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THE RELEASE OF ENDOTOXIN AND INTERLEUKIN-6 DURING TREATMENT WITH DIFFERENT ANTIBIOTICS ALONE OR IN COMBINATION WITH TAUROLIDINE OF RATS WITH EXPERIMENTAL GRAM-NEGATIVE SEPSIS

A.S.M. DOFFERHOFF[1*], H.L. BARTELS[2], H.G. DE VRIES-HOSPERS[3], J.H. NIJLAND[1], J. BIJZET[1], V.J.J. BOM[1], P.O.M. MULDER[1], J. WEITS[1], D. VAN DER WAAIJ[3], and R.P. BLEICHRODT[4]

[1]Department of Internal Medicine, [2]Central Animal Laboratory, [3]Laboratory of Medical Microbiology, and [4]Department of General Surgery, University Hospital Groningen, Groningen, The Netherlands.

SUMMARY

To evaluate the role of different antibiotics in the release of endotoxin and the production of interleukin 6 (IL-6) during the treatment of experimental *Escherichia coli* septical peritonitis, we obtained serial blood samples from septic rats treated with placebo, ceftazidime, aztreonam or imipenem. We also studied the effect of taurolidine, given alone or in combination with aztreonam, on the release of endotoxin and IL-6. In rats treated with placebo or taurolidine, we demonstrated a correlation between viable *E. coli* counts and the levels of free endotoxin and IL-6. Despite decreasing levels of viable *E. coli* counts after treatment with ceftazidime, aztreonam or imipenem, levels of free endotoxin increased in all animals. We did not notice any significant differences in the extent of endotoxin release between the different treatment groups. However, we did find significant differences in the IL-6 production between the different treatment groups. After two hours of treatment, IL-6 levels had increased in all animals with the highest levels in the imipenem treated animals, whereafter IL-6 levels decreased again in the rats treated with imipenem or ceftazidime. IL-6 levels further increased in the rats treated with placebo or aztreonam. The increase in IL-6 levels was associated with poor outcome. The increase in IL-6 levels in the aztreonam treated animals is thought to be the result of the formation of long bacterial filaments in the abdominal cavity. In the present study, treatment with taurolidine could not prevent or inhibit the release of endotoxin or IL-6. Unexpectedly, treatment with taurolidine alone or in combination with aztreonam caused a dramatic increase in IL-6 levels, which was associated with an increased mortality. We conclude that antibiotics can cause the release of endotoxin in spite of decreasing levels of bacteraemia *in vivo*. We have demonstrated an antibiotic type-dependent increase in plasma IL-6 levels, and we found an association between the level of IL-6 and mortality.

*Present address: Department of Internal Medicine, Canisius-Wilhelmina Hospital, Nijmegen, The Netherlands.

INTRODUCTION

The mortality rate due to severe Gram-negative sepsis and in particular that associated with shock, is still up to 50% in spite of appropriate antimicrobial therapy and optimum supportive care (*Kreger* et al., 1980). From the beginning of the antibiotic era it has been suggested that in some circumstances shock might be precipitated by bacterial cell lysis and the sudden release of endotoxin (LPS) from Gram-negative bacteria exposed to antibiotics (*Galpine*, 1949; *Spink* et al., 1948; *Hopkin,* 1977; 1978; 1985). These findings are supported by *in vitro* studies that have shown an enhanced endotoxin release from Gram-negative bacteria after treatment with antibiotics (*Cohen* and *McConnell*, 1985; 1986; *McConnel* and *Cohen*, 1986; *Dofferhoff* et al., 1991a). In addition, animal studies have shown that antibiotic therapy for experimental Gram-negative sepsis and/or meningitis can promote endotoxin release *in vivo* (*Shenep* et al., 1985; *Tauber* et al., 1987; *Rokke* et al., 1988; *Andersen* and *Solberg*, 1984). Recently, also antibiotic-induced endotoxin liberation during the treatment of human septicaemia has been demonstrated (*Shenep* et al., 1988; *Dofferhoff* et al., 1991a). Endotoxin toxicity is considered to be largely mediated by the monocyte/macrophage derived tumour necrosis factor-α (TNF) (*Michie* et al., 1988; *Waage* et al., 1989; *Cannon* et al., 1990; *Michalek* et al., 1980; *Tracey* and *Lowry*, 1990). It is also known that endotoxin can further promote the lethal effects of TNF suggesting that both factors determine the clinical outcome (*Rothstein* and *Schreiber*, 1988). It has also been suggested that IL-6, either by itself or by interactions with TNF, interleukin-1 (IL-1) or LPS, is involved in the pathogenesis of septic shock (*Waage* et al., 1989; *Fong* et al., 1989; *Jirik* et al., 1989; *Tracey* and *Lowry*, 1990).

In a previous study (*Dofferhoff* et al., 1991a), we have demonstrated the release of endotoxin from *Escherichia coli* by several newer (β-lactam) antibiotics nowadays frequently used in the management of patients with septic shock and found an antibiotic type- and dose dependent increase in the release of endotoxin from an *in vitro* culture of *E. coli.* In a subsequent study, we have analysed the influence of antibiotic-induced release of endotoxin from *in vitro* cultured *E. coli* on TNF production by human monocytes and also found an antibiotic type- and dose dependent increase in TNF production by monocytes as the result of antibiotic-induced release of endotoxin (*Dofferhoff* et al., 1991b). To study the release of endotoxin and the cytokine IL-6 *in vivo*, we analysed the release of these mediators in rats treated for an experimental Gram-negative sepsis with different antibiotics. Also the influence of treatment with taurolidine, an endotoxin-binding agent, alone or together with antibiotic treatment, was evaluated in the present study.

MATERIALS AND METHODS

Organisms

For the endotoxin release studies *E. coli* strain ATCC 25922 was used. Organisms were stored at -70˚C and prior to use subcultured onto blood agar. Subsequently one colony was inoculated into 9 ml Brain Heart Infusion (BHI) broth (Difco laboratories) and incubated at 37˚C overnight. This overnight culture was centrifuged at 5,000

RPM for 15 minutes. The supernatant was discarded and the bacteria were resuspended in 9 ml fresh BHI broth and incubated at 37°C for another two hours. This suspension was centrifuged at 5,000 RPM for 15 min., the supernatant was discarded and the bacteria were resuspended into 8 ml sterile NaCl 0.9%. The MIC's for this *E. coli* strain were as follows: ceftazidime, 0.25 mg/l; aztreonam, 0.125 mg/l; imipenem, 0.0675 - 0.125 mg/l.

Antibiotics

Antibiotics used were ceftazidime (Glaxo BV, Nieuwegein, The Netherlands), aztreonam (Squibb BV, Rijswijk, The Netherlands), and imipenem/cilastatin (Merck, Sharp & Dome BV, Haarlem, The Netherlands). Also taurolidine 2% (Multipharma BV, Weesp, The Netherlands), an antibacterial agent with anti-endotoxin properties was used. This solution was made isotonic through the addition of 5 ml of a 50% glucose solution.

Sepsis model and experimental protocol

Male Wistar rats (200 - 300 g), locally bred at the Central Animal Laboratory of the University of Groningen, were used throughout the study. During the experiments, the animals were allowed to drink and eat *ad libitum* and were housed at 21°C in labelled cages. Prior to the start of the experiment, the bacterial suspension was divided into four equal aliquots. The challenge dose for the different pairs of rats ranged from 2 to 5 x 10^9 CFU of *E. coli.* One aliquot (2 ml) was administered intraperitoneally (i.p.) to each of the paired rats. Two hours after the bacterial challenge four rats were randomly assigned to receive either 1 ml sterile NaCl 0.9% i.v., 200 mg/kg ceftazidime or aztreonam in NaCl 0.9% (1 ml infusion solution) i.v., or 50 mg/kg imipenem in NaCl 0.9% (1 ml) intra-veneously (i.v.). It was not possible to administer imipenem in a dose of 200 mg/kg because precipitation occurred above a concentration of 10 mg/ml. These experiments were repeated four times and were performed on five different days. In the experiments with taurolidine, 1 ml of isotonic 2% solution of taurolidine was administered i.v. as a single agent or together with aztreonam. The experiments with taurolidine were performed in quadruplicate. The antibiotics were given as a bolus i.v. injection, while taurolidine was administered i.v. hourly. For the administration of the drugs the tail vein or the penile vein was used. It was verified that the plasma concentrations of the antibiotics two hours after the bolus injection were >10 mg/l (data not shown). Heart blood samples were taken aseptically from anaesthetised animals just before the administration of the antibiotics (t = 0) and 2, 4, and 6 hours after the administration of the drugs. From each of the rats, 1 ml of blood was transferred into pyrogen free Falcon tubes (Becton Dickinson, New Jersey, USA) containing 50 units of heparin (Leo Pharmaceutical Products BV, Weesp, The Netherlands). These tubes were immediately immersed in melting ice. Plasma was prepared at 4°C by centrifugation at 2,000 RPM for 10 min. Plasma samples of 0.1 ml were removed for the IL-6 assay. For the endotoxin assay, 0.1 ml plasma was transferred to pyrogen free Falcon tubes containing 0.9 ml pyrogen free NaCl 0.9% and thoroughly mixed. Free endotoxin was separated from bacterial cell-bound endotoxin by filtration of an aliquot of each sample through a 0.45 μm pyrogen free filter (Millex HA, Millipore SA, Molsheim, France). All samples were stored at -80°C. At the end of the experiment (the time of death or 6 hours after the administration of the antibiotics) the abdomi-

nal cavity was opened and fluid was aspirated for microscopical examination. During the administration of the drugs and during the heart punctures, the animals were anaesthetised with halothane.

Viable counts

Twenty-five μl aliquots of the whole blood or the dilutions were plated onto blood agar in duplicate and colony counts were done after overnight incubation. The minimal detectable number of CFU/ml was 40.

Endotoxin assay

Endotoxin was measured using the chromogenic limulus amoebocyte lysate (LAL) assay (KabiVitrum BV, Amsterdam, The Netherlands) according to the prescriptions of the manufacturer and adapted to a microtiter scale with minor modifications. In brief, 25 μl aliquots of the test sample were added in duplicate to 25 μl LAL in a pyrogen free microtiter tray. After incubating at 37°C for 10 min., 50 μl of a prewarmed chromogenic substrate (S 2423)-buffer solution was added. After incubating at 37°C for 4 min., colour development was terminated by addition of 100 μl 20% acetic acid. The optical density was measured at 405 nm and the endotoxin level calculated from a calibration curve using *E. coli* 0111:B4 as the standard (12 EU/ml = 1 ng/ml). Appropriate standards and controls were included within each assay. The between-assay coefficient of variation was 4.7%. Detection level was 0.05 EU/ml.

IL-6 assay

The biologic activity of IL-6 was determined using an IL-6 dependent cell line B9 as previously described (*Helle* et al., 1988) (control values < 5 U/ml).

Statistical analysis

Differences between variables were tested with the Wilcoxon signed rank test or the Mann-Whitney U test. Differences between treatment groups were tested by the Kruskal-Wallis analysis by ranks. Correlations were analysed with the Spearman rank correlation test. A p-value < 0.05 (two-tailed) was considered statistically significant. Viable counts, endotoxin levels and cytokine levels are expressed as median values.

RESULTS

Viable counts

The viable counts of control and antibiotic treated rats of E. *coli* ATCC 25299 at different time intervals are shown in Figure 1. Of the 28 rats tested, 24 rats had a positive blood culture with *E. coli* two hours after the i.p. administration of the bacteria (median level for the whole group: 3×10^3 cfu/ml, range: $0 - 5 \times 10^4$ cfu/ml). In control rats there was during the following 6 hour period (or until the time of death) a significant increase in viable counts from 4×10^3 cfu/ml (range: $0 - 14 \times 10^3$) to 2×10^5 (range: $0.4 - 12 \times 10^5$) cfu/ml. Two hours after the administration of the different antibiotics the median levels of viable organisms were significantly decreased ($p < 0.05$), except for taurolidine monotherapy. In the animals treated with ceftazidime, viable counts slightly increased after two hours of treatment from 20 cfu/ml (range: 0 - 280) to 160 cfu/ml (range: 0 - 700). In the animals treated with aztreonam alone or in combination with taurolidine, viable counts decreased persistently until the animals died (2 animals

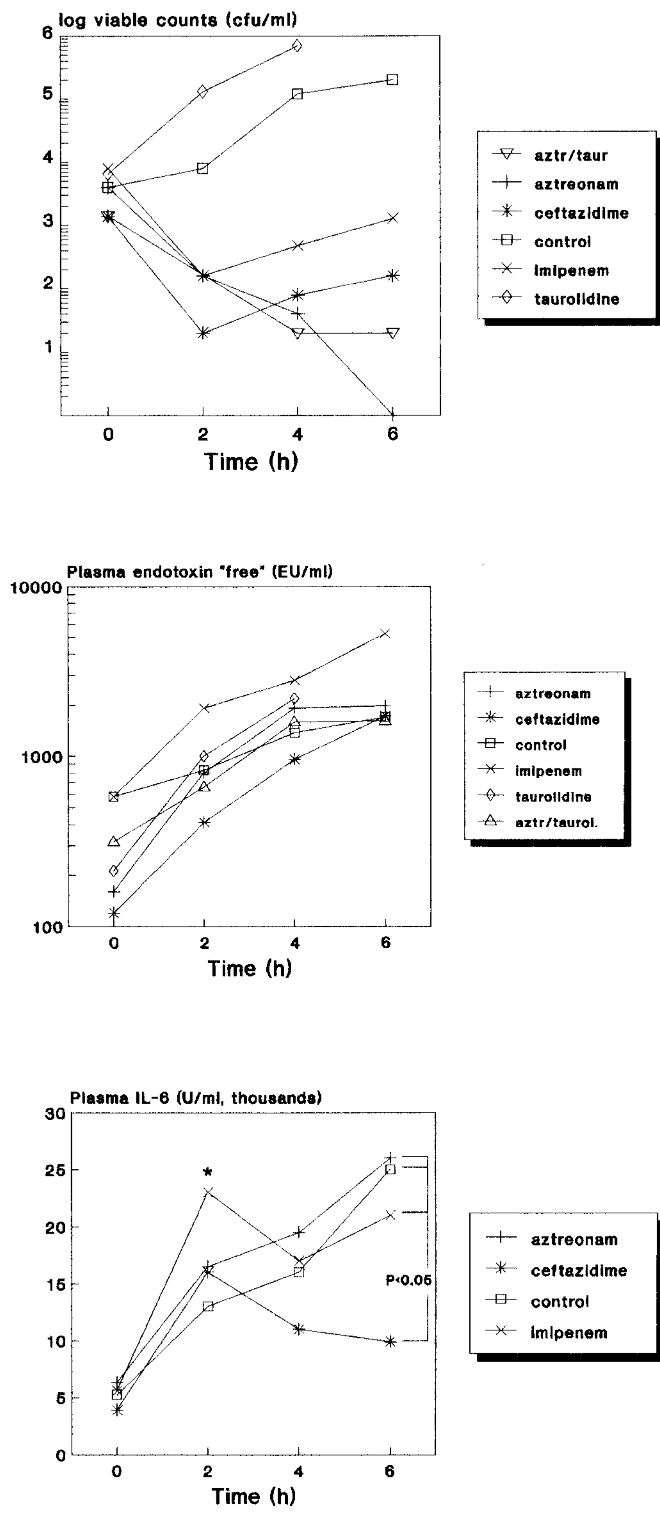

Figure 1: Median levels of viable counts (top), free endotoxin (middle) and plasma IL-6 (bottom) during the treatment of experimental Gram-negative sepsis ($^{*}p < 0.05$).

in the aztreonam group and 3 animals in the aztreonam/taurolidine group) or until the end of the study period. Median levels at the end of the study period were 0 cfu/ml (range: 0 - 40) and 20 cfu/ml (range: 0 - 40), respectively. In the imipenem treated animals, viable counts started to increase again after two hours of treatment, probably as the result of the lower initial dose and the short half-life of this drug in animals. Taurolidine monotherapy resulted in a steady and significant increase in viable counts up to 7 x 10^5 cfu/ml (range: 0 - 1 x 10^6) (Figure 1) at 4 hours of treatment. Six hours after the first injection of taurolidine only one animal was still alive. After 2 and 4 hours of treatment viable counts were significantly higher in the taurolidine treated animals than in the control rats ($p < 0.05$).

Plasma levels of free endotoxin during antibiotic treatment

Two hours after the i.p. injection of *E. coli* plasma levels of free endotoxin were detectable in all rats (median level free endotoxin: 223 EU/ml, range: 0.2 - 1,710 EU/ml) (Figure 1). Plasma levels of free endotoxin increased during the 6 hours following the administration of placebo or antibiotics in all but one animal. The maximal increases in the levels of free endotoxin in the control rats varied from four-fold to 15-fold. Despite the decreases in viable counts, in all but one rat treated with either ceftazidime, aztreonam or imipenem, plasma levels of free endotoxin increased significantly during the treatment period ($p < 0.05$) (Figure 1). During this period, there were no statistically significant differences in the levels of free endotoxin between the different treatment groups (Figure 1). In the rats treated with antibiotics, maximal increases in the levels of free endotoxin varied from two-fold to 25-fold. In one rat treated with aztreonam blood cultures were sterile during the entire study period. In this rat plasma levels of free endotoxin decreased from 8.5 EU/ml on admission to 3 EU/ml 6 hours after the administration of the antibiotic.

In the rats treated with taurolidine alone, plasma levels of free endotoxin also significantly increased during the treatment period from a median level of 212 EU/ml (range: 0.2 - 276 EU/ml) to 2175 EU/ml (range: 1 - 3,600 EU/ml). During the study period the levels of free endotoxin in the taurolidine treated rats did not significantly differ from the levels of free endotoxin in the control rats. In the rats treated with a combination of aztreonam and taurolidine, plasma levels of free endotoxin were not different from those in rats treated with taurolidine or aztreonam alone (Figure 1). In none of the different groups we found an association between the levels of free or total endotoxin and survival.

Levels of interleukin-6 during antibiotic treatment

Two hours after the bacterial challenge, plasma levels of IL-6 were increased in all animals, median level 4850 U/ml (range: 300 - 12,000 U/ml). In control animals, IL-6 levels significantly ($p < 0.05$) increased during the study period from 5,250 U/ml (range: 3,000 - 6,600 U/ml) to 25,000 U/ml (range: 2,600 - 38,000 U/ml) (Figure 1). In both control animals and antibiotic treated animals, after two hours of treatment there was an increase in median IL-6 levels (Figure 1). This initial rise in IL-6 levels between 0 and 2 hours after the administration of the drugs, was significantly higher in the imipenem treated group than the control rats ($p < 0.05$). After 2 hours of treatment with ceftazidime, median plasma levels of IL-6 decreased from 16,000 U/ml (range: 7,600 - 25,000 U/ml) at two hours to 11,000 U/ml (range:

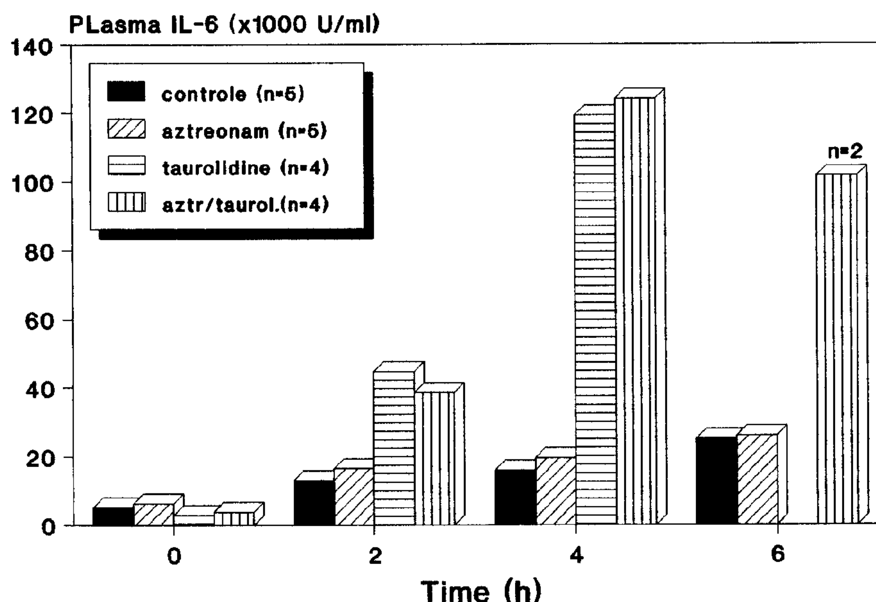

Figure 2: Median plasma levels of IL-6 during the treatment of septic rats with placebo and aztreonam, and the influence of the administration of taurolidine.

7,800 - 15,000 U/ml) at 6 hours of treatment (Figure 1). In the imipenem treated animals also a decrease was seen between 2 and 4 hours of treatment, however between 4 and 6 hours of treatment with imipenem IL-6 levels started to rise again from 17,000 U/ml (range: 9,700 - 31,000 U/ml) at 4 hours to 23,000 U/ml (range: 11,000 - 25,000 U/ml) at 6 hours (Figure 1). Treatment with aztreonam resulted in a significant increase in median plasma levels of IL-6 from 6300 U/ml (range: 5,100 - 7,100 U/ml) at the start of the treatment to 26,000 U/ml (range: 15,000 - 29,000 U/ml) at the time of death or at 6 hours of treatment (Figure 1). At the end of the study period median IL-6 plasma levels were significantly higher ($p < 0.05$) in the control animals and the aztreonam treated animals than the ceftazidime treated animals.

In the animals treated with taurolidine, alone or in combination with aztreonam, we noticed a dramatic increase in the plasma IL-6 levels from 2,970 (range: 300 - 5,260 U/ml) to 119,200 U/ml (range: 105,000 - 200,000 U/ml) in the taurolidine group and from 3,850 U/ml (range: 2,600 - 7,200 U/ml) to 101,550 U/ml (range 3,100 - 200,000 U/ml) in the rats treated with a combination of aztreonam and taurolidine (Figure 2). The increases in plasma IL-6 levels in the taurolidine group and the aztreonam/taurolidine group were significantly higher than in the control group and the aztreonam group, respectively ($p < 0.05$) (Figure 2).

The kinetics of IL-6 production was associated with survival. In the whole study group IL-6 levels increased from t=0 to 2 hours of treatment whereafter in the surviving animals IL-6 levels stabilised, while in the non-surviving animals IL-6 levels increased up to the time of death (Figure 3).

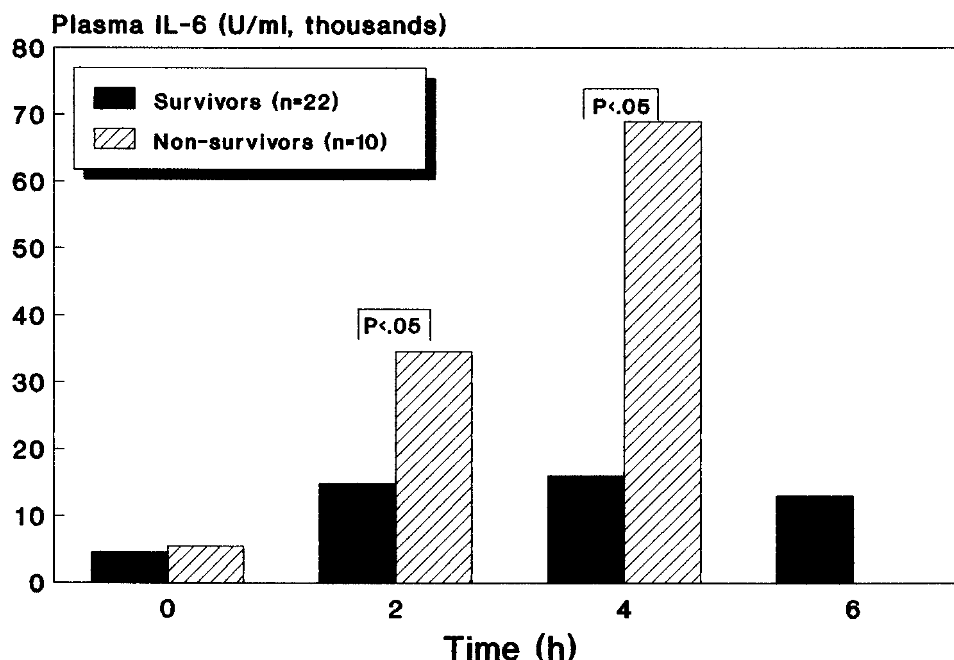

Figure 3: Plasma IL-6 levels in septic rats, surviving versus non-surviving animals.

Correlations between viable counts, plasma levels of free endotoxin and plasma IL-6 levels in septic rats

At the start of the treatment, we found a strong correlation between viable counts and the levels of free endotoxin ($r=0.82$, $p < 0.001$). There was also a correlation between the plasma levels of IL-6 and viable counts ($r=0.51$, $p=0.01$), and between levels of IL-6 and levels of free endotoxin ($r=0.53$, $p < 0.01$). During the 6 hours following the administration of saline in the control animals, we found a correlation between viable counts and the levels of free endotoxin ($r=0.88$, $p < 0.001$) and between levels of IL-6 and free endotoxin ($r=0.87$, $p < 0.001$). In the animals treated with taurolidine alone, similar correlations were found. In the animals treated with ceftazidime or imipenem only a weak correlation was found between IL-6 levels and the levels of free endotoxin, while in the rats treated with aztreonam such a relation was not found.

Mortality

Of the 28 animals tested, 10 died before the end of the study period (= 6 hours after the administration of the antibiotics or the saline). Three out of the five animals treated with saline died at 5 hours of treatment. Two of the five animals treated with aztreonam died at 4 and $5^1/_4$ hours of treatment, respectively. Treatment with taurolidine or the combination taurolidine with aztreonam resulted in death of 3 and 2 animals, respectively, at approximately 4 hours of treatment. In the animals treated with either ceftazidime or imipenem, no mortality occurred.

Morphological studies

In the animals treated with saline normal Gram-negative bacteria were

seen in the abdominal fluid, while after treatment with ceftazidime only cellular debris was seen. After treatment with imipenem we noticed cellular debris together with normal looking Gram-negative bacteria. Treatment with aztreonam resulted in the formation of long filaments of non-septating bacteria.

DISCUSSION

In the present study we demonstrate the release of free endotoxin during the treatment with both placebo or different antibiotics of rats with experimental *E. coli* septical peritonitis. In the control rats, the levels of free endotoxin remained more or less proportional to the level of blood viable counts as observed in previous studies (*Shenep* et al., 1985; *Dofferhoff* et al., 1991a). Median levels of free endotoxin increased concordantly after the administration of the different antibiotics in spite of decreasing levels of bacteraemia. Although in the imipenem treated animals levels of endotoxin tended to be highest, we found no significant differences in the extent of endotoxin release between the different antibiotic treated groups, the taurolidine group or the control group. However, we did find significant differences in the IL-6 production between the different treatment groups. All animals had elevated IL-6 levels at the start of the therapy, and median IL-6 levels correlated with the level of viable counts and the level of endotoxin. In the control group and in the rats treated with taurolidine alone, the level of IL-6 remained proportional to the level of viable counts and the level of endotoxin.

In the antibiotic treated groups there was a rise in IL-6 levels from the start of the treatment to two hours after the start of the antibiotic treatment. At this time, imipenem had caused the greatest rise in IL-6. From this point IL-6 levels started to decrease again in the imipenem and ceftazidime treated rats, while in the rats treated with aztreonam IL-6 levels further increased. The increase in IL-6 levels in the imipenem group between 4 and 6 hours after the start of the therapy is probably the result of an increase in viable counts. The difference in IL-6 production between aztreonam treated rats and the rats treated with either ceftazidime or imipenem is unexplained, but may be related to the formation of bacterial filaments in the aztreonam treated animals. The antibiotics used in this study cause different modes of bacterial cell damage by binding to and inhibition of the different penicillin-binding proteins (PBP's). The three penicillin-binding proteins of *E. coli* that are essential for antibacterial activity have been assigned PBP-1, PBP-2, and PBP-3. It has been shown that inhibition of PBP-1 is associated with rapid killing and lysis, whereas inhibition of PBP-2 produces spherical non-growing cells and that of PBP-3 produces long filaments (*Neu*, 1985; *Tuomanenet* al., 1986).

In previous studies, we have demonstrated that treatment of *in vitro* cultured *E. coli* with ceftazidime (high dose) or imipenem resulted in the formation of non-growing spherical cells and rapid bacterial cell lysis, which resulted in a rapid increase in the levels of free endotoxin, while treatment with aztreonam (or cefuroxime) resulted in the formation of long filaments, which resulted in a more gradual but much higher increase in the levels of free and total endotoxin. The observed effects of cefuroxime and aztreonam and low dose ceftazidime are consistent with their reported binding to PBP-3, while the effects of ceftazidime (high dose) and

imipenem are consistent with binding to PBP-1 and PBP-2, respectively (*Neu*, 1985; *Hanberger* et al., 1990). In the present study, the higher initial rise in IL-6 levels in the imipenem group may be the result of rapid cell lysis within the abdominal cavity, while the rise in IL-6 levels between 4 and 6 hours of therapy probably results from the increase in viable counts due to low antibiotic concentrations. The persistent rise in IL-6 levels in the aztreonam treated groups may be the result of the formation of long filaments of non-septating bacteria within the abdominal cavity. These long filaments may have caused the prolonged activation of the cytokine cascade. In a previous study (*Dofferhoff* et al., 1991b) we have demonstrated that treatment with antibiotics that cause the formation of long filaments, like aztreonam or cefuroxime can cause higher levels of TNF from *E. coli* stimulated human monocytes than treatment with drugs that cause rapid cell lysis or the formation of non-growing spherical cells like ceftazidime or imipenem.

We have also studied the effects of the endotoxin-binding agent taurolidine in the management of Gram-negative sepsis. *Thomas* and colleagues (1985) demonstrated a dose dependent inactivation of endotoxins by taurolidine. They showed that the endotoxin inactivation by taurolidine was irreversible and that there was no interaction of taurolidine with the chromogenic limulus lysate assay they used. We have also studied the effects of taurolidine on the production of TNF by *E. coli* human monocytes and demonstrated a decrease in TNF production by these monocytes as the result of a nearly complete neutralisation of the endotoxin released upon treatment with aztreonam and imipenem and that this neutralisation lasts for at least 24 hours. In the present study we found no influence of the administration of taurolidine on the release of endotoxin but did find a dramatic increase in IL-6 levels upon treatment with taurolidine, alone or in combination with aztreonam, which was associated with an increased mortality.

From the beginning of the antibiotic era it has been suggested that antibiotics may cause the massive liberation of endotoxins in the bloodstream as the result of bacterial lysis and this may actually aggravate the endotoxin shock (*Galpine*, 1949; *Spink* et al., 1948; *Hopkin*, 1977; 1978; 1985). So far, the endotoxin liberating effect of penicillin (*Andersen* and *Solberg*, 1984), moxalactam (*Shenep* et al., 1985), cefotaxime (*Tauber* et al., 1987), ceftriaxon (*Mustafa* et al., 1989), cefuroxime, ceftazidime, aztreonam and imipenem (*Dofferhoff* et al., 1991a; 1991b), the aminoglycosides kanamycin (*Johnston* and *Greisman*, 1984), tobramycin (*Dofferhoff* et al., 1991a; 1991b) and gentamicin (*Shenep* et al., 1985; *Rokke* et al., 1988), several quinolones (*Cohen* and *McConnell*, 1986; 1985; *McConnel* and *Cohen*, 1986) and chloramphenicol (*Shenep* et al., 1985; *Tauber* et al., 1987; *Dofferhoff* et al., 1991a) has been studied *in vitro*. There are, at present, little data on the effect of antibiotic treatment on the release of mediators like TNF and/or IL-6 *in vivo*. In rabbits with experimental *Haemophilus influenzae* type b meningitis, treatment with ceftriaxon resulted in increased concentrations of endotoxin as well as TNF in the cerebrospinal fluid, which was associated with an enhanced meningeal inflammatory response (*Mustafa* et al., 1989a). *Mustafa* and colleagues (1989b) also demonstrated that in children with Gram-negative meningitis treated with a combination of ampicillin intravenously and gentamicin intraventricular, cerebro-spinal fluid concentrations of endotoxin, interleukin-1β were significantly higher than

in children treated with ampicillin i.v. alone. In these children high levels of IL-1β were associated with poor outcome.

In conclusion, we have demonstrated antibiotic-induced release of endotoxin from *E. coli* by the different antibiotics *in vivo*. We also found an antibiotic type dependent release of IL-6 during the treatment of experimental Gram-negative septic peritonitis, in which high levels of IL-6 were associated with mortality. These data suggest that the effects of antibiotic treatment on the release of endotoxin from Gram-negative bacteria and the subsequent increase in the release of other mediators like IL-6 (and probably TNF and IL-1 as well) may be of clinical importance. In this study, the addition of taurolidine could not prevent the release of endotoxin and/or IL-6, but rather enhanced IL-6 production.

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