Bacterial Susceptibility Study Of Zinc Containing Compounds With Respect To Gram Positive And Gram Negative Microorganisms

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Abstract

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Zinc compounds are commonly used as an over the counter treatment for colds and flu in the form of throat lozenges. The purpose of this study is to determine the effectiveness of zinc compounds at inhibiting the growth of common infectious microorganisms. We tested seven different zinc compounds, along with the corresponding anion or ligand contained in the zinc compounds to determine whether a correlation exists between the zinc ion content of a compound, and the antimicrobial activity. The anion or ligand of our zinc compounds was also tested in an attempt to determine if the zinc ion was responsible for the antimicrobial activity. Microbial zone inhibition studies were carried out using two gram positive organisms (*Staphlococcus aureus*, *Streptococcus pyogenes*), two gram negative (*Salmonella typhimerium, Eschrichia coli*), and one common yeast (*Candida albicans*). We found that the selected zinc compounds are effective at inhibiting the growth of these microorganisms. This study not only supports the use of zinc compounds for the treatment of infectious microorganisms, but also warrants further study in this area of research. We are currently studying the MIC (minimum inhibitory concentration) of several zinc compounds against several microorganisms.

Introduction

With nutritional status of zinc well established by 1934 [1], it has taken nearly the rest of the 20th century to determine what the biological functions of zinc are. It has been estimated that zinc is essential to at least 200 enzymes [1] involved in cellular biochemistry. It has been noted that the function of zinc in biological systems can be separated into three main categories which include: catalytic activity, structural integrity, and regulatory processes for proteins and peptides with some overlap in these three functions [2]. Along with respect to the well established biological function of zinc, supplements are being sold in today's marketplace for many different uses including helping alleviate symptoms of the common cold[10]. Zinc lozenges are being marketed as a treatment for the common cold, but yet we still have limited research to support this use[3]. The supportive role of zinc with respect to immune function and zinc deficiency has been well established[4]. There is however some controversy as to how to effectively measure immune response with respect to zinc [1]. Zinc is also known to be toxic at high doses. Our study is directed towards examining what effect Zn^{+2} ions have in inhibiting the growth of common infectious microorganisms. The study was designed to test whether the Zn^{+2} ions of common zinc sources have an effect on inhibiting the growth of gram positive, gram negative bacteria and a common strain of yeast. If zinc is shown to poses anti-microbial properties, it would be an added benefit to the many nutritional properties of zinc.

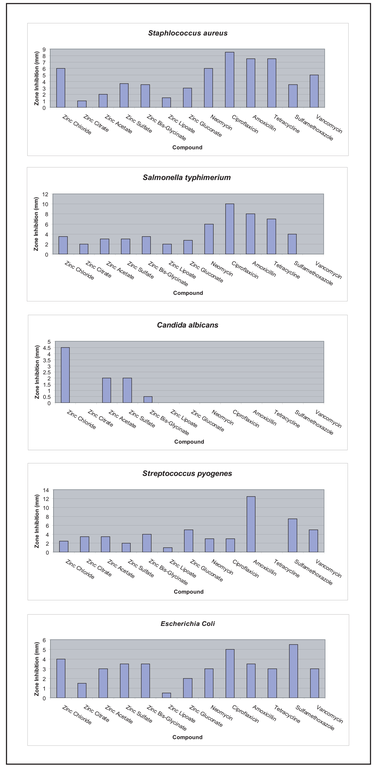
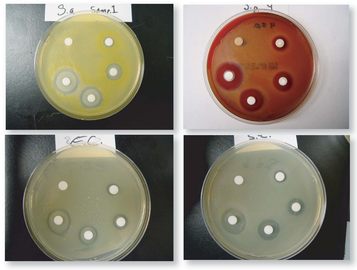


Table 2. Initial Screening of Zinc Compounds for anti-microbial properties



Procedure

To test the anti-microbial properties of Zn^{+2} ions from common zinc sources, we obtained samples of the most commonly used zinc sources and their sodium analogs. Two of the compounds tested were zinc chelates produced by Albion Advanced Nutrition. Table 1 is a list of the compounds tested along with their zinc percent composition as tested.

Compound	Supplier	Cas #	%Zn
Zinc Chloride	Sigma Chemicals	7646-85-7	46.5%
Zinc Citrate	Specturm Chemicals	5990-32-9	32.3%
Zinc Acetate	Sigma Chemicals	5970-45-6	29.5%
Zinc Sulfate	ACROS	7446-20-0	22.7%
Zinc Bisglycinate (Zinc chelazome)	Albion Advanced Nutrition	14281-83-5	21.2%
Zinc Lipoate	Albion Advanced Nutrition	N/A*	14.4%
Zinc Gluconate	Specturm Chemicals	4468-02-4	12.9%
Sodium Chloride	Fisher Chemicals	7647-20-0	0.0%
Sodium Citrate	Mallinckrodt	64-04-2	0.0%
Sodium Acetate	Sigma Chemicals	127-09-3	0.0%
Sodium Sulfate	EM Science	7757-85-6	0.0%
Sodium Glycinate	Specturm Chemicals	6000-44-8	0.0%
Delta-Glucono-lactone	Jungbunzlauer	90-80-2	0.0%

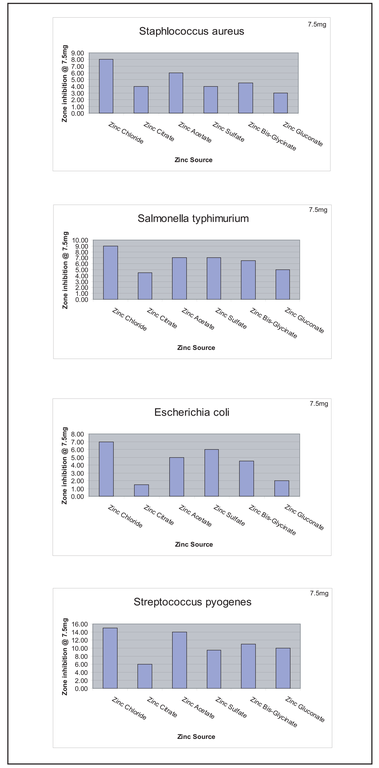
* Albion Advanced Nutrition Research Department

Table 1

Each of the chemical compounds was analyzed by ICP-AES for the amount of zinc present[5,6]. The sodium salts were also analyzed to confirm there was no zinc present. All percentages presented in Table 1 are data from these ICP analyses. Unfortunately some of our zinc compounds were old and therefore saturated with water, which was not discovered until this study was is progress. Delta-glucono-lactone was analyzed as gluconic acid due to hydrolysis of this compound when dissolved in water[7]. All samples were then randomly assigned a number and delivered to an outside laboratory for testing. Five organisms were selected for initial studies to test for anti-microbial activity. Bacterial inhibition studies were carried out using ATCC registered cultures[8] and NCCLS protocols[9]. These organisms included *Staphlococcus aureus*, *Streptococcus pyogenes, Salmonella typhimerium, Escherichia coli,* and *Candida albicans.* Organisms were chosen to include both gram positive and gram negative bacteria along with a common strain of yeast. Initial testing was performed with a wide variety of zinc compounds and to determine if zinc containing compounds exhibited any antimicrobial effects on these organisms. Sodium salts of each zinc compound were also tested to establish that the activity was due to the Zn^{+2} ions present in each zinc compound. There was one exception with gluconic acid tested as the free acid. Two of the compounds tested were Zn chelates, in which the Zn^{+2} ion has coordinate covalent bonds to the ligand present. There were no inhibition zones present with any of the Na Salts or individual ligands tested. Having established activity with select zinc compounds, we then repeated the inhibition study with antibiotic controls to determine how effective these compounds were at inhibiting growth of the organisms tested. Sterile discs were soaked in 100 mg/ml solutions of each compound and then placed on a well established bacterial lawn of each individual organism. Cultures were incubated for 24 hours, inhibition zones were measured for each compound with respect to the antibiotic controls for each organism. Results of the second antibiotic controlled study appear on Table 2. Zinc lipoate proved to be very difficult to work with in this study due to its hydrophobic properties, and it was eliminated from further testing. We did not get very broad activity with the *Candida albicans* studies either, and consequently this organism was eliminated from further study. It must be noted that the zinc compounds tested are not as effective as the antibiotic controls per unit mass at inhibiting the growth of the organisms tested. We do however have clear anti-microbial activity as indicated by the initial zone inhibition studies, and evidence that the activity is due to the Zn^{+2} ions. We then tried to estimate Minimum Inhibitory Concentrations (MIC) using only the zinc containing compounds from Table 1, with the exception of zinc lipoate. As with the initial zone inhibition studies all samples were delivered to an outside laboratory, with only a six digit number corresponding to this research project for identification. A modified NCCLS[9] protocol was used to generate data for this presentation. All samples were dissolved into sterile de-ionized water at an initial concentration of 250mg/ml. sterile discs were than placed on to an established bacterial lawn for each organism tested. Each compound was then micro-pipetted onto the sterile discs at volumes of 30µl, 20µl , 10µl, 5µl. Aliquots were chosen carefully so that each sterile disc would not be overloaded and bleed out into the bacterial culture. The aliquots were chosen to correspond with 7.5, 5.0, 2.5, and 1.25 mg of the zinc compounds respectively. All cultures were allowed to incubate for 24 hours and inhibition zones were measured from the edge of the disc to the edge of the zone in mm. MIC were estimated using simple linear regressions Antibiotic controls were again used for zone comparison in the MIC study. The amount of material present in the antibiotics is presented in Table 3. Having established a range of activity for all compounds and with respect to four micro-organisms, further study is warranted in the determination of more precise MIC's for each organism. The study of additional organisms may be an area of interest.

Antibiotic Controls	*Staphlococcus aureus*	*Eschrichia coli*	*Salmonella typhimerium*	*Streptococcus pyogenes*
Amoxicillin/Clavulanic Acid 30 ug disc BD	20.0mm	2.0mm	8.0mm	11.0mm
Ciprofloxacin 5 ug disk BD	6.5mm	7.0mm	8.5mm	2.0mm
Vancomycin 30 ug disk BD	3.5mm	0.0mm	0.0mm	6.0mm
Sulfamethoxazole 23.75 ug BD	5.0mm	6.5mm	7.0mm	0.0mm

Table 3. Inhibition Zones Produced in Minimum Inhibitory Concentration Screening Assay



Conclusion

The results of our study show that zinc has the ability to inhibit the growth of certain gram positive and gram negative micro-organisms. Our study also shows that the anti-microbial properties of zinc are not as effective as modern antibiotics. This study does however indicate that at typical zinc lozenge doses of 10-30mg, it is possible for the Zn^{+2} ions present to have an effect on the microbial growth of organisms they come in contact with. The anti-microbial properties of zinc also seem to be present with respect to both gram positive and gram negative micro-organisms. MIC estimates proved to be more difficult than originally anticipated. Our data clearly shows that the MIC for each compound is well below our smallest dose of 1.25mg for all organisms tested, but we found it difficult to do the MIC estimations because of linearity problems and differing inhibition ranges for each organism. We had also hoped to be able to show a linear relationship with respect to the percent zinc in the different compounds tested with respect to zone inhibition for each level tested. While there is clearly a trend with respect to the amount of zinc present in each compound tested with respect to zone inhibition, our data is not linear. This trend, while different for each organism, is indeed the same for each organism and level tested. We do feel that we have established a good range of activity for all zinc compounds with respect to all four micro-organisms, and further study is warranted in the determination of precise MIC's for each organism. The study of additional organisms and metal ions may also be an area of interest.

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Acknowledgments

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