HOW DOES ESTROGEN ENHANCE ENDOTOXIN TOXICITY?
LET ME COUNT THE WAYS


ABSTRACT

The relationship among gender, lipopolysaccharide (LPS), and liver disease is complex. Accordingly, the effect of estrogen on activation of Kupffer cells by endotoxin was studied. All rats given estrogen intraperitoneally 24 h before an injection of a sublethal dose of LPS died. Mortality was prevented totally by pretreatment with gadolinium chloride, a Kupffer cell toxicant. Peak serum tumor necrosis factor-α (TNF-α) values as well as TNF-α mRNA in the liver after LPS were twice as high in the estrogen treated group as in the untreated controls. Plasma nitrite levels and inducible nitric oxide synthase in the liver were also elevated significantly in estrogen-treated rats 6 h after LPS. Furthermore, Kupffer cells isolated from estrogen-treated rats produced about twice as much TNF-α and nitrite as controls did in response to LPS. In addition, Kupffer cells from estrogen-treated rats required 15-fold lower amounts of LPS to increase intracellular Ca²⁺ than controls did, and Kupffer cells from estrogen-treated animals expressed more CD14, the receptor for LPS/LPS binding protein, than controls. Moreover, estrogen treatment increased LPS binding protein mRNA dramatically in liver in 6-24 h. It is concluded that estrogen treatment in vivo sensitizes Kupffer cells to LPS, leading to increased toxic mediator production by the liver.

COMMENTS

Endotoxin is in the minds of many who study liver disease. Lipopolysaccharide (LPS) has been implicated in the pathogenesis of many types of liver injury, including cholestatic disorders, alcoholic hepatitis, and nonalcoholic steatohepatitis. In many of these conditions there is a distinct gender inequity; women can be two or more times as likely to develop disease than men. The reason for this discrepancy is uncertain, but if endotoxin is involved, then gender-related differences in endotoxin responses may be important in defining disease pathogenesis. In 1968, Nolan and Ali reported that pharmacological doses of endotoxin enhance endotoxin-induced mortality in rats. The mechanism underlying this effect, particularly as it relates to liver, had not been fully explored until recently. In the article abstracted above, Ikejima et al. address the connection between estrogens and endotoxin and conclude that estrogens influence the pathogenicity of LPS in several ways.

Before discussing the specifics of the article, it is important to review the fate of endotoxin in vivo (reviewed in Fenton and Golenbock). When endotoxin gains access to the circulation, it complexes with lipopolysaccharide binding protein (LBP), a 65-kd polypeptide that derives primarily from hepatocytes. The LPS/LBP complex can then bind to one of several LPS receptors. CD14, which is present on macrophages, is a signaling receptor; upon binding to CD14, LPS/LBP promotes phagocytosis, cytokine production, and generation of reactive oxygen species. LPS can also bind another leukocyte receptor designated CD11/CD18; this is also a signaling receptor, but in contrast to CD14, CD11/CD18 does not appear to require LBP to bind LPS. LPS/LBP can also bind scavenger receptors. These do not signal, and thus serve to limit endotoxin toxicity. LBP also facilitates the entry of LPS into lipoprotein particles (such as chylomicron remnants and high-density lipoproteins), which neutralize endotoxin and direct it for uptake by hepatocytes.

Because LPS/LBP complexes can follow either signaling or nonsignaling routes in vivo, the biological consequences of an episode of endotoxemia are likely to depend on the relative abundance of signaling and nonsignaling molecules. In support of this notion, transgenic mice that overexpress the signaling receptor CD14 are rendered exquisitely sensitive to endotoxin shock. Conversely, rats treated with intravenous lipid emulsions, which can neutralize LPS, are less susceptible to endotoxin-induced mortality than untreated controls. Based on this concept of competing pathways for endotoxin trafficking, estrogens, which enhance endotoxin toxicity, could be acting either by stimulating a signaling pathway or inhibiting a neutralizing pathway. Estrogens are known hypolipidemic agents: they increase the number of hepatic low density lipoprotein receptors, which in turn increase clearance of circulating lipoproteins. In 1995, Feingold et al. proposed that estrogen-induced hypolipidemia was the major factor underlying endotoxin toxicity in estrogen-treated rats. These authors showed that ethynylestradiol, when administered at 5 mg/kg to female rats for 4 days, reduced serum lipids by over 50%. It also increased endotoxin-induced mortality from 28% to 100%. Interestingly, estrogen-treated rats could be completely rescued from endotoxin toxicity by lipoprotein infusions, which restored circulating lipids to normal levels.

If Feingold et al. were completely correct in their conclusion that estrogens enhance endotoxin toxicity by causing hypolipidemia, then the effect of estrogen should only be manifest in vivo. Stated alternatively, if estrogens act solely by tipping the balance of endotoxin pathways in vivo away from those that promote neutralization toward those that promote toxicity, then there should be no specific effect of endotoxin on the signaling pathways themselves. Ikejima et al., how-
ever, find that estrogens do have specific effects that promote endotoxin toxicity independently of any effect on serum lipids.

Ikejima et al. began their study by confirming that estrogen-treated rats are extremely sensitive to endotoxin toxicity. They showed that female rats, treated with estradiol to achieve plasma concentrations of 6 nmol/L at 24 hours, had an LPS-induced mortality of 100%, as opposed to control rats, which had a mortality of 0%. Mortality was associated with high plasma levels of TNF and nitric oxide as well as induction in the liver of genes encoding TNF, inducible nitric oxide synthase, interferon-γ, and interleukin-12. They then examined the effects of LPS in vitro on Kupffer cells isolated from estriol-treated rats; interestingly, they found that Kupffer cells from estriol-treated rats produced more TNF and more nitric oxide and exhibited greater increases of intracellular calcium in response to LPS than Kupffer cells isolated from control rats. The fact that isolated Kupffer cells from estriol-treated rats exhibited a clear enhancement of LPS effects, even after culture for 24 hours, indicates that estrogen has a specific effect on LPS signaling by these cells. The authors went on to explore the mechanism by which estradiol enhances LPS signaling in Kupffer cells; they found that it increases CD14 expression, and that CD14 remains high even during Kupffer cell culture. In addition, they found that estrogen increases hepatic production and secretion of LBP, which accentuates LPS binding to cells that express CD14.

One other observation by Ikejima et al. deserves specific mention. Although Kupffer cells isolated from estriol-treated rats exhibited increased responsiveness to LPS, LPS sensitivity could not be induced in normal Kupffer cells by treating them with estradiol in culture. This suggests that in vivo, estrogens are acting indirectly on Kupffer cells to increase CD14 expression, and possibly also indirectly on hepatocytes to increase LBP secretion. The mechanism of this effect remains to be determined; it should be noted, however, that similar observations have been made previously in mice, with estrogen receptors being implicated as important factors.

In summary, it appears that estrogens enhance the toxicity of endotoxin in vivo by at least three independent mechanisms. First, estrogens reduce serum lipoproteins, thereby impairing an important pathway for endotoxin neutralization. Second, estrogens increase CD14 expression on macrophages, which increases the capacity for these cells to bind endotoxin and produce cytokines and reactive oxygen intermediates. Estrogens also increase synthesis of LBP; this could have positive or negative effects on endotoxin toxicity, because LBP facilitates LPS trafficking to both signaling (toxic) and nonsignaling (neutralizing) pathways. However, in the context of the two previous alterations, which tip the balance in favor of signaling pathways, LBP would serve to amplify LPS sampling even further.

Do these data mean that women are more susceptible to the toxic effects of endotoxin, both in the liver and the body as a whole? To answer this question, one must consider the amount of estrogen required to enhance endotoxin toxicity. In the current study, as well as in previous studies showing estrogen-enhanced endotoxin toxicity, estrogens were administered in doses large enough to suppress ovulation or mimic pregnancy. Smaller doses of estrogen have not been extensively studied; the available data suggest they have little effect on endotoxin responses. Based on current information, it is difficult to draw firm conclusions regarding the role of estrogens and endotoxin as an explanation for female susceptibility to certain liver diseases. The study by Ikejima et al. opens the door to further investigation in this important area.

REFERENCES

IS URSEDOXYCHOLATE AN ANTIAPOPTOTIC DRUG?


ABSTRACT
The hydrophilic bile salt ursodeoxycholic acid (UDCA) protects against the membrane-damaging effects associated with hydrophobic bile acids. This study was undertaken to (a) determine if UDCA inhibits apoptosis from deoxycholic acid (DCA), as well as from ethanol, TGF-β1, Fas ligand, and okadaic acid; and to (b) determine whether mitochondrial membrane perturbation is modulated by UDCA. DCA induced significant hepatocyte apoptosis in vivo and in isolated hepatocytes determined by terminal transferase-mediated dUTP-digoxigenin nick end-labeling assay and nuclear staining, respectively (P < 0.001). Apoptosis in isolated rat hepatocytes increased 12-fold after incubation with 0.5% ethanol (P < 0.001). HuH-7 cells exhibited increased apoptosis with 1 nM TGF-β1 (P < 0.001) or DCA at (≥)100 μM (P < 0.001), as did Hep G2 cells after incubation with anti-Fas antibody (P < 0.001). Finally, incubation with okadaic acid induced significant apoptosis in HuH-7, Saos-2, Cos-7, and HeLa cells. Coadministration of UDCA with each of the apoptosis-inducing agents was associated with a 50-100% inhibition of apoptotic changes (P < 0.001) in all cell types. Also, UDCA reduced the mitochondrial mem-