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ANTIBACTERIAL STUDIES OF CEFIXIME COPPER, ZINC AND CADMIUM COMPLEXES

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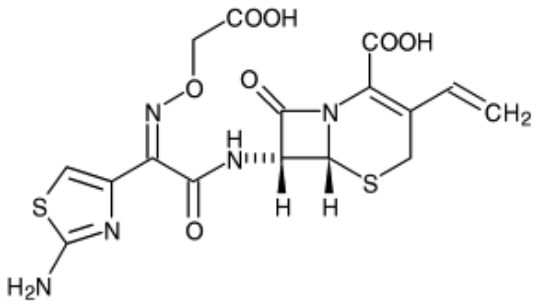
ABSTRACT

Antibacterial studies of metal complexes of cefixime, with copper zinc and cadmium have been carried out by observing the Minimum Inhibitory Concentration (MIC) and by measuring the Zone of Inhibition of the complexes and compared with the parent cephalosporin against both Gram negative and Gram positive microorganisms. Various microorganisms used were *Staphylococcus aureus, E. coli, Klebsiella and Proteus* species. For MIC observation, serial dilution method was employed and zone sizes were determined by diffusion disk method.

Our investigations divulge that formation of complexes results in decrease in antimicrobial activity of cefixime and MIC values were increased.

INTRODUCTION

Cefixime is a semisynthetic, third generation cephalosporin antibiotic for oral administration. Chemically, it is 7-2[2-(amino-4-thiazolyl)-2-(carboxymethoxyimino)acetamido]-3-vinyl-3-cephem-4-carboxylic acid acid [1], having molecular weight 507.50 as the trihydrate (Budavari 1987).



[1]

Its synthesis was reported by Takaya & Kawabata *et al*. (Takaya, 1981 & Kawabata *et al.,* 1983) and Yamanaka *et al.* (Yamanaka *et al.,*1985), synthesis and activity of (E)- isomer by Kawabata *et al.* (Kawabata *et al.,* 1986), mechanism of action by Shigi *et al.* (Shigi *et al.,* 1984), comparative antibacterial spectrum *in vitro* and *in vivo* by Kamimura *et al.* (Kamimura *et al.,* 1984), β-lactamase stability by Neu *et al.* (Neu *et al.,* 1984), pharmacokinetics in humans by Guay *et al.* (Guay *et al.,* 1986) and clinical trials in urinary tract infections by Levenstein *et al.* and Irvani *et al.* (Levenstein *et al.*, 1986; Irvani *et al.,* 1988) and in respiratory infections by Kiani *et al.* (Kiani *et al.,* 1988).

As a rule, the third-generation cephalosporins are less active than the older cephalosporins against Gram-positive cocci but are much more active against the Enterobacteriaceae, including multiply-resistant isolates. A subset of the third generation agents (cefoperazone, ceftazidime and cefsulodin) also exhibits marked activity among these new agents.

There are number of reported drug interactions of cephalosporins (McMahon 1980; New & Prince 1980; Foster *et al.,* 1980; Portier *et al.,* 1980; Reeves & Davies 1980; Drummer *et al.,* 1980; Brown *et al.,* 1982; Witt 1983; Elenbaas *et al.,* 1982; Buening *et al.,* 1981) and in particular, cefixime has a reported drug interaction with nifedipine, a calcium channel blocker (Duverne 1992). Cefixime also forms complexes with many essential and trace elements present in the body. Present studies comprise of antibacterial studies of metal complexes of cefixime, with copper zinc and cadmium and changes in microbiological activity of the parent cephalosporin after complexation has been studied. These studies were carried out by observing the minimum inhibitory concentration (MIC) and by measuring the zone of inhibition of the complexes and compared with the parent cephalosporin against both Gram negative and Gram positive microorganisms. Various microorganisms used were *Staphylococcus aureus, E. coli, Klebsiella* and *Proteus* species. For MIC observation, serial dilution method was employed and zone sizes were determined by diffusion disk method.

MATERIALS AND METHODS

Materials

Cefixime reference standard was obtained from GlaxoWellcome laboratories Karachi with a care that it has expiry date not earlier than 365 days. Copper, zinc and cadmium complexes of cefixime, the synthesis of which has been reported elsewhere were used in these studies (Ali 2002). The organisms employed in these studies were *Staphylococcus aureus, Escheracia coli, Pseudomonas aeruginosa, Klebsiella and Proteus mirabillus*.
Sterile swabs available commercially were used for the inoculation of the organsim into the petri dishes.

Methods

Sample Preparation

0.016 g of cefixime reference standard was dissolved in minimum amount of methanol in a 10 ml volumetric flask and volume was made up to the mark with the same solvent. This standard solution was used for disc spiking and was diluted to 16 μg/ml for MIC. Similarly, 0.016 g of each of the cefixime metal complex was individually dissolved in minimum amount of methanol in a 10 ml volumetric flask and volume was made up to the mark with the same solvent. This sample solution was used for disc spiking and was diluted to 16 μg/ml for MIC.

Cultures and Media

The cultures of the organisms mentioned above were collected and were further checked morphologically and culturally. All the media were autoclaved for 15 minutes at 15 psi pressure at 121°C. Media used for the primary isolation of the organism comprised of peptone from meat (5.00 g), meat extract (3.00 g), agar-agar (12.00 g) and pH was maintained at 7.0 ± 0.2. 20 g of dehydrated medium was suspended in 1 liter of distilled water, heated on boiling water bath till dissolved completely.

Preliminary Identification of the Cultures

The Brolacin agar (Bromothymol-blue-lactose cystine agar) was used for the further confirmation of identified organisms (National Committee for Clinical Laboratory Standards

1984). It consisted of peptone (7.00 g), yeast extract (2.00 g), meat extract (2.00 g), L-cystine (0.128 g), lactose (10.00 g), bromothymol blue (0.03 g) and agar-agar (12.00 g), while pH was maintained at 7.3 ± 0.1.

33 g of Broclain agar powder was suspended in 1 liter of distilled water, heated on boiling water bath till dissolved completely.

Subculturing and Maintenance of Different Organisms

The 'Stock Culture Agar' was used for the maintenance and subculturing of different organisms. It is a soft, almost semisolid medium. The success of the medium probably lies in the fact that the medium contains a small quantity of dextrose, which serves as a readily available source of energy. This medium, support luxuriant growth of pathogenic bacteria and preserve their viability over a long period of time.

Antimicrobial Susceptibility

Trypto-Soy Broth used for determining the sensitivity of bacteria against different antimicrobial agents contained bacto tryptone (17 g), bacto soytone (3 g), bacto dextrose (2.5 g), sodium chloride (2.0 g) and dipotassium pohaphate (2.5 g).

The Muller-Hinton broth (MBH) (National Committee for Clinical Laboratory Standards 1973) used for preparaing inoculum consisted of meat infusion (2.0 g), casein hydrolysate (17.50 g) and starch (1.50 g). pH was maintained at 7.4 ± 0.2.

The MHB was prepared by suspending 21 g of MHB in 1 liter of distilled water, heated on boiling water bath till dissolved completely and then transferred into test tubes (4-5 ml each). It was then autoclaved for 15 minutes at 121°C.

Assay Plates

Flat bottom petri dishes with internal diameter of 150 mm were used in the procedure. Bottles contiaining MHA were allowed to equilibrate at 50°C, on a water bath. The medium was poured into sterile petri dishes on a level surface to a depth of 4 mm and allowed to harden. The pH of the medium was maintained and allowed to dry for 15 to 30 minutes before use or storage at 4°C. Agar plates to be stored for longer than 7 days were wrapped in plastic to prevent excessive evaporation of moisture.

Antimicrobial Disks

The antimicrobial impregnated filter paper disk of sample and standard were used. Both sample and standard disc had concentration of 32 μg/ml were used.

Test Procedure of Antimicrobial Susceptibility Testing

Portions of each of four or five well isolated colonies of same morphological type were touched with a sterile wire loop, suspended into tubes containing 5 ml of Trypt-Soyn Broth. This medium was then incubated at 35°C for 2-8 hours until the turbidity reached. If turbidity exceeded than standard, it was diluted with broth until it was visually comparable with standard.

Streaking Agar Surface

A sterile swab was dipped into a broth suspension of organsims within 15 minutes of inoculum standardization. Excess inoculum was removed by rotating the swab several times against the wall of the tube above the fluid level. The entire surface of a Muller-Hinton Agar plate was then streaked evenly in three/two directions approximately 60 degree from each other. The lid was then replaced and was allowed to dry for 3 to 5 minutes.

Disk Placement

The antimicrobial-impregnated disks were placed with sterile forceps on to the agar surface. The disks were distributed such that they were at least 24 mm apart so as to avoid overlapping during incubation. The plates were then incubated at 37°C for 18-24 hours.

After 18-24 hours of incubation, the diameter of zones of inhibition around each disk was measured with a vernier calliper on the back of the plates, with reference light against a dark non-refelective background.

RESULTS AND DISCUSSION

Selection of an antibiotic for therapy of bacterial infection often depends on knowledge of the susceptibility of the infecting organism (Gennaro 1985). Usually, it is possible to determine susceptibility by *in vitro* tests. When these tests are properly standardised, the result obtained correlate well with the response to therapy observed in clinical practice (Rayan 1970).

As with other cephalosporins, bactericidal action of cefixime results from inhibition of cell-wall synthesis in presence of β-lactamase enzymes. It was verified during present *in vitro* studies (table 1) that cefixime is active against both Gram positive and Gram negative strains of organisms. Table 1 indicate that *Escherichia coli* and *Staphylococcus aureus* were susceptible to cefixime, while on the other hand *Proteus mirabillus* and *Klebsiella aureginosa* were resistance at 10-8 μg/ml concentrations, however susceptible at 64, 32, 16 and 12 μg/ml concentrations. *Pseudomonas aureginosa* was found resistant to cefixime.

Antimicrobial activity of cefixime copper complex as shown in table 2 reveal that *Escherichia coli* susceptibility had not been effected by the copper complexation while activity of the complex was reduced towards *Staphylococcus aureus*, *Proteus mirabillus*, *Klebsiella aureginosa* and *Pseudomonas aureginosa*.

Cefixime zinc complex was susceptible against *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabillus* and *Klebsiella aureginosa* at concentrations of 64, 32 and 16 μg/ml as shown in table 3. *Escherichia coli* however, was susceptible even at lower concentrations, which means that even after complex formation it had lower MIC and showed zones, which were equivalent to that of standard drug cefixime. While other organisms showed moderate activity as zone sizes were smaller in comparison to standard. Cefixime zinc complex showed activity against *Pseudomonas aureginosa*, at 64 and 32 μg/ml concentrations only that even higher MIC value which was further confirmed by the smaller zone sizes.

Whilst in cefixime cadmium complex organisms *Escherichia coli*, *Staphylococcus aureus* and *Proteus mirabillus* as shown in table 4 were susceptible at higher MIC which was found 64 and 32 μg/ml. Zone sizes for *Escherichia coli* were small as compared to standard but with *Staphylococcus aureus* and *Klebsiella aureginosa*; they were more smaller and were at lower zone limit; though for *Klebsiella aureginosa* MIC was lower, organisms showed susceptibility at 64, 32 and 16 μg/ml. *Pseudomonas aureginosa* showed resistance and higher MIC values were observed.

The antimicrobial susceptibilities of these cefixime copper, zinc and cadmium complexes against various organisms are given in table 5 and are compared with the standard cephalosporin. These results further reveal that formation of complexes results in decrease in antimicrobial activity of cefixime and MIC values were increased. Consequently, it is suggested that cefixime should not be co-administered with preparations containing these essential and trace elements.

Table 1
Antimicrobial activity of cefixime

Organism	Time (Hrs)	Concentration μg/ml					
		64	32	16	12	10	8
Escherichia coli	48	S	S	S	S	S	S
	72	S	S	S	S	S	S
Staphylococcus aureus	48	S	S	S	S	S	S
	72	S	S	S	S	S	S.S
Proteus mirabillus	48	S	S	S	S	S	S
	72	S	S	R	R	R	R
Klebsiella aureginosa	48	S	S	S	S	S	S
	72	S	S	R	R	R	R
Pseudomonas aureginosa	48	S	S	S.S	R	R	R
	72	R	R	R	R	R	R

Table 2
Antimicrobial activity of cefixime copper complex

Organism	Time (Hrs)	Concentration μg/ml					
		64	32	16	12	10	8
Escherichia coli	48	S	S	S	S	S	S.S
	72	S	S.S	S	R	S	R
Staphylococcus aureus	48	S	S	S	S	S	S.S
	72	S	R	S	R	S	R
Proteus mirabillus	48	S	S	S	S	S	S
	72	R	R	R	R	R	R
Klebsiella aureginosa	48	S	S	S	S	S	S
	72	R	R	R	R	R	R
Pseudomonas aureginosa	48	S	S	R	R	R	R
	72	R	R	R	R	R	R

S= susceptible; R = resistant; S.S = slightly susceptible; S.R = slightly resistant

Table 3
Antimicrobial activity of cefixime zinc complex

Organism	Time (Hrs)	Concentration μg/ml					
		64	32	16	12	10	8
Escherichia coli	48	S	S	S	S	S	S
	72	S	S	S	S	S	S
Staphylococcus aureus	48	S	S	S	S	S.R	S.R
	72	R	R	R	R	R	R
Proteus mirabillus	48	S	S	S	S	S	S
	72	R	R	R	R	R	R
Klebsiella aureginosa	48	S	S	S	S	S	S
	72	R	R	R	R	R	R
Pseudomonas aureginosa	48	S	S	S	S	R	R
	72	R	R	R	R	R	R

Table 4
Antimicrobial activity of cefixime cadmium complex

Organism	Time (Hrs)	Concentration μg/ml					
		64	32	16	12	10	8
Escherichia coli	48	S	S	S	S	R	R
	72	R	R	R	R	R	R
Staphylococcus aureus	48	S	S	S.R	R	R	R
	72	R	R	R	R	R	R
Proteus mirabillus	48	S	S	S.R	S.R	R	R
	72	R	R	R	R	R	R
Klebsiella aureginosa	48	S	S	S	S	S	S
	72	R	R	R	R	R	R
Pseudomonas aureginosa	48	S	S	R	R	R	R
	72	R	R	R	R	R	R

S= susceptible; R = resistant; S.S = slightly susceptible; S.R = slightly resistant

Table 5
Antimicrobial susceptibility of cefixime metal complexes

Zone of inhibition (mm) → Organisms ↓	Copper complex		Zinc complex		Cadmium complex	
	Sample	Standard	Sample	Standard	Sample	Standard
Escherichia coli	15.45 - 17.00	22.85 - 23.74	22.41 - 23.49	22.98 - 23.21	23.26 - 24.07	22.71 - 24.20
Staphylcoccus aureus (ATCC 25923)	13.90 - 15.04	13.85 - 21.45	12.31 - 13.62	19.21 - 19.69	09.97 - 13.99	15.27 - 15.77
Proetus mirabillus	28.10 - 29.61	29.21 - 20.62	24.60 - 25.03	21.20 - 26.92	24.02 - 25.10	17.76 - 25.20
Klebsilla aureginosa	19.55 - 22.91	29.93 - 19.55	14.96 - 15.13	18.39 - 23.99	18.14 - 19.02	18.35 - 23.76
Pseudomonas aureginosa	No Zone	No Zone	11.24 - 11.88	10.79 - 13.08	10.56 - 11.66	No Zone
Staphylcoccus aureus	13.90 - 15.04	13.99 - 18.57	13.50 - 16.43	18.48 - 21.20	11.02 - 12.03	18.76 - 19.92

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