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Bacterial Lipopolysaccharide Exposure Alters Aflatoxin B_1 Hepatotoxicity: Benchmark Dose Analysis for Markers of Liver Injury

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Aflatoxin B_1 (AFB_1) is a fungal toxin that causes both acute hepatotoxicity and hepatocellular carcinoma in humans and experimental animals. Previous studies demonstrated that a small, noninjurious dose of bacterial lipopolysaccharide (LPS) augments the hepatotoxicity of AFB_1 through activation of inflammatory cells and production of soluble inflammatory mediators (Barton *et al*., 2000b, 2001). This study was conducted to examine the effect of LPS on the dose-response relationship for AFB_1-induced liver injury. Male Sprague-Dawley rats (250–350g) were treated with AFB_1 (0.1 mg/kg–6.3 mg/kg, ip) and 4 h later with a noninjurious dose of *E. coli* LPS (7.4×10^6 EU/kg, iv). Twenty-four h after AFB_1 administration, hepatic parenchymal cell injury was estimated from elevations in serum alanine aminotransferase and aspartate aminotransferase activities. Injury to intrahepatic bile ducts was evaluated from increased serum γ-glutamyl transferase and alkaline phosphatase activities. Based on benchmark dose (BMD) analysis, the AFB_1 BMD for parenchymal cell injury was decreased 10-fold by LPS cotreatment, whereas AFB_1 BMDs for bile duct injury were decreased nearly 20-fold. The data suggest that concurrent inflammation renders the liver considerably more sensitive to the hepatotoxic effects of AFB_1.

Key Words: **lipopolysaccharide; aflatoxin B_1; liver injury; risk assessment; benchmark dose.**

Aflatoxin B_1 (AFB_1) is a mycotoxin produced by *Aspergillus flavus* and *Aspergillus parisiticus*. It is a common contaminant of grain foods for both human and animal consumption. Human exposure to AFB_1 is greatly influenced by quality of grain storage, climate, and culinary customs (Hall and Wild, 1994; Wilson and Payne, 1994). Indeed, in contrast to the United States, human exposure to AFB_1 in developing countries can be quite large. Consumption of contaminated corn is probably the most important mode of exposure (Wood, 1989). In the Guangxi province of the People's Republic of China, where corn is a dietary staple, AFB_1 contamination of corn has been measured at 460 mg/kg (Li *et al.*, 2001). Human exposure in this region has been estimated to be between 50 to 75 mg/day (Groopman *et al*., 1992).

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AFB_1 causes acute hepatotoxicity and liver carcinomas in people and laboratory animals (Roebuck and Maxuitenko, 1994). It is metabolized to a highly reactive 8,9-epoxide that binds to cellular macromolecules, primarily in the periportal region of the liver. AFB_1-induced liver injury manifests itself as periportal parenchymal cell necrosis, hemorrhage, and injury to intrahepatic bile ducts. Clinical manifestations of acute AFB_1 exposure in humans include abdominal pain, pulmonary edema, and liver necrosis, and these are collectively referred to as aflatoxicosis (Cullen and Newberne, 1994).

Identification of populations susceptible to chemical toxicity is an integral component of risk assessment. Epidemiological studies of AFB_1 exposure have proved to be crucial in identification of "at risk" populations for hepatotoxicity and liver carcinoma. In regions where AFB_1 exposure is commonplace, there is a strong correlation between hepatocellular carcinoma incidence and hepatitis B infection, a defining feature of which is inflammation of the liver (Groopman *et al*., 1993). Strong association can be seen between expression of hepatitis B viral proteins and an inflammatory response, and it has been suggested that this may enhance the action of certain hepatocarcinogens by increasing rates of hepatocellular injury and proliferation (Jin *et al*., 2001; Sell *et al*., 1991). Moreover, strong positive correlations have been found in rats between AFB_1-induced acute liver injury and preneoplastic lesions (Maxuitenko *et al*., 1996).

Supporting the correlations identified in people are studies in experimental animals, which suggest that modest inflammation increases the hepatotoxic response to AFB_1 (Barton *et al*., 2000a). Endotoxic lipopolysaccharide (LPS) is an outer cell-wall component of gram-negative bacteria. It is a potent inflammagen and contributes significantly to the pathogenesis of gram-negative bacterial infections by activating toll-like receptors on inflammatory cells, which in turn precipitate the expression of numerous soluble inflammatory mediators. Exposure to large amounts of LPS during conditions such as sepsis is associated with fever, circulatory shock, disseminated intravascular coagulation, and injury to several organs, including the liver (Ghosh *et al*., 1993). In contrast, small doses of LPS do not cause overt tissue injury but can nevertheless lead to

tissue accumulation of inflammatory cells and release of inflammatory mediators. Episodes of modest inflammation, although benign on their own, are probably commonplace in people and have the ability to augment the toxicities of several xenobiotic agents (Ganey and Roth, 2001; Roth *et al*., 1997).

The aim of this study was to quantify the ability of LPS to shift the dose-response relationship for AFB_1-induced liver injury. AFB_1 was given at various doses in the presence or absence of a small, noninjurious dose of LPS, and liver injury was determined via analysis of serum enzyme markers. Benchmark dose (BMD) analysis was used to estimate the magnitude of LPS-induced shifts in sensitivity to AFB_1 hepatotoxicity.

MATERIALS AND METHODS

Animals and materials. Male Sprague-Dawley rats (CD-Crl: CD-(SD)BR VAF/Plus; Charles River, Portage, MI) weighing 250–350 grams were used for these studies. Reagent kits used to measure serum markers of liver injury (Infinity-ALT, Infinity-AST, ALP, GGT) were purchased from Sigma Chemical Co. (St. Louis, MO), as was lipopolysaccharide derived from *E. coli* serotype 0128:B12 with an activity of 1.7×10^6 EU (endotoxin units)/mg. A colorometric, kinetic limulus amebocyte lysate (LAL) assay was used to estimate LPS-specific activity using a kit (#50-650U) purchased from Biowhittaker (Walkersville, MD). Unless otherwise stated, all chemicals were purchased from Sigma Chemical Co.

Treatment protocol. In preliminary studies, 24-h fasting had minimal effect on the magnitude of hepatotoxicity but decreased variability in response among animals. Rats fasted for 24 h were given a dose of AFB_1 ranging from 0.1 mg/kg to 6.3 mg/kg intraperitoneally in a vehicle comprising 8% DMSO in sterile water. Four h later they were given 7.4×10^6 EU LPS/kg or sterile saline via the tail vein. This dose of LPS was not overtly hepatotoxic when administered alone (Barton *et al*., 2000a). Twenty-four h after AFB_1 administration, rats were anesthetized with sodium pentobarbital (50 mg/kg, ip), and blood was drawn from the dorsal aorta, allowed to clot, and centrifuged to separate serum.

Serum markers of liver injury. Commercial reagent kits (see above) were used to measure serum enzyme activities. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured spectrophotometrically by the methods of Wroblewski and LaDue (1956) and Karmen (1955), respectively. Serum γ-glutamyl transferase (GGT) and alkaline phosphatase (ALP) activities were measured by the methods of Szasz (1974) and Bowers and McComb (1966), respectively.

Benchmark dose (BMD) analysis. Background and technical information on BMD analysis were obtained from the most recent United States Environmental Protection Agency guidance publication (U.S. EPA, 1995). Dose-response curves were analyzed using the EPA's Benchmark Dose Response software (Version 1.3), which was developed by the National Center for Environmental Assessment to aid in computer analysis of dose-response data (information and software available at http://cfpub.epa.gov/ncea/cfm/nceahome.cfm). A continuous Hill model was employed to calculate benchmark doses for individual dose-response curves. This model was utilized because it provided an adequate fit to data for each of the toxicity endpoints measured. The benchmark response was defined as the response corresponding to one control standard deviation from the control mean. Assuming serum ALT activities above the 99th percentile of the control mean (control mean + 2.33 standard deviations) are considered adverse, this benchmark response identifies an AFB_1 dose at which 10% of treated animals would have serum ALT activities above the 99th percentile (Crump, 1995). Assumption of equal slopes was confirmed via calculation of the Hill slope from a best-fit, four-parameter logistic model for each curve. Hill slopes for individual curves were compared statistically using Student's *t*-test.

Statistical analysis. Results are expressed as mean ± SE. *N* for treatment groups was 4–11; vehicle-treated rats were included on each experimental day such that *n* for combined animals was 24. Data were analyzed using a one-way ANOVA, with group comparisons made with Tukey's test. The Kolmogorov-Smirnov test was applied to test homogeneity of variance. Data with nonhomogenous variance were analyzed using Kruskal-Wallis one-way ANOVA on ranks, with Dunn's *post hoc* test for multiple comparisons. The criterion for significance was $p < 0.05$ for all studies.

RESULTS

Hepatic Parenchymal Cell Injury

Animals received either intravenous saline or 7.4×10^6 EU/kg LPS 4 h after AFB_1 administration, and hepatic parenchymal cell injury was assessed 24 h after AFB_1 treatment. Doses for the AFB_1/Veh curve ranged from 0.63 mg/kg to 6.3 mg/kg AFB_1, whereas doses for the AFB_1/LPS curve ranged from 0.1 mg/kg to 1.0 mg/kg AFB_1. In the AFB_1/saline and AFB_1/LPS groups given the largest dose of AFB_1, mortality was approximately 30% and 50%, respectively, whereas survival at the other AFB_1 doses was between 90–100%. Blood samples were taken from surviving rats for determination of biomarkers of liver injury. Increases in ALT (Fig. 1A) and AST (Fig. 1B) activities in AFB_1/Veh-treated animals were dose-dependent, with a sharp increase in activity near 4.0 mg/kg AFB_1 for both markers. No observed adverse-effect levels (NOAELs) for ALT and AST were 4.0 mg/kg and 2.0 mg/kg AFB_1, respectively. Significant increases in both markers were observed at markedly smaller AFB_1 doses in animals cotreated with LPS. The NOAEL for both ALT and AST in AFB_1/LPS-cotreated animals was 0.25 mg/kg. Thus, using the NOAEL as a marker of hepatic parenchymal cell injury, LPS cotreatment resulted in an 8–16-fold increase in AFB_1 toxicity.

Bile Duct Injury

Consistent with markers of parenchymal cell injury, appreciably larger AFB_1 doses were required to cause significant increases in ALP (Fig. 2A) and GGT (Fig. 2B) activities in the AFB_1/Veh group than in the AFB_1/LPS cotreated group. NOAEL values for increases in ALP activity were 4.0 mg/kg and 0.25 mg/kg AFB_1 for AFB_1/Veh- and AFB_1/LPS-treated animals, respectively. The NOAEL value for GGT in AFB_1/LPS cotreated animals was 0.4 mg/kg, whereas no significant increases in GGT were seen for animals treated with AFB_1 alone (i.e., NOAEL $\geq$ 6.3 mg/kg). Therefore, the NOAEL dose for markers of bile duct injury in AFB_1/LPS cotreated animals was about 16-fold less than the NOAEL for animals treated with AFB_1 alone.

Benchmark Analysis of AFB_1 Dose Response

Due to limitations of NOAEL as an estimation of threshold response, benchmark dose analysis was performed (U.S. EPA, 1995). Table 1 illustrates the BMD calculated for a continuous Hill model with a benchmark response defined as the response

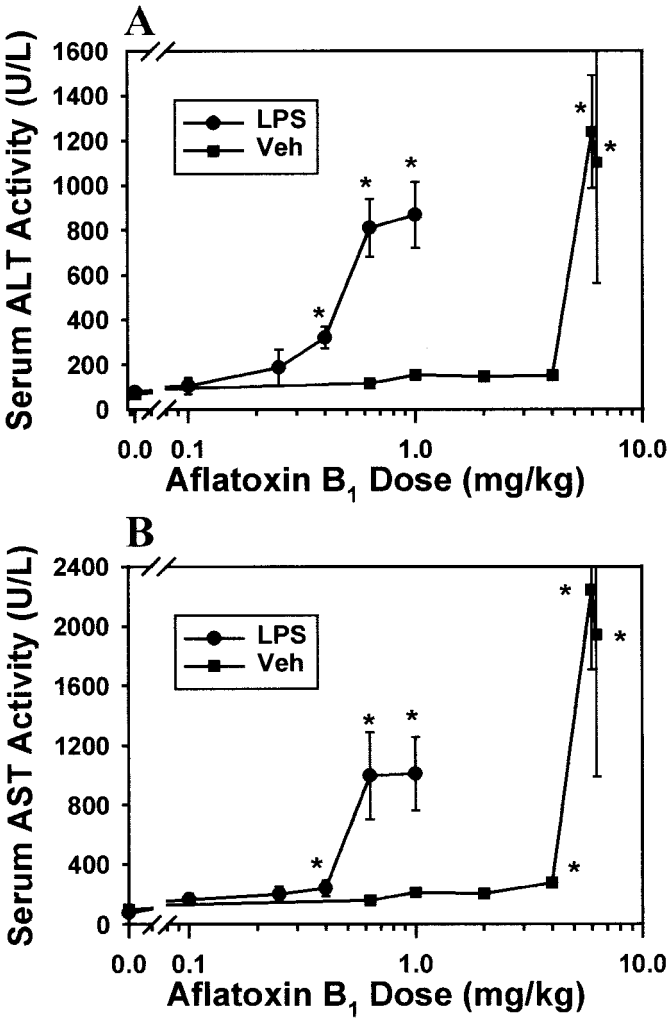

FIG. 1. AFB_1 dose response for serum markers of hepatic parenchymal cell injury. Rats were treated with various doses of AFB_1, then 4 h later with 7.4×10^6 EU/kg LPS or saline. AFB_1 doses used for saline-treated animals were 0.63, 1.0, 2.0, 4.0, 6.0, and 6.3 mg/kg. AFB_1 doses used for LPS-treated animals were 0.1, 0.25, 0.4, 0.63, and 1.0 mg/kg. Hepatic parenchymal cell injury was estimated by measuring the serum activities of (A) alanine aminotransferase (ALT) and (B) aspartate aminotransferase (AST). Data are expressed as means ± SEM; $n = 4$–24 animals; *significantly different from respective control group not treated with AFB_1 ($p < 0.05$).

corresponding to one control standard deviation above the control mean. AFB_1 BMD values for ALT and AST in animals treated with AFB_1 alone were 3.97 mg/kg and 3.71 mg/kg, respectively. AFB_1 BMD values for ALT and AST activities in LPS-cotreated animals were markedly decreased to 0.36 mg/kg and 0.38 mg/kg, respectively. Thus, the BMD calculation indicated a 10-fold leftward shift in the BMD for enzyme markers of hepatic parenchymal cell injury. AFB_1 BMD values for ALP were 4.61 mg/kg and 0.18 mg/kg for AFB_1/Veh- and AFB_1/LPS-treated animals, respectively. The benchmark response for GGT activity was not achieved within the dose range for animals treated with AFB_1 alone. However, cotreated animals displayed an AFB_1 BMD of 0.22 mg/kg, signifying a pronounced leftward shift in BMD for GGT activity. Hence, AFB_1 BMDs for markers of bile duct injury were decreased ≥ 20-fold.

DISCUSSION

Liver lesions associated with LPS-enhanced AFB_1 hepatotoxicity mimic those occurring with a large dose of AFB_1 and are characterized by periportal necrosis and bile duct epithelial cell injury (Barton *et al*., 2000a; Kalengayi and Desmet, 1975).

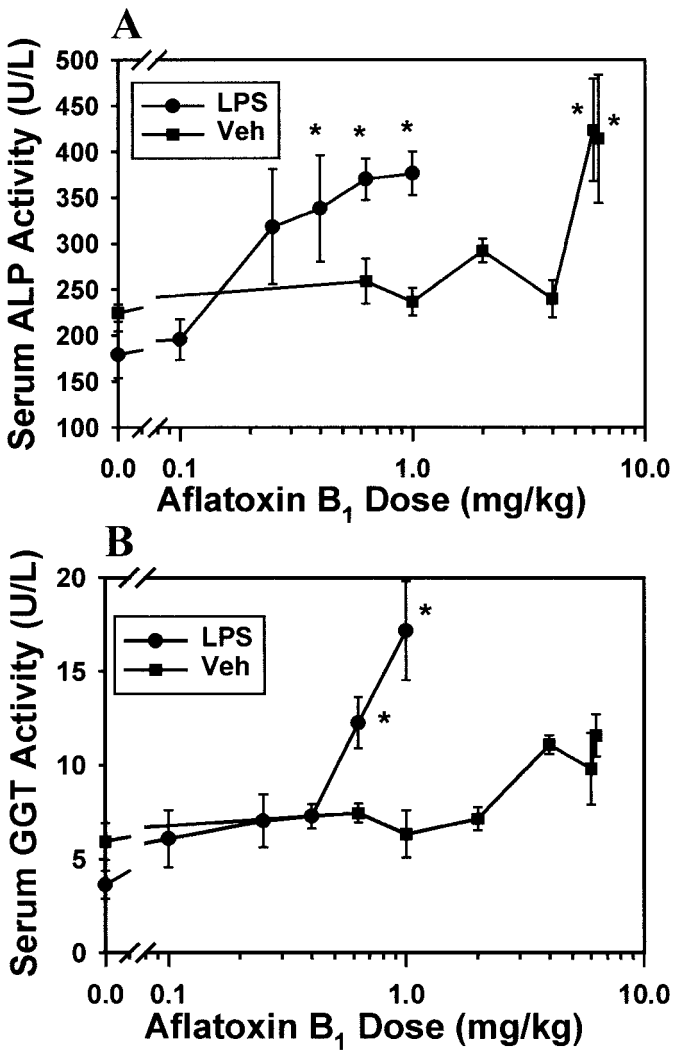

FIG. 2. AFB_1 dose response for serum markers of bile-duct injury. Rats were treated with various doses of AFB_1, then 4 h later with 7.4×10^6 EU/kg LPS or saline. AFB_1 doses used for saline-treated animals were 0.63, 1.0, 2.0, 4.0, 6.0, and 6.3 mg/kg. AFB_1 doses used for LPS-treated animals were 0.1, 0.25, 0.4, 0.63, and 1.0 mg/kg. Bile duct injury was estimated by measuring the serum activities of (A) alkaline phosphatase (ALP) and (B) γ- glutamyltransferase (GGT). Data are expressed as means ± SEM; $n = 4$–24 animals; *significantly different from respective control group not treated with AFB_1 ($p < 0.05$).

TABLE 1
Effect of LPS Administration on AFB_1 Benchmark Doses

	AFB_1 +Veh			AFB_1 +LPS		
Serum enzyme	BMD (mg/kg)	95% CI[a]	GOF[b] *p*-value	BMD (mg/kg)	95% CI	GOF *p*-value
ALT	3.97	1.53	0.96	0.36	0.11	0.79
AST	3.71	1.57	0.89	0.38	0.08	0.90
ALP	4.61	3.59	0.39	0.18	0.10	0.88
GGT	ND	ND	0.81	0.22	0.16	0.58

Note. Rats were treated with various doses of AFB_1, then 4 h later with 7.4×10^6 EU/kg LPS or saline. Dose-response data were fit using a continuous Hill model. The BMD refers to an AFB_1 dose (mg/kg) required to cause an increase in enzyme activity one control standard deviation above the control mean. ND, not determined.

[a]Lower 95% confidence limit of the BMD (mg/kg).

[b]Goodness of fit (GOF) *p*-value provides a measure of the degree to which the predicted incidence and observed incidence are the same (U.S. EPA, 2001). A *p*-value greater than 0.05 indicates that the model effectively describes the data.

Thus, based on the nature of the lesions, LPS appears to enhance AFB_1 hepatotoxicity (Barton *et al*., 2000a). Dose-response curves for markers of AFB_1-induced hepatic parenchymal cell and bile duct injury showed a marked leftward shift in animals coexposed to a nontoxic dose of LPS. We used both NOAEL and BMD analysis to estimate shifts in thresholds for toxicity. Application of the traditional NOAEL analysis to dose-response data for non-cancer health effects comes with several disadvantages, and NOAEL values often differ markedly from derived BMDs (Allen *et al*., 1994; Gaylor *et al*., 1998). For example, the assignment of a NOAEL relies critically on data from only one dose, whereas BMD analysis takes all of the dose-response relationship into account, including slope, in determining the BMD. Moreover, in contrast to BMD analysis, NOAEL values are highly dependent on sample size and tend to be larger in studies with a smaller sample size (U.S. EPA, 1995). In an effort to overcome the shortcomings of NOAEL analysis, numerous investigators have used BMD analysis to analyze dose-response toxicity data for diverse effects including neurotoxicity, developmental toxicity, and effects on the endocrine system (Mantovani *et al*., 1998; Rabovsky *et al*., 2001; Zhou *et al*., 2001). In this study, benchmark doses determined for each curve indicated that the dose required to achieve the predetermined benchmark response was 10–20-fold less in LPS-cotreated animals. NOAEL values for the dose-response curves were decreased 8–16-fold by LPS cotreatment. Thus, as measures of toxicity threshold, both the NOAEL and BMD analyses showed similar estimates of the increase in AFB_1 toxicity in response to LPS cotreatment.

Inflammatory events initiated by LPS are responsible for its ability to augment AFB_1 toxicity. Elevations in plasma tumor necrosis factor-α (TNF-α) are seen before the onset of AFB_1/LPS-induced liver injury, and neutralization of TNF-α protects against the augmentation of both parenchymal cell injury and bile duct injury (Barton *et al*., 2001). Similarly, neutrophils (PMNs) accumulate early in the livers of AFB_1/LPS-treated rats, and PMN depletion prior to AFB_1/LPS treatment causes a significant reduction in hepatocellular injury. By contrast, PMN depletion does not alter bile duct epithelial cell (BDEC) injury in this model (Barton *et al*., 2000b). This result suggests that two different mechanisms are operative, one for hepatocellular injury that depends on PMNs and another for bile-duct damage that is independent of PMNs. In the present dose-response analysis, LPS produced a greater leftward shift in biliary injury markers than in markers of hepatocellular damage. This result is consistent with different mechanisms underlying injury to parenchymal cells vs. BDECs. Another possibility is that the same mechanism contributes to injury in both cell types but that parenchymal cells are less easily damaged than BDECs and require an additional, PMN-dependent insult for the expression of overt injury.

AFB_1 hepatotoxicity requires metabolic activation of AFB_1 to its toxic 8,9-epoxide (Eaton *et al*., 1994). Formation of the AFB_1 8,9-epoxide is catalyzed by cytochrome (CYP) P450 family members including CYP1A2 and CYP3A4 (Eaton *et al*., 1994). One possible explanation for the enhancing effects of LPS on AFB_1 toxicity is that LPS might increase production of reactive 8,9-epoxide. However, LPS treatment has been shown to cause a decrease in hepatic CYP450 levels (Liu *et al*., 2000). Additionally, inflammatory cytokines, including TNF-α and IL-1, decrease expression of CYP450 isoforms responsible for AFB_1 metabolism, both *in vitro* and *in vivo* (Muntane-Relat *et al*., 1995; Pous *et al*., 1990). Accordingly, it seems unlikely that LPS enhanced hepatotoxicity in this model via increased production of reactive AFB_1 metabolites. Nevertheless, in some human studies, evidence suggests that chronic inflammation of the liver caused by disease states (e.g., hepatitis) causes upregulation of the CYP1A2 and CYP3A4 isoforms of CYP450 (Kirby *et al*., 1996).

One important route of AFB_1 8,9-epoxide detoxification is via conjugation to glutathione (GSH) (Degen and Neumann, 1978; Eaton *et al*., 1994). It is conceivable that LPS might decrease liver GSH concentration, thereby decreasing capacity

to detoxify the AFB_1 8,9-epoxide, and resulting in enhanced hepatotoxicity. However, LPS given to rats at a hepatotoxic dose nearly 13-fold greater than that used in this study did not cause significant reduction in hepatic GSH concentration (Sneed *et al*., 1997). Therefore, it is unlikely that the small dose of LPS used in this study enhances AFB_1 toxicity via alteration of hepatic GSH levels. Nevertheless, to minimize variation in responses, we used rats that had been fasted for 24 h, a procedure shown to decrease liver GSH content (Maruyama *et al*., 1968). The possibility of a synergistic action of LPS and fasting on hepatic GSH levels cannot be ruled out, but preliminary studies indicated that fasting decreased variability among animal responses, with little effect on the magnitude of hepatotoxicity produced after AFB_1/LPS cotreatment (data not shown).

People with hepatitis B who are exposed to AFB_1 are at greater risk for hepatocellular carcinoma (Qian *et al*., 1994). Localized expression of hepatitis viral proteins in periportal regions of the liver strongly correlates with periportal inflammation, and Jin *et al*. (2001) have suggested that inflammation induced by these proteins during hepatitis may contribute to multistage carcinogenesis by increasing rates of cellular damage and proliferation. Indeed, partial hepatectomy or pretreatment with carbon tetrachloride in AFB_1-treated rats increases the quantity and size of placental glutathione *S*-transferase (GST-P)-positive, preneoplastic foci (Hiruma *et al*., 1996). Additionally, in AFB_1-treated rats, the incidence of GST-P-positive preneoplastic lesions in livers strongly correlates with increases in biomarkers of acute AFB_1 hepatotoxicity (Maxuitenko *et al*., 1996). These results suggest that physical or chemical injury to the liver may increase sensitivity to the carcinogenic effects of AFB_1 by inducing hepatocellular proliferation. Our results indicate that the sensitivity to AFB_1-induced parenchymal cell injury is markedly enhanced by modest inflammation caused by LPS, and previously reported results indicated that enhanced hepatocellular proliferation follows the liver injury (Barton *et al*., 2000b, 2001). Thus, our results are consistent with the hypothesis that LPS or other inflammagens predispose individuals to the hepatocarcinogenic effects of AFB_1 by enhancing hepatocellular proliferation.

In conclusion, modest inflammation induced by LPS exposure causes a pronounced leftward shift in dose-response curves for AFB_1-induced liver injury. Considering the commonplace nature of exposure of humans to LPS and other inflammagens, concurrent inflammation should be considered as a potentially important risk factor for hepatotoxic effects of AFB_1 and other xenobiotic agents. Concurrent inflammation is only one of many determinants of individual sensitivity to chemical toxicity. Others include age, gender, metabolic polymorphisms, diet, and coexposure to other xenobiotics. The present observation that a single one of these has the potential to increase sensitivity by 10–20-fold is remarkable, especially when one considers the potential for additivity or synergy among such factors. Inflammation should be considered along with other determinants of sensitivity in the setting of safety factors or estimations of risk in risk assessment paradigms.

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REFERENCES

Allen, B. C., Kavlock, R. J., Kimmel, C. A., and Faustman, E. M. (1994). Dose-response assessment for developmental toxicity: II. Comparison of generic benchmark dose estimates with no observed adverse effect level. *Fundam. Appl. Toxicol*. **23,** 487–495.

Barton, C. C., Barton, E. X., Ganey, P. E., Kunkel, S. L., and Roth, R. A. (2001). Bacterial lipopolysaccharide enhances aflatoxin B1 hepatotoxicity in rats by a mechanism that depends on tumor necrosis factor-alpha. *Hepatology* **33**, 66–73.

Barton, C. C., Ganey, P. E., and Roth, R. A. (2000a). Lipopolysaccharide augments aflatoxin B(1)-induced liver injury through neutrophil-dependent and -independent mechanisms. *Toxicol. Sci*. **58,** 208–215.

Barton, C. C., Hill, D. A., Yee, S. B., Barton, E. X., Ganey, P. E., and Roth, R. A. (2000b). Bacterial lipopolysaccharide exposure augments aflatoxin B(1)-induced liver injury. *Toxicol. Sci*. **55,** 444–452.

Bowers, G. N., Jr., and McComb, R. B. (1966). A continuous spectrophotometric method for measuring the activity of serum alkaline phosphatase. *Clin. Chem*. **12,** 70–89.

Crump, K. S. (1995). Calculation of benchmark doses from continuous data. *Risk Anal*. **15,** 79–89.

Cullen, J. M., and Newberne, P. M. (1994). Acute hepatotoxicity of aflatoxins. In *The Toxicology of Aflatoxins* (D.L. Eaton and J.D. Groopman, Eds.), pp. 3–26. Academic Press, San Diego.

Degen, G. H., and Neumann, H. G. (1978). The major metabolite of aflatoxin B1 in the rat is a glutathione conjugate. *Chem. Biol. Interact*. **22,** 239–255.

Eaton, D. L., Ramsdell, H. S., and Neal, G. E. (1994). Biotransformation of Aflatoxins. In *The Toxicology of Aflatoxins* (D. L. Eaton and J. D. Groopman, Eds.), pp. 45–72. Academic Press, San Diego.

Ganey, P. E., and Roth, R. A. (2001). Concurrent inflammation as a determinant of susceptibility to toxicity from xenobiotic agents. *Toxicology* **169,** 195–208.

Gaylor, D., Ryan, L., Krewski, D., and Zhu, Y. (1998). Procedures for calculating benchmark doses for health risk assessment. *Regul. Toxicol. Pharmacol*. **28,** 150–164.

Ghosh, S., Latimer, R. D., Gray, B. M., Harwood, R. J., and Oduro, A. (1993). Endotoxin-induced organ injury. *Crit. Care Med*. **21**(2 Suppl.), S19–S24.

Groopman, J. D., Wild, C. P., Hasler, J., Junshi, C., Wogan, G. N., and Kensler, T. W. (1993). Molecular epidemiology of aflatoxin exposures: Validation of aflatoxin-N7-guanine levels in urine as a biomarker in experimental rat models and humans. *Environ. Health Perspect*. **99,** 107–113.

Groopman, J. D., Zhu, J. Q., Donahue, P. R., Pikul, A., Zhang, L. S., Chen, J. S., and Wogan, G. N. (1992). Molecular dosimetry of urinary aflatoxin-DNA adducts in people living in Guangxi Autonomous Region, People's Republic of China. *Cancer Res*. **52,** 45–52.

Hall, J. H., and Wild, C. P. (1994). Epidemiology of aflatoxin-related disease. In *The Toxicology of Aflatoxins* (D. L. Eaton and J. D. Groopman, Eds.), pp. 223–258. Academic Press, San Diego.

Hiruma, S., Qin, G. Z., Gopalan-Kriczky, P., Shinozuka, H., Sato, K., and Lotlikar, P. D. (1996). Effect of cell proliferation on initiation of aflatoxin B1-induced enzyme altered hepatic foci in rats and hamsters. *Carcinogenesis* **17,** 2495–2499.

Jin, Y. M., Yun, C., Park, C., Wang, H. J., and Cho, H. (2001). Expression of hepatitis B virus × protein is closely correlated with the high periportal inflammatory activity of liver diseases. *J. Viral Hepat.* **8,** 322–330.

Kalengayi, M. M., and Desmet, V. J. (1975). Sequential histological and histochemical study of the rat liver during aflatoxin B1-induced carcinogenesis. *Cancer Res*. **35,** 2845–2852.

Karmen, A. (1955). A note on the spectrophotometric assay of glutamic-oxalacetic transaminase in human blood. *J. Clin. Invest*. **34,** 131.

Kirby, G. M., Batist, G., Alpert, L., Lamoureux, E., Cameron, R. G., and Alaoui-Jamali, M. A. (1996). Overexpression of cytochrome P-450 isoforms involved in aflatoxin B1 bioactivation in human liver with cirrhosis and hepatitis. *Toxicol. Pathol*. **24,** 458–467.

Li, F. Q., Yoshizawa, T., Kawamura, O., Luo, X. Y., and Li, Y. W. (2001). Aflatoxins and fumonisins in corn from the high-incidence area for human hepatocellular carcinoma in Guangxi, China. *J. Agric. Food Chem.* **49,** 4122–4126.

Liu, J., Sendelbach, E., Parkinson, A., and Klaassen, C. D. (2000). Endotoxin pretreatment protects against the hepatotoxicity of acetaminophen and carbon tetrachloride: Role of cytochrome P450 suppression. *Toxicol*ogy **147,** 167–176.

Mantovani, A., Maranghi, F., Ricciardi, C., Macri, C., Stazi, A. V., Attias, L., and Zapponi G. A. (1998). Developmental toxicity of carbendazim: Comparison of no-observed-adverse-effect level and benchmark dose approach. *Food Chem. Toxicol*. **36,** 37–45.

Maruyama, E., Kojima, K., Higashi, T., and Sakamoto, Y. (1968). Effect of diet on liver glutathione and glutathione reductase. *J. Biochem*. (*Tokyo)* **63,** 398–399.

Maxuitenko, Y. Y., Curphey, T. J., Kensler, T. W., and Roebuck, B. D. (1996). Protection against aflatoxin B_1-induced hepatic toxicity as a short-term screen of cancer chemopreventive dithiolethiones. *Fundam. Appl. Toxicol*. **32,** 250–259.

Muntane-Relat, J., Ourlin, J.-C., Domergue, J., and Maurel, P. (1995). Differential effects of cytokines on the inducible expression of CYP1A1, CYP1A2, and CYP3A4 in human hepatocytes in primary culture. *Hepatology* **22,** 1143–1153.

Pous, C., Giroud, J. P., Damais, C., Raichvarg, D., and Chauvelot-Moachon, L. (1990). Effect of recombinant human interleukin-1 beta and tumor necrosis factor alpha on liver cytochrome P-450 and serum alpha-1-acid glycoprotein concentrations in the rat. *Drug Metab. Dispos*. **18,** 467–470.

Qian, G. S., Ross, R. K., Yu, M. C., Yuan, J. M., Gao, Y. T., Henderson, B. E., Wogan, G. N., and Groopman, J. D. (1994). A follow-up study of urinary markers of aflatoxin exposure and liver cancer risk in Shanghai, People's Republic of China. *Cancer Epidemiol. Biomarkers Prev*. **3,** 3–10.

Rabovsky, J., Fowles, J., Hill, M. D., and Lewis, D. C. (2001). A health risk benchmark for the neurologic effects of styrene: Comparison with NOAEL/LOAEL approach. *Risk Anal.* **21,** 117–126.

Roebuck, B. D., and Maxuitenko, Y. Y. (1994). Biochemical mechanisms and biological implications of the toxicity of aflatoxins as related to aflatoxin carcinogenesis. In *The Toxicology of Aflatoxins* (D. L. Eaton and J. D. Groopman, Eds.), pp. 27–43. Academic Press, San Diego.

Roth, R. A., Harkema, J. R., Pestka, J. P., and Ganey, P. E. (1997). Is exposure to bacterial endotoxin a determinant of susceptibility to intoxication from xenobiotic agents? *Toxicol. Appl. Pharmacol.* **147,** 300–311.

Sell, S., Hunt, J. M., Dunsford, H. A., and Chisari, F. V. (1991). Synergy between hepatitis B virus expression and chemical hepatocarcinogens in transgenic mice. *Cancer Res*. **51,** 1278–1285.

Sneed, R. A., Grimes, S. D., Schultze, A. E., Brown, A. P., and Ganey, P. E. (1997). Bacterial endotoxin enhances the hepatotoxicity of allyl alcohol. *Toxicol. Appl. Pharmacol*. **144,** 77–87.

Szasz, G. (1974). New substrates for measuring gamma-glutamyl transpeptidase activity. *Z. Klin. Chem. Klin. Biochem*. **12,** 228.

U.S. EPA (1995). *The Use of the Benchmark Dose Approach in Health Risk Assessment*. Office of Research and Development, Environmental Protection Agency. Washington, DC. EPA/630/R94/007. February.

U.S. EPA (2001). *Help Manual for Benchmark Dose Software*. Office of Research and Development, Environmental Protection Agency. Washington, DC. EPA 600/R-00/014F. March.

Wilson, D. M., and Payne, G. A. (1994). Factors affecting *Aspergillus flavus* group infection and aflatoxin contamination of crops. In *The Toxicology of Aflatoxins* (D. L. Eaton and J. D. Groopman, Eds.), pp. 309–325. Academic Press, San Diego.

Wood, G. E. (1989). Aflatoxins in domestic and imported foods and feeds. *J. Assoc. Off. Anal. Chem*. **72,** 543–548.

Wroblewski, F., and LaDue, J. S. (1956). Serum glutamic-pyruvic transaminase in cardiac hepatic disease. *Proc. Soc. Exp. Biol. Med*. **91,** 569.

Zhou, T., Ross, D. G., DeVito, M. J., and Crofton, K. M. (2001). Effects of short-term *in vivo* exposure to polybrominated diphenyl ethers on thyroid hormones and hepatic enzyme activities in weanling rats. *Toxicol. Sci*. **61,** 76–82.