Ranitidine Treatment during a Modest Inflammatory Response Precipitates Idiosyncrasy-Like Liver Injury in Rats

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Received May 12, 2003; accepted June 16, 2003

ABSTRACT

Drug idiosyncrasy is an adverse event of unknown etiology that occurs in a small fraction of people taking a drug. Some idiosyncratic drug reactions may occur from episodic decreases in the threshold for drug hepatotoxicity. Previous studies in rats have shown that modest underlying inflammation triggered by bacterial lipopolysaccharide (LPS) can decrease the threshold for xenobiotic hepatotoxicity. The histamine-2 (H2)-receptor antagonist ranitidine (RAN) causes idiosyncratic reactions in people, with liver as a usual target. We tested the hypothesis that RAN could be rendered hepatotoxic in animals undergoing a modest inflammatory response. Male rats were treated with a nonhepatotoxic dose of LPS (44 × 10⁶ endotoxin units/kg i.v.) or its vehicle and then 2 h later with a nonhepatotoxic dose of RAN (30 mg/kg i.v.) or its vehicle. Liver injury was evident only in animals treated with both RAN and LPS as estimated by increases in serum alanine aminotransferase, aspartate aminotransferase, and γ-glutamyl transferase activities within 6 h after RAN administration. LPS/RAN cotreatment resulted in midzonal liver lesions characterized by acute necroinflammatory hepatitis. Famotidine (FAM) is an H2-antagonist for which the propensity for idiosyncratic reactions is far less than RAN. Rats given LPS and FAM at a dose pharmacologically equipotent to that of RAN did not develop liver injury. In vitro, RAN sensitized hepatocytes to killing by cytotoxic products from activated neutrophils, whereas FAM lacked this ability. The results indicate that a response resembling human RAN idiosyncrasy can be reproduced in animals by RAN exposure during modest inflammation.

Adverse drug reactions of unknown etiology that occur in a small fraction of the treated population are defined as idiosyncratic. These reactions are typically unpredictable, show no obvious relation to dose, and display a variable time to onset in relation to start of drug therapy. A variety of drugs with different pharmacological properties cause idiosyncratic toxicity, with a common target organ being the liver. Idiosyncratic reactions not only cause human suffering but also sometimes result in curtailing use of otherwise effective therapeutic agents.

Idiosyncratic drug responses are commonly thought to arise either from drug metabolism polymorphism or from an allergic response to a drug or its metabolite(s). However, for the majority of drugs, supporting evidence for either of these hypotheses is lacking. The ability of modest inflammation to potentiate the toxicity of numerous xenobiotic agents led us to hypothesize that some drug idiosyncrasies may result from episodes of mild inflammation occurring during drug therapy (Ganey and Roth, 2001; Buchweitz et al., 2002). For example, with respect to chemical-induced hepatotoxicity, induction of inflammation in rats by exposure to a nonhepatotoxic dose of bacterial lipopolysaccharide (LPS) results in a 10- to 20-fold increase in sensitivity to liver injury from the fungal toxin aflatoxin B₁ (Luyendyk et al., 2002). This increase in sensitivity is dependent on an LPS-stimulated inflammatory response involving neutrophils (PMNs) and cytokines (Barton et al., 2000a, 2001). Such augmentation of hepatotoxicity by LPS coexposure seems to occur with at least some pharmaceutical agents as well. For example, treatment of rats with LPS precipitates liver injury in hypoxic rats exposed to halothane (Lind et al., 1984). A recent study demonstrated that coadministration of nontoxic doses of LPS and the antipsychotic drug chlorpromazine results in...
hepatotoxicity and other effects that occur in idiosyncratic reactions to chlorpromazine in people (Buchweitz et al., 2002). Thus, it seems that inflammation alters hepatotoxicity thresholds for some xenobiotics and pharmaceutical agents.

Another widely used drug associated with idiosyncratic hepatotoxicity is the histamine-2 (H2)-receptor antagonist ranitidine (RAN). RAN is available over-the-counter for oral administration or by prescription for parenteral administration (s.c., i.m., and i.v.) for treatment of duodenal ulcers, gastric hypersecretory diseases, and gastroesophageal reflux disease. Idiosyncratic RAN hepatotoxicity occurs in less than 0.1% of people taking the drug (Vial et al., 1991). Most liver reactions are mild and reversible; however, extensive liver damage and death have occurred in individuals undergoing RAN therapy (Ribeiro et al., 2000). Idiosyncratic hepatotoxicity to RAN typically manifests as elevations in serum markers of hepatocellular injury with more modest increases in indicators of cholestatic injury. These reactions are typical of idiosyncrasy as the time of onset of hepatotoxicity relative to initiation of therapy varies greatly, and rechallenge with RAN does not necessarily result in a reoccurrence of toxicity (Halparin, 1984; Hiesse et al., 1985). Indeed, in some cases the adverse response resolves despite continued therapy (Barr and Piper, 1981). Although direct evidence implicating inflammation as a contributing factor to these idiosyncratic reactions is lacking, it is interesting that examination of 24 reports describing 34 human cases of RAN idiosyncratic hepatotoxicity revealed promodal signs consistent with endotoxemia (i.e., increased LPS burden in blood) or inflammation (e.g., diarrhea, fever, nausea/vomiting, and/or abdominal pain) in over 60% of the cases.

The purpose of this study was to test the hypothesis that underlying inflammation triggered by a nonhepatotoxic dose of LPS renders RAN hepatotoxic in rats, revealing a response resembling human RAN idiosyncrasy. Additionally, the hypothesis was tested that the H2-antagonist famotidine (FAM), for which the propensity to cause idiosyncratic reactions is markedly smaller, would not be rendered hepatotoxic by LPS. Finally, the ability of RAN to influence the killing of inflammatory cells was explored in vitro.

Materials and Methods

Materials. Unless otherwise noted, all chemicals were purchased from Sigma-Aldrich (St. Louis, MO). Lipopolysaccharide derived from Escherichia coli serotype O55:B5 with an activity of $6.6 \times 10^6$ EU/mg was used for these studies. This activity was determined using a colorometric, kinetic limulus amebocyte lysate assay (kit 50-650U) purchased from Cambrex Corp. (East Rutherford, NJ).

Animals. Male Sprague-Dawley rats (Crl:CD [SD]IGS BR; Charles River, Portage, MI), weighing 250 to 350 g (in vivo studies) or 90 to 150 g (in vitro studies), were used for these studies. Animals were fed standard chow (Rodent Chow/Tek 8640; Harlan Teklad, Madison, WI) and allowed access to water ad libitum. They were allowed to acclimate for 1 week in a 12:12-light-dark cycle before use.

Experimental Protocol. Rats fasted for 24 h were given 44.4 $\times 10^6$ EU/kg LPS or its saline vehicle i.v. Two hours later 30 mg/kg RAN, 6 mg/kg FAM, or sterile phosphate-buffered saline vehicle was administered i.v. RAN solution was administered at 2 ml/kg at a rate of approximately 0.15 ml/min. Three, 6, 12, and 24 h later, separate groups of rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and killed by exsanguination. Blood was allowed to clot at room temperature, and serum was collected and stored at $-20{}^\circ C$ until use. Representative (3–4 mm) slices of the ventral half of the left lateral liver lobe were collected and fixed in 10% neutral buffered formalin.

Clinical Chemistry. Selected serum enzymes were analyzed using a Beckman Coulter, Inc. (Brea, CA) Synchrone CX7 random access clinical analyzer powered by version 6.4 CX7 software. Hepatic parenchymal cell injury was estimated as increases in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities. Biliary injury was estimated from increases in $\gamma$-glutamyl transferase (GGT) activity.

Histopathology. Formalin-fixed liver samples (3–4 samples/rat) were embedded in paraffin, sectioned at 5 $\mu$m, stained for H&E, and examined by light microscopy. All tissue sections were examined by the pathologist without knowledge of treatment (i.e., performed in blinded manner). All lesions were assigned a score of 0 to 5, with 0 representing no significant lesion and increasingly higher numbers representing progressively more severe lesions.

Hepatocyte Isolation. Hepatocytes were isolated using the hepatocyte product line (Invitrogen, Carlsbad, CA), including liver perfusion medium, liver digest medium, and hepatocyte wash buffer (cataloging at 17790, 17703, and 17704). All reagents were warmed to $37{}^\circ C$ before perfusion. Rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.), and the portal vein was cannulated. The liver was initially perfused with 150 ml of perfusion medium at a rate of 12 ml/min with excess medium draining from the severed vena cava. This was followed immediately by perfusion with 100 ml of liver digestion medium at a rate of 12 ml/min. The liver was transferred carefully to a culture dish containing hepatocyte wash medium and gently scraped to separate cells. The resulting liver digest was passed through sterile gauze and spun briefly at 50 g to pellet hepatocytes. The resulting pellet was washed two additional times with 50-ml volumes of hepatocyte wash medium. Hepatocytes were then resuspended at a density of $2.5 \times 10^6$ cells/ml in Williams’ medium E (Invitrogen) containing 10% fetal bovine serum (Atlanta Biologicals, Norcross, GA) and plated in 12-well cell culture plates (Corning, Palo Alto, CA) at 0.80 ml/well. Cells were allowed to attach for 4 to 5 h before treatment.

Polymorphonuclear Leukocyte (PMN) Isolation and Conditioned Medium (CM) Preparation. Rat PMNs were isolated by glycogen elicitation, as described previously (Ganey et al., 1994). Washed PMNs were suspended at a concentration of $2.5 \times 10^6$ cells/ml in Williams’ medium E. CM-conditioned medium (PMN-CM) was prepared by treating PMNs with cytochalasin B (final concentration, 5 $\mu$g/ml) and then 1 min later with the PMN activator f-Met-Leu-Phe at a concentration of 100 nM. PMNs were then incubated at $37{}^\circ C$ for 30 min. They were removed by centrifugation and the supernatant (PMN-CM) collected. Aliquots were stored at $-80{}^\circ C$ until use.

Effect of RAN and FAM on PMN-CM-Induced Cytotoxicity. Serum-free Williams’ medium E containing various concentrations of PMN-CM (0, 25, or 50%) and either RAN (0, 175, 526, or 877 $\mu$g/ml) or FAM (0, 35, 105.2, or 175.4 $\mu$g/ml) was added after hepatocytes were attached (4–5 h). After 16 h, the medium was collected, and the (catalyzed) attached cells were lysed with 1% Triton X-100 followed by brief sonication. Media and lysates were centrifuged at 600 g for 5 min. Cytotoxicity was assessed by measuring ALT release into the medium using Sigma Diagnostics Infinity ALT reagent (Sigma-Aldrich). ALT activity in the medium was expressed as a percentage of total ALT activity (i.e., medium activity plus lystate activity).

Statistical Analyses. Results are presented as mean ± S.E.M. For studies in vivo, one-way or two-way analysis of variance was used as appropriate. Histopathology scores were compared using a rank sum test. For hepatocyte studies in vitro, one- or two-way repeated measures analysis of variance was applied after appropriate data transformation. All individual group comparisons were made using Tukey’s test. The criterion for significance was $p < 0.05$ for all studies.
Results

Dose-Ranging Studies. Rats were given LPS (4.44 x 10^6 EU/kg) 2 h before various doses of RAN (0, 10, 20, 25, or 30 mg/kg). Serum ALT activity was evaluated 24 h after RAN administration. A statistically significant increase in ALT activity was not observed in rats cotreated with LPS and RAN at doses of less than or equal to 25 mg/kg, whereas ALT activity was significantly increased in animals cotreated with LPS and 30 mg/kg RAN (Fig. 1A). No dose of RAN alone caused a significant increase in ALT activity, and doses above 30 mg/kg resulted in significant mortality (data not shown). A similar dose-response study was performed for LPS at 30 mg/kg RAN (Fig. 1B). Rats were given various doses of LPS (0, 7.4, 14.8, 22.2, or 44.4 x 10^6 EU/kg) 2 h before 30 mg/kg RAN, and ALT activity was evaluated 24 h after RAN. A significant increase in ALT activity was observed in animals cotreated with RAN and an LPS dose as low as 14.8 x 10^6 EU/kg. Based on these results, doses of RAN and LPS were selected to be 30 mg/kg and 44.4 x 10^6 EU/kg, respectively, for the remaining studies.

Development of Hepatotoxicity after LPS/RAN Co-treatment. Rats were given LPS or its vehicle 2 h before RAN or its vehicle. RAN or LPS given alone was without significant effect on ALT activity compared with control at any time evaluated (Fig. 2A). Treatment with RAN caused a slight but statistically significant increase in AST activity at 12 h but was without effect at other times (Fig. 2B). Because AST is not specific for liver injury and RAN caused no change in serum ALT activity or liver histopathological change (see below), the small increase in AST activity likely arose from an extrahepatic source. Cotreatment of rats with LPS/RAN resulted in a 6- to 10-fold increase in ALT (Fig. 2A) and a 7- to 14-fold increase in AST (Fig. 2B) activities that were significant as early as 6 h after RAN treatment and remained elevated through 24 h. Biliary injury, as reflected in serum GGT activity, increased by 6 h after administration of LPS/RAN and remained elevated by at least 1.5-fold through 24 h. RAN or LPS treatment alone had no effect (Fig. 2C).

Histopathological Examination. Acute, multifocal, midzonal hepatic necrosis developed in LPS/RAN-cotreated rats as early as 3 h and increased in severity and incidence through 24 h (Fig. 3). Necrotic foci varied in size and number and were characterized by hepatocellular cytoplasmic eosinophilia and nuclear pyknosis. Variable numbers of infiltrating PMNs, many of which were necrotic, were consistently present within hepatocellular necrotic foci. Qualitatively similar lesions developed in the same time frame in LPS/Veh-treated rats, but with much less severity and frequency compared with LPS/RAN-cotreated rats. This lesion was not present in any other treatment group. Table 1 presents a summary of the severity of the liver lesion in rats treated with LPS and or RAN over 24 h.

Additional histopathological changes included vasculitis of mild-to-moderate severity in livers of all rats treated with LPS, irrespective of drug treatment. This began by 3 h in LPS/RAN-treated rats and somewhat later (6 h) in rats treated only with LPS. Diffuse sinusoidal hypercellularity occurred within 3 h to a similar degree in all three groups treated with RAN and/or LPS. This comprised hypertrophy of Kupffer cells and increased numbers of sinusoidal PMNs.

Comparison of RAN and FAM. The antisecretory potency of FAM is at least 5 times greater than RAN in rats (Scarpignato et al., 1987). For these studies, doses of RAN and FAM that inhibit gastric acid secretion to a similar degree in rats (30 mg/kg RAN and 6 mg/kg FAM) were used. This dose of FAM was not hepatotoxic when administered alone (data not shown). Significant increases in markers of hepatic parenchymal cell injury were not observed in animals cotreated with LPS/FAM after 24 h, whereas marked elevations were observed in animals given LPS/RAN (Fig. 4, A and B). Similar results were observed for GGT activity (Fig. 4C). Histopathological evaluation revealed midzonal hepatocellular necrosis in livers of rats treated with LPS/RAN, but this was absent in LPS/FAM-treated rats. Compared with rats treated with LPS alone, no significant increase in ALT activity was observed in rats cotreated with LPS and a dose of FAM that was equimolar to that of RAN (data not shown).

Fig. 1. Dose-ranging studies with RAN and LPS. A, rats were treated with 4.44 x 10^6 EU/kg LPS (i.v.) and then 2 h later with various doses of RAN (0, 10, 20, 25, or 30 mg/kg) (i.v.), n = 3 to 10 animals/group. B, rats were treated with various doses of LPS (0, 7.4, 14.8, 22.2, or 44.4 x 10^6 EU/kg) (i.v.) and then 2 h later with 30 mg/kg RAN (i.v.), n = 4 to 7 animals/group. Hepatic parenchymal cell injury was estimated 24 h after RAN administration from increases in serum ALT activity. Data are expressed as mean ± S.E.M. *, significantly different from respective control group (p < 0.05).
PMN-CM alone caused a concentration-dependent increase in ALT release (Fig. 5), as reported previously (Ho et al., 1996). By itself, RAN did not cause significant ALT release at any concentration used (Fig. 5A). However, it enhanced the hepatocellular killing activity of PMN-CM in a concentration-dependent manner. Pharmacologically equipotent concentrations of FAM were tested for comparison. FAM alone at the largest concentration used caused a very slight, but statistically significant increase in ALT release (Fig. 5B). The cytotoxicity of PMN-CM was unaffected by FAM at any concentration of the drug tested.

Discussion

It is widely accepted that idiosyncratic drug reactions arise from production of reactive drug metabolites capable of causing tissue damage or from a specific immune response to drug or drug metabolite haptens. These modes of action have been proposed for RAN idiosyncrasy (Vial et al., 1991), but supporting evidence is lacking, and neither seems to explain easily all features observed in clinical cases of RAN hepatotoxicity. For example, time of onset of hepatotoxicity relative to the initiation of RAN therapy varies greatly: some episodes occur as early as 1 week, whereas others do not occur until months after the start of maintenance therapy (Halparin, 1984; Hiesse et al., 1985; Ramrakhiani et al., 1998). Furthermore, elevations in markers of hepatocellular damage resolve despite continued RAN therapy (Barr and Piper, 1981), which seems unlikely to occur if accumulation of reactive metabolites is necessary. A role for a specific immune response in RAN idiosyncrasy is equally unsupported. Clinical signs of allergic responses such as eosinophilia have been observed in some cases (Souza Lima, 1984; Devuyst et al., 1993) but are not a consistent finding (Barr and Piper, 1981; Hiesse et al., 1985). Anti-RAN antibodies have not been identified. Additionally, an autoimmune component of RAN idiosyncrasy has not been identified conclusively. Of 14 cases for which the presence of autoantibodies was assessed, only one described the presence of anti-smooth muscle antibodies, albeit at a very low concentration (Barr and Piper, 1981). Additionally, rechallenge with RAN does not always result in a recurrence of toxicity (Graham et al., 1985), as might be expected with drug allergy. Thus, current hypotheses regarding mechanisms of RAN-induced liver injury are not consistent with all of the clinical features of these reactions.

Interestingly, prodromal indicators consistent with inflammation and endotoxemia are observed in many cases of RAN idiosyncrasy. Evaluation of 34 cases of RAN-related liver injury revealed accounts of diarrhea, fever, nausea/vomiting, and/or abdominal pain in nearly 60% of the cases. Exposure of people to innumogens such as LPS is episodic and commonplace (Roth et al., 1997). Indeed, health care providers have noted associations between transient illness characterized by signs consistent with inflammation/endotoxemia and increases in liver enzymes in serum in RAN-treated patients (Barr and Piper, 1981; Halparin, 1984). In addition, factors known to cause translocation of endogenous LPS across the gastrointestinal lumen such as excessive alcohol consumption and surgery have been noted in some cases of RAN idiosyncrasy (Halparin, 1984; Hiesse, 1985). Thus, although no definitive studies in humans have been reported, it seems
that hepatotoxic responses to RAN are often accompanied by signs consistent with inflammation. Modest inflammation can markedly increase sensitivity to hepatotoxic effects of xenobiotic agents (Ganey and Roth, 2001; Luyendyk et al., 2002). Thus, it is likely that the liver may emerge as a target organ if an inflammatory episode occurs during RAN treatment. The studies presented herein showed that a normally nonhepatotoxic dose of RAN is rendered hepatotoxic when administered to rats undergoing acute inflammation triggered by LPS. Rats cotreated with LPS/RAN showed a larger change in serum markers of parenchymal cell injury (e.g., ALT and AST) compared with cholestasis (e.g., GGT). These data are consistent with observations made in clinical cases of human RAN idiosyncrasy, in which increases in serum markers of hepatocellular injury were usually greater than markers of cholestatic injury. Thus, the nature of alterations in serum markers of liver damage after treatment of rats with LPS/RAN is similar to RAN idiosyncrasy in people. It should be borne in mind, however, that this model involves acute administration of RAN and may represent one of several mechanisms of idiosyncratic liver injury.

Fig. 3. Representative photomicrographs of liver after LPS/RAN cotreatment. Rats were treated with $4.4 \times 10^6$ EU/kg LPS or its vehicle (i.v.), then 2 h later with 30 mg/kg RAN or its vehicle (i.v.). Livers were removed 3 (A), 6 (B), 12 (C), or 24 h (D) after RAN administration, fixed in 10% neutral-buffered formalin, stained with H&E, and examined by light microscopy. Acute, multifocal, necropurulent hepatitis (arrows) was present at each time and increased in severity and frequency from 3 to 24 h. The inset in D shows infiltrating PMNs, many of which are necrotic themselves. CV, central vein.

TABLE 1
Midzonal hepatic necrosis after LPS/RAN cotreatment
Rats were treated with $4.4 \times 10^6$ EU/kg LPS or its vehicle (i.v.), then 2 h later with 30 mg/kg RAN or its vehicle (i.v.). Livers were removed 3, 6, 12, or 24 h after RAN treatment, fixed in 10% neutral buffered formalin, and evaluated by light microscopy. Lesions characterized by midzonal hepatocellular necrosis were assigned a histopathology score based on the following scale: 0, no significant lesion; 1, minimal; 2, mild; 3, moderate; 4, marked; 5, severe. n = 6 to 17 rats per group. Data are expressed as mean and range of scores for each group.

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<th>Treatment</th>
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* Significantly different from Veh/Veh-treated rats at that time (p < 0.05).
lar necrosis (Lauritsen et al., 1984; Ribeiro et al., 2000).

Consistent with the elevated serum ALT and AST activities, lesions in LPS/RAN-treated rats were characterized by acute midzonal hepatocellular necrosis accompanied by large numbers of infiltrating PMNs. The elevation in GGT activity did not have an obvious histological correlate. Although several confounding factors impinge on comparing lesions observed in severe cases of RAN idiosyncrasy in humans and LPS/RAN-treated rats (e.g., time of liver sampling), similar features such as marked inflammatory cell infiltrates and severe hepatocellular necrosis are found in both.

To our knowledge, there have only been three published reports linking FAM administration to hepatotoxicity compared with the 34 published reports for RAN. One report described a hepatotoxic response that occurred greater than 2 months after FAM therapy was discontinued, leading the authors to question whether FAM was responsible (Jimenez-Saenz et al., 2000). The link between FAM and idiosyncratic hepatotoxicity in another case was confounded by earlier RAN treatment, and the authors noted that a RAN contribution could not be ruled out (Ament et al., 1994). FAM-associated hepatotoxicity has been observed in a third patient who also developed hepatotoxicity after treatment with the H2-antagonist cimetidine (Hashimoto et al., 1994), suggesting a general sensitivity to H2-receptor antagonists. Accordingly, FAM has been associated with few published reports of idiosyncratic hepatotoxicity, and in those cases the contribution of FAM to hepatotoxic responses described was not clear. Thus, unlike RAN, FAM has little propensity to cause idiosyncratic hepatotoxicity.

We tested the hypothesis that FAM would not have the same hepatotoxic interaction with LPS that occurs with RAN. For people the recommended dose of FAM is less than that for RAN, because FAM is a more potent H2-receptor blocker (Lin, 1991). Accordingly, pharmacologically equipotent doses of the two drugs were selected based on their relative antisecretory effect and pharmacological potencies in rats (Scarpignato et al., 1987). Cotreatment of rats with LPS and RAN resulted in the expected hepatocellular damage as marked by increases in serum ALT and AST activities, whereas cotreatment of rats with FAM and LPS was without significant effect. Furthermore, LPS/FAM did not cause an elevation in GGT activity. These results suggest that the ability of inflammation to cause a drug in this class to produce liver injury may be selective for those drugs that have a propensity to cause idiosyncratic reactions in humans.

Rats treated with large doses of LPS develop acute liver injury characterized by midzonal necrosis accompanied by Kupffer cell swelling and marked PMN accumulation (Hewett et al., 1992). Liver lesions resulting from chemical-LPS synergy can resemble those produced by hepatotoxic doses of the chemical or LPS or both (Barton et al., 2000b; Yee et al., 2000). LPS/RAN treatment caused an acute, midzonal, suppurative, necrotizing hepatitis that resembled lesions in animals treated with a hepatotoxic dose of LPS. This result suggests that RAN may increase hepatic parenchymal cell sensitivity to an LPS-like hepatotoxic response. In livers of rats treated with LPS/RAN, inflammatory infiltrates comprised predominately PMNs, suggesting the possibility of a role for these cells in LPS/RAN liver injury. In other models of interaction between xenobiotic agents and LPS, PMNs are present in the liver lesions and contribute to the hepatotoxic response (Barton et al., 2000a; Yee et al., 2003). PMNs are also critically involved in the hepatotoxic response to large, toxic doses of LPS and probably act through the release of cytotoxic factors when these cells are activated (Hewett et al., 1992; Ho et al., 1996).
The exact role of PMNs in LPS/RAN liver injury has not been evaluated. Interestingly, RAN attenuates liver injury after ischemia-reperfusion, probably by inhibiting release of cytotoxic factors by PMNs (Okajima et al., 2002). Previous studies demonstrated that RAN was nontoxic to hepatocytes even at high (e.g., 5 mM) concentrations (Zimmerman et al., 1986), and our results confirmed these findings (Fig. 5). However, hepatocytes treated with RAN were rendered more sensitive to killing by cytotoxic factors released by activated PMNs. In the context of observations in LPS/RAN-treated rats, these results suggest that RAN may act by increasing hepatocellular sensitivity to PMN-derived factors. In contrast, FAM did not increase the sensitivity of hepatocytes to killing by PMN-derived cytotoxic factors. Because pharmacologically equipotent concentrations were used, this suggests that the sensitizing effect of RAN on hepatocytes is independent of H2-receptor blockade. Further studies are needed to understand the mechanism by which RAN alters hepatocyte sensitivity to PMN-derived products.

In summary, RAN was rendered hepatotoxic in rats undergoing a mild inflammatory response triggered by LPS. LPS/RAN-cotreated animals developed midzonal necroinflammatory hepatitis and a liver-related clinical chemistry pattern resembling human cases of RAN idiosyncrasy. In contrast, animals cotreated with LPS and a pharmacologically equipotent or equimolar dose of FAM did not develop liver damage, a result consistent with the far lesser (and debatable) propensity of FAM to cause idiosyncrasy in people. Treatment of hepatocytes in vitro with RAN, but not with FAM, increased hepatocellular sensitivity to cytotoxicity from PMN-derived factors. Overall, our demonstration that modest inflammation causes the emergence of liver as a target for RAN toxicity in rats suggests a role for inflammation in idiosyncratic reactions to this H2-antagonist. In addition, the results raise the possibility of developing animal and cell-based models for predicting which drug candidates are more or less likely to cause idiosyncratic reactions in people and for studying the underlying mechanisms by which these reactions occur.

Acknowledgments
We thank Dave Blakeman for technical assistance.

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