ORIGINAL ARTICLE

IN VITRO ACTIVITY OF CEFADROXIL, CEPHALEXIN, CEFATRIZINE AND CEFPIROME IN PRESENCE OF ESSENTIAL AND TRACE ELEMENTS

NAJMA SULTANA AND M. SAEED ARAYNE*

Department of Pharmaceutical Chemistry, Research Institute of Pharmaceutical Sciences, Faculty of Pharmacy, University of Karachi, Karachi-75270, Pakistan

**Department of Chemistry, University of Karachi, Karachi-75270, Pakistan*

ABSTRACT

Evidences supporting the introduction of metallic elements in several biological processes are rapidly accumulating. Likewise, many drugs possess modified toxicological and pharmacological properties when in the form of metal complexes. In order to ascertain the role of various essential and trace element complexation on the antibacterial activity of various cephalosporins, the synergistic or antagonistic behavior of cefadroxil, cephalexin, cefatrizine and cefpirome in presence of essential and trace elements has been studied and compared with the parent drug. The essential and trace elements comprised of magnesium, calcium, chromium, manganese, ferric, cobalt, nickel, copper, zinc and cadmium in the form of their chloride. These studies were carried out by observing the minimum inhibitory concentration (MIC) using agar dilution method and compared with the MIC'S of the standard cephalosporins against various species of Gram (+) and Gram (–) microorganisms such as *Staphylococcus aureus***,** *Staphylococcus epidermidis, Streptococcus faecalis, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi* and *Shigella dysenteriae.* Different dilutions of cephalosporins and salts of essential and trace elements were used in these studies. The ratio of the drug and metal salts was 1**:**1 and the reactions were carried out at two different temperatures as 37^{o}C and 60^{o}C in order to study the complex formation.

The aim of our study was on one hand to evaluate the changes in microbiological activity of the standard cephalosporins after *in vitro* metal interactions to study the synergetic or antagonistic behavior of the later through the difference in MICs values of these cephalosporins and on the other hand to access the bioassay directed extent of drug metal complexations. Our investigation reveal that interaction of above cephalosporins with essential and trace elements cause antagonistic effect in many cases which was shown by decrease in antimicrobial activity of cephalosporins and MIC values were increased.

Keywords: Cefadroxil; cephalexin; cefatrizine; cefpirome; metal interactions; essential and trace elements.

INTRODUCTION

Cephalosporins, astride of penicillins comprise of most widely used β-lactam antibiotics. They can be modified at a number of positions; alterations at C-3 position tend to affect the pharmacokinetic and metabolic properties, while modifications at C-7 yield enhanced β-lactamase stability especially for the cephalosporinases of certain *bacteriodes* (O'Grady *et al*., 1997). Cephalosporins may be classified by their chemical structure, clinical pharmacology, resistance to β-lactamase or antimicrobial spectrum, but the well accepted system of classification by generation is very useful and it is based on general features of antimicrobial activity (Karchmer, 1995).

Cefadroxil is a first-generation semi-synthetic cephalosporin with a broad antibacterial spectrum and a high chemotherapeutic potential when administered orally. The inhibitory activity of this compound was similar to that of cephalexin and cephradine when tested against 602 clinical isolates on Mueller-Hinton medium. In the oral treatment of experimental infections of mice, cefadroxil was more effective than cephalexin against *Streptococcus pyogenes*, and comparably effective against *Streptococcus pneumoniae, Staphylococcus aureus*, and several Gram-negative species. In regard to other properties which were investigated, the behavior of cefadroxil compared favorably to that of cephalexin (Buck and Price 1980). This may be because of similarity in structure as it is *p*-hydroxy derivative of cephalexin (fig. 1).

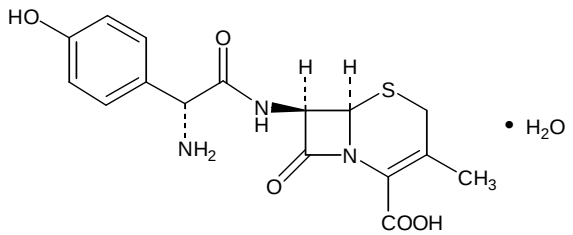

Fig. 1

Corresponding author: Email: lab9@gawab.com

Cephalexin is a first-generation cephalosporin and has good activity against Gram-positive bacteria and relatively modest activity against Gram-negative microorganisms. Many Gram-positive microorganisms release relatively large amounts of β-lactamase into the surrounding medium which can destroy the β-lactamic antibiotics by hydrolysis of the β-lactam ring; this is the most prevalent mechanism of resistance (O'Grady *et al*., 1997 and Karchmer, 1995). The structure of cephalexin is shown in fig. 2.

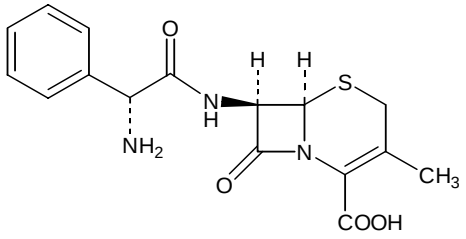

Fig. 2

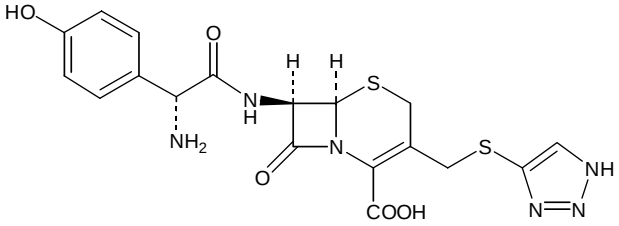

Fig. 3

Cefatrizine (fig. 3) has excellent activity against Gram-positive cocci, inhibiting all except enterococci at minimal inhibitory concentrations below 1 µg/ml. Cefatrizine inhibited the majority of *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis*, and *Salmonella* at concentrations below 12.5 µg/ml. Although cefatrizine was not hydrolyzed by many ß-lactamases, it did not inhibit a number of strains of *Enterobacter, Serratia*, or indole-positive *Proteus*. Cefatrizine was more active than cephalothin or cephalexin against *E. coli, Klebsiella, Enterobacter, Citrobacter, Salmonella*, and *Shigella*. Its overall activity was less than that of cefoxitin against strains resistant to cephalothin, but its activity against cephalothin-susceptible strains was equivalent to that of cefamandole **(**Harold and Kwung 1979).

Burno *et al* (Burno *et al*., 1990) examined the absorption kinetics of cefatrizine. Due to the innovative feature of the model incorporating the Michaelis-Menten equation, simulations of the effect of altering the model parameters and the dose administered on the concentration-time profile, were performed. The observation of saturable absorption kinetics was consistent with a carrier-mediated transport previously reported to occur in the gastrointestinal tract of rats.

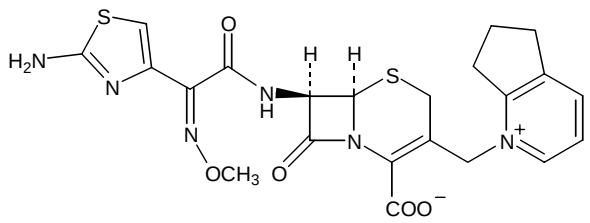

Fig. 4

Cefpirome (fig. 4) is an injectable extended-spectrum or 'fourth generation' cephalosporin. Its antibacterial activity encompasses many of the pathogens involved in hospital-acquired infections such as *Enterobacteriaceae*, methicillin-susceptible *Staphylococcus aureus*, coagulase-negative *staphylococci* and viridans group *streptococci*. Cefpirome also has *in vitro* activity against *Streptococcus pneumoniae* regardless of penicillin susceptibility. It is stable against most plasmid- and chromosome-mediated β-lactamases, with the exception of the extended-spectrum plasmid-mediated SHV enzymes. cefpirome has shown clinical efficacy comparable to that of ceftazidime in the treatment of hospitalized patients with moderate to severe infections. Clinical response and bacteriological eradication rates were similar in patients with severe pneumonia or septicaemia treated with either cefpirome or ceftazidime. Cefpirome appeared more effective than ceftazidime in the eradication of bacteria in patients with febrile neutropenia in 1 study; however, clinical response rates were similar in the 2 treatment groups. The tolerability of cefpirome appears similar to that of ceftazidime and other third generation cephalosporins, diarrhoea being the most frequently observed event. Thus, cefpirome is likely to be a valuable extended-spectrum agent for the treatment of severe infections. Cefpirome offers improved coverage against some Gram-positive pathogens and *Enterobacteriaceae* producing class I β-lactamases compared with the third generation cephalosporins, although this has yet to be demonstrated in clinical trials (Wiseman and Lamb, 1997). Antimicrobial activity and MIC of cefpirome had been tested and compared with other cephalosporins by a number of workers (Jones *et al*., 1991; Spangler *et al*., 1994).

There are number of reported drug interactions of cephalosporins with essential and trace elements (Ahmed *et al*., 2000; Arayne et. al., 2001; Arayne et. al., 2006; Arayne et. al., 2007; Deppermann *et al*., 1989; Domenico *et al*., 1992; Sultana et. al., 2003; Sultana et. al., 2005; Thomas and Burns 1998). The aim of the present study was to evaluate the changes in antimicrobial activity of cefadroxil, cephalexin, cefatrizine and cefpirome after interaction with salts of essential and trace elements at 37 and 60°C by observing changes in MIC values, determined by agar dilution susceptibility test method.

MATERIALS AND METHODS

Materials

Cefadroxil and cefatrizine were a kind gift from Bristol-Meyers-Squibb, cephalexin from Eli-Lilly and cefpirome from Hoechst Marion Roussel Ltd Karachi. Isolate strains of different Gram (+) and Gram (-) organisms used in these studies were *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus faecalis, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi and Shigella dysenteriae.*

Interaction of antimicrobial agents with essential and trace elements

Different dilutions of each antimicrobial agent and essential and trace element salts in concentration from 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25 and 0.125µg/ml (for antibiotic dilutions 2 concentrations i.e. 0.25 and 0.125µg/ml were prepared only in case of cefpirome) were prepared according to the reported MIC values. The ratio of each antibiotic and metal/element salts solution were 1:1 and the reaction were carried out at two different temperatures i.e., 37°C and 60°C for 30 minutes on water bath. The

Table 1: MIC of reference cephalosporins

Organisms	MIC (µg/ml)			
	cefadroxil	cephalexin	cefatrizine	cefpirome
Staphylococcus aureus	4	4	1	0.5
Staphylococcus epidermidis	8	8	1	4
Streptococcus faecalis	32	R	R	32
Escherichia coli	8	8	4	1
Proteus vulgaris	R	R	32	2
Pseudomonas aeruginosa	R	R	R	8
Salmonella typhi	16	4	8	0.25
Shigella dysenteriae	4	4	2	0.25

Table 2: MIC(µg/ml) of cefadroxil when reacted with essential and trace elements

Organisms	Mg	Ca	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd
Staphylococcus aureus	16(64)	8(32)	16(64)	16(16)	8(32)	8(32)	8(8)	8(32)	8(32)	16(32)
Staphylococcus epidermidis	32(R)	32(R)	32(R)	32(64)	16(32)	16(32)	8(16)	16(32)	16(16)	16(32)
Streptococcus faecalis	64(R)	R(R)	64(R)	64(R)	64(R)	64(64)	64(64)	32(64)	32(32)	32(64)
Escherichia coli	32(64)	64(64)	32(64)	16(32)	32(64)	16(32)	8(16)	32(64)	16(32)	32(64)
Proteus vulgaris	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)
Pseudomonas aeruginosa	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)
Salmonella typhi	32(64)	32(64)	32(64)	16(64)	32(32)	16(32)	16(16)	16(32)	16(32)	32(64)
Shigella dysenteriae	16(32)	8(32)	16(32)	16(16)	16(32)	16(32)	8(16)	16(32)	8(16)	8(16)

When reacted at 37°C; Values in parenthesis are when reacted at 60°C.

Table 3: MIC(µg/ml) of cephalexin when reacted with essential and trace elements

Organisms	Mg	Ca	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd
Staphylococcus aureus	16(32)	8(16)	16(32)	8(32)	8(32)	4(8)	8(32)	8(16)	16(32)	8(32)
Staphylococcus epidermidis	64(64)	16(32)	32(64)	16(32)	16(64)	16(32)	8(32)	16(64)	16(32)	8(64)
Streptococcus faecalis	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)
Escherichia coli	32(64)	16(32)	32(64)	16(64)	16(32)	16(64)	16(32)	32(R)	16(64)	32(64)
Proteus vulgaris	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)
Pseudomonas aeruginosa	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)
Salmonella typhi	16(32)	16(32)	8(32)	16(32)	16(32)	8(16)	4(8)	4(16)	8(16)	8(16)
Shigella dysenteriae	16(32)	8(16)	8(64)	16(32)	8(16)	32(32)	8(16)	8(32)	16(32)	16(16)

When reacted at 37°C; Values in parenthesis are when reacted at 60°C

Table 4: MIC(µg/ml) of cefatrizine when reacted with essential and trace elements

Organisms	Mg	Ca	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd
Staphylococcus aureus	4(16)	2(8)	4(32)	16(32)	4(16)	4(16)	2(16)	4(8)	8(32)	4(16)
Staphylococcus epidermidis	2(8)	4(4)	4(8)	2(4)	1(2)	4(4)	2(4)	4(4)	4(4)	4(4)
Streptococcus faecalis	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)
Escherichia coli	16(64)	16(32)	64(R)	32(64)	16(64)	8(64)	32(64)	8(32)	8(32)	16(64)
Proteus vulgaris	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)
Pseudomonas aeruginosa	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)	R(R)
Salmonella typhi	32(32)	16(64)	16(64)	16(32)	16(64)	16(32)	16(64)	16(64)	32(64)	32(64)
Shigella dysenteriae	4(16)	16(32)	16(32)	8(16)	8(32)	8(32)	8(16)	8(32)	4(16)	8(32)

When reacted at 37°C; Values in parenthesis are when reacted at 60°C

Table 5: MIC(µg/ml) of cefpirome when reacted with essential and trace elements

Organisms	Mg	Ca	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd
Staphylococcus aureus	4(32)	4(8)	4(8)	4(16)	2(4)	8(32)	2(8)	8(32)	2(8)	4(16)
Staphylococcus epidermidis	8(16)	8(16)	8(8)	8(8)	4(8)	16(32)	8(16)	8(64)	8(32)	4(8)
Streptococcus faecalis	32(32)	32(64)	32(64)	32(32)	32(32)	32(64)	64(64)	R(R)	32(R)	32(64)
Escherichia coli	1(1)	4(8)	4(8)	2(4)	2(4)	2(16)	4(8)	4(16)	2(8)	2(16)
Proteus vulgaris	8(8)	4(16)	8(32)	16(32)	16(16)	8(32)	32(R)	16(64)	8(32)	4(16)
Pseudomonas aeruginosa	32(64)	32(R)	16(16)	32	16(32)	32(R)	32(64)	16(64)	32(16)	32(64)
Salmonella typhi	2(8)	1(2)	1(8)	0.5(1)	1(1)	2(8)	1(4)	2(16)	1(4)	0.5(2)
Shigella dysenteriae	1(2)	1(2)	1(2)	0.5(2)	0.5(1)	2(4)	1(2)	0.5(2)	2(4)	0.5(1)

When reacted at 37°C; Values in parenthesis are when reacted at 60°C

complex formation of each antibiotic with essential and trace elements resulted in changes in the MIC values of the antibiotic.

Standardization and preparation of inoculum

The density of viable cells in the inoculum is most important variable that influence the results of susceptibility tests. To obtain reproducible results the inoculum density was carefully prepared and standardized (Lorian 1991) by McFarland 0.5 turbidity standard (Bertina and Wentworth 1987).

Susceptibility tests

To determine the MIC of the antibiotics, agar dilution technique was used and the drug was incorporated in a liquefied agar medium (45 – 50^{o}C), which was then mixed and poured into a petri dish and allowed to solidify (Snyder *et al*., 1976). A series of petri dishes were prepared with increasing concentration of the drug and with the aid of a inoculating device, eight different species of microorganisms were inoculated one by one on to each plate. After overnight incubation at 37^{o}C, the MIC end point was observed as the lowest concentration that completely inhibits growth, disregarding a single colony or growth (Washington, 1985).

RESULTS AND DISCUSSION

There have been numerous reports that cephalosporins bind with essential and trace elements to form metal chelates (Anacona and Rodriguez 2004; Chohan and Jaffery 2000). These studies have shown involvement of the α-amino group present C-7 substitution and the carboxylic group at C-3 or the participation of carboxylic group at C-3 and bridgehead nitrogen between dehydrothiazine and β-lactam forming a six membered metal chelate (Sultana *et al*., 2003; Sultana *et al*., 2005). These complexes have shown a square planar or octahedral geometry.

Since the presence of complexing ligand may not only affect the bioavailability of essential and trace elements in the blood or tissues but also the efficacy of the antibiotic; therefore, in order to study the probable interaction of cefadroxil, cephalexin, cefatrizine and cefpirome with essential and trace elements present in human body, these cephalosporins were reacted with magnesium, calcium, chromium, manganese, ferric, cobalt, nickel, copper, zinc and cadmium in the form of their chlorides, at human body temperature (37°C) and at accelerated temperature (60°C) and changes in antimicrobial activities with reference to parent drugs were investigated against

various species of Gram positive and Gram negative microorganisms such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus faecalis, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhi and Shigella dysentery*.

The agar dilution method was employed for determining the susceptibility of individual cephalosporins taken as reference standard as shown in table 1 as well as by interaction with essential and trace elements at two different temperatures (37°C and 60°C) as given in *table*s 2-5 and observed the synergetic or antagonistic changes by measuring the differences in MIC values. The lowest concentration that completely inhibited bacterial growth was taken as MIC. This is the most common measure for bacterial sensitivity and reflects only results after overnight incubation and do not account for interactions occurring between the time of inoculation and the final reading.

Cefadroxil, the first generation orally active semisynthetic cephalosporin, available as monohydrate or trihydrate is highly active against Gram positive *cocci* and because of its mode of action has a slow bactericidal action to Gram negative *bacilli* (Akimoto *et al*., 1994; Casewell and Bragman 1987; Stromberg *et al*., 1987). Comparative studies of cefadroxil alone and after interaction with essential and trace elements salts at 37°C and accelerated temperature at 60°C (table 2) clearly shows a extraordinary change in MIC values and reveal antagonistic behavior.

Metal complexes of cephalexin and cephradine with essential elements have been extensively studied. Cephalexin is first generation orally active semi-synthetic cephalosporin supplied as monohydrate. Its mode of action is very much similar to that of other first generation cephalosporins against Gram positive *cocci* and to some extent Gram negative *bacilli* (Spangler *et al*., 1994). The comparative studies of cephalexin after interaction with essential and trace elements salts at body temperature (37°C) and accelerated temperature (60°C) are given in table 3, which clearly show antagonistic effect of interaction of metal salts by the increase in MIC values in most of the reactions.

Cefatrizine categorized as second generation semi-synthetic cephalosporin has approximately the same spectrum as that of cephalexin. A wide strain variations in susceptibility have been reported; activity is affected by medium composition and inoculum size (Pfeffer *et al*., 1983). The MIC values of cefatrizine after interaction with essential and trace element salts at 37°C and accelerated temperature (60°C) are given in table 4, which also shows similar antagonistic behavior of essential and trace elements.

Anacona and Rodriguez (Anacona and Rodriguez 2004) proposed a structure (fig. 5) of cephalexin metal complexes showing the involvement of α-amino group of acyl side chain in metal complexation. Cefadroxil, cephalexin and cefatrizine, all contain a α-amino group in the acyl side chain which may possible be involved in metal chelation, which has resulted in the antagonistic behavior of these cephalosporins.

Cefpirome, is a fourth generation semi-synthetic injectable cephalosporin. Its spectrum includes Gram negative organisms e.g. *Ps. aeruginosa* as well as Gram positive organism that makes it suitable for the empiric treatment for severe infections (Mitsukude *et al*., 1989). The comparative studies data of cefpirome after interaction with essential and trace element salts at both temperatures is given in table 5, which also shows an antagonistic effect of these elements on cefpirome activity by the increase in MIC values in most of the reactions. In Cefpirome the primary α-amino group of acy side chain has been replaced with a tertiary nitrogen, which again reveal similar results as outlined in earlier three cases.

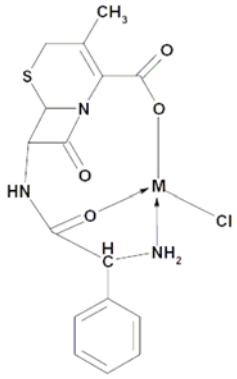

Fig. 5: Tentative structure of the cephalexin metal complexes (Anacona and Rodriguez, 2004).

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