



Study of the Interactive Effect between Tylosin Tartrate and Colistin for its Use in the Therapy and Prevention of Poultry Diseases in Veterinary Medicine

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ARTICLE INFO	ABSTRACT
Published Online: 09 March 2022	The pharmacological interaction of antibiotic combinations of tylosin and colistin was studied. For this purpose, an in vitro challenge method was used. The results were used to calculate their interaction using the graphic representation known as ISOBLOGRAM. The pure salts of tylosin tartrate and colistin sulfate were obtained from the manufacturer of the product, Laboratorios Ecozoo SA de CV Mexico. The bioassay method was used in agar wells and to quantify the results, the following criteria were used: A synergistic effect was defined when the real/theoretical effect (SMF) was greater than 1. The additive effect was defined when the SMF was equal to 1. The area between the antagonism and additive effect was defined as the zone of indifference. When studying the interaction between tylosin and colistin it was observed that the best SMF of this antibiotic combination was 3 to 2 and 2 : 4 (tylosin colistin respectively). It was concluded that the combination of tylosin with colistin exerts a synergistic effect and can be recommended for oral administration in poultry for the treatment of infections by disease producing germs sensitive to the formula.
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INTRODUCTION

Since the beginning of chemotherapy when sulfonamide was first used and penicillin was discovered, veterinarians have been looking for ways to combine them to increase antimicrobial effectiveness against diseases produced by infectious germs susceptible to each one of them. In this particular case the interaction of colistin with tylosin tartrate was studied.

At present, there are laboratory and graphical methodologies (1) that study trends in antimicrobial effectiveness of antibiotics, when used alone or in combination (2, 3, 4).

First a small resume of the pharmacological properties of the antibiotics here used:

TILOSIN

It is an antibiotic substance of the macrolide group, produced by a strain of *Streptomyces fradiae*, different from the one that produces neomycin. Aqueous solutions of tylosin are stable at pH 5.5 to 7.5 at temperatures of 25°C for periods of up to three months. At acidic pH (4.5 or less) it degrades, first to desmicosin and then to inert products. Tylosin base is poorly water soluble (5 mg/ml at 25°C), but dissolves readily in organic solvents. It combines with minerals and organic

acids to form highly soluble tylosin salts. It is approved for use in domestic animals; mainly in dogs, cats, cows and pigs. It has a pKa of 7.1.

ANTIBACTERIAL SPECTRUM

It attacks gram-positive microorganisms, especially *Mycoplasma gallisepticum* S6. In vitro attacks PPLO of chickens, turkeys, pigs, cows and goats. It also attacks several strains of spirochetes and leptospirae. It is useful against the causal agent of swine erysipelas; *Haemophilus pertussis*, *Moraxella bovis*, *Vibrio* and some gram-negative bacteria.

BACTERIAL RESISTANCE TO TILOSIN

Microbes develop little resistance against this antibiotic. When it occurs, especially in *Staphylococcus aureus*, partial cross-resistance has been observed with erythromycin. However, the author has had the experience that tylosin loses all its effectiveness as a function of the resistance that develops to the compound due to its indiscriminate use as a growth promoter. It is present in many mixtures of feed concentrates for various species of domestic animals.

MECHANISM OF ACTION

It is bacteriostatic and interferes with the production of bacterial proteins by inhibiting the function of the 30S subribosomal unit.

ABSORPTION, METABOLISM AND EXCRETION:

The digestive tract of chickens, turkeys and pigs easily absorb tartrate salt. In hens this salt can be applied subcutaneously. Sometimes, phosphate salt is mixed with pig feed, but it seems to be absorbed with more difficulty than tartrate salt. It is administered parenterally, but intramuscular administration is preferred. To the preparation for intramuscular application, 4% V/V benzyl alcohol is added as a bactericide to the base tylosin, dissolved in propylene glycol and water. After oral or parenteral administration, it is efficiently distributed in organic tissues. It does not cross the brain barrier. But it passes into the lungs and milk in concentrations higher than those of plasma. It is excreted by the liver and kidneys. The LD50 (Lethal Dose 50%) in pigs is 5 g/kg orally and 1 g/kg intramuscularly.

USES OF TYLOSINE

Tartrate salt is effective in the treatment or prevention of chronic respiratory disease (CRD or ECR). Tylosin is useful after vaccinations or any other stress. In turkeys it is useful as support in infectious sinusitis and in prevention of respiratory forms of the same disease. In pigs tylosin can be administered with tartrate in water or as phosphate salt in feed, when treating or preventing enteritis by *Vibrio*. It is recommended to continue treatment with tylosin phosphate, even if the acute symptoms of the disease have disappeared. For cows and calves, tylosin base injected intramuscularly is useful in pneumonia, scabies and metritis. In pigs it is applied intramuscularly against erysipelas, pneumonia and dysentery. In the cat and the dog, tylosin base is applied intramuscularly in upper respiratory infections, otitis externa, cellulitis, metritis, leptospirosis and secondary infections, produced in the normal course of viral affections. It is also used in postoperative treatment.

CONTRAINDICATIONS IN THE USE OF TYLOSIN

Tylosin is not administered to hens in production, because the egg can carry high concentrations of the antibiotic. For human consumption, hens should not be slaughtered for at least three days after the last parenteral application of tylosin, or during the previous 24 hours, if they received the drug orally. In turkeys, once the antibiotic has been administered, it is necessary to wait five days to slaughter them for human consumption. Lactating cows should be removed from the milking line for ninety-six hours, so that their milk is not consumed. Pigs should not be slaughtered during the 21 days following the administration of tylosin. It is also used in intestinal bacterial overpopulation in dogs, in which it is administered with feed three times a day. When administered

orally to cows it can produce considerable diarrhea, in horses it also produces diarrhea that can lead to death, when administered by any route. DOSAGE: BIRDS, hens and turkeys: 0.5 g/l of water for as long as the case requires. Hens: subcutaneously, 1 ml/kg. of weight of 50 mg/ml or 200 mg/ml solution, depending on the severity of the infection. In this case, the total dose should not exceed 2.5 milliliters. If inflammation persists, it can be treated a second time after 10 days. DOG, CAT: 2 to 10 mg/kg/day intramuscularly for three consecutive days. If there is no response, continue the medication; in addition, laboratory tests should be performed. SWINE: 9 mg/kg twice a day, treatment should not exceed three days.

CATTLE AND HORSES

4 to 10 mg/kg/day intramuscularly for three consecutive days, in parallel; antibiograms will be performed.

CONTRAINDICATED IN HORSES. SHEEP

10 mg/kg per day, treatment should not exceed 5 days. In case of abortion by *Vibrio*, tylosin tartrate salt can be administered. It is applied intramuscularly in a total dose of 400 mg per day. Only two applications are given to control the outbreak in about 10 days. In Mexico, indiscriminate use allows the development of resistance in most bacteria. At present, its clinical medication needs reevaluation. INTERACTIONS: Tylosin may increase the toxicity of cardiac glycosides.

COLISTIN BELONGS TO THE POLYMYXIN GROUP.

A group of antibiotics produced by various strains of *Bacillus polymyxin*. There are several types of polymyxins that are designated according to the following nomenclature: A, B, C, D, and E. Types B and E are useful. In America, B is preferentially used, and in Great Britain, E (Colistin). These antibiotics must be used with care because they are polypeptides and therefore potentially toxic. They are cationic detergents. They occur as white or yellowish flakes soluble in water and saline solutions at concentrations no greater than 25 mg/ml of water. They are stable as salts acidic for long periods of time, even in solution. They are easily destroyed by alkalis and the purity of commercial preparations is limited to 65 to 75%. Where 1 microgram is equal to 10 units of polymyxin.

ANTIBACTERIAL SPECTRUM

They mainly attack gram-negative bacteria. In order of importance, their efficacy is reflected in the following bacteria: *Aerobacter*, *Ebertella*, *Escherichia coli*, *Haemophilus*, *Klebsiella*, *Pasteurella*, *Salmonella*, *Shigella*, *Vibrio*, *Pseudomonas*, *Brucella*, *Proteus*, *Neisseria*. They are bactericidal in vitro and are not affected by the presence of serum, blood or pus. They are antagonized by cationic surfactants. When administered orally they can eradicate

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Pseudomonas, which is present in a great variety of tissue infections; it seems that polymyxins are one of the antibiotics that most attack *Pseudomonas*. Their bacterial resistance occurs very little, so they are used in combination with other antibiotics.

MECHANISM OF ACTION

They are cationic surfactants. Polymyxins are absorbed into the bacterial cell where they combine with the structures responsible of osmotic equilibrium.

They alter permeability by allowing purines and pyrimidines to escape and provoke cell lysis. This effect is similar to that produced by cationic detergents. It is possible that they bind to polyphosphate groups near or on the cell surface as they. The effect is similar to that of quaternary ammonium detergents, and they may bind to polyphosphate groups near or on the cell surface, as there is known competitive antagonism between polymyxins and quaternary ammonium detergent cations. Polymyxin B has synergistic action when combined with antibiotics such as oxytetracycline, chloramphenicol, carbenicillin, sulfamethoxazole and tetracycline. Gram-negative bacteria are more sensitive to polymyxins than gram + bacteria.

ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION

It is absorbed slowly by enteric route, but rapidly by intramuscular route and produces maximum concentrations in one or two hours, and an average concentration in 12 hours or more. Its excretion is slow by the kidneys.

MATERIAL AND METHODS

Drugs: Tylosin and colistin in their pure salts were obtained from Laboratorios Ecozoo SA de CV, Tepatitlan Jalisco, Mexico.

The in vitro tests were carried out according to the National Committee for Clinical Laboratory Standards of the United States of America. And adapted and modified according to the methodology suggested by Bennet et al. (1966). Suspensions of *B. subtilis* were made by adding the contents of two ampoules of *Bacillus subtilis* spore suspension (DIFCO) to 100 ml of sterile normal saline and 2.5 ml of a solution containing 0.3825 dibasic potassium phosphate and 0.0833 g of monobasic potassium phosphate to bring the pH of the *B. subtilis* solution to a value of 7.0.

The antibiotic standards tested were vacuum dried for a minimum of 48 hours and then carefully weighed and added to a solution to achieve a concentration of 1000µg/ml. This concentration functions as a stock solution from which the corresponding dilutions are made for the in vitro tests.

The combination of tylosin and colistin was tested by making serial dilutions of the two antimicrobial agents, which were mixed in such a way that each row and column consisted of a fixed amount of one agent and increasing amounts of the

other antimicrobial. The concentration ranges used were based on the MICs obtained for each of the anti-infective agents.

Each of the anti-infective agents used and the bacteria used as a test. Dilutions covered 4 x MIC (antagonistic action) and 0.25 x MIC (synergistic action): 75 µL aliquots of bacteria (c. 1 x 10⁶ cfu/mL) and 75 µL of each antibiotic were added to each microtiter plate. As controls, MIC of each antibiotic alone and their combinations were determined on each plate. Plates were incubated overnight at 37°C and bacterial growth was visually inspected and then confirmed by photometer (Bausch & Lomb) at an optimal density of 540 nm.

The results were collated and in cases where synergistic trends were observed, the changes in MIC were plotted and their trend observed with the resulting isobolograms.

RESULTS AND DISCUSSION

Figure 1 shows the experimental arrangement to study the antibiotic properties of tylosin with colistin; it represents an agar plate with 96 wells in which the reference germs and different concentrations of antibiotics were deposited and from which six solutions were prepared.

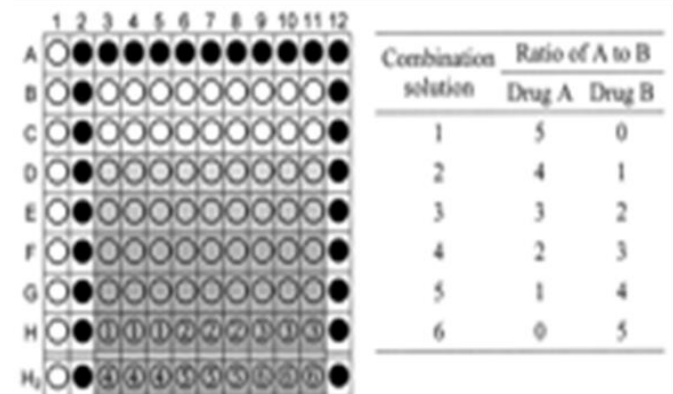


Figure 1. Plate arrangement

The uncolored or clear wells functioned as controls (no antibiotic and no bacteria), the dark wells functioned as controls for bacteria (no antibiotic 0% growth inhibition) and the wells labeled 1 to 6 functioned as medicated wells for six combinations of antibiotic dilutions, in triplicate, while the wells in row H received the highest concentration of antibiotic combination. Two 96-well plates were made with row H2 representing solutions 4 to 6 of the second 96-well agar plate.

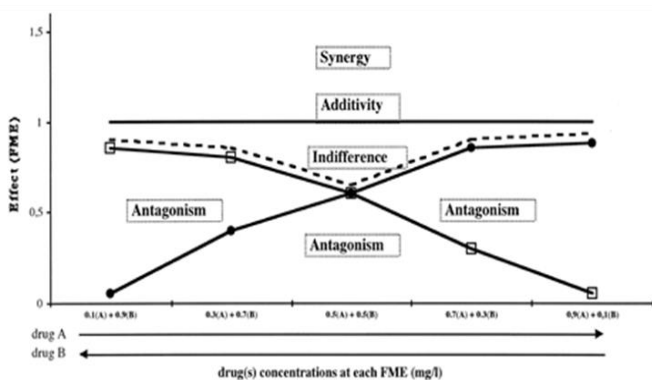
In the antibiotic combinations the proportions used A corresponds to Tylosin while B corresponds to Colistin. These results can be seen in the following table:

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Table 1. The values of the graphical representation of the changes in MIC and observe their trend with the resulting isobolograms in a combination of Tylosin with colistin in the solutions with the mentioned proportions

	Solution ratio of A to B		
	Antibiotic A	antibiotic B	MIC value
1	5	0	1.0
2	1.0	4	1
3	1.1	3	2
4	1.1	2	3
5	1.0	1	4
6	1.0	0	5

The graph of our results was made according to the following scheme (7):



When the corresponding results observed in Table 1 are applied, the resulting graph is as shown in Figure 2, and in which the trends of the combinations can be observed in terms of the proportions used in the interaction tests.

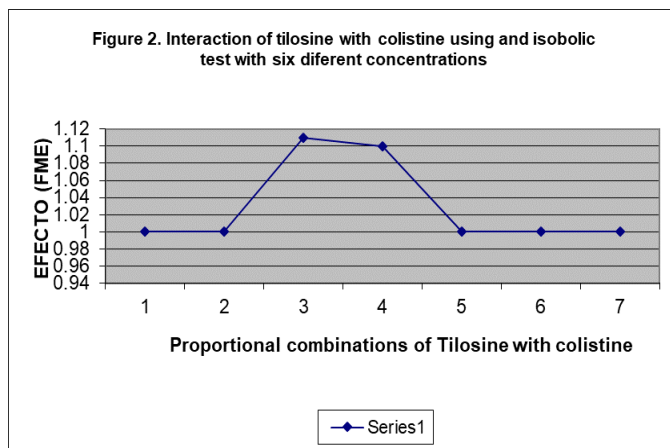


Figure 2. Combination trend

It can be argued that the view of the graph does not agree with what is expressed in the introduction, for this interpretation the effect of the antibiotic combinations used should be taken into account according to the scheme presented as a reference. (7).

It can be observed that there is a repetitive line related to an additive state of tylosin with colistin, respectively, results that

agree with other similar studies with different chemical compounds and antibiotics (8) against E. coli...

Based on the results obtained with this study it can be postulated that the antibiotic combination used between the proportions studied tend to establish an additive effect between the two antibiotics studied but the one that tends to a synergistic effect is in the solution 3 and 4.

CONCLUSION

The antibiotic combination of tylosin with colistin in the proportion of 3 : 2 and 2 : 3 respectively, present an additive and slightly synergistic effect, therefore it will be usefull for therapy of susceptible infections in poultry

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