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EFFECTS OF BACTERIAL ENDOTOXIN ON DRUG PHARMACOKINETICS

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ABSTRACT

Bacterial endotoxin (lipopolysaccharide) has a variety of biological and immunological activities. Endotoxin-induced physiological changes in several organs might modify the pharmacokinetic behavior, including the biliary and urinary excretions and hepatic metabolism, of various drugs. We have conducted a series of studies as part of a program for the development of guidelines for the safe use of various drugs in patients with Gram-negative bacterial infections. We have found that endotoxin isolated from *Klebsiella pneumoniae* dramatically reduces renal and biliary excretion of organic anionic drugs actively secreted into the urine and bile, respectively. More recently, we found that *K. pneumoniae* endotoxin decreases the activity of cytochrome P450-mediated drug-metabolizing enzymes in a time-dependent manner.

This article reviews recent progress in the description of pharmacokinetic properties of drugs during conditions of endotoxemia, focusing especially upon the effects of *K. pneumoniae* endotoxin on the hepatic metabolism and biliary excretion of drugs, and the relationship between pharmacokinetic changes and various endotoxin-induced mediators.

Key Words: *Klebsiella pneumoniae* endotoxin, drug pharmacokinetics, metabolism, renal and biliary excretion

GENERAL INTRODUCTION OF ENDOTOXIN

Bacterial endotoxin, which is a major component of the outer membrane of Gram-negative bacteria, is believed to play an important role in the pathogenicity of Gram-negative sepsis, shock, and the development of multiple organ failure, and is mainly responsible for the high mortality by Gram-negative bacterial infections. Endotoxin derived from various bacterial families shares a common architecture.[1,2] The general chemical structure of endotoxin is shown in **Fig. 1**. The molecule comprises the O-antigenic polysaccharide, which is linked to the core oligosaccharide (R-core), which in turn is linked to the lipid portion, called lipid A.[1-3] The structure of the O-antigenic polysaccharide moiety appears to be variable among species and strain of bacteria, whereas that of the core oligosaccharide is similar. Endotoxin administered systemically is rapidly distributed into various tissues, such as the liver, lungs, spleen and kidneys, and is mainly eliminated by the liver. Its metabolites are excreted into the urine with long half-life.

Antibiotics, including those of the β-lactam and carbapenem familes, are often used in the treatment of patients suffering Gram-negative bacterial infections and these may enhance the liberation of endotoxin from bacteria to the body.[4,5] It is well known that endotoxin released from bacteria induces a variety of pathophysiological and immunological changes in the body

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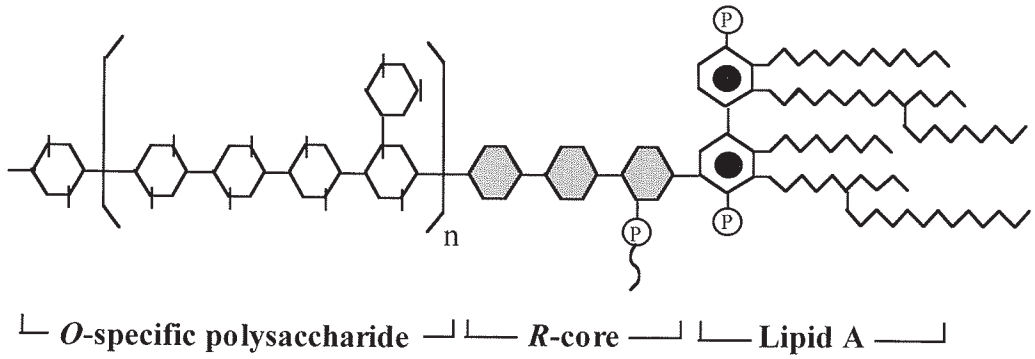

Fig. 1. General chemical structure of endotoxin.

including circulatory shock, disseminated intravascular coagulation (DIC), and damages to numerous organs such as the central nervous system (CNS), liver, kidney, heart, gastrointestinal tract, and lungs. These endotoxin-mediated activities may contribute to the development of tissue injury and consequently lead to shock and death. It is likely that the lipid A moiety is the most responsible for endotoxin's biological activities, because evidence exists suggesting that both purified lipid A from endotoxin and synthetic lipid A induce a number of effects similar to those of endotoxin.[6,7]

It is generally difficult to distinguish the biolgical effects mediated by endotoxin from those of other contaminations derived from intact bacteria. The animal model appears to be useful for the prediction of various changes occuring in the human body during Gram-negative bacterial infections, because purified endotoxin from various Gram-negative bacteria can be prepared and injected into the animals either intravenously or intraperitoneally avoiding the possible confound of the various contaminations derived from living bacteria. The choice of animal model must be carefully considered, however, because humans are known to be much more sensitive to endotoxin than animals, and dfferent species and strains of experimental animals have large variations in their susceptibility to the lethal toxicity induced by endotoxin.[8,9] For example, mice and rats are relatively resistant, but rabbits generally exhibit a relatively higher susceptibility to endotoxin. The lethal toxicity of endotoxin in guinea pigs is reported to be stronger than that in rats and mice since guinea pigs have more Kupffer cells in their livers than do rats and mice.[9] Moreover, there are a number of factors influencing the effects of endotoxin in the body, such as the dose, routes and schedules of endotoxin injection. Lastly, endotoxin (O55 and O111) purified from *Escherichia coli* is widely used in animal experiments since *E. coli* is the most frequent Gram-negative bacterial pathogen in patients with sepsis, although there are strain- and species-related differences in potency among Gram-negative bacteria.

Thirty years ago, Nakashima and colleagues in the Department of Bacteriology, Nagoya University School of Medicine discovered that endotoxin isolated from the culture supernatant of *Klebsiella pneumoniae* Kasuya strain, which is clinically isolated in their laboratory, possesses a much stronger ajuvant effect on antibody responses and delayed-type hypersensitivity to protein antigens in mice than other known adjuvants including endotoxins from *E. coli* O55, O111 and O127 and *salmonella enteritidis*.[10-16] More recently, Hasegawa and colleagues determined the chemical structure of the O-antigenic polysaccharide isolated from *K. pneumoniae* endotoxin to be based on mannan, having α-mannosyl-(1-3)-α-mannosyl-(1-2)-α-mannosyl-(1-2)-α-mannosyl-(1-2)-α-mannose units joined through α-mannosyl (1-3)-linkages.[17,18] This O-antigen polysaccha-

ride fraction, isolated by acid hydrolysis, is essentially non-toxic. We have since performed comparative studies on the antitumor activity of *K. pneumoniae* endotoxin and its polysaccharide fraction in mice. Endotoxin dramatically suppressed the growth of Sarcoma-180, Meth-A and MM2 tumors in a dose-dependent manner and showed complete regression, whereas the antitumor effect of the polysaccharide fraction, although significant, was much weaker than that of endotoxin.[19,20] These findings suggest that *K. pneumoniae* endotoxin and its polysaccharide fraction, which can be obtained by the acid hydrolysis, possess antitumor activity on both allogeneic tumors and syngeneic tumors, and their antitumor activity is probably due to host-mediated actions.

EFFECTS OF ENDOTOXIN ON RENAL EXCRETION OF DRUGS

Drug pharmacokinetics (absorption, distribution, metabolism and excretion; 'ADME') and pharmacodynamics are altered in some disease states, due to functional changes occuring in the body, especially in the kidney and liver. Endotoxin could modify the pharmacokinetics and pharmacodynamics of drugs since it induces physiological and pathological changes in several organs. The kidney is the most important organ for excretion of drugs and their metabolites from the body. Excretion of drugs and their metabolites into the urine involves three processes including glomerular filtration, active tubular secretion, and passive tubular reabsorption. Endotoxin induces adverse and toxic effects on the kidneys and causes a number of functional changes including decreases in renal plasma flow (RPF), glomerular filtration rate (GFR), and blood pressure.[21-25] The impairment of renal function induced by endotoxin is rapid in onset and possibly reversible, in which case the renal failure is described as acute.[26]

We have studied the effects of endotoxin on renal excretion using the model drugs of enprofylline, a xanthine derivative, and tobramycin, an aminoglycoside antibiotic. Enprofylline is primarily excreted into the urine by an anion tubular secretion mechanism in animals and in humans.[27-29] In contrast to enprofylline, aminoglycoside antibiotics, such as tobramycin, gentamicin and amikacin, are not bound to plasma proteins and are therefore distributed throughout the extracellular fluid, and excreted into the urine by glomerular filtration and tubular reabsorption. That is, aminoglycoside antibiotics are dependent upon the glomeular filtration rate (GFR), making them useful for evaluating renal function.[30,31] Given that endotoxin isolated from *E. coli* O111:B4 and *Salmonella minnesota* R595 distributes rapidly throughout the body organs soon after an intravenous injection and then slowly disappear from the blood with a half-life of 12 h,[32] all of our experiments studying the effect of *Klebsiella* endotoxin on renal excretion of drugs were performed 2 h after an intravenous injection of the endotoxin. The results of our experiments demonstrated that *K. pneumoniae* endotoxin decreased the glomerular filtration rate (GFR) in a dose-dependent manner, such that 250 μg/kg was enough to reduce renal function in rats.[33] We therefore chose 250 μg/kg of endotoxin as a standard dose for studying the renal excretion of drugs. This dose significantly decreased the renal clearance (CL_R), the glomerular filtration rate (GFR) and the clearance ratio (CL_R/GFR) of tobramycin by 40%, 25% and 20%, respectively. These findings suggest that endotoxin increases the tubular reabsorption of tobramycin in addition to decreasing GFR. The enhanced renal reabsorption of the positively charged tobramycin, may be due to its binding to negatively charged phospholipids in the renal brush border membrane (BBM) surface, by the presence of negatively charged endotoxin or lipid A.

K. pneumoniae endotoxin (250 μg/kg) also affected the renal handling of enprofylline, decreasing the apparent maximum capacity of transport (V_{max}), the Michaelis-Menten constant (K_m), and the glomerular filtration rate (GFR) as estimated by inulin clearance.[34] These findings

suggest that the endotoxin decreases both the affinity and capacity of the tubular transport system. On the other hand, *K. pneumoniae* endotoxin modified the pharmacokinetics of the xanthine derivative 1-methyl-3-propylxanthine (MPX), which is a highly hydrophobic, binds strongly to plasma albumin in a concentration-dependent manner, and is completely metabolized in the liver.[28,29,35,36] That is, endotoxin significantly increased the systemic clearance and volume of distribution for total (unbound plus bound) MPX, but these parameters for unbound MPX were unchanged, indicating that endotoxin may change the protein binding behavior of drugs, particularly those drugs which are highly bound to albumin in plasma. Unbound drug can diffuse across biological membranes and alterations in the degree of protein binding can therefore influence drug distribution and elimination kinetics. Thus, we also studied the contribution of plasma protein binding to endotoxin-induced changes in renal excretion of drugs, using as a model compound the β-lactam antibiotic cefazolin, which is highly bound to plasma proteins and is mainly excreted into the urine by tubular organic anion transport.[37,38] Endotoxin was found to decrease the plasma protein binding potency of cefazolin, but did not change the volume of distribution for unbound cefazolin. The estimated protein binding parameters for cefazolin suggested that endotoxin-induced decreases in the protein binding potency may be due to an altered conformation of the albumin molecule (**Fig. 2**). Cefazolin is localized in the extra-

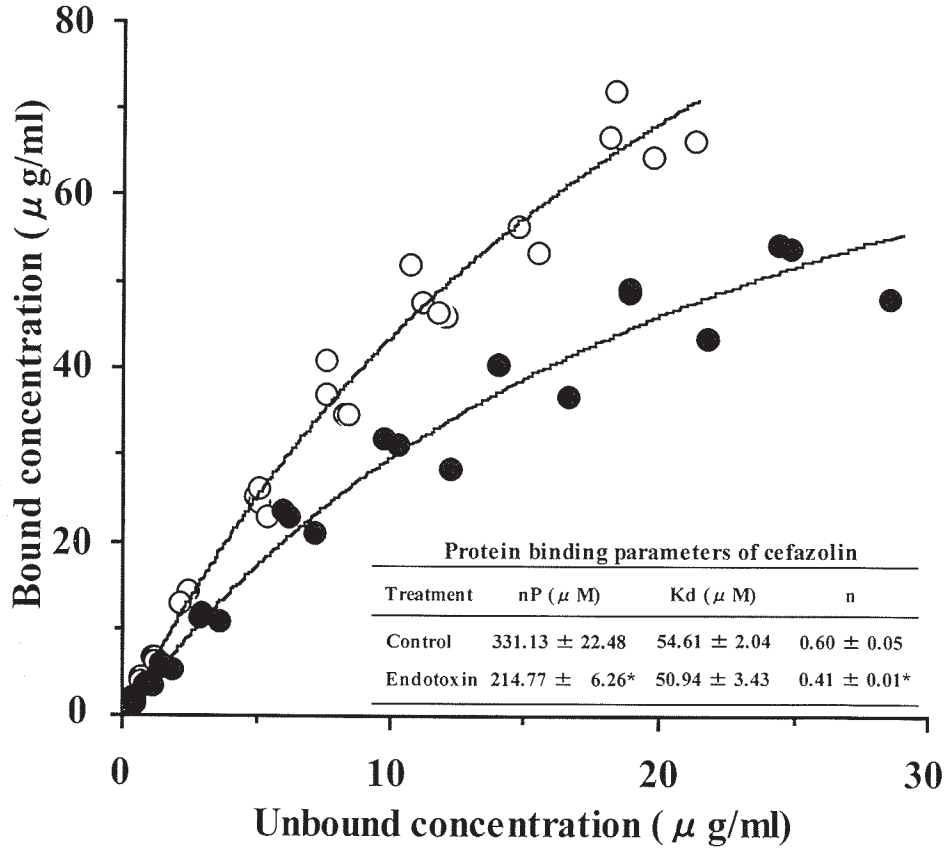

Fig. 2. Effect of endotoxin on the protein binding behavior of cefazolin highly bound to fresh plasma obtained from control (○) and endotoxin-treated (●) rats. Solid lines represent computer-fitted curves.

cellular water space and binds to plasma protein in the intravascular and interstitial fluids in nondisposing organs.[39,40] We have also demonstrated that *K. pneumoniae* endotoxin does not change the extracellular fluid volume, which is estimated as the volume of distribution of inulin.[33] Based on these observations, it may be suggested that endotoxin decreases the binding potency of cefazolin to albumin in the interstitial fluid, as well as in plasma, and that the actual distribution volume is not changed. The systemic and renal clearances for unbound cefazolin and the glomerular filtration rate (GFR) were significantly decreased by endotoxin. Moreover, the clearance ratio of unbound cefazolin (renal clearance divided by glomerular filtration rate) dropped to 70% of that in untreated rats, and the net tubular secretion of cefazolin was also reduced. Because cefazolin is one of the most widely used β-lactam antibiotics for preoperative and postoperative prevention of Gram-negative bacterial infections, these findings are especially important determining the appropriate dosage of cefazolin.

We further investigated the time-dependent changes in renal excretion of enprofylline in endotoxemic rats.[41] Significant changes in the renal clearance for enprofylline were observed in rats pretreated 2 or 10 h earlier with endotoxin (250 μg/ml), but no such changes were observed in rats pretreated 24 h earlier with endotoxin (Fig. 5). It is interesting to note that endotoxin-induced reductions in both the renal clearance of enprofylline and the glomerular filtration rate (GFR) occurred immediately (within 30 min) and proceeded gradually, whereas both parameters returned to normal levels within 24 h. Moreover, endotoxin did not induce any histopathological changes in the kidney.[34] Accordingly, intravenous administration of *K. pneumoniae* endotoxin at a dose of 250 μg/kg induces acute renal failure in a dose-dependent and time-dependent manner as a result of reduced GFR, and it dramatically reduces renal excretion of enprofylline by decreasing both glomerular filtration and tubular secretion (**Figs. 3 and 4**). Such observations suggest that endotoxin-induced decreases in renal functions appear to be transient events and, at least at the dose used in this experiment, endotoxin has no renal cell cytotoxicity.

It is well known that anionic drugs are actively taken into the tubular cells across the basolateral membranes (BLM) and pass through the brush border membranes (BBM) into the

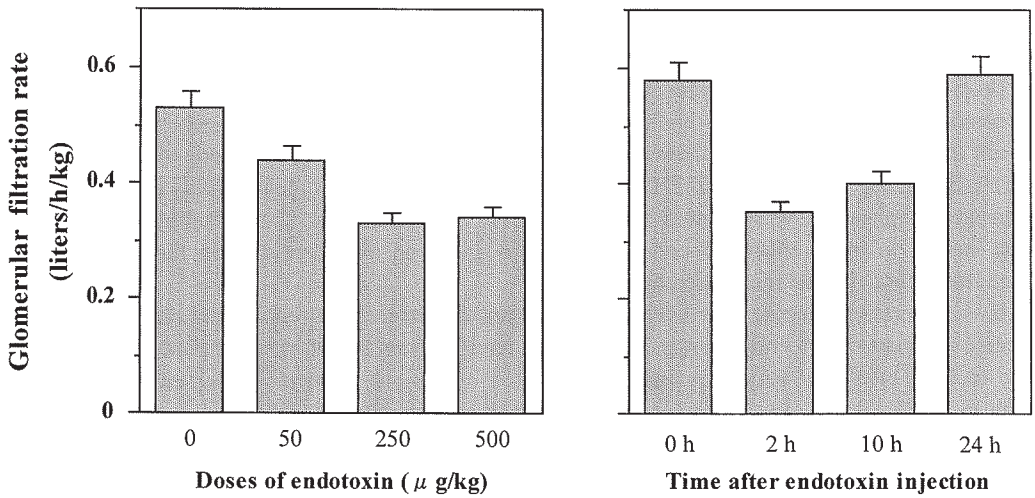

Fig. 3. Dose- and time-dependent changes in renal function induced by endotoxin. Glomerular filtration rate (GFR) was estimated as inulin clearance.

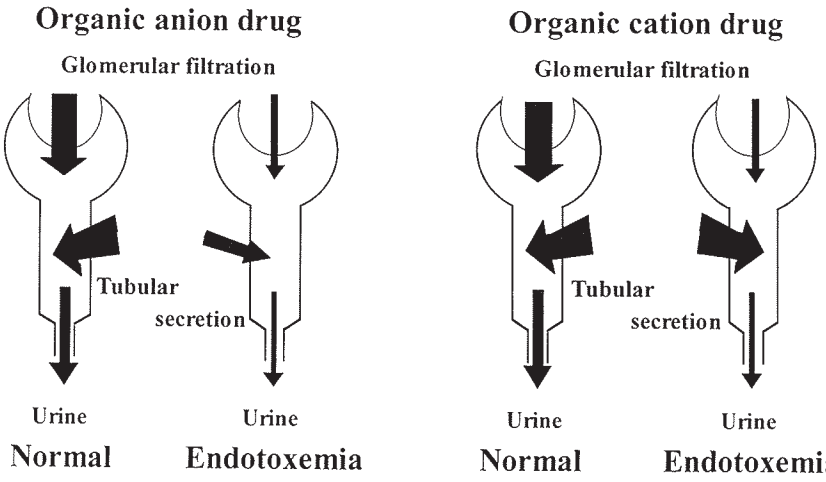

Fig. 4. Effect of endotoxin on renal handling of organic anions and cations.

urine by facilitated diffusion. In order to study the possible effect of endotoxin on these processes, we performed renal uptake kinetic experiments in mice using enprofylline as a model drug.[42] We demonstrated that this drug appears to share a common transport system with endotoxin or lipid A in the tubular cells and that the endotoxin-induced reduction in tubular secretion is likely to be caused by competition for renal uptake into the tubular cells. We have also studied the effect of *K. pneumoniae* endotoxin on renal excretion of organic cations using as a model compound famotidine, which is secreted into the urine by an active tubular cation secretory mechanism in the renal proximal tubules.[43,44] Endotoxin increased the clearance ratio of famotidine (CL_R/GFR), but not the net tubular secretion, indicating that the endotoxin has little or no effect on the active tubular cation secretory system.[45] Based on these observations, we proposed that endotoxin influences the renal uptake system at the basolateral membrane (BLM) rather than excretion at the brush border membranes (BBM).

More recently, we have studied the contribution of lipid A, isolated from endotoxin by acid hydrolysis, to the endotoxin-induced reduction of renal excretion and the intrarenal accumulation of drugs, using the aminoglycoside antibiotic gentamicin as a model compound.[46] No significant diference was observed in the tubular reabsorption or intrarenal accumulation of gentamicin between endotoxin and lipid A. However, both endotoxin and lipid A induced, to the same degree, significant decreases in the GFR and systemic clearance of gentamicin, and significant increases in plasma levels of urea nitrogen (BUN) and creatinine. These findings demonstrate that, similar to other effects of endotoxin, lipid A plays an important role in the endotoxin-induced reduction in the renal excretion of drugs.

It is generally accepted that endotoxin does not directly effect renal function, although the precise mechanism of endotoxin-induced acute renal failure is not yet completely understood. The indirect effects of endotoxin may be mediated by various molecules released by endotoxin, such as certain cytokines, certain arachidonic metabolites, platelet activating factor (PAF).[47-50] For instance, experiments using isolated perfused rat kidney suggested that endotoxin has no direct effect on renal functions, including GFR, Na^+ reabsorption or K^+ content.[51] The indirect mechanism is most likely due to vasoconstriction, which may be mediated by arachidonic acid metabolites, thromboxane, prostaglandins, leukotrienes, platelet activating factor (PAF) and

endothelin.[52] Tumor necrosis factor (TNF-α) and its related cytokines may also be involved in the indirect actions of endotoxin since intravenous injection of TNF-α induces a state similar to endotoxin shock.[53] On the contrary, reactive oxygen metabolites seem not to be important mediators of endotoxin-induced acute renal failure because neither the oxygen radical scavenger superoxide dismutase (SOD) or catalase has a protective effect against endotoxin-induced renal failure.[54] On the other hand, it has been reported that adenosine antagonists, such as theophylline, 8-phenyltheophylline and 8-cyclopentyl-1,3-dipropylxanthine, can protect against the nephrotoxicity induced by glycerol, indicating that adenosine acts as a modulator in the hemodynamic and pathophysiological changes in the kidney induced by glycerol.[55,56] Adenosine also appears to be involved in the renal hemodynamic changes induced by renal ischemia in animals.[57] Similarly, there is *in vivo* evidence that pretreatment with theophylline, at concentrations high enough to antagonize adenosine, can reverse the endotoxin-induced decrease in glomerular filtration (GFR), thus suggesting that adenosine may also play an important role in endotoxin-induced acute renal failure.[45] The role of adenosine in endotoxin-induced renal failure may thus be similar to its role in glycerol-induced acute renal failure.

EFFECT OF ENDOTOXIN ON BILIARY EXCRETION OF DRUG

The liver consisting of hepatic parenchymal cells, vascular endothelial cells and Kupffer cells (hepatic macrophage), plays an important role in the specific elimination and detoxification of endogenous (e.g. bilirubin) and exogenous (e.g. drug, xenobiotics) substances. For example, organic anions widely used for diagnosis of hepatic function such as indocyanine green (ICG) and dibromosulfophthalein (DBSP), are eliminated from blood to bile by three processes including uptake into the sinusoidal membrane, intracellular transport, and excretion into the bile canalicular membrane. The biliary excretion of such anionic drugs might be influenced by endotoxin.

Systemically administered endotoxin is rapidly taken into the liver and localized in both hepatocytes and Kupffer cells. It induces morphological and functional hepatic changes in the early stages, and may subquently cause severe damage to the liver.[58] The endotoxin-induced functional alterations of the liver include decreases of hepatic blood flow and protein synthesis, cholestasis, hyperbilirubinemia, and increases of acute phase proteins; the morphological changes include Kupffer cell swelling, formation of platelet thrombi, and accumulation of neutrophils (PMNs) in hepatic sinusoids. Endotoxin appears to localize and accumulate PMNs at sites of Gram-negative bacterial infections. PMNs play an important role in the defence of the host against Gram-negative bacterial infections whereas they also contributes to the pathogenesis of the associated.[58,59] PMN infiltration is observed in the early stages of morphologic changes in the liver, suggesting that the accumulation of PMNs in the liver may contribute to endotoxin-induced liver injury. Indeed, this theory is supported by the demonstration that pretreatment with the immunoglobulin fraction from rabbit serum immunized with rat PMNs (anti-PMN Ig) reduces *E. coli* endotoxin-induced increase in the number of PMNs in the liver and protects against liver injury.[60] These PMN-mediated changes generally occur within 1 h after endotoxin injection and precede degenerative effect in parenchymal cells. The actions endotoxin on hepatic parenchymal cells, on the other hand, which result in cholestasis and hyperbilirubinemia, appear to be direct and independent of PMNs. For example, endotoxin has been reported to cause cholestatic jaundice with concomitant elevations in plasma concentrations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST)[52,60] in patients with Gram-negative bacterial infections. There is evidence that endotoxin directly decreases bile secretion by inhibiting Na^+, K^+-ATPase on the bile canalicular membrane of hepatocytes.[61]

We have investigated the characteristics of biliary and renal excretions of certain anionic drugs in a spontaneous hyperbilirubinemic rat with conjugated hyperbilirubinemia (Eisai hyperbilirubinemic mutant rats; EHBRs).[62,63] EHBRs exhibit abnormalities in the excretion of glutathione disulfate, organic anions, bile glucuronide, and sulfate. These abnormalities appear to be similar to those observed in human with Dubin-Johnson syndrome and in TR^- and GY rats. We have demonstrated, in experiments using the β-lactam antibiotic cefpiramide as a model compound, that EHBRs abnormally excrete this drug which usually is almost completely recovered in the urine and bile in the unchanged form (>90%). The biliary clearance of cefpiramide in EHBRs markedly decreased to less than 10% of that in normal rats, while total urinary recovery and renal clearance of cefpiramide increased. Hyperbilirubinemia appears to increase the urinary excretion of cefpiramide while reducing the biliary excretion.[62] However, the effects of hyperbilirubinemia on the protein binding of drug must also be taken into account: it is known that bilirubin binds strongly to plasma albumin and that only drug unbound by plasma albumin is capable of diffusing across various bilogical membranes to be distributed in the body and this subject to metabolism and renal excretion. It is possible that bilirubin competes for the binding of drugs which are normally highly bound by plasma proteins. To investigate this possibility, we investigated the effects of hyperbilirubinemia, using EHBRs, on the renal handling of enprofylline which is normally highly bound by albumin.[63] The pharmacokinetics of enprofylline in EHBRs were changed due to the altered protein binding behavior induced by hyperbilirubinemia, but the renal handling of this drug, including glomerular filtration and tubular secretion, was unchanged. Biliary excretion of the organic anion dyes indocyanine green (ICG) and dibromosulfophthalein (DBSP) are also remakedly decreased in EHBRs becausee of the reduced intracellular transport rate for ICG and the impaired transport rate of DBSP across the canalicular membrane.[64] These observations suggest that endotoxemia might also modify the pharmacokinetics of drugs that strongly bind to plasma albumin.

Bile comprises bile salt-dependent and bile salt-independent fractions.[65] Na^+, K^+-ATPase on the bile canalicular membrane of hepatocytes contributes to the latter. Endotoxin reduces Na^+, K^+-ATPase activity on the canalicular membrane and thus decreases bile formation and flow by inhibiting the bile salt-independent fraction, but it does not change the active secretion of bile acids.[61] Because basal bile flow is predominantly driven by the secretion of anions rather than by bile acids in rats.[66] The endotoxin-induced decrease in the bile acid-independent flow rate is attributable to decreased activity of the ATP-dependent canalicular multiple organic anion transporter (cMOAT). Although the precise mechanism responsible for endotoxin-induced liver injury remains unknown, drugs primarily excreted into the bile may be influenced by endotoxin-induced impairments of hepatic function and liver elimination. For example, endotoxin decreases the biliary excretion of the organic anion dyes sulfobromophthalein (BSP) and ICG, which are primarily excreted into the bile by an active transport system.[67] Endotoxin is likely to impair the excretion process of organic anions at the stage of transport from intracellular storage to bile via the canalicular membrane, rather than at the stage of transport from blood to hepatocytes via the sinusoidal membrane.

Endotoxin stimulates macrophages and Kupffer cells, all of which produce eicosanoids and cytokines including TNF-α and interleukin-1 (IL-1).[68] TNF-α is likely to contribute to the endotoxin-induced decrease in sodium-dependent bile acid uptake via basolateral membranes since anti-TNF-α prevents against the endotoxin-induced decrease in bile flow rate.[69,70] On the other hand, there is also evidence that anti-TNF-α can not prevent the endotoxin-induced reduction of taurocholate uptake in mixed hepatocyte membranes.[71] We recently found that granulocyte colony-stimulating factor (G-CSF), which suppresses TNF-α production by endotoxin, can not protect against the decrease in the bile flow rate induced by *K. pneumoniae* endotoxin.[72] It is

thus unlikely that TNF-α alone plays the major role in endotoxin-induced decreases in bile flow and in the biliary transport of organic anions, and the precise mechanism remains to be elucidated.

To establish guidelines for the use of antibiotics in patients with hepatobiliary infections by Gram-negative bacteria, we investigated the effects of *K. pneumoniae* endotoxin on the hepatic elimination of a β-lactam antibiotic, cefoperazone. This drug is often used in the treatment of Gram-negative bacterial infections, and is actively secreted from blood to bile, via three processes: active uptake into the hepatocytes through a sinusoidal membrane, intracellular translocation, and excretion by a carrier-mediated transport system through the canalicular membrane into the bile. *K. pneumoniae* endotoxin dramatically reduced both biliary excretion of the β-lactam antibiotic and bile flow rate, due to changes occurring in the biliary secretory system.[72] From these observations, it appears that endotoxin may decrease the ATP-dependent transport of organic anions, including cefoperazone, through the canalicular membrane as a result of decreased Na^+, K^+-ATPase activity. A recent report suggests that endotoxin decreases the activity of the ATP-dependent canalicular multiple organic transporter (cMOAT), which is a transporter for cefoperazone and an important player in the generation of the bile salt-independent bile flow.[73] In our study, the amount of biliary excretion of cefoperazone in rats pretreated with endotoxin was significantly correlated with the mean bile flow rate, suggesting that cMOAT activity is an important determinant of both of these changes.[72] We also found that pretreatment with an anti-inflammatory drug, dexamethazone, can protect against the endotoxin-induced decrease in biliary excretion of cefoperazone, suggesting that some inflammatory mediators released by endotoxin contibute to decreased hepatic functions (unpublished data). Endotoxin-induced decrease in the biliary excretion of organic anions may be caused by inhibiting the anion transport system across the sinusoidal and/or bile canalicular membrane (**Fig. 5**). Further studies are needed to calrify the precise mechanism.

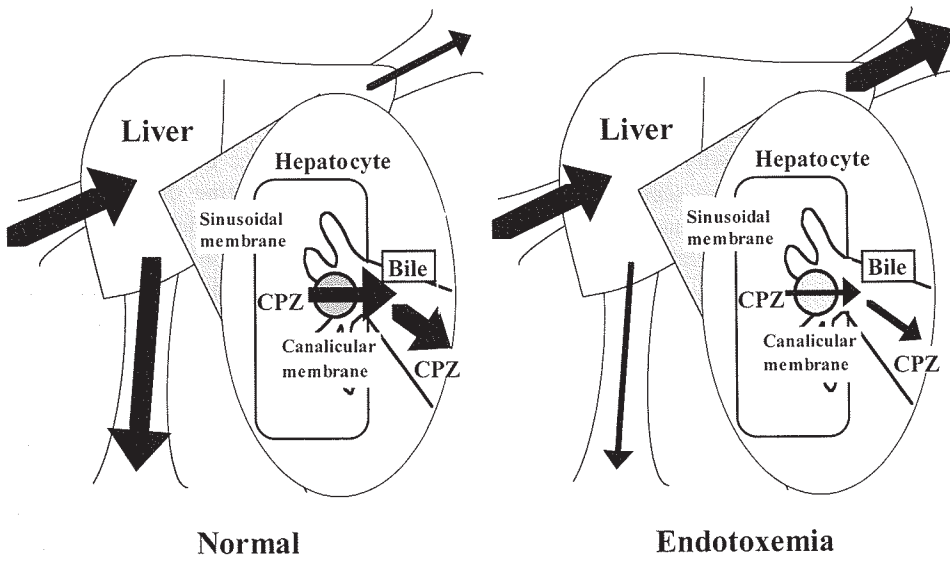

Fig. 5. Effect of endotoxin on biliary excretion of cefoperazone (CPZ), an organic anion.

EFFECT OF ENDOTOXIN ON HEPATIC DRUG METABOLISM

Bacterial endotoxin plays a key role in decreased hepatic drug-metabolizing enzyme activity in animals and in humans during Gram-negative bacterial infections. Endotoxin reduces both the content and activity of hepatic cytochrome P450-mediated drug metabolizing enzymes, and thus can delay the elimination of drugs, which are almost completely metabolized in the liver. The activity of hepatic drug-metabolizing enzymes in rats decreases 2 h after endotoxin administration and the activity of the hepatic type O form of xanthine oxidase in mice is decreased by IL-1, a cytokine released by endotoxin stimulation.[74] On the other hand, we previously found that 2-h pretreatment with endotoxin has little or no effect on the metabolism of theophylline catalyzed by the cytochrome P450 monooxygenase system in rats.[75] This discrepancy could be due to a relatively small contribution of the liver to theophylline metabolism in rats or to a time-dependent effect of endotoxin on drug pharmacokinetic behavior which went undiscovered after the 2-h pretreatment. There is little information currently available on the time-dependent effects of endotoxin on hepatic drug-metabolizing enzyme activity in animals and humans, and the remainder of this review focusses on this measure in rats.

There is evidence that the turnover time of P450 in rats is 2 to 4 days[76] and that endotoxin decreases the amount of mRNA for the hepatic cytochrome P450 isozymes 2C11, 2E1, and 3A2 from 6 to 48 h after its administration in rats.[77] In recent study using antipyrine as a model substrate in rats, we determined that *K. pneumoniae* endotoxin time-dependently reduces the activity of hepatic cytochrome P450-mediated drug-metabolizing enzymes.[78] Antipyrine is almost completely metabolized by the hepatic cytochrome P450 isozymes (CYP1A2, CYP2B6, CYP2C8, CYP2C18 and CYP3A4).[79] The systemic clearance of antipyrine represents the entire capacity of its metabolism, because the protein binding potency of this drug in plasma is negligible and its elimination is independent on hepatic blood flow. We evaluated the time-dependent effects of endotoxin on the activity of hepatic drug metabolizing enzymes, including aniline hydroxylase (also known as the P450 isozyme CYP2E), aminopyrine *N*-demethylase (CYP3A), benzphetamine *N*-demethylase (CYP2B) and *p*-nitoroanisole-O-demethylase (several isozymes), and on the content of hepatic cytochrome P450 and b_5.[80-83] Both the systemic clearance of antipyrine and the activity of hepatic cytochrome P450-dependent drug-metabolizing enzymes were significantly decreased 24 h after a single intraperitoneal injection of endotoxin (1 mg/kg body weight), but these parameters returned to control levels by 96 h.[78] Reductions in antipyrine clearance and enzyme activity after repeated endotoxin treatments (once daily for 4 days) were to the same degree as those seen 24 h after a single injection, indicating that repeated endotoxin treatments induce tolerance to endotoxin or to inflammatory stimulation. The systemic clearance of antipyrine correlated significantly both with the activity of all hepatic microsomal enzymes tested and with the content of cytochrome P450 and b_5, suggesting that changes in the systemic clearance of antipyrine reflect changes in the activity of hepatic P450-mediated drug-metabolizing enzymes. Only moderate hypertrophy of Kupffer cells was observed histopathologically in rats sacrificed 24 h after either the single and or repeated treatments, suggesting the absence of severe liver-tissue damage. Based on these histopathological examinations, it is unlikely that the changes in the activity of hepatic cytochrome P450-mediated drug-metabolizing enzymes are caused directly by endotoxin- or cytokine-induced hepatic damage. Whereas it was previously thought that endotoxin affected hepatic cytochrome P450 by endotoxin is by increasing the degradation of cytochrome P450 haem proteins,[84,85] it is now believed that endotoxin-induced depression of hepatic P450-mediated drug metabolism is likely to be caused by suppression of protein translation and mRNA transcription for cytochrome P450 isozymes, mediated by certain inflammatory cytokines.[86-90]

Cytokines, polypeptide mediators, are released from Kupffer cells in the liver, and by circulating monocytes and macrophages. TNF-α induces IL-1, and both TNF-α and IL-1 induce IL-6 production. It is well known that endotoxin increases release of certain inflammatory cytokines, such as IL-1, IL-2, IL-6, TNF-α and γ-interferon (IFN-γ), which contribute to the physiological effects and lethal toxicity associated with endotoxemia. The circulating concentration of TNF-α peaks 1 to 2 h after injection of endotoxin and those of IL-1 and IL-6 peak 2 to 6 h after endotoxin injection. These cytokines appear to play an important role in endotoxin-induced changes in the activity of hepatic drug-metabolizing enzymes. For example, the main biological activity of IL-6 is to regulate protein synthesis by hepatocytes. Both TNF-α and IL-1 increase an acute phase protein, α_1-acid glycoprotein (AGP) in plasma, whereas this effect of IL-6 is very weak. Similarly, TNF-α and IL-1 dramatically suppress both the hepatic P450 contents and activity of ethoxycoumarin-O-deethylase (represented as P450 subfamilies CYP1A and 2B) and the activity of aniline hydroxylase (CYP2E), whereas these effects of IL-6 are weak.[87,91-93] In addition, IL-1 and IL-6 dramatically decrease ethylmorphine-*N*-demethylase activity (CYP3A) to the same extent as does endotoxin itself.[94,95] There is an interesting report that systemic clearance of antipyrine correlates significantly with the peak concentrations of TNF-α or IL-6 after endotoxin injection in humans, suggesting the possibility that measurement of peak serum concentrations of TNF-α and IL-6 may yield predictions of the activity of hepatic P450-mediated drug-metabolizing enzymes during Gram-negative bacterial infections.[96] Although it has been demonstrated that G-CSF prevents endotoxin-induced TNF-α release, we recently reported that pretreatment with G-CSF can not block the suppressed hepatic cytochrome P450-mediated drug-metabolizing activity induced by *K. pneumoniae* endotoxin, suggesting that the synergistic action of other cytokines[78] in addition to TNF-α must be involved in this effect.

Although the mechanism mediating suppression of hepatic drug-metabolizing enzyme activity in endotoxemia remains to be fully elucidated, one important factor may be a free radical gas, nitric oxide (NO). NO is synthesized from the enzyme, NO synthase (NOS), which enzymatically converts the amino acid, L-arginine, into L-citrulline and NO. NOS exists as several isozymes including Ca^{2+}-calmodulin activated, constitutive NOS in neurons (nNOS) and endothelial cells (eNOS), and endotoxin- and cytokine-inducible Ca^{2+}-independent NOS (iNOS) in macrophages, Kupffer cells and PMNs. NO possesses the biological activity of endothelium-derived relaxing factor (EDRF) and has an inhibitory activity of platelet aggregation. It has been demonstrated that injection of endotoxin induces iNOS mRNA expression and up-regulated iNOS enzyme activity in several cell types, including macrophages, smooth muscle cells, endothelial cells, hepatocytes and Kupffer cells.[52,97-103] In the liver, the major organ of NO production, endogenous NO appears to play multiple roles in the physiological control of hepatic functions, immune responses, host defenses against bacterial infection, and inflammatory disease. The physiological properties of NO allow it to react with various sustances, including heme, iron, superoxide, thiols and oxygen. In particular, NO appears to bind reversibly to the heme-iron moiety of P450, forming an inactive P450-NO complex.[104,105] Heme oxygenase also plays an important role in degradation of cytochrome P450 and liberation of heme, since it is induced immediately after endotoxin injection. There are also reports suggesting that NO decreases the activity of various cytochrome P450 isozymes, including CYP2B1, CYP1A1, and CYP1A2.[106-109]

Our laboratory has recently focused on the role of NO in the suppression of cytochrome P450-mediated drug-metabolizing enzymes activity induced by *K. pneumoniae* endotoxin in rats, as illustrated by following experiments. Plasma concentrations of nitrite plus nitrate (NO_2^-/NO_3^- ; NOx) were found to increase, beginning 4 h after a single intraperitoneal injection of endotoxin (1 mg/kg), to peak at approximately 400 μM after 12 h, and to returned to undetectable levels 24 h after the injection of endotoxin, indicating that production of NO is enhanced for a

prolonged period by endotoxin. We assessed the contribution of NO to endotoxin-induced suppression of hepatic drug-metabolizing enzyme activity using two tools: an exogenous source of NO, (±)-(E)-ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexenamide (FK-409), and a selective iNOS inhibitor, *S*-methylisothiourea (SMT).[110-112] There is a evidence that selective iNOS inhibitors, including SMT, do not increase endotoxin-induced liver injury, but nonselective iNOS inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME), increases.[113] In fact, SMT suppresses the manifestation of acute lung injury due to endotoxin-induced overproduction of NO, reduced endotoxin-related tissue damages and lethal toxicity, and blocks endotoxin-induced vascular contractility.[114] First, we examined *in vivo* whether SMT can suppress both the endotoxin-induced overproduction of NO and the decrease in the systemic clearance of antipyrine, which is representative of hepatic cytochrome P450 activity. Indeed, a single intraperitoneal injection of SMT at a dose of 5 mg/kg in rats, 2 h after endotoxin injection, completely suppressed the overproduction of NO and protected against the endotoxin-induced decrease in the systemic clearance of antipyrine, suggesting that the selective iNOS inhibitor suppresses the endotoxin-induced decrease in the activity of cytochrome P450-mediated drug-metabolizing enzymes (**Fig. 6**). These results indicate that NO is involved in the effect of endotoxin. Second, we studied *in vivo* whether the NO donor FK-409 can suppress the activity of cytochrome P450-mediated drug-metabolizing enzymes. We adjusted the dosing schedule of the NO donor on the basis of the disappearance curve of NOx after a single intraperitoneal injection of this drug because NO, a potent vasodilator, induces immediate hypotension. For example, a single intraperitoneal injection of FK-409 in rats initially decreases the mean arterial blood pressure (MAP), which recovers to baseline levels after 30 min. Repeated injections of FK-409 (10 mg/kg), administered in a pattern demonstrated to mimic the prolonged overproduction of NO following *K. pneumoniae* endotoxin injection, dramatically reduced the systemic clearance of antipyrine when the antipyrine was

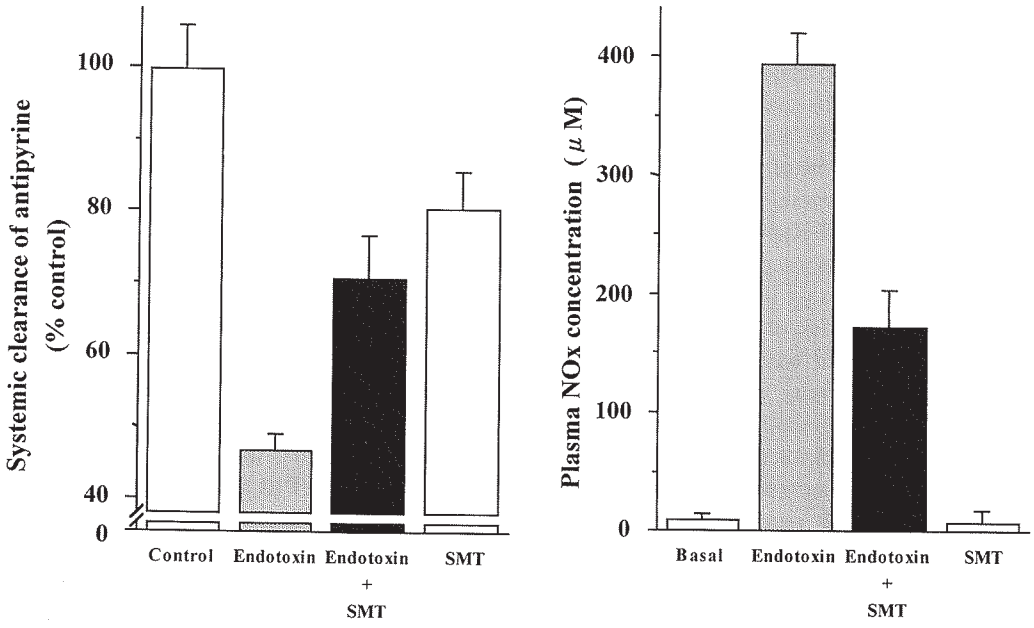

Fig. 6. Protective effect of a selective iNOS inhibitor against the endotoxin-induced overproduction of NO and decreased antipyrine clearance, the latter of which reflects hepatic drug-metabolizing enzyme activity.

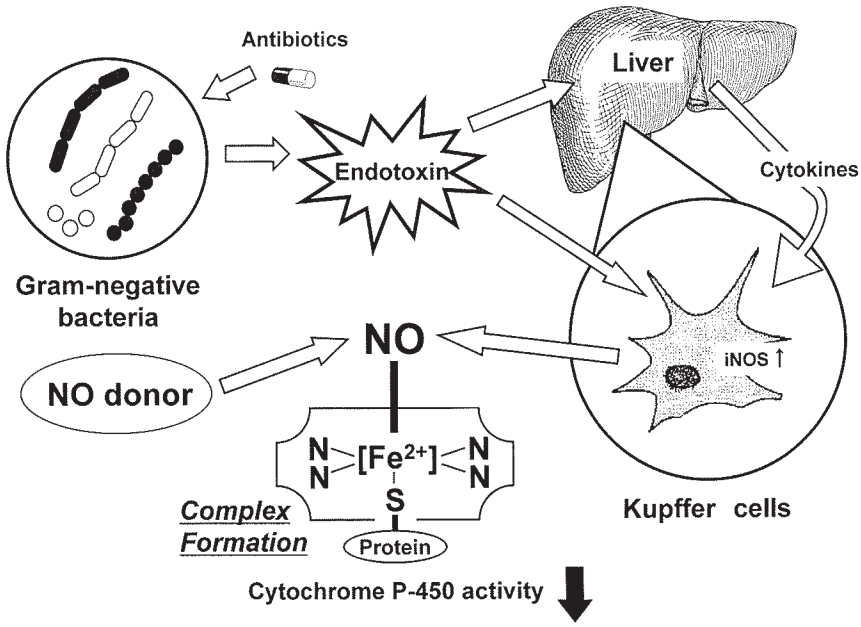

Fig. 7. Possible mechanism by which endotoxin decreases cytochrome P450-mediated drug-metabolizing enzyme activity.

administered 24 h after endotoxin injection 10 h after final dose of FK-409. The decrease was to the same degree as that seen after endotoxin injection, indicating that *in vivo* treatment with the exogenous source of NO reduces the activity of hepatic drug-metabolizing enzymes. These findings suggest that the overproduction of NO, caused by endotoxin itself or by some endotoxin-induced cytokines, mediates the suppression of cytochrome P450-mediated drug metabolizing enzyme activity induced by endotoxin. A possible mechanism for the endotoxin-induced decrease in hepatic drug-metabolizing enzyme activity is shown in **Fig. 7**. Our data also suggest that selective inhibition of iNOS may prevent a variety of pathophysiological changes associated with bacterial sepsis and septic shock.

CONCLUSION

This article reviews bacterial endotoxin induced changes in renal and biliary drug excretion mechanisms and in hepatic drug-metabolizing enzyme activity, although the precise mechanisms responsible for these changes are unclear. Endotoxin may induce clinical complications due to changes occurring in the pharmacokinetics (renal and biliary excretions and metabolism) of certain dugs, thus increasing the risk for developing serious adverse effects. With respect to hepatic drug-metabolizing enzyme activity during Gram-negative bacterial infections, endotoxin-induced overproduction of NO plays a key role in reducing the activty of cytochrome P450-mediated drug-metabolizing enzymes. This report provides further characterization of endotoxin-induced decreases in hepatic drug-metabolizing enzyme activity and should provide useful information for designing drug regimens in patients with Gram-negative bacterial infections.

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