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Study of the Interactive Effect between Fosfomycin and Tylosin for Use in the Therapy and Prevention of Disease in Veterinary Medicine

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ABSTRACT

The pharmacological interaction of the combination of Fosfomycin and tylosin was studied. An in vitro challenge method was used for this purpose. The results were used to calculate their interaction using the graphical representation known as ISOBOLOGRAM. The agar well bioassay method was used. The following criteria were used to quantify the results: A synergistic effect was defined when the actual/theoretical effect (SMEF) was greater than 1. The additive effect was defined when the SMEF was equal to 1. The area between the antagonism and additive effect was defined as the zone of indifference. When studying the interaction between Fosfomycin and Tylosin, it was observed that the best SMF of this antibiotic combination was 2 : 3. This result allows postulating that the use of this combination of Fosfomycin and Tylosin establishes a well recognised and documented synergistic effect between them and can be recommended in pigs and poultry for the treatment of infections by gram + and gram - germs and other infectious agents sensitive to this combination.

KEYWORDS: Fosfomycin, Tylosin, Veterinary Medicine

INTRODUCTION

Fosfomycin is an antibiotic formulated in the 1960s, used primarily for the treatment of infectious diseases of broiler chickens and piglets.

Over the years it has maintained its activity and has shown stable rates of resistance, Fosfomycin has emerged as a candidate antibiotic to treat infections caused by carbapenem-, tigecycline-, and polymyxin-resistant bacteria (Gobernado 2003,Han et al, 2021). Fosfomycin shows promising during septic states related to lung injury by infectious agents, and for its antioxidant and anti-inflammatory properties.

Tylosin is a commonly used in-feed antimicrobial and is approved in several countries to reduce the incidence of liver abscesses in beef cattle (Caser et al, 2020). In Mexico, combinations of tylosin with fosfomycin are common and recommended for various infections in domestic animals, which justifies synergy studies between fosfomycin and tylosin.

The aim of this study is to investigate the antibiotic interaction (antagonism, synergism) of the combination of Fosfomycin and Tylosin, which allows for a short review of the therapeutic properties of these antibiotics as follows:

FOSFOMYCIN (phosphonomycin)

This bactericidal antibiotic is used in difficult infections in human medicine, however, it is not yet approved by any pharmacopoeia. But it has been used in productive animals for 40 years (Perez et al. 2014). It is L-(cis)-1,epoxypropyl phosphonic acid. It is obtained from strains of Streptomyces fradiae. However, it is currently manufactured by industrial synthesis. In humans it has been used in therapy of localised peritonitis, localised brain abscesses, severe soft tissue infections, cystitis and other infections. In Asia and Latin America it is widely used in swine infections.

Fosfomycin does not bind to plasma proteins and is cleared via the kidneys. Due to its extensive tissue penetration, fosfomycin may be indicated for infections of the CNS, soft tissues, bone, lungs, and abscesses (Dijkmans et al. 2017). It is highly soluble in hard or soft water.

MECHANISM OF ACTION: Interferes with bacterial cell wall formation, it is actively transported into the bacterium. For which it can use two pathways (in E.coli) using the transport systems l-alpha-glycerophosphate and hexose-6-phosphate.

Fosfomycin decreases bacterial adherence to urinary tract epithelial cells, especially Streptococcus and Haemophilus. It is synergistic with beta-lactams, aminoglycosides,

chloramphenicol, tetracyclines, erythromycin, co-trimoxazole and quinolones.

ANTIBACTERIAL SPECTRUM: It is considered broad spectrum, showing effectiveness against Staphylococcus aureus, Streptococcus and Gram. Negative bacilli of Enterobacteriaceae. It is also considered to be relatively active against Pseudomonas aeruginosa.

It has the ability to penetrate biofilms, structures that prevent access to antibiotics. Rifampin and fosfomycin may be antagonistic.

Can be synergistic with Linezolid, ciprofloxacin, ceftriaxone, ciprofloxacin, rifampin, Cefamandole, cefazolin, vancomycin, rifampin, carbapenems, cefmetazole, cefoperazone-sulbactam, linezolid, quinupistin-dalfopristin, fusidic acid, minocycline, tigecycline, daptomycin.

BACTERIAL RESISTANCE: This can be easily realised especially when administered alone. This makes it necessary to use high doses, especially when treating patients affected by Gram. Negative germs.

The structure and mechanism of action of fosfomycin are unique, which prevents cross-resistance with other antibiotics.

It crosses the brain barrier, especially in the presence of inflammation, and reaches cortical and callosal bones.

DOSE: These only apply to humans and are as follows: capsules, injectable and suspension. 50 mg/kg/day in children weighing less than 20 kg is 100 mg/kg. However, kinetic profiles have been performed in hens, rabbits, cows, dogs, horses and piglets.

In dogs, doses of 40 mg/kg fosfomycin calcium per os and iv sodium salt were used.

In cats 20 mg/kg of calcium salt per os and Na salt per iv, in piglets 15 mg/kg.

In veterinary medicine, its use in Mexico and other countries has been oriented to the treatment of infections in broilers and piglets, it is eliminated in 2 to 7 days, the withdrawal time is 2 to 3 days after intramuscular or per os administration.

Other effects In addition to antibacterial activity, FOS have other properties, such as inhibition of bacterial adhesion to epithelial cells, biofilm penetration of exopolysaccharide penetration, immunomodulation, phagocytosis promotion and protection against nephrotoxicity caused by other drugs (Perez et al.2014).

THYLOSINE

Antibiotic of the macrolide group, produced by a strain of Streptomyces fradiae, different from that which produces neomycin. Aqueous solutions of tylosin are stable at pH 5.5 to 7.5 at temperatures of 25°C for 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 25oC), 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 dogs, cats, cattle and pigs. It has a pKa of 7.1.

ANTIBACTERIAL SPECTRUM: Attacks gram-positive microorganisms, especially Mycoplasma gallisepticum S6. In vitro attacks PPLO of chickens, turkeys, pigs, cows and goats. Also various strains of spirochetes and leptospires. It is useful against the causative agent of swine erysipelas; Haemophilus pertussis, Moraxella bovis, Vibrio and some gram-negative bacteria. BACTERIAL RESISTANCE TO THYLOSINE: Microbes develop little resistance to this antibiotic. When it does occur, especially in Staphylococcus aureus, partial cross-resistance with erythromycin has been observed. However, it has been the author's experience that tylosin loses all effectiveness as resistance develops to the compound through indiscriminate use as a growth promoter. It is found in many blends of feed concentrates for various species of domestic animals. MECHANISM OF ACTION: It is bacteriostatic and interferes with bacterial protein production by inhibiting the function of the 50S subribosomal unit. Inhibits protein synthesis by interfering with mRNA.

ABSORPTION, METABOLISM AND EXCRETION: Tartrate salt is readily absorbed by the digestive tract of chickens, turkeys and pigs. In hens, this salt can be applied subcutaneously. Phosphate salt is sometimes mixed with pig feed, but seems to be more difficult to absorb 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 organ tissues. It does not cross the brain barrier. But it passes into the lungs and milk in concentrations higher than 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). Tylosin is useful after vaccinations or any other stress. In turkeys it is useful as a 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 Vibrio enteritis. 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 cats and dogs, tylosin base is used intramuscularly for upper respiratory infections, otitis

externa, cellulitis, metritis, leptospirosis and secondary infections caused in the normal course of viral diseases.

CONTRAINDICATIONS IN THE USE OF TYLOSIN

Tylosin is not administered to hens in production, because the egg may carry high concentrations of the antibiotic. For human consumption, hens shall not be slaughtered for at least three days after the last parenteral application of tylosin, or for 24 hours before if they received the drug orally. In turkeys, after administration of the antibiotic, five days must be allowed before slaughter for human consumption. Lactating cows should be removed from the milking line for 96 hours so that their milk is not consumed. Pigs should also not be slaughtered for 21 days after tylosin administration. It is also used in intestinal bacterial overpopulation in dogs, where it is administered with feed three times daily. When administered orally to cows it can cause severe diarrhoea, in horses it also causes diarrhoea which 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 body 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 ml. If inflammation persists, a second treatment can be given 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 carried out. SWINE: 9 mg/kg twice daily, treatment should not exceed three days. CATTLE: 4 to 10 mg/kg/day intramuscularly for three consecutive days, in parallel; antibiograms should be carried out.

CONTRAINDICATED IN HORSES

SHEEP: 10 mg/kg per day, treatment should not exceed 5 days. In case of Vibrio abortion, tylosin tartrate salt can be administered. It is applied intramuscularly in a total dose of 400 mg per day. Only two applications will be 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 re-evaluation.

INTERACTIONS: Tylosin may increase the toxicity of cardiac glycosides. Combined with florfenicol it has a synergistic effect. As a growth promoter it has been used in pigs, accelerating the maturation of the intestinal microbiota with similar effect in piglets combined with colistin and/or florfenicol. DOI: 10.5433/1679-0359.2016v37n4p1947

. DOI:10.4014/jmb.1512.12004 In poultry, tylosin has also been reported as an effective growth promoter.

TYLOSIN RESIDUES AND WITHDRAWAL TIME

Residues of tylosin depend on the route of administration.

In poultry the withdrawal time of tylosin is considered to be at least 6 days.

In dairy cows it is recommended not to use milk from 6 milkings, tylosin residues in dairy cows were no longer detectable at 108-144 hours.

In pigs tylosin 100g/ton in feed no amounts were observed within lps MRLs and not detectable at the recommended 21 days withdrawal.

MATERIAL AND METHODS FOR THE DEVELOPMENT OF THIS STUDY

Medicines: Fosfomycin and Tylosin in their pure salts were obtained from Farmacom de Mexico S. A. de C .V., Mexico.

In vitro tests were performed 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 dried under vacuum for a minimum of 48 hours and then carefully weighed and added to a solution to achieve a concentration of 1000µg/ml. This concentration serves as a stock solution from which dilutions are made for in vitro testing.

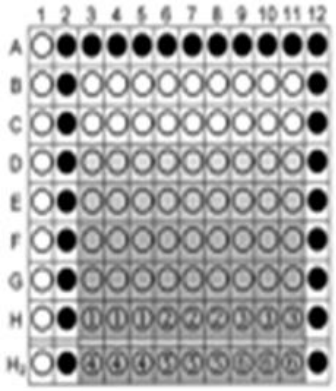
The effect of the combination of Fosfomicin and Tylosin was tested by making serial dilutions of the two antimicrobial agents, which were mixed in such a way that each row and column consisted of a fixed amount of one agent and increasing amounts of the other antimicrobial. The concentration ranges used were based on the MICs obtained from 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): Aliquots of 75 µL of bacteria (c. 1 x 106 cfu/mL) and 75 µL of each antibiotic were added to each micro titration plate. As controls, the 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 optimum density of 540 nm.

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

RESULTS AND DISCUSSION

In the following figure,



Combination solution	Ratio of A to B	
	Drug A	Drug B
1	5	0
2	4	1
3	3	2
4	2	3
5	1	4
6	0	5

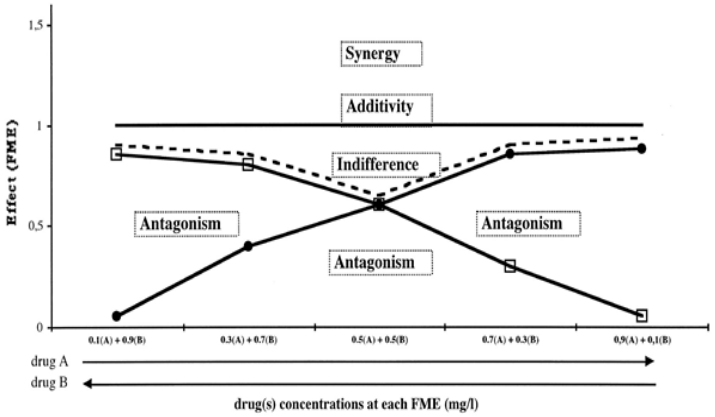
the experimental arrangement to study the antibiotic properties of Fosfomycin and Tylosin can be observed; it represents an agar plate with 96 wells in which the reference germs and the different concentrations of Fosfomycin and Tylosin of which six solutions were prepared. The uncoloured or clear wells functioned as controls (no antibiotic and no bacteria), the dark wells functioned as controls for bacteria (no antibiotic 0% growth inhibition) and the wells labelled 1 to 6 functioned as medicated wells for six combinations of Fosfomycin and Tylosin dilutions, in triplicate, while the wells in row H received the highest concentration of the Fosfomycin + Tylosin 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 in A correspond to Fosfomycin while B corresponds to Tylosin. These results can be seen in the following table:

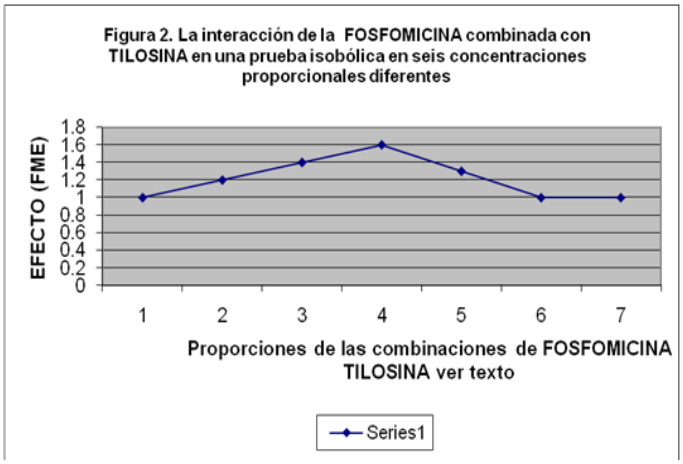
Table 1. The values of the graphical representation of the changes in MIC and observe their trend with the resulting isobolograms in a combination of Fosfomycin and Tylosin in the solutions with the mentioned ratios.

Solution	proportion of A with B		
	Antibiotic A	antibiótico B	
Value of MIC			
1	5	0	
1.0			
2	4	1	
1.2			
3	3	2	
1.4			
4	2	3	
1.6			
5	1	4	
1.3			
6	0	5	
1.0			

The graph of our results was made according to the following scheme (:Desbiolles and Cols. 2000).



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.



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 (Desbiolles et al. 2000).

In this study it can be observed that there is a tendency towards synergy in all combinations, but it becomes apparent that combination 2 is the most representative of a desirable antibacterial synergy. In this combination a ratio of 1 to 1.5 is used for Fosfomycin and Tylosin respectively, results which are in agreement with other similar studies with different chemical compounds and antibiotics (Ulvatne, 2001) against E. coli.

Based on the results obtained with this study, it can be postulated that the combination of Phosphomycin and Tylosin in a 3:2 ratio is suitable for use in pig and poultry diseases caused by germs susceptible to the combination studied. To finally conclude that the combination of Fosfomycin in the ratios of 3:2, 2:3 with Tylosin has the best synergistic effect. Although it is well established that the range of effective combinations can be very wide in

the case of these substances, it should be noted that the antibacterials studied here per se have an activity of 1.0 when not combined with others as can be seen in the table of results and in the corresponding graph.

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