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Effect of Zinc Salts on the Activity of Various Antimicrobial Agents

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SUMMARY

Antimicrobial activity of ofloxacin, cephradine, cloxacillin erythromycin, chloramphenicol and amoxycillin was determined against three bacterial strains, Staphylococcus aureus, Streptococcus pyogenes and Salmonella typhi using them separately and in conjunction with zinc citrate or zinc chloride. The activity of all the antibiotics remained unchanged with the addition of zinc salts with the exception of cloxacillin. S. aureus was found resistant to cloxacillin and its activity was restored with the supplementation of zinc salts. Clotrimazole and nystatin activity against Candida albicans was unaltered by zinc salts. From the results it can be concluded that addition of zinc to antibiotics does not decrease their activity, rather it might restore the activity of bacterial resistant drugs.

INTRODUCTION

Trace elements are essential for optimal growth and development of the humans. An inadequate intake of these elements may lead to impaired cellular and physiological functions and prolong illnesses of many kinds[1]. Among the established nine trace elements, zinc has been investigated most extensively and found to play far-reading role in glucose[2], fatty acid and nucleic acid metabolism[3]. Severely decreased zinc serum titre in patients with lobular pneumonia, malignant neoplasms, liver diseases, chronic poly-arthritis rheumatic, and chronic nephritis is suggestive of its involvement to help combat infections[4]. An unambiguous evidence exists in the literature about antimicrobial activity of zinc[5]. The activity of antibacterial prostatic factor, present in the prostatic fluid, is directly related to the amount of zinc in the fluid[6]. Published literature greatly supports the fact that beneficial effects can be achieved by using zinc in combination with antimicrobials. Moreover, synergistic effect was obtained by adding zinc to triclosan[7]. Also potentiation of antimicrobial effects of butylated hydroxyanisole and propylgallate has been reported with the supplementation of zinc[8].

Similarly the antiplaque activity of Tridosan against existing plaque and new plaque formation is increased in the presence of zinc[9]. The present work was initiated with the objective to investigate the effect of zinc supplementation on antimicrobial activity of different antibiotics commonly used in daily clinical practice.

MATERIALS AND METHODS

Zinc chloride and zinc citrate were received as gift from PCSIR Laboratories Lahore. All the antibiotics, cephradine (Glaxo), erythromycin (Abbot), chloramphenicol (Parke-Davis), clotrimazole (Bayer), nystatin (Cyanamid), amoxycillin and cloxacillin (Beechan) and ofloxacin (Hoechst) were obtained from respective pharmaceutical companies based in Pakistan. Muller Hinton Agor and nutrient broth were purchased from local market.

Bacterial test strains, Streptococcus pyogenes, Staphylococcus aureus, and Salmonella typhi, and fungus Candida albicans were obtained from microbiology laboratory, Shaikh Zayed Hospital. All the glass apparatus and other laboratory equipment were sterilized by autoclaving at 121°C for 30 minutes.

Concentrations A (6.25 $\mu g/\mu l$) and B (12.5

$\mu g/\mu l$) of zinc chloride and zinc citrate were prepared in water and dimethyl formamide (DMF) respectively. The zinc salts solutions were sterilized by passing through 0.2μm millipore filter paper.

Preparation of Muller Hinton Agar Media

Media (38 gm) was accurately weighed and dissolved in distilled water (1000ml). The solution, after gently boiling, was autoclaved for fifteen minutes at 121°C. The pH of the media was kept at 7.4±0.2.

Preparation of Standard Inocclum

Inocculum of each test micro-organism was prepared by transferring a loop-full of micro-organisms from a 24 hours incubated culture to a 10ml sterilized nutrient broth and concentrating it till its turbidity matched with that of tube No.10 of Mcfarland nephelometer. This gave a viable count of $30x10^8/ml$[10].

Determinations of Antimicrobial Activity

Antimicrobial activity was determined using the well method[11]. Two millilitre standard inoculum was pippetted into each petri-dish containing Muller Hilton Agar media. Petri-dishes were rotated gently clock-and anticlock-wise to mix the inocculum with media thoroughly. The Petri dishes were set aside for solidification. Seven wells or holes, each measuring 8mm in diameter, were made in each petri dish. Each well was seeded with 20μl of either vehicle, single antimicrobial agent or combination of agents. The whole process was carried out under strict sterile conditions. The petri-dishes were incubated at 37°C for 24 hours and zone of inhibition was measured in millimeters (mm).

Statistical Analysis

Non-paired Student's `t' test was used to assess the level of significance between the two treatment groups. P value ≤ 0.05 was considered as significant.

RESULTS

The effect of addition of zinc salts on the activity of cephradine, cloxacillin and ofloxacin against Staphylococcus aureus is presented in Table 1. The data show that this strain was equally sensitive to cephradine and ofloxacin and resistant to cloxacillin. Zinc salts did not alter the activity of active antibiotics. In case of cloxacillin, the addition of both zinc salts, significantly increased the effect of cloxacillin. The activity was increased about 54% when the combinations of cloxacillin with zinc chloride or zinc citrate with employed. Table 2 shows the activity of ofloxacin, erythromycin and amoxicillin when used separately and in combined forms with zinc salts against Streptococcus pyogenes. It is clear from the table that this bacterial strain was most sensitive to ofloxacin and least to amoxicillin whereas to erythromycin, the sensitivity was in the middle of the two extremes. The addition of zinc citrate and zinc-chloride at both concentrations neither altered the activity of the three antibiotics nor any additive effect was achieved. Table 3 delineates the growth inhibiting effect of different antibiotics applied separately and in combination with the two salts of zinc at two concentration levels against Salmonella typhi. The results indicate that this bacterium had nearly the same degree of sensitivity towards chloramphenicol amoxicillin and ofloxacin. The addition of zinc neither affected the antimicrobial activity of the antibiotics nor it contributed its own antibacterial effect in combination form.

Table 1: The antimicrobial effect (measured as zone of inhibition in mm) of Cephradine, Cloxacillin, Ofloxacin and zinc salts (A & B concentrations) per se and in combination forms against Staphylococcus aureus.

Antimicrobial agent(s)	*Antibiotics per se*	*Zinc citrate*	*Zinc chloride*
DMF	0	-	-
Conc. A	-	9.0±0.31	12.0±0.31
Conc. B	-	11±0.0	13±0.31
Cloxacillin	0*	-	-
Cephradine	30.0±0.31	-	-
Ofloxacin	30.2±0.37	-	-
Cloxacillin+Conc. A	-	18.0±0.31*	20.0±0.31*
Cloxacillin+Conc. B	-	16.0±0.31*	20.0±0.31*
Cephradine+Conc. A	-	30.0±0.31	30.0±0.00
Cephradine+Conc. B	-	30.0±0.0	30.0±0.31
Ofloxacin+Conc. A	-	30.0±0.00	33.0±0.31
Ofloxacin+Conc. B	-	30.0±0.31	33.0±0.31

(Each value represents the mean±SEM of 5 experiments)

*when compared with respective value of Cloxacillin alone and incombination with the A and B concentrations of the zinc salts

Table 2 The antimicrobial effect (measured as zone of inhibition in mm) of Amoxicillin, Ofloxacin, Erythromycin and zinc salts (A & B concentrations) per se and in combination forms against Streptococcus pyogenes

Antimicrobial agent(s)	*Antibiotics per se*	*Zinc citrate*	*Zinc chloride*
Control	0	-	-
Conc. A	-	0	0
Conc. B	-	13.0±0.0	11.0±0.31
Amoxicillin	22.2±0.37	-	-
Ofloxacin	41.2±0.37	-	-
Erythromycin	30.2±0.37	-	-
Amoxicillin+Conc. A	-	21.0±0.31	22.0±0.0
Amoxicillin+Conc. B	-	20.4±0.40	22.0±0.31
Ofloxacin+Conc. A	-	40.0±0.0	41.0±0.0
Ofloxacin+Conc. B	-	36.6±0.24	41.0±0.54
Erythromycin+Conc. A	-	28.0±0.54	30.0±0.37
Erythromycin+Conc. B	-	29.0±0.54	31.0±0.54

(Each value represents the mean±SEM of 5 experiments)
No statistically significant difference was observed when anti-biotics per se and their combinations with zinc salts were compared.

Table 3: The antimicrobial effect (measured as zone of inhibition in mm) of Amoxicillin, Ofloxacin, Chloramphenicol and zinc salts (A & B concentrations) per se and in combination forms against Salmonella typhi

Antimicrobial agent(s)	*Antibiotics per se*	*Zinc citrate*	*Zinc chloride*
DMF (Control)	0	-	-
Conc. A	-	9.0±0.54	11.0±0.54
Conc. B	-	12.0±0.6	13.0±0.00
Chloramphenicol	20.2±0.37	-	-
Amoxicillin	18.80±0.73	-	-
Ofloxacin	21.0±0.31	-	-
Amoxicillin+Conc. A	-	18.0±0.63	18.0±0.00
Amoxicillin+Conc. B	-	18.0±0.63	18.0±0.04
Chloramphenicol+Conc.A	-	20.0±0.54	20.8±0.58
Chloramphenicol+Conc.B	-	20.0±0.00	21.0±0.63
Ofloxacin+Conc. A	-	20.0±0.54	22.0±0.00
Ofloxacin+Conc. B	-	21.0±0.31	22.0±0.54

(Each value represents the mean±SEM of 5 experiments)
Comparison in zone of inhibition of antibiotic alone and with their combination with zinc showed no statistically significant difference

The antifungal effect of nystatin, cotrimazole and zinc salts, used separately and in combinations, against Candida albicans is presented in Table 4. The data show that zinc citrate was active at only C (25μg/μl) and D (37.5μg/μl) concentrations whereas zinc-chloride had poor antifungal activity at concentration D. Nystatin was found inactive against this micro-organism. When mystatin was used in combination with zinc citrate and zinc chloride, the antifungal effect was equal to the individuals effects, obtained with zinc chloride or zinc citrate separately. Although this fungal strain remained resistant to nystatin yet, zinc salts did show their activity in combination forms. When clotrimazole and zinc salts were used against the fungus, the total effect of the combinations was not significantly different from that when clotrimazole was used per se. It suggests that zinc salts failed to show any synergistic or additive effect when employed with clotrimazole.

Table 4: Antifungal effect (measured as zone of inhibition in mm) of Nystatin, Clotrimazole, and zinc salts (A, B, C & D concentrations) per se and in combination forms against Candida albicans

Antimicrobial agent(s)	*Antibiotics per se*	*Zinc citrate*	*Zinc chloride*
DMF	0	-	-
Conc. A	-	0	0
Conc. B	-	0	0
Conc. C	-	15.0±0.31	0
Conc. D	-	16.0±0.31	12.6±0.40
Nystatin	0	-	-
Clotrimazole	20±0.54	-	-
Nystatin+Conc. A	-	0	0
Nystatin+Conc. B	-	0	0
Nystatin+Conc. C	-	14.6±0.24	0
Nystatin+Conc. D	-	17.0±0.31	14.0±0.31
Clotrimazole+Conc. A	-	20.0±0.31	19.0±0.31
Clotrimazole+Conc. B	-	20.0±0.31	18.0±0.31
Clotrimazole+Conc. C	-	20.0±0.00	19.0±0.31
Clotrimazole+Conc. D	-	20.0±0.31	19.0±0.31

(Each value shows the mean±SEM of 5 experiments)
No statistically significant difference was seen when anti-fungals per se and their combinations with zinc salts were compared.

DISCUSSION

The antibiotics belonging to various groups, with different mechanism of actions, were used against three bacterial and one fungal strain. Two zinc salts, zinc chloride and zinc citrate were selected for studying interaction between antibiotics and these salts. The activity of all the antibiotics, with the exception of cloxacillin, remained unaltered with the supplementation of zinc chloride and zinc citrate hence, phenomena of antagonism, addition and synergism were not noticed. This aspect of observation seems beneficial for clinical use. The role of zinc in improving humoral and cell-mediated immunity is well established and is gaining more importance now-a-days[12,13,14]. Thus the use of zinc supplementation towards elimination of infectious diseases by independently elevating the immune response without having any activity lowering effect on antibiotics. The achievement of adequate antibiotic concentration at the site of infection is very essential to achieve therapeutic goal. Some cations, like iron, are found to reduce the activity of tetracycline and penicillin groups of drugs significantly[15]. Whereas our data show that zinc salts are devoid of such activity-curtailing effect.

Staphylococcus aureous was found resistant to cloxacilin when used per se. However, when cloxacillin was employed in conjunction with zinc salts, inactive cloxacillin was rejuvenated and its marked activity was noticed against the previously resistant test micro-organism. This means that zinc salts made the micro-organism sensitive to antimicrobial to which it was resistant previously. Our data on this aspect is in full agreement with the findings of Bradshaw et al. who reported that synergistic effect can be achieved by adding zinc to triclosan[7]. An identical observation was reported by McCarthy et al when zinc was added to antioxidants like butylated hydroxyanisole and propylgallate[8]. Our finding about activation of cloxacillin is strongly supported by results of Holl et al. who found that appropriate concentration of erythromycin and zinc inhibited the growth of resistant propioni bacterium acne cells[16].

In the present findings, the activation of cloxacillin is an indication of involvement of zinc to overcome the mechanism of resistance to the drug. Among the so many possibilities, it might be due to activation of autolytic enzymes, as zinc being either essential for the structure or for the biological activity of more than 200 enzymes may enhance activity[3]. Alternatively, zinc might have helped the drug to get access to PBRs or there is possibility of generation of PBRs. Also it can be suggested that some mechanism by which bacteria expelled the drug in an attempt not to let it reach MIC5 might have been blocked by the zinc salts.

Though it is premature to say yet rejuvenation of inactive antimicrobials will be a real break through to overcome the ever-increasing problem of drug-resistance.

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