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# MINIREVIEW

# Clinical Relevance of Antibiotic-Induced Endotoxin Release

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## INTRODUCTION

Considerable mortality is associated with gram-negative infections, especially when they are complicated by shock (45). Although appropriate antibiotic treatment significantly reduces this mortality (45), it was already known in the early days of antibiotic treatment that fatal vasomotor collapse may occur after administration of a loading dose of chloramphenicol for typhoid fever (13). James Reilly hypothesized that the adverse clinical effects that were associated with destruction of the typhoid bacilli could be caused by an overwhelming release of endotoxin. He subsequently advised that one should start with a low dose of antibiotics: "*frappez doucement*" ("hit gently") instead of "*frappez fort et vite*" ("hit vigorously") (13).

During the last 10 years a renewed interest in the possible deleterious effects of liberation of endotoxin (lipopolysaccharide [LPS]) during bacterial cell death has emerged.

In this minireview, we review the current knowledge of the mechanisms of antibiotic-induced endotoxin release as well as their possible clinical implications.

## ENDOTOXIN

Endotoxins are LPS constituents of the outer membrane of the cell wall of gram-negative bacteria. It is considered to be the most important bacterial factor in the pathogenesis of the gram-negative septic syndrome. Once in the circulation, the lipid A moiety of endotoxin prompts the release of endogenous mediators, such as tumor necrosis factor alpha (TNF-α), interleukin 1, interleukin 6, and other cytokines, from mononuclear phagocytes and other cells. These cytokines induce a cascade of secondary inflammatory mediators, eventually leading to endothelial damage and severe hemodynamic and metabolic derangements (10).

Endotoxins can be quantitated by a chromogenic *Limulus* amebocyte lysate (LAL) assay, which is based on activation of the *Limulus* lysate by endotoxin. This assay is sensitive (detection limit, 5 to 10 pg/ml) and reflects the biological activity of endotoxin. Less sensitive methods of measuring endotoxin are immunoassays with monoclonal antibodies directed against the core polysaccharide or the O antigen. Gas chromatography measures, e.g., the β-$OHC_{14:0}$ content, a chemical marker of lipid A. The results that are obtained are dependent on the form of endotoxin studied. Related to its β-$OHC_{14:0}$ content, shed endotoxin was shown to be the most *Limulus*-active form of LPS, while purified LPS was the most antigen-active form (50).

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## THE FATE OF ENDOTOXIN IN THE HOST

Although the human organism is able to detoxify endotoxins, little is known about the fate of endotoxins in the clinical setting. In rats, the clearance rate of endotoxin from blood is dependent on the physicochemical properties of the LPS injected: the more aggregated preparations have shorter half-lives, and rough endotoxin (which lacks the outer polysaccharide chain) is cleared much faster than smooth LPS (34, 49). After intravenous injection of LPS into rabbits, two elimination phases can be distinguished. The first is a rapid clearance phase (half-life, <30 min), with uptake mainly by the liver and the spleen. This is followed by a second phase, which has a half-life of 12 h (49), that is characterized by interaction of LPS with lipoproteins, in particular high-density lipoproteins (34). The high-density lipoprotein–LPS complex is more slowly internalized by mouse macrophages, which may be related to this increase in the half-life in serum. Many serum proteins, including albumin, immunoglobulins, complement component C3, and several unidentified proteins, are also able to bind endotoxin (75). The interaction of endotoxin with two related proteins, lipopolysaccharide-binding protein (LBP) and bactericidal–permeability-increasing protein (BPI), importantly alters its biological effects. LBP is an acute-phase serum protein that is synthesized by hepatocytes and that is able to bind to the lipid A part of the endotoxin molecule (64). The presence of LBP in serum potently amplifies the ability of endotoxin to stimulate TNF-α production by monocytes (64), and this effect is dependent on the presence of CD14 on the stimulated cells (82). Hence, it seems that binding of the LPS-LBP complex to CD14 significantly increases the responsiveness of monocytes to endotoxin. Monocytes can be stimulated by endotoxin, however, in the complete absence of LBP (64), and it is therefore likely that other endotoxin receptors exist. BPI is a constituent of the azurophilic (primary) granules of neutrophils (26) and shares significant homology with LBP (64), in particular, in the region that is thought to be involved in binding to lipid A. In contrast to LBP, binding of BPI to endotoxin inhibits the capacity of endotoxin to stimulate cytokine production by monocytes (26, 48). BPI is expressed on the surfaces of activated neutrophils (48), but investigators are uncertain to what extent neutrophils secrete BPI in vivo. It is likely, however, that substantial amounts of BPI are released from dying and disintegrating neutrophils in abscesses. Several other proteins that are present in neutrophils also have endotoxin-binding abilities and may be involved in endotoxin neutralization in vivo.

In conclusion, the removal and detoxification of endotoxin by the human host is a complex phenomenon that depends on many factors. In addition to the fact that humans, in contrast to many animals, including most subhuman primates, are ex-

tremely sensitive to endotoxin, these observations explain the difficulty in establishing a clear-cut dose-response relationship between the amount of circulating endotoxin (activation of the *Limulus* lysate) and clinical outcome in patients with sepsis. For similar reasons, data on endotoxin release obtained in animal models should be cautiously extrapolated to the clinical setting.

## SPONTANEOUS ENDOTOXIN RELEASE

During bacterial growth in culture, LPS is continuously shed. This phenomenon has been observed in, among other organisms, *Escherichia coli* and other members of the family *Enterobacteriaceae* (27, 42, 50), *Pseudomonas aeruginosa* (14, 27), and *Neisseria meningitidis* (3, 52). In meningococci, it was observed that isolates from patients had higher endotoxin-liberating activities than strains isolated from carriers (3). During normal growth, until the stationary phase, most endotoxin remains bound to the cell (4, 25, 65, 69), and a stable ratio of bacterial counts and endotoxin concentration (total and shed) is usually observed (4, 25, 41, 68, 78).

Normal serum is bactericidal for many gram-negative bacteria, resulting in the release of LPS (74). Hence, culture conditions may poorly reflect the fate of bacteria in blood. Therefore, endotoxin release may occur spontaneously during in vitro and in vivo growth of several bacterial species, and not all endotoxin measured during antibiotic treatment is necessarily released by the bactericidal actions of the antibiotics.

In addition, the mere fact that endotoxin is released from bacteria in vivo does not necessarily prove that this is the only important mechanism of disease. For instance, compared with *E. coli*, a dissociation between the level of endotoxemia and the severity of septicemia was found for *P. aeruginosa* (22). During treatment of *Haemophilus influenzae* type b meningitis, peptidoglycans released from the bacterial cell wall into the cerebrospinal fluid (CSF) also contribute to meningeal inflammation (12). Jarisch-Herxheimer reactions that occur during treatment of secondary syphilis were proven not to be mediated by endotoxin (66). These findings support the concept that bacterial products other than endotoxin may also be contributors in the pathogenesis of septic shock. Thus, in animal and clinical studies, not all deteriorations in the severity of disease during treatment of gram-negative infections may automatically be ascribed to the effects of liberated endotoxin.

## ENDOTOXIN RELEASE IN VITRO

Several investigations studying various gram-negative bacteria addressed the question of endotoxin release during exposure to antibiotics in vitro (17, 25, 37, 44, 51, 52, 78, 79) (those investigations are summarized in Table 1, which was adapted and extended from the data of Hurley [40]). In all control cultures, a proportional increase in both bacterial counts and endotoxin levels was observed. In all cultures treated with an effective antibiotic, a decrease in bacterial counts occurred after antibiotic treatment, with an increase in total or free endotoxin concentrations. This rise in endotoxin level was as great as a 2-$\log_{10}$-unit change, i.e., a 100-fold increase (25). These data show that endotoxin release can be induced by bacterial death caused by exposure to antibiotics.

In two studies, the biological activities of the released endotoxin were investigated. Simon et al. (70) measured TNF-$\alpha$ release from mononuclear cells exposed to filtrates of antibiotic-killed *E. coli*, and Arditi et al. (5) measured TNF-$\alpha$ release from human whole blood ex vivo in response to antibiotic-exposed cultures of *H. influenzae* type b. Whereas low levels of TNF were produced in response to viable bacteria, exposure to filtrates of antibiotic-treated cultures produced a significant increase in TNF levels. A comparable induction of TNF was provoked by purified endotoxin, and the addition of polymyxin B (which binds endotoxin; see below) inhibited TNF generation. This strongly suggests that biologically active endotoxin is released during antibiotic killing of *E. coli* or *H. influenzae* type b.

The question of which mechanisms are responsible for this increase in endotoxin levels remains unanswered. The time course of endotoxin release does not always parallel the bacterial killing curve. In two of the in vitro studies (44, 79), most of the bacteria were killed within 2 h, whereas endotoxin levels still increased after 6 h. In two studies (52, 78), the increase in endotoxin concentrations leveled off after 1 to 2 h, whereas bacterial counts still decreased. In two other studies (9, 25), the time course of bacterial killing and endotoxin release occurred in parallel.

These different patterns of endotoxin release are possibly related in part to the bacterial strain and the antibiotic studied, but in general, consideration of the increase in endotoxin levels to be the direct result of bacterial cell wall disintegration seems to be a conclusion that is too simple.

In some studies, a distinction was made between total and free endotoxin levels. Confusion exists on the biological implications of this distinction. Free endotoxin levels are usually defined as the levels measured after passing culture samples through a filter (in general, a 0.45-$\mu$m-pore-size Millipore filter). In this manner, a physical separation is made between bacterial cell-bound endotoxin (which will not pass through the filter) and non-bacterial cell-bound or free endotoxin. The reliability of this method for differentiating between free and cell-bound endotoxin has been questioned (40), and indeed, in one study (78), no differences were detected in the levels of total (unfiltered) and free (filtered) endotoxin either in untreated cultures or in cultures after exposure to antibiotics. However, a shift from cell-bound to free endotoxin was observed in four studies (25, 65, 67, 69), and in one clinical study (4), total endotoxin levels decreased after antibiotic treatment, whereas free endotoxin levels increased. The cell-bound endotoxin of enterobacteria is less reactive, as measured by the LAL-based endotoxin assay, and is less easily recognized by cross-reactive monoclonal antibodies (29, 50, 68). By inference, one would expect free endotoxin to be more toxic for the host. In a model of experimental meningitis, free LPS induced more inflammation than an equal amount of cell-bound endotoxin did (81). In conclusion, most investigations have indicated that free endotoxin is biologically more active than membrane-bound endotoxin, and therefore, it does make sense to distinguish these two forms of endotoxin in experimental and clinical studies.

## STUDIES IN ANIMALS

Studies on endotoxin release during antibiotic treatment of experimental bacteremia may have more clinical relevance than culture data. In general, such studies showed a decrease in bacterial counts concurrent with an increase in endotoxin levels in antibiotic-treated animals or a larger increase in endotoxin levels in treated than in untreated animals. This was demonstrated in experimental *E. coli* sepsis in rabbits (65, 69) and pigs (63), *H. influenzae* sepsis in rats (79), meningococcal sepsis in mice (20), and *E. coli* (35, 73) or *H. influenzae* type b (54, 57) meningitis in rabbits. On the other hand, in three studies on *E. coli* sepsis in rats or dogs (1, 23, 58), no differences in endotoxin levels between control and antibiotic-

TABLE 1. Endotoxin release in vitro

| Reference | Organism | Antibiotic | Concn (mg/liter [MIC]) | Duration of exposure (h) | $Log_{10}$ change from baseline | | |
|---|---|---|---|---|---|---|---|
| | | | | | Total endotoxin | Free endotoxin | Bacterial titer |
| 37 | *E. coli* | Control | | 3 | +0.43 | | +0.6 |
| | | Ampicillin | 50 (8× MIC) | 3 | +0.90 | | −1.7 |
| | | Streptomycin | 50 (8× MIC) | 3 | +0.93 | | −1.8 |
| | | Tetracycline | 50 (8× MIC) | 3 | +0.36 | | −1.1 |
| | | Polymyxin B | 6 (7.5× MIC) | 3 | +0.26 | | −1.8 |
| 51 | *E. coli* | Control | | 2 | | +0.69 | +1.0 |
| | | Enoxacin | 3.7 (23× MIC) | 2 | | +1.05 | −2.9 |
| | | Ofloxacin | 5.3 (265× MIC) | 2 | | +1.05 | −4.0 |
| | | Pefloxacin | 4.3 (54× MIC) | 2 | | +0.92 | −3.4 |
| | | Norfloxacin | 1.5 (37.5× MIC) | 2 | | +0.92 | −3.4 |
| | | Ciprofloxacin | 2.3 (115× MIC) | 2 | | +0.69 | −3.4 |
| 17 | *E. coli* | Control | | 2 | | +0.85 | +1.0 |
| | | Gentamicin | 8 (133× MIC) | 2 | | +0.40 | −3.6 |
| | | Ciprofloxacin | 5 (250× MIC) | 2 | | +1.04 | −4.0 |
| 79 | *H. influenzae* | Control | | 2 | | +0.48 | +0.8 |
| | | Ampicillin | 100 (1,000× MIC) | 2 | | +0.98 | −1.8 |
| | | Chloramphenicol | 25 | 2 | | +1.18 | −1.8 |
| | | Colistin | 5 | 2 | | +0.48 | +0.9 |
| | | Polymyxin B | 7 (2,300× MIC) | 2 | | −0.82 | No growth |
| 25 | *E. coli* | Control | | 4 | +2.3 | +1.3 | +2.3 |
| | | Tobramycin | 8 (16× MIC) | 4 | +0.7 | +1.2 | −3.4 |
| | | Chloramphenicol | 100 (25× MIC) | 4 | +1.9 | +1.4 | +1.7 |
| | | Cefuroxime | 75 (37.5× MIC) | 4 | +1.3 | +2.1 | −2.0 |
| | | Ceftazidime | 100 (400× MIC) | 4 | +0.6 | +1.3 | −3.0 |
| | | Aztreonam | 100 (800× MIC) | 4 | +1.7 | +2.3 | −2.0 |
| | | Imipenem | 100 (800× MIC) | 4 | +0.2 | +0.9 | −3.0 |
| 52 | *N. meningitidis* | Penicillin | (1× MIC) | 1 | | +0.05 | −1.3 |
| | | Penicillin | (100× MIC) | 1 | | +0.10 | −2.7 |
| | | Chloramphenicol | (1× MIC) | 1 | | +0.02 | −0.7 |
| | | Chloramphenicol | (100× MIC) | 1 | | +0.05 | −1.7 |
| 44 | *P. aeruginosa* | Control | | 4 | | +2.12 | +2.0 |
| | | Imipenem | 0.5 (0.5× MIC) | 4 | | +1.70 | +0.0 |
| | | Imipenem | 2 (2× MIC) | 4 | | +1.40 | −3.4 |
| | | Ceftazidime | 0.8 (0.5× MIC) | 4 | | +2.39 | +1.3 |
| | | Ceftazidime | 4 (2× MIC) | 4 | | +2.34 | −4.3 |
| 78 | *E. coli* | Control | | 6 | +3.33 | | +4.0 |
| | | Gentamicin | 4 (16× MIC) | 6 | +0.51 | | −4.9 |
| | | Amoxicillin | 64 (16× MIC) | 6 | +0.34 | | −3.9 |
| | | Ciprofloxacin | 0.128 (16× MIC) | 6 | +1.63 | | −3.8 |

treated animals were observed. Nevertheless, in those studies the endotoxin/CFU ratio increased in the antibiotic-treated animals because of a sharp decrease in bacterial counts.

Parallel with a higher increase in endotoxin levels in antibiotic-treated animals, some studies reported higher TNF levels (35, 54, 57), but a correlation between a rise in endotoxin level and interleukin 6 levels could not be demonstrated (23). Although corticosteroids appear to have no influence on the process of antibiotic-induced endotoxin release (31, 57), the TNF response of the host could be attenuated by dexamethasone (54, 57).

In several studies, higher endotoxin levels were associated with an adverse outcome, such as higher mortality (2), deterioration of hemodynamic parameters (63), or, in the case of meningitis, brain edema (73) and higher leukocyte counts and protein and lactate levels in CSF (35, 54, 57).

In conclusion, these data from studies in animals are in agreement with data from the in vitro studies discussed above, and several studies demonstrated that endotoxin release is paralleled by a deterioration in the parameters of disease severity. However, this increase in endotoxin and cytokine levels is seen early in treatment; after a few hours, free endotoxin and TNF fall to low levels, while they continue to rise in untreated animals (35, 54, 57).

## CLINICAL STUDIES

Apart from observations in the early 1950s of patients with typhoid fever mentioned above (13), in only a limited number of clinical studies was the phenomenon of antibiotic-induced endotoxin release investigated.

In two studies (25, 67), endotoxin levels were serially assessed in patients with suspected gram-negative sepsis before and after the institution of antibiotic treatment. In 7 of 11

patients with positive blood cultures, a 2- to 50-fold increase in free endotoxin levels following administration of antibiotics occurred (67). Dofferhoff et al. (25) recorded a 2- to 15-fold increase in total endotoxin levels in 3 of 10 patients with detectable endotoxemia on admission, with a concomitant decrease in blood pressure and a rise in serum lactate levels. Both studies also reported that endotoxin shifted from the cell-bound to the free form after antibiotic treatment.

In patients treated for systemic meningococcal disease, a decrease in total lipooligosaccharide levels was seen in all patients after the institution of antibiotic therapy (11). In these patients, lipooligosaccharide levels were extremely high at the start of therapy, however, and this made assessment of any further rise in endotoxin levels difficult.

Children with gram-negative meningitis, who, in addition to intravenous antibiotics, received gentamicin intrathecally, had higher endotoxin and interleukin 1β levels in their CSF and a higher rate of mortality than children who received only intravenous antibiotics (56). It was suggested that the intraventricular gentamicin caused a greater release of endotoxin, resulting in increased inflammation. However, only a subgroup of patients (i.e., the patients who survived long enough for multiple CSF samples to be obtained) was studied, and bacterial numbers, substantiating a faster rate of killing, were not given. Also, gentamicin has endotoxin-neutralizing capacities (see below). Therefore, other explanations for the increased rate of mortality are possible, for instance, a direct toxic effect of gentamicin.

Arditi et al. (4) monitored children with *H. influenzae* meningitis treated with ceftriaxone. In eight patients, in whom a second lumbar puncture was performed, a sharp decrease in bacterial numbers was noted, with a decrease in the total endotoxin level and a shift to and increase in the free endotoxin level. The increase in the free endotoxin level was paralleled by an increase in mean lactate and lactate dehydrogenase levels in CSF and a decrease in the glucose level in CSF, suggesting (but not proving) an enhanced inflammatory response. The results of that study once more underscored the importance of distinguishing free from total endotoxin levels.

Finally, antibiotic-induced endotoxin release was studied in chronically bacteriuric patients (41). These patients received a low dose of various antibiotics, which resulted in an average 0.93-$\log_{10}$-unit decrease in CFU and an average 0.59-$\log_{10}$-unit increase in total endotoxin levels in urine. In nontreated patients, the bacterial counts and endotoxin levels remained unchanged during the period of observation. No important differences in the capacity to cause endotoxin release were noticed between the antibiotics. As was seen in some in vitro studies, maximal bacterial lysis preceded a maximal increase in endotoxin levels by 1 h.

Although clinical data are still sparse, the available evidence reviewed here indicates that treatment with various antibiotics may cause systemic or local endotoxin liberation. To date, however, there is no conclusive evidence that this effect is related to an adverse clinical outcome.

## DO ANTIBIOTICS DIFFER IN POTENTIAL FOR ENDOTOXIN LIBERATION?

Results of in vitro, animal, and clinical studies support the hypothesis that endotoxin is liberated during antibiotic treatment of gram-negative infections, but appropriate antibiotic treatment significantly reduces mortality (45). Hence, the question with the most clinical relevance is whether equally effective antibiotics differ in the amounts of endotoxin release that they induce.

Such differences have been most extensively studied in vitro (Table 1); as has been discussed before, many confounding variables may preclude direct extrapolation of these results to the clinical setting.

As far as bactericidal and bacteriostatic antibiotics are concerned, ampicillin and streptomycin caused more killing of *E. coli* and more endotoxin release than tetracycline (37), and penicillin induced a faster rate of killing of meningococci coincident with more endotoxin release than chloramphenicol (52). In experiments with *H. influenzae* type b, ampicillin and chloramphenicol treatment resulted in an equal rate of killing and endotoxin release (79). Dofferhoff et al. (24, 25) recorded significantly more endotoxin release by cefuroxime and aztreonam in comparison with that by ceftazidime and tobramycin. Imipenem was the least potent inducer of endotoxin release, despite comparable bactericidal potential. The addition of tobramycin to cefuroxime resulted in a significant decrease in endotoxin liberation in comparison with that from cefuroxime monotherapy (24, 25). This decrease was also found when amikacin was added to ampicillin or cefotaxime monotherapy (9).

At 0.5 to 50 times the MIC, i.e., much lower concentrations than those used in the studies of Dofferhoff et al. (24, 25), ceftazidime released much more endotoxin than imipenem (44).

Quinolones had equal bactericidal effects, but caused more endotoxin release than gentamicin and amoxicillin (17, 78). In this respect, no differences between the various quinolones were observed (51).

These differences may vary with the bacterial strain studied; for *E. coli* and *Klebsiella pneumoniae*, treatment with gentamicin, the quinolones, and, especially, imipenem resulted in the release of less endotoxin than treatment with ceftazidime, aztreonam, and chloramphenicol did; for *P. aeruginosa* treatment with imipenem and ceftazidime produced equal amounts of endotoxin (27).

The biological effects of endotoxin are in large part mediated by cytokines, and it is therefore important to compare the cytokine-inducing potentials of endotoxins that are released after antibiotic treatment. Mononuclear cells released significantly more TNF when they were exposed to filtrates of cultures treated with ceftazidime, cefotaxime, or aztreonam than when they were exposed to filtrates of cultures treated with amikacin or imipenem. Treatment with ciprofloxacin gave intermediate levels of TNF release (70). In a comparable study, cefuroxime, low-dose ceftazidime, and aztreonam induced greater production of extracellular TNF than high-dose ceftazidime, imipenem, or tobramycin (24). High concentrations of cephalosporins resulted in less TNF production than low concentrations (24). TNF production by human whole blood ex vivo was measured during antibiotic killing of *H. influenzae* type b (5). Treatment with ceftriaxone resulted in the production of significantly more TNF-α than treatment with imipenem did. In all three studies (5, 24, 70), there was a strong correlation between endotoxin levels and TNF production. Injection of filtrates of *P. aeruginosa* cultures exposed to ceftazidime caused much more lethality in LPS-hypersensitive mice than filtrates of imipenem-exposed or untreated cultures did (44). This finding was expected in view of the in vitro differences between imipenem and ceftazidime in the amounts of free endotoxin that they liberate (44).

Only a few studies in animals have addressed the biological significance of antibiotic-induced endotoxin release. In rabbits with *E. coli* sepsis, treatment with gentamicin and moxalactam resulted in equal killing of bacteria, but there was a 7- to 20 fold higher endotoxin release resulting from moxalactam treat-

ment than from gentamicin treatment (65); this difference in endotoxin release was not associated with differences in survival, however. In a comparable study (23), no differences in endotoxin release between aztreonam, ceftazidime, and imipenem could be shown. In *E. coli* meningitis, cefotaxime and chloramphenicol induced equal bacterial killing, but there was more release of endotoxin after cefotaxime treatment than after chloramphenicol treatment, and in that study more brain edema was observed in the cefotaxime-treated animals (73).

Finally, in the only clinical study in which the endotoxin-liberating potentials of antibiotics were compared in humans, no differences were found in the potential of ticarcillin, cephalothin, ceftazidime, gentamicin, or ciprofloxacin to induce endotoxin release in the urine of chronically bacteriuric patients (41).

In summary, slower bacterial killing by bacteriostatic antibiotics was accompanied by less endotoxin release in some studies. Significant differences in endotoxin-liberating potential have been demonstrated between various β-lactam antibiotics. Imipenem in particular and also the aminoglycosides seem to be antibiotics with only modest endotoxin-liberating abilities. In most studies, treatment with the quinolones resulted in the release of relatively greater amounts of endotoxin. With one exception, in the few animal and clinical studies that have been performed, these differences were not parallelled by differences in parameters of illness.

## HOW DO ANTIBIOTICS INDUCE ENDOTOXIN RELEASE?

Penicillin-binding proteins (PBPs) are the primary biochemical targets of β-lactam antibiotics in bacteria. These PBPs are enzymes that are located in the bacterial plasma membrane. They catalyze the terminal stages in the assembly of the peptidoglycan network of the bacterial cell wall (76). The PBPs of a given organism are numbered in order of molecular weight, but the activity and numbering system of the PBPs of gram-positive bacteria or *H. influenzae* bear no relation to those of members of the family *Enterobacteriaceae* (53, 59, 61).

Treatment of growing cultures of *E. coli* with β-lactam antibiotics that have a high selective affinity for PBP 1a and especially PBP 1b causes rapid and extensive killing of the bacteria, with the degradation of cell wall material and cellular lysis. In contrast, antibiotics with selective affinity for PBP 2 cause conversion of the bacilli to round cells (also called spheroplasts); this is followed by the loss of viability but is not accompanied by extensive cell wall degradation. Inhibitors of PBP 3 cause selective inhibition of bacterial septation, which leads to the formation of long filaments, but only limited bactericidal activity and lysis take place (59, 70, 71, 76).

Examples of antibiotics that are selective for PBP 1 of *E. coli* are cephaloridine and cefsulodin (59, 61, 76). Antibiotics selective for PBP 2 are mecillinam, clavulanic acid, and imipenem (at higher concentrations, imipenem also inhibits PBP 1b [60]). Antibiotics selective for PBP 3 are aztreonam, piperacillin, and mezlocillin and, at low concentrations, cephalexin, cefotaxime, ceftazidime, and cefuroxime. At higher concentrations, these antibiotics also have affinities for PBP 1a (59, 61).

Antibiotics that target PBPs 1a and 3 induce filamentous changes as well as killing without lysis (38, 77). In addition, the formation of spheroplasts has been described when PBPs 1a and 3 are inhibited simultaneously (38). These differences have been ascribed to the sum of individual inhibitory effects (38), which may also explain the different effects of low and high concentrations of cephalosporins (24, 38). The combined inhibition of PBPs 2 and 3 also causes lysis of *E. coli* (71).

The higher level of endotoxin release in PBP 3-specific β-lactam antibiotics presumably results from the increased cell mass and the ongoing production of LPS by the cell during continued bacterial growth because of filamentation. The lower level of endotoxin release resulting from treatment with PBP 1- or PBP 2-specific antibiotics may be explained by their rapid bactericidal actions, which prevent an increase in total cell mass (25, 44, 78).

Theoretically, antibiotics that are not primarily cell wall active might be expected to release less endotoxin than the β-lactam antibiotics. However, quinolones (which inhibit the enzyme DNA gyrase) have been reported to be relatively potent inducers of endotoxin release. The effects of treatment with ciprofloxacin were very similar to the effects of treatment with PBP 3-specific antibiotics, i.e., filamentation with an increase in nonviable bacterial biomass and an important increase in endotoxin production (78).

Gentamicin treatment resulted in a stabilization of bacterial numbers, with the loss of viability of bacteria in the absence of lysis. The concomitant endotoxin release (albeit much less than that after ciprofloxacin treatment) was thought to be due to the continuation of LPS synthesis (78). It should be noted that an additional property of some aminoglycosides is the ability to bind to endotoxin (see below).

In conclusion, differences in the endotoxin-releasing abilities of various antibiotics exist and are a likely result of their different modes of antibacterial activities.

## ENDOTOXIN BINDING BY ANTIBIOTICS

The prototype endotoxin-binding antibiotic is polymyxin B (55). Polymyxin B specifically binds to lipid A, and after binding, LPS particles of bacteria are disintegrated into small fragments, as seen by electron microscopy (47). In vitro, this results in a diminished bioactivity in the LAL assay and other endotoxin tests (6, 7, 16, 18, 33, 37).

In vitro, polymyxin B also blocks several biological effects induced by endotoxin, including the release of lysosomal enzymes by neutrophils (8), the neutrophil respiratory burst (21), LPS-enhanced tumor cell killing by macrophages (15), and LPS-induced release of TNF-α from alveolar macrophages (72), human whole blood ex vivo (5), or malignant mononuclear cells (70).

In animals with experimental endotoxemia, polymyxin B reduced the levels of mortality (7, 16, 32, 36, 39, 44), acidosis (7), hypotension and other hemodynamic changes (36, 39, 62), diffuse intravascular coagulation (19), leukocytopenia and thrombocytopenia (16, 19), inflammatory skin changes (43), the generalized Shwartzman reaction (33), increased permeability of the blood-brain barrier (81) and the TNF response (7).

In experimental *Pasteurella multocida* sepsis in animals, polymyxin B reduced the endotoxin level, which was associated with less severe neutropenia and thrombocytopenia (20). At subinhibitory levels, it reduced mortality in rats with *H. influenzae* type b infection (79), it reduced the effects of antibiotic-induced endotoxin release in a meningitis model (73), and it attenuated acidosis and hemodynamic changes in rabbits with experimental *E. coli* sepsis (30).

The widespread clinical use of polymyxin B is precluded by its substantial neurotoxicity and nephrotoxicity (28).

The polymyxin B nonapeptide is less toxic, but unfortunately, it is also less potent than its parent compound, polymyxin B (21, 55). Two other polymyxins that are included as

oral agents in several selective bowel decontamination protocols, colistin and colistin nonapeptide, also have some antiendotoxin activities in vitro (80).

Gentamicin and other aminoglycosides can inhibit LPS synthesis (46), and they inhibited the release of LPS in certain nongrowing bacteria (46).

Gentamicin can also bind endotoxin (55, 65), and gentamicin, amikacin, and tobramycin have been shown to neutralize the effects of endotoxin, as measured by the LAL and other assays (6, 33). These neutralizing effects were less than those of polymyxin B. When amikacin or tobramycin was added to β-lactam antibiotics, bacterial lysis was accelerated, but endotoxin release and endotoxin-induced TNF production by macrophages or monocytes in vitro was less (9, 24, 25). In mice, the addition of gentamicin and amikacin in clinically tolerable concentrations reduced the endotoxin-induced generalized Shwartzman reaction (33). Teicoplanin also had endotoxin-binding properties in vitro (32), and it decreased mortality in LPS-challenged mice (32).

No endotoxin binding could be demonstrated for chloramphenicol (6, 18, 33), ampicillin or carbenicillin (18), cefixim or aztreonam (33), imipenem (44), netilmicin (33), ofloxacin (33), or sulfisoxazole and tetracycline (18).

## CONCLUSION

Results of in vitro, animal, and clinical studies support the hypothesis that endotoxin is liberated during antibiotic treatment of infections caused by gram-negative organisms.

In several animal and clinical studies, endotoxin release paralleled deterioration of the parameters of illness. Significant differences in the endotoxin-liberating potentials of various antibiotics have been demonstrated. In particular, imipenem and the aminoglycosides seem to be antibiotics with only modest endotoxin-liberating abilities. Probably because of their endotoxin-neutralizing abilities, the addition of aminoglycosides to β-lactams reduces the amount of endotoxin measured compared with that measured after monotherapy with these β-lactams. In part, the differences in endotoxin-liberating potentials between antibiotics can be related to their different modes of antibacterial activity.

Because of the limited number of experimental and clinical studies, future comparative studies in well-defined populations are indicated. In particular, the issue of whether different antibiotics vary in their capacities to release endotoxin in the clinical setting needs to be addressed. Finally, it remains to be demonstrated that the capacity of antibiotics to induce endotoxin release is related to an adverse clinical outcome.

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