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Evidence for Lipid Peroxidation in Endotoxin-Poisoned Mice

DUANE L. PEAVY[1]* AND EDWARD J. FAIRCHILD II[2]

Program in Medical Technology/Cytogenetics, School of Allied Health Sciences,[1] and the Division of Environmental Sciences, School of Public Health,[2] University of Texas Health Science Center, Houston, Texas 77225

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Ethane has been identified and quantitated in air exhaled by mice following intraperitoneal injection of 20, 40, or 200 mg of *Escherichia coli* O111:B4 lipopolysaccharide (LPS) per kg. Significant increases in ethane concentration occurred within 1 to 5 h after LPS administration. In addition, increased concentrations of malondialdehyde were found in crude homogenates of livers obtained from mice 16 h after administration of 20 mg of LPS per kg. These results suggest that lipid peroxidation may be an important mechanism responsible for LPS toxicity.

When injected into experimental animals or when generated by gram-negative infection, bacterial lipopolysaccharide (LPS) or endotoxin elicits a diverse array of pathophysiologic effects, including lymphocyte transformation, macrophage activation, and initiation of the complement, kinin, and blood coagulation pathways (15, 16, 18). Although these pathways appear responsible for the increased vascular permeability observed during hypovolemic shock, a satisfactory explanation for the capacity of LPS to injure or kill host cells has yet to be identified.

Recent experimental evidence suggests that mononuclear phagocytic cells play a central role in mediating the toxic effects of bacterial LPS (21). After intravenous administration, LPS accumulates in the liver and spleen, organs rich in reticuloendothelial cells (5). Hyperreactivity, characterized by a 100- to 10,000-fold decrease in the 50% lethal dose for LPS, occurs in mice 10 to 14 days after infection with *Mycobacterium bovis* BCG or *Corynebacterium parvum* or after multiple, daily injections of zymosan, glucan, or muramyldipeptide (3, 6, 28). Macrophages obtained from BCG-infected mice demonstrate similar increases in sensitivity to the cytotoxic effects of LPS in vitro (19, 20). Shortly after BCG-activated macrophages are incubated with trace amounts of LPS, these cells acquire tumoricidal activity, in part due to their ability to secrete H_2O_2 and free radicals of O_2 (17). These products have been shown to damage and destroy normal cells by electrophilic attack upon proteins and nucleic acids and initiate peroxidation of polyunsaturated fatty acids. We examined, therefore, expired air from LPS-poisoned mice for the presence of ethane, a specific product of lipid peroxidation reactions (8, 9, 24).

Adult (20 to 25 g), female, Swiss Webster (Texas Inbred Mouse Co.) and C3H/HeJ (Jackson Laboratory, Bar Harbor, Maine) mice were used in this investigation. Standard laboratory chow and water were available ad libitum at all times except when mice were housed in the inhalation chamber. An air-tight, moisture-free chamber was constructed with an 8.75-liter, plastic, vacuum desiccator equipped with a two-way stopcock. A wire mesh screen (6 by 6 in. [~15 by 15 cm]) was placed over a Drierite bed (W. A. Hammond Drierite Co., Xenia, Ohio) for support. Immediately after each treatment, five of the Swiss Webster mice were placed inside the desiccator, and the lid was tightly sealed with vacuum grease and clamps. The chamber was then flushed with high-purity O_2 (>99.99%; Scott Specialty Gases, Houston, Tex.) for 30 s at 15 ml/min. Air samples were withdrawn through the stopcock with a 1-ml gastight syringe (series 1000; The Hamilton Co., Reno, Nev.) and were injected immediately into a gas chromatograph (model 910; The Perkin-Elmer Corp., Norwalk, Conn.) fitted with a stainless steel column (6 ft. by 0.125 in. [~1.83 m by 0.318 cm]) containing 100/120 mesh Poropak R. Trace ethane analysis required use of an He carrier (30 ml/min), a flame ionization detector, and maximal-sensitivity electrometer settings. Injector, oven, and detector temperatures were 100, 50, and 200°C, respectively. Ethane was identified by retention time, using a certified standard of 8 ppm of ethane in N_2 (Linde Specialty Gases, Houston, Tex.), and quantitated by comparison to peak areas produced by serial dilutions of the standard. Results were expressed as picomoles of ethane per milliliter. Data from control and experimental groups were analyzed by Student's *t* test. The apparatus was examined for leaks by completely filling the chamber with standard ethane and sampling the atmosphere every 2 h for 16-h period. After 12 h, the loss was less than 5% of the original ethane concentration.

Various amounts of LPS, extracted from *Escherichia coli* O111:B4 by the phenol-water technique were resuspended in saline and administered intraperitoneally (i.p.) in 0.2 ml. Results demonstrated that ethane was exhaled by LPS-poisoned mice in a dose- and time-dependent manner (Fig. 1). Ethane concentrations increased to a statistically significant ($P < 0.05$) level within 5 h after 20 mg of LPS per kg, within 3 h after 40 mg of LPS per kg, and within 1 h after 200 mg of LPS per kg. Comparison of ethane concentrations at each time point revealed a gradual increase, indicating that a continued accumulation had occurred at all LPS doses tested. Maximal concentrations were observed after 7 h, reaching levels 2.5- to 3-fold greater than concentrations obtained at time zero. A response of this magnitude compared favorably with a sixfold increase measured 7 h after i.p. administration of 3,000 mg of carbon tetrachloride in mineral oil per kg (data not shown).

Further biochemical evidence for the occurrence of lipid peroxidation was obtained by quantitating malondialdehyde (MDA) in livers of endotoxin-poisoned mice. At 16 h after i.p. administration of LPS or saline, Swiss Webster mice

* Corresponding author.

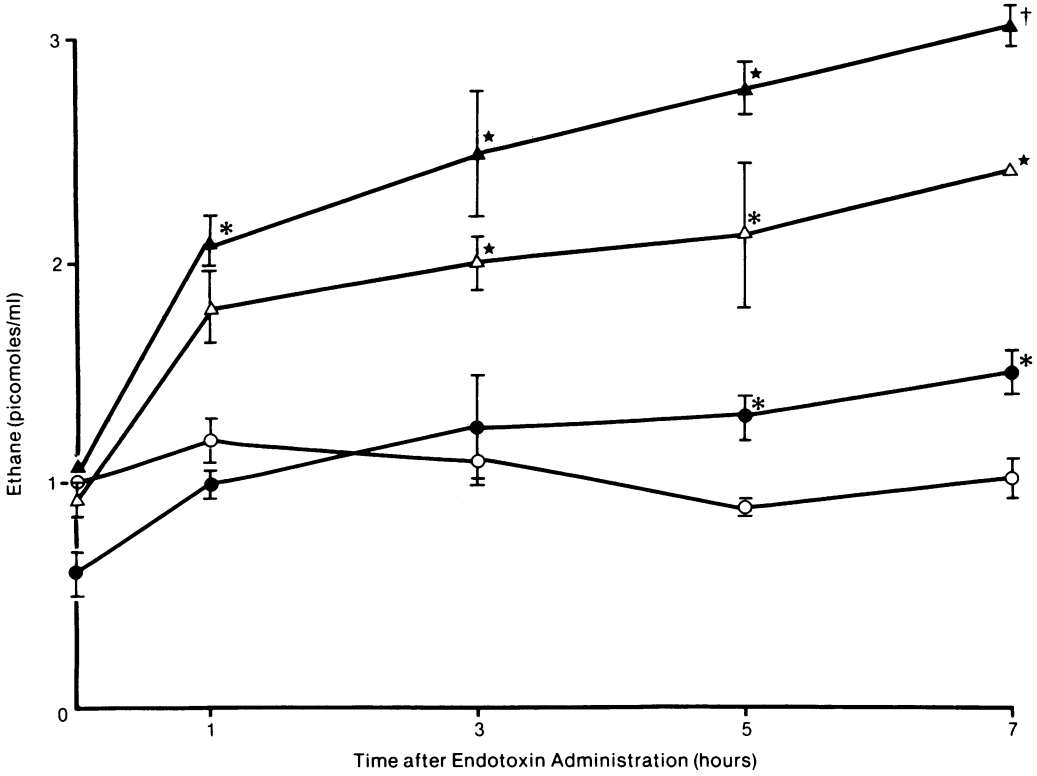

FIG. 1. Kinetics of ethane production by endotoxin-poisoned mice. Mice received 20 (●), 40 (△), or 200 (▲) mg/kg LPS or saline (○) i.p. Ethane was measured in 1-ml air samples at the indicated times after LPS administration. Results obtained from three experiments are expressed as the mean ± standard error. Asterisks, stars, and crosses denote $P < 0.05$, 0.01, and 0.001, respectively.

were sacrificed by cervical dislocation. Each liver was removed, rinsed in 5 ml of 0.25 M TMN-sucrose buffer, pH 7.4, and placed in a tissue homogenizer containng 10 ml of TMN-sucrose buffer. After homogenation at 4°C, a sample was diluted in 0.1 M phosphate-buffered saline, pH 7.4, to approximately 1 mg/ml (Lowry method, using bovine serum albumin as a standard). MDA in 1-ml samples of the crude homogenate was measured spectrophotometrically by reaction with 2-thiobarbituric acid, in accordance with the method of Stohs et al. (17, 26). Results of these experiments, expressed as the mean nanograms of MDA per milligram of protein derived from groups of five mice, are illustrated in Fig. 2. A dose of 2 mg of LPS per kg had no appreciable effect; however, 20 mg of LPS per kg, a dose shown to enhance ethane evolution, increased the MDA concentration to a statistically significant level ($P < 0.05$). In contrast, pretreatment of mice with α-*dl*-tocopherol, 100 mg/kg i.p. daily for 4 days before administration of 20 mg of LPS per kg, entirely inhibited the observed increase in MDA (Fig. 2). Additional experiments demonstrated that enhancement of MDA is a specific property of LPS since neither 20 nor 80 mg of LPS per kg increased MDA when administered to LPS-resistant, C3H/HeJ mice (Fig. 3).

These results provide evidence that lipid peroxidation may contribute to the pathophysiologic effects of bacterial LPS. Although the target organ(s) and the initiating molecular species have not, as yet, been identified, evolution of the light hydrocarbons ethane and pentane has been used frequently to identify chemical-, metal-, and drug-induced lipid peroxidations in vivo and in vitro (8, 9, 24). Lipid peroxidation is extremely damaging to biological systems, since the structure and function of cell membranes and membranous organelles may be greatly impaired by a reaction that, once initiated, proceeds autocatalytically (22).

Previously reported evidence suggests that the liver may be a target for lipid peroxidation. Severe hypoglycemia, impaired gluconeogenesis, and elevated serum transaminase levels are observed in LPS-poisoned animals and in patients with gram-negative sepsis (6). Administration of CCl_4 in amounts sufficient to produce centrilobular necrosis renders mice hyperreactive to the lethal effects of LPS (5). The mechanism underlying the pathogenesis of CCl_4 poisoning is lipid peroxidation arising from the metabolic conversion of CCl_4 by hepatic microsomal enzymes of the cytochrome $P\text{-}_{450}$ system (22, 23). Electron microscopy of parenchymal cells obtained from LPS- and CCl_4-poisoned animals has revealed several striking similarities, including plasma membrane alterations, mitochondrial swelling, and dissolution of rough endoplasmic reticulum (7, 12, 25). Losses in membrane-associated enzyme activity have also been observed (22). These morphologic and functional alterations are characteristic features of cell damage resulting from lipid peroxidation (1, 2, 10, 11, 26, 27).

Lipid peroxidation arises primarily from the metabolic conversion of a parent compound into a free radical or from reaction of the parent compound with cell constituents (dissolved O_2), yielding free radicals. To date, there are no available data to suggest that LPS is involved in either mechanism. However, LPS interaction with macrophages, especially macrophages activated by BCG infection, results in secretion of H_2O_2 and free radicals of O_2, hydroxyl radical ($OH \cdot$), and singlet oxygen ($^1O_2$) (17). All these extracellular

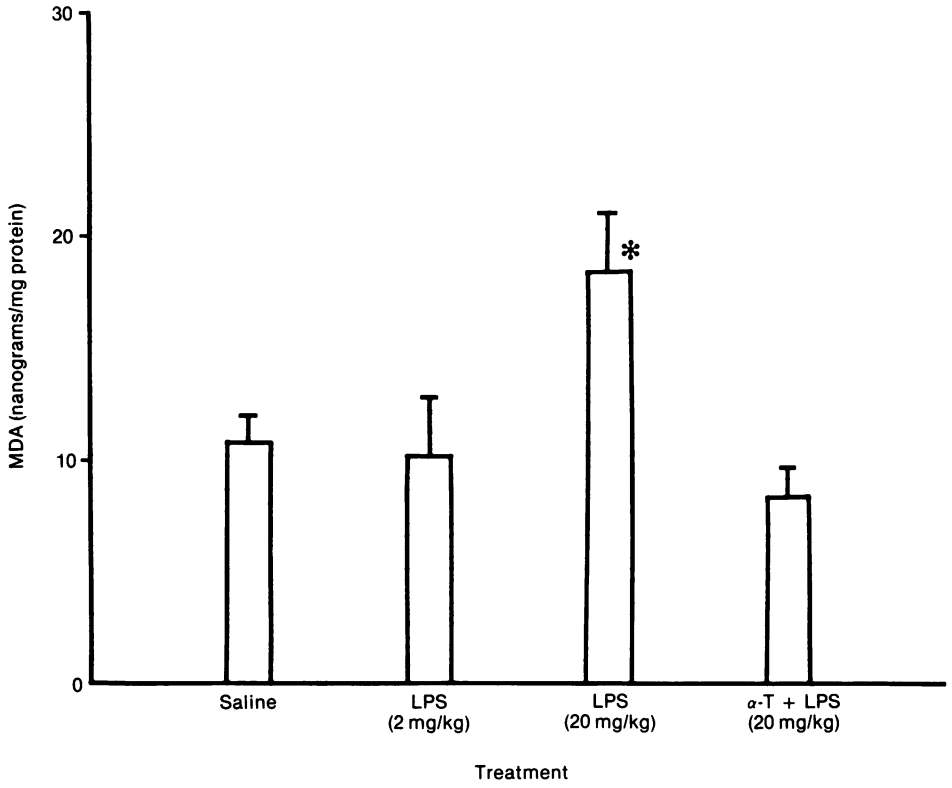

FIG. 2. Accumulation of MDA in livers of endotoxin-poisoned mice. Mice received a single i.p. injection of LPS or saline. A daily i.p. 100-mg/kg dose of α-*dl*-tocopherol was administered for 4 days prior to LPS. MDA was quantitated in liver homogenates 16 h after LPS administration. Results are expressed as the mean ± standard error derived from five mice per treatment group. Asterisks denote $P < 0.05$.

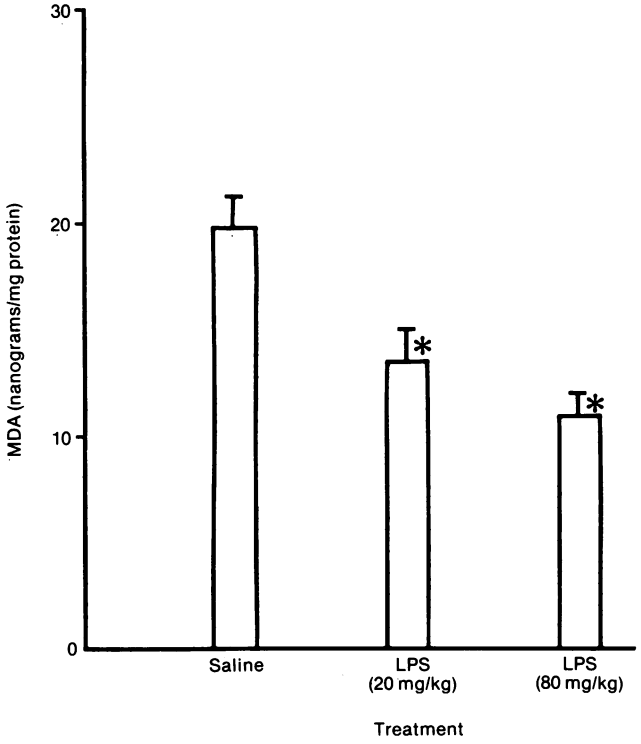

FIG. 3. Failure of endotoxin to increase MDA in CeH/HeJ mice. Mice received LPS or saline via a single i.p. injection. MDA was quantitated in liver homogenates 16 h after LPS administration. Results are expressed as the mean ± standard error obtained from five mice per treatment group. Asterisks denote $P < 0.05$.

products are well-known initiators of lipid peroxidation reactions (14, 22).

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