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Brief Report

Antimicrobial-induced Endotoxaemia in Patients with Sepsis in the Field of Acute Pyelonephritis

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Abstract:

BACKGROUND: In vitro results have shown that antimicrobial agents may induce the Gram-negative bacteria to release endotoxins (LPS), which in turn, could trigger the secretion of cytokines from monocytes. AIMS: To compare the effect of cefuroxime, netilmicin or ciprofloxacin on serum levels of LPS and tumour necrosis factor-alpha (TNFα). METHODS: Seventy-four patients with acute pyelonephritis caused by Gram-negative bacteria and signs of sepsis were randomly assigned to receive one of three intravenous regimens of cefuroxime, netilmicin or ciprofloxacin. Blood samples were collected before therapy and at specified time intervals for 96 hours after the initiation of treatment for the determination of serum levels of LPS and of TNFα. RESULTS: Patients treated with cefuroxime presented an early peak of LPS and of TNFα in serum two hours after the initiation of treatment compared to the other study groups. After that time interval, concentrations of LPS and TNFα were similar in all the study groups. Fever accompanied by endotoxaemia was still detected for 48 hours after the start of therapy in 36, 37.5 and 36% of patients treated with cefuroxime, netilmicin and ciprofloxacin respectively. The corresponding figures for these agents at 72 hours were 28, 12.5 and 24%, respective and 12, 4.2 and 4% at 96 hours (P value not significant). CONCLUSIONS: With the exception of an early peak in the serum levels of LPS and TNFα in patients treated with cefuroxime, no significant difference could be detected amongst the study groups as far as their effect on serum levels of LPS and TNFα were concerned. This suggests that these three antimicrobial agents may be administered safely at the early stages of sepsis. (J Postgrad Med 2003;49:118-122)

Key Words: Cefuroxime, netilmicin, ciprofloxacin, endotoxins, sepsis, antibiotics

In-vitro results on antimicrobial-induced endotoxaemia have raised fears of a probable perpetuation of the systemic inflammatory response after the start of antimicrobial chemotherapy. Administration of antimicrobial agents leads to bacterial killing and release of endotoxins from the bacterial cell wall. Published data indicates that administration of beta-lactam anti-bacterial agents is associated with a higher release of endotoxins (LPS) from the bacterial cell wall as compared to that observed in association with the fluoroquinolones.[1-5] It is feared that this release of endotoxins may trigger mononuclear cells to secrete pro-inflammatory cytokines.[6] Although in-vitro results have been verified in experimental animals,[7] clinical studies in human subjects are scarce and have mainly concentrated on imipenem and

ceftazidime.[8-10]

The present study was undertaken to compare three different classes of antimicrobial agents for their ability to cause endotoxin release and to trigger the secretion of tumour necrosis factor alpha (TNFα). The agents selected were cefuroxime (as a representative for β-lactam antimicrobial agents), netilmicin, an aminoglycoside and ciprofloxacin, which belongs to the fluoroquinolone group. Such a study has been performed in experimental animals,[11] but to the best of our knowledge, this is the first such study undertaken in human beings.

Patients and Methods

Consecutive patients presenting with pyelonephritis and sepsis were enrolled prospectively in this study that was carried out between January 1998 and January 2001 after receiving an approval from the Ethics Committee of the General Hospital of Athens "G. Gennimatas". The subjects' epidemiological and clinical data are shown in Table 1. The diagnosis of pyelonephritis and sepsis was made on the basis of the following criteria:[11-13] a) case history compatible with acute pyelonephritis comprising at least two spikes of fever above 38°C and presence of lumbar tenderness on examination, b) pyuria defined as presence of more than 10 polymorphs per high-power field under a light microscope, c) positive urinary culture with the colony count exceeding 10^4 cfu/ml for a single Gram-negative bacterial species, and d) the presence of at least two signs of the systemic inflammatory response syndrome i.e. fever above 38°C, tachypnoea (more than 20 breaths/ minute), tachycardia (more than 90 beats per minute) and presence of leucocytosis or leucopenia.[14] Patients with history of taking any antimicrobial agent in the one-month period prior to presentation were excluded. All patients were subjected to a renal ultrasound examination. The findings of ultrasound examination are depicted in Table 1.

Patients were randomly assigned to receive an intravenous antimicrobial agent (cefuroxime 1.5g tid or netilmicin 150mg bid or ciprofloxacin 400mg bid for a total of seven days). Three blood samples were collected by venipuncture from a forearm vein at fifteen-minute intervals before the start of therapy. Mean values of LPS and of TNFα of these determinations were applied as indicators of steady state levels before therapy. Blood samples were then drawn at exactly 0.5, 1, 1.5, 2, 3, 8 and 24 hours after the administration of the first dose of each antimicrobial agent. Samples were then collected daily and at 24-hour intervals only if fever (axillary temperature above 37.5°C) persisted. The time period of blood sampling was selected in analogy to former studies in humans[8,9] and in accordance to microbiological data of the kinetics of LPS release by bacteria after addition of antimicrobial agents in the growth medium.[2]

One aliquot of 5ml of each sample was cultured into broth-containing tubes (Becton Dickinson, Cockeysville Md) and incubated for a total of seven days at 35°C. Another aliquot of 3ml was placed into a pyrogen-free tube (Oxoid Ltd, London, UK) and it was centrifuged for five minutes at 2,500g and 4°C. The serum samples were kept refrigerated at -70°C until being assayed. Identification of bacterial isolates from blood or urine was made by the API 20E system (bioMérieux, Paris, France).

Serum samples were processed for the determination of LPS and of TNFα as follows: Before being assayed for LPS, the serum sample was diluted 1:10 with pyrogen-free water (BioWhitaker, Walkersville, Maryland, USA) and incubated for five minutes at 70°C in a shaking water bath. LPS level was then measured by the LAL QCL-1000 colorimetric assay (BioWhitaker, Walkersville, Maryland, USA, lower detection limit 0.1 EU/ml). False-positive results were excluded by the using serum samples obtained from healthy volunteers. The inter-day coefficient of variation of the assay was 10.2%. Concentrations of TNFα were determined by an enzyme-immunoassay (Amersham, London, UK, lower detection limit 10pg/ml). All determinations were performed in duplicate. The difference between the levels were analysed by one-way analysis of variance (ANOVA).[15] Values of *P* below or equal to 0.05 were considered as significant. To avoid random significances, a Bonferroni correction was applied.

Endotoxaemia was defined as any concentration of LPS in serum above 0.1 EU/ml.[16] Comparisons for the presence of endotoxemia among patients with fever persisting for 48 hours post-therapy initiation were performed by Fischer's exact test ($P<0.05$).

Results

Bacteremia was detected pre-therapy in five, one and six patients in the cefuroxime, netilmicin and ciprofloxacin groups, respectively (Table 1). None of the patients had bacteremia after the institution of therapy, irrespective of the antimicrobial agent used. The median concentrations of LPS before the start of

therapy were 0.91, 0.64 and 1.33 EU/ml, respectively. The differences in these levels were not statistically significant. Signs of multiple organ failure with respiratory distress and intra-vascular coagulation were present in only one patient treated in the cefuroxime group, who was admitted in the ICU.

The concentrations of LPS and of TNFα of each study group over time after the initiation of treatment are shown in Table 2. The levels of these substances in 2-hour sample were significantly higher in the cefuroxime group than those in the netilmicin and ciprofloxacin groups. For all other timed-samples, there was no significant difference amongst the three groups.

Fever persisted for 48 hours after the start of therapy with cefutoxime, netilmicin and ciprofloxacin in 12, 10 and 11 patients, respectively. Endotoxaemia was detected in nine patients in each group. The difference amongst the three groups on this count was not statistically significant. All the patients who were detected to have endotoxemia presented with signs of sepsis. In contrast, patients who persisted to have fever for at least 48 hours after the initiation of therapy did not demonstrate any signs of sepsis. Table 3 depicts the correlation between persistence of fever and the rate of endotoxaemia.

Discussion

In-vitro results on the release of LPS by antimicrobial agents acting on Gram-negative bacteria[3-5] raise a lot of dilemma whether antimicrobial chemotherapy might amplify the systemic inflammatory response. To clarify that hypothesis, cefuroxime, netilmicin and ciprofloxacin were administered intravenously to patients who presented with acute pyelonephritis caused by Gram-negative bacteria and signs of sepsis. The patients were closely monitored clinically and readings of LPS and TNFα serum levels were obtained at pre-determined intervals. Although all patients enrolled presented with sepsis, not all of them were detected to have bacteraemia. Other authors have demonstrated similar findings.[17] It should also be noted that previously reported clinical trials on the comparative effect of imipenem and ceftazidime on serum LPS of patients with urosepsis did not comprise patients with bacteraemia alone.[8]

Results revealed that concentrations of LPS in serum did not differ significantly among the three study groups over the first 24 hours after start of treatment with the exception of the two-hour sample. This sample was characterized by a peak of serum LPS and TNFα of patients treated with cefuroxime compared to patients receiving netilmicin or ciprofloxacin. A similar peak of LPS in serum was reported two hours after the administration of ceftazidime, which is an inhibitor of PBP-3 like cefuroxime.[9] Attenuation of the release of TNFα and of interleukin-6 (IL-6) has been shown in mice pre-treated with ciprofloxacin before challenge with LPS,[18] but similar results were not found in the present study. Irrespective of changes in the levels of LPS and TNFα throughout the study, none of the patients showed signs of sepsis after 48 hours of therapy and none of the patients had detectable bacteremia, demonstrating the success of the antimicrobial therapy.

No direct evidence was found that persistence of fever after 48 hours of therapy might be attributed to antimicrobial-induced release of LPS since the frequency of endotoxaemia was similar in patients at 48, 72 or 92 hours after the start of treatment, irrespective of the agent used.

Three studies have attempted to investigate the effect of antimicrobial agents on the in-vivo release of LPS in patients with infections caused by Gram-negative bacteria. Maury et al enrolled 18 patients with various infections. These patients were treated with a variety of β-lactam agents and LPS levels in the serum were determined prior to and at one- and four- hours after the start of therapy.[19] The other two studies investigated the comparative effect of imipenem and ceftazidime on 68 patients with melioidosis[9] and on 33 patients with urosepsis.[8] These patients were followed up for a short period of six- to eight- hours after the initiation of therapy.

On the basis of findings obtained in this study, the authors agree with others that the pathogenesis of sepsis is a very complicated process,[20] so that it could not be expected to obey to the simplistic rules of the in-vitro conditions where single bacterial cells are exposed to a certain concentration of an anti-microbial agent in the close proximity of a mononuclear cell culture.

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Expert's Comments - Antimicrobial-induced endotoxemia in patients with sepsis in the field of acute pyelonephritis

Endotoxin or Lipopolysaccharide (LPS), a major player in Gram-negative sepsis, is released from disintegrating cell walls of gram-negative pathogens. The causal relationship between endotoxin and sepsis has been successfully demonstrated in cell and animal experiments. However in patients admitted in the intensive care units sepsis is a result of an interaction amongst multiple factors. Urosepsis may be an ideal disease to study antibiotic-induced endotoxin release as only Gram-negative pathogens are responsible for most of the cases.[1] Antibiotic-induced endotoxin release, a basic research topic for the past 20 years, gained the attraction of clinicians when in-vitro experiments showed that antibiotics differ in their potential to release endotoxin.[2-4] In surgical intensive care patients, Penicillin-binding protein (PBP) 2-specific imipenem was associated with endotoxin negative tests whereas PBP 3-specific Beta-lactam antibiotics induced endotoxin release.[5] In patients with acute pyelonephritis, increased endotoxin levels correlated with plasma levels of cefuroxime.[6] In a randomised follow-up study, endotoxin levels were significantly increased two hours after infusion of cefuroxime as compared to those after the administration of aminoglycoside or quinolone antibiotics. However at later time-points, endotoxin levels did not differ and body temperature in these patients showed no correlation when checked two to four days later.[7] The

presence of Endotoxin may be determined directly or indirectly by the Limulus-Amebocyt-Lysate (LAL) test, the Endotoxin Core Antibody (EndoCAb) test or by detection of TNF-alpha release from LPS-activated monocytes; the LAL test may reflect the bioactivity best.[8-10] Contamination of the sample, variable reliability, possibility of false-negative and false-positive test results have been major concerns in the context of endotoxin assays.[11] This may be caused by activation or deactivation of the LAL-reaction by ingredients of the sample, e.g., plasma proteins, drugs, and candida. Most commercially available tests lack an internal standard in each individual sample. This would have made excluding some, though not all, of the interference induced by the factors referred to above.[12] Heparin or EDTA may influence endotoxin and TNF-alpha assays[13] and there are major inter-assay differences in commercial TNF-alpha ELISA kits, which make the interpretation of test results difficult.[14] These difficulties may drive us to focus more again on the classic clinical parameters, e.g., temperature, blood-pressure, heart rate which may indicate endotoxin release shortly after exposure to the antibiotic, together with LPS related mediators, e.g., CD 14, BPI protein. The individual host response seems to be decisive in overcoming sepsis. The release of bioactive cell membrane compounds of other pathogens and fungi may influence test system as well. Patients with a severe form of sepsis (APACHE II score above 15) may not possess enough capability to neutralize endotoxin and to balance the inflammatory response triggered by endotoxin. More patients older than 65 years, treated in intensive care units with antibiotics, could benefit from new evidence about how antibiotics may influence the immune balance.

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