral products can precipitate inflammatory responses. Of these, we will focus below on endotoxin produced by gram-negative bacteria, since it seems to be a particularly important inflammagen and is one which we and others have used experimentally to induce inflammation. Endotoxin is released from bacteria when they divide or are damaged by antibiotics or other factors. Bacterial infection is an obvious source of systemic endotoxin exposure; however, modest exposure also occurs when it is released into the intestinal lumen by indigenous, gram-negative flora and translocates across the intestinal mucosa into the portal venous circulation. Research in humans over the last couple of decades has revealed that mild endotoxemia is a normal but episodic occurrence in people and that numerous conditions enhance endotoxin concentrations in the plasma (illustrated schematically in Fig. 1A). These conditions include alterations in diet, alcohol consumption, gastrointestinal distress or disease, liver disease, anesthesia, surgical trauma, exposure to xenobiotic agents and others (reviewed by Roth et al., 1997). The magnitude of exposure from intestinal translocation is typically too small to cause overt illness but may be sufficient to initiate a modest inflammatory response in tissues that includes influx of inflammatory cells and release of proinflammatory mediators.

Endotoxin and Inflammation. The principal, biologically active component of endotoxin is lipopolysaccharide (LPS), a potent inflammagen. Recently, Toll-like receptors (TLRs) have been identified on mammalian inflammatory cells, at least one of which (TLR4) binds LPS to initiate signaling mechanisms that lead to stimulation of inflammatory cells, activation of transcription factors such as nuclear factor kappa-B (NFkB) and synthesis and release of

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numerous proinflammatory mediators. The latter include cytokines (e.g., tumor necrosis factor- α [TNF], interleukin-1, various chemokines), cyclooxygenase-2 (COX2) products and other lipid metabolites (e.g., prostaglandins, platelet activating factor), reactive oxygen species (e.g., superoxide, nitric oxide and their derivatives), toxic proteases (e.g., elastase, cathepsin G), etc. (Arbour et al., 2000; Beutler, 2000). These mediators can be similarly evoked upon exposure to inflammagens other than LPS. They are essential in defense against pathogens but are also capable of altering the homeostasis of host cells.

Enhancement of Hepatotoxicity by a Modest Inflammatory Response Induced by LPS. The hepatotoxicities of several xenobiotic agents are augmented by coexposure to LPS (Roth et al., 1997). In our studies in rats, we have typically used LPS doses that incite modest inflammation (e.g., cytokine and COX2 expression) but cause no tissue injury. For example, a small, noninjurious dose of LPS converts nontoxic doses of monocrotaline (MCT), a pyrrolizidine alkaloid plant toxin, into ones that are markedly hepatotoxic (Yee et al., 2000). Similar results occur with other hepatotoxicants that act by various mechanisms and produce different hepatic lesions. Our recent studies in rats indicate that the hepatotoxic effects of allyl alcohol and aflatoxin B₁ are markedly enhanced by coadministration of a small dose of LPS (Sneed et al., 1997; Barton et al., 2000a). In the case of aflatoxin B₁, the threshold for toxicity is decreased by more than 10-fold (Fig. 2); both the biliary injury (as marked by increased GGT in plasma) and the hepatocellular necrosis (as marked by increased alanine aminotransferase [ALT] in plasma) that this fungal toxin produces at large doses become apparent when a smaller, normally nontoxic dose is coadministered with LPS.

These and other results (see Roth et al., 1997) indicate clearly that the toxicities of several xenobiotic agents are enhanced by LPS administration. It is important to note, however, that this appears not to apply to all chemicals. For example, we have been unable in preliminary studies to enhance the hepatotoxicity of moniliformin by LPS coadministration

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(unpublished results). Thus, a small, nontoxic dose of LPS can increase the sensitivity of the liver to injury from some, but not all xenobiotic agents. It is noteworthy that LPS coadministration can influence tissue targets qualitatively. For example, deoxynivalenol (vomitoxin) typically produces dose-related toxicity in the GI tract and lymphoid tissue in rats; however, when a nontoxic dose is coupled with a small dose of LPS, the liver emerges as a target organ (unpublished results). The timing of exposures to toxicant and inflammagen can also influence targets for injury. When LPS was given either 4 hr before or 4 hr after a small dose of MCT, 85% of animals survived until 24 hrs and liver injury was apparent; by contrast, when the same doses of MCT and LPS were given simultaneously, animals began to die within 6 hr and only 30% survived until 24 hr (Yee et al., 2002). The animals appeared to expire from extrahepatic effects (possibly circulatory collapse), since the deaths were rapid and the liver injury was no worse than when the administrations were 4 hr apart. Accordingly, temporal differences in exposure may produce *qualitative* changes in tissue targets. Drug idiosyncrasy is similar in that not every human responder experiences the same type of adverse reaction to a drug.

It is clearly the inflammation-inducing property of LPS that is responsible for its ability to augment hepatotoxicity. The doses of LPS used in our studies produce little or no hepatic injury by themselves but do cause appearance of TNF in plasma as well as neutrophil accumulation and expression of mRNA for COX2 and TNF in liver (Barton et al., 2000b; Barton et al., 2001; Ganey et al., 2001). Either prior depletion of neutrophils or neutralization of TNF with antibodies markedly reduced hepatocellular injury from aflatoxin B₁/LPS cotreatment, indicating that these inflammatory factors play a causal role in the injury (Barton et al., 2000b; Barton et al., 2001). However, inhibition of COX2 did not afford protection. This contrasts with the effects of LPS on allyl alcohol toxicity. As with aflatoxin B₁, LPS cotreatment enhanced the hepatotoxicity of allyl alcohol and enhanced COX2 expression. In this case, however, COX2 inhibition reduced the

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injury, but TNF neutralization did not (Sneed et al., 2000; Ganey et al., 2001). These results suggest that xenobiotic agents that perturb liver homeostasis by different mechanisms respond to different inflammatory factors in order to generate liver injury. Interestingly, although neutrophil depletion protected against hepatocellular injury in the aflatoxin B₁/LPS model, it did not protect against injury to bile duct epithelium (Barton et al., 2000b), suggesting that inflammatory mechanisms contributing to injury may vary with the specific cellular target.

In summary, we and others have found that inflammation induced by small doses of LPS markedly augments responses to toxic chemicals in the liver and other organs of experimental animals (Roth et al., 1997; Sneed et al., 1997; Fanucchi et al., 1998; Barton et al., 2000a; Rumberiha et al., 2000; Yee et al., 2000a; Zhou et al., 2000). In some cases, animals not only become more sensitive to toxic insult during LPS exposure, but the major tissue target for toxicity may change. Furthermore, this change appears to depend on the chemical agent and on the exposure paradigm. Thus, the qualities of these augmented responses bear similarity to many idiosyncratic drug reactions, in which affected individuals respond with unusual sensitivity and with involvement of tissue targets that seem atypical (i.e., unrelated to a drug's pharmacologic or "dose-related" toxicologic targets).

The Hypothesis. As noted above, modest inflammatory episodes in people are commonplace, occurring sporadically and varying in magnitude. When an inflammatory episode of sufficient magnitude occurs during drug therapy, it may decrease the threshold for drug toxicity and thereby render an individual susceptible to a toxic reaction that would not otherwise occur (i.e., an "idiosyncratic" response). In Figure 1B, this is illustrated hypothetically during maintenance drug therapy as a threshold for toxic drug concentration that changes inversely with exposure to LPS (compare Figs. 1A and 1B). The episodic and variable nature of exposure to LPS and other inflammagens could explain the infrequency of idiosyncratic reactions and their erratic temporal relationship to the onset of drug therapy.

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Studies with Drugs. The studies demonstrating inflammation-augmented sensitivity to toxicity that formed the basis for the idiosyncrasy hypothesis were performed largely with nondrug agents that were known to be hepatotoxic at large doses. What about drugs? The first potentially relevant study in animals appeared almost two decades ago. Lind et al. (1984) found that a nontoxic dose of LPS markedly enhanced hepatotoxicity in hypoxic rats exposed to halothane. This result suggested the possibility of developing an animal model for halothane idiosyncrasy, but the idea of a general connection between inflammation and drug idiosyncrasy seems not to have been pursued until recently.

As noted above, human idiosyncratic reactions associated with antipsychotic drugs such as clozapine and chlorpromazine (CPZ) include increased plasma creatine kinase activity and cholestatic liver injury. Clinical markers of the latter include increased activities of enzymes such as GGT, ALT and alkaline phosphatase (ALP) released into the plasma. In a recent study (Buchweitz et al., 2002), rats were given a noninjurious dose of LPS and then 2 hr later a sedating but otherwise nontoxic dose of CPZ. Neither CPZ nor LPS when given alone caused changes in plasma markers of tissue injury; by contrast, the LPS/CPZ combination resulted in significant increases in serum activities of each of the enzymes listed above, suggesting a reaction that resembles, at least in part, idiosyncratic responses to antipsychotic drugs in people. In the livers of LPS/CPZ-treated rats, inflammatory infiltrates characterized the lesions as they do in people with idiosyncratic reactions to this class of drugs (Bianchi and Scheuer, 1974; Larrey and Erlinger, 1988; Moradpour et al., 1994).

Appearing in this issue (Luyendyk *et al.*, 2003) are the results of a recent animal study with ranitidine (RAN), another drug that causes idiosyncratic liver injury in people. RAN or its vehicle was given to rats 2 hr after either a nontoxic dose of LPS or its vehicle. No liver toxicity appeared in animals treated with either RAN or LPS alone, but plasma ALT increased within 6 hr in animals that received both RAN and LPS. Plasma AST activity was similarly elevated,

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while gamma-glutamyl transpeptidase activity showed a significant but smaller increase relative to controls. This result mirrors what is seen in human RAN idiosyncrasy: elevated markers of hepatocellular injury accompanied by more modest changes in biliary injury markers (Ramrakhiani et al., 1998; Ribeiro et al., 2000). Next, famotidine was used as a “negative comparator.” Like RAN, famotidine is an H₂-receptor antagonist and has been marketed over-the-counter longer than has RAN. Despite its widespread use, only 3 reports of liver injury associated with famotidine exposure have appeared (Ament et al., 1994; Hashimoto et al., 1994; Jimenez-Saenz et al., 2000), and the contribution of FAM to liver injury described in these reports is not clear (Luyendyk et al., 2003). Accordingly, it can be considered a drug for which idiosyncratic reactions are extraordinarily rare or essentially nonexistent. Interestingly, whereas the RAN/LPS combination caused liver injury when given to rats, famotidine (given at a pharmacologically equipotent dose) coadministered with LPS produced no liver injury. Thus, not only did LPS coexposure cause the emergence of a response in rats that resembles human RAN idiosyncrasy, but a drug in the same pharmacologic class that is less associated with human idiosyncratic reactions was less responsive in this animal model.

As noted below, additional work in animals and humans is clearly needed to extend these findings. Nevertheless, the results suggest that it may be possible to reproduce certain human idiosyncratic responses in animals by coupling drug administration with a small dose of LPS that produces modest, concurrent inflammation.

Dose-response Considerations. Because of the results described above and the nature of idiosyncratic drug reactions in people, we have come to think of these reactions in terms of relative sensitivities of organs to toxicity and whether LPS or other inflammagens may selectively enhance the sensitivity of one organ vs. another. Consider a hypothetical example

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of drug A, for which the kidney was identified in preclinical studies as a target organ for toxicity. As depicted in Fig 3., the dose-response curves for pharmacologic effect and toxicity are well separated, rendering drug A a “good drug” from the standpoints of efficacy and consideration of typical, dose-related toxic responses. Operationally, the latter means that the kidney was more sensitive than other organs to toxicity, and animals died at doses smaller than those capable of producing injury in other organs. If we accept that “the dose makes the poison” (ala Paracelsus), then a corollary is that every chemical can be viewed as being toxic to every organ at some dose. It is death’s intervention that prevents us from observing toxicity in less sensitive organs, for which the dose response curve for toxicity lies to the right of the lethality curve (e.g., see “Liver Tox” curve in Fig. 3). If underlying inflammation were to shift selectively the dose/response curve of a “nontarget” organ to the left of the lethality curve, then this organ would appear as a target organ for toxicity. In the example above, if concurrent inflammation (e.g., from LPS exposure) caused the liver to become much more sensitive to injury from Drug A, then its curve might suddenly move to the left of that of the lethality curve, rendering it a “target” for drug A toxicity (i.e., see “Liver Tox + LPS” curve in Fig. 3). If the leftward shift were pronounced enough, the patient would experience hepatotoxicity at the therapeutic dose (see asterisk in Fig. 3). According to this hypothesis, drugs for which the dose-response relationship for insensitive (“nontarget”) organs is either unaffected or minimally affected by inflammagen exposure would not produce an idiosyncratic reaction, as long as the dose-response curve for organ injury remained considerably to the right of the curve for pharmacologic effect.

By this paradigm, the inflammation-potentiated toxic response (eg, liver injury) would be expected to be unrelated to the pharmacologic effect of the drug, and its relationship to drug dose would be obscured by the ability of inflammation to change toxic potency and thereby cause the emergence of an otherwise unrecognized organ target. Moreover, since episodes of modest inflammation happen sporadically and probably with frequencies that vary considerably

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within and among individuals, inconsistent temporal relationships between drug exposure and adverse responses would be expected. Finally, the liver appears to be the most frequent target for idiosyncratic reactions. This may be in part because it contains most (80-90%) of the body's fixed macrophages (i.e., Kupffer cells), which are highly sensitive to activation by LPS and other inflammagens and are the likely initiators of inflammatory cascades that result in enhanced tissue sensitivity to xenobiotic agents. Moreover, the liver is the first organ to contact LPS translocating from the intestinal lumen into the portal circulation and would be exposed to larger concentrations than other organs. Hepatic Kupffer cells are not only activated by LPS but remove it from the circulation, thereby potentially protecting other organs from its inflammatory effects. Accordingly, this paradigm accounts for the characteristics of "drug idiosyncrasy."

It is important to point out that this hypothesis can embrace the conventional thinking about drug metabolism polymorphisms and allergic reactions as players in drug idiosyncrasy. Drug metabolism polymorphisms could play a role in determining the extent to which a drug or its metabolite alters target cell homeostasis to render cells susceptible to injury from inflammatory factors. Allergic reactions typically culminate in activation of the innate immune system, and as such they may be considered one of several paths to a modest inflammatory response.

Knowledge Gaps and Research Needs. At this stage, the perspective outlined above amounts to a hypothesis, with the beginnings of support in a few animal studies. Clearly, there are many results that need to be generated before it should be widely accepted. These include but are not limited to the following:

--In a recent, thought-provoking commentary, Alden et al. (2003) take issue with prevailing opinion that preclinical animal studies do not predict idiosyncratic potential of drugs in humans. The authors point to examples in which preclinical studies revealed liver changes

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during drug treatment of animals; however, because these changes were subtle, inconsistent across species and/or not reproduced in all studies, they were deemed insufficient cause for abandoning development and did not preclude FDA approval. The authors suggest further that, if interpreted in a different light, these subtle hepatic effects in animals might predict idiosyncratic injury potential in humans. Indeed, it could be that such subtle changes reflect alterations in hepatic homeostasis that could be the basis for some human idiosyncrasy and that could be converted into consistent, overt injury in animals in the face of a concurrent, modest inflammatory episode. Our results with RAN and CPZ are consistent with this hypothesis and might represent a platform on which predictive models could be developed. Clearly, further exploration and validation are needed to determine how universally applicable the hypothesis is and whether predictive animal models are plausible. Such studies should include the use of a wide variety of drugs from numerous pharmacologic classes that have various propensities to cause idiosyncratic reactions in people.

--*In vitro*, cell-based systems that mimic the drug-inflammation interaction *in vivo* are needed to explore mechanisms of the interactions. Inflammation-potentiated liver injury is likely to occur when a drug or its metabolite alters cellular homeostasis in a way that permits inflammatory mediators to initiate cell death pathways. If so, then useful cell-based systems will comprise the target cell (eg, hepatic parenchymal cells (HPCs) exposed to various drugs and inflammatory mediators. Our finding that RAN potentiates the ability of PMN-derived products to kill HPCs may represent a beginning in the development of such systems (Luyendyk et al., 2003). As noted above, results of studies with several hepatotoxic agents have indicated that the particular inflammatory factors critical to the potentiation response *in vivo* vary with the toxicant. This is presumably because xenobiotic agents interfere with target cell homeostasis by diverse mechanisms, each of which may require a different inflammatory factor to generate a potentiated toxic response. Accordingly, the development of widely applicable models *in vitro*

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may not be a simple task. However, the payoff in terms of increased understanding of mechanisms of potentiation and of developing a high-throughput, preclinical screening assay for use in pharmaceutical development could be large.

--Clinical/epidemiological studies of the association between inflammation and drug idiosyncrasy are needed to determine the applicability of findings in animals to human idiosyncrasy. We have reviewed published clinical reports for evidence of underlying inflammation in association with idiosyncratic episodes. In 68% of chlorpromazine cases, there were reported prodromal signs consistent with inflammation or endotoxin exposure (eg, fever, diarrhea, vomiting, etc.). Similarly, 60% of ranitidine cases mentioned such signs. The actual frequency may be higher, since prodromal signs were not considered in all reports. Although such figures do not by themselves prove much, they are at least consistent with the overall hypothesis. Unfortunately, better data in humans do not yet exist to our knowledge. The design and execution of epidemiological studies to address the inflammation-idiosyncrasy association will be challenging with respect to choice of study design, study populations and endpoints for evaluation. Although systemic exposure to LPS within a few hours of a xenobiotic agent appears to cause a potentiated response, LPS exposure one or two days earlier is more likely to produce tolerance to the potentially toxic agent. For example, people experiencing *chronic* inflammation might be less, rather than more susceptible to drug idiosyncrasy due to development of tolerance. Similarly, people with bacterial infection might develop tolerance to LPS exposure before administration of an antibiotic, and this might temporarily reduce the potential for idiosyncratic responses to antibiotic drugs. Accordingly, relatively small differences in the temporal relationship between an inflammatory episode and drug exposure could determine whether an idiosyncratic response occurs, and this as well as other subtleties should be taken into account in the design of epidemiological studies.

--"Inflammation polymorphisms" may be important as risk factors for idiosyncratic

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reactions. These include genes that encode for or control the expression of inflammatory factors such as cytokines, reactive oxygen species, lipid mediators, proteases, adhesion molecules, coagulation and complement factors, Toll-like receptors, etc. Such genes are expected to control the magnitude of the proinflammatory response to a particular amount of LPS or other inflammatory stimuli. Of interest in this regard is the recent finding of Schwartz and colleagues that polymorphisms in the gene expressing the Toll-like 4 receptor not only occur in people but control the pulmonary functional response to inhaled LPS (Arbour et al., 2000). Other genes that would be expected to have important influences are those that determine target cell (eg, HPC) sensitivity to inflammatory factors. As examples, genes controlling hepatocellular glutathione or other antioxidants, proliferative repair and signal transduction might fall into this category. If the inflammation-idiosyncrasy connection proves to be correct, then genetic differences would render some people more likely than others to develop an idiosyncratic response from the same exposure to drug and inflammagen. Looking to the future, genotypic analysis could be used to identify at-risk individuals before drug therapy is initiated.

--The focus of this perspectives article has been on hepatic idiosyncratic reactions, since the liver is the most frequent target. However, numerous other targets exist (heart and other striated muscle, bone marrow, etc.), and consideration should be given to the possibility that modest, concurrent inflammation may also underlie their involvement in idiosyncrasy.

Summary. In this short commentary, we have proposed that the basis for at least some drug idiosyncrasy is a modest episode of inflammation occurring during drug therapy, which could lower the hepatotoxic threshold to the drug enough to precipitate a toxic reaction. Inasmuch as episodes of modest inflammation occur sporadically in people, they could explain the characteristics of drug idiosyncrasy if one considers their influence on toxic thresholds in the context of basic principles of pharmacology and toxicology. By broadening our thinking about

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the basis for drug idiosyncrasy, doors may open to increased understanding of mechanisms and to ways to predict or avoid such untoward reactions to otherwise useful drugs.

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References

- Alden C, Lin J and Smith P (2003) Predictive toxicology technology for avoiding idiosyncratic liver injury. *Preclinica* **May/June**: 27-35.
- Ament PW, Roth JD and Fox CJ (1994) Famotidine-induced mixed hepatocellular jaundice. *Ann Pharmacotherapy* **28**: 40-42.
- Arbour NC., Lorenz E, Schutte BC, Zabner J, Kline JN, Jones M, Frees K, Watt JL and Schwartz DA (2000) Tlr4 mutations are associated with endotoxin hyporesponsiveness in humans. *Nat Genet* **25**: 187-191.
- Barton CC, Hill DA, Yee SB, Barton EX, Ganey PE and Roth RA (2000a) Bacterial lipopolysaccharide exposure augments aflatoxin B₁-induced liver injury. *Toxicol Sci* **55**: 444-452.
- Barton CC, Ganey PE and Roth RA (2000b) Lipopolysaccharide augments aflatoxin B₁-induced liver injury through neutrophil-dependent and independent mechanisms. *Toxicol Sci* **58**: 208-215.
- Barton CC, Barton EX, Ganey PE, Kunkel SL and Roth RA (2001) Bacterial lipopolysaccharide enhances aflatoxin B₁ hepatotoxicity in rats by a mechanism that depends on tumor necrosis factor- α . *Hepatology* **33**: 66-73.
- Beutler F (2000) Tlr4: central component of the sole mammalian LPS sensor. *Curr Opin Immunol* **12**: 20-26.
- Bianchi L and Scheuer PJ (1974) Guidelines for diagnosis of therapeutic drug-induced liver injury in liver biopsies. *Lancet* **1 (7862)**: 854-857.
- Caroff SN (1980) The neuroleptic malignant syndrome. *J Clin Psychiatry* **41**: 79-83.
- Fanucchi MV, Hotchkiss JA and Harkema JR (1998) Endotoxin potentiates ozone-induced mucous cell metaplasia in rat nasal epithelium. *Toxicol Appl Pharmacol* **152**: 1-9.

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- Fukano M, Amano S, Sato J, Yamamoto K, Adachi H, Okabe H, Fujiyama Y and Bamba T (2000) Subacute hepatic failure associated with a new antidiabetic agent, troglitazine: a case report with autopsy examination. *Hum Pathol* **31**: 250-235.
- Ganey PE, Barton Y-W, Kinser S, Sneed RA, Barton CC and Roth RA (2001) Involvement of cyclooxygenase-2 in the potentiation of allyl alcohol-induced liver injury by bacterial lipopolysaccharide. *Toxicol Appl Pharmacol* **174**: 113-21.
- Goldman SA (1996) Lithium and neuroleptics in combination: the spectrum of neurotoxicity. *Psychopharmacol Bull* **32**: 299-309.
- Gurumurthy P, Krishnamurthy MS, Nazareth O, Parthasarathy R, Sarma GR, Somasundaram PR, Tripathy SP and 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.
- Hashimoto F, Davis RL and Egli D (1994) Hepatitis following treatments with famotidine and then cimetidine. *Ann Pharmacother* **28**: 37-39.
- Ishak KG and Irey NS (1972) Hepatic injury associated with the phenothiazines. Clinicopathologic and follow-up study of 36 patients. *Arch Pathol* **93**: 283-304.
- Jimenez-Saenz M, Arguelles-Arias F, Herrerias-Gutierrez JM and Duran-Quintana JA (2000) Acute Cholestatic Hepatitis in a Child Treated with Famotidine. *Am J Gastroenterol* **95**: 3665-6.
- Kitteringham NR, Kenna JG and Park BK (1995) Detection of autoantibodies directed against human hepatic endoplasmic reticulum in sera from patients with halothane-associated hepatitis. *Br J Clin Pharmacol* **40**: 379-86.
- Khouri MR, Saul SH, Dlugosz AA and Soloway RD (1987) Hepatocanalicular injury associated with vitamin A derivative etretinate. An idiosyncratic hypersensitivity reaction. *Dig Dis Sci* **32**: 1207-1211.

JPET # 41624

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

Lazarou J, Pomeranz BH and Corey PN (1998) Incidence of adverse drug reactions in hospitalized patients— a meta-analysis of prospective studies. *JAMA* **279**: 1200-1205.

Lind RC, Gandolfi AJ, Sipes IG and Brown BR (1984) The involvement of endotoxin in halothane-associated liver injury. *Anesthesiol* **61**: 544-550.

Luyendyk JP, Maddox JF, Cosma GN, Ganey PE, Cockerell GL and Roth RA (2003) Ranitidine Treatment during a Modest Inflammatory Response Precipitates Idiosyncrasy-like Liver Injury in Rats. *J Pharmacol Exp Ther*, **in press**.

Luyendyk JP, Shores KC, Ganey PE and Roth RA (2002) Bacterial lipopolysaccharide exposure alters aflatoxin B(1) hepatotoxicity: benchmark dose analysis for markers of liver injury. *Toxicol Sci* **68**: 220-5.

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

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

Murphy EJ, Davern TJ, Shakil AO, Shick L, Masharani U, Chow H, Freise C, Lee WM, Bass NM and the Acute Liver Failure Study Group. (2000) Troglitazone-induced Fulminant Hepatic Failure. *Dig Dis Sci* **45**: 549-553.

Nankivell BJ, Bhandari PK and Koller LJ (1994) Rhabdomyolysis induced by thioridazine. *Br Med J* **309**: 378.

Njoku DB, Greenberg RS, Bourdi M, Borkowf CB, Dake EM, Martin JL and Pohl LR (2002) Autoantibodies associated with volatile anesthetic hepatitis found in the

JPET # 41624

- sera of a large cohort of pediatric anesthesiologists. *Anesth Analg* **94**: 243-249.
- Ramrakhiani S, Brunt EM and Bacon BR (1998) Possible cholestatic injury from ranitidine with a review of the literature. *Am J Gastroenterol* **93**: 822-826.
- Ribeiro JM, Lucas M, Baptista A and Victorino RMM (2000) Fatal hepatitis associated with ranitidine. *Am J Gastroenterol* **95**: 559-560.
- Roth RA, Harkema JR, Pestka JP and Ganey PE (1997) Is exposure to bacterial endotoxin a determinant of susceptibility from xenobiotic agents? *Toxicol Appl Pharmacol* **147**: 300-311.
- Rumbeiha WK, Fitzgerald SD, Braselton WE, Roth RA and Kaneene JB (2000) Potentiation of mercury-induced nephrotoxicity by endotoxin in the Sprague-Dawley rat. *Toxicology* **149**: 75-87.
- Singapore Tuberculosis Service/British Medical Research Council (1977) Controlled trial of intermittent regimens of rifampin plus isoniazid for pulmonary tuberculosis in Singapore. *Am Rev Respir Dis* **116**: 807-820.
- Sneed RA, Grimes SD, Schultze AE, Brown AP and Ganey PE (1997) Lipopolysaccharide enhances the hepatotoxicity of allyl alcohol. *Toxicol Appl Pharmacol* **144**: 77-87.
- Sneed RA, Buchweitz JP, Jean PA and Ganey PE (2000) Pentoxifylline attenuates bacterial lipopolysaccharide-induced enhancement of allyl alcohol hepatotoxicity. *Toxicol Sci* **56**: 203-210.
- Stevens GJ, Payton M, Sim E and McQueen CA (1999) N-acetylation of the heterocyclic amine batracylin by human liver. *Drug Metab Dispos* **27**: 966-971.
- Utrecht JP (1999) New concepts in immunology relevant to idiosyncratic drug reactions: The "danger hypothesis" and innate immune system. *Chem Res Toxicol* **12**: 387-395.

JPET # 41624

Weber WW, Hein DW, Litwin A and Lower GM (1983) Relationship of acetylator status to isoniazid toxicity, lupus erythematosus, and bladder cancer. *Fed Proc* **42**: 3086-3091.

Weber WW (1999) Populations and genetic polymorphisms. *Mol Diagn* **4**: 299-307.

Yee SB, Kinser S, Hill DA, Barton CC, Hotchkiss JA, Harkema JR, Ganey PE and Roth RA (2000) Synergistic hepatotoxicity from coexposure to bacterial endotoxin and the pyrrolizidine alkaloid monocrotaline. *Toxicol Appl Pharmacol* **166**: 173- 185.

Yee SB, Copple BL, Ganey PE and Roth RA (2002) The temporal relationship between bacterial lipopolysaccharide and monocrotaline exposures influences toxicity: shift in response from hepatotoxicity to nitric oxide-dependent lethality. *J Toxicol Environ Health* **65**: 961-76.

Zhou HR, Harkema JR, Hotchkiss JA, Yan D, Roth RA and Pestka JJ (2000) Lipopolysaccharide and the trichothecene vomitoxin (deoxynivalenol) synergistically induce apoptosis in murine lymphoid organs. *Toxicol Sci* **53**: 253-263.

Zimmerman HJ (1993) Hepatotoxicity. *Dis Mon* **39**: 675-787.

Figure Legends

Figure 1. Exposure to LPS and Other Inflammagens as a Determinant of Drug

Idiosyncrasy. Systemic exposure to LPS and other inflammagens is commonplace and episodic. For example, LPS exposure can occur from a locus of infection or from translocation of LPS from the intestine into the portal circulation. The latter can be enhanced by numerous factors, as noted in the text and schematically in Figure 1A. Results of animal studies indicate that modest inflammation from LPS exposure can lower the threshold for toxicity to a variety of agents. In Figure 1B, the threshold for drug toxicity is pictured hypothetically to be inversely proportional to plasma LPS concentration (compare toxicity threshold with Fig. 1A). A patient on maintenance drug therapy would experience an hepatotoxic response if the threshold for toxicity reaches the plasma concentration of the drug. Such a paradigm could explain the bizarre characteristics of drug idiosyncrasy.

Figure 2. LPS Coadministration Enhances Aflatoxin B₁ (AFB₁) Hepatotoxicity. Rats were treated with various doses of AFB₁, with or without coadministration of a nontoxic dose of LPS, and examined 24 hr later. LPS coadministration decreased the threshold for AFB₁ hepatotoxicity more than 10-fold. (from Luyendyk et al., 2002)

Figure 3. Hypothetical Relationship between Inflammation and Drug Idiosyncrasy. Drug A appears to be a clinically useful drug, because it produces its desired, pharmacological effect at doses much lower than those that are lethal or cause injury to target organs, in this case the kidney. Asterisk indicates the usual therapeutic dose. The liver does not normally appear as a target for Drug A toxicity, since doses required to injure the liver exceed those that cause death. However, upon coexposure to an inflammagen such as LPS, the liver becomes much more sensitive to Drug A, rendering it a “target organ.” Since systemic exposure to LPS occurs only

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sporadically in people, the resultant shift in the hepatotoxicity dose-response curve appears as an idiosyncratic reaction.

Figure 1

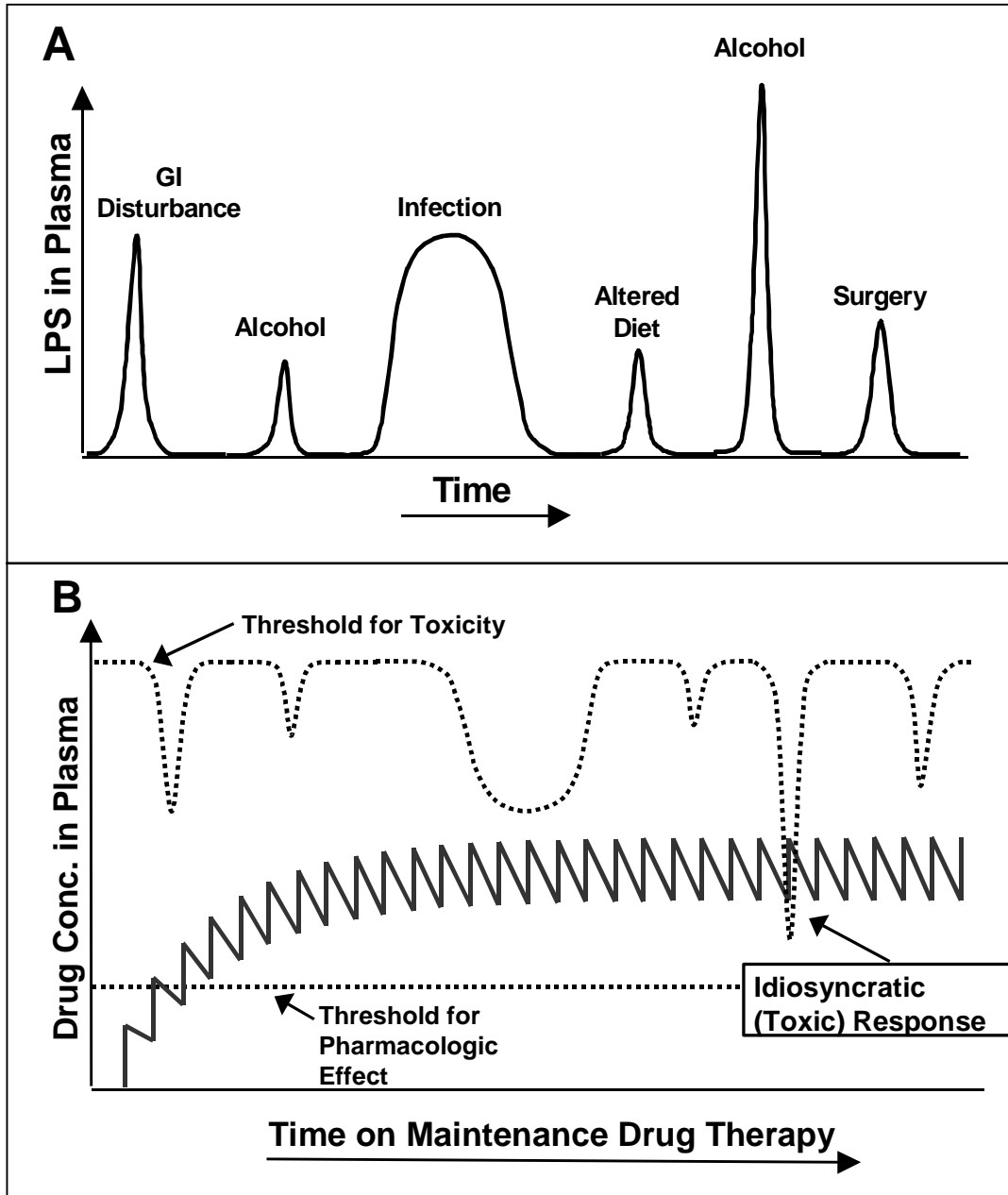


Figure 2

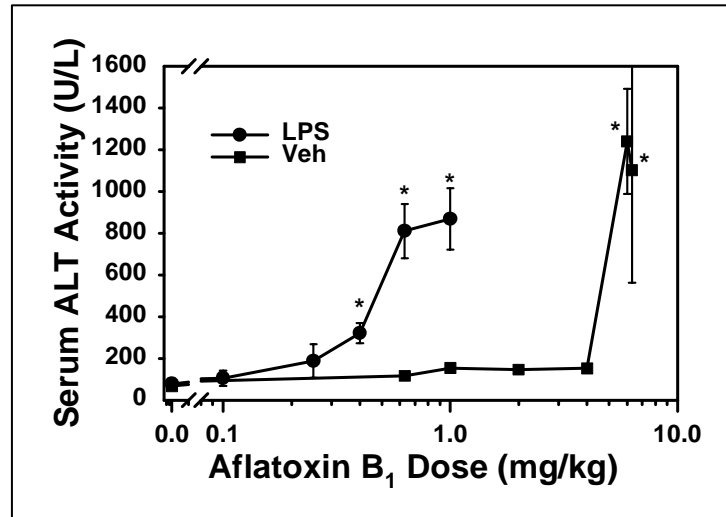


Figure 3

