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IN VITRO ACTIVITY OF CEFTIZOXIME AND CEFTAZIDIME IN PRESENCE OF ESSENTIAL AND TRACE ELEMENTS

M. SAEED ARAYNE, NAJMA SULTANA* AND KIRAN RAFIQ*
Department of Chemistry, University of Karachi, Pakistan
**Department of Pharmaceutical Chemistry, Research Institute of Pharmaceutical Sciences, Faculty of Pharmacy, University of Karachi, Pakistan*

ABSTRACT

The research work comprises of the study of the changes in antimicrobial activity of ceftizoxime and ceftazidime after interaction with essential and trace elements. The minimum inhibitory concentration (MIC) was observed and thereafter compared with the standard MIC's of the respective drug by agar dilution method against both Gram positive and Gram-negative organisms so as to evaluate changes in antimicrobiological activity of the standard cephalosporins after metal interactions.

It was observed that these metal elements that are essential for our body, either present within the body or coadminstered with vitamins markedly influence the MIC's of antibiotics by producing synergism or antagonism.

INTRODUCTION

Ceftizoxime is a third generation cephalosporin with a broad spectrum of activity; it is highly stable to hydrolysis by most β-lactamases. It has greater activity against Gram- negative organisms than first and second-generation cephalosporins. It is used in lower respiratory tract, urinary tract, intra-abdominal, skin, bone and joint infection as well as in the infections caused by mixture of organisms resistant to other cephalosporins, aminoglycosides or penicillins (Neu *et al.,* 1982). The most common adverse effects are hypersensitivity reactions including skin rashes, urticaria eosinophilia, fever, haemolytic anaemia and positive response to the Coomb's test (Neu 1982).

Ceftazidime is a third generation cephalosporin with an extended antimicrobial activity (Neu & Labthavikul 1981; Clumeck *et al.,* 1983; Quinn *et al.,* 1987; Ergova *et al.,* 2000; PoiatÄ*ƒ et al.,* 2002). It is used in the treatment of respiratory-tract infections (Varotto *et al.,* 2001), urinary tract, skin, bone and joint cystic fibrosis, and meningitis caused by *Haemophilus influenzae* and *Neisseria menengitidis* (Joel *et al.,* 1996). It has a higher activity (83% approximately; 79% susceptibility) against *Pseudomonas aeruginosa* (Xu *et al.,* 2000; Lambert *et al.,* 2001; Robinson *et al.,* 2001; Karako & GerÃ§eker 2001). It is recommended in the treatment of Peritonitis (Korzets & Lang 2001; Rusthoven *et al.,* 2001) and anti-HIV-1 activity has been observed *in vitro* of ceftazidime degradation products (Hobi *et al.,* 2001). However, it is found to be less active than other third generation agents against gram-positive cocci. Seizures may produce in patients receiving higher doses especially in renal impairment. It has potential for colonization and super infection with resistant organisms.

The present studies comprises of the study of changes in antimicrobial activity of two cephalosporins, ceftizoxime and ceftazidime after interaction with salts of essential and trace elements. The agar dilution method was adopted and the MIC was observed and thereafter compared with the standard MIC's of the respective drug against both Gram-positive and Gram-negative organisms so as to evaluate changes in microbiological activity of the standard cephalosporins after metal interactions.

It was concluded that metal elements that are essential for our body mechanism either present in the body or coadminstered with vitamins markedly influence the MIC's of antibiotics by producing synergism or antagonism.

EXPERIMENTAL

Materials

GlaxoWelcome Karachi gifted Ceftizoxime and Ceftazidime reference standards. The essential and trace elements used were in the form of their hydrated or anhydrous salts as magnesium chloride, calcium chloride, chromium chloride, manganese chloride, ferric chloride, cobalt chloride, nickel chloride, copper chloride, zinc chloride and cadmium chloride.

Various Gram-positive and Gram-negative organisms used were *Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli*, *Salmonella typhii*, *Proteus vulgaris* and *Staphylococcus epidermidis*.

Methods

I Preparation of Dilutions

Dilutions of different concentrations of both drug and metal salts were prepared in pH 5 sterilized buffer; (prepared by taking 0.1M sodium citrate and then adding 0.1M HCl to it until the pH was maintained) (Washington 1985). Required amount of each antimicrobial agent was calculated from its potency (92 & 94%) and 0.054 g and 0.053 g of ceftizoxime and ceftazidime were dissolved in 50 ml of pH 5 buffer individually so as to obtain 50 mg / 50 ml concentration.

According to the reported MIC's of each drug different dilutions were prepared as for ceftizoxime 128 to 2 µg/ml and for ceftazidime 256 to 4 µg/ml (Thomas 1988; Jaime & William 1991). Same procedure was adopted for preparing stock solutions and dilutions of metal salts i.e., 0.05 g was dissolved in 50 ml of pH 5 buffer to obtain 1 mg/ml solution and then dilutions were prepared accordingly from 256 to 4µg/ml (Ales 1997).

II Preparation of Mueller – Hinton Agar (MHA) and Nutrient Broth

34g of agar in 1 liter of deionized water was dissolved completely by heating on a boiling water bath then sterilized in an autoclave for 15 minutes at 15 psi (121°C) and cooled to 45–50°C. This was stored in a dry and tightly closed container at 15-25°C and protected from light. pH was 7.4 ± 0.2 at 25°C.

Nutrient broth was prepared by dissolving 8g in a liter of deionized water completely, autoclaved for 15 minutes at 121°C (15 psi) and stored in clean test tubes either screw capped or closed with cotton plugs at 15-25°C and protected from light, pH 7.0 ± 0.2 at 25°C. The density of viable cells in the inoculum is the most important variables that influence the results of susceptibility tests. In order to obtain reproducible results the inoculums density was carefully standardized by the using the McFarland turbidity standard (Lorian 1991). This was prepared by adding 0.5 ml of 0.048 M barium chloride ($BaCl_2$) to 99.5 ml of 0.35N sulfuric acid (H_2S0_4) and distributed into 4, 5 ml screw capped tubes of the same size for growing culture inoculation. The test tubes were tightly sealed and stored in the dark at room temperature. These turbidity standards were vigorously agitated on a vortex mixer just before use (Bertina & Wentworth 1987).

III Preparation of inoculums

The inoculum was prepared by touching the tops of 4 to 5 colonies of the same morphological type with a wire loop and suspending them in a tube containing 4-5 ml of nutrient broth. This was incubated at 35°C for 4–6 hours until the turbidity reached or exceeded that of 0.5 McFarland standard. If the turbidity exceeded, the suspension was diluted with broth until it was visually comparable to McFarland 0.5 turbidity standard (Snyder 1976).

IV Agar Dilution Susceptibility Test

The drug was incorporated in a liquefied agar medium (45–50°C), which was then after mixing, poured into a petri dish and allowed to solidify (Snyder 1976; Barry 1976). A series of petri dishes were prepared with increasing concentration of the drug (Steers 1959) and with the aid of a inoculating device, 8 different species of microorganisms were spotted and inoculated one by one on to each plate. After overnight incubation at 37°C, the MIC end point was read as the lowest concentration that completely inhibited growth (Barry 1976; Washington 1985; Ericson 1971).

V Preparation and Inoculation of Antimicrobial Plates

The different antimicrobial dilutions ranging from 256 to 2 μg/ml were added under aseptic conditions to the melted and cooled agar (45-50°C) in a ratio of 1 part antimicrobial agent to 9 parts medium (2 ml of drug to 18 ml of agar for each petri dish), the medium was then mixed gently. The contents were then poured into the appropriate number of petri dishes (Ericson 1971) and allowed to solidify without any disturbance. By the help of dispenser 6 different species of the microorganisms under aseptic conditions were inoculated as spots of 5-8 mm in diameter (Barry 1976; Ericson 1971), the plates were allowed to stand until the drug was absorbed and then these were incubated at 37°C for overnight. The MIC end point was read as the lowest concentration that inhibited bacterial growth.

VI Interaction of antibiotic with essential and trace element salts

Different dilutions of ceftizoxime and essential and trace element salts ranging in concentration from 128 to 2 μg /ml and for ceftazidime concentrations ranging from 256 to 4 μg /ml were prepared. The ratio of each antibiotic and metal/element salts solution was 3:1.

For this interaction, stock solution of each antibiotic was prepared in double concentration i.e., 100mg/50ml in order to have the same proportion of each antibiotic in respective antibiotic-trace element salt complexes. Accordingly same concentration was used in reference standards of each antibiotic solution. The two solutions of metal salt and antibiotic were mixed in two different sets and each heated separately at 37 and 60°C for 30 minutes (on water bath) and incorporated in a liquefied agar medium (45–50°C), which was then after mixing, poured into a petri dish and allowed to solidify. A series of petri dishes were prepared with increasing drug concentration (Joel *et al.,* 1996) and with the aid of a inoculating device, 6 different species of microorganisms were spotted and inoculated one by one on to each plate. After overnight incubation at 37°C, the MIC end point was read as the lowest concentration that completely inhibited growth (Washington 1985). This was compared with respective reference standard of each antibiotic by observing the change in MIC.

RESULTS AND DISCUSSION

The results of (MIC's) of reference drugs i.e. ceftizoxime and ceftazidime against various

Gram-positive and Gram-negative organisms are given in table 1. They are also compared with the reported values. In the current research work the MIC's of ceftizoxime and ceftazidime were found to increase against *S. aureus, E. coli, S. typhii* and *Pr. Vulgaris* up to 128µg/ml than the reported values because of increased resistance of organisms as we used isolated strains from blood and urine.

All the metal salts were found to be inactive or resistant. Different synergetic and antagonistic effects of drug metal complexations were observed and compared with standard drug after the interaction.

I Ceftizoxime

In the interaction reactions of ceftizoxime with the metal elements a wide range of variable annotations were observed (table 2 & 3) at both temperatures. The interaction with magnesium chloride led to the synergetic effect due to decrease in MIC against *S. aureus, E. coli, S. typhii, Pr. vulgaris* and *S. epidermidis* at both the temperatures. The presence of calcium chloride and chromium chloride produced similar effects but only against *S. aureus* and *S. epidermidis* in the former and against *E. coli* and *Pr. vulgaris* in the later case. Furthermore, resistance was exhibited by *S. aureus* and *S. typhii* against the later.

Ceftizoxime has produced antagonistic effect due to rise in MIC after interaction with manganese chloride against *S. faecalis*, *E. coli* and *Pr. vulgaris* at 37^{o}C while *S. aureus* and *S. epidermidis* were inhibited at lower concentrations at 60^{o}C thus leading to synergetic effect. Antagonism was exhibited against *E. coli* at 37^{o}C due to the presence of iron chloride while synergetic behavior was prominent against *S. aureus*, *S. typhii*, *S epidermidis* and *E. coli* with iron chloride and nickel chloride at both the temperatures.

The interaction of drug with cobalt has produced synergism against *S. epidermidis, S. aureus* and *S. typhi* at both the temperatures whereas antagonism was exhibited against *Pr. vulgaris* at 60^{o}C. The presence of nickel chloride at 37 and 60^{o}C has displayed synergetic effects against *S. aureus*, *E. coli*, *S. typhii* and *S. epidermidis* whereas at 60^{o}C antagonist effect was exhibited by *S. aureus* and *S. faecalis*. When the drug was reacted with copper chloride at both the temperatures synergetic behavior was found against *S. aureus*, *E. coli*, *S. typhii* and *S. epidermidis*.

In the interaction reaction between drug and zinc chloride at 37^{o}C synergism was noted against *S. aureus* and *Pr. vulgaris* while antagonism was shown against *S. faecalis* and *E. coli*, wheras reaction with cadmium chloride has caused decrease in values of MIC of the drug against *S. aureus*, *E. coli*, *S. typhii* and *S. epidermidis* at both 37 and 60^{o}C.

II Ceftazidime

The interaction of ceftazidime with the trace elements has also led to the variation in the efficacy of drug, as show in table 5 & 6. When the drug was reacted with calcium chloride and magnesium chloride at 37°C and 60°C, the synergetic effect was found due to decrease in MIC against *E. coli.*

The presence of chromium chloride thus caused synergism against *E.Coli* and *S. typhi* at 37° C and also against *Pr. vulgaris* at 60^{o}C. The interaction of drug with manganese chloride, iron chloride and cobalt chloride has led to synergism against *S. aureus, Pr. vulgaris* and *S. epidermidis* at both the temperatures. Nickel chloride has caused decrease in MIC's thus resulting in synergism against *Pr. vulgaris* and *S. epidermidis* at both the temperatures.

Copper chloride changed the efficacy of drug producing synergism against *E. coli* and *S. typhii,* while antagonism against *S. faecalis* and *S. epidermidis* was observed at 37 and 60°C. Interaction of the drug with zinc chloride and cadmium chloride at both the temperatures produced synergetic effect against all remaining organisms except *S. faecalis* due to decrease in MIC's.

It is concluded that these *in vitro* interactions may affect the pharmacokinetics of cephalosporins (NyhlÃ©n *et al.,* 2001) and hence a basic understanding of the drug interaction of ceftizoxime and ceftazidime with essential and trace elements is required in order to coadminister these drugs simultaneously (LÃpezDÃaz *et al.,* 2001).

Table 1
MIC's (µg/ml) of reference cephalosporins against various organisms

Organisms	Ceftizoxime		Ceftazidime	
	Reported	Observed	Reported	Observed
Staphylococcus aureus	2-4	128	4-8	256
Streptococcus faecalis	–	2	–	4
Escherichia coli	0.03	32	0.1	128
Salmonella typhii	0.25	64	0.25	256
Proteus vulgaris	0.06	32	0.1	256
Staphylococcus epidermidis	–	128	-	256

Table 2
MIC's of ceftizoxime when reacted with essential and trace elements
at 37°C against various microorganisms

Organisms	MICs (µg/ml)									
	Mg	Ca	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd

Staphylococcus aureus	16	32	R	128	16	128	32	16	64	8
Streptococcus faecalis	2	2	2	64	2	2	2	2	16	2
Escherichia coli	4	32	64	128	64	64	8	16	64	2
Salmonella typhii	4	R	R	R	16	64	2	16	64	2
Proteus vulgaris	4	32	128	64	16	64	32	32	16	32
Staphylococcus epidermidis	4	32	128	128	16	16	64	32	128	64

Table 3
MIC's of ceftizoxime when reacted with essential and trace elements
at 60°C against various microorganisms

Organisms	MICs (µg/ml)									
	Mg	Ca	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd
Staphylococcus aureus	16	64	R	32	32	32	128	16	16	8
Streptococcus faecalis	2	2	2	32	2	2	2	2	8	2
Escherichia coli	2	32	64	128	32	32	2	16	64	2
Salmonella typhii	2	R	R	R	8	32	2	16	16	2
Proteus vulgaris	64	32	64	32	16	16	16	32	16	8
Staphylococcus epidermidis	64	32	128	16	8	8	16	16	128	64

Table 4
MIC's of ceftazidime reacted with essential and trace elements
at 37°C against various microorganisms

Organisms	MICs (µg/ml)									
	Mg	Ca	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd

Staphylococcus aureus	R	256	R	128	128	128	256	256	128	128
Streptococcus faecalis	4	4	4	4	256	128	4	64	4	4
Escherichia coli	4	64	4	R	128	128	128	64	64	16
Salmonella typhii	256	R	4	R	64	128	256	64	128	4
Proteus vulgaris	R	256	256	16	4	4	4	256	32	32
Staphylococcus epidermidis	R	R	R	128	4	4	4	64	128	64

Table 5
MIC's of ceftazidime reacted with essential and trace elements
at 60°C against various microorganisms

Organisms	MICs (µg/ml)									
	Mg	Ca	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd
Staphylococcus aureus	R	256	R	128	128	64	128	128	128	128
Streptococcus faecalis	4	4	4	4	256	128	4	64	4	4
Escherichia coli	4	32	4	R	128	64	32	64	32	16
Salmonella typhii	256	R	4	R	32	128	128	16	32	4
Proteus vulgaris	R	256	128	16	4	4	4	64	64	32
Staphylococcus epidermidis	R	R	R	128	64	4	4	16	16	32

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