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# ROXITHROMYCIN ANTAGONISM WITH ESSENTIAL AND TRACE ELEMENTS

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**ABSTRACT**

In order to ascertain the role of various essential and trace element complexation on the antibacterial activity of various macrolide antibiotics, the synergistic or antagonistic behavior of roxithromycin metal complexes have been studied and compared with the parent drug. Metal complexes of roxithromycin with magnesium, calcium, chromium, manganese, iron, cobalt, nickel, copper, zinc and cadmium have been investigated for their antibacterial activity and compared with roxithromycin by observing the changes in minimum inhibitory concentration (*MIC*) and by measuring the zone of inhibition of complexes against both Gram-negative and Gram-positive microorganisms. Various microorganisms used were *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Salmonella typhi, Proteus vulgaris, Shigella dysentery, Klebsiella pneumoniae and Staphylococcus epidermidis.* For *MIC* observation, serial dilution method was employed and zone sizes were determined by diffusion disk method.

Our investigations divulge that formation of roxithromycin complexes results in antagonistic behavior against few microorganisms where in *MIC* is increased with respect to the parent drug, while against most of the microorganism, there was no effect on the *MIC* of the roxithromycin metal complexes with respect to parent roxithromycin.

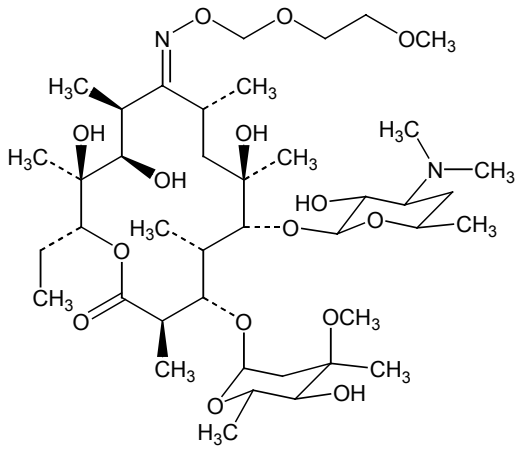
## INTRODUCTION

Roxithromycin is erythromycin 9-{O-((2-methoxyethoxy) ethyl) oxime} (**"**RULID" 1998; British Pharmacopoeia 2002) or erythromycin 9-{O-(2-methoxyethoxy)methyl)oxime}; oxacyclotetradecane erythromycin derivative-9-(2',5'-dioxa-hexyloxyimino)erythromycin (Merck Index 2000); or 3R,4S,5S,6R,7R,9R,11S,12R,13S,14R) -4-[(2,6-dideoxy-3-C,3-O-dimethyl-α-L-ribo-hexopyranosyl)oxy]-14-ethyl-7,12,13-trihydroxy-10-[(E)-[(2-ethoxyethoxy)methoxy]imino]-3,5,7,9,11,13-hexamethyl-6-[(3,4,6-trideoxy-3-dimethylamino-β-D-xylo-hexopyranosyl)oxy] oxa-cyclotetradecan-2-one, $C_{41}H_{76}N_2O_{15,}$ molecular weight 837.1 and has the following structure (British Pharmacopoeia 2002).

Roxithromycin like that of erythromycin contains a 14-member lactone ring to which two sugar residues are attached. It is unique amongst the macrolide antibiotics in having an ether oxime grouping linked to its lactone ring. This unique substituent confers favorable pharmacokinetic properties as compared to the parent and henceforth an activity similar but much more *in vivo* is observed (Merck Index, 2000).

Roxithromycin exerts its bacteriostatic antibacterial action in the same manner as erythromycin, and clarithromycin (Florey, 1996). The antibacterial activity of roxithromycin has extensively been studied against a number of Gram-negative and Gram-positive microorganisms, and compared with those of other macrolides. Methicillin-resistant *Staphylococci*, which are usually resistant to macrolides are inhibited by roxithromycin at readily achievable tissue levels

(Markham & Faulds 1994; Williams & Sefton 1993; Nalin *et al.,* 1995; Ploy *et al.,* 1994; Bauernfeind *et al.,* 1990; Garcia-Rodriquez *et al.,* 1993; Forsgren & Walder 1994; Rampianesi *et al.,* 1993; Shinichi *et al.,* 1992; Loza *et al.,* 1992; Koji and Keiko 1996; El Mostafa *et al.,* 1995; Jenes *et al.,* 1998; Dianne *et al.,* 1999). The pharmacokinetics of roxithromycin has also extensively been reviewed (Markham & Faulds 1994; Portier *et al.,* 1994; Birkett *et al.,* 1990).




Roxithromycin

There are several interactions of roxithromycin reported in the literature (Huynh *et al.,* 2002; Bertho *et al.,* 1998; Lauby *et al.,* 1996; Rosensteil & Adam 1995). These include *in vivo* interactions like interactions at peptidyl transferase center (Schlunzen *et al.,* 2001), in respiratory tract infections (Zhanel *et al.,* 2001), with neutrophils (Noma *et al.,* 1998; Anderson *et al.,* 1996; Tamaoki *et al.,* 1996; Ras *et al.,* 1992), its effect on tumor necrosis (Koch *et al.,* 2000; Oyama *et al.,* 2000), on cytochrome P-450 (Bertho *et al.,* 1998; Delaforge *et al.,* 1992) and pharmacokinetic interactions (Periti *et al.,* 1992). Other drug interactions include interaction with ciprofloxacin (Rozenberg-Arska *et al.,* 1989), phospholipids (Montenez *et al.,* 1999; Feldman *et al.,* 1997), propton pump inhibitors (Kees *et al.,* 2000; Klotz 2000; Uygan *et al.,* 1999; Malizia *et al.,* 1998), cyclosporin (Billaud *et al.,* 1990; Moravek 1990), digoxin (Bizjak & Mauro 1997; Corallo & Rogers 1996), warfarin (Paulsen *et al.,* 1988), phenytoin (al-Humayyd 1997), clofazimine (Roman *et al.,* 1989), theophylline (Honma 1996; Hashiguchi *et al.,* 1992; Upton 1991), carbamazepine (Saint-Salvi *et al.,* 1987), phentoxifylline (Hand & Hand 1995), midazolam (Backman *et al.,* 1994; Mattila *et al.,* 1993), oral contraceptives (Meyer *et al.,* 1990; Archer & Archer 2002; de la Rey et. al. 1993) and phagocytes (Carlone *et al.,* 1991). There have been no roxithromycin-metal interactions reported in the literature except a report on the formation of cytochrome P-450 iron II nitrosoalkane complex. There are however, interactions of erythromycin reported with antacids containing di- and trivalent metal cations (Arayne & Sultana, 1993).

Present studies comprise of antibacterial studies of metal complexes of roxithromycin with magnesium, calcium, chromium, manganese, ferric, cobalt, nickel, copper, zinc and cadmium and changes in microbiological activity of the parent roxithromycin 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 roxithromycin against both Gram-negative and Gram-positive microorganism. For *MIC* observation, serial dilution method was employed and zone sizes were determined by diffusion disk method.

## EXPERIMENTAL

***Materials***

Roxithromycin metal complexes (Table-1) used for antibacterial studies were synthesized in Lab-9 of the department of chemistry, University of Karachi. The synthesis and characterization of these complexes are reported elsewhere.

The organisms used in the antimicrobial studies of roxithromycin metal complexes were *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Salmonella typhi, Proteus vulgaris, Shigella dysentery, Klebsiella pneumoniae* and *Staphylococcus epidermidis.* Media was Muller-Hinton agar and broth. Routine laboratory chemicals like barium chloride, sulfuric acid, sodium carbonate, mercuric chloride, sodium citrate and hydrochloric acid, organic polar and non-polar solvents; de-ionized water and pH 2 buffer solutions were used in these experiments.

**Table 1**
Roxithromycin metals complexes

| S. No. | Code | Complex | M.P. °C |
|---|---|---|---|
| 1 | Rox | Roxithromycin | 118 |
| 2 | Rox-Mg | Roxithromycin magnesium | 150 |
| 3 | Rox-Ca | Roxithromycin calcium | 120 |
| 4 | Rox-Cr | Roxithromycin chromium | 158 |
| 5 | Rox-Mn | Roxithromycin manganese | 98 |
| 6 | Rox-Fe | Roxithromycin ferric | 170 |
| 7 | Rox-Co | Roxithromycin cobalt | 90 |
| 8 | Rox-Ni | Roxithromycin nickel | 96 |
| 9 | Rox-Cu | Roxithromycin copper | 105 |
| 10 | Rox-Zn | Roxithromycin zinc | 100 |
| 11 | Rox-Cd | Roxithromycin cadmium | 162 |

***Methods***

1. *Preparation of pH 2 buffer solution*

Citrate buffer of pH 2 was prepared by mixing 300 ml of 0.1M sodium citrate solution and 150 ml of 0.1M hydrochloric acid in a liter beaker and the final pH was adjusted by either of the two solutions. This buffer was sterilized by autoclaving at 121°C and at 15 psi pressure for 15 minutes.

2. *Preparation of roxithromycin solutions*

The stock solution of roxithromycin was prepared by dissolving 0.025 gram of drug in distilled ethanol in a 25 ml volumetric flask and the final volume was made up with the same solvent. Aliquots were diluted between 0.25 μg/ml to 1 μg /ml to give the required concentrations of 0.5, 1, 2, 4, 8, 16, 32, 64 and 128 μg/ml.

For subsequent dilutions of drug highest concentration was taken first i.e., 128 μg/ml was made by diluting 6.4 ml of stock solution in a 50ml volumetric flask up to the mark with the buffer of pH 2. Aliquots of 64, 32, 16, 8, 4, 2, 1 and 0.5 μg/ml concentrations were prepared by serially diluting the later solution in the same solvent.

3. *Preparation of solutions of metals*

The stock and primary standard solutions of metal salts were prepared exactly in the same manner as those prepared for the antibiotics in the required concentrations (128, 64, 32, 16, 8, 4, 2, 1 and 0.5 μg/ml). The stock and primary standard solutions of roxithromycin metal complexes (as given in table 1) were also prepared in the same manner as those for roxithromycin in the same concentrations.

4. Preparation of Mueller–Hinton agar (MHA), Mueller-Hinton broth (MHB), preparation of inoculums, controlling inoculum's density, MacFarland turbidity standards and agar dilution susceptibility tests were carried out according to standard procedures reported elsewhere [Sultana *et al.,* 2001].

5. *Preparation, inoculation and Incubation of antimicrobial plates*

The agar medium prepared in conical flask was allowed to cool to 50°C on a water bath. Petri dishes were sterilized by placing them in an oven at 150°C for one and a half hour and labeled according to their concentrations (0.5 μg/ml to 128 μg/ml). Various dilutions of roxithromycin and roxithromycin metal complexes were prepared according to the procedures described above. These were added to the melted and cooled medium in a ratio of 1 part dilution to 9 part medium (2 ml of dilution of each to 18ml of agar for each petri dish). The medium was mixed by gently shaking the flask several times and the contents were poured into appropriate number of petri dishes marked, set aside on a flat horizontal surface and allowed to harden undisturbed till the contents solidified (Bertina, 1987).

An inoculum (1 - 2 μl) of each organism was applied to the surface of each antimicrobial petri dish with the help of a sterilized wire loop. The inoculum was applied as a spot that made a circle (Bertina, 1987; National Committee For Clinical Laboratory Standards 1990). The inoculated petri dishes were not disturbed until the spot of inoculum was absorbed completely, after which they were then inverted and incubated at 37°C for 24 hours to obtain the growth of the test organism. Incubation under increased $CO_2$ atmosphere was avoided because of the resulting increase in surface pH, which might adversely affect some antimicrobial agents. The petri dishes were then examined for the presence or absence of growth. The lowest concentration of each antimicrobial that inhibited growth was considered the MIC (single colony or haze growth was ignored) (American Public Health Association 1987).

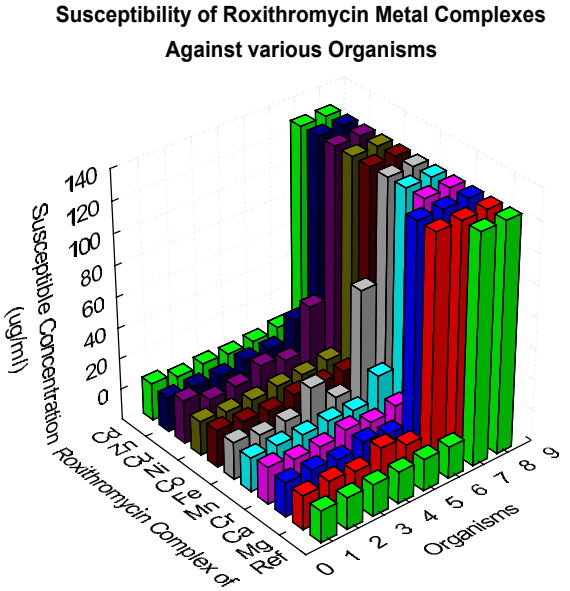
## RESULT AND DISCUSSION

Selection of an antibiotic for therapy of bacterial infection often depends on knowledge of the susceptibility of the infecting organism. Usually, it is possible to determine susceptibility by *in vitro* tests. When they are properly standardized, the result obtained correlate well with the response to therapy observed in clinical practice. Like the rest of the macrolide group, roxithromycin exerts its bacteriostatic antibacterial action by binding to the 50S ribosomal subunit of susceptible organisms and by inhibiting protein synthesis through translocation of aminoacyl transfer-RNA (Florey, 1996; Goodman, Gilman's, 1996). Some *Staphylococci* are sensitive to erythromycin, the range of MIC is very high for *Staphylococcus epidermidis*, 8 to > 32 μg/ml, and

for *Staphylococcus aureus*, 0.12 to > 128 µg/ml. Erythromycin resistant strains of *Staphylococcus aureus* are also resistant to roxithromycin.

Reference standard of roxithromycin during present *in vitro* studies verified that roxithromycin is active against both Gram positive and Gram-negative strains of organisms. Table-2 indicates that *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Salmonella typhi, Proteus vulgaris, Shigella dysentery* were susceptible at 0.5 µg/ml concentrations. However *Klebsiella pneumoniae, Staphylococcus epidermidis* were resistant to roxithromycin at 128 µg/ml concentrations. Some *Staphylococci* are sensitive to erythromycin, the range of inhibitory concentration is very high for *Staphylococcus epidermidis* (8 to > 32 µg/ml) and *Staphylococcus aureus* (0.12 to > 128 µgm/ml).

The antibacterial susceptibility of all the roxithromycin metal complexes are compared with the parent drug in Fig. 1. Antibacterial activity of roxithromycin magnesium complex as shown in Table-2 reveal that *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, were susceptible at* 2.0 µg/ml concentration while *Shigella dysentery Klebsiella pneumoniae* and *Staphylococcus epidermidis* were susceptible at higher concentrations of 128 µg/ml while on the other hand *Enterococcus faecalis* was susceptible at 4 µg/ml and *Salmonella typhi* at 8 µg/ml concentration.




Organisms:

| | |
|---|---|
| 1 = *Staphylococcus aureus* | 2 = *Enterococcus faecalis* |
| 3 = *Escherichia coli* | 4 = *Salmonella typhi* |
| 5 = *Proteus Vulgaris* | 6 = *Shigella dysentery* |
| 7 = *Klebsiella pneumoniae* | 8 = *Staphylococcus epidermidis* |

**Table 2**
Susceptibility of Roxithromycin and its metal complexes

| S. No | Organisms ↓ Complex → | Susceptible concentration (μg/ml) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Rox | Rox-Mg | Rox-Ca | Rox-Cr | Rox-Mn | Rox-Fe | Rox-Co | Rox-Ni | Rox-Cu | Rox-Zn | Rox-Cd |
| 1. | *Staphylococcus aureus* | 0.5 | 2 | 2 | 4 | 2 | 4 | 4 | 2 | 8 | 2 | 4 |
| 2. | *Enterococcus faecalis* | 0.5 | 4 | 2 | 2 | 2 | 1 | 2 | 0.5 | 1 | 2 | 1 |
| 3. | *Escherachia coli* | 0.5 | 2 | 0.5 | 2 | 1 | 2 | 2 | 0.5 | 2 | 1 | 2 |
| 4. | *Salmonella typhi* | 0.5 | 8 | 4 | 4 | 2 | 16 | 16 | 2 | 8 | 4 | 1 |
| 5. | *Proteus Vulgaris* | 0.5 | 2 | 1 | 1 | 1 | 2 | 4 | 2 | 4 | 4 | 2 |
| 6. | *Shigella dysentery* | 0.5 | 128 | 128 | 4 | 16 | 64 | 64 | 4 | 32 | 16 | 4 |
| 7. | *Klebsiella pneumoniae* | 128 | 128 | 128 | 128 | 128 | 128 | 128 | 128 | 128 | 128 | 128 |
| 8. | *Staphylococcus epidermidis* | 128 | 128 | 128 | 128 | 128 | 128 | 128 | 128 | 128 | 128 | 128 |

Roxithromycin calcium complex was susceptible against *Escherachia coli* at 0.5 µg /ml concentration similar to that of reference drug. *Staphylococcus aureus* and *Enterococcus faecalis* were susceptible at 2 µg/ml concentrations. On the other hand *Salmonella typhi* was susceptible at 4 µg/ml, *Proteus vulgaris* at 1 µg/ml, while *Shigella dysentery, Klebsiella pneumoniae* and *Staphylococcus epidermidis* all were susceptible at higher concentration of 128 µg/ml.

The susceptibility of roxithromycin chromium complex against *Staphylococcus aureus, Salmonella typhi* and *Shigella dysentery* was higher at 4 µg/ml while *Enterococcus faecalis* and *Escherachia coli* were susceptible at 2 µg/ml and *Proteus vulgaris* at 1 µg/ml concentration. *Klebsiella pneumoniae* and *Staphylococcus epidermidis* were susceptible at higher concentration 128 µg/ml concentrations in case of all the complexes of roxithromycin like the reference drug.

Roxithromycin manganese complex was susceptible at concentrations of 2 µg/ml against *Staphylococcus aureus, Enterococcus faecalis* and *Salmonella typhi,* while against *Escherachia coli* and *Proteus vulgaris* at 1µg/ml concentrations. *Shigella dysentery* on the contrary had a higher susceptibility value of 16 µg/ml.

Roxithromycin iron complex exhibited a varied type of behavior against these organisms. *Salmonella typhi* was susceptible at 16 µg/ml while the activity of the complex was reduced against *Staphylococcus aureus, Escherachia coli* and *Proteus vulgaris* at 4, 2 and 2 µg/ml concentrations respectively. *Enterococcus faecalis* was susceptible at lower concentration of 1 µg/ml.

Roxithromycin cobalt complex was susceptible at moderate concentration of 4 µg/ml against *Staphylococcus aureus, Salmonella typhi, Proteus vulgaris* and *Shigella dysentery and at low concentration* of 1 µg/ml concentrations against *Escherachia coli*. There was no effect of roxithromycin nickel complexation on the susceptibility of *Enterococcus faecalis* and *Escherachia coli* while *Staphylococcus aureus, Salmonella typhi, Proteus vulgaris* and *Shigella dysentery* were susceptible at 2-4 µg/ml concentrations respectively.

Roxithromycin copper complex was found susceptible against *Staphylococcus aureus, Salmonella typhi* and *Shigella dysentery,* at higher concentrations of 8, 8 and 32 µg/ml concentrations whereas *Enterococcus faecalis* and *Escherachia coli* were susceptible at lower concentrations of 1, 2 µg/ml concentration respectively.

Roxithromycin zinc complex was susceptible at moderate concentrations against all organisms except *Proteus vulgaris* where it was the susceptible concentration was 16 µg/ml concentrations. The susceptibility of roxithromycin cadmium complex was found similar to that of zinc complex except in case of *Shigella dysentery* where it was lower at 4 µg/ml concentrations.

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