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The Interactive Effect between Kitasamycin Tartrate and Doxycycline Hydrate for Use in the Therapy and Prevention of Diseases in Veterinary Medicine

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ABSTRACT

An in vitro test on the pharmacological interaction of the antibiotic combination between kitasamycin tartrate and Doxycycline hydrate was carried out. 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 pure salts of Kitasamycin tartrap and Doxycycline Hydrate were used and were obtained from the manufacturer of the product, Lapi - Doxi.

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 (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 florfenicol and tylosin tartrate it was observed that the best SMF of this antibiotic combination was 1.45 when the concentration ratio between the antibiotics was 1 : 4 (Kitasamycin tartrate and doxycycline hydrate respectively). Consequently it can be postulated that the combination of kitasamycin tartrate with doxycycline hydrate showed a synergistic effect and can be recommended in poultry and swine for the treatment of mycoplasma infections, most gram + and gram- germs and other infectious agents sensitive to the formulation.

KEYWORDS: Isobologram. Kitasamycin, Doxycyclin

INTRODUCTION

The use of antibiotics in farm animals is a continuous problem, farmers use them in beef, pork and poultry. In USA 29 millions pounds of antibiotics are used per year. And VMDs some times have to use combinations of antibiotics as therapy of emerging diseases, where one antibiotic alone is unable to solve outcoming infections. This latter attituded of VMDs need laboratory tests that show posible combinations of antimicrobials, for this purpose there are now both laboratory (Bennet et al. 1966) and graphical (King, 1981; Hamilton, 1985; Rahal, 1978) methodologies available to establish the antimicrobial trends of antibiotics, alone or in combination. Before studying their interaction, a brief review of the pharmacological properties of the antibiotics studied is presented:

DOXYCICLIN CALCIUM, DOXYCICLIN HICLATE, DOXYCICLIN MONOHIDRATE

It is a semi-synthetic tetracycline derived from oxytetracycline. The hydrate salt is used for solution for injection and also for oral presentation, it is a yellow crystalline powder that is soluble in water and slightly soluble in alcohol. Once reconstituted in water it has a pH of 1.8 to 3.3, the hyclate salt is also called doxycycline hydrochloride. While the monohydrate salt is present in powder form for oral administration, it is a yellow crystalline powder with very slight solubility in water and poor solubility in alcohol. During the manufacturing process a calcium salt is formed, which is present in the syrup sold commercially. Doxycycline has a longer half-life with greater CNS penetration, making it the tetracycline of choice for minor species, especially in azotemic patients. In poultry, doxycycline is considered

the tetracycline of choice for oral treatment of psittacosis, especially when the number of birds to be treated is small.

PHARMACOKINETICS Doxycycline is well absorbed after it is administered orally. In humans its bioavailability is 100%, food does not greatly decrease the absorption of doxycycline. Doxycycline is more fat-soluble, which allows it to pass into body tissues and fluids more easily than tetracycline and oxytetracycline. It penetrates more efficiently into prostatic fluid, cerebrospinal fluid and the eye. The volume of distribution in dogs is 1.5 L/kg. It is 25 to 93% bound to plasma proteins in humans, 76 to 86% in dogs and about 93% in cattle and swine. Doxycycline has a unique mode of excretion, primarily excreted in the bile in an inactive form, it is thought that the antibiotic is inactivated in the lumen of the intestine by chelate formation and then eliminated in the faeces. In dogs 75% of the dose is handled in this way, with 25% excreted renally, making it the drug of choice in cases of renal failure. **PRECAUTIONS**. It may retard skeletal development in the foetus and may also cause discolouration of deciduous teeth, so it should only be used during the latter half of pregnancy when the benefits outweigh the risks to the foetus. **ADVERSE EFFECTS**. The most common side effects after oral administration in the dog and cat are nausea and vomiting, to reduce this effect it can be administered with food without diminishing the effect. Rapid intravenous injection of doxycycline has produced transient collapse and cardiac arrhythmias in several species, this is likely to be caused by the calcium ion chelating action of tetracyclines..

MEDICINAL INTERACTIONS: Sodium bicarbonate, kaolin, pectin or bismuth subsalicylate may prevent the absorption of tetracyclines when administered together orally. Tetracyclines may antagonise the bactericidal antibiotic effect of penicillins, cephalosporins and aminoglycosides. Tetracyclines may increase the bioavailability of digoxin, especially in humans, precipitating digoxin toxicity, an effect that may persist for months after the antibiotic is administered. It should not be administered to patients who have been anaesthetised with methoxyflurane, because it increases the nephrotoxic effect of the anaesthetic.

DOSE: DOGS AND CATS, 5 mg/kg. orally every 24 hours in acute infections every 12 hours. **HORSES**, when administered orally it has been associated with death of the patient and intravenously there are experiences of deaths of horses 15 minutes after administration, so it is recommended not to use doxycycline in horses. POULTRY, In Psittacides for the treatment of calmidiosis (psittacosis) 25 mg/kg twice daily. In critically ill animals 20 mg/kg. intravenous one dose, followed by oral medication.

LEUCOMYCIN (KITASAMYCIN)

Macrolide antibiotic, obtained from Streptomyces kitasatoensis, Hata. Its broad spectrum allows it to attack: staphylococci, streptococci, diplococci, Leptospira, Klebsiella, Pseudomonas, Escherichia coli, Salmonella, Vibrio, Haemophilus, spirochetes, some large viruses. It is chemically similar to Josamycin (Leucomycin A3). Its antibacterial performance is similar to that of erythromycin, and it has a strong inhibitory effect on Gram-positive bacteria, such as Staphylococcus, Streptococcus pyogenes, Streptococcus viridans, Streptococcus pneumoniae, Bacillus tetanus, Bacillus diphtheria, etc. It also has a considerable inhibitory effect on Gram-negative bacteria such as Neisseria gonorrhoeae and Bacillus pertussis. In addition, it has an inhibitory effect on Mycoplasma, Leptospira and Rickettsia. This product is characterised by its efficacy against most penicillin- and erythromycin-resistant Staphylococcus aureus bacteria.

Kitasamycin tatrate was developed by the Japanese Kitasa Institute in 1960 from Streptomyces kitasatoensis. This antibiotic has many other names including: Selectomycin, Leucomycin, Ayermicin, Sineptin, Josamycin, etc.

It has been approved for use since 2001 as a growth promoter in poultry and swine and also as a preventive for digestive and respiratory diseases.

Kitasamycin can help control SD in pigs infected with susceptible isolates of B. hyodysenteriae. It has several active components (A1, A2, A3, A4, A5, A6, A7, A8, A9, B1, B2, B3 and B4. Among these B1. B2, B3 and B4 have half the potency of A but are more toxic. All components of kitasamycin are basic, some are soluble in organic solvents, and poorly soluble in water.

Kitasamycin is stable in neutral and slightly alkaline media. Its antibiotic activity decreases when the pH rises above 5.5.

Its antimycobacterial activity is similar to tylosin, erythromycin, spiramycin and oleandomycin. Its mechanism of action involves inhibition of protein synthesis. Its antibacterial spectrum includes Mycoplasmas, gram-positive, some major negatives, Leptospires and Rickettsiae. It also inhibits most bacteria resistant to penicillin, oxytetracycline, chlortetracycline, erythromycin and chloramphenicol. It can be used safely and effectively as a growth promoter in poultry and pigs.

Josamycin is used clinically, mainly for the treatment of respiratory diseases (e.g. Mediterranean spotted fever and pneumonia), and also as a veterinary drug for sinusitis, synovitis and arthritis in pigs and poultry. Combining kitsamycin with enrofloxacin reported an antagonistic effect and combined with oxolinic acid it showed a synergistic effect.

MATERIAL AND METHODS

Drugs: Kitasamycin tartrate and doxycycline hydrate in their pure salts were obtained from LAPISA SA de CV.

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.

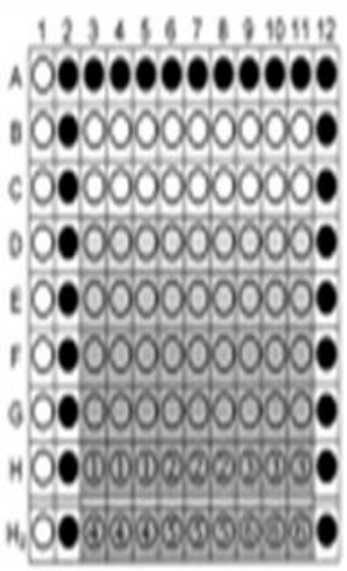
The effect of the combination of Kitasamycin tatratate and Doxycyline hydrate was tested by making serial dilutions of the two antimicrobial agents, which were mixed in such a way that each row and column consisted of a fixed amount of one agent and increasing amounts of the other antimicrobial. The 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 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**.**

RESULTS AND DISCUSSION

In figure 1 below, the experimental setup to study the antibiotic properties of Kitasamycin combined with doxycycline can be observed; it represents an agar plate with 96 wells in which the reference germs and the 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 1-6 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.



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

In the antibiotic combinations the ratios used: A corresponds to Kitasamycin while B corresponds to Doxycycline.

These results can be seen in the following table:

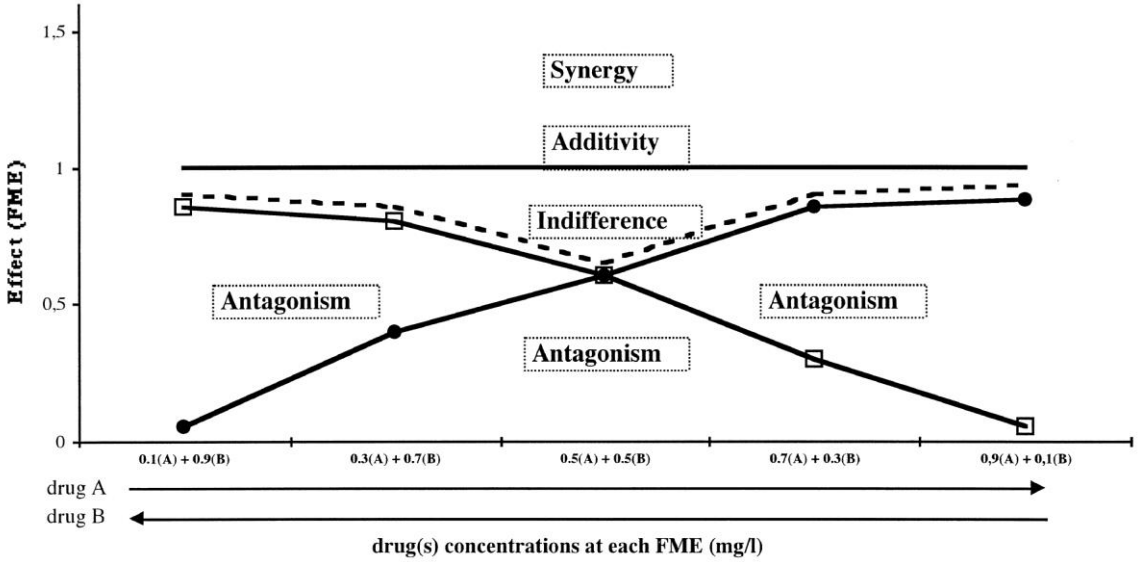
Table 1 The values of the graphical representation of the changes in MIC and their trend with the resulting isobolograms in a combination of Kitasamycin tartrate and Doxycycline hydrate in the solutions with the mentioned ratios.

Solution ratio of A to B

	Antibiotic A	Antibiotic B	MIC Value
1	5	0	1.0
2	4	1	1.1
3	3	2	1.3
4	2	3	1.15
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)

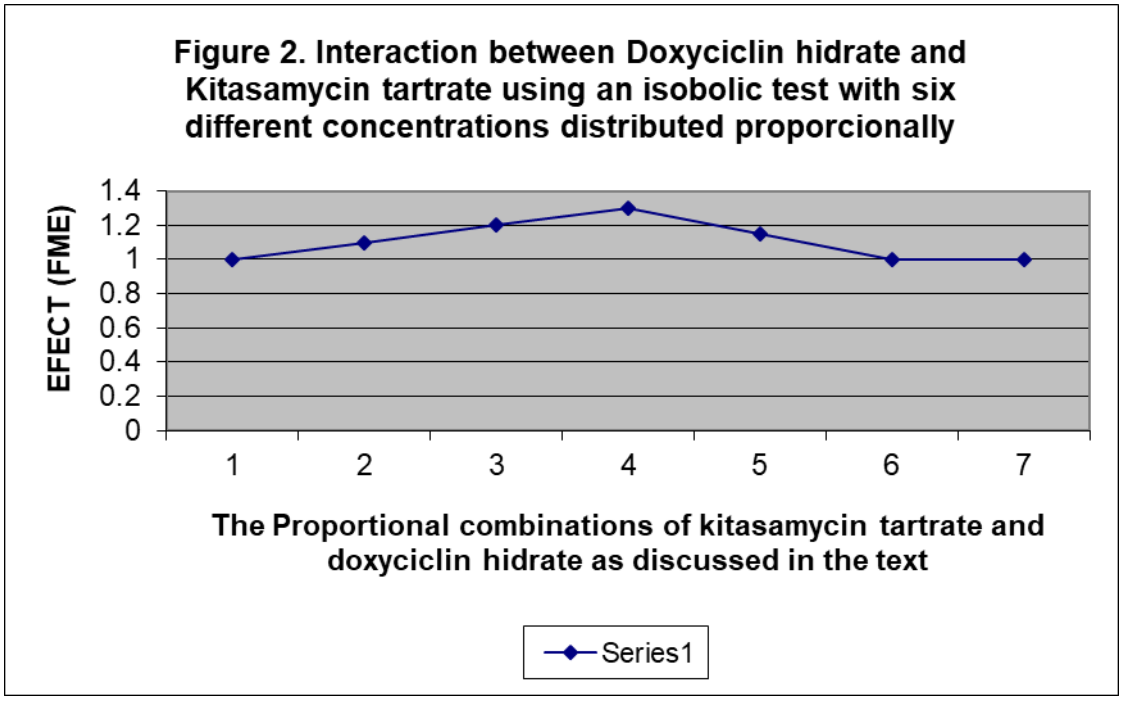
Figure 2



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 of the antibiotics 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 combinations 2 to 5, but it becomes apparent that combinations 3 and 4 are the most representative of a desirable antibiotic synergy. In this combination, a ratio of 1 to 4 is used for Kitasamycin tartrate and doxycycline hydrate, respectively, results which are in agreement with other similar studies with different chemical compounds and antibiotics (Ulvatne, 2001) against E. coli.



CONCLUSIONS

Based on the results obtained in this study, it can be postulated that the antibiotic combination used between the ratios 3 and 4 is suitable for use in pig and poultry diseases caused by germs susceptible to the combination studied. Finally, it can be concluded that the antibiotic

combination of Kitasamycin tartrate and Doxycycline hydrate in a ratio of 3 : 2 and 2 : 3 respectively, have the best synergistic effect.

BIBLIGRAPPHY

1. Bennet, J. V., Brodie J. L.; Benner, E. J.,Kirby, W. M.M. 1966 Simplified, accurate method for antibiotic assay of clinical specimens. App. Microbiol. 14: 170 -177
2. Desbiolles N, Piroth P, Lequeu C, Neuwirth C, Portier H, Chavanet P. Fractional Maximal Effect Method for In Vitro Synergy between Amoxicillin and Ceftriaxone and between Vancomycin and Ceftriaxone against Enterococcus faecalisand Penicillin-Resistant Streptococcus pneumonia. Antimicrobial Agents and Chemotherapy 45, 12, 1 2001, 3328-3333.
3. Gaynor M. and Mankin A.S**.** (2005). Macrolide antibiotics: binding site, mechanism of action, resistance. Front. Med. Chem. 2, 21-35.
4. Hamilton-Miller, J. M. T**.** 1985. Rationalization of terminology and methodology in the study of antibiotic interaction. J. Antimicrob. Chemother. **15:**655-658
5. King, T. C., D. Schlessinger, and D. J. Krogstad**.** 1981. The assesment of antimicrobial combinations. Rev. Infect. Dis. **3:**627-633
6. Lewicki K. 2006 TYLOSIN A review of pharmacokinetics, residues in food animals and analytical methods. ftp://ftp.fao.org/ag/agn/food/tylosin_2006.pdf
7. National Committee for Clinical Laboratory Standards**.** 1994. Performance for antimicrobial susceptibility testing. Standard M100-S5. National Committee for Clinical Laboratory Standards, Villanova, Pa.
8. Rahal, J. J**.** 1978. Antibiotic combinations: the clinical relevance of synergy and antagonism. Medicine **57:**179-195
9. Ruiz, J. G. Antibioticos 2000. En Farmacología Veterinaria. Autor: Victor O. Fuentes, ISBN 970-27-0165-1 Comision Editorial de la Universidad de Guadalajara Mexico p 60 - 150
10. Ulvatne, H., Karoliussen, S., Stiberg, T., Rekdal, O., Svendsen, J., 2001**.** Short antibacterial peptides and erythromycin act synergically against *Escherichia coli.* Journal of Antimicrobial Chemotherapy (2001) **48**, 203-208
11. Zhanel G.G., Dueck M., Hoban D.J., Vercaigne L.M., Embil J.M., Gin A.S. and Karlowsky J.A**.** (2001). Review of macrolides and ketolides. Focus on respiratory tract infections. Drugs 61, 443-498.