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Comparative Analysis of Cefotaxime Sodium and Cefoperazone Sodium Drugs Activity and their Phytochemical Combinations Against Bacteria

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Among the emerging classes of antibiotics, Cephalosporins have been in lime light throughout the last decade. Consisting of many generations the cephalosporins are well known for their broad spectrum of activity against many gram positive and gram negative microorganisms, either used alone or in combination with other antibiotic classes. But the combination of two cephalosporins has never been checked yet. In this research work, two cephalosporins. Cefotaxime sodium and cefoperazone sodium were tested individually, in combination with each other and also in combination with honey against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Increase in activity of these drugs in combination was observed in case of *Escherichia coli*. Antagonistic effects were predominant in these drug combinations. However, the combination of drug with honey resulted in dominant additive effect.

Keywords: Cefotaxime sodium, Cefoperazone sodium, Synergy, Honey.

INTRODUCTION

The cellular and protein epoch of 1980s was followed by an immense increase in the knowledge about molecular biology. Life sciences such as bacteriology has been largely affected by these emerging techniques in molecular biology and many molecular bacteriologists are now using these techniques to support their particular field of interest[1]. The last ten years of twentieth century have also observed a great increase in infectious diseases[2,3]. Evolutionary changes and plethora of many microbes are the main problems in the fight against these pathogens[4].

Escherichia coli; a gram negative microbe, is usually a dweller of human and animal's intestinal tract and is normally not pathogenic, however it may cause some infections of urinary tract such as pyelonepheritis, cystis and pyelitisetc[5]. Similarly another gram negative pathogen *Pseudomonas aeruginosa* is a cause of bacteremia, urinary tract infections, pneumonia and wound infections[6]. Its ability to become mutant to antimicrobial agents and its frequent reoccurrence in serious infections also make it a severe threat to many hospitals and it is a cause of major nosocomial pneumonia infections particularly in cystic fibrosis patients[7]. In addition to many gram negative microbes, many gram positive pathogens are a source of trouble to human beings, one being the multidrug resistant *Staphylococcus aureus* which is also a source of many hospital acquired infections[8,9].

Microorganisms can be combated in two ways: (i) Their inactivation inside the organism and (ii) microbial control of the environment. Unfortunately the microbes responsible for these infectious diseases are present everywhere in this universe; also the methods used to control them are not always as efficient and environmental friendly[10]. Antibiotics produced by many pharmaceutical companies have been used worldwide to control these microorganisms inside the organisms[11,12] but the microbes have acquired the tendency to alter their genetic makeup and to become resistant to these antibiotics[13]. In view of all this, antimicrobial combination therapy can be used as a last line of defense in reducing the emerging prevalence of multidrug resistance in these infective pathogens[14].

The basic principle behind the use of combination therapy is that use of two or more drugs (usually with dissimilar mechanism of action) can result in improved pharmacodynamic properties of these drugs. In this regard, many antibiotic combinations have been tested[15-17]. Also the combination of drugs with phytochemicals is a novel idea[18-20].

Combinations of cehalosporins with many other classes of antibiotics have resulted in an increase in activity against many gram positive and gram negative microorganisms[21]. However, not a single combination of two cephalosporin has been tested yet. The action of cephalosporins is attributed to their irreversible inhibition of enzymes and prevention of cell wall synthesis[22]. The purpose of this study was to evaluate the

antibacterial combination of two drugs (with the same mechanism of action, here the cephalosporins) against the above listed microbes. All possible combinations of cefoperazone sodium (CFP) and cefotaxime sodium (CTX)were tested in equal ratios at four different concentrations. Both cephalosporins were also checked in combination with honey at these four concentrations. All analysis was performed using well known disc diffusion method.

EXPERIMENTAL

Cefotaxime sodium and cefoperazone sodium were purchased from Aries Pharmaceuticals (Pakistan). Bacterial strains used were *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027) and *Staphylococcus aureus* (ATCC 6528). Both drugs were used without further purification and sterilization.

Preparation of sample solutions: Drug sample solutions were prepared for concentrations 40 and 43 μg/mL. These solutions were then diluted to 20 and 21.5 μg/mL. All possible combinations of these two drugs were prepared in 50:50 ratios at these four concentrations 40, 43, 20 and 21.5 μg/mL. The sample solutions were also prepared in these four concentrations for two drugs in combination with a 6 % (v/v) honey solution.

Susceptibility tests: The susceptibility of three microbes against these drugs, drug combinations and combination of drugs with honey was tested with disc diffusion techniqueusing filter discs about 5 mm in diameter. The filter discs were soaked in sample solutions and placed on the pre-incubated and inoculated plates. The plates were incubated overnight and the results were recorded by measuring the diameter of the inhibition zone. All results were further confirmed by statistical analysis.

RESULTS AND DISCUSSION

The class of antibiotic containing a β-lactam ring fused with a six member dihydrothiazine ring and resembling the β-lactam class of antibiotics is called Cephalosporin. Until now there have been five generations of cephalosporins based on their spectrum of activity against many gram positive and gram negative microorganisms[22]. From the view point of combination therapy, it can be seen that the third and fourth generation cephalosporins could be much effective against many gram positive and gram negative microbes when used in combination with each other. The present research work aims at looking for the better and effective cephalosporin combinations against *E. coli*, *P. aeruginosa* and *S. aureus*. For this purpose, two semi-synthetic and broad spectrum cephalosporins, cefotaxime sodium and cefoperazone sodium were selected. Also the ancient history tells us a lot about the use of honey as an anti infective agent, wound dressings and a remedy for many gastrointestinal illnesses[23,24]. Keeping in view the anti infective properties of honey, we also focused to check the activity of honey in combination with these cephalosporins against the test organisms.

The combinations were labeled as synergic, additive or antagonistic according to the literature[25]. The effect of individual drugs at four different concentrations is mentioned in Table-1. Out of all possible drug combinations tested against *E. coli*, only one combination was found to be synergic. For all other drug combinations antagonisms was predominant over additive effect. Not a single synergic combination was found against *P. aeruginosa*, rather antagonism was also prominent in this case. Similar results were obtained when all 16 combinations were analyzed for *S. aureus* (Table-2). The main cause of this antagonistic effect may be due to the interaction of a drug in the physiological action of the other drug having similar chemical structure. The two drugs in combination having similar chemical structure may compete for the active site of the enzyme and both of them may fail to perform their action efficiently.

Considerable improvement in the activity of both drugs was observed when tested in combination with honey. Individual effect of honey against these three microbes is given in Table-3. Out of a total of 8 combinations of drugs with honey tested against *E. coli* showed additive effect, while one combination showed antagonistic effect. In case of *P. aeruginosa* only two combinations were found to be antagonistic, while 6 combinations were additive in nature. *S. aureus*, however showed an exactly opposite effect to that of *P. aeruginosa*, where six combinations exhibited the antagonistic effect and only two were additive in nature (as shown in Table-4).

Conclusion

All these results help us concluding that both these drugs *i.e.*, cefotaxime sodium and cefoperazone sodium are not much effective when used in combination with each other. Combining these drugs with honey also could not lead to any synergistic effect. However the struggle for the development of better and effective combinations against many drug resistant microbes can be aided by *in vitro* analysis of different cephalosporins in unequal ratios. There is still a need to test the drug combinations of different cephalosporin generations and also the analysis of cephalosporins with many other phyto-chemicals requires attention.

TABLE-1
INHIBITION ZONE DIAMETER (IZD) VALUES OF INDIVIDUAL DRUGS AGAINST THREE MICROBES MEAN IZD (mm)

Sample No.	Sample Name	Conc. (μg/mL)	*E. coli*	*P. aeruginosa*	*S. aureus*
C_1	Cefotaxime sodium	40.0	0.00	20.30	10.30
C_2	Cefotaxime sodium	43.0	8.00	13.30	12.30
(½)C_1	Cefotaxime sodium	20.0	0.00	9.30	9.30
(½)C_2	Cefotaxime sodium	21.5	6.70	9.70	8.70
D_1	Cefoperazone sodium	40.0	8.30	14.70	26.00
D_2	Cefoperazone sodium	43.0	12.30	28.70	32.00
(½)D_1	Cefoperazone sodium	20.0	8.70	26.00	27.70
(½)D_2	Cefoperazone sodium	21.5	6.30	29.00	29.30

TABLE-2
INHIBITION ZONE DIAMETER (IZD) VALUES OF CEFOTAXIME SODIUM AND CEFOPERAZONE SODIUM COMBINATIONS AGAINST THREE MICROBES MEAN IZD (mm)

Sample No.	Sample name	Conc. (µg/mL)	*E. coli*	*P. aeruginosa*	*S. aureus*
C_1D_1	CTX + CFP	40 + 40	8.00 (I)	24.7 (I)	18.7 (A)
C_1D_2	CTX + CFP	40 + 43	6.70 (A)	20.7 (I)	24.7 (A)
C_2D_1	CTX + CFP	43 + 40	0.0 (A)	11.3 (A)	25.7 (I)
C_2D_2	CTX + CFP	43 + 43	13.0 (I)	10.0 (A)	23.0 (A)
$C_1(1/2)D_1$	CTX + CFP	40 + 20	6.70 (A)	19.3 (A)	16.0 (A)
$C_1(1/2)D_2$	CTX + CFP	40 + 21.5	0.0 (A)	18.7 (A)	17.7 (A)
$C_2(1/2)D_1$	CTX + CFP	43 + 20	0.0 (A)	21.3 (A)	17.7 (A)
$C_2(1/2)D_2$	CTX + CFP	43 + 21.5	7.30 (I)	20.3 (A)	24.3 (A)
$(½)C_1D_1$	CTX + CFP	20 + 40	8.30 (I)	19.0 (I)	19.7 (A)
$(½)C_1D_2$	CTX + CFP	20 + 21.5	7.30 (A)	23.7 (I)	19.7 (A)
$(½)C_2D_1$	CTX + CFP	21.5 + 40	7.70 (I)	23.7 (I)	16.0 (I)
$(½)C_2D_2$	CTX + CFP	21.5 + 21.5	8.00 (A)	21.3 (A)	18.0 (A)
$½(C_1D_1)$	CTX + CFP	20 + 20	7.70(A)	20.0 (A)	11.7 (A)
$½(C_1D_2)$	CTX + CFP	20 + 21.5	0.0(A)	22.7 (A)	18.0 (A)
$½(C_2D_1)$	CTX + CFP	21.5 + 20	0.0(A)	14.0 (A)	16.7 (A)
$½(C_2D_2)$	CTX + CFP	21.5 + 21.5	12.7(S)	20.7 (A)	16.0 (A)

S = Synergy, I = Indifference or Additive effect, A = Antagonistic; CTX = Cefotaxime sodium; CFP = Cefopeiazone microbes sodium

TABLE-3
IZD VALUES OF HONEY (6 % v/v) AGAINST THREE MICROBES

Organisms	Conc. of honey used (% v/v)	Mean IZD (mm)
Escherichia coli	6	16.7
Pseudomonas aeruginosa	6	24.3
Staphylococcus aureus	6	22.7

TABLE-4
IZD (mm) VALUES OF HONEY AND DRUG COMBINATIONS AGAINST THREE MICROBES MEAN IZD (mm)

Sample No.	Conc. (µg/mL)	*E. coli*	*P. aeruginosa*	*S. aureus*
HC_1	6 % v/v + 40.0	15.3(I)	22.0(I)	11.7(A)
HC_2	6 % v/v + 43.0	20.7(I)	21.7(I)	12.7 (A)
$H(1/2)C_1$	6 % v/v + 20.0	14.3(I)	24.0(I)	00.0(A)
$H(1/2)C_2$	6 % v/v + 21.5	9.30(A)	21.3(I)	15.0(A)
HD_1	6 % v/v + 40.0	15.0(I)	21.7(I)	22.7(I)
HD_2	6 % v/v + 43.0	17.0(I)	26.7(I)	27.7(I)
$H(1/2)D_1$	6 % v/v + 20.0	13.0(I)	20.7(I)	24.0(I)
$H(1/2)D_2$	6 % v/v + 21.5	16.7(I)	18.3(A)	28.0(I)

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