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Study of the Interactive Effect between Gentamicin and Sulfa-Trimethoprim for Use in the Therapy and Prevention of Disease in Veterinary Medicine

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ABSTRACT

The pharmacological interaction of the combination of Gentamicin sulphate and sulfamethoxazole-Trimethoprim 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 pure salts of Gentamicin sulphate and Sulfamethoxazole-Trimethoprim were used and were obtained from the manufacturer DIPROFA'Q Productos Farmaceuticos Veterinarios, Mexico. 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 Gentamicin and Sulfamethoxazole-Trimetroprim 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 Gentamicin and Sulfamethoxazole-Trimethoprim establishes a well recognised and documented synergistic effect between them and can be recommended in Canids and Felids for the treatment of enteric infections by gram + and gram- germs and other infectious agents sensitive to this combination.

KEYWORDS: Gentamicin and Sulfa-Trimethoprim, Veterinary Medicine

INTRODUCTION

Synergy between antibiotics is a strictly defined microbiological phenomenon, requiring two bioactive agents to exhibit enhanced bacterial killing when the two are combined (Bush, 2017). Trimethoprim (TMP)-sulfamethoxazole (SMX) is a widely used synergistic antimicrobial combination to treat a variety of bacterial and certain fungal infections TMP potentiates SMX activity through the disruption of a previously unrecognized metabolic feedback loop and the cyclic mutual potentiation of these disruptions results in amplified depletion of the essential cofactor THFolate, (minato et al., 2018). Since the early 1950s synergic combinations of antibiotics such as penicillin streptomyxin gave the pad to the use of combinations of different numbers of antibiotics (Acar, 2000). Combinations of antibiotics with antiparasitic drugs has also been used (Sangaré et all, 2016).

The aim of this study is to investigate the isobolic antibiotic interaction of the combination Getamycin-Sulfamethoxazole-Trimethoprim. The study starts with a summary of the pharmacological properties of the drugs used (Fuentes 2000).

GENTAMYCIN

An aminoglycoside antibiotic. It inhibits bacterial protein synthesis by binding to the 30S ribosome. Gentamicin is concentration-dependent bactericidal. It has a broad spectrum of activity that includes most bacterial isolates in animals including staphylococci and gram-negative bacilli of the Enterobacteriaceae. It is not very active against streptococci and anaerobic bacteria.

This antibiotic is obtained from Micromonospora purpurea, from which three very similar compounds were isolated: gentamicins C1, C2 and C1A. It is water-soluble and heat-stable, resists various pH and does not need to be refrigerated. ANTIBACTERIAL SPECTRUM: It is the most active of the fast-acting aminoglycosides.
It is broad-spectrum, attacks aerobic gram-negative germs including enterobacteria, among which are: Pseudomonas aeruginosa, Escherichia coli, Klebsiella, Aerobacter,

Staphylococcus aureus, streptococci, diplococci, Pasteurella multocida, Haemophilus influenzae, Proteus, Mima, Mycoplasma pneumoniae, Mycoplasma tuberculosis. When combined with ampicillin and kanamycin, or both, there appears to be an additive effect against Proteus. Combined with colistin there is an additive effect against Pseudomonas. Chloramphenicol antagonises gentamicin**.**

MECHANISM OF ACTION AND RESISTANCE: It causes an error in the metabolic reading and transcription of genetic codes. Gentamicin-resistant bacteria show cross-resistance to neomycin, kanamycin, paromomycin and streptomycin. ABSORPTION, METABOLISM AND EXCRETION: It is not absorbed orally and when applied intramuscularly maximum blood levels are achieved in 60 minutes; it decreases after six to eight hours. The higher the dose, the longer the duration of blood levels, and it can be applied intravenously and intrathecally. Dosage in the cow is 0.4 L/kg. A dose of 4.4 mg/kg intramuscularly in the horse produces a maximum concentration in 30 minutes as it diffuses into the synovial fluid, where it reaches its maximum concentration after two hours; it has a plasma persistence of eight hours in horses. The duration of therapeutic levels is maintained for up to twelve hours and after 1-2 days of therapy blood levels can be maintained for up to 24 hours with a single dose. It is excreted by glomerular filtration (40%). The body accumulates or sequesters up to 60% of the total of a dose. It diffuses into the cerebrospinal fluid up to 40% in cases of meningeal inflammation, but when the meninges are normal it does not cross the brain barrier. It shows ototoxicity mainly against the vestibular portion, and at large doses can cause renal damage and it is recommended that the dose be adjusted strictly because three times the normal dose in dogs is reported to produce nephrotoxicity. Its ability to easily cross the placenta predisposes foetuses to its toxic effect. In very rare cases it causes respiratory paralysis due to its neuromuscular blocking capacity, which is antagonised by the administration of calcium and neostigmine. USES: Preferably as prophylaxis before genitourinary or gastrointestinal operations. It is also useful in possible cardiac infections (endocarditis). It should not be used as a first-line treatment. The author uses it in dogs for acute respiratory infections accompanied by pyrexia that do not respond to common antipyretics. DOSE: In dogs and cats, 4 to 6 mg/kg every 6 to 8 hours. In horses, 4 to 5 mg/kg every 8 to 12 hours. COWS 5 mg/kg. every 8 hours. BIRDS OF PREY: 2.5 mg/kg. every 8 hours im.

SULFAMETHOXAZOLE TRIMETHOPRIM (STMP). SULPHAS COMBINED WITH TRIMETHOPRIM OR BOOSTED SULPHAS

The pharmacological properties of trimethoprim will be discussed later, but in this section some of its properties will be mentioned in order to complement the information related to the combination of sulphas. Sulfonamides combined with trimethoprim are called co-trimoxazole, and among the sulfonamides combined with trimethoprim, sulfadiazine, sulfamethoxazole and others such as sulfadimidine have been used. Sulfonamides alone are bacteriostatic while trimethoprim is bactericidal, when combined with trimethoprim the sulphonamides become bactericidal. Potentiated sulphonamides sequentially inhibit enzymes involved in the manufacture of folic acid, inhibiting bacterial thymidine synthesis. The sulphonamide blocks the transformation of para amino benzoic acid (PABA) into dihydrofolic acid (DFA), while Trimethoprim blocks the conversion of DFA to tetrahydrofolic acid because it inhibits dihydrofolate reductase. The optimal in vitro ratio for attacking most susceptible bacteria is 1:20, i.e. one part trimethoprim to 20 parts sulfa. But effective synergistic action has been reported with ratios of 1:1 and up to 1:40. The serum concentration of trimethoprim is considered to be more important than the sulfa concentration. The trimethoprim MIC for most susceptible bacteria is above 0.5 mcg/ml. The antibacterial spectrum of potentiated sulfa drugs is quite broad, and susceptible gram+ bacteria include most streptococci, many strains of staphylococci and Nocardia. A large number of gram-positive bacteria are also susceptible, including Enterobacteriaceae, but Pseudomonas aeruginosa is not affected. The combination of antibacterials may also affect some protozoa such as Pneumocystis carinii, coccidia and toxoplasma. It appears that boosted sulphas are not effective against most anaerobic germs. Resistance is difficult to establish, and when it occurs it is plasmid-mediated. PHARMACOKINETICS: Trimethoprim sulfa (S+TMP) is well absorbed when administered orally, giving peak levels in 1 to 4 hours after administration. It is more slowly absorbed when administered subcutaneously. In ruminants, TMP is reported to be trapped in the rumen-reticulum and undergoes some degree of breakdown when administered orally. The organic distribution of S+TMP is quite good, and when the meninges are inflamed it crosses them, achieving concentrations in the cerebrospinal fluid in concentrations of up to 50% in relation to the plasma concentration. Both drugs cross the placenta and diffuse into milk. The volume of distribution in different species is 1.49 L/kg in dogs and 0.59 to 1.51 L/kg in horses. The volume of distribution of sulfadiazine in dogs is 1.02 L/kg. S+TMP is excreted unchanged in the urine by glomerular filtration and tubular secretion, and undergoes some

metabolism in the liver. Sulphas are acetylated and conjugated with glucuronic acid and trimethoprim gives rise to hydroxylated oxides and metabolites. It is possible that TMP is metabolised more extensively in the liver of ruminants. The half-life of TMP in different species is 2.5 hours in dogs, 1.91 to 3 hours in horses and 1.5 hours in cows. The half-life of sulfadiazine in different species is 9.84 hours in dogs, 2.71 hours in horses and 2.5 hours in cows. Although TMP disappears rapidly from plasma, it remains longer in tissues.

ADVERSE EFFECTS: Adverse effects have been observed in dogs including keratoconjunctivitis sicca which can sometimes be irreversible; acute neutrophilic hepatitis accompanied by jaundice has also been observed; on other occasions vomiting, anorexia, diarrhoea, fever, haemolytic anaemia, urticaria, polyarthritis, inflammation of the face, polydipsia, polyuria and cholestasis have been observed. It is possible that acute hypersensitivity reactions may occur, manifesting as Type I or anaphylaxis, or Type III reaction or serum sickness. Hypersensitivity reactions seem to be more common in larger breeds of dogs. Doberman Pinschers appear to be the most susceptible. In cats, hypersensitivity reactions manifesting as anorexia, leukopenia and anaemia have been reported. In horses, a pruritic reaction has been observed following intravenous injection; and when oral therapy with S + TMP is used, diarrhoea may occur in some horses.

DOSAGE

DOGS: 30 mg/kg every 12 hours for 3 to 5 days, when Nocardia is present then the dose should be doubled. In cases of mastitis 30 mg/kg oral twice daily for 7 days.GATOS: 30 mg/kg. cada 12 horas oral por 3 a 5 días y cuando Nocardia está presente se duplica la dosis.

CATTLE: 25 mg/kg i.v., i.m. every 24 hours for 3 to 5 days and in calves 50 mg/kg every 24 hours.

HORSES: 15 mg every 8 to 12 hours for 3 to 5 days. In foals 15 mg/kg i.v. every 12 hours for 3 to 5 days.

SWINE: 48 mg/kg.

POULTRY: Oral suspension is used which has 240 mg in 5 ml and two ml are administered orally twice a day, it is good for the treatment of enteric infections produced by gram- and gram+. Vomiting may occur in Macaos. For enteric and respiratory infections in Psittacidae, the 24% injectable suspension is used, administering 0.22 ml per kg intramuscularly once or twice daily. For coccidiosis in toucans and mines the oral suspension is used with 240 mg in 5 ml at a rate of 2.2 ml/kg. but it can also be mixed in feed. For enteric and respiratory infections in hand-fed Psittacidae infants, oral suspension is also used at 0.22 ml/30 g live weight. There are many other sulphonamides on the commercial market, some of which have no support in the scientific technical literature to study their advantages and disadvantages. Others are commonly used sulphonamides whose literature was booming several decades ago and their therapeutic properties correspond to those of sulphonamides in general, e.g. Sulfadimethylpyrimidine, Sulfadoxine, Sulfametopyrazine, Sulfamethopyrazine, Sulfamonomethoxine, Sulfatolamide, Sulforthymidine.

MATERIAL AND METHODS FOR THE DEVELOPMENT OF THIS STUDY

Medicines: Gentamicin Sulphate and Sulfamethoxazole Trimethoprim in their pure salts were obtained from DIPROFA'Q Productos Farmaceuticos Veterinarios, Mexico.

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.

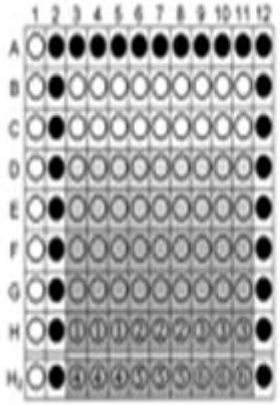
The antibiotic standards tested were dried under vacuum for a minimum of 48 hours and then carefully weighed and added to a solution to achieve a concentration of 1000µg/ml. This concentration serves as a stock solution from which dilutions are made for in vitro testing.

The effect of the combination of Gentamicin with Sulfamethoxazole-Trimethoprim 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.

The 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



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 figure 1 below, the experimental setup to study the antibiotic properties of Gentamicin and Sulfamethoxazole Trimethoprim can be observed; it represents an agar plate with 96 wells in which the reference germs and different concentrations of Gentamicin and Sulfamethoxazole Trimethoprim were deposited, 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 the dilutions of Gentamicin and Sulfamethoxazole Trimethoprim, in triplicate, while wells in row H received the highest concentration of the Gentamicin + Sulfamethoxazole Trimethoprim combination. Two 96-well plates were made with row H2 representing solutions 4 to 6 of the second 96-well agar plate.

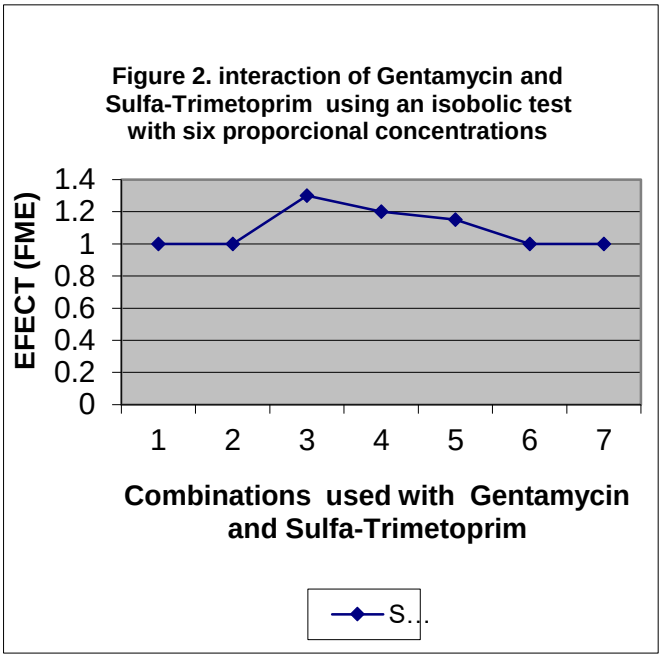
In the antibiotic combinations the proportions used in A correspond to Gentamicin while B corresponds to Sulfamethoxazole Trimethoprim. These results can be seen in the following table**:**

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 Gentamicin and Trimethoprim Sulfamethoxazole 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	1.2
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)Cuando se aplican los resultados correspondientes observados en el cuadro 1 la gráfica resultante es como se puede observar en la figura 2, y en la cual se pueden observar las tendencias de las combinaciones en cuanto a las proporciones de los mismos utilizados en las pruebas de interacción



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 1:1.5 is used for Gentamicin with Sulfamethoxazole Trimethoprim 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 Gentamicin with Sulfamethoxazole Trimethoprim in a 3:2 ratio is suitable for use in canid and felid diseases caused by germs susceptible to the combination studied. To finally conclude that the combination of Gentamicin in the ratios of 3:2, 2:3 with Sulfamethoxazole Trimethoprim 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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