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Regulation of gene expression of hepatic drug metabolizing enzymes and transporters by the Toll-like receptor 2 ligand, lipoteichoic acid ☆

Romi Ghose *, Tao Guo, Nadia Haque

Department of Pharmacological and Pharmaceutical Sciences, College of Pharmacy, University of Houston, 1441 Moursund Street, Houston, TX 77030, USA

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ABSTRACT

Expression of hepatic drug metabolizing enzymes (DMEs) is altered in infection and inflammation. However, the role of Gram+ve bacterial components and their receptor, Toll-like receptor (TLR) 2 in regulation of hepatic DMEs is unknown. Gene expression of DMEs is regulated by members of the nuclear receptor superfamily (PXR, CAR and RXRα). The TLR2 ligand, lipoteichoic acid (LTA) reduced RNA levels of CAR and its target genes, Cyp2b10, Cyp2a4 and Sultn in mouse liver (~60–80% reduction). Hepatic genes regulated by PXR and CAR, Cyp3a11 and Mrp2 were moderately reduced by LTA, along with ~50% reduction of PXR RNA and nuclear protein levels of RXRα. The effects of LTA were significantly attenuated by pre-treatment with the Kupffer cell inhibitor, gadolinium chloride, indicating that Kupffer cells contribute to LTA-mediated down-regulation of hepatic genes. These results indicate that treatment with Gram+ve bacterial components preferentially down-regulate CAR and its target genes in the liver.

During infection and inflammation, expression and activity of key phase I and phase II drug metabolizing enzymes (DMEs)[1] and drug transporters are altered in the liver, leading to impaired drug metabolism and clearance [1,2]. Gene expression of these enzymes and transporters are regulated by members of the nuclear receptor (NR) superfamily, pregnane X receptor (PXR), constitutive androstane receptor (CAR) and retinoid X receptor (RXRα) [3,4]. Inflammation-mediated alterations in DME and transporter gene expression is associated with reduced expression and activity of the regulatory NRs [1,6–12]. However, the mechanism of suppression of hepatic DME and transporter genes in inflammation is not fully understood. Inflammatory responses in the liver are mediated by Toll-like receptors (TLRs) present on Kupffer cells (KCs) which recognize microbial components and endogenous ligands from damaged or stressed cells [13–17]. TLR2 and TLR4 are activated by components of Gram+ve (lipoteichoic acid (LTA)), and Gram-ve (lipopolysaccharide (LPS)) bacteria, respectively [15,16,18,19]. We have recently reported that the Gram-ve bacterial endotoxin, lipopolysaccharide (LPS) can modulate expression of hepatic DMEs by Toll-like receptor (TLR) 4-dependent mechanism [8]. However, the role of Gram+ve bacterial components and their receptor, TLR2 in regulation of hepatic gene expression has never been investigated. In addition to infections by Gram-ve bacteria, there is also a high incidence of infection by Gram+ve bacteria which can induce inflammatory responses leading to potential alterations in metabolism, distribution and elimination of drugs and toxins. In fact, it has been reported that Gram+ve organisms account for ~50% cases of sepsis from 1979 to 2000 [20]. LTA, derived from the cell-wall components of Gram+ve bacteria like *Staphylococcus aureus* are known to cause systemic inflammation [21]. LTA has been shown to induce release of interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF) α and other inflammatory mediators from immune cells, including macrophages [22,23].

It has been previously shown that LPS-mediated induction of pro-inflammatory cytokines results in the activation of a variety of cell-signaling kinases including c-Jun N-terminal kinase (JNK) and NF-κB [8,9,24–26]. These cell-signaling components are involved in regulation of DMEs and transporters by modulating the activity of some of their regulatory NRs [26–28]. It has been shown that curcumin, a known inhibitor of JNK, can block LPS-mediated down-regulation of Cytochrome P450 (Cyp) enzymes, although the underlying mechanism is not known [29]. Furthermore, we have shown that activation of JNK by LPS or IL-1β results in modification and nuclear export of RXRα, leading to suppression of RXRα-dependent hepatic genes [9,25]. JNK inhibits glucocorticoid receptor (GR) activity, resulting in suppression of CAR gene expression

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* Corresponding author. Fax: +1 713 795 8305.
E-mail address: rghose@uh.edu (R. Ghose).

[1] *Abbreviations used:* DMEs, drug metabolizing enzymes; NR, nuclear receptor; PXR, pregnane X receptor; CAR, constitutive androstane receptor; RXRα, retinoid X receptor ; TLRs, Toll-like receptors; KCs, Kupffer cells; LTA, lipoteichoic acid; LPS, lipopolysaccharide; JNK, c-Jun N-terminal kinase; GR, glucocorticoid receptor.

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[26]. Reduction in CAR gene expression in inflammation has also been attributed to the disruption of GR-mediated transactivation of the CAR gene by NF-κB [24,30]. Recent work has demonstrated that NF-κB can interact with PXR–RXR complex, leading to the suppression of Cyp3a4 gene expression by LPS [31].

In this study, we sought to determine whether the Gram+ve bacterial component, LTA has a role in regulating gene expression of key phase I and II DMEs and drug transporters in the liver. Our results indicate that LTA treatment resulted in altered gene expression of DMEs, transporters and their regulatory nuclear receptors in the liver. Expression of TLR2 and the pro-inflammatory cytokines were significantly induced by LTA, and the cell-signaling components, JNK and NF-κB were activated in the liver. Expression of the NR, CAR and its target genes were rapidly and profoundly repressed by LTA, indicating that CAR is preferentially targeted by TLR2-dependent mechanisms in inflammation. The effects of LTA on hepatic genes were attenuated by the Kupffer cell inhibitor, gadolinium chloride ($GdCl_3$), which indicates that cytokines released by KCs may play a role in mediating the effects of LTA.

Materials and methods

Materials

Lipoteichoic acid (*S. aureus*) was purchased from InvivoGen (San Diego, CA) and freshly diluted to the desired concentration in pyrogen-free 0.9% saline before injection. Anti-JNK (#9252), anti-phospho-JNK (#9251) (Cell-Signaling, Beverly, MA), anti-RXRα (D-20) (#sc-553) and anti-TLR2 (H-175) (#sc-10739) (Santa Cruz Biotechnology, CA) were used according to manufacturer's instructions. Oligonucleotides were obtained from Sigma Genosys, Houston, TX. All reagents for real-time PCR were purchased from Applied Biosystems (Foster City, CA).

Animals and treatments

Adult male C57BL/6 mice were obtained from The Jackson Laboratory, Maine. The animals were maintained in a temperature- and humidity-controlled environment and were provided with water and rodent chow ad lib. Mice were given intraperitoneal (IP) injection with 6 mg/kg body wt. LTA in saline or saline alone. Livers were removed at the time indicated in the figure legends (0–16 h) after treatment. For 0 h treatment, mice were IP-injected with LTA, and sacrificed immediately.

To inactivate Kupffer cells, mice were given a single dose of $GdCl_3$ intravenously (10 mg/kg), followed by IP injection of LTA (6 mg/kg) 24 h after $GdCl_3$ treatment. This concentration of $GdCl_3$ has been previously shown to inhibit and deplete Kupffer cells from the liver [6,32]. All animal protocols were approved by the Institutional Animal Care and Use Committee. Experiments were performed in triplicate and repeated 3–4 times.

Preparation and analysis of nuclear and whole cell extracts and membrane fractions

Nuclear and whole cell extracts were prepared as described previously [9,33]. Membrane fractions were prepared from mouse liver using the Mem-PER® Eukaryotic Membrane Protein Extrac-

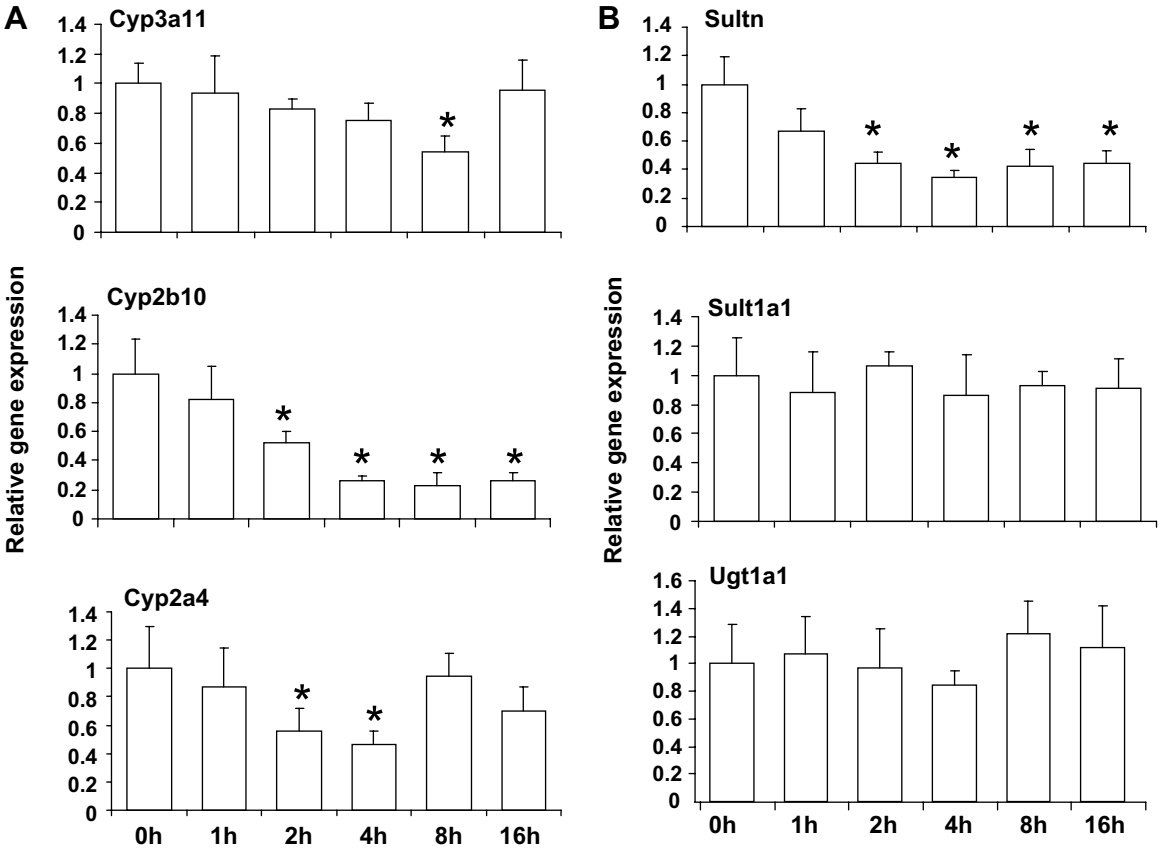

Fig. 1. Regulation of DME RNA levels in mouse liver following LTA treatment. Mice were IP-injected with 6 mg/kg LTA and livers were harvested from 0 to 16 h (n = 6–8 per group). RNA was isolated from the livers and analyzed by TaqMan real-time PCR as described in Materials and methods. RNA levels of phase I (A) and phase II (B) enzymes were determined. All data were presented as ±SD and standardized for cyclophilin RNA levels. Expression in 0h LTA-injected mice was set to 1, fold change after LTA treatment from 1 to 16 h was compared to the 0 h LTA-injected controls. The asterisks indicate significant difference ($p < 0.05$).