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Pharmacochemical Aspects of the Interaction between Chlortetracycline and Tiamulinfor Use in the Therapy and Prevention of Swine Diseases in Veterinary Medicine

Victor Octavio Fuentes-Hernández
(https://orcid.org/000-0003-0777-1791)
Centro Universitario de los Altos
Universidad de Guadalajara México 47600

***For Correspondence**

Victor Octavio Fuentes-Hernández

Centro Universitario de los Altos
Universidad de Guadalajara
México 47600

victoroctaviof@yahoo.com

vfuentes@cualtos.udg.mx

ABSTRACT
In this work, the pharmacological interaction of the antibiotic combination between chlortetracycline and thiamulin was studied. For this purpose, an in vitro challenge method was used. The results obtained were used to calculate their interaction using the graphical representation known as ISOBOLOGRAM. The pure salts of chlortetracycline and tiamulin were obtained from the product manufacturer ASTRONOVA SA DE CV. The agar bioassay method was used. The following criteria were used to quantify the results: A synergistic effect was defined when the actual/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 chlortetracycline and tiamulin; and it was observed that the best SMF of this antibiotic combination was 3.1 and 3.0 when the concentration ratio between the antibiotics was 4 : 1 and 3 : 2 (chlortetracycline tiamulin respectively) corresponding to solutions 2 and 3 of this test. Consequently it can be postulated that the use of this combination of chlortetracycline with thiamulin exerts a synergistic effect and can be recommended in pigs for the treatment of infections by infectious agents sensitive to the formulation. NOTE: Because this formulation is well known for its synergistic effect and for being elaborated and recommended in veterinary therapeutics in Mexico. The results of this isobologram are similar and in some cases equal to those previously reported for other laboratories in Mexico.

Keywords: Chlortetracyclin, Tiamulin, Interactio, Isobologram

I. INTRODUCTION

It has been demonstrated that chlortetracycline, when simultaneously administered with tiamulin, shows a synergistic antimicrobial effect. This might imply that a combination therapy with these 2 antimicrobial products might allow a reduction of the tiamulin dosage necessary for M. gallisepticum disease control (Garmyn et al 2017). When thia combination was uswd in Fatening pigs, was successful at controlling respiratory and enteric disease and, consequently, improved growth performance and carcass weight of grow-finish pigs (puls et al 2019).

The combination of chlortetracyclin and tyamulin has been documented since the 1970^{s} , And it should be coninually screened to insure that after so many years of use, maintains its synergistic effect.

There are both laboratory (Bennet et al. 1966) and graphical (King, 1981; Hamilton, 1985; Rahal, 1978) methodologies that allow the antimicrobial tendencies of antibiotics, alone or in combination, to be established. Although similar in their mechanism of action.

In this study a review of the pharmacological properties off Tyamulin and chlortetracyclin wil be included:

II. TIAMULIN

Semi-synthetic antibiotic derived from pleuromutilin produced by Pleurotus mutilis. For oral administration hydrogen fumarate is used and for parenteral application tiamulin base is used. It is especially active against Treponema hiodysenteriae, which causes dysentery in pigs, and Haemophilus pleuropneumoniae, which causes pneumonia. Gram-positive bacteria such as Staphylococcus, Streptococcus and gram-negative bacteria such as Shiguella, Klebsiella and E. Coli. It is mentioned to attack Haemophilus pleuropneumoniae. It also attacks gram-positive germs and has good action against Mycoplasma. It is well absorbed orally, and tiamulin-hydrogen-fumarate dissolves effectively in drinking water for pigs and poultry. DISTRIBUTION AND METABOLISM: The hydrogen fumarate salt is highly soluble in water. It is well absorbed when administered orally. It is well distributed to all tissues and especially to the lungs. The drug and its metabolites are excreted in the bile and some in the urine. In ruminants it is well absorbed orally. As a weak, lipophilic organic base, tiamulin penetrates into cells producing several times higher concentrations in milk and tissues. In dogs it has a half-life of 4.7 hours and when administered subcutaneously it has a longer half-life. When administered orally in monogastric animals, it is completely absorbed.

.**INTERACTIONS:** Do not administer to animals receiving ionophore polyether such as monensin, lasalocid, narasin or salinomycin because adverse effects may occur. May be antagonised when combined with clindamycin, lincomycin, erythromycin and tylosin because they compete for the same site of action. DOSE: 9 mg/kg/day. Do not mix with monensin because it prevents biotransformation of monensin and causes toxic effects. Do not administer to pigs weighing more than 125 kg. DO NOT ADMINISTER TO HORSES AND SPECIES WITH EQUINE-LIKE GASTROINTESTINAL TRACTES. USES: In pigs it is administered against mycoplasma pneumonia and dysentery. In cattle, sheep and goats it has the same indications as tylosin. In sheep against Rickettsial keratoconjutivitis in doses of 20 to 30 mg/kg. IV it is necessary to repeat after three days and possible to repeat after 6 to 9 days. In pigs it is used as a growth promoter and also against dysentery (30 ppm in feed, in water 40 to 60 ppm 3 days) and chronic pneumonia. To eradicate dysentery, tiamulin is used at 10 mg/kg. IM to carriers for 5 days plus rodent control, in growing sows orally for 10 days followed by carbadox for 42 days. In enzootic pneumonia due to mycoplasma, it was used at 200 ppm in feed for 10 days at weaning, significantly reducing lung lesions (Fuentes, 2020).

III. CLORTETRACICLINA (Aureomicina, Fermicin)

Polycyclic compound amphoteric and that fluoresce when exposed to ultraviolet light. Most are prepared as the hydrochloride salt. They form insoluble chelates with cations such as Ca2+, Mg2+, Fe3+, and Al3+.

Accumulates in growing teeth and bones forming a tetracyclin calcium complex in the enamel and dentine. Is potentially nephrotoxic, care should be considered in patients with kidney disfunction

Mechanism of action. Tetracyclines reversibly inhibit bacterial protein synthesis by binding to the 30S ribosome and preventing attachment of aminoacyl tRNA to the mRNA-ribosome complex. The addition of amino acids to the growing peptide chain is interrupted. They are bacteriostatic and broad spectrum. Their antimicrobial spectrum includes Gram(+) and Gram(–) aerobes and anaerobes, Rickettsiae, Spirochetes, Chlamydiae, Mycoplasma, and some protozoans such as Anaplasma spp. and Haemobartonella spp

Formulations

• Chlortetracycline is available as a powdered feed additive in 25 g/lb or 64 g/lb. It is also available as an anaplasmosis block in 2.5 g/lb and in 25- and 500-mg tablets. (A range of concentrations exists for premix.)

Stability and Storage

Store in a tightly sealed container, protected from light, and at room temperature. Do not mix with ions that may chelate tetracyclines (calcium, magnesium, iron, aluminum, etc.).

Small Animal Dosage

Dogs and Cats

• 25 mg/kg q6-8h PO.

Large Animal Dosage

Cattle

• Prophylaxis for anaplasmosis: 0.36-0.7 mg/kg/day. (Approximately one block per 10 animals.)

• Tablets: 11 mg/kg q12h for 3-5 days PO.

• Powdered feed additive: 22 mg/kg/day added to water. Actual dose will be affected by feed and water consumption for each animal.

Pigs• Powdered feed additive: 22 mg/kg/day added to water. Actual dose will be affected by feed and water consumption for each animal.

Regulatory Information

Cattle withdrawal time for meat: Withdrawal times vary from product to product from 1, 2, 5, or 10 days. Most products list a withdrawal time of 1 day for cattle.

Pig withdrawal time for meat: 1-5 days.

Note that for chlortetracycline, withdrawal times may vary considerably from one product to another. One should consult specific product packaging to determine exact withdrawal time.

In cáncer research tetracyclins (chlortetracyclin) is mentioned as a a potent chemical inhibitor of Arf6 activity and Arf6-dependent cancer cell invasion in vitro (Macia et al. 2021).

Based on the anti-infective properties and the very similar mechanism of action of these two antibiotics, it was considered important to study the combination of chlortetracycline with thiamulin in a standard culture of a sensitive germ, in order to graphically represent their interaction and to determine whether or not the combination has an additive or synergistic effect on the test germs.

IV. MATERIAL AND METHODS

Medicinal products:

Chlortetracycline HCL and TIAMULIN in its pure salts were obtained from LABORATORIOS ASTRONOVA SA DE CV. Lot PP1607004.

During the development process of this test, the person in charge of quality control of the applicant laboratory was present to attest the studies carried out.

The in vitro tests were carried out in accordance with 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 standard antibiotics 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.

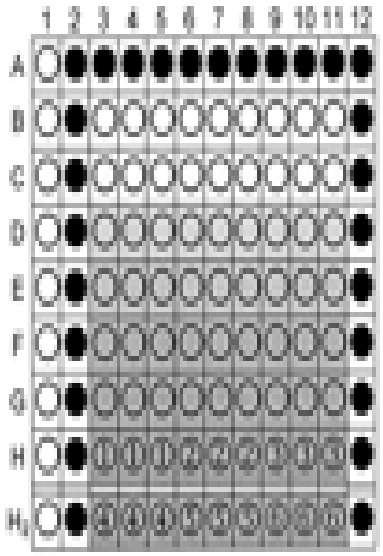
The effect of the combination of chlortetracycline and Tiamulin was tested by making serial dilutions of the two antimicrobial agents, which were mixed in such a way that each row and column consisted of a fixed amount of one agent and increasing amounts of the other antimicrobial. The concentration ranges used were based on the MICs obtained for each of the anti-infective agents used and the bacteria used as test bacteria. 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 microtitre 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 optimal density of 540 nm.

The. Results were collated and where synergistic trends were observed, they were plotted by noting the changes in MIC and observing their trend with the resulting isobolograms.

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

This represents an agar plate with 96 wells in which the reference germs and different concentrations of the antibiotics are deposited and from which six solutions were prepared.

The uncoloured or clear wells were used 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 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.

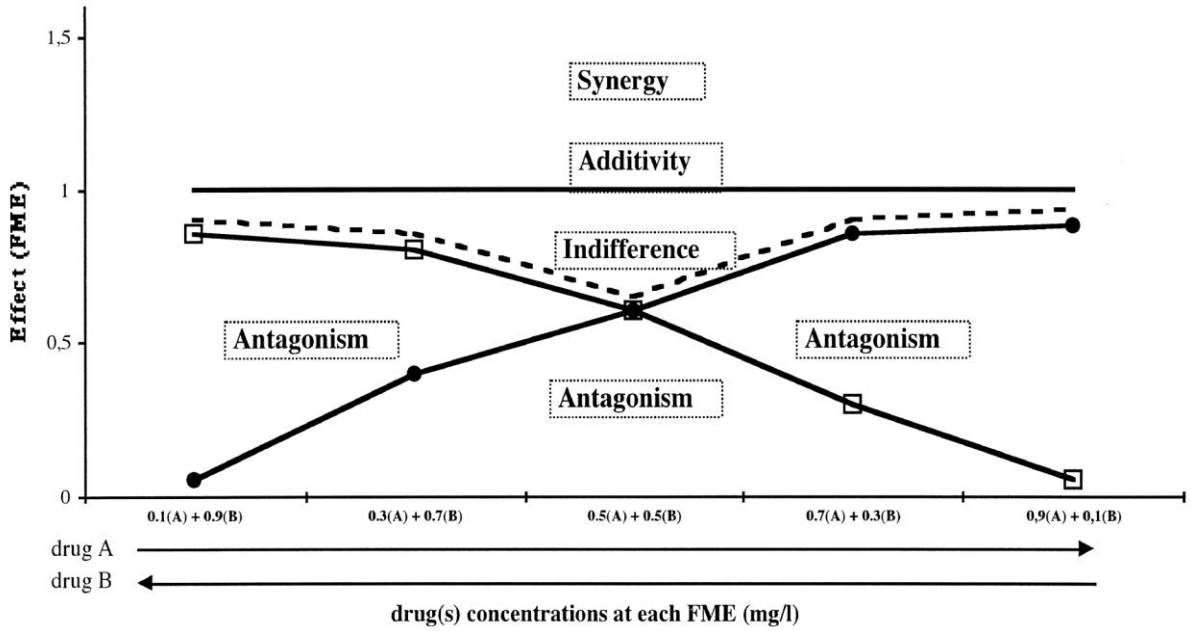
In the antibiotic combinations the ratios used A corresponds to chlortetracycline while B corresponds to tiamulin.

These results can be seen in the following table:

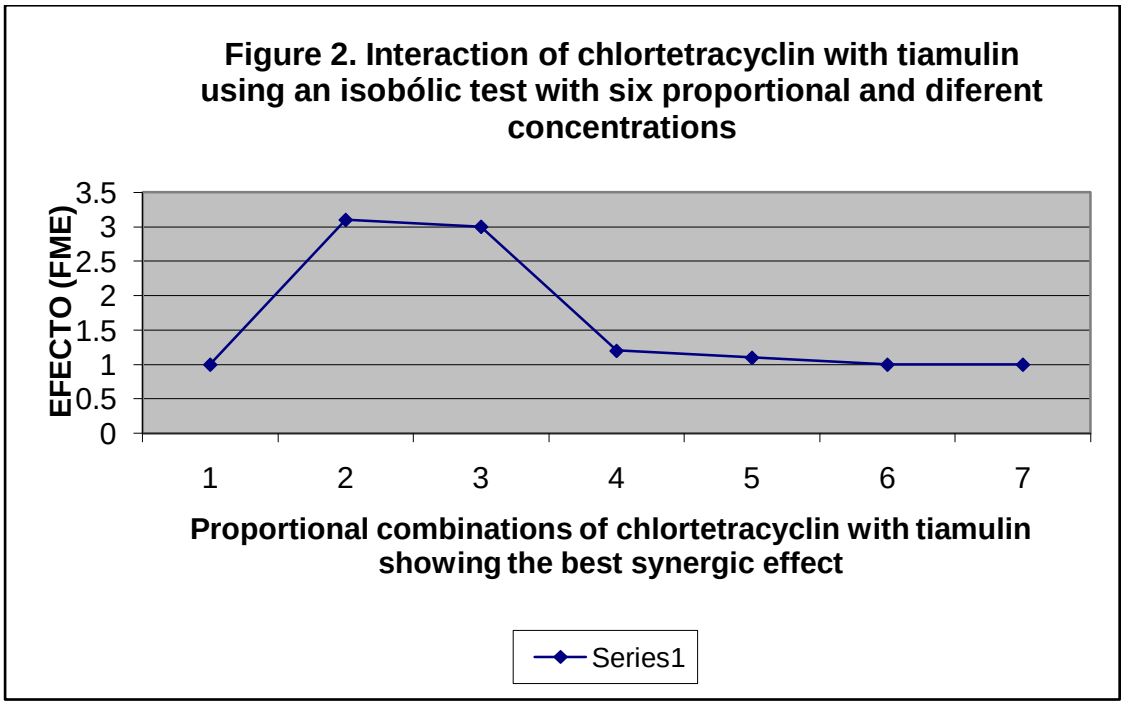
Table 1 The values of the graphical representation of the changes in MIC and observe their trend with the resulting isobolograms in a combination of chlortetracycline with tiamulin in the solutions with the mentioned ratios.

Solución	proporción de A con B		
	Antibiótico A	antibiótico B	Valor de MIC
1	5	0	1.0
2	4	1	3.1
3	3	2	3.0
4	2	3	1.2
5	1	4	1.11
6	0	5	1.1

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:



The graph does not seem to 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 combination, it can be observed that the combination falls within ranges 2 and 3 of the solutions used for chlortetracycline and tiamulin, results that 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 antibiotic combination used between the ratios 4 : 1 and 3 : 2 is suitable for use in pig diseases caused by germs susceptible to the combination studied. To finally conclude that the antibiotic combination of chlortetracycline thiamulin in the ratio of 3 : 1 respectively, is the one with the best synergistic effect because it falls within the average range of the results of this study. This isobologram has been performed in our laboratories at the request of other laboratories with similar or equal results.

It is also observed that there is a synergistic effect between the components of this formulation, an observation confirmed by other authors (Fuentes, 2016; Islam et al., 2008).

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VoBo
Gerente de Control de Calidad

LABORATORIOS ASTRONOVA SA DE CV