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Different endotoxin release and IL-6 plasma levels after antibiotic administration in surgical intensive care patients

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Summary Despite the use of broad-spectrum antibiotics, aggressive fluid resuscitation, vasopressor support, the mortality associated with Gram-negative sepsis and septic shock has not decreased significantly in the last two decades. The consequences of host exposure to endotoxin and the relationship of antibiotic administration to endotoxin release have become important areas of intense interest. In vitro studies have demonstrated that there was a difference in endotoxin release between PBP-3 specific antibiotics (β-lactam antibiotics) and PBP-2 specific antibiotics (carbapenems). This is the first clinical report of surgical patients admitted to the surgical and anaesthesiology intensive care unit on the missing endotoxin release after imipenem treatment; however cefotaxime and ceftriaxone showed significantly more positive endotoxin tests in the plasma when compared to imipenem. Ciprofloxacin and vancomycin were intermediate in endotoxin release and tobramycin did not cause endotoxin release. There were also significant differences in endotoxin neutralizing capacity. IL-6 levels were decreased after imipenem faster than after ceftriaxone or cefotaxime; ciprofloxacin seemed to increase IL-6. Endotoxin may be harmful in patients where the immune system has been continuously challenged. Timing, dosage, or combination with other compounds as well as the effect of antibiotics on macrophages need to be tested in larger clinical trials. In this respect a consecutive study was started.

INTRODUCTION

Despite improvement of intensive care, the mortality after Gram-negative infections is still very high. 500,000 patients suffer from sepsis in the US every year and 175,000 die. In Gram-negative infections, endotoxin is recognized as a trigger substance for the release of TNFα and IL-6. Endotoxin or lipopolysaccharides are constituents of the outer membrane of Gram-negative bacteria and can be released following disintegration or after antibiotic administration. Several reports demonstrated a difference in endotoxin release after administration of different antibiotics. Especially certain types of β-lactam antibiotics seem to release more endotoxin than others. There is a strong correlation with penicillin binding protein 3 (PBP-3) inducing antibiotics and endotoxin release.[1]

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It was demonstrated by us that even small amounts of endotoxin are able to activate pro- and anti-inflammatory cytokines in elective aortic aneurysm repair.[2] These patients tolerate the endotoxin release well, but it may be harmful in critical care patients where the delicate balance of the immune response may be tipped to deterioration. There are only two studies in clinical settings concerning the endotoxin release after antibiotic administration;[3,4] most the studies were done in vitro or in animals. However, sepsis is occurring in different patterns, especially in surgical patients (two-hit-model).[5] It seems necessary to study the effects of different antibiotics in surgical intensive care patients in regard to endotoxin, endotoxin neutralizing capacity and IL-6.

MATERIALS AND METHODS

In a prospective study we investigated the effect of antibiotic administration (cefotaxime 3 × 2 g, ceftriaxone 1 × 2 g, ciprofloxacin 2 × 0.4 g, tobramycin 2 × 80 mg, imipenem 3 × 0.5 g, and vancomycin 3 × 0.5 g) in 56 patients of a surgical intensive care unit. Patients were already treated for infection with antibiotics. The majority of antibiotics were given mono, according to the susceptibility results. If a combination therapy was administered, in most instances metronidazole was the drug of choice. The interval between the infusion of the first drug and the second drug was 3–4 h.

The following parameters were recorded: age, gender, diagnosis, leukocytes, blood pressure, heart rate, temperature, albumin, and APACHE II score. Temperature, blood pressure, heart rate, and leukocytes were recorded during or immediately after the blood sampling.

Blood samples were collected immediately before antibiotic administration (8 h after antibiotic administration as baseline level), 60, 120, 180, 240 and 300 min after antibiotic infusion. The blood was collected mainly via the central venous catheter in vacuum tubes (Chromogenix Endotube, Haemochrom Diagnostica, Essen, Germany) and centrifuged within 30 min. The tubes were stored at −30°C until further processing.

Endotoxin assay

For determination of endotoxin and endotoxin neutralizing unit we used the method described by Urbaschek et al.[6] This is a turbidimetric, kinetic LAL-assay with internal standardization. Endotoxin standard [NP-3 (KSE) endotoxin standard, *Salmonella abortus equi*, 100 ng/ml] and lysate [Pyrospektro, *Limulus* amoebocyte lysate (LAL) Cape Cod] were provided by Pyroquant Diagnostik, Walldorf, Germany.

Each sample was spiked by a known concentration of endotoxin, the kinetic reaction was read continuously in an ELISA plate reader (Molecular Devices, MWG Biotech, Ebersberg, Germany) and the endotoxin concentration was calculated by a special software program. While the heated samples were used for endotoxin determination, the unheated samples were tested in the same test for endotoxin neutralizing capacity.

IL-6

For IL-6 determination we used a commercially available IL-6 ELISA (R&D, Minneapolis, MN, USA (DPC, Bad Nauheim, Germany)). Sensitivity was 3 pg/ml.

Statistical analysis

For qualitative analysis of endotoxin results, the chi-square analysis was performed using a statistical package (Graphpad, Santa Monica, CA, USA). The quantitative comparison of IL-6 was performed using the Mann-Whitney analysis. Results were considered significantly different when $P < 0.05$.

RESULTS

In 56 patients of the surgical intensive care unit, we investigated the effect of ceftriaxone, cefotaxime, imipenem, vancomycin, ciprofloxacin and tobramycin on endotoxin release, endotoxin neutralizing capacity and IL-6. The patients were mainly admitted to the intensive care unit for pneumonia (22), peritonitis (13), polytrauma (10) after intraabdominal operations and had to be treated with antibiotics. The isolated pathogens are shown in Table 1.

Table 1 Isolated pathogens of surgical intensive care patients treated for infections

Enterococcus	23%
E. coli	13%
Coagulation negative *Staphylococci*	10%
Ps. aeruginosa	8%
Klebsiella	8%
Candida albicans	8%
Streptococcus	6%
Bact. fragilis	6%
Others	18%

The temperature at the end of the antibiotic administration was comparable in all antibiotics (range 37–38°C). Leucocytes varied from 6900 (median) in the cefotaxime group to 15300 (median) in the vancomycin group. Albumin level range was 2.9 g/dl (ciprofloxacin) to 3.5 g/dl (tobramycin). The median heart rate was 101 (ciprofloxacin), 94 (cefotaxime), 110 (tobramycin), 104 (ceftriaxone), 109 (vancomycin), and 95 (imipenem). The median of the

Table 2 Temperature, leukocytes, albumin, heart rate (pulse), blood pressure (RR) at the end of the antibiotic administration (2–4 h after start)

	Temperature	APACHE II	Leukocytes	Albumin	Pulse	RR
Ciprofloxacin	38.1/0.28	17.3/1.4	11400/1900	2.8/0.16	102/5	84/5
Cefotaxime	37.6/0.1	13.4/1.7	7900/1000	2.98/0.17	93/5	82/4
Tobramycin	37.9/0.29	16/2.1	13200/2300	3.7/0.17	105/6	91/3
Ceftriaxone	37.9/0.2	13/1.1	12100/1400	3.06/0.32	103/5	82/3
Vancomycin	38/0.2	13.4/2.5	16200/4100	2.88/0.3	110/3	90/10
Imipenem	37.7/0.36	16.3/2.3	12800/2000	3.29/0.23	97/4	85/3

Table 3 Endotoxin results after antibiotic administration: indicated are endotoxin positive/endotoxin negative results in patients at the different time points, $^*P < 0.05$ in comparison to imipenem

	Baseline	60 min	120 min	180 min	240 min	300 min
Ciprofloxacin	1/9	1/9	1/9	1/9	2/8	1/9
Cefotaxime	2/9	4/7	4/7	5/6*	5/6*	4/7
Tobramycin	0/7	0/7	0/7	1/6	2/5	1/6
Ceftriaxone	2/9	5/6*	3/8	4/7	5/6*	5/6*
Vancomycin	0/5	0.25	0.25	2/3	2/3	2/3
Imipenem	0/11	0/11	0/11	0/11	0/11	0/11

mean blood pressure was 84 (ciprofloxacin), 78 (cefotaxime), 89 (tobramycin), 80 (ceftriaxone), 90 (vancomycin), and 86 (imipenem) (*see* Table 2).

The endotoxin release after antibiotic administration was different in regard to the type of antibiotic. During ciprofloxacin treatment, endotoxin was only detectable in one case, which was comparable to tobramycin. In the vancomycin group, 2 patients revealed positive endotoxin after antibiotic infusion. Ceftriaxone and cefotaxime had positive endotoxin tests in 5 of 11 patients. This was significantly different from imipenem were none of the patients had positive endotoxin detectable (Table 3).

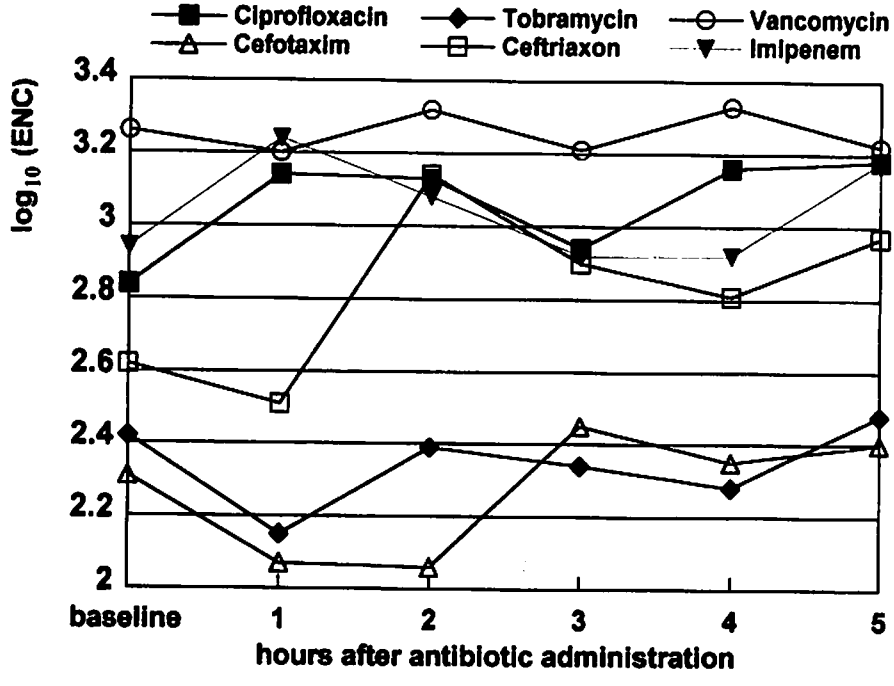

Fig. 1 Endotoxin neutralizing capacity (log_{10}) after antibiotic administration. ENC was significantly different between imipenem and cefotaxime 60 min after antibiotic treatment $P < 0.05$. The difference between ceftriaxone and imipenem was almost significantly different $P = 0.09$.

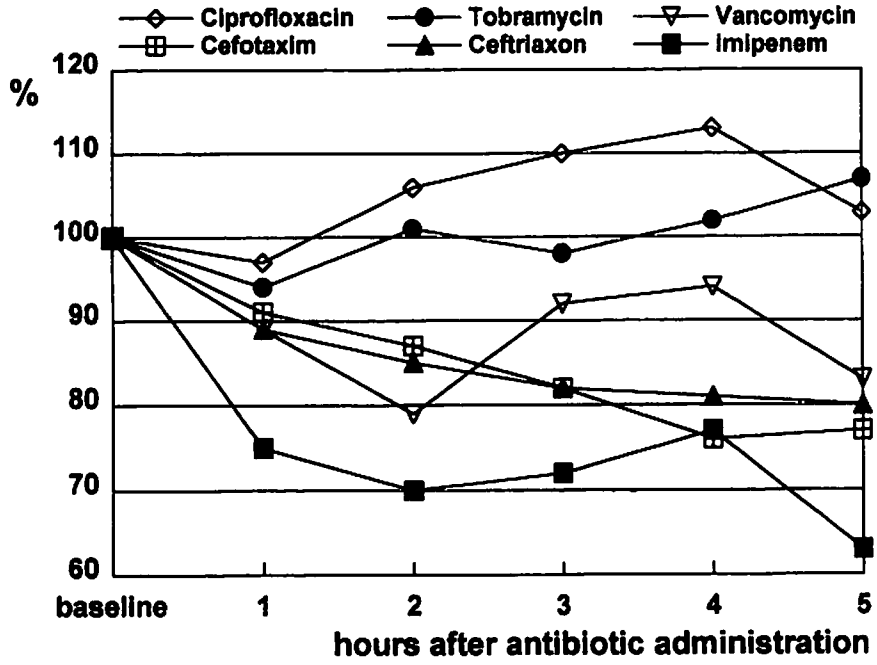

Fig. 2 IL-6 levels (in % of baseline level) after antibiotic administration. IL-6 levels were significantly decreased at 60 min (imipenem $P < 0.01$), at 120 min (ceftriaxone $P < 0.05$), at 240 min (cefotaxime $P < 0.05$). IL-6 was almost significantly increased at 180 min (ciprofloxacin $P = 0.051$).

Endotoxin neutralizing units were below baseline after antibiotic administration in cefotaxime, tobramycin, ceftriaxone and vancomycin. However, ciprofloxacin and imipenem had increasing levels of endotoxin neutralizing units after antibiotic administration.

Endotoxin neutralizing unit after 60 min increased after imipenem ($P < 0.01$) compared to cefotaxime. Baseline levels were not different between antibiotics (Fig. 1).

IL-6 levels which are indicated as % of base line level are different after antibiotic administration. While IL-6 is decreased after imipenem already after 60 min ($P < 0.05$), it takes 120 min for ceftriaxone and 240 min for cefotaxime to decrease IL-6 significantly. Tobramycin showed no effect on IL-6, but ciprofloxacin seemed to increase IL-6 ($P = 0.051$ after 180 min). Imipenem was the compound which decreased IL-6 maximally (63% of baseline level); vancomycin decreased IL-6 to 83%, cefotaxime to 77%, ceftriaxone to 80% (Fig. 2).

DISCUSSION

Despite the use of broad-spectrum antibiotics, aggressive fluid resuscitation, vasopressor support, the mortality associated with Gram-negative sepsis and septic shock has not decreased significantly in the last two decades.[7]

It became evident that the pathogenic mechanism leading to sepsis may be different and influence the outcome of therapy.[8] Although it is well recognized that TNFα is a major player in the inflammatory cascade, it has been demonstrated that TNFα production may decrease after trauma and consecutive sepsis (double-hit), further indicating that intraabdominal sepsis may differ from other forms of sepsis.[5]

The consequences of host exposure to endotoxin and the relationship of antibiotic administration to endotoxin release have become important areas of intense interest. Endotoxins are highly heterogenous lipopolysaccharides that are components of Gram-negative bacterial cell walls. Administration of endotoxin to normal humans activates the acute phase response and can qualitatively reproduce many features of sepsis.[9] Endotoxin, a potent activator of the coagulation, complement, and kallikrein pathways, initiates the septic cascade by stimulating the production of various cytokines (including TNFα, IL-1 and IL-6) that can cause fever, widespread circulatory changes, end organ dysfunction, multiple organ system failure and death.[7,10-12]

In this study we report for the first time that there may be an in vivo difference in endotoxin release after antibiotic administration in surgical patients. The patients were mainly treated for peritonitis or had infections after operations of the gastrointestinal tract. We did not observe any case of septic shock. The isolated pathogens were mainly *Enterococci*, *Escherichia coli* and

coagulase negative *Staphylocci*; only in 6% of all isolated pathogens were detected as *Bacteroides fragilis*. In the majority of cases, the antibiotic therapy was a monotherapy according to the susceptibility results. When combination therapy was applied, metronidazole was the drug of choice in most instances. Metronidazole therapy was started 3–4 h after the first drug was started. *Bact. fragilis*, and related bacteria, are also endotoxin-containing organisms. However, we did not observe any changes in endotoxin release which could be related to an effect of the metronidazole infusion. Tobramycin and imipenem did not release endotoxin, however, after cefotaxime and ceftriaxone treatment, there were more endotoxin positive patients when compared to imipenem ($P < 0.05$). Endotoxin release after vancomycin and ciprofloxacin was intermediate. The chance to detect positive endotoxin was best when blood samples were obtained 180 or 240 min after antibiotic administration. In some instances, however, endotoxin was detectable already 60 min after the start of infusion. These in vivo results have confirmed the results of several in vitro experiments. Induction of LPS in *Pseudomonas aeruginosa* cultures suggested ceftazidime (PBP-3 specific) induced filamentation released larger quantities of bioreactive endotoxin than non-filamentous fast-lyzing imipenem.[1] Total endotoxin levels increased after single treatment with cefuroxime or aztreonam, whereas ceftazidime, tobramycin or a combination of tobramycin with cefuroxime released less endotoxin. The increase in free endotoxin was higher than in total endotoxin.[3] In whole blood assays (ex vivo), endotoxin was higher when cells were treated by ceftazidime, ciprofloxacin than by imipenem or gentamicin.[13] Crosby et al.[14] reported that cefotaxime, ciprofloxacin and piperacillin caused significant endotoxin release in in vitro cultures of *Enterobacter cloacae* and *E. coli*. Little endotoxin was released when bacteria were exposed to tobramycin. The activity of aminoglycosides to release less endotoxin or to neutralize the toxin was reported in several studies.[3,15–18]

The reports on quinolones indicate that there may be limited endotoxin release,[18] intermediate release[19] or high endotoxin release.[13,14,20] In our study, endotoxin release after ciprofloxacin infusion was less often positive than after PBP-3 specific antibiotics. Despite this decrease, the IL-6 release was upregulated after ciprofloxacin when compared to PBP-3 specific antibiotics. There was no correlation of endotoxin release with Gram-negative organisms or any specific organism. The majority of infections were polymicrobial.

The changes observed in plasma endotoxin and IL-6 could not be related to outcome of the patients due to the relatively small patient population in this preliminary pilot study. The detection level of endotoxin in this assay was 0.5 pg/ml. Although the levels of endotoxin were between 0.5–18 pg/ml, the majority of these results were between 1–3 pg/ml. We know from studies in aortic aneurysm repair that small amounts of endotoxin are sufficient to trigger a proinflammatory and antiinflammatory response. To highlight this effect of endotoxin, we have focussed the analysis of endotoxin on the determination of endotoxin positive versus endotoxin negative results. It should be kept in mind that, even in endotoxin negative results, endotoxin might have triggered the inflammatory response somewhere before our study phase.

In in vitro studies it is generally agreed that ceftriaxone and cefotaxime support the release of endotoxin.[1,3,13–15,18,19,21] However, most of these studies were performed in vitro. Prins et al. measured endotoxin release in urosepsis after 4, 24, 48 and 72 h antibiotic administration.[4] Hurley et al. had indicated, already in 1991, that chronically bacteriuric patients can be considered as a model to study endotoxin release after antibiotic administration.[22] Endotoxin increased 2 h after antibiotic administration, which was also confirmed by Dofferhoff et al.,[3] who measured increased free endotoxin and increased IL-6 levels 2 h after antibiotic treatment. This was somewhat different from our results. With the exception of ciprofloxacin, all IL-6 levels decreased, with imipenem showing the most dramatic and fastest IL-6 reduction. Due to the relatively small number of patients we could not observe a correlation between IL-6, endotoxin and outcome.

Endotoxin is known to activate IL-6 secretion, which is a good marker of acute phase response. Callery et al. have demonstrated that endotoxin stimulated the IL-6 production by human Kupffer cells.[27] IL-6 determination in plasma was more consistent and reliable than TNFα (unpublished observations). Increased IL-6 production could also be attributed to an effect of antibiotics on macrophage metabolism. It is known that antibiotics can suppress cytokine production (IL-1, TNFα).[28] However, it was also stated that antibiotics could induce proliferation or affect the degranulation of neutrophils.[29,30] The different effect on IL-6 release could also be due to a another direct effect of antibiotics on macrophages. Subinhibitory concentrations of antibiotics may have influenced differently phagocytosis or other macrophage functions (e.g. cytokine production).[31] These effects could, at least partially, explain the controversial endotoxin and IL-6 levels after ciprofloxacin treatment in this study. Another important mechanism to consider is a different signal transduction pathway for IL-1 and TNFα in macrophages.[32] Finally, the increased IL-6 production after ciprofloxacin may reflect a feedback mechanism. It was demonstrated by Denis, that TNFα activity was decreased by in vivo IL-6 treatment and enhanced by in vivo neutralization with anti-IL-6.[33] The inhibitory role of IL-6 in macrophage proliferation was substantiated by Riedy and

Stewart.[34] Nevertheless, the results of most in vitro experiments are controversial; the effects on different cytokines and of different cytokines could be divergent at the same time.[35]

Endotoxin neutralizing capacity is rarely measured, although there are several reports on neutralizing capacity of antibiotics (e.g. aminoglycosides or polymyxin B).[13,16,17,23] Most of the studies were performed in vitro or they used indirect measurement of endotoxin neutralizing capacity (e.g. TNFα release), to demonstrate the effect of antibiotics. Endotoxin neutralizing capacity is an index which indicates the capability of plasma to neutralize the known amounts of endotoxin added to the assay. The neutralization of endotoxin can have vital effects in patients with severe sepsis. Endotoxin can be metabolized or neutralized in patients with a normal immune system; however, in critical care patients this defense may be damaged. This may be detected by a decreasing index in the follow-up. In our study, baseline levels of endotoxin neutralizing capacity were comparable. However, 60 min after antibiotic treatment, there were differences between cefotaxime and imipenem. Although LAL is a sensitive method for detecting endotoxin, its value as a diagnostic test when applied to plasma remains critical. Plasma contains incomplete defined factors that interfere with the detection of endotoxin or that confound the reading of the assay.[25] However, in our system, the interference of the plasma factors is taken into account by introducing an internal standardization in each plasma sample.[26] Also quality controls assured us that the results are reproducible and comparable. In a consensus conference (New Jersey, USA, January 1996) it was agreed that despite of the limitations, the LAL assay is the only method to detect minute amounts of bioreactive endotoxin.

We conclude that the different mechanisms of penicillin binding proteins may have a clinically observable effect on endotoxin release. PBP-3 specific antibiotics resulted more often in endotoxin positive determinations than do PBP-2 specific antibiotics. However, also different mechanism than cell wall synthesis blocker, may be responsible for endotoxin release (e.g. quinolones). Antibiotics could have directly influenced macrophage metabolism. IL-6 levels, which were decreased after most of the antibiotics, were increased after quinolone treatment. Subinhibitory concentrations or metabolic effects of antibiotics could have triggered a differential cytokine release. To our knowledge, this is the first report on endotoxin release by antibiotics in surgical patients admitted to the intensive care unit. While endotoxin is metabolized and neutralized in patients with normal immune system, it may be harmful in patients where the immune system has been continuously challenged (double-hit). Timing, dosage, and combination of antibiotics or other compounds need to be tested in larger clinical trials. In this respect we have started already with a consecutive study.

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