Aluminium – Magnesium Silicate enhances antibacterial activity of Ampicillin trihydrate, against *Salmonella gallinarum*

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Abstract

Solutions of different concentrations, of Ampicillin trihydrate (AT) and of a formulation of AT in Aluminium Magnesium Silicate (AMS), were used for sensitivity test on *Salmonella gallinarum* cultures. Also, *S. gallinarum*-infected chicks were treated with ; 10 mg / Kg (AT), 10 mg / Kg (AT in AMS), 7.5 mg / Kg (AT), 7.5 mg /Kg (AT in AMS). Mean inhibition area, 28.39 $\pm$ 2.07 mm produced by AT did not vary significantly (P= 0.0581) from 26.36 $\pm$ 2.05 mm produced by AT in AMS. However, 17.5 $\times 10^5$ *Salmonella gallinarum* culture forming units per ml of bile of the untreated chicks and 3.4 $\times 10^5$ (80.58 % reduction), 2.5 $\times 10^5$ (85.7 % reduction) , 5.4 $\times 10^5$ (69.2 % reduction) and 0.38 $\times 10^5$ (97.8 % reduction) of the respective treated groups, showed AMS significantly (P =0.01) improved AT`s ability to clear the infection, in vivo.

Key words: Antibiotic resistance,Synthetic Aluminium – Magnesium Silicate , Stabilization, *Salmonella gallinarum.*

Background

Salmonellosis (typhoid fever) is still a leading human health challenge in the world. In 2,000 alone, typhoid fever caused over 216,500 human deaths[1]. Though incidence of salmonellosis in humans is highest in less developed countries of Asia, Africa and Latin America[2], it is still a public health concern even in the USA[3]. It is a zoonosis and leads to condemnation of meat at meat inspection. So, it is a concern both for public health and as a cause of loss of investment to livestock farmers and to meat processing factories[4].

Development of resistance by bactria to antibiotics often results if the antibiotics fail to ellinate the bacterial infections[5].For Ampicilline, its antibacterial activity depends on its bioavailability and on lenght

of time it is able to maintain high concentration in blood of treated animals[6]. Differences in therapeutic actions of drugs made from same medicinal active ingredients but manufactured by different companies are reported to be due to interactions of components used in formulating the drugs and on stabilizing agents used[7].

Alluminium – Magnesium Silicate (AMS) is used as stabilizing agent for drugs used for treatment of diseases of man and of animals[8,9]. It is safe for use even in food animals[10] and was also decleared safe in a recent assessment that involved topical and oral administration to laboratory animals[11]. Its molecules have one of their ends positively charged and the other negatively charged[9]. These electrical charges make AMS, when in solution, to hydrate and form three dimentional coloidal structures which stabilize drugs` active ingredients[9] .

To stabilize means to protect against destruction. So,if AMS is used to make a formulation of Ampicillin trihydrate, it may protect the antibiotic from being rapidly degraded by metabolic processes. If high concentration of Ampicillin is retained in blood of treated animals for a long time, it may lead to better clearance of Salmonella organisms. Enhanced bacterial clearance by antibiotics could reduce incidence of antibiotic resistance by bacteria, including the Salmonella species.

The natural AMS contains many impurities[9]. If it is used at doses higher than those currently employed, the impurities it contains could lead to adverse side effects on treated animals or humans. To overcome this problem posed by impurities in the natural AMS, we reacted Aluminium Silicate and Magnesium Silicate to get a Synthetic Aluminium – Magnesium Silicate[11].

Summary of methods

Ampicillin trihydrate powder and a 2.5 % of the same Ampicillin powder, formulated in a synthetic AMS[11] were tested for antibacterial effect, against *S. gallinarum,* in vitro. One gramm of the 2.5 % Ampicillin in AMS and 0.1 g of the Ampicilin powder were disolved in 1 ml and in 4 ml of normal saline respectively, to obtain Ampicillin concentration of 25 mg / ml in each of the two solutions. The solutions were then, each, serially double diluted to get Ampicillin concentrations of 12.5 mg / ml, 6.25 mg / ml, 3.125 mg / ml and 1.5625 mg / ml for each of the two solutions.The five Ampicillin trihydrate concentrations of each of the two solutions were then used for sensitivity test on pure cultures of *S. gallinarum*. Areas of inhibition on replicate cultures tested, were measured and their means recorded as areas of inhibition for the concentrations of Ampicillin in the two AT drug preparations. Mean inhibition diameters for the Ampicillin powder and for the Ampicillin-AMS drug formulation were compared by the student T – test.

In the in vivo experiment, fifty chicks infected with *S. gallinarum* were randomly divided into five groups. Two groups were treated, for five days, at dose rates of 10 mg and 7. 5 mg of Ampicillin per Kg body weight respectively, with the Ampicillin powder. Two other groups were similarly treated with the Ampicillin–AMS drug formulation while the fifth group served as control. Clinical signs and lessions of salmonellosis in the five groups were recorded. Also, *S.gallinarum* Colony Forming Units per ml (CFU/ml) of bile of chicks in the different treatment categories were determined and tested for statistical difference by Analysis Of Variance.

Findings

Mean diameter of inhibition of *S.gallinarum* by Ampicillin trihydrate was 28.30 ± 2.07 with the Ampicillin powder and 26.36 ± 2.05 with the Ampicillin–AMS preparation (P= 0.0581) . There was no mortality even in the untreated group. However, greenish diarrhae persisted in the untreated group while it ceased in all the treated chicks. Livers of the untreated chicks were congested, haemorrhagic and friable.Those of the group treated with 7.5 mg / Kg Ampicillin powder had only congestion . The group treated with 10 mg / Kg Ampicillin in AMS had haemorrhagic livers. There was no gross lessions on livers of the chicks treated with 10 mg / Kg Ampicillin powder and those treated with 7.5 mg / Kg Ampicillin in AMS. *Salmonella gallinarum* load in bile of the control chicks was 17.5×10^5 CFU / ml while those of the groups treated with 10 mg / Kg (Ampicillin),10 mg / Kg (Ampicillin in AMS), 7.5 mg / Kg (Ampicillin) and 7.5 mg / Kg (Ampicillin in AMS) were 3.4×10^5, 2.5×10^5, 5.4×10^5 and 0.38×10^5 repectively. Rates of reduction in bacterial load in the respective groups treated with Ampicillin powder and with the Ampicillin-AMS drug formulation were: At 10 mg / kg, 80.5 % and 85.7 % (P = 0.01) and at 7.5 mg / kg, 69.2 % and 97. 8 % (P = 0.01) .

Discussion

Detection of *S.gallinarum* infection level of 17.5 CFU/ml of bile of chicks that were apparently healthy is of publichealth concern, because such infected chicken can be passed as wholesome for human consumption, at meat inspection.

AMS improved antibacterial action of Ampicillin both at 10 mg / Kg and at 7.5 mg / Kg. However, its effect on 7.5 mg / Kg was significantly (P=0.01) better than its effect on 10 mg / Kg . It is possible that by delaying degradation of Ampicillin,the AMS caused the normaly used dose of Ampicillin (10 mg / Kg) to become overdose and thus led to toxicity in treated chicks. Antibiotic toxicity can lead to immune supression hence the relative higher CFU/ml of bile. Haemorhages on livers of the chicks treated with 10 mg / Kg Ampicillin in AMS while lessions were absent in chicks treated with 10mg / Kg Ampicillin alone,even when the 10 mg / kg Ampicillin group had higher CFU/ml of bile, supports the suspision that toxicity and not *Salmonella* infection caused the relative reduction in effect of the AMS.

That 10 mg / Kg of Ampicillin powder which is the recomended dose of Ampicillin achieved only 80.58 % reduction of the bacterial load may be one of the causes of antibiotic resistance against the drug by *Salmonellae*.Clearance of the infection by as much as 97.8 % when treatment was with 7.5 mg /Kg Ampicillin in AMS suggests that incorporating Ampicillin trihydrate in AMS may help to reduce incidence of Ampicillin resistant *S.gallinarum.*The 2.2 % left, could be elliminated by immune mechanisms of the chicks.

Use of lower doses of Ampicillin potentiated by AMS, to treat salmonellosis in food animals, has in addition to better bacterial clearance as demonstrated in this study, advantages of reduction in cost of treatment and reduction in level of antibiotic residues in meat of treated animals.

AMS could not enhance ability of Ampicillin trihydrate to inhibit growth of *S. gallinarum* in vitro, but it significantly reduced the bacterial load in bile of treated chicks. This suggests that mechanism by which the AMS potentiated antibacterial action of the antibiotic against the bacterium may not be by increasing its potency but by reducing rate of degradation of the drug. Brent *et al* [6] had already observed that when high concentration of Ampicillin was retained in blood of treated animals, improved antibacterial effects resulted. Vanderbilt[9] reported that AMS is a stabilizing agent. To stabilize means to protect against destruction. So, what AMS does may be to protect drugs against degradation by metabolic processes so that high concentrations are retained in the blood for longer periods after treatment.

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