EVALUATION OF SYNERGISTIC EFFECT OF CEFUROXIME AND CLAVULANIC ACID AGAINST ESCHERICHIA COLI

Abdul Jalil, Abdul Wadood, Riaz Naseem

ABSTRACT

Background: β-lactamase mediated resistance is the most common mechanism which renders the β-lactam antibiotics ineffective against bacteria including Escherichia coli. The development of resistance has led to the widespread empirical use of higher generation cephalosporins and it is likely that the third generation cephalosporins are also losing their efficacy by the same mechanism. The present study was conducted to evaluate the and microbial effect of cefuroxime with and without a β-lactamase inhibitor named clavulanic acid, against Escherichia coli.

Research Methodology: An institution based analytic observational study was carried out at Department of Microbiology, Khyber Medical College, Peshawar. β-lactamase producing Escherichia. coli were identified and cultured in Mueller Hinton medium in petridishes. The antibiotic disks containing cefuroxime alone and in combination with clavulanic acid were placed in the same petridishes about 24-30 mm apart. The zones of inhibition were measured according to NCCLs zone diameter criteria using disk diffusion method.

Results: A total of 40 β-lactamase producing bacteria were isolated. The zones of inhibition surrounding the disks containing both cefuroxime and clavulanic acid were larger than those containing cefuroxime alone.

Conclusion: In this study it was found that the synergistic antibacterial effect of the combination of cefuroxime and clavulanic acid was far more than the cefuroxime alone against Escherichia coli.

Key words: Cefuroxime, Resistant Escherichia coli, Clavulanic acid.

INTRODUCTION

Penicillin, cephalosporins and other antibiotics contain a four membered ring in their structure constitute β-lactam antibiotics. The β-lactam antibiotics kill the bacteria by competitively binding to transpeptidases and thus interfering with peptidoglycan cell-wall, which completely surrounds the bacterium and protects it from its own pressure. The peptidoglycan is also an essential element in maintaining the shape and integrity of cell wall in both gram positive and gram negative bacteria. Despite the discovery of other groups of antibiotics and addition of new members in each group, the β-lactams account for approximately 50% of antibiotics used in the world.

Cefuroxime is a second generation cephalosporin, which possesses greater activity against gram negative organisms as compared to the first generation cephalosporins. Due to improper use of β-lactams including cefuroxime, resistance has developed against these antibiotics. Although there are also other factors involved in the development of resistance against β-lactam antibiotics, β-lactamase production is by far the most important and the commonest cause of bacterial resistance to penicillins, cephalosporins and related β-lactam compounds. The gram negative bacteria produce a wider variety of β-lactamas than do the staphylococci. The novel therapy of combining an established β-lactam antibiotic with β-lactamase inhibitor, neutralizes the effect of β-lactamase. It thus allows, the β-lactam antibiotic to act as if the organism was fully sensitive. This approach has largely overcome the resistance which was developed in many organisms including Escherichia coli. Clavulanic acid is naturally occurring potent inhibitor of β-lactamase obtained by, fermentation from streptomyces clavuligerus. It has negligible antimicrobial activity but is a “suicidal inhibitor” of β-lactamases produced by a wide range of gram positive and gram negative microorganisms. Amoxicillin plus clavulanic acid is effective in-vitro and in-vivo antibiotic for β-lactamase producing strains of Streptococci, H. influenzae, Gonococci and E. coli. Addition of clavulanic acid to a β-lactam, like cefuroxime is likely to have a synergistic effect on its activity.
RESEARCH METHODOLOGY

Escherichia coli isolated from the clinical laboratory specimens were cultured and identified by their morphology, cultural characteristics and biochemical activity.11 Out of these isolates, 40 β-lactamase producing strains were isolated by a rapid chromogenic method using chromogenic cephalosporin sticks.12 The impregnated end of the stick was moistened and touched with a bacterial colony, picking up a small cluster of cells.

The tip was examined for up to five minutes. The development of pink/red colour indicated a positive reaction.13 For preparation of media, Mueller Hinton agar14 was used. It has now become the standard for Baur-Kirby method and its performance is specified by NCCLS. The E. coli that had been subcultured on nutrient agar and re-identified, were inoculated in 2 ml of Mueller Hinton broth in Bijou bottles and incubated at 37°C aerobically for 18 hours. Then turbidity of the suspension was adjusted as 10^5-10^6 CFUs/ml15 with sterile Mueller Hinton broth and checked against McFarlands turbidity standard barium sulphate.16 This broth was poured over the standard Mueller Hinton agar plates.

For preparation of the antibiotic disks, 6mm diameter filter paper chips were sterilized at 160°C in a hot air oven and after allowing the disks to cool, 20mL of the required concentration of cefuroxime solution were poured on each disk.17

Disks of 20, 30 and 40 µg concentration of cefuroxime alone and with clavulanic acid were prepared and placed in the Mueller Hinton culture plates with about 24 mm distance between two consecutive disks. The plates were incubated at 35-37°C for about 16-18 hours. The diameter of zone of inhibition around each disk was measured which corresponded to the activity of each disk.19 Zone diameter of 14-18 mm was taken as sensitive.

RESULTS

The susceptibilities of all 40 β-lactamase producing isolates were recorded by using antibiotic disks of 20, 30 and 40 µg cefuroxime and the disks containing same concentration of this antibiotic with β-lactamase inhibitor clavulanic acid in 1:1 and 2:1 ratios. The results obtained by measuring the zone of inhibition of different disks are shown in Table 1 and Fig. 1.

| Table 1: Synergistic effect of cefuroxime and clavulanic acid against E. Coli |
|----------------------------------|-----------------|-----------------|-----------------|
|                                  | Conc (µm)       | Resistant (%)   | Sensitive (%)   | Resistant (%)   | Sensitive (%)   |
| Cefuroxime (Control)            | 20              | 29 (3%)         | 11 (1%)        | 4               | 36 (3%)         | 6               | 34 (2%)         |
|                                 | 30              | 27 (2%)         | 13 (1%)        | 1               | 39 (2%)         | 1               | 39 (1%)         |
|                                 | 40              | 18 (2%)         | 22 (1%)        | 1               | 39 (1%)         | 1               | 39 (1%)         |
| Cefuroxime + clavulanic acid (1:1) | 20              | 29 (3%)         | 11 (1%)        | 4               | 36 (3%)         | 6               | 34 (2%)         |
|                                 | 30              | 27 (2%)         | 13 (1%)        | 1               | 39 (2%)         | 1               | 39 (1%)         |
|                                 | 40              | 18 (2%)         | 22 (1%)        | 1               | 39 (1%)         | 1               | 39 (1%)         |
| Cefuroxime + clavulanic acid (2:1) | 20              | 29 (3%)         | 11 (1%)        | 4               | 36 (3%)         | 6               | 34 (2%)         |
|                                 | 30              | 27 (2%)         | 13 (1%)        | 1               | 39 (2%)         | 1               | 39 (1%)         |
|                                 | 40              | 18 (2%)         | 22 (1%)        | 1               | 39 (1%)         | 1               | 39 (1%)         |
DISCUSSION

Table 1 and Fig. 1 delineate the sensitivity of Escherichia coli against cefuroxime alone and in combination with clavulanic acid. The cefuroxime alone has some antibacterial activity against Escherichia coli. But by combining 20, 30 and 30 µg cefuroxime with clavulanic acid in 1:1 ratio, the sensitivity is increased by 62.5%, 65% and 42.5% respectively. In 2:1 ratio, the combination produced 57.5% 65% and 42.5% increase in sensitivity.

This synergistic effect of the clavulanic acid with cefuroxime in this ratio is parallel to that with combination in 1:1 ratio, specially in higher concentration (30 µg and 40 µg). This shows that combination of cefuroxime with clavulanic acid has a significant synergistic effect.

The combinations of clavulanic acid with amoxicillin and ticarcillin are already in use. In several others studies, the β-lactamase mediated resistance and its control with β-lactamase inhibitors has been evaluated.20

Our study shows that the scope of the use of β-lactam/β-lactamase inhibitors can be extended to the higher generation cephalosporins. This can prove to be a cheaper and a more appropriate approach to deal with the increasing resistance to cephalosporins.

Although indiscriminate use of β-lactam antibiotics including cephalosporins has been implicated as the major factor responsible for development of β-lactamase mediated resistance, it is worth mentioning here that β-lactam antibiotics often exhibited resistance as soon as they were first introduced. For instance in 1944, when benzyl penicillin was newly introduced in the market, it was active against 95% of Staphylococcus aureus isolates, but the remainder of 5% Staphylococcus aureus had β-lactamase and were resistant. Within 5 years, the proportion of enzyme producers had grown to 50%, reflecting gene transfer and strain selection. Subsequently the resistant proportion had risen to around 90%.3

In order to overcome the menace of resistance against β-lactam antibiotics, we can either think of developing new and extended spectrum β-lactams or using β-lactamase inhibitors in combination.

But the extended spectrum cephalosporins e.g. third generation cephalosporins have been correlated with appearance of extended spectrum β-lactamases, plasmid mediated enzymes that confer resistance to oxyimino cephalosporins and monobactams.21 Moreover this option is also not much practicable due to huge financial liabilities in evaluating, synthesizing and market-

ing new drugs. More than a decade ago, it was estimated that enormous costs from $ 100 million to over $ 500 million were involved in the development of a single successful drug;22 that is why very few new antibiotics were developed in 1990s.23 Another disadvantage of the newer β-lactamase resistant cephalosporins is that they are quite expensive and usually available only by injection.24

Thus more than anything, the opportunities for control of resistance lie in the careful and prudent use of the power compounds that are available, rather than in undue optimism about any next generation of β-lactams.3

Some β-lactam/β-lactamase inhibitors combinations are already available in the market.7 These are amoxicillin and ticarcillin with clavulanic acid and ampicillin and cefoperazone with sulbactam.5

The combination in the present in-vitro study also shows significant degree of success at least against Escherichia coli.

REFERENCES


Address for correspondence:
Dr. Abdul Jalil,
Assistant Professor,
Department of Pharmacology,
Khyber Medical College,
Peshawar