Post-antibiotic and post-β-lactamase inhibitor effects of ceftazidime plus sulbactam on extended-spectrum β-lactamase-producing Gram-negative bacteria

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Objectives: To measure the in vitro post-antibiotic effect (PAE) and post-β-lactamase inhibitor effect (PLIE) of a ceftazidime–sulbactam combination on bacteria producing extended-spectrum β-lactamases (ESBLs).

Methods: PAE and PLIE were studied for ESBL-producing strains of Escherichia coli and Klebsiella pneumoniae. Two ATCC β-lactamase-negative strains of E. coli and K. pneumoniae were used as controls. The MICs of a ceftazidime–sulbactam combination were determined with a fixed concentration of sulbactam (8 mg/L). The organisms were exposed to the antibiotics at twice the MIC for 2 h before removal of the antibiotics by filtration of the culture. Bacteria on the filter were resuspended in drug-free medium to determine the PAE and in medium containing ceftazidime, at the same concentration as originally present, to determine the PLIE.

Results: The PAE of ceftazidime was similar for bacteria producing the same ESBL except for E. coli producing CTX-M-1. PLIE values varied according to the type of β-lactamase but similar results were observed for the strains producing the same ESBLs. PLIEs were longer than PAEs and were longer when the MICs of ceftazidime were lower.

Conclusions: To the best of our knowledge, we describe here for the first time an in vitro PLIE for a ceftazidime–sulbactam combination on different bacteria producing different ESBLs. These findings indicate that suicide inhibitors may be used in combination with third-generation cephalosporins.

Keywords: ESBLs, PAE, PLIE, ceftazidime–sulbactam, cephalosporins, suicide inhibitors

Introduction

Bacterial resistance is a growing phenomenon and causes therapeutic difficulties in everyday practice. The production of β-lactamases is still the main mechanism for resistance of Gram-negative bacilli to β-lactams. Recently, new varieties of β-lactamases have been described, notably TEMs which are resistant to suicide inhibitors (inhibitor-resistant TEMs or IRTs).1,2 However, other β-lactamases which have previously been described, such as extended-spectrum β-lactamases (ESBLs), have become progressively more widespread among many species of Gram-negative bacilli. It would thus seem necessary to find new therapeutic weapons against this growing threat.1 The aim of this work was therefore to assess the in vitro activity of a ceftazidime–sulbactam combination on bacteria producing ESBLs and also to measure post-antibiotic effect (PAE) and post-β-lactamase inhibitor effect (PLIE) of this combination. The PLIE was described for the first time in 1992 and published in 1996 by Thorburn et al.4 This persistent inhibition of growth of the bacteria is the result of a prolonged lag phase and represents the time before production of enough β-lactamase following inhibition by sulbactam. So far, the PLIE has only been proven for an in vitro combination of amoxicillin plus suicide inhibitor.4-6

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Materials and methods

Compounds

Ceftazidime pentahydrate was kindly supplied as laboratory reference material by GlaxoSmithKline (Durham, UK). Sulbactam sodium was kindly supplied as laboratory reference material by Pfizer Inc. (Groton, CT, USA).

Organisms

Nine strains of bacteria were used in this work. Two strains (one strain of Escherichia coli, one strain of Klebsiella pneumoniae) were ATCC strains that did not produce β-lactamases. Seven were ESBL-producing strains (four strains of E. coli of which one was wild and the other three transconjugants, and three wild strains of K. pneumoniae). The susceptibility of these organisms was determined using a standard method. The MICs of the ceftazidime–sulbactam combination were determined for a fixed sulbactam concentration of 8 mg/L.

Inocula

For all the strains, 40 µL bacterial culture incubated at 37°C for 18 h was diluted in Mueller–Hinton broth (Oxoid, Basingstoke, UK) to obtain a starting inoculum between 10⁵ and 10⁷ cfu/mL.

PAE and PLIE determinations

As previously described by Thorburn et al., the organisms were exposed to the antibiotics at twice the MIC in Mueller–Hinton broth for 2 h before removal of the antibiotics by filtering the culture through a 0.25 µm pore size nylon filter (Corning Costar, New York, USA). The untreated control culture was also filtered. Two washes with 10 mL of a pre-warmed, drug-free test medium followed filtration. The bacteria on the filter were resuspended in 20 mL of a pre-warmed drug-free medium to determine the PAE and 20 mL of pre-warmed test medium containing ceftazidime, at the same concentration as originally present, to determine the PLIE. All the cultures were incubated at 37°C in an environmental orbital shaker.

Viable bacterial counts

The number of live bacteria was determined by taking samples every hour during the 2 h of pre-exposure to antibiotics, immediately after filtering, up to 9 h after exposure, and finally at 24 h. Samples were diluted 10-fold serially in a sterile, drug-free Mueller–Hinton broth, and 100 µL drops of each were plated on nutrient agar. The number of cfu was determined after 24 h of incubation.

The PAE was calculated as T–C, where T was the time for the number of viable organisms in the test culture to increase by 1 log₁₀ above the number observed immediately after filtration, and C was the time for the number of viable organisms in the untreated control culture to increase by 1 log₁₀ above the number observed immediately after filtration.

The PLIE was calculated as T–C, where T was the time for the number of viable organisms in the test culture to increase by 1 log₁₀ above the number observed immediately after filtration, and C was the time for the number of viable organisms in the untreated control culture containing ceftazidime after filtration to increase by 1 log₁₀ above the number observed immediately after filtration.

The PAE and PLIE were assayed three times. Means and standard deviations (S.D.) for all the experimental data were calculated.

Results

MICs

The MICs of ceftazidime and the ceftazidime–sulbactam combination are shown in Table 1.

PAEs and PLIEs

PAE and PLIE values for each strain are shown in Table 1. Example curves are shown in Figure 1.

Similar PAE results were observed in different ESBL-producing strains of K. pneumoniae. Similar observations were made with the ESBL-producing strains of E. coli except for the CTX-M-1-producing strain of E. coli. The ATCC strains (K. pneumoniae ATCC 13883 and E. coli ATCC 25922) that were ESBL-negative were clearly different to the ESBL-producing strains (Table 1). The addition of sulbactam...
PLIE values varied according to the type of β-lactamase. Similar results were observed for the strains which produced the same ESBLs (Table 1). The PLIEs were longer than the PAEs and were longer when the MICs of ceftazidime were lower.

For the E. coli strain TrMEN-1 which produces CTX-M-1, the PAE (0.3 h) turned out to be much shorter and the PLIE (5 h) longer than for the other strains of E. coli. These results seemed logical owing to the lower ceftazidime MICs for this strain.

Discussion

Several authors have demonstrated in vitro the existence of a PLIE for a co-amoxiclav combination with different bacteria producing plasmidic or chromosomal β-lactamases. To the best of our knowledge, ours is the first study in which a PLIE for a ceftazidime–sulbactam combination has been clearly demonstrated on wild bacteria (K. pneumoniae, E. coli) and transconjugant bacteria (E. coli). These β-lactamases were chosen for their increasing prevalence among certain bacterial species (i.e. Enterobacter spp. or E. coli), which are often encountered in routine clinical practice, and thus responsible for the therapeutic failure of third-generation cephalosporins.

The PAEs of ceftazidime, with or without sulbactam, on strains that did not produce ESBLs, were non-existent or negative; this is in agreement with data already published. The PAEs on strains producing ESBLs proved to be homogeneous depending on which strain was being studied: 0.8–0.9 h for K. pneumoniae, 0.5–0.7 h for the E. coli except for the strain producing CTX-M-1. For this strain, the MICs of ceftazidime were the lowest (32 mg/L). The greater susceptibility of this strain would explain the lower PAE. In all cases, the PAEs were less than 1 h long, and this short time span is consistent with data in the literature.

The concentration of sulbactam used in this study was 8 mg/L, corresponding to the standard dose used for carrying out the antibiogram. For each strain, the concentration of ceftazidime corresponded to twice the MICs of the combination (as recommended by Thorburn et al.). Most of the concentrations of ceftazidime used were consistent with the serum concentrations measured in vivo during discontinued intravenous perfusions of 1 or 2 g every 8 h or as a continuous perfusion of 100 mg/kg per 24 h.

The PLIEs varied according to the type of β-lactamase. The ceftazidime plus sulbactam combination tested on K. pneumoniae CF1104 and E. coli CF1124 producing TEM-24 gave PLIEs of 0.7 and 0.8 h, respectively. For K. pneumoniae KP210–2 and E. coli CF1004 producing SHV-4, the PLIEs were 2.2 and 2.5 h, respectively. Similar results were obtained with TEM-3 (Table 1). The PLIE therefore seems more dependent on the product of the concentrations of the ceftazidime plus sulbactam combination than the concentration of sulbactam alone in the culture medium. We noted that the shortest PLIE was obtained with bacteria for which the MIC of ceftazidime was the highest: K. pneumoniae CF1104 and E. coli CF1004. This would seem to be in relation to the bacteria producing the most β-lactamases. These data are in agreement with findings by Thorburn et al. for K. pneumoniae and E. coli. This combination, the cephalosporin and suicide inhibitor combination has already been assessed in vitro and in vivo on bacteria producing ESBLs. The interest in using sulbactam as a suicide inhibitor lies in the fact that it is available independently from β-lactams. As early as 1985, Jones et al. had successfully tested in vitro a combination of a third-generation cephalosporin, cefoperazone, and sulbactam on β-lactamase-producing Enterobacteriaceae. This combination,
later used in man for intra-abdominal infections, fever in neutropenic cancer patients, lower respiratory infections and complicated urinary tract infections, has proved its efficacy.²⁻¹² Fantin et al. demonstrated the efficacy of a ceftriaxone plus sulbactam combination against *E. coli* producing SHV-2 in a rabbit endocarditis model.²³ Other authors showed results for this combination in the same experimental model, with the infection from *Pseudomonas aeruginosa*.²⁴ By combining sulbactam with ceftazidime or cefotaxime, the activity of these third generation cephalosporins could be improved *in vitro* against various strains (*E. coli*, *E. cloacae*, *C. freundii*, Acinetobacter spp.) producing ESBLs.²⁵ However, the combination of ceftriaxone plus sulbactam used in the rabbit model for infectious endocarditis with *K. pneumoniae* producing TEM-3 did not satisfactorily sterilize the valvular vegetations. The authors cited a high level of β-lactamase in the vegetations as being the cause of this failure.²⁶ This would therefore confirm the need to maintain sufficient residual concentrations of the two components for as long as possible within the seat of the infection. Assessing the PLIE for such a combination could help to optimize this relationship between pharmacokinetics and pharmacodynamics.

We described, in this study, an *in vitro* PLIE associated with long PAE and PLIE of a ceftazidime–sulbactam combination on different bacteria producing different ESBLs. This interesting finding merits further work particularly using animal experimental models. Such work could lead to new associations of suicide inhibitors with third-generation cephalosporins as well as new ways of administering them, in order to circumvent the ever increasing problem of bacterial resistance linked to β-lactamases.

References


