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Endotoxin from various gram-negative bacteria has differential effects on function of hepatic cytochrome *P*450 and drug transporters

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Abstract

The differential effects of endotoxin derived from *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli* on hepatic cytochrome *P*450 (CYP)-dependent drug-metabolizing enzyme activity and on the expression of hepatic CYP3A2, CYP2C11, P-glycoprotein and multidrug resistance-associated protein 2 (Mrp2) was investigated in rats. Endotoxin from all three different pathogens significantly decreased the systemic clearance of antipyrine, reflecting reduced hepatic drug-metabolizing enzyme activity 24 h after intravenous injection (0.5 mg/kg). The degree of the decreased systemic clearance by *P. aeruginosa* endotoxin was smaller than that by both *K. pneumoniae* and *E. coli* endotoxin. Western blot analysis revealed that the down-regulation of CYP3A2 by *K. pneumoniae* and *E. coli* endotoxin was greater than that by *P. aeruginosa* endotoxin. However, the down-regulation of CYP2C11 by all three different endotoxin was almost the same. Both *K. pneumoniae* and *P. aeruginosa* endotoxin significantly down-regulated P-glycoprotein, but did not down-regulate Mrp2. *E. coli* endotoxin had no effect on the expression of either P-glycoprotein or Mrp2, probably due to the low dose used. The down-regulation of CYP3A2 by endotoxin was parallel to the decreased systemic clearance of antipyrine. These results suggest that endotoxin has a differential effect on the hepatic CYP-mediated drug-metabolizing enzyme activity, and on the protein levels of hepatic CYP3A2 and P-glycoprotein, probably due to bacterial source-differences in the production of some proinflammatory mediators. Endotoxin appears to regulate coordinately CYP3A2, CYP2C11 and P-glycoprotein, but not Mrp2.

Keywords: Endotoxin; CYP3A2; CYP2C11; P-glycoprotein; Antipyrine clearance; (Rat)

1. Introduction

Endotoxin, an active component in the outer membrane of gram-negative bacteria, indirectly secretes various inflammatory cytokines (mediators) such as platelet activating factor (PAF), tumor necrosis factor-α (TNF-α), interleukin-1β, interleukin-6, and interferons (Cassatella et al., 1993; Crawford et al., 1997; Evans et al., 1993) from activated Kupffer cells (Bertini et al., 1989; Freudenberg et al., 1986), which are resident macrophages in the liver. These inflammatory cytokines are believed to induce various pathophysiological changes in the body, such as damage to the liver and kidney (Hewett and Roth, 1993; Hirata et al., 1980).

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The liver plays a major role in the detoxication (phase I and phase II metabolism) and elimination of various hydrophobic drugs and their metabolites. Endotoxin decreases hepatic cytochrome *P*450 (CYP)-dependent metabolism in experimental animals, including rats, as well as humans (Shedlofsky et al., 1994). Among various mediators, the release of nitric oxide (NO) is enhanced after endotoxin injection, subsequent to the expression of inducible NO synthase (iNOS) (Bredt and Snyder, 1994; Khatsenko and Kikkawa, 1997; Khatsenko et al., 1993; Moncada et al., 1991; Sewer et al., 1998), suggesting that NO may be involved in reducing hepatic CYP-dependent drug-metabolizing enzyme activity by endotoxin. In fact, *Escherichia coli* endotoxin was reported to reduce CYP-dependent drug metabolism and a reduction possibly due to the overexpression of various inflammatory cytokines and/or NO (Minamiyama et al., 1998; Morgan, 1997; Sewer et al., 1996). Previously we also found that *Klebsiella pneumoniae* endotoxin reduces hepatic drug-metabolizing enzyme activity, due in part to the over-production of NO in plasma (Kitaichi et al., 1999; Nadai et al., 1998). Otherwise, endotoxin has been reported to induce cholestasis and hyperbilirubinemia by down-regulating multidrug resistance-associated protein 2 (Mrp2) for bile acids and bilirubin, due to secretion of some cytokines from activated Kupffer cells (Green et al., 1996; Nakamura et al., 1999; Trauner et al., 1997). Like Mrp2, the ATP-binding cassette transport protein, P-glycoprotein, is expressed in many eliminating organs such as the liver and kidney (Cordon-Cardo et al., 1990; Thiebaut et al., 1987) and acts as efflux transport protein for endogenous and exogenous toxic substances (Thiebaut et al., 1987; Schinkel et al., 1996, 1997). These two drug transport proteins, P-glycoprotein and Mrp2, might have a protective function of excluding various lipophilic substrates from the liver as well as CYP. Considering that the numerous substrates of CYP3A, P-glycoprotein and Mrp2 overlap (Mayer et al., 1995; Oude Elferink et al., 1995; Wacher et al., 1995), and that some drugs such as rifampicin, dexamethazone and cyclosporin up-regulate both P-glycoprotein and CYP3A in the liver (Jette et al., 1996; Schuetz et al., 1996), CYP3A, P-glycoprotein and Mrp2 might be regulated coordinately.

There are several reports on the effect of endotoxin on the expression and function of P-glycoprotein and Mrp2 in the liver. For example, *E. coli* endotoxin down-regulates both P-glycoprotein and Mrp2 (Nakamura et al., 1999; Hartmann et al., 2001, 2002; Tang et al., 2000; Vos et al., 1998). Recent studies in our laboratories reported that *K. pneumoniae* endotoxin impaired the P-glycoprotein-mediated transport of P-glycoprotein substrates (Ando et al., 2001; Nadai et al., 2001; Zhao et al., 2002). These findings suggest the possibility that endotoxin might down-regulate simultaneously hepatic P-glycoprotein, Mrp2 and CYP3A. However, to our knowledge, there is no information confirming whether endotoxin simultaneously regulates the expression of CYP3A4, P-glycoprotein and Mrp2. In addition, the differential effects of endotoxin derived from various gram-negative bacteria on the expression of CYP3A, P-glycoprotein and Mrp2 is not fully understood.

Endotoxin derived from various gram-negative bacterial families shares a common architecture (Rietschel et al., 1993). The molecule consists of the O-antigenic polysaccharide, which is linked to the core oligosaccharide (R-core), which in turn is linked to the lipid portion lipid A (Rietschel et al., 1993; Westphal et al., 1983). The structure of the O-antigenic polysaccharide moiety is known to vary among species and strains of bacteria, whereas that of the core oligosaccharide is similar. In a series of our studies using *K. pneumoniae* endotoxin, Kato and colleagues demonstrated that *K. pneumoniae* endotoxin (O3 lipopolysaccharide) exhibits much stronger adjuvant activity in augmenting antibody responses and delayed-type hypersensitivity to protein antigens than other kinds of endotoxin from *E. coli* O55, O111, O127, and *Salmonella* enteritidis (Kato et al., 1984, 1985; Ohta et al., 1982a,b). They suggested that the extraordinarily strong adjuvant activity of *K. pneumoniae* endotoxin is due primarily to the binding of mannan as the O-specific polysaccharide moiety of *K. pneumoniae* endotoxin to the mannose-binding protein on the surface of macrophages (Kato et al., 1985). Considering that endotoxin has differential cytokine-inducing activity (Flad et al., 1993; Frieling et al., 1997; Mathiak et al., 2003; Netea et al., 2001), and that the mannose receptor is present on Kupffer cells (Magnusson and Berg, 1993), we presume that *K. pneumoniae* endotoxin, which possesses mannan as the O-specific polysaccharide moiety, might induce stronger down-regulation of the hepatic CYP3A, P-glycoprotein and Mrp2 than other endotoxin, including *E. coli* endotoxin.

The aim of the present study was to investigate the differential effects of endotoxin derived from *K. pneumoniae*, *Pseudomonas aeruginosa* and *E. coli*, which are the most frequent gram-negative bacterial pathogens in patients with sepsis, on the systemic antipyrine clearance, which represents the entire capacity of its hepatic CYP-dependent drug-metabolism (Kitaichi et al., 1999; Nadai et al., 1998), and on the expression of hepatic CYP3A2, CYP2C11, P-glycoprotein and Mrp2 by Western blot analysis.

2. Methods

2.1. Chemicals

Endotoxin was isolated from *K. pneumoniae* LEN-1 (O3:K1$^-$), which was identical to that used in previous studies (Ueyama et al., 2004; Nadai et al., 1998; Ando et al., 2001; Zhao et al., 2002). Endotoxin from *E. coli*