*Cefotaxime – Recent Experiences**

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Resistance to Third Generation Cephalosporins: the Current Situation

Summary: Newer β-lactam antibiotics, notably the third generation cephalosporins (3 GC) have been designed for providing high intrinsic potency against a large variety of microorganisms. Bacterial resistance can occur however, and nowadays, clinicians are concerned by novel situations where even most recently developed compounds can be ineffective. A first situation is generated by bacteria which produce great amounts of chromosomal cephalosporinase. The resistance emerges during therapy, in hospital isolates which are classified as susceptible with conventional susceptibility testing. The prevalence of 3 GC resistance among these gram-negative rods with inducible β-lactamase seems to increase in some institutions but the significance of susceptibility testing in this regard is doubtful. It is probably more important to note that the prevalence of gram-negative rods with inducible β-lactamases remains stable. A second problem arose with the abrupt development of plasmid mediated β-lactamases markedly active against 3 GC. This resistance is underestimated because some strains fall into susceptibility range of 3 GC as determined by MICs or inhibition zone sizes. These extended spectrum enzymes are now distributed over four continents and represent a growing threat.

Zusammenfassung: *Resistenz gegen Cephalosporine der dritten Generation: Gegenwärtige Situation.* Neuere β-Laktamantibiotika, vor allem die Cephalosporine der dritten Generation, wurden im Hinblick auf hohe intrinsische Aktivität gegen ein breites Erregerspektrum konzipiert. Dennoch können Bakterien gegen diese Substanzen resistent werden, und die Ärzte stehen besorgt vor der Situation, daß selbst die neuesten Substanzen wirkungslos sein können. Das eine Problem ist die Produktion chromosomal kodierter Cephalosporinasen in großen Mengen durch bestimmte Bakterien. Die Resistenzentwicklung tritt unter der Therapie auf, betroffen sind Krankenhausstämme, die bei konventioneller Empfindlichkeitstestung sensibel sind. Diese gramnegativen Bakterien mit induzierbarer β-Laktamase scheinen in einigen Kliniken zuzunehmen, die Resistenztestung ist in dieser Hinsicht jedoch von fraglichem Wert. Wichtiger erscheint die Feststellung, daß die Prävalenz von gramnegativen Bakterien mit induzierbarer β-Laktamase stabil bleibt. Ein zweites Problem ergab sich aus dem plötzlichen Auftreten plasmid-kodierter β-Laktamasen, die beträchtliche Wirkung gegen Cephalosporine der dritten Generation haben. Diese Resistenz wird unterschätzt, da einige Stämme noch im Empfindlichkeitsbereich der Cephalosporine der dritten Generation bei MHK-Bestimmung oder im Hemmhoftest liegen. Diese Breitspektrum-Enzyme haben sich inzwischen über vier Kontinente ausgebreitet und stellen eine zunehmende Bedrohung dar.

Introduction

Third generation cephalosporins (3 GC) exhibit very potent activity against a large variety of gram-negative bacteria, and the microorganisms had to develop original mechanisms for avoiding the lethal effects of these recent antibiotics. The first mechanism involves the production of inducible, chromosomally mediated β-lactamases by certain non fastidious gram-negative aerobic rods. This ability existed before the introduction of 3 GC and is essentially limited to nosocomial bacteria. Its prevalence probably remains stable, despite apparent increases in some institutions. The second problem arose in 1983, in Germany, with appearence of a plasmid-mediated extended-spectrum β-lactamase (ESBla). A dozen types of ESBlas are now known, and strains producing ESBlas have been found all over the world. Bacterial mechanisms, and practical consequences of these two varieties of resistance are analyzed in this article.

Non Transferable Resistance Emerging During Therapy with Third Generation Cephalosporins and Monobactams.

Certain non-fastidious gram-negative bacilli that possess inducible β-lactamase have shown the ability to develop resistance during therapy by 3 GC and monobactams.

Mechanisms

A murine model has been developed to allow the detection and quantification of the resistance that may arise during short-term therapy [1, 2]. The model taught us that the development of resistance depended on the bacterial species inoculated into the animal and on the antibiotic

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administered. *Enterobacter cloacae* was most prone to produce resistance, followed by *Pseudomonas aeruginosa, Serratia marcescens* and *Citrobacter freundii.* Susceptibility of *Klebsiella pneumoniae* and *Escherichia coli* remained unchanged during β-lactam therapy. These observations were in good accordance with clinical experience where, in addition, indole-positive *Proteus* species, *Providencia* species, *Morganella* species and other non fermentative gram-negative bacilli have been shown to possess the same aility [3]. In the murine model, 3 GC (cefotaxime, ceftriaxone, ceftazidime, cefpirome were studied) were the most prone to produce resistance. A lesser risk was found with the monobactams (aztreonam and carumonam). It was very uncommon or impossible to obtain MIC increases during therapy with imipenem, cefepime or piperacillin [4].

For understanding the mechanisms by which some bacteria escape the lethal effects of potent β-lactams, one must consider the β-lactamase activity together with the penetration rate of the antibiotic molecules. All the bacteria involved in the resistance process produce a chromosomally encoded cephalosporinase belonging to the type I in the Richmond and Sykes' classification [5], or to the molecular class C [6]. The most convincing indication that cephalosporinase is associated with resistance was provided by transfer of the chromosomal *bla* gene from a stably derepressed mutant of *E. cloacae* into *E. coli,* leading to acquisition of β-lactam resistance by the recipient *E. coli* [7]. However, bacterial populations involved in the resistance are heterogeneous with regard to β-lactamase production. The wild type cells produce basic low levels of enzyme. This level can be greatly increased by the addition of β-lactam antibiotics acting as inducers (for instance cefoxitin or imipenem). Induced enzymatic hyperproduction ceases as soon as the inducer is removed from the growth medium, and the enzyme levels get down abruptly back to the basic production [8]. Linked to this inducibility, is the ability to undergo mutation to high level constitutive β-lactamase production [9]. Such mutant clones occur at a high frequency (10^{-4} to 10^{-7}) and appear to be highly resistant to most β-lactam antibiotics, except the penems. As reviewed recently [9], the inducible cephalosporinase is mediated by the chromosomal *ampC,* which is governed by *ampR* regulatory gene. Other regulatory genes, *ampD* (which negatively controls *ampC* expression) and *ampE* have been found. Mutations in *ampD* have been associated with constitutive enzyme overproduction. Whether the resistance is caused by induced wild type cells or by constitutive hyperproducer mutants has been matter of controversy, but several lines of evidence seem to favor the second alternative [10, 11, 12].

When cephalosporins with great stability to chromosomally encoded β-lactamases became available, initial hydrolysis studies generated with type I β-lactamase showed that compounds such as cefotaxime and ceftriaxone were poor substrates but potent inhibitors [13]. Binding to, rather than hydrolysis by, β-lactamases was postulated to account for resistance. However, more recent studies with lower substrate concentrations showed that *E. cloacae* β-lactamase hydrolyzed newer cephalosporins at a measurable rate [14]. A strong indication of hydrolysis was provided by HPLC experiments showing 3 GC decay in the presence of intact cells of *E. cloacae,* in conditions thought to be physiologically relevant [11], in good accordance with an *in vitro* membrane model [15] and convincing enzymologic studies [16].

However, slow entry of the antibiotic into the bacterial cell is a fundamental assumption for a proper understanding of resistance. In gram-negative bacteria, and notably in those considered here, the β-lactamase molecules are located in the periplasmic space, i.e. between the outer and inner membranes. The outer membrane is asymmetrically composed of an inner layer of phospholipids and an outer layer, built mainly with proteins and lipopolysaccharides. As a whole, the outer membrane constitutes a strong permeability barrier to the hydrophilic molecules, such as the β-lactam compounds. However, the outer membrane contains a set of proteins, the porins, which form water-filled channels, allowing the non specific penetration of hydrophilic small molecules, including the β-lactams. Since the capacity of hydrolysis and the number of β-lactamase molecules present in the periplasmic space are necessarily limited, the number of β-lactam molecules penetrating into this space becomes the determining factor. When the enzyme capacity is overloaded, the antibiotic gains access to its target molecule (located within the inner membrane) and the bacterium dies; when the outer membrane permeability is reduced to the point where the β-lactamases eliminate all antibiotic molecules arriving in the periplasmic space, resistance occurs. Indeed, changes in outer membrane permeability is now a well-established mechanism of resistance, which affects the susceptibility of the organisms viewed in this discussion, synergistically with hydrolysis by cephalosporinases [11, 17, 18].

Practical Aspects

a) Susceptibility Testing

E. cloacae and other inducible, non-fastidious aerobic bacteria are often falsely classified as susceptible to 3 GC before therapy, and failure to detect resistance is a "very major" error, potentially harmful for the patient. The conventional susceptibility testing uses typically a bacterial inoculum of 10^5 cfu or less. Since the bacterial resistance is associated with the presence of small numbers of resistant cells within the wild-type population, inaccurate detection of resistance is probably caused by an insufficient inoculum. Other studies [19] have shown that mutational resistance in strains of *Enterobacter* was not only inoculum dependent but also time dependent, which might create other difficulties when using rapid automatic systems for testing bacterial susceptibility [20].

To avoid such errors, different solutions have been proposed: detection of β-lactamase inducibility by a cefoxitin disc-induction test [21], population analysis on antibiotic

gradient agar [1], or on agar containing the β-lactam to be tested at a concentration equivalent to the superior break point [4]. None of these methods has been evaluated with regard to clinical relevance. For a patient with an infection caused by a potentially inducible organism, for instance *E. cloacae,* it might be advisable to avoid the use of a 3 GC whatever the results of conventional susceptibility testing.

b) Prevalence of Resistance

Prevalence of this type of resistance is difficult to assess. Several angles should be considered. First, the rates of emergence of resistance in patients infected with organisms possessing inducible β-lactamases and treated with newer cephalosporins have been evaluated at 14 to 56%, [22]. In patients in whom the resistance emerged, failures and relapses appear in 25 to 75% of the cases. Some risk factors have been identified, and for example, resistance appears more often in respiratory, bone or soft tissue infections [23] or in patients with deep neutropenia [24] or cystic fibrosis [25]. Surprisingly, and despite numerous bacterial populations, this type of resistance does not occur commonly in urinary tract infections [26]. It is also very clear that the phenomenon is essentially nosocomial, and rarely affects community acquired bacteria (which typically are not inducible organisms). Another way of looking at the problem is the evolution of this resistance over the course of the time. Some reports are alarming [27], showing dramatic increases of resistance to 3 GC during the present decade. However, it should be emphasized that failure to detect resistance properly, as discussed earlier, definitively hampers the validity of such reports. Indeed the inducible non-fastidious gram-negative rods should be regarded as potentially resistant anyway, whether or not they were classified as such by the laboratory, today as in 1980. So, rather than the percentage of isolates of gram-negative bacilli resistant to 3 GC, it might be preferable to take into account the prevalence of gram-negative bacilli with inducible β-lactamases for epidemiological surveys. This marker seems to remain stable in most institutions [28], so that it may well be that there is no actual increase of this type of resistance.

c) Dosing Schedules

In the murine model [1, 4] emergence of resistance after therapy with aztreonam or ceftriaxone was dose related. These observations support the idea of avoiding underdosing patients, especially at initiation of therapy when the bacterial populations are high. Animal models have shown that combined antimicrobial therapy may limit the emergence of resistance. Any dual combination of amikacin, pefloxacin and ceftriaxone produced less acquired resistance than did monotherapy [28]. In rabbits with pseudomonal endocarditis, the addition of amikacin to ceftazidime reduced the occurrence of ceftazidime-resistant strains five days after therapy – but not at the end of therapy [29]. Combinations of amikacin with piperacillin or ceftazidime, but not the combination piperacillin + ceftazidime, decreased emergence of β-lactam resistant isolates in *P. aeruginosa* infections of neutropenic rats [30]. Limiting effects of combinations on resistance rates have not been recognized in some clinical series [3] but these investigations concerned only limited numbers of patients. Again, using antibiotic combinations empirically to prevent the emergence of resistance during therapy would be especially justified at the beginning of therapy, when the bacterial populations are numerous.

Plasmid-mediated β-Lactamase Markedly Active Against Third Generation Cephalosporins

a) Description

Usually, Klebsiellae produce low levels of a chromosomal penicillinase, practically unable to significantly alter the newer enzyme stable β-lactam antibiotics. The most widely distributed β-lactamase among these isolates is SHV-1, conferring resistance to ampicillin and carbenicillin [31]. Some *Klebsiella oxytoca* strains make an exception to the rule, when they synthetize a chromosomal enzyme with significant hydrolytic activity against 3 GC [32]. Since the seventies, however, progressive development of resistance to β-lactams emerged in *Klebsiella* species. Resistance to older generation cephalosporins was first observed [33] involving plasmid encoded TEM type and SHV-1 β-lactamases (the SHV-1 enzyme being then encoded by a plasmid, and produced at higher levels). In 1983, in Germany, a transferable resistance to cefoxitin and 3 GC was described in *K. pneumoniae* isolates [34], associated to [35] the production of SHV-2, structurally derived from SHV-1. Another outbreak showed up a little later in France [36], involving *K. pneumoniae* isolates producing a plasmid-mediated ESBla, markedly active against 3 GC, primarily called CTX-1. Later, DNA-DNA hybridization analysis indicated that actually CTX-1 belonged to the TEM-type family enzymes, and was subsequently called TEM-3. Extension of hydrolytic activity of TEM-3 compared to TEM-1 and TEM-2 was caused by point mutations in the TEM-type gene [37]. Other modified enzymes were found afterwards, and now a dozen of ESBlas have been recognized belonging to the TEM or SHV family of structures. As to CEP-1, the first ESBla to be described in the literature [38], apparently did not spread widely. All these enzymes differ by their isoelectric points and kinetic profiles. They confer a high level of resistance to ampicillin, ticarcillin, piperacillin and cephalothin, and increased MICs (10- to 800-fold) to 3 GC and aztreonam. Susceptibility to cephamycin and imipenem is not affected. Also, the ESBlas are more or less inhibited by clavulanate and sulbactam, which produce a synergy with the β-lactams affected by the resistance. The plasmids coding for the new β-lactamases are now harboured by several enterobacteriaceae including *E. coli* and Salmonella [39], and have been found in several countries of the world, not only

France and Germany, but also Greece, Tunisia, black Africa, United Kingdom, Chile, Argentina, etc.

Clinical Responses

a) Susceptibility Testing

Some of the strains producing the ESBlas are hardly detected by conventional disk-diffusion method in agar because the inhibition diameters still fall within the susceptible zone. However, clinical failures have been observed during therapy with the antibiotics concerned by the problem, so that, again, there is a risk of "very major" errors. For avoiding this serious inconvenience, a double-disk synergy test performed with cefotaxime and augmentin disks (placed 30 mm apart, center to center) has been found useful [40].

b) Therapy

The combination of cefotaxime plus sulbactam against various *Klebsiella* isolates producing extended broad spectrum plasmid encoded β-lactamases has recently been evaluated in a murine sepsis [41]. Experiments showed that the *in vitro* synergy of the two compounds was also found *in vivo* but, in terms of therapeutic efficacy, better results were obtained with a single drug not grossly affected by the enzymatic hydrolysis such as a penem.
Several plasmids encoding for ESBla also mediated resistance for aminoglycosides (including amikacin), tetracycline and trimethoprim [42, 39, 40], an observation which indeed may account for additional therapeutic difficulties.

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