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REPORT

EFFECT OF MAGNESIUM AND ZINC ON ANTIMICROBIAL ACTIVITIES OF SOME ANTIBIOTICS

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Mg and Zn are essential elements in biological system. They are essential for enzymatic activity, maintaining three-dimensional structure of proteins, for the synthesis of nucleic acids and proteins etc. Deficiency of Mg and Zn causes different life threatening diseases. That is why the optimum level of Mg and Zn must be maintained for all biological systems. The experiment was aimed to evaluate the effect of magnesium and zinc on the efficacy of antibiotic agents against different microorganisms. It has been observed that the antibiotic activity of an antibiotic agent increased significantly with concomitant use of Mg salt at a concentration ranging from 3-9 µg per antibiotic disc. Similarly, Zn salt increased the activity of an antibiotic at a concentration ranging from 9-15 µg per antibiotic disc. The experiment revealed that concomitant administration of antibiotic with Mg or Zn salt not only will supplement the deficiency of these electrolytes but also will increase the activity of antibiotics against different bacterial strains.

Keywords: Magnesium, zinc, antimicrobial activities, antibiotics.

INTRODUCTION

Magnesium is one of the most abundant minerals in the body and is a cofactor in over 300 enzymatic reactions. It is required for protein and nucleic acid synthesis, the cell cycle, cytoskeletal and mitochondrial integrity and the binding of substances to the plasma membrane. Magnesium

is essential for almost all hormonal reactions in the body because Mg is required for adenylate cyclase activity, which is crucial for the transmission of extracellular hormonal signals to intracellular targets (Boyd *et al.*, 1983). Total body Mg content is approximately 1.15 mol of Mg, in an average 70 kg adult. Only the free ionized Mg fraction is biologically active (Wacker and Parisi, 1968).

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Table 1
Zone of inhibition of different antibiotics against different microorganisms with and without magnesium salt
(3 µg/disc of magnesium sulphate)

Name of the Microorganism	Name of antibiotic (µg/disc)	Zone of inhibition (mm)		
		Antibiotic only	Antibiotic + Mg salt	Only Mg salt (6 µg/disc)
<i>Bacillus megaterium</i>	Ciprofloxacin(5)	27.8±1.76	30.5±0.87	No effect
	Ceftriaxone (30)	13.8±0.83	12.2±2.02	
	Gentamicin (10)	22.3±0.57	24.0±0.50	
	Nalidixic acid (30)	20.3±0.57	24.2±0.29	
<i>Sarcina lutea</i>	Tetracycline (30)	18.0±1.0	22.5±0.50	No effect
	Cephadrine (25)	23.7±1.15	24.2±0.76	
	Amoxycillin (30)	14.0±1.00	16.5±0.87	
	Ciprofloxacin (5)	28.5±1.80	31.2±0.76	
<i>Salmonella typhi</i>	Ciprofloxacin (5)	34.7±0.57	38.5±0.50	No effect
	Ceftriaxone (30)	36.0±1.00	39.8±0.29	
	Cephadrine (25)	27.5±0.50	28.2±0.57	
	Amoxycillin (30)	41.7±1.15	41.8±1.04	
<i>Staphylococcus aureus</i>	Cloxacillin (1)	21.0±1.00	21.2±1.04	No effect
	Gentamicin (10)	23.0±1.01	24.8±0.29	
	Nalidixic acid (30)	17.8±0.76	18.8±0.57	
	Ciprofloxacin (5)	29.6±0.57	30.5±0.98	
<i>Escherichia coli</i>	Gentamicin (10)	24.0±1.00	27.2±0.57	No effect
	Tobramycin(10)	22.1±1.02	25.0±1.01	
	Ciprofloxacin (5)	36.7±0.57	39.0±0.01	
	Nalidixic acid (30)	32.7±0.57	34.4±0.29	
<i>Bacillus subtilis</i>	Ciprofloxacin (5)	26.0±0.02	30.5±0.50	No effect
	Cephadrine (25)	23.0±1.73	24.8±1.76	
	Nalidixic acid (30)	18.3±0.57	22.5±1.32	
	Amoxycillin (30)	12.0±1.00	16.8±0.29	
<i>Bacillus cereus</i>	Ciprofloxacin (5)	25.3±1.83	25.3±0.57	No effect
	Gentamicin (10)	21.7±0.57	22.5±0.50	
<i>Pseudomonas sp.</i>	Carbencillin(100)	21.7±1.36	23.5±.25	No effect
	Ciprofloxacin (5)	29.7±0.57	29.8±0.76	
	Amoxycillin (30)	17.5±0.50	17.7±0.52	
	Nalidixic acid (30)	29.0±1.02	25.5±2.60	
	Gentamicin (10)	26.8±3.33	27.2±2.75	
<i>Klebsiella pneumoniae</i>	Ciprofloxacin (5)	34.3±1.15	37.8±1.76	No effect
	Cephadrine (25)	23.0±1.77	23.5±0.87	
	Nalidixic acid (30)	27.0±0.02	28.2±0.29	
	Amoxycillin (30)	21.7±0.57	21.3±1.61	
<i>Shigella dysenteriae</i>	Ciprofloxacin (5)	24.7±1.53	25.2±0.76	No effect
	Amoxycillin (30)	17.5±0.50	16.1±1.73	
	Cephadrine (25)	24.2±0.76	23.8±1.04	
	Nalidixic acid (30)	22.3±0.57	22.7±0.29	

There are some reports of health benefits of magnesium in human body. The mineral Mg helps coordinating the activity of the heart muscle as well as the functioning of the nerves that initiate the heartbeat. Cardiovascular problems associated with magnesium deficiency can be solved by magnesium supplements (Resnick *et al.*, 1984, John *et al.*, 1992). Mg plays a role in reducing elevated blood pressure by relaxing the muscles that control blood vessels allowing blood to flow more freely. Because magnesium can help to lower blood pressure and inhibit dangerous arrhythmias,

two common complications in those with congestive heart failure, a weakened heart may benefit from extra doses of this mineral (Rubenowitz *et al.*, 1999). It has been observed that sufficient amount of Mg may protect against non-insulin dependent (type 2) diabetes and its complications, such as eye disease (Resnick *et al.*, 1984). Magnesium's role in relaxing contracted or stiff muscles makes it helpful for relieving the pain associated with fibromyalgia, a chronic rheumatic disorder (Jing *et al.*, 1995). For proper muscle contraction and relaxation, magnesium and calcium

Table 2
Zone of inhibition of different antibiotics against different microorganisms with and without magnesium salt
(6 µg/disc of magnesium sulphate)

Name of the Microorganism	Name of antibiotic (µg/disc)	Zone of inhibition (mm)		
		Antibiotic only	Antibiotic + Mg salt	Only Mg salt (6 µg/disc)
<i>Bacillus megaterium</i>	Ciprofloxacin(5)	36.7±0.62	38.8±0.30	No effect
	Ceftriaxone (30)	09.3±0.10	09.7±0.57	
	Gentamicin (10)	22.8±0.31	28.1±1.70	
	Nalidixic acid (30)	22.1±1.05	24.8±1.76	
<i>Sarcina lutea</i>	Tetracycline (30)	18.5±0.52	22.8±0.57	No effect
	Cephadrine (25)	24.33±0.57	28.7±0.76	
	Amoxycillin (30)	15.8±0.76	19.5±0.50	
	Ciprofloxacin (5)	29.7±0.57	31.3±0.57	
<i>Salmonella typhi</i>	Ciprofloxacin (5)	33.7±0.57	40.6±0.76	No effect
	Ceftriaxone (30)	33.3±4.20	37.7±3.78	
	Cephadrine (25)	31.7±2.10	30.0±1.50	
	Amoxycillin (30)	41.0±1.02	47.0±0.01	
<i>Staphylococcus aureus</i>	Cloxacillin (1)	21.7±0.57	22.33±1.5	No effect
	Gentamicin (10)	23.2±1.90	24.3±1.15	
	Nalidixic acid(30)	16.0±2.20	18.7±1.53	
	Ciprofloxacin (5)	30.3±0.57	33.2±1.53	
<i>Escherichia coli</i>	Gentamicin (10)	24.5±0.50	28.0±0.50	No effect
	Tobramycin(10)	22.7±0.57	27.1±0.76	
	Ciprofloxacin (5)	38.0±1.21	42.8±0.29	
	Nalidixic acid (30)	30.0±0.05	34.5±0.50	
<i>Bacillus subtilis</i>	Ciprofloxacin (5)	24.7±0.57	27.3±0.57	No effect
	Cephadrine (25)	30.0±0.10	29.0±0.87	
	Nalidixic acid (30)	16.3±0.33	16.8±0.29	
	Amoxycillin (30)	14.7±0.57	17.5±0.50	
<i>Bacillus cereus</i>	Ciprofloxacin (5)	24.7±0.57	25.7±0.57	No effect
	Gentamicin (10)	21.0±0.06	21.0±1.01	
<i>Pseudomonas sp.</i>	Carbencillin (100)	11.5±0.50	11.5±0.50	No effect
	Ciprofloxacin (5)	26.0±1.02	27.7±0.50	
	Amoxycillin (30)	25.0±4.00	25.8±3.75	
	Nalidixic acid (30)	17.33±0.57	20.7±0.76	
	Gentamicin (10)	26.0±1.00	29.0±0.50	
<i>Klebsiella pneumoniae</i>	Ciprofloxacin (5)	30.5±0.50	31.7±2.31	No effect
	Cephadrine (25)	23.3±0.57	23.2±0.76	
	Nalidixic acid (30)	25.0±1.00	31.0±0.50	
	Amoxycillin (30)	25.5±0.5	23.3±1.53	
<i>Shigella dysenteriae</i>	Ciprofloxacin (5)	26.8±0.29	30.0±0.87	No effect
	Amoxycillin (30)	20.0±0.02	23.0±0.01	
	Cephadrine (25)	18.7±0.57	22.7±0.57	
	Nalidixic acid (30)	17.3±0.57	17.8±0.76	

need to be present in balanced amounts. A supplement containing these minerals, taken regularly, may lessen the pain from sports injuries or excessive physical activity (Jing et al., 1995). Magnesium is used to prevent migraines, relieve premenstrual syndromes (Shiels, 1998), minimizes the severity of asthma attack and prevents osteoporosis

(Nadler and Rude, 1995) etc. Zinc is also essential for over 100 enzymatic reactions in the human body.

Zinc is present in all the tissues and fluids of the body. The total body content has been estimated to be approximately 2-3 g. Whole blood contains about 900 µg/100 ml, of which

Table 3

Paired T-test for two related samples (for control antibiotic and antibiotic with magnesium sulphate solution)

Amount of Mg sulphate per antibiotic disc (μg)	P-value
0.9	0.099
1.5	0.149
3.0	0.000
6.0	0.000
9.0	0.000
12.0	0.000

Table 4

Paired T-test for two related samples (for control antibiotic and antibiotic with zinc sulphate solution)

Amount of Zn sulphate per antibiotic disc (μg)	P-value
3	0.109
6	0.08
9	0.000
15	0.000
30	0.000

85% in RBC, 12% in plasma and 3% in leukocytes and 70% plasma Zn is bound to albumin (Jahan and Rahman, 2000). Zinc is not accumulated in any tissue, so there is no store of Zn in the body. Zinc supplementation can fight colds and flu, accelerate healing of canker sores and sore throat, promote healing of skin wounds, eczema, burns and other irritations, control acne and diabetes, protect against osteoporosis and treat hormone-related infertility problems (Sazawal *et al.*, 1998).

Rationale of the work

From the literature survey it has been observed that magnesium and zinc are essential elements to maintain the activity of different biological processes. Both play important part in various biochemical reactions by acting as a cofactor for many enzymes. In many cases without Mg or Zn, protein cannot maintain its active conformation. The same is true for other biological systems as in microorganisms. With this view in mind we designed this experiment. Most of the antibiotics target essential proteins of microorganisms. After binding with antibiotics essential proteins become inactive resulting in the death of microorganisms. In some cases antibiotics interfere in the process of protein synthesis in microorganism. Therefore, if Mg or Zn help to maintain the active conformation of the protein, obviously more drug will be able to bind with that particular protein and as a result more microorganisms will die. The data generated from this study will focus on the potentiality of concomitant administration of magnesium or zinc salt with different antibiotics.

MATERIALS AND METHODS

Both gram positive and gram negative organisms were selected to observe the activities of different antibiotics in the presence and the absence of Mg salt. The following organisms were used to conduct the experiment; *Sarcina lutea*, *Salmonella typhi*, *S. aureus*, *E. coli*, *Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas sp.*, *Klebsiella pneumoniae*, *Shigella dysenteriae*. The same experiment was repeated for Zn salt. Nutrient agar medium was used to prepare fresh culture and Mueller-Hinton agar medium was used for antibacterial sensitivity test. Media and salts were purchased from Sigma. Different concentrations of Mg and Zn salts were added with antibiotic disc. Only Mg salt or Zn salt was used as control. Salts were added to the disc using micropipette. Antibacterial activity was observed using disc diffusion method and each experiment was repeated thrice.

RESULTS AND DISCUSSIONS

Antibacterial activities of different antibiotics in the presence and the absence of magnesium sulphate were observed. In this experiment different amount of Mg sulphate was used for each antibiotic disc e.g. 0.9 μg , 1.5 μg , 3 μg , 6 μg , 9 μg and 12 μg . Different antibiotic discs used in this experiment are mentioned in table 1 and 2. In each experiment we analyzed randomness test and normality test and found that samples were random and came from a normally distributed population ($P > 0.05$). Then we analyzed samples with paired T-test for two related samples. P-values less than 0.05 means the null hypothesis is rejected that is the result is significantly different due to presence of Mg or Zn salt. Therefore, Mg or Zn salt has increased the activities of different antibiotics. P value more than 0.05 means Mg or Zn has no effect on antibiotic activity. From the experiment we observed that Mg or Zn salt itself did not show any activity against 10 selected organisms. Mg salt increased the activity of some antibiotics against some specific organisms at a concentration of 3 $\mu\text{g}/\text{disc}$ or higher (table 1 and 2). At Mg salt concentration of 3 $\mu\text{g}/\text{antibiotic disc}$, the activities of nalidixic acid, ciprofloxacin, ceftriaxone, gentamicin and tobramycin was found to be increased against *Bacillus megaterium*, *Sarcina lutea*, *Salmonella typhi*, *E. coli* and *Bacillus subtilis* respectively. At Mg salt concentration of 6 $\mu\text{g}/\text{antibiotic disc}$, the activities of gentamicin, tetracycline, ciprofloxacin, tobramycin and nalidixic acid was increased against *Bacillus megaterium*, *Sarcina lutea*, *Salmonella typhi*, *E. coli* and *Klebsiella pneumoniae* respectively. When the Mg salt concentration was increased to 9 $\mu\text{g}/\text{antibiotic disc}$, the activities of maximum antibiotics increased irrespective of the type of microorganism. At Mg salt concentration of 12 $\mu\text{g}/\text{antibiotic disc}$ the influence of Mg salt upon the antimicrobial activities of maximum antibiotic remained the same as was shown for concentration of 9 $\mu\text{g}/\text{disc}$. From the p values (table 3) it can be concluded that 3-9 μg Mg salt

per antibiotic disc was the optimum concentration for increased antibacterial activities of different antibiotics. From the experimental data it may be possible to optimize the amount of Mg salt required for concomitant administration with different antibiotics. Before that it is imperative to repeat the experiment in animal model. Similar experiment was repeated for Zn salt in different concentrations. The experiment was started with 3 µg Zn salt per antibiotic disc and continued until 30 µg Zn salt per antibiotic disc. From the p values it was observed that optimum result was obtained at Zn sulphate concentration of 9-15 µg/disc (table 4). At Zn salt concentration >15 µg/antibiotic disc, no increase in the antibacterial activities of different antibiotic agents were observed. Zn salt concentration less than 9 µg/antibiotic disc did not produce any significant effect on antibacterial activities of the antibiotics. Conformational change of protein molecule brought about by Zn or Mg and subsequent interaction of antibiotic-protein can be held responsible for such increased activity.

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