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Comparison of Inhibitory and Bactericidal Activities of Cefoperazone + Sulbactam Against *Pseudomonas aeruginosa* Strains, from 1992 to 1994

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Abstract: In this study; inhibitory and bactericidal activities of cefoperazone + sulbactam against *Pseudomonas aeruginosa* strains were determined in two studies first in 1992 and second in 1994. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were determined by following the microdilution method recommended by NCCLS (Approved Standard M7-A2 1990) protocol. *P.aeruginosa* strains were isolated from different clinical materials. The results of the studies were then compared to point out if the difference between MIC and MBC values were significant and if there was resistance development following two years of use. In the first study in 1992: MIC90 and susceptibility percentage were 8 μgr/ml and 99%; MBC90 and susceptibility percentage were 64 μg/ml and 90%, respectively. In the second study: MIC90 was 128 μg/ml; susceptibility percentage was 71%; MBC90 and susceptibility percentage were 256 μg/ml and 28%, respectively. When the susceptibilities were compared according to MIC90 and MBC90 values; the difference was not significant in 1992 (χ^2= 0.43, p>0.05), but significant in 1994 (χ^2= 18.8, p<0.001). The difference between the susceptibilities according to MIC90 values was statistically significant (χ^2= 4.6, p<0.05), where according to MBC90 values very significant (χ^2= 32.56, p<0.001). These results indicated that the antipseudomonal activity of cefoperazone+sulbactam reduced since 1992 to 1994 and MBC determinations were necessary to evaluate the antibiotic susceptibility of *P.aeruginosa* rather than MIC determinations.

Key Words: *Pseudomonas aeruginosa*, cefoperazone+sulbactam, MIC, MBC, susceptibility determination.

Introduction

Cefoperazone+sulbactam is an effective agent against *Pseudomonas aeruginosa* which is one of the most common cause of nosocomial infections particularly in patients with impaired defence mechanisms who therefore require bactericidal rather than bacteriostatic therapy (1,2).

The antimicrobial susceptibility of bacteria is in general evaluated by the relationship between minimum inhibitory concentration (MIC) and the blood level of the drug. This evaluation considers only the bacteriostatic activity of the drug and assumes that minimum bactericidal concentration (MBC) does not differ from MIC significantly which is principally acceptable for bactericidal antibiotics (3).

In this study; the inhibitory and the bactericidal activities of cefoperazone+sulbactam against *P.aeruginosa* strains were determined in two studies first in 1992 and second in 1994. The results of the studies were then compared in order to point out if the difference between inhibitory and bactericidal activities were significant and if there was resistance development following two years of use.

Materials and Methods

Each year, 100 *P.aeruginosa* strains, isolated from various clinical materials were identified by conventional methods and confirmed by API 20NE (bioMerieux) in the Microbiology Laboratory of Refik Saydam Hygiene Center. The MIC and MBC values of these strains were determined by standard methods recommended by NCCLS (Approved Standard M7-A3,1993) (4).

The antimicrobial powder with known potence was obtained from the pharmaceutical manufacturer. Stock solutions of the antimicrobial were prepared in Mueller-Hinton broth (MHB) (Oxoid) and used on the day of testing and kept frozen at -70°C for maximum 15 days (4).

MHB was used in the microdilution method. Antibiotic solution was dispensed in the first well and serially diluted within the wells of disposable plastic U-shaped microtiter plates obtaining 50 μg/well. Suspensions of the

test organisms were prepared by 1:1000 dilution of overnight cultures. The suspensions were dispensed in 50µl volumes into the antibiotic containing wells. The ranges were arranged between 256-0.125. The final inoculum concentration was about $5x10^5$ cfu/ml. The plates were incubated overnight at 37°C (4).

The MIC was defined as the lowest concentration of the antibiotic that inhibited the growth of the organism as detected by lack of visual turbidity. All the wells that had no turbidity and the last well with turbidity were subcultured to sheep blood agar as 10µl and incubated at 37°C for 24 hours. The colonies of *P.aeruginosa* were counted in order to determine the MBC values which were defined as the lowest concentration of the antibiotic that allowed more than 99.9% of the original inoculum devitalization (4).

According to NCCLS breakpoints, including the moderate susceptibilities, ≤16µg/ml were susceptible, ≥64µg/ml were resistant. The combination of sulbactam and cefoperazone were 1:1. *P.aeruginosa* ATCC 27853 was included in each test as reference strain (4).

Results

The activity of cefoperazone+sulbactam against *P.aeruginosa* strains were evaluated by means of MIC, MBC values and the susceptibility percentages of the agent in two studies in 1992 and 1994 on the table.

When the susceptibilities were compared according to MIC90 and MBC90 values; the difference was not significant in 1992 (χ^2= 0.43, p>0.05), but significant in 1994 (χ^2= 18.8, p<0.001). The difference between the susceptibilities according to MIC90 values between two years 1992 and 1994 were statistically significant (χ^2= 4.6, p<0.05). The difference between the susceptibilities according to MBC90 values between two years 1992 and 1994 were statistically very significant (χ^2= 32.56, p<0.001).

Discussion

Cefoperazone is a third generation cephalosporin that has a wide antibacterial spectrum. As a beta-lactam antibiotic; it should penetrate through the bacterial cell wall, be resistant to beta-lactamase enzymes and bind to the penicillin binding proteins. When cefoperazone is combined with a beta-lactamase inhibitor like sulbactam, its in vitro activity increases. This combination is found to be effective against *P.aeruginosa* strains (1). For an antibiotic to have a particular effectiveness against a species, at least 50% of the strains should have MIC values below the mean concentrations attained at the site of infection or in the blood (3).

In 1992, we determined that *P.aeruginosa* strains were susceptible to cefoperazone+sulbactam with MIC and MBC values which were similar with the other studies of that year in our country (5,6). In 1994, it was susceptible with MIC but resistant with MBC values that made it difficult to be qualified as an effective antipseudomonal antibiotic. Therefore if MIC would be taken as the reference point for the laboratory designations "susceptible or resistant", MBC determinations would be more certain for the prediction of susceptibility in the cases that required bactericidal therapy rather than bacteriostatic therapy such as impaired defence mechanisms were there (7,8).

In the first study in 1992, the susceptibility was very high, because it was the first year that cefoperazone+sulbactam was commercialy available in our country. However, using the same breakpoints it gave a very different susceptibility pattern in 1994. Especially when MBC values were considered, it was obvious that the spectrum of activity reduced against *P.aeruginosa*. Resistance rates for two different periods were significantly different and higher in the second year. We had observed the resistance development against cefoperazone+sulbactam in two years' time (9).

The data indicated that MBC determinations were necessary to evaluate the antibiotic susceptibility of *P.aeruginosa* rather than MIC determinations at least in patients with impaired defence mechanisms which required bactericidal therapy. It was also indicated that there was resistance development against cefoperazone+sulbactam among *P.aeruginosa* since 1992 to 1994 because of the uncontrolled wide usage of the antibiotic.

Table: MIC90, MBC90 and susceptibility percentages of cefaperazone+sulbactam against *P.aeruginosa* strains in 1992 and 1994

P.aeruginosa	(n=100)				
Years	Ranges	MIC_{90}	Susceptibility %	MBC_{90}	Susceptibility %
1992	0.125-256	8	99%	64	90%
1994	0.125-256	128	71%	256	28%

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