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Study of the Interactive Effect between Erythromycin Thiocyanate and Colistin for use in the Therapy and Prevention of Poultry 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

Correspondence Author

Victor Octavio Fuentes-Hernández

vfuentes@cualtos.udg.mx

ABSTRACT

The pharmacological interaction of the antibiotic combination between erythromycin and colistin was studied. An in vitro method was used for this purpose. The results were used to calculate their interaction using the graphical representation known as ISOBOLOGRAM. The agar bioassay method was used. 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 erythromycin and colistin, it was observed that the best SMF of this antibiotic combination was 3 : 2 and 1 : 4 (erythromycin colistin respectively).It can be postulated that the use of this combination of erythromycin with colistin exerts a synergistic effect and can be recommended for oral administration in poultry for the treatment of infections by infectious agents sensitive to the formulation.

I. INTRODUCTION

Since the beginning of chemotherapy when sulphonamide was first used and penicillin was discovered, veterinarians have been looking for ways to combine them to increase the effectiveness against diseases caused by infectious germs susceptible to each of them. In this particular case it is necessary to mention florfenicol and tylosin tartrate.

Nowadays, there are laboratory (Bennet et al. 1966) and graphical (King, 1981; Hamilton, 1985; Rahal, 1978) methodologies that allow to establish the antimicrobial tendencies of antibiotics, alone or in combination.

To achieve the objective of this study, the pharmacological properties of each of the compounds studied will be briefly described.

II. ERYTHROMYCIN

Produced by Streptomyces erytreus isolated from a soil sample from the Philippine archipelago. It has a macrocyclic lactone at its core. It is water soluble at 2 mg/ml and very soluble in organic solvents; unstable in acidic solutions, very stable in refrigerated aqueous solutions, and moderately stable at room temperature. Unstable in gastric acid. In vitro it is very active in alkaline medium, has a pKa of 8.9. ANTIBACTERIAL SPECTRUM: Erythromycin, depending on the nature of the microorganism and the concentration of the antibiotic, can be bactericidal or bacteriostatic. Its antibiotic activity is very similar to that of benzylpenicillin. In vitro it is quite effective against gram-positive cocci such as: Staphylococcus aureus, group A; Pasteurella multocida; Streptococcus pyogenes; Brucella suis; Streptococcus faecalis; Rickettsia; Pneumococci: Treponemas; Neisseria gonorrheae; Erisipelothrix; Haemophilus influenzae; Bacillus anthracis; Corynebacterium; Clostridium; Listeria; Mycoplasma pneumoniae. The following are resistant: Proteus; Pseudomonas; Escherichia coli; Aerobacter aerogenes; Brucella abortus; Salmonella; Klebsiella pneumoniae. It is also effective against penicillin G-sensitive strains of staphylococci, resistant strains of staphylococci and streptomycin-resistant organisms. Resistance to erythromycin can be produced by serial culture without cross-resistance to other antibiotics. One mechanism causing bacterial resistance to erythromycin is alteration of the protein components of the 50S ribosomal subunit, which decreases the affinity for erythromycin and probably other macrolides and lincosamides. This type of resistance results in a demonstrable chromosomal mutation in Bacillus subtilis, Streptococcus piogenes, Escherichia coli and even Staphylococcus aureus. It has also been observed that enterobacteria sometimes decrease their membrane permeability against erythromycin. Exposure of bacteria to amounts well below minimum inhibitory levels can lead to resistance to all macrolides and lincosamides. It has better antimicrobial capacity if the pH is alkaline

MECHANISM OF ACTION: Combines with 50S ribosomal subunits. Prevents polymerisation of phenylalanine into the polyuridylic acid systems of ribosomes and enzymes by blocking or decreasing the binding ability of tRNA to phenylalanine and ribosomal complexes; this inhibits the synthesis of polymerised homopeptides. Gram-positive bacteria accumulate up to 100 times more erythromycin than gram-negative bacteria.

In vitro experiments combining erythromycin, chloramphenicol and lincosamides, an overlap in the acceptance point of erythromycin with the above antibiotics was observed; this indicates that despite unequal binding sites, conjugation of chloramphenicol or lincosamides may inhibit erythromycin function and vice versa. METABOLISM: It is well absorbed in the early part of the small intestine and diffuses rapidly through all tissues. The non-ionised form is more permeable because it readily crosses the bacterial cell membrane. Sometimes the acid pH of the stomach destroys some of the antibiotic; this is avoided by the use of enteric coatings; however, it is possible to use powders, especially stearate, which is more stable. After administration per os in monogastric animals it produces adequate blood concentrations. Large amounts of ingestion delay the absorption of the antibiotic. When the patient has received food, erythromycin stolate is better absorbed. Intramuscular administration of erythromycin 15 mg/kg lasts eight to nine hours. It is distributed in semen, prostatic and bladder fluids at 1/3 of the plasma concentration. Diffuses into peritoneum, pleural fluids and placenta at therapeutic concentrations.

LA COLISTINA PERTENECE AL GRUP DE LAS POLIMIXINAS

Grupo de antibióticos producido por varias cepas de Bacillus polimixa[38]. Existen varios tipos de polimixinas que se designan según la siguiente nomenclatura: A, B, C, D, y E. Son útiles los tipos B y E. En América se le da uso preferentemente a la B, y en la Gran Bretaña a la E (**Colistina**). Estos antibióticos se deben utilizar con

cuidado por ser polipéptidos y por tanto, potencialmente tóxicos. Son detergentes catiónicos. Se presentan en forma de escamas blancas o amarillentas solubles en agua y soluciones salinas a concentraciones no mayores de 25 mg/ml de agua. Son estables como sales ácidas durante largos periodos de tiempo, aun en solución. Los álcalis las destruyen con facilidad y la pureza de las preparaciones comerciales está limitada en un 65 a 75%. Donde 1 microgramo es igual a 10 unidades de polimixina.**ESPECTRO ANTIBACTERIANO**: Atacan principalmente a bacterias gramnegativas. En orden de importancia, su eficacia se refleja en las siguientes bacterias: Aerobacter, Ebertella, Escherichia coli, Haemophilus, Klebsiella, Pasteurella, Salmonella, Shigella, Vibrio, Pseudomona, Brucella, Proteus, Neisseria. Son bactericidas in vitro y no son afectadas por la presencia de suero, sangre ni pus. Son antagonizadas por los tensioactivos catiónicos. Cuando se administran por vía bucal pueden erradicar Pseudomonas, la cual se encuentra presente en una gran variedad de infecciones tisulares; tal parece que las polimixinas son uno de los antibióticos que más atacan a Pseudomonas. Su resistencia bacteriana ocurre muy poco, por lo cual se utilizan en combinación con otros antibióticos.**MECANISMO DE ACCION**: Son agentes tensioactivos catiónicos. Las polimixinas son absorbidas hacia el interior de la célula bacteriana donde se

combinan con las estructuras causantes del mantenimiento del equilibrio osmótico; alteran la permeabilidad al permitir el escape de las purinas y pirimidinas y provocan lisis celular. Este efecto es similar al que producen los detergentes catiónicos. Es posible que se unan a grupos polifosfato cerca de

la superficie celular o en ella, pues se sabe de un antagonismo competitivo entre las polimixinas y los cationes de los detergentes de amonio cuaternario. La polimixina B posee acción sinérgica cuando se combina con antibióticos como la oxitetraciclina, cloranfenicol, carbenicilina, sulfametoxazol y tetraciclina. Las bacterias gram negativas son más sensibles a las polimixinas que las gram +.

ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION: Absorbed slowly by the enteric route, but rapidly by the intramuscular route, producing peak concentrations in one to two hours, and a mean concentration in 12 hours or more. Excretion is slow by the kidneys.

III. MATERIAL AND METHODS

Drugs: Erythromycin and colistin in their pure salts were obtained from Laboratorios AVILAB, Tepatitlan Jalisco, 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 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 serves as a stock solution from which dilutions are made for in vitro testing.

The effect of the combination of erythromycin 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): 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 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 the resulting isobolograms were used to observe the trend.

IV. RESULTS AND DISCUSSION

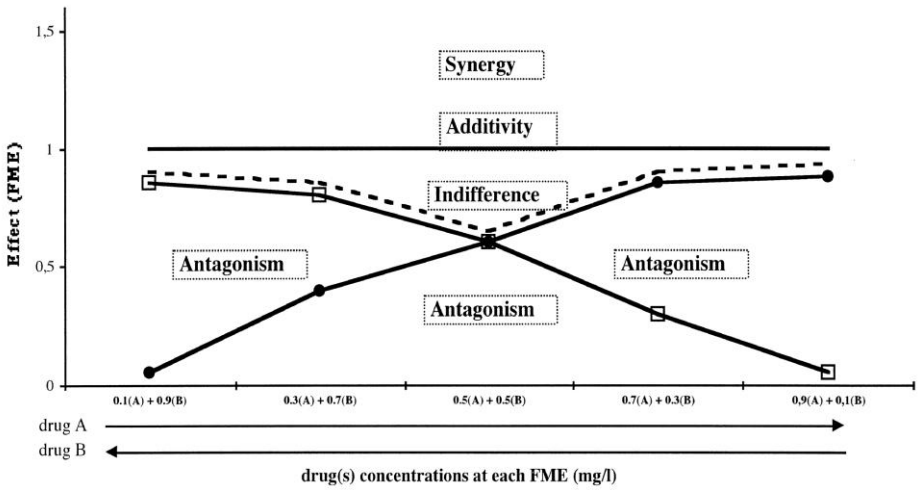
The experimental setup for studying the antibiotic properties of erythromycin with colistin can be seen; it represents an agar plate with 96 wells in which the reference germs and different concentrations of the antibiotics were deposited and from which six solutions were prepared. The uncoloured or 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 antibiotic dilutions, in triplicate, while the wells in row H received the highest combination concentration.of antibiotics. Two 96-well plates were made in which row H2 represents solutions 4 to 6 of the second 96-well agar plate.

In the antibiotic combinations the ratios used A corresponds to erythromycin while B corresponds to colistin. 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 erythromycin with colistin in the solutions with the mentioned ratios.

Solution	proportion of A with B		
	Antibiotico A	antibiotico B	Valor de MIC
1	5	0	1.0
2	4	1	1.0
3	3	2	1.1
4	2	3	1.1
5	1	4	1.0
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 1, 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 repetitive line related to an additive status of erythromycin with colistin, respectively, 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 in this study, it can be postulated that the antibiotic combination used between the ratios studied tends to establish an additive effect between the two antibiotics studied, but the one that tends to have a synergistic effect is solution 3 and 4.

V. CONCLUSION

concluded that the antibiotic combination of erythromycin with colistin in the ratio of 3 : 2 and 2 : 3 respectively, are the ones that present an additive and slightly synergistic effect.

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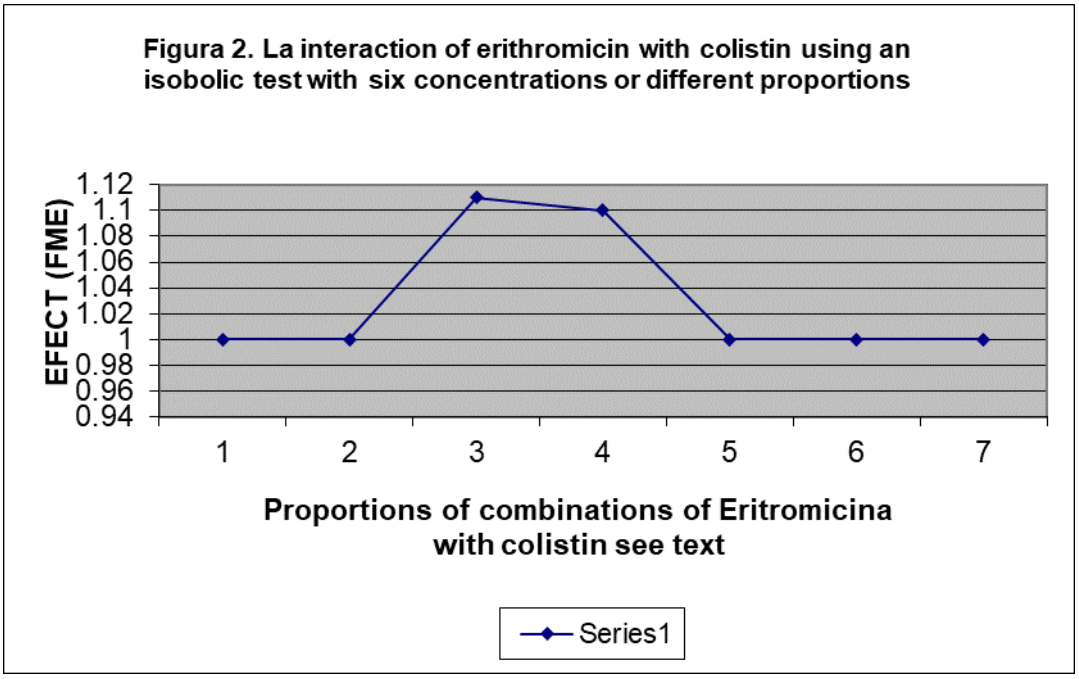
Figura 2. La interaction of erithromicin with colistin using an isobolic test with six concentrations or different proportions
EFECT (FME)
1.12
1.1
1.08
1.06
1.04
1.02
1
0.98
0.96
0.94
1
2
3
4
5
6
7
Proportions of combinations of Eritromicina with colistin see text
Series1