Trovafoxacin Enhances the Inflammatory Response to a Gram-Negative or a Gram-Positive Bacterial Stimulus, Resulting in Neutrophil-Dependent Liver Injury in Mice

Patrick J. Shaw, Patricia E. Ganey, and Robert A. Roth

Department of Pharmacology and Toxicology, National Food Safety and Toxicology Center, Center for Integrative Toxicology, Michigan State University, East Lansing, Michigan

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ABSTRACT

Trovafoxacin (TVX), a fluoroquinolone antibiotic, has been strongly linked with several cases of idiosyncratic hepatotoxicity in humans. Previous studies showed that a modest inflammatory stress induced by a Gram-negative bacterial stimulus [i.e., lipopolysaccharide (LPS)] rendered nontoxic doses of TVX hepatotoxic in mice. This study compared the interaction of TVX with Gram-negative and Gram-positive stimuli. Mice were given TVX 3 h before LPS (Gram-negative stimulus) or a peptidoglycan-lipoteichoic acid (PGN-LTA) mixture isolated from *Staphylococcus aureus* (Gram-positive stimulus). Administration of TVX, LPS, or PGN-LTA alone was nonhepatotoxic. However, TVX administration before PGN-LTA or LPS resulted in significant liver injury that occurred with similar time courses. TVX/PGN-LTA-induced hepatocellular necrosis was primarily localized to centrilobular regions, whereas that caused by TVX/LPS was predominantly midzonal. Administration of either LPS

or PGN-LTA alone led to increased plasma concentrations of several cytokines and chemokines at a time near the onset of liver injury. TVX administration before LPS enhanced the concentrations of all of these cytokines, whereas TVX treatment before PGN-LTA increased all of the cytokines except tumor necrosis factor (TNF)- α and interferon- γ . Liver injury was reduced in TVX/LPS- and TVX/PGN-LTA-treated mice given an antibody to CD18 and also in mice deficient in neutrophil [polymorphonuclear neutrophil (PMN)] elastase. Hepatic PMN accumulation and TNF- α production after TVX/PGN-LTA-, but not after TVX/LPS-coexposure, was CD18-dependent. In summary, TVX significantly enhanced the murine inflammatory response to either a Gram-negative or a Gram-positive stimulus and caused hepatotoxicity that developed similarly and was dependent on PMN activation in mice but that differed in lesion location and cytokine profile.

In 1997, a new fluoroquinolone antibiotic, trovafoxacin (TVX), was released to market. Initially, TVX was preferred over other antibiotics due to a broader spectrum of activity against Gram-negative and Gram-positive bacteria and its extended half-life (Gooding and Jones, 1993; Spangler et al., 1994). These properties made TVX an attractive antibiotic for the treatment of several bacterial infections.

Subsequently, severe hepatic reactions were associated with TVX use in 140 patients after ~2.5 million courses of therapy (Stahlmann, 2002). The severity of hepatotoxicity from TVX led to restrictions being placed on its use in 1999.

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The hepatotoxicity of TVX is unrelated to its pharmacologic action, inasmuch as hepatotoxicity is not seen extensively with other fluoroquinolones. Based on the rare and erratic occurrence, TVX hepatotoxicity was classified as an idiosyncratic adverse drug reaction.

The mechanisms underlying idiosyncratic toxicities remain unknown. We and others have hypothesized that an inflammatory stress might interact with some xenobiotics to precipitate an idiosyncratic adverse drug reaction. The sporadic occurrence of acute inflammatory episodes could explain the variable onset of idiosyncratic reactions during a course of drug therapy. Inflammatory stress can be initiated by the recognition of microbial products in the plasma by the host, and these products can be increased by several events, including alcohol consumption, surgery, gastrointestinal disturbances, and others (Hellman et al., 2002). Bacterial components of both Gram-positive bacteria (peptidoglycan [PGN])

ABBREVIATIONS: TVX, trovafoxacin; PGN, peptidoglycan; LTA, lipoteichoic acid; LPS, lipopolysaccharide; TLR, toll-like receptor; NF- κ B, nuclear factor- κ B; PMN, polymorphonuclear neutrophil; ALT, alanine aminotransferase; NE, neutrophil elastase; Veh, vehicle; IL, interleukin; TNF, tumor necrosis factor; IFN, interferon; VEGF, vascular endothelial growth factor; MCP, monocyte chemoattractant protein; KC, keratinocyte chemoattractant; MIP, macrophage inflammatory protein.

and lipoteichoic acid [LTA]) and Gram-negative bacteria (lipopolysaccharide [LPS]) activate Toll-like receptors (TLRs) to induce inflammation. PGN and LTA activate TLR2 and induce NF- κ B activation through MyD88-dependent mechanisms (Schwandner et al., 1999; Majcherczyk et al., 2003; Moreillon and Majcherczyk, 2003; Weber et al., 2003). LPS activates TLR4 and induces NF- κ B activation through both MyD88-dependent and -independent mechanisms (Janssens and Beyaert, 2003). These isolated bacterial agents can be used to induce inflammatory stress, and they elicit the majority of the clinical manifestations of each type of bacterial infection (Yoshimura et al., 1999; Hellman et al., 2002; Heumann and Roger, 2002; Moreillon and Majcherczyk, 2003).

In previous studies, we showed that TVX synergized with a nonhepatotoxic dose of LPS to cause liver injury in mice that was dependent on several inflammatory cytokines (Shaw et al., 2007, 2009a,b). Here, we explored whether TVX synergized with a Gram-positive bacterial stimulus (PGN-LTA) to cause liver injury. In addition, the possibility that TVX enhanced the inflammatory stress induced by LPS or PGN-LTA was evaluated. Furthermore, neutrophils [polymorphonuclear neutrophils (PMNs)] commonly contribute to inflammatory tissue injury in various models, and the role of PMNs in TVX/LPS- and TVX/PGN-LTA-induced liver injury was determined.

Materials and Methods

Materials. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise noted. LPS from *Escherichia coli* 055:B5 (lot 075K4038) with an activity of 3.3×10^6 endotoxin units/mg was used for these studies. Endotoxin activity was measured using the *Limulus* amebocyte assay (Cambrex, East Rutherford, NJ). PGN and LTA from *Staphylococcus aureus* were used for all studies. Rabbit anti-murine CD18 antiserum designed against amino acids 89 to 100 was purchased from New England Peptide (Gardner, MA). TVX was synthesized by Cayman Chemical (Ann Arbor, MI). Infinity alanine aminotransferase (ALT) reagent used to measure ALT activity was purchased from Thermo Electron Corp. (Louisville, CO).

Animals. Male C57BL/6J mice purchased from The Jackson Laboratory (Bar Harbor, ME) were used at an age of 9 to 11 weeks and weighed between 21 and 26 g. Wild-type and neutrophil elastase [NE(-/-)] knockout (B6.129X1-Ela2/J) mice were purchased from The Jackson Laboratory, used at an age of 12 to 14 weeks and weighed 25 to 35 g. Mice had continual access to Global Rodent diet 2018 (Harlan Teklad, Madison, WI) and spring water. Upon arrival, mice were acclimated for 1 week in a 12-h light/dark cycle.

Experimental Protocols. Mice fasted 12 h were given TVX (150 mg/kg p.o.) or vehicle (saline) 3 h before an intraperitoneal administration of either LPS (2×10^6 endotoxin units/kg), PGN-LTA (30 mg/kg each), or Veh (saline). The doses and timing were based on a previous study (Shaw et al., 2007). The dose of TVX was selected based on its ability to cause liver injury upon LPS cotreatment without significant mortality. Mice were fasted to minimize variability of the response to LPS or PGN-LTA. Food was returned immediately after this latter dosing. Mice were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and killed at various times after the administration of LPS, PGN-LTA, or Veh for various measurements. Blood was drawn from the vena cava into a syringe containing sodium citrate (final concentration, 0.76%). The left lateral lobe of the liver was fixed in 10% neutral-buffered formalin and paraffin-blocked.

For some studies, mice were treated with CD18 antiserum. CD18 antiserum or rabbit control serum (0.25 ml i.p.) was administered

when food was removed and then again 2 h after LPS, PGN-LTA, or Veh administration.

Histopathology. Paraffin-embedded left lateral liver lobes were sectioned and stained with hematoxylin and eosin. These sections were then examined by light microscopy. Histopathology figures presented are sections from mice with plasma ALT activity approximating the average of the respective treatment group.

Cytokine and Chemokine Analysis. The plasma concentrations of interleukin (IL)-1 β , tumor necrosis factor (TNF)- α , IL-10, IL-6, IL-18, interferon (IFN)- γ , vascular endothelial growth factor (VEGF), monocyte chemoattractant protein (MCP)-1, keratinocyte chemoattractant (KC), macrophage inflammatory protein (MIP)-2, and MIP-1 α were measured with custom Bio-Plex cytokine assays purchased from Bio-Rad Laboratories (Hercules, CA) using the Bio-Plex 200 System (Bio-Rad Laboratories).

Neutrophil Staining. Paraffin-embedded left lateral liver lobes were stained for PMNs using a rabbit anti-PMN Ig isolated from the serum of rabbits immunized with rat PMNs (Hewett et al., 1992). Immunohistochemical staining of PMNs was done using the protocol described by Yee et al. (2003). The slides were coded, randomized, and then visualized without knowledge of treatment using a light microscope. Neutrophil accumulation was quantified by counting PMNs in 10 high-power fields, and the averaged values were considered a replication.

Statistical Analyses. Results are presented as mean $\pm$ S.E.M. A Student's *t* test or a two-way analysis of variance was used as appropriate after data normalization. All pairwise comparisons were made using Tukey's test, with the criterion for significance at $p < 0.05$.

Results

TVX Coexposure with PGN-LTA Causes Hepatotoxicity. TVX alone was not hepatotoxic up to doses of 1000 mg/kg (data not shown). In addition, administration of PGN-LTA (30 mg/kg each) alone was not hepatotoxic (Fig. 1). However, pretreatment with TVX (150 mg/kg) 3 h before the PGN-LTA mixture caused a significant elevation in plasma ALT activity as early as 4.5 h, which continued to increase through 15 h after PGN-LTA administration (Fig. 1). TVX (150 mg/kg) administered 3 h before a nonhepatotoxic dose of LPS was shown previously to cause hepatotoxicity with a similar time course (Shaw et al., 2007, 2009a).

Histopathologic examination of liver sections from mice treated with TVX/PGN-LTA revealed significant hepatocellular necrosis primarily in centrilobular regions (Fig. 1). In addition, hepatocytes exhibiting apoptotic morphology such as nuclear chromatin margination and apoptotic bodies were observed primarily in centrilobular regions. This finding was in contrast to TVX/LPS coexposure, which induced hepatocellular oncosis and apoptosis primarily in midzonal regions (Shaw et al., 2007).

TVX Enhances Cytokine Induction Differently for TLR2 and TLR4 Agonists. Administration of either LPS or PGN-LTA caused a significant increase at 4.5 h in plasma concentrations of the following cytokines: IL-1 β , TNF- α , IL-10, IL-6, IL-18, IFN- γ , VEGF, and MCP-1 (Fig. 2). TVX pretreatment significantly increased the LPS induction of all cytokines listed above (Fig. 2). In contrast, TVX treatment before PGN-LTA caused an increase in all of the cytokines except for TNF- α and IFN- γ . Several chemokines were also evaluated. LPS or PGN-LTA alone caused a significant increase in the plasma concentrations of KC, MIP-2, and MIP-1 α at 4.5 h (Fig. 3). TVX treatment 3 h before either

Fig. 1. Time course of TVX/PGN-LTA-induced liver injury. Mice were treated with TVX 3 h before PGN-LTA as described under *Materials and Methods*. Mice were killed various times after PGN-LTA, and plasma ALT activity was measured. $n = 5$ to 8 animals/group. *, significantly different from 0 h within treatment group; #, significantly different from all other treatment groups at same time. A representative photomicrograph was taken of liver at 15 h from a mouse treated with TVX/PGN-LTA. The arrows highlight lesions of oncotic necrosis.

inflammatory stimulus caused a significant increase in the induction of these chemokines (Fig. 3).

Effect of TVX on LPS- or PGN-LTA-Induced Hepatic PMN Accumulation. Hepatic PMN accumulation was evaluated immunohistochemically 4.5 h after LPS or PGN-LTA. TVX alone did not cause any PMN accumulation (Fig. 4). Both LPS and PGN-LTA alone increased the numbers of PMNs present in the liver, and this response was unaffected by TVX pretreatment (Fig. 4).

TVX/LPS- and TVX/PGN-LTA-Induced Liver Injury Depends on PMN Activation. Wild-type and PMN elastase knockout [NE(–/–)] mice were treated with TVX/LPS or TVX/PGN-LTA as described under *Materials and Methods*. NE(–/–) mice had a smaller increase in plasma ALT activity after TVX/LPS or TVX/PGN-LTA coexposure compared with wild-type mice (Fig. 5). Histopathologic examination of liver sections from NE(–/–) mice treated with TVX/LPS- or TVX/PGN-LTA revealed reduced frequency and size of necrotic lesions compared with similarly treated wild-type mice (data not shown).

CD18 integrin is involved in the infiltration of PMNs into parenchyma and activation of these cells to release elastase and other factors. A neutralizing antiserum to CD18 was administered to mice to prevent PMN activation (Jaeschke et al., 1993). CD18 antiserum significantly attenuated both TVX/LPS- and TVX/PGN-LTA-induced liver injury (Fig. 6) as measured by plasma ALT activity. Histopathologic examination of liver sections from mice treated with TVX/LPS or TVX/PGN-LTA revealed reduced frequency and size of ne-

crotic lesions in CD18 antiserum-cotreated mice (data not shown).

The hepatic PMN accumulation caused by TVX/LPS coexposure was unaffected by CD18 antiserum administration (Fig. 7). In contrast, CD18 antiserum significantly reduced hepatic PMN accumulation induced by TVX/PGN-LTA coexposure (Fig. 7).

Effect of CD18 Neutralization on TVX/LPS- and TVX/PGN-LTA-Induced Cytokine Production. As noted above, TVX/LPS and TVX/PGN-LTA coexposure induced significant increases in the plasma concentrations of IL-6, IL-10, IFN- γ , TNF- α , IL-1 β , MCP-1, KC, and MIP-1 α (Fig. 8). Administration of CD18 antiserum did not attenuate the TVX/LPS-induced increase in any of these cytokines. In contrast, CD18 neutralization did reduce the TVX/PGN-LTA-induced increases in the plasma concentrations of TNF- α and MCP-1 (Fig. 8).

Discussion

The inflammatory stress induced by Gram-positive and Gram-negative bacterial products is slightly different. Because LPS and PGN-LTA activate different TLRs, the resultant intracellular signaling also differs. For example, LPS induces type I interferon (IFN- α and IFN- β) production, whereas PGN-LTA does not (Kaisho and Akira, 2003). Despite some differences in downstream signaling and in inflammatory mediator production, TVX synergized with a nonhepatotoxic dose of either of these inflammagens to cause liver injury. That TVX interacted with either TLR2- or TLR4-activating ligands to cause liver injury proves that the TVX/inflammation-induced liver injury shown previously (Shaw et al., 2007) is not specific to LPS-induced TLR4 activation. Indeed, this finding in conjunction with a previous study in which TVX interacted with a nonhepatotoxic dose of recombinant TNF- α (Shaw et al., 2009b) suggests that TVX interacts with inflammatory stress, irrespective of its initiating stimulus, to precipitate liver injury.

TVX/LPS-treated mice developed lesions of hepatocellular oncotic necrosis and apoptosis primarily localized to mid-zonal regions (Shaw et al., 2007), whereas TVX/PGN-LTA lesions were primarily centrilobular. Such a difference in localization might be due to a difference in lobular expression of TLR2 and TLR4 in the mouse liver, of which little is known. Alternatively, the difference might reflect dissimilar mechanisms of injury, which could be related to different inflammatory cytokine profiles.

Inflammatory cytokines were measured at the time of onset of liver injury to examine the effect of TVX on LPS- and PGN-LTA-induced inflammatory stress. TVX enhanced the LPS- and PGN-LTA-induced increases in a number of cytokines, but TNF- α and IFN- γ were only enhanced in LPS-treated mice. The difference in IFN- γ induction between LPS and PGN/LTA could be due to a difference between TLR2- and TLR4 activation. TLR4 but not TLR2 ligation activates dendritic cells (Gerosa et al., 2008; Perrin-Cocon et al., 2008), which can stimulate natural killer and T cells to produce IFN- γ (Del et al., 2007). Thus, it is possible that TVX acts to enhance steps in the pathway to IFN- γ production induced by LPS that are not activated by PGN-LTA. We showed previously that IFN- γ is involved in TVX/LPS-induced liver injury (Shaw et al., 2009a). In addition, the difference in

Fig. 2. Effect of TVX on LPS- and PGN-LTA-induced increases in plasma cytokines. Mice administered Veh or TVX were treated with vehicles, LPS, or PGN-LTA as described under *Materials and Methods*. Plasma concentrations of IL-1 β , TNF- α , IL-10, IL-6, IL-18, IFN- γ , VEGF, and MCP-1 were measured 4.5 h after LPS or PGN-LTA administration. $n = 4$ to 6 animals/group. *, significantly different from Veh control not treated with inflammagen; #, significantly different from TVX control not treated with inflammagen; ϕ , significantly different from Veh group within the same inflammagen treatment.

Fig. 3. Effect of TVX on LPS- and PGN-LTA-induced increases in plasma chemokines. Mice administered Veh or TVX were treated with vehicles, LPS, or PGN-LTA as described under *Materials and Methods*. Plasma concentrations of KC, MIP-2, and MIP-1 α were measured 4.5 h after LPS or PGN-LTA administration. $n = 4$ to 6 animals/group. *, significantly different from Veh control not treated with inflammagen; #, significantly different from TVX control not treated with inflammagen; ϕ , significantly different from Veh group within the same inflammagen treatment.

IFN- γ production might relate to the difference in histopathologic lesion localization from TVX/LPS or TVX/PGN-LTA coexposure.

Chemokine production induced by LPS or PGN/LTA was enhanced by TVX pretreatment. The production of these chemokines and several of the other cytokines that were changed can be up-regulated as a result of NF- κ B activation (Ouaaz et al., 1999). Thus, it is likely that TVX acts by increasing NF- κ B activation or by enhancing downstream signaling that culminates in cytokine production.

KC, MIP-2, and MIP-1 α all have chemotactic activity for

neutrophils; therefore, we examined whether TVX enhanced PMN accumulation in the liver. Despite the increases in several chemokines, TVX pretreatment did not affect LPS- or PGN-LTA-induced neutrophil accumulation in the liver. In other studies, neutrophils arrested in hepatic postsinusoidal venules after LPS/galactosamine treatment independently of KC or MIP-2, but extravasation and activation of neutrophils into the parenchyma were significantly reduced by neutralization of MIP-2 or KC (Li et al., 2004). It is possible that although the numbers of PMNs present are not different, PMN activation was enhanced in the presence of TVX and

Fig. 4. Effect of TVX on LPS- and PGN-LTA-induced hepatic PMN accumulation. Mice administered Veh or TVX were treated with vehicles, LPS, or PGN-LTA as described under *Materials and Methods* and killed 4.5 h after LPS or PGN-LTA. Paraffin-embedded livers were stained for PMNs, and the number of PMNs was quantified as described under *Materials and Methods*. HPF, high-power field. $n = 4$ to 6 animals/group. *, significantly different from respective control without inflammagen treatment.

Fig. 5. Role of NE in TVX/LPS- and TVX/PGN-LTA-induced liver injury. Wild-type and NE^{-/-} mice were treated with TVX 3 h before LPS or PGN-LTA as described under *Materials and Methods*. Mice were killed 15 h after LPS or PGN-LTA, and plasma ALT activity was measured. $n = 4$ to 6 animals/group. *, significantly different from wild-type group.

that this activation plays a role in the development of hepatotoxicity.

PMNs are a common mediator of inflammatory tissue injury. PMN accumulation in the hepatic sinusoids can be stimulated by several inflammatory stimuli. However, in general, extravasation of the PMNs into the parenchyma is required for them to cause tissue damage (Chosay et al., 1997). Extravasation from liver sinusoids into the parenchyma is facilitated by β_2 -integrins, such as CD18 (Jaeschke and Hasegawa, 2006). Upon activation, PMNs release myeloperoxidase, reactive oxygen species, and proteases such as cathepsin G and NE. Activated PMNs are cytotoxic to hepatocytes, and this cytotoxicity is dependent on protease activity (Ho et al., 1996).

NE^{-/-} mice had significantly reduced plasma ALT activity after TVX/LPS or TVX/PGN-LTA coexposure compared

Fig. 6. Role of CD18 in TVX/LPS- and TVX/PGN-LTA-induced liver injury. Mice were treated with control serum or CD18 antiserum along with TVX/LPS or TVX/PGN-LTA as described under *Materials and Methods*. Mice were killed 15 h after LPS or PGN-LTA, and plasma ALT activity was measured. $n = 4$ to 6 animals/group. *, significantly different from control serum group.

Fig. 7. Role of CD18 in TVX/LPS- and TVX/PGN-LTA-induced hepatic PMN accumulation. Mice were treated with control or CD18 antiserum along with TVX/LPS or TVX/PGN-LTA as described under *Materials and Methods*. They were killed 4.5 h after LPS or PGN-LTA. Paraffin-embedded livers were stained for PMNs, and the number of PMNs was quantified as described under *Materials and Methods*. HPF, high-power field. $n = 4$ to 6 animals/group. #, significantly different from control serum group.

with wild-type mice. The mechanism by which NE is involved in the progression of liver injury is unknown, and there are several possibilities. As noted above, NE is directly cytotoxic to hepatocytes (Ho et al., 1996). However, NE can also play a role in PMN adhesion to endothelium and transmigration into parenchyma (Chin et al., 2008; Kaynar et al., 2008). Nevertheless, that NE^{-/-} mice were protected suggests that PMNs are critical for TVX/LPS- and TVX/PGN-LTA-induced liver injury. Further evidence for this suggestion is the observation that pretreatment with a neutralizing antibody to CD18, a β_2 -integrin critical for PMN activation (Jaeschke et al., 1993; Deng et al., 2007), attenuated hepa-

Fig. 8. Role of CD18 in TVX/LPS- and TVX/PGN-LTA-induced inflammatory mediator production. Mice were treated with control or CD18 antiserum along with TVX/LPS or TVX/PGN-LTA as described under *Materials and Methods*. Mice were killed 4.5 h after LPS or PGN-LTA. Plasma concentrations of IL-1 β , TNF- α , IL-10, IL-6, IFN- γ , MCP-1, KC, and MIP-1 α were measured as described under *Materials and Methods*. $n = 4$ to 6 animals/group. #, significantly different from control serum group treated with the same inflammagen.

toxicity induced by either TVX/LPS or TVX/PGN-LTA co-exposure. Accordingly, PMN activation seems to be a common pathway required for the progression of liver injury. Although critical for PMN activation, CD18 is not always needed for neutrophil accumulation in tissue. Indeed, CD18 neutralization did not affect TVX/LPS-induced hepatic PMN accumulation, a result consistent with previous reports exploring LPS-induced hepatic neutrophil accumulation (Jaeschke et al., 1996). In contrast, hepatic PMN accumulation after TVX/PGN-LTA coexposure was significantly reduced by CD18 antiserum. This is a novel finding that requires further study to understand how mechanisms of PMN accumulation differ after LPS or PGN-LTA administration.

Because TVX/LPS-induced liver injury is dependent on several cytokines (Shaw et al., 2007, 2009a), inflammatory cytokines were measured after CD18 neutralization in TVX/LPS- and TVX/PGN-LTA-treated mice. In mice treated with TVX and either LPS or PGN-LTA, the plasma concentrations of some cytokines in control serum-treated mice were slightly different from those reported for vehicle-treated mice (compare Figs. 2 and 3 with Fig. 8), probably due to the large volume of control serum injected. Nevertheless, CD18 neutralization did not significantly reduce the TVX/LPS or TVX/PGN-LTA induction of several cytokines measured (Fig. 8). Macrophages, as well as PMNs, can express CD18. However, because Kupffer cells are the major producers of IL-6, KC, and IL-10 and these cytokines were unchanged by CD18

neutralization, it is unlikely that Kupffer cell activation was affected.

CD18 neutralization did attenuate the increase in TNF- α and MCP-1 after TVX/PGN-LTA treatment. The attenuation of TNF- α and MCP-1 is probably due to the reduction in hepatic PMN accumulation. PMNs can produce and release MCP-1 (Shiratsuchi et al., 2007; Yoshimura and Takahashi, 2007). In addition, PMNs express TNF- α converting enzyme on their plasma membranes (Walcheck et al., 2006), which is critical for the conversion of proTNF- α to active TNF- α . TVX/PGN-LTA-induced liver injury probably requires TNF- α because TVX can directly interact with TNF- α to cause liver injury (Shaw et al., 2009b). If TNF- α is involved, it is unknown whether protection by CD18 neutralization is due to prevention of PMN activation or to reduced TNF- α concentration.

TVX interacted with either TLR2 or TLR4 agonists to enhance cytokine production. This effect of TVX is likely on inflammatory cells such as macrophages, NK cells, and neutrophils. However, little is known about direct effects of TVX on hepatocytes. It is possible that TVX sensitizes hepatocytes to cytokine- or PMN protease-induced cell death. TVX affects RNA processing and mitochondrial function in human hepatocytes (Liguori et al., 2005). Therefore, it seems probable that one mechanism by which TVX interacts with an inflammatory stress to cause liver injury in mice is by disrupting

the homeostasis of hepatocytes, thereby sensitizing them to a normally nontoxic insult.

In summary, TVX synergized with a modest inflammatory stress induced by either a Gram-positive or a Gram-negative stimulus to cause liver injury in mice. TVX enhanced the LPS- and PGN-LTA-induced increases in proinflammatory cytokines. However, TVX did not enhance the hepatic PMN accumulation driven by either of these stimuli. NE(−/−) mice were significantly protected from TVX/LPS- and TVX/PGN-LTA-induced liver injury. In addition, PMN inactivation with CD18 antiserum attenuated TVX/LPS- and TVX/PGN-LTA-induced liver injury. These results suggest that PMNs play a critical role in injury progression in both models through a mechanism involving PMN elastase. CD18 neutralization attenuated TVX/PGN-LTA-induced increases in hepatic PMN accumulation and in TNF- α and MCP-1 plasma concentrations. In contrast, it did not affect hepatic neutrophil accumulation or proinflammatory cytokine increases induced by TVX/LPS. Thus, it is possible that CD18 neutralization protects mice from TVX/LPS- or TVX/PGN-LTA-induced liver injury by different mechanisms. Despite the possible mechanistic difference, PMNs play a pathogenic role in both models of TVX-induced hepatotoxicity.

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Address correspondence to: Dr. Robert Roth, 221 Food Safety Bldg., Michigan State University, East Lansing, MI 48824. E-mail: rothr@msu.edu