Combined Activities of Colloidal Silver Concentrate and Cephalexin on *Staphylococcus aureus* using the Agar Diffusion Technique

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Abstract

The study of combined activity of colloidal silver concentrate and cephalexin against Staphylococcus aureus was carried out using agar diffusion technique. The results of the in vitro study showed that colloidal silver concentrate increases the MIC of cephalexin thereby reducing the efficacy of cephalexin. Also, the test microorganism Staphylococcus aureus was highly sensitive to the cephalexin used, but resistant to the colloidal silver concentrate. This shows that in the treatment of infections of Staph. aureus the two antimicrobial agents should be used together with caution.

Keywords: Combined activity, colloidal silver, cephalexin, *Staphylococcus aureus.*

Introduction

Antimicrobial agents have since their discovery been used in the treatment of diseases of man and animal. However, the need for safety and efficacy of these antimicrobial agents in chemotherapeutic cases calls for continuous information about the nature and mode of actions of these agents both *in vivo* and *in vitro* (Genarro, 1990).

Several investigations have been carried out on both colloidal silver concentrate and cephalosporins. The history of microbial activity of silver started at least 1200 years ago when people found out that silver cans prevented diseases and this was the basis of the saying that "disease could not be transmitted by drinking from a silver cup". Silver coins were commonly dropped into a jar to prevent the spoilage of milk and other drinks and silver containers were used to prolong the freshness of foods in general (Crooks, 1995).

In a laboratory test, Crooks (1995) found that all fungus, virus, bacterium, *Streptococcus*, *Staphylococcus* and other pathogenic organisms are killed by colloidal silver in six minutes or less in a dilution as little as 0.005 mg/ml. The silver acts as a catalyst and is not consumed in the process. This may be the reason why bacteria cannot develop resistance to silver easily as they do to other antibiotics, because silver does not destroy them directly, but rather destroys the enzymes that they depend on (Crooks, 1995)

Cephalosporium acremonium, the first source of the cephalosporins, was isolated in 1948 by Brotzu from the sea near a sewer outlet off the Sardinian coast (William, 2001). Crude filtrates from cultures of this fungus were found to inhibit the *in vitro* growth of *Staphylococcus aureus* and to cure staphylococcal infections and typhoid fever in human beings. Culture fluids in which the Sardinian fungus was cultivated were found to contain three distinct antibiotics, which were named cephalosporin P, N and C, with the isolation of the active nucleus of cephalosporin C, 7-amino-cephalosporic acid, and with the addition of side chain, it became possible to produce semi-synthetic components with antibiotic activity very much greater than that of the parent substance. The antibiotic acts on bacteria by inhibiting cell wall formation leading to cell lyses (Cheesbrough, 1985).

Currently, microbial resistance to antibiotics is a threat to chemotherapy worldwide. Some of the resistance results from the misuse of antibiotics including improper combination. However, multidrug therapy is encouraged to combat drug resistance. This study is aimed at evaluating the combined effects of two antimicrobial agents.

Materials and Methods

Test organism: The test microorganism used for this experimental study was a clinical isolate of *Staphylococcus aureus.*

Reagents: The following reagents were used: colloidal silver concentrate (for MOR International, USA) and cephalexin (Ranbaxy, India). The culture media were manitol salt agar and nutrient agar (Oxoid).

Preliminary test with *Staphylococcus aureus* and cephalexin: From Table 1 the preliminary culture and sensitivity study that was done shows that *Staphylococcus aureus* was highly sensitive to cephalexin. This antibiotic was then combined with colloidal silver concentrate to study their combined interactions on the *Staphylococcus aureus* under study.

MIC of cephalexin: The determination of MIC of cephalexin was carried out as follows: A sterile Petri dish was aseptically seeded with 0.1 ml of freshly prepared suspension of *Staphylococcus aureus* using a sterile standard pipette. A 20 ml aliquot of sterile molten nutrient agar at 45 °C in

McCartney bottle was poured into the plate and swirled clockwise and anticlockwise for even distribution. The agar plate was marked into four sections and labeled 1-4 with an indelible marker in a randomized manner representing the four dilutions of the cephalexin. Using a sterile 8 mm cork-borer, cups were made in the agar plate in each of the four divisions. The four different dilutions of the cephalexin were aseptically added in the cup using standard sterile droppers starting with the dilution with the least concentration to that with highest concentration.

Table 1: Preliminary culture and sensitivity tests

Drug	*Staphylococcus aureus*
1. Colloidal silver concentrate	–
2. Cephalosporin	++++
3. Colloidal silver concentrate and cephalosporin	++

The results shown in the Table above were interpreted as follows: (i) - Staphylococcus aureus was resistant to colloidal silver concentrate (ii) +++ Staphylococcus aureus was highly sensitive to cephalosporin (iii) ++ Staphylococcus aureus was intermediately sensitive to combined colloidal silver concentrate and cephalosporin

The plate was incubated at room temperature for 24 h and then the zones of inhibition were measured. This was repeated three times and the average values of the zones of inhibition were determined. The graph of the logarithm of the concentration of the dilutions used against the inhibition zone diameters was plotted. The MIC of the cephalexin was then determined from the slope of the resultant graph.

The combined effect of colloidal silver concentrate and cephalexin on *Staphylococcus aureus:* The combined activity of these antimicrobial agents on *Staphylococcus aureus* was carried out using cup agar diffusion technique. The experimental study followed these procedures:

500 mg Cephalexin was dissolved in 1 ml quantity of sterile distilled water and later made up to 10 ml while 1 ml of colloidal silver concentrate was made up to 4 ml with sterile distilled water.

One in 20 dilutions of the 10 ml solution of cephalexin was made followed by three, two-fold serial dilutions and their concentrations noted. With the 1 in 4 dilution of the colloidal silver concentrate, a further three, two-fold serial dilutions were also made and their concentrations also noted.

Three sterile Petri dishes were used for this study. A 0.1 ml culture suspension of *Staphylococcus aureus* containing 10^4 cfu/ml was aseptically seeded in each three sterile Petri dishes using a sterile pipette. A 20 ml quantity of sterile nutrient agar in a McCartney bottle at 45 °C was poured into each seeded plate. This was swirled clockwise and anticlockwise to obtain an even distribution of the organism in the nutrient agar. This was done for the remaining two plates and they were allowed to cool.

The agar plates were then marked into four sections and numbered 1–4 with a marker. Using a sterile 8 mm cork-borer, a cup was made in each of the four sections in each agar plate.

The solutions of the diluted colloidal silver concentrate in the four test tubes were then put in drops into the cups 1–4 using a sterile standard dropper. The solutions of the diluted cephalexin and colloidal silver concentrate were mixed in the four test tubes and were then added in the cups using a sterile standard pipette for each drug. The plates were labelled accordingly and incubated at room temperature for 24 h. The zones of inhibition were measured with their different concentrations noted. This experiment was repeated three more times for a better and more reliable result and statistical analysis.

Results

Calculation of MIC of cephalexin: The average inhibition zone diameters produced by the various dilutions of the test antibiotic were translated into log concentrations from the straight line of the standard plot. The corresponding concentrations were multiplied by the respective dilution factors. The average of the products gives the MIC of the cephalexin. From the graph (Fig. 1), 0.07 mg/ml was determined as the MIC of the cephalexin.

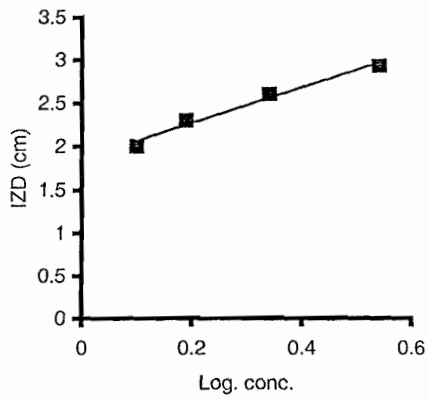

Fig. 1.Graph of activity of cephalexin agaisnt Staph. aureus

Calculation of MIC of combined colloidal silver concentrate and cephalexin: In calculating the MIC of the combined activity of these drugs, the average of the inhibition zone diameters of their interaction were plotted against the logarithm of their corresponding concentrations. Then, MIC was therefore determined as explained in the above.

From the graph (Fig. 2), the MIC of cephalexin was reduced to 0.39 mg/ml. The higher MIC of the combined drugs is indicative of reduced efficacy of cephalexin compared to 0. 07 mg/ml for the drug when used singly.

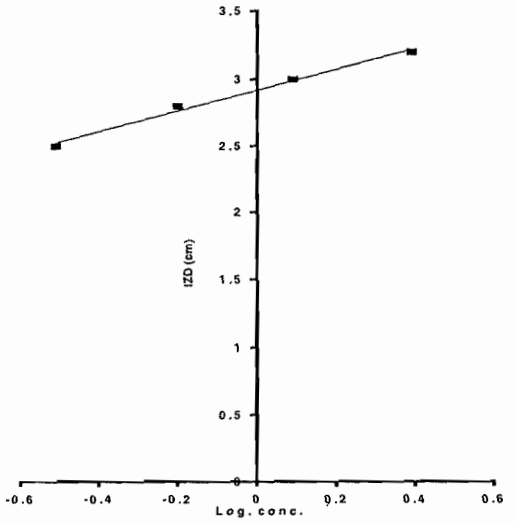

Fig.2. Graph of combined activity of colloidal silver concentrate and cephalexin against Staph. aureus

Discussion

Germs that survive antibiotics and other drugs have become a global menace. Scores of additional antibiotics have been developed, both those that are natural and those that are made synthetically. Yet, bacteria have developed ways of resisting many of these antibiotics, thus, increasing the global medical problem. Thus the research on combined chemotherapy should be a continuous one.

The use of an antimicrobial agent in the treatment of bacterial infections caused by single pathogen is usually effective provided that the substance or the agent can reach the site of the infection in sufficient concentration. However, the simultaneous use of two or more antimicrobial agents has a certain rationale and is recommended in specifically defined situations. Besides, the combined administration of these agents could lead to extending of the antibacterial spectrum because it is possible that two or more infectious microorganisms with different sensitivity patterns have to be dealt with. In a similar vein, it could lead to reduction of the likelihood of selecting mutant strains resistant to one or the other of the antimicrobial agent. The advantages are more marked when the antimicrobial agents exhibit inhibitory or bactericidal synergy as this can lead to complete eradication of the invading microorganisms.

The interactions above are when two or more antimicrobial agents are involved. However, the interaction between an antimicrobial agent and a non-antimicrobial agent may also enhance the killing or inhibiting effect of the antimicrobial agents on the microorganisms. On the other hand, combined interaction of antimicrobial agents may impair the overall antimicrobial activity.

Combined interactions involving antimicrobial agents and other drugs have some importance. This forms the basis of treatment of stubborn diseases with combined drug administration.

Some of these interactions are pharmacokinetic in origin while others are pharmacodynamic (Davis, 1991). For instance, flucytosine when combined with Amphotericin B permits lowering the dose of Amphotericin B and results in a 6-week rather than a 10-week duration of therapy with similar cure rates and less toxicity in non-HIV infected patients with cryptococcal meningitis. Sulphonamides when combined with trimethoprim results in synergism due to the blocking of sequential steps in microbial folate synthesis which is enhanced by an inhibitor of dihydrofolate reductase (trimethoprin). Amoxycillin when combined with potassium clavulinate inhibits penicillinase enzyme thereby enhancing the efficacy of amoxycillin. The enzyme inhibition is produced by the potassium clavulinate.

Some of this combinations may also result in adverse interactions. Adverse interactions of drugs are potential threat to life. Antimicrobial combinations also can be disadvantageous due to the risk of toxicity from two or more agents, the selection of multiple-drug-resistant microorganisms, and the increased cost to the patient (Genarro, 1990).

It is worth noting that most studies on drug interactions are done in order to identify adverse consequences. Usually, concern over drug interaction only arises when an adverse event is caused. Drugs such as sulphaphenazole inhibits the oxidation of tolbutamide leading to hypoglycaemic crisis. Rifampicin is an inducer of microsomal enzymes and therefore, decreases the efficacy of oral contraceptives that are largely metabolized by microsomal enzymes.

The recognition that the efficacy of certain drugs can be altered in the presence of other drugs calls attention to the possible investigation of the involvements of drug interactions in both pharmacokinetics and pharmacodynamics. This helps in therapeutics since if a drug has a very high efficacy, it will need only a small fraction of the receptors to evoke the maximum response of which the tissue is capable, but if a drug has a low efficacy, then a high dose may occupy all the receptors, and yet the maximum response produced is less than that of which the tissue is capable (Akah and Odita, 2001).

From Table 2 it was observed that at all concentrations of the combined colloidal silver concentrate and cephalosporin, there was decrease in the inhibition zone diameters when compared to that of only cephalexin. This means that their interactions were antagonistic to each other. This caused the increase in MIC of the cephalexin when combined with colloidal silver concentrate. It should however, be noted that this is an *in vitro* study and that the likelihood of possibility of changes in activities of colloidal silver concentrate may occur in *vivo* study. For instance, there is the possibility that colloidal silver concentrate combined with ingredient of the nutrient agar thereby inhibiting the rate of diffusion of cephalexin through the nutrient agar when they were administered simultaneously. This

is because even in drug administration, there is indication that other ingested substances may complex with orally administered drugs to yield non-absorbable or inactive derivatives (Davies, 1991). All these interactions, however, occur only if the interacting agents are administered simultaneously or within 30–60 minutes of each other that was applicable in this study. Finally, the low MIC of cephalexin as seen in the graph Fig. 1 shows that cephalexin can be used alone in treatment of diseases due to *Staphylococcus aureus*.

Table 2: Table of the test with cephalexin and with the combined agents

Cephalexin		Combined	
Conc (mg/ml).	Inhibition Zone (mm)	Conc (mg/ml)	Inhibition Zone(mm)
2.5	32	3.45	29
1:25	30	2.2	26
0.63	28	1.58	23
0.31	25	1.26	20

Conclusion: The combined activity of colloidal silver concentrate and cephalexin on *Staphylococcus aureus* done in this work was only by an *in vitro* method. It is, therefore, not enough to explain all the *in vivo* interactions of these two drugs in human being or animals. This is because colloidal silver concentrate does not attack bacteria directly, but rather decomposes the enzymes that the anaerobic bacteria, viruses, yeasts and molds require for their metabolic processes thereby killing them. Thus an *in vivo* study should be done to confirm the interaction observed in this study.

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