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The Effect of Zinc On Microbial Growth

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Abstract: The antibacterial activity of zinc acetate against *Staphylococcus auerus, Staphylococcus epidermidis* and *Pseudomonas aeruginosa* isolates was measured in Mueller-Hinton broth containing different amounts of zinc acetate using spectrophotometry. *Staphylococcus auerus, Staphylococcus epidermidis* and *Pseudomonas aeruginosa* isolates were inhibited by zinc acetate ($p<0.05$). The effect on *Staphylococcus auerus* and *Staphylococcus epidermidis* was greater than on *Pseudomonas aeruginosa* ($P<0.05$).

Key Words: Zinc, antibacterial activity

Introduction

Zinc ion concentrations of 10^{-5}-10^{-7} M are required for optimal bacterial growth of most microorganisms in vitro (1). However, it is claimed that high zinc ion concentrations may have some antibacterial properties (2). The antibacterial effect of adhesive zinc tapes on streptococci and staphylococci were described by Gilje as early as 1949 (3). Zinc oxide is used extensively as a component of cements and periodontal dressing and as filles in endodontic gutta percha cones. In dentistry, the antibacterial effects of zinc oxide have been reported (4). The aim of this study was to investigate in vitro the antibacterial effect of zinc acetate using spectrophotometry.

Materials and Methods

Staphylococcus auerus, Staphylococcus epidermidis and *Pseudomonas aeruginosa* strains (n=7 for each group) were obtained from the Department of Microbiology, Faculty of Medicine, Dicle University. Fresh isolates were grown on Mueller-Hinton broth for 24 hours at 37°C before use. Bacterial isolates were adjusted to an optimal density of 0.5 McFarland standard (10^8 cfu/ml) with sterile saline and then further diluted to achieve a final bacterial concentration of 10^7 cfu/ml. In microbiological experiments zinc oxide has been used as a source of zinc ion. However, in this study zinc acetate $(CH_3COO)_2$ Zn was used as the source of Zn^{+2} because zinc acetate is readily dissolved in water whereas zinc oxide is more difficult to dissolve in water. Zinc acetate was dissolved and diluted with sterile distilled water to obtain 2.8, 5.5, 11, 16 and 22 mmol/l zinc acetate solutions. For each test, 30 µl bacterial suspension and 50 µl Mueller-Hinton broth were added to 20 µl zinc acetate solution. The experiments were set up in wells of different zinc acetate concentrations containing bacterial suspension, and in the last stage the zinc acetate solution contained 22 mmol/l zinc acetate, which was the highest zinc acetate concentration. The control wells contained only 30 µl bacterial suspension and 70 µl Mueller-Hinton broth without the addition of zinc acetate solution. Microplate wells were incubated aerobically at 37°C in an incubator. After 24 hours, the absorbance, as the measure of growth, was determined at 450 nm using a spectrophotometer. The final pH was measured using a pH/ion meter. Statistical analysis was performed by analysis of variance unpaired student's t -tests when appropriate. $P<0.05$ was considered to be significant.

Results

The mean absorbance values were obtained from the control and zinc acetate wells containing bacterial suspension. There were satistically significant differences in the absorbance values of *S.aureus,* which were parallel to the increment of the zinc acetate concentrations ($P<0.05$). The gradually increasing differences in the absorbance values of *S.aureus* were observed in wells which had 11 mmol/l zinc acetate solution ($P<0.05$). For

Table. The absorbance values of suspensions of *S.aureus, S.epidermidis* and *P.aeruginosa* strains incubated for 24 hours with various zinc concentrations. Each figure represents the mean±SEM of seven experiments.

		Mean Absorbance		
		S.aureus	*S.epidermidis*	*P.aeruginosa*
	Mueller-Hinton broth without bacterial suspension and zinc acetate solution	0.086	0.081	0.068
A	Bacterial suspension without zinc acetate (control)	0.678±0.065	0.628±0.055	1.399±0.031
B	Bacterial suspension +2.8 mmol/l zinc acetate containing solution	0.377±0.049	0.271±0.032	1.427±0.048
C	Bacterial suspension +5.5 mmol/l zinc acetate containing solution	0.396±0.055	0.253±0.082	1.493±0.033
D	Bacterial suspension +11 mmol/l zinc acetate containing solution	0.177±0.043	0.182±0.016	1.246±0.121
E	Bacterial suspension +16 mmol/l zinc acetate containing solution	0.169±0.041	0.178±0.015	1.272±0.052
F	Bacterial suspension +22 mmol/l zinc acetate containing solution	0.174±0.037	0.194±0.029	1.238±0.018

S.aureus F (0.05, 5.36)= 2.507; ab, ac, ad, ae, af, be, bd, bf, cd, ce, cf, P<0.05; bc, de, df, ef, insignificant
S.epidermidis F (0.05, 5.36)= 4.482; ab, ac, ad, ae, af, P<0.05; bc, bd, be, bf, cd, ce, cf, de, df, ef, insignificant
P.aeruginosa F (0.05, 5.36)= 2.507; cd, ce, cf, P<0.05; ab, ac, ad, ae, af, bc, bd, bf, de, df, ef, insignificant

S.epidermidis the mean absorbance values were reduced from 0.628±0.055 to 0.271±0.032 by the addition of 2.8 mmol/l zinc acetate solution (P<0.05). However, no further difference in absorbance values was observed with increasing zinc acetate concentrations (P>0.05).

Although there were differences in the absorbance values of zinc acetate containing *P.aeruginosa*, these were insignificant when compared to the control (P>0.05). There were some statistically significant diferences between absorbance values of the wells containing 5.5 mmol/l zinc acetate and the absorbance values of the wells with higher zinc values (P<0.05) but, as mentioned above, the absorbance values of all the zinc wells were not statistically different from the control wells (P>0.05). When the differences were compared in the absorbance values related with the zinc acetate concentrations of the *S.aureus, S.epidermidis* and *P.aeruginosa* suspension wells, it was observed that the effect of zinc acetate on *S.aureus* and *S.epidermidis* was greater than on *P.aeruginosa* (P<0.05) (data not shown).

Discussion

The results of this study showed that zinc acetate has an antibacterial effect on *S.aureus, S.epidermidis* and *P.aeruginosa.* Although the inhibitory activity of zinc acetate on *P.aeruginosa* was statistically significant, this effect was moreevident on *S.aureus* and *S.epidermidis* than on *P.aeruginosa* (P<0.05). This effect was not relevant to the changes in mean pH because zinc acetate solution had no effect on the mean pH.

There are several possible mechanisms for the antibacterial action of zinc ion. It has been suggested that zinc binds to the membranes of microorganisms, similar to mammalian cell (1) zinc, prolonging the lag phase of the growth cycle and increasing the generation time of the organisms so that it takes each organism more time to complete cell division (5).

In a study by Södeberg et al. (2), it was found that gram-positive bacteria were the most susceptible bacterial group to zinc ion but gram-negative aerobic bacteria were usully not inhibited even at the highest concentration (1024 µl/ml). In addition, it was reported that a combination of zinc oxide and rosin or resin acids has synergistic effects on antibacterial activity against gram-positive bacteria but not against gram-negative bacteria (6).

Although it is not clear why zinc exhibits different bacterial affinity with gram-positive and gram-negative

bacteria, it may be ascribed to the difference in the protein constituents of their cell walls (1).

In a comparative stduy of different dressigns applied to excised wounds in rats, the number of bacteria in the granulation tissue was significantly less under zinc oxide adhesive tape than under gauze or hydrocolloid dressings (7). In conclusion, we have shown that zinc acetate exhibits antibacterial activity in S.aureus, *S.epidermidis* and *P.aeruginosa.* This effect was greater on *S.aureus* and *S.epidermidis* than on *P.aeruginosa.* Future studies

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