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Antibiotic Induced Endotoxin Release and Clinical Sepsis: a Review

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Summary

Sepsis and peritonitis have not lost much of their danger for patients. The mortality rate in peritonitis has only marginally decreased during the last 30 years despite aggressive surgical and sophisticated intensive care treatment. In intra-abdominal infection and peritonitis source control remains the mainstay of treatment, although general principles and denominators of successful source control need to be established.

Endotoxin has been recognized as a major player in the pathogenesis of sepsis and its significance in clinical disease has been investigated in clinical studies for more than 20 years. Since the Sixties there is a growing interest in the effect of antibiotics and other compounds on the release of endotoxin. The effect of antibiotics on the release of endotoxin and inflammatory parameters, e.g., cytokines, remains to be clarified despite a growing body of *in-vitro* studies, animal studies and a few clinical studies. The purpose of this review is to evaluate the evidence of endotoxin release in clinical studies and the effect that antibiotic treatment may have *in-vitro, in-vivo* and in clinical studies on endotoxin and cytokine release.

***In-vitro* antibiotic-induced endotoxin release may depend on antibiotic class, presence of serum, type of organism, site of antibiotic action and Gram-stain. Endotoxin release may be different in late or early lysis, proportional to the number of killed pathogens. Morphology of bacteria may have an impact on endotoxin release and phagocytosis.**

Antibiotic-treated animals may show higher endotoxin levels with a higher survival rate than untreated animals. Plasma endotoxin may increase despite decreasing bacteremia. There may be a similar killing rate by different antibiotics but a difference in endotoxin release. Intestinal endotoxin does not necessarily correlate to the level of Gram-negative bacteria. However, the alteration of the gut content by pretreatment may be associated with reduced endotoxemia and increased survival. Antibiotic-induced endotoxin release may be different depending on the type of infection, the location of infection, the virulence of strains, Gram-stain, mode of application and dosage of antibiotic. Different antibiotics may induce the release of different forms of endotoxin

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which may be lethal for sensitized animals. The combination of antibiotics with inhibitors of endotoxin or the pro-inflammatory response may be responsible for increased survival by decrease of endotoxin release.

The clinical significance of antibiotic-induced endotoxin release is documented only in a few clinical disorders, e.g., meningitis, urosepsis. The difference in endotoxin release by PBP 2-specific antibiotics, e.g., imipenem, and PBP 3-specific antibiotics, e.g., ceftazidime, may not be visible in each study. Patients with increased multi-organ failure (MOF) scores may profit from treatment with antibiotics known to decrease endotoxin.

In conclusion, the clinical significance of antibiotic-induced endotoxin release remains to be clarified. Type of pathogen and its virulence may be more important than recently suggested. Gram-positive pathogens were just recently recognized as an important factor for the development of the host response. In case of fever of unknown origin in intensive care patients either failure of treatment, e.g., failure of source control in intra-abdominal infection, or a side effect of antibiotic treatment, e.g., endotoxin release, should be considered as a cause of the fever.

Key words: **Endotoxin release, sepsis, antibiotic-induced endotoxin.**

INTRODUCTION

Sepsis and peritonitis have not lost much of their danger for patients. The mortality rate in peritonitis has only marginally decreased during the last 30 years despite aggressive surgical and sophisticated intensive care treatment. In intra-abdominal infection and peritonitis source control remains the mainstay of treatment, although general principles and denominators of successful source control need to be established [1]. Endotoxin has been recognized as a major player in the pathogenesis of sepsis and its significance in clinical disease has been investigated in clinical studies for more than 20 years [2,3]. Since the Sixties there is a growing interest in the effect of antibiotics and other compounds on the release of endotoxin [4]. It has been demonstrated that antibiotics may induce endotoxin release from bacteria on intravenous inline filters [5]. The possibility of severe side effects of antibiotic treatment is known and obvious even in antibiotics used for minor infections, e.g., sinusitis, and otitis media [6]. The analysis of parameters of betalactam antibacterial activity, antibiotic-induced release of bacterial endotoxin and the interrelationship between pharmacokinetics and pharmacodynamics of antibiotics has been recently published [7].

The effect of antibiotics on the release of endotoxin and inflammatory parameters, e.g., cytokines, remains to be clarified despite a growing body of *in-vitro* studies, animal studies and a few clinical studies. The purpose of this review is to evaluate the evidence of endotoxin release in clinical studies and the effect antibiotic treatment may have *in-vitro, in-vivo* and in clinical studies on endotoxin and cytokine release.

THE SIGNIFICANCE OF ENDOTOXIN IN CLINICAL INFECTION AND SEPSIS

Endotoxin has been studied in a variety of clinical disorders, e.g., peritonitis, infections in neutropenic patients, obstructive jaundice, and trauma/hemorrhage.

In peritonitis and septic shock there seems to be an obvious relationship due to the pathogenesis, e.g., bowel perforation and Gram-negative pathogens, to endotoxin release. Beger has observed endotoxin in 63% of peritonitis patients. In 8 of 9 patients with a fatal outcome within 72 hours endotoxin was detected [8]. But even in patients with multiple organ failure due to sepsis there may be no difference in endotoxemia between survivors and non-survivors and endotoxemia may be present in patients with Gram-positive infections [9]. Endotoxin plasma levels in patients with sepsis syndrome were significantly higher in non-survivors compared to controls, but not to survivors [10]. Peritoneal rather than plasma endotoxin levels were significantly higher in patients

with severe secondary peritonitis, levels may differ between survivors and non-survivors and may be part of an immuncompartmentalized inflammatory response [11]. Endotoxin was detected in 51.2% of patients with severe sepsis or septic shock and was associated with a higher incidence of bacteremia; however, it did not correlate with organ dysfunction or mortality [12]. This is in agreement with a recently published European multicenter study on the significance of immunological surrogate parameters as a prognostic model for multi-organ failure and death. Endotoxin did not predict the development of severe complications or fatal outcome. Endotoxin neutralizing capacity (ENC) in surgical intensive care patients indicated complications and outcome [13].

Immuno-suppressed patients or patients with hematological differences may differ in their response to endotoxin due to a lack of granulocyte activity. Endotoxemia may be found in infected patients with fever, in patients with fever of unknown origin, or even in afebrile patients without any cause for endotoxemia [14]. Gram-negative pathogens did not always produce endotoxemia in these patients [15]. One study reported a correlation of endotoxemia with fever in septic neutropenic patients [16]. Endotoxin determination may be influenced by many factors in serum of patients [17]. Endotoxin test systems may have a sensitivity of 69.7% and a specificity of 96.3% for Gram-negative infections in febrile patients with hematological malignancies, but only 39.7% of endotoxemic samples grew Gram-negative pathogens [18]. However, endotoxin determinations are not recommended for routine diagnosis of sepsis in patients with hematological malignancy [19].

Endotoxin may be released during major surgical procedures. Endotoxemia was common before (68%), during (70%), and after (81%) surgery in jaundiced patients [20]. Although 60% of patients with surgery for obstructive jaundice were endotoxemic, there was no correlation with clinical sepsis or Gram-negative infection detectable [21]. In liver transplant patients, Hamilton *et al.* observed only a transient endotoxin elevation during clamping of liver vessels without any correlation to TNF-alpha or the occurrence of complications [22]. Increased plasma endotoxin levels were observed during and after elective abdominal aortic aneurysm repair with a significant and simultaneous increase of pro- and anti-inflammatory cytokine release but there was no correlation with clinical or laboratory parameters [23]. Translocation of bacteria and endotoxin could be detected in mesenteric lymph nodes, but systemic concentrations of endotoxin and inflammatory parameters did not correspond to levels within mesenteric lymph nodes [24]. Translocation of endotoxin may be related to a failure in the barrier function of the bowel mucosa. Endotoxemia was found in 88% of patients with ulcerative colitis and 94% with Crohn's disease, which correlated to disease activity, disease extent and endotoxin core antibodies [25]. The clinical significance, however, remains unclear [26]. Trauma and hemorrhage are known to be associated with endotoxin release. CD14 expression in injured patients may discriminate between infected and non-infected patients [27]. Patients with urinary tract infections had higher endotoxin levels but there was a lack of correlation with temperature, leukocytes and CRP [28]. Translocation of endotoxin has been demonstrated in some studies after thermal injury; however, in a recent report by Carsin the significance of translocation of bacteria and endotoxin has been challenged [29]. Intravascular coagulation and endotoxin concentrations may reflect septic DIC [30].

ANTIBIOTIC-INDUCED ENDOTOXIN RELEASE IN *IN-VITRO* STUDIES

It was demonstrated that antibiotic administration may induce the release of endotoxin from bacteria retained on intravenous inline filters in 1975 by Rusmin *et al* [5]. Rosenthal revealed by scanning and transmission electron microscopy that antibiotics caused the formation of numerous protrusions or blebs on the surface of E. *coli* with apparent release of membrane vesicles from cells [31], which was confirmed by Goodell who demonstrated that penicillin greatly increased the shedding of lipid and LPS into the medium [32]. In case penicillin did not liberate free endotoxin, the peptidoglycan layer was present after 2 h of treatment with penicillin, but was undetectable after 20 h [33]. Cohen stated that in antibiotic-induced endotoxin release the rate of cell death is less important than the site of antibiotic effect. Ciprofloxacin induced endotoxin release,

whereas gentamicin did not, despite an equally rapid bactericidal effect. Ciprofloxacin is thought to act mainly on DNA gyrase; however, these results indicated that it might also act on the bacterial wall [34]. Kusser and Ishiguro further demonstrated the inhibitory action of aminoglycosides on LPS release [35] but there is evidence that gentamicin enhanced rather than inhibited TNF production [36].

NORMAL HUMAN SERUM INDUCES ENDOTOXIN

Normal human serum may by itself induce endotoxin release. It should further be noted that the composition of LPS subunits in the outer membrane appeared to influence serum-mediated LPS release. Pre-treatment of *Escherichia coli* with various antibiotics prior to treatment with normal human serum did not modulate the LPS release. This may suggest that a serum factor *in vivo* may contribute to the effect of antibiotic-induced endotoxin release, which is not yet detected [37]. The isolation process may induce differences among *in-vitro* studies, as mononuclear cells may be less responsive to further physiologic manipulation [38].

RELEASE OF ENDOTOXIN AND BACTERIAL LYSIS

Imipenem or imipenem plus tobramycin induced an early increase of total endotoxin from *E. coli* at 1 h. Total endotoxin levels increased 5-fold after treatment with ceftazidime, tobramycin, or tobramycin plus cefuroxime, 22-fold after treatment with cefuroxime and 49-fold with aztreonam. Free endotoxin levels increased 6-fold after 1 h treatment with imipenem or imipenem plus tobramycin or ceftazidime concomitantly with rapid bacterial lysis. Cefuroxime or aztreonam induced much higher endotoxin release causing the formation of filamentous structures in pathogens [39]. Rapid killing of *E. coli* by amikacin and imipenem has been associated with less endotoxin release than slow killing by ceftazidime, aztreonam and cefotaxime. Rapid killing produced less TNF release in mononuclear cells [40]. Bingen *et al* did not observe an increase in endotoxin release when amikacin was added to ampicillin and cefotaxime, which resulted in accelerated killing of *H. influenzae* [41]. Evidence against lysis-correlated LPS release was presented by van den Berg *et al* who demonstrated that after gentamicin bacterial killing free limulus amebocyte lysate (LAL) activity increased only moderately whereas ciprofloxacin which was associated with filamentation induced a 43- and 68-fold increase in free LAL activity. The authors concluded that LPS is released as long as *E. coli* remains structurally intact [42]. Following treatment of *E. coli* with cefuroxime, aztreonam, and low dose ceftazidime late bacterial lysis occurred associated with high levels of endotoxin, high dose ceftazidime and imipenem caused rapid lysis, formation of spheroblasts and low endotoxin levels. There was no difference in TNF release in aztreonam or imipenem treated cultures. Low dose treatment seems to induce higher TNF release [43]. Cefotaxime, ciprofloxacin, and piperacillin caused significant endotoxin release correlating with their ability to affect cell-wall morphology, filamentation, and lysis. Tobramycin induced almost no endotoxin release and morphological changes when bacteria were exposed to bactericidal concentrations [44]. Treatment of *Enterohemorrhagic Escherichia coli (EHEC)* strains with imipenem resulted in less endotoxin release than after treatment with ceftazidime [45]. Despite a rapid bactericidal effect cefotaxime treatment is followed by endotoxin release which may be reduced up to 66% by the addition of immunoglobulin G [46]. Endotoxin liberation was found to be proportional to the number of killed bacteria for cefuroxime and tobramycin at each concentration level, justifying the endotoxin-liberating potential to be expressed as release of endotoxin per killed bacterium, which is independent of inoculum size. Highest release was found after cefuroxime and lower after tobramycin or the combination of both. With increasing doses there was a significant reduction in the endotoxin release. An unspecific binding of endotoxin to tobramycin did not reduce endotoxin release [47]. There may be a difference in bacterial cell wall products released by different antibiotics. Ceftriaxone-induced killing of *Haemophilus influenzae* resulted in the release of bacterial wall products, which were more pro-inflammatory than that released by imipenem [48].

ANTIBIOTIC-INDUCED RELEASE OF CYTOKINES

Antibiotics may differ in their effect on cytokine release in human monocytes and lymphocytes. Sulbactam-ampicillin and cefamandole induced IFN-γ production, clindamycin TNF and IL-6 release, lincomycin released IL-4 and teicoplanin TNF, IL-1 alpha and IL-6 [49]. Teicoplanin decreased in human whole blood the endotoxin-induced release of IL-8, TNF and IL-1beta [50]. Cephalosporin antibiotics increased histamine release induced by *E. coli* and *Staphylococcus aureus*, whereas synthesis of leukotrienes, IL-6 and TNF decreased [51]. Erythromycin and roxithromycin inhibited TNF-release whereas ofloxacin, penicillin G, minocycline or josamycin failed to inhibit TNF release and may not have affected endotoxin release [52]. Cefodizime induced a significant increase in GM-CSF release, but did not affect IL-8 production in cultured human bronchial epithelial cells. Ceftriaxone had no effect on cytokine production in this model [53]. In human monocytes ceftriaxone and ceftazidime failed to have an effect on TNF, IL-6, or IL-8 release. Cefodizime, however, significantly decreased TNF and IL-6 release and stimulated IL-8 release [54]. Vancomycin downregulated TNF-alpha production and inhibited TNF mRNA accumulation in LPS stimulated monocytes [55]. RO-23-9424 significantly enhanced TNF release from PMN compared with controls, but reduced IL-1 and did not influence IL-8 release. Cefotaxime significantly increased IL-1beta and reduced IL-8 after 24 h. Lower concentrations of cefotaxime reduced LPS-stimulated IL-8, whereas fleroxacin enhanced IL-8 release [56]. *(Table 1)*

DIFFERENCE IN STRAINS

Many studies focussed on the endotoxin release in *E. coli*. However, antibiotic-induced endotoxin release may also depend on the type of strain. An increased liberation of endotoxin after adding penicillin was observed in six of the strains of *Neisseria meningitidis*, which were known to have an enhanced capacity for spontaneous endotoxin liberation [57]. Eng *et al* have discovered that the effect of antibiotics on endotoxin release was organism dependent.

TABLE 1 - *Antibiotic-induced effects on cytokine release.*

Antibiotic	IFN-γ	TNF-α	IL-1	IL-4	IL-6	IL-8	GM-CSF
Sulbactam-ampicillin	↑						
Cefamandole	↑						
Clindamycin		↑			↑		
Lincomycin				↑			
Teicoplanin		↑↓	↑↓		↑	↓	
Erythromycin		↓					
Roxythromycin		↓					
Ofloxacin		0					
Pencillin G		0					
Minocycline		0					
Josamycin		0					
Cefodizime		↓			↓	↑0	↑
Ceftriaxone		0			0	0	0
Vancomycin		↓					
Cefotaxime			↑			↑↓	
Fleroxacin						↑	

Antibiotics showed no effect on *S. aureus*. Imipenem, ofloxacin, ciprofloxacin and gentamicin released less endotoxin compared to ampicillin-sulbactam, chloramphenicol, and aztreonam in *E. coli* cultures. The highest levels were detected in controls and after ceftazidime treatment. In *Pseudomonas aeruginosa* cultures ceftazidime and imipenem released small amounts of endotoxin, all other antibiotics higher amounts. It could be demonstrated that the amounts of endotoxin released were related to the rate of bacterial killing [58]. Antibiotic class, presence of serum and type of organism may influence bactericidal activity and endotoxin release. Ceftazidime induced the highest rate of endotoxin release in *E. coli*; amikacin and ofloxacin had a favorable rate of endotoxin release in relation to the amount of bacterial killing. In *P. aeruginosa*, imipenem and ofloxacin showed similar low ratios of endotoxin release to bacterial killing [59]. Supernatants from *Staphylococcus epidermidis* incubated with beta-lactam antibiotics induced higher TNF-levels than culture medium alone, vancomycin or clindamycin. Human serum potentiated supernatant-induced TNF-release. Soluble peptidoglycan and teichoic acid contents were proportional to TNF release [60]. No increase in endotoxin release during antibiotic killing of *meningococci* was observed with penicillin or ceftriaxone [61]. Differences in cytokine release between Gram-positive and Gram-negative pathogens were detected in ceftazidime and imipenem induced killing of *P. aeruginosa* and *S. aureus* [62]. Morphology of pathogens and endotoxin release may have a relationship. *P. aeruginosa* exposed to biapenem showed a longer oval-centered form and released more endotoxin than imipenem, panipenem (spherical form) or meropenem (shorter oval-centered cells). In all strains except *P. aeruginosa* carbapenems induced significantly less endotoxin than ceftazidime [63]. In contrast, Trautmann *et al* did not detect a difference in endotoxin release between imipenem and meropenem, although meropenem binds to PBP-2 and -3 [64]. Trautmann concluded that morphological changes in bacteria in the presence of antibiotics do not predict their LPS-liberating effect. Ciprofloxacin caused low levels of endotoxin despite filament formation [65]. Horii *et al* reconfirmed in another study that time-course and magnitude of endotoxin release induced varied among carbapenems and also with regard to morphological changes [66]. Kishi *et al* reported that treatment of *E. coli* with ceftazidime transforms the rod shaped bacteria into filament structures, which may be prevented by pretreatment with clindamycin [67]. LAL and ELISA confirmed low endotoxin releasing activity of ciprofloxacin. LPS released had low bioactivity in terms of TNF induction [68]. In *P. aeruginosa* imipenem showed a strong bactericidal effect with less liberation of free LPS, but free LPS levels increased after 8 h accompanied by a regrowth of the organism [69]. Round *P. aeruginosa* cells treated with imipenem became susceptible to phagocytosis by peritoneal cells, whereas long filamentous cells induced by ceftazidime treatment were hardly phagocytized. The morphology was correlated to plasma endotoxin levels [70].

RELEASE OF LIPOTEICHOIC ACID (LTA)

Inhibition of growth and lysis of a pathogen may be induced via the inhibition of different penicillin-binding proteins. Lysis of penicillin-susceptible and -resistant strains was attributed in part to the extensive loss of acylated lipoteichoic acid from *Enterococcus faecium* into the growth medium [71]. Beta-lactam antibiotics enhanced the release of lipoteichoic acid (LTA) and peptidoglycan (PG) in *S. aureus* cultures whereas protein synthesis inhibitors did not affect LTA and PG release. This was associated with increased release of TNF-alpha and IL-10 in human whole blood [72]. The sensitivity of antibiotics may change with high temperature. Cell wall antibiotics were only effective against *P. aeruginosa* when added to cells growing at 46°C prior to a temperature shift to 37°C [73]. Rapid release of teichoic acid and LTA was detected after ceftriaxone and meropenem treatment of different strains of *Streptococcus pneumoniae*, low release after rifampicin and quinupristine/dalfopristin [74]. Antimicrobial mechanism of action may affect the macrophage pro-inflammatory mediator production after stimulation with *S. pneumoniae*. Oxacillin treatment leads to a significantly higher release of TNF than clindamycin [75]. Endotoxin activity was many times higher with the *Bacteroides fragilis sensu stricto* than with the rest of the *Bacteroides spp.*, which may

explain why this strain is associated with clinical infections and higher morbidity. At 4 times MIC cefoxitin and piperacillin/tazobactam induced small amounts of endotoxin after 48 h exposure whereas trovafloxacin, imipenem, meropenem induced high levels of endotoxin release [76].

PENICILLIN BINDING PROTEIN SPECIFIC ANTIBIOTICS

In 1992 Jackson and Kropp reported that in *P. aeruginosa* cultures ceftazidime-induced filamentations were associated with larger quantities of bioreactive LPS than nonfilamentous fast-lysing imipenem. The filtrated ceftazidime-induced endotoxin release induced a higher mortality rate in mice when compared to imipenem-induced endotoxin. The authors concluded that *in-vitro* endotoxin release depends on the site of action of the antibiotic and that PBP 2-specific antibiotics induce less endotoxin than PBP 3-specific antibiotics [77]. There was a quantitative but not qualitative difference in the efficacy of ceftazidime and imipenem in mediating endotoxin release. In a mouse model in which the animals were made sensitive to the effects of endotoxin imipenem provided a greater level of protection when compared to ceftazidime [78]. Yokochi *et al* reported that treatment of bacteria induced much lower levels of endotoxin release than meropenem, both carbapenems and PBP 2-specific antibiotics. Exposure of filtrates of *P. aeruginosa* treated with imipenem to cells caused low-level TNF-production and nitric oxide, whereas meropenem induced high levels [79]. This finding is supported by Arditi *et al* who reported a difference in imipenem-induced IL-6 release in human vascular endothelial cells when compared to meropenem and ceftriaxone [80]. The mechanism of antibiotic-induced LPS release is independent of PBP affinities for ceftazidime or imipenem. Once *Salmonella typhi* is killed by either imipenem or ceftazidime, the rate of LPS release from *S. typhi* does not differ [81].

ANTIBIOTIC CONCENTRATION

LPS release may increase as a function of the antibiotic concentration, reaching a maximum at the concentration that kills most of the pathogens. Ceftazidime released 61.9%, imipenem 51.1%, gentamicin 9.8% and ciprofloxacin 12.7% of LPS within the first hour of incubation. It was suggested that antibiotic-released and cell-bound LPS had a similar O-polysaccharide content [82]. The minimal inhibitory concentration (MIC) of the studied antibiotic may affect the quantity of endotoxin released. At 50 times the MIC ceftazidime and ciprofloxacin resulted in significantly higher levels of endotoxin, TNF and IL-6 than those of imipenem and gentamicin. However, at 0.5 times the MIC the differences were small, comparable to untreated controls [83].

In summary, antibiotic-induced endotoxin release may depend on antibiotic class, presence of serum, type of organism, site of antibiotic action and Gram-stain. Endotoxin release may be different in late or early lysis, proportional to the number of killed pathogens. Morphology of bacteria may have an impact on endotoxin release and phagocytosis. *(Table 2)*

ANTIBIOTIC-INDUCED ENDOTOXIN RELEASE - ANIMAL MODELS

Van Miert demonstrated that polymyxin B produced an antipyretic effect in endotoxin-induced fever by interaction with endotoxin [84]. Although polymyxin B delayed the dynamic effects of endotoxin, e.g., systemic hypotension, decrease in cardiac output, it did not prevent the initial metabolic acidosis following endotoxin and the acute systemic hypotension [85]. Endotoxemia may correlate with mortality. However, the level of endotoxemia in antibiotic treated animals (kanamycin) was significantly greater than in animals dying without any antibiotic therapy. It was concluded that aminoglycosides might shift the lethal mechanism during *Pseudomonas mirabilis* peritonitis from those involving bacterial proliferation and low levels of endotoxemia to those involving bacterial death and the release of large amounts of endotoxin. Corticosteroids may be able to protect those animals when endotoxin was added [86]. Clindamycin, lincomycin and ceftazidime treatment in an *E. coli* sepsis rat model is associated with a 20-fold increase of plasma endotoxin concentration when compared to no treatment [87]. When methylprednisolone was

TABLE 2 - *Antibiotic-induced endotoxin release - in-vitro studies.*

Antibiotic	Number of studies	High endotoxin	Intermediate endotoxin	Low endotoxin	Filamentation
Ciprofloxacin	9	4	1	4	Yes
Gentamicin	7	-	2	5	No
Tobramycin	6	-	-	6	No
Imipenem	26	1	4	21	No
Ceftazidime	20	18	1	1	Yes
Meropenem	11	1	8	2	Indeterminate
Cefotaxime	5	5	-	-	Yes

tested in a model with intraperitoneal administration of *E. coli*, there was no difference between methylprednisolone-treated and placebo-treated animals in bacteremia, endotoxemia, and physiologic, metabolic and hematological parameters [88]. This was further analyzed by Shenep *et al* who demonstrated that in animals treated with placebo the concentration of free endotoxin was proportional to the level of bacteremia. However, in animals treated with antibiotics the plasma levels of free endotoxin increased 10- to 2000-fold in spite of decreasing levels of bacteremia [89].

Endotoxin liberation during therapy for sepsis caused by Gram-negative bacteria may be dependent upon the class of antibiotics and not necessarily correlated with the rate of bacterial killing. Despite similar rates of bacterial killing, mean levels of endotoxin were up to 20-fold higher in rabbits treated with moxalactam than in paired rabbits receiving gentamicin [90]. Oral administration of antibiotics, e.g., polymyxin B, aztreonam, temocillin, cephalothin and neomycin, may decrease endotoxin concentration in fecal supernatants to 1%. Antibiotics did not interfere with the LAL test. It was concluded that in mice 90% of the feces derived endotoxin was released by intestinal aerobic Gram-negative pathogens [91]. Intestinal endotoxin level does not necessarily correlate with the level of Gram-negative bacteria but corresponds to the proliferative activity of these bacteria [92]. Pretreatment with agents that alter gut content in animals reduced endotoxemia, maintained normal glutamine and ammonia levels, and thereby increased survival [93]. Total parenteral nutrition has been associated with translocation of endotoxin and pathogens, which resulted in increased TNF production by alveolar macrophages. The addition of polymyxin B reduced endotoxemia and inhibited TNF release [94]. Ampicillin decreased significantly the rate of bacteremia in surviving animals but at the same time increased the activity of free endotoxin compared to controls [95]. A comparison of endotoxin release by different antimicrobial agents in experimental *E. coli* meningitis revealed that antibiotic therapy irrespective of the agent might result in an increase in free endotoxin and enhancement of inflammation. The amount of endotoxin released may be smaller than in untreated animals [96]. Depending on the inoculum level, cefoxitin alone and not anti-TNF antibodies may prevent death. However, when anti-TNF antibodies are added to cefoxitin treatment in a murine model of mixed *E. coli* and *B. fragilis* peritonitis, it abrogated the rise in TNF and increased the survival rate [97]. Imipenem provided a greater level of protection than ceftazidime in a mouse bacteremia model [98]. However, the protective effect may not be due to an inhibition of the endotoxin induced proinflammatory cytokine release. Elevated TNF after cecal ligation and puncture (CLP) in rats treated with antibiotics was associated with enhanced hemodynamic response to CLP, but did not increase mortality. Despite increases in circulating TNF after antibiotic administration, the mortality rate decreased after both bactericidal and bacteriostatic antibiotics. TNF levels and cardiac output were elevated to a greater extent in bactericidal treated rats than bacteriostatic or untreated rats [99]. Evidence for endotoxin-induced pro-inflammatory response and its effect on survival were reported by Yao *et al* who prevented the increase of TNF and IL-1 activities of peritoneal macrophages by low dose polymyxin B which

was followed by an enhanced survival rate [100]. Differences in protective efficacy among ceftazidime, imipenem, meropenem, were reported for *E. coli* and *P. aeruginosa* infection but not in *S. aureus* infections [101]. Changing outcome with different forms of strains – virulent versus nonvirulent – cannot be solely attributed to endotoxin release. Viable virulent strains caused less endotoxin but more harm. Virulence factors associated with *E. coli* strains may be more important in the outcome of septic shock than endotoxin levels [102]. In rabbit Gram-negative sepsis the administration of rBPI12 in conjunction with cefamandole prevented the cefamandole-induced endotoxin release, accelerated bacterial clearance, ameliorated cardiopulmonary dysfunction and thereby prevented death [103].

Further evidence for antibiotic-mediated endotoxin release as a contributing factor to lethality in Gram-negative sepsis has been presented by Morrison and Bucklin [104]. In the early phase of therapy antibiotic-induced endotoxin release may be influenced by the mode of action of the antibiotics. Nitsche *et al* reported that pharmacodynamics, e.g., dosage, might have an impact on endotoxin release as well. Lowest levels of endotoxin in a peritonitis rat model were measured in high dose ciprofloxacin when compared to imipenem, gentamicin, cefotaxime and low dose ciprofloxacin. Except in the high dose ciprofloxacin group, the endotoxin increase was associated with a decrease in mean arterial pressure [105]. Topical applied imipenem in a rat peritonitis model induced endotoxin and TNF release despite profound bactericidal activity. The survival rate was higher in the imipenem treated group than the taurolidine group or controls [106]. Unlike the dramatic increase in endotoxin concentration after antibiotic treatment in the cerebrospinal fluid during experimental *H. influenzae* meningitis, there was no endotoxin release visible subsequent to the antibiotic treatment of experimental otitis media [107]. *In-vitro* treatment of *P. aeruginosa* with imipenem caused low-level release of free endotoxin, which was not lethal for D-GalN-sensitized mice. Treatment with ceftazidime, meropenem, and cefozopran caused high-level release of endotoxin, which was lethal for D-GalN-sensitized mice. There was a close relationship between free endotoxin and mortality [108]. In experimental pneumococcal meningitis trovafloxacin delayed the increase in pro-inflammatory cytokines TNF and IL-1beta when compared to ceftriaxone [109]. Doxycycline protected mice from lethal endotoxemia by reducing nitric oxide synthesis via an IL-10 independent mechanism [110]. Infections with *E. coli*, *Serratia marcescens*, *K. pneumoniae*, *P. aeruginosa*, *Proteus vulgaris*, *and Proteus mirabilis* resulted in an increase of plasma endotoxin concentration after antibiotic treatment. Except for *P. aeruginosa*, the endotoxin levels were lower after carbapenem treatment than ceftazidime treatment. Treatment of *P. aeruginosa* sepsis with meropenem or biapenem induced more endotoxin release than other carbapenems [111]. Different levels of endotoxin in platelet-rich plasma according to differential affinity for penicillin-binding protein (PBP) were detected in *E. coli*-inoculated mouse whole blood *ex vivo* [112]. Inflammatory response to viable killed pathogens may differ depending on the bacterium, the host sensitivity to TNF and possible Gram-stain classification. *S. aureus* killed during imipenem or ceftazidime treatment elicited an early release of TNF. Protection with dexamethasone was not seen when animals were challenged with viable organisms but without concurrent administration of antibiotics [113].

In summary, antibiotic-treated animals may show higher endotoxin levels with a higher survival rate than untreated animals. Plasma endotoxin may increase despite decreasing bacteremia. There may be a similar killing rate by different antibiotics but a difference in endotoxin release. Intestinal endotoxin does not necessarily correlate with the level of Gram-negative bacteria. However, the alteration of the gut content by pretreatment may be associated with reduced endotoxemia and increased survival. Antibiotic-induced endotoxin release may be different depending on the type of infection, the location of infection, the virulence of strains, Gram-stain, mode of application and dosage of antibiotic. Different antibiotics may induce the release of different forms of endotoxin which may be lethal for sensitized animals. The combination of antibiotics with inhibitors of endotoxin or the pro-inflammatory response may be responsible for increased survival due to decreased endotoxin release.

ANTIBIOTIC-INDUCED ENDOTOXIN RELEASE - CLINICAL STUDIES

The information gathered by clinical studies that antibiotic-induced endotoxin release may have a significant effect on outcome leaves space for speculation. It should be noted, however, that the treatment of a subgroup of patients with sepsis or septic shock with immunoglobulin preparations which are known to interact with endotoxin has been successful [114]. Arditi *et al* reported that ceftriaxone induced a marked increase in free endotoxin in the cerebrospinal fluid, which correlated with number of bacteria killed. Initial CSF total endotoxin concentrations correlated both with the Herson-Todd clinical severity score and the number of febrile days [115]. Mean peak endotoxin and IL-1 beta concentrations were significantly higher in infants who received intraventricular gentamicin and i.v. antibiotics than infants given i.v. antibiotics alone. Mean IL-1beta concentrations in CSF correlated with adverse outcome and endotoxin concentrations [116]. Hurley *et al* demonstrated that the antibiotic treatment of chronically bacteriuric patients was associated with a decrease of CFU 2h after antibiotic administration and an increase of endotoxin [117]. Erythromycin caused a significant reduction in neutrophil numbers, IL-8/albumin ratio and neutrophil elastase/albumin ratio in bronchoalveolar lavage fluid of patients with chronic airways disease [118]. In a post-hoc analysis from a randomized study to evaluate the efficacy of interferon gamma in trauma patients it was observed that mortality in the PBP 3/TNF group (17%) was higher than in the non-PBP 3/TNF group (8%) although the injury severity score was similar in both groups [119]. Prins *et al.* detected that in patients with urosepsis blood endotoxin levels decreased after treatment with imipenem but increased when patients received ceftazidime [120]. In patients with acute pyelonephritis of Gram-negative origin cefuroxime administration caused a rise in endotoxin, which was associated with persistence of fever in up to 50% of patients. A positive correlation between endotoxin and drug levels was detected 6 h after initiation of therapy [121]. Clinical antibiotic-induced endotoxin release did not occur after hepatic resection regardless of the antibiotic used, probably due to scavenging of endotoxin in peripheral blood [122]. In a randomized clinical study comparing imipenem versus ceftazidime in patients with Gram-negative urosepsis there was no difference in plasma endotoxin, IL-6 or TNF levels detectable [123]. In contrast, ceftazidime treated patients with severe melioidosis had greater endotoxin levels than imipenem treated patients after the first dose of antibiotics. Mortality was 35% in both groups and not different [124]. In surgical intensive care patients in a German university center there were significantly more patients with endotoxemia when treated with cefotaxime/ceftriaxone when compared to patients treated with imipenem. However, there were no differences among the groups in temperature, APACHE II scores, leukocytes, albumin, blood pressure and heart rate after antibiotic administration. Endotoxin neutralizing capacity (ENC) was significantly different in patients treated with imipenem when compared to patients with cefotaxime treatment. This is in agreement with a significantly greater decrease of IL-6 in the imipenem group compared to the cefotaxime and ceftriaxone group [125]. In a European multi-center study in surgical intensive care patients there was no difference in plasma endotoxin levels in patients treated with imipenem compared to PBP 3-specific antibiotics. However, patients who were treated with imipenem were in general associated with higher MOF scores and IL-6 levels decreased faster when compared to patients treated with PBP 3-specific antibiotics. Despite higher MOF scores the mortality was similar in both groups [126].

In summary, the clinical significance of antibiotic-induced endotoxin release is documented only in a few clinical disorders, e.g., meningitis, urosepsis. The difference in endotoxin release by PBP 2-specific antibiotics, e.g., imipenem, and PBP 3-specific antibiotics, e.g., ceftazidime, may not be visible in each study. Patients with increased MOF scores may profit from treatment with antibiotics known to decrease endotoxin.

CONCLUSIONS

In conclusion, the clinical significance of antibiotic-induced endotoxin release remains to

be clarified. Type of pathogen and its virulence may be more important than recently suggested. Gram-positive pathogens were just recently recognized as an important factor for the development of the host response. Multi-drug strategies are necessary to inhibit the synergistic mechanism causing tissue damage and organ failure in sepsis and peritonitis [127]. In case of fever of unknown origin in intensive care patients either failure of treatment, e.g., failure of source control in intra-abdominal infection, or a side effect of antibiotic treatment, e.g., endotoxin release, should be considered as cause of the fever.

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