THE STABILITY OF ENROFLOXACIN

A Senior Thesis

By

Meagan A. Dodge

1997-98 University Undergraduate Research Fellow

Texas A&M University

Group: Biology

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MEAGAN A. DODGE

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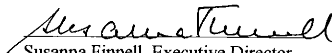
Approved as to style and content by:

Dawn Boothe
Department of Veterinary Clinical Pharmacology



Susanna Finnell, Executive Director
Honors Programs and Academic Scholarships

Fellows Group: Biology

ABSTRACT

The Stability of Enrofloxacin

Meagan Dodge, (Dr. Dawn Boothe) University Undergraduate Fellow, 1997-1998, Texas A&M University, Department of Veterinary Clinical Pharmacology

Enrofloxacin is an antimicrobial drug approved for veterinary use only. Enrofloxacin is administered orally or parenterally. Veterinarians may dilute the drug for injection or topical use in a commercial physiological solution. The injectable commercially used Enrofloxacin, Baytril, is sometimes too strong, causing vomiting, and much smaller concentrations are effective topically. It is hypothesized that the dilution of enrofloxacin in some commercially used solutions will not affect the drug's efficacy. The purpose of my study was to test the afore stated hypothesis. In the experiment, stock solutions of enrofloxacin in physiological saline solution, dextrose 5% in water, lactated Ringer's solution, Epi-otic solution, and vinegar. *E. coli* 2592-2 (standardized solution in saline) was the microorganism chosen to be tested because it is known to be susceptible to enrofloxacin. At time 0, 1 day and 7 days and temperatures of 25 C and 4 C, decreasing tube dilutions (0.125 to 16 μg/ml) of each stock solution and a standard enrofloxacin solution (control) were made using Mueller-Hinton broth. Each dilution of the enrofloxacin solution and an equal amount of bacterial solution were combined and incubated for 24 hours. The resulting growth was read using ultraviolet spectrophotometry and the minimum inhibitory concentration (MIC) of enrofloxacin in each solution was determined and compared to the control. Results showed that the MIC's at time 0 were: control - 8 μg/ml, saline - 0.25 μg/ml, dextrose - 4 μg/ml, ringer - 1 μg/ml, epi-otic - 4 μg/ml, and vinegar - 2 μg/ml. The results for both temperatures at 1 day followed the same pattern. At 7 days, crystals of enrofloxacin were observed in all solutions at both temperatures, hence these solutions were not tested by tube dilution. Therefore, it can be concluded that because the commercial solutions caused a decrease in MIC, they all appear to increase rather than decrease the efficacy of enrofloxacin. The experiment shows that some physiological solutions can alter the efficacy of enrofloxacin when the two are combined for clinical use, but this alteration is favorable.

Background

Enrofloxacin (ENRO) is a fluoroquinone quinoline carboxylic acid derivative. As an antibiotic, this antimicrobial drug is given orally or parenterally to patients suffering from susceptible infections.[1] Enrofloxacin inhibits DNA gyrase, a bacterial type II topoisomerase, responsible for bacterial replication.[1] The drug is rapidly bactericidal.

ENRO is approved for use by veterinarians only. This antibiotic is highly effective against aerobic gram-negative rods and coccobacilli, such as *Pseudomona aeruginosa, Aeromonas spp.* and *Pasteurella spp.* Selected gram-positive and acid fast bacteria, including *Staphylococcus aureus and Mycobacteria sp.*, are also susceptible.[3]

ENRO can also be administered parenterally. The pharmaceutical product is prepared as a 2.27% solution in n-butyl alcohol, potassium hydroxide, and water, which serve only as solvents. However, administered from the bottle, concentrations are often too strong, causing the patient to vomit. In addition, much smaller concentrations are effective topically. Thus, veterinarians may chose to add a small amount of a commercial physiological solution to dilute the drug before injection, or they may add the drug to a solution intended for topical use (as in the ears). This experiment will determine whether the addition of various dilutents will alter the efficacy of the antimicrobial. In addition, the effects of storing the diluted drug up to 1 week at room temperature and in the refrigerator (25°C and 4°C, respectively) will be studied.

Objectives

The objectives of this study are to determine the inhibitory effects of selected commercially available solutions on the antibacterial activity of ENRO.

Hypothesis

Our hypothesis is that the efficacy of the ENRO will not be affected by diluting or storing the drug in these various solutions.

Materials and Methods

The tube dilution method was used to find the minimum inhibitory concentration (MIC) of each solution. MIC is the lowest concentration of drug that yields no bacterial growth. The solutions tested were physiological saline solution (PSS) [0.9% NaCl], dextrose 5% in water (D5W), lactated ringer solution (LRS) [Na, Cl, Ca^{2+}, and K^{+} as an isotonic fluid], epi-otic© fluid [2.7% lactic acid and 0.1% salicylic acid], vinegar [acetic acid], used as an ear cleaning solution, and water as a control. The concentration of ENRO added to the first three solutions (PSS, D5W, and LRS) was based on a 5 mg/kg dose (labeled dose) for a twenty kilogram dog. In such a case, 100 mg of ENRO would be administered parenterally. For all dilutions, the commercially available drug was used, Baytril©. To create stock solutions following these guidelines, 2.2 ml of Baytril© was added to 2.8 ml of solution, yielding 5 ml of stock. For the epi-otic solution and the vinegar, which are administered topically (in the ear), a 1 to 9 dilution

is generally recommended. 1 ml of Baytril was added to 8 ml of solution, yielding 9 ml of stock. These five ENRO spiked stock solutions were then tested immediately (time 0) and day 1 and day 7 following storage at 25°C and 4°C. At each time period and at each temperature, duplicate dilutions were made using pure ENRO and each of the stock solutions with ENRO added. The dilutions used were 16, 8, 4, 2, 1, 0.5, 0.25, and 0.125 micrograms/mL. (Figure 1) The dilutions were made in Mueller-Hinton broth. These dilutions were selected based on the clinical response of susceptible microorganisms. 120 microliters of each dilution were added to a microplate containing 96 wells (Figure 1). The bacteria chosen was *E. coli* 2592-2, which is known to be susceptible to ENRO at the above concentrations. 120 microliters of a solution of this bacteria were added to the wells containing the dilutions of each solution. The plate was incubated at 37°C overnight. The next day, the microplate was read using ultraviolet spectrophotometry. The MIC of each solution was that well which contained the lowest concentration of ENRO and no growth of organism following a 24 hour incubation. The MIC's of each solution was compared to that of the pure ENRO.

Discussion

The microbiological assay method will be used to try to gain more convincing results in an easier to decipher manner. A concentration of each solution shown to be appropriate for this method will be compared to a stock solution of ENRO on a plate of bacteria, *E. coli* 2592-2.

Small metal wells, penicylinders, will be placed on top of a lawn of bacteria. The spiked solutions and stock solution are added to the wells. The plate is incubated at 37 degrees overnight. The next day, the zones of no growth will be measured. Solutions showing the same inhibition diameter as the standard are assumed to have retained complete efficacy of the drug. This method will also be repeated at zero, one and seven days.

Results

The ENRO in each solution crystallized after 7 days of storage. Thus, MIC tube dilutions were not done for day 7, since it is assumed that the crystals were of drug. The drug must be soluble (that is in solution) to be effective. No clearly satisfactory results could be determined from repetition of this method at time 0 and time 1 day. Figures 2-7 cue graphs of log optical density versus log concentration of ENRO for each solution at time zero, including the standard ENRO solution. The MIC is represented by the point at the baseline of the graph (that is, no bacterial growth). Similar graphs were produced from data after one day of incubation. Table 1 is a summary of the MIC for each solution at each time and temperature. From these data, it is apparent that only PSS and LRS affected the efficacy of the drug because the MIC decreased as compared to the standard MIC. Hence, the effect was an increased efficacy. However, the points on duplicate graphs do not match perfectly. Also, there are points on many graphs that show growth at higher concentrations than the MIC point determined (where there is no growth).

Ideal graphs would show no growth at the MIC and at all points of greater concentration. Therefore, these data are not precise enough to be reported as results to my problem.

Discussion

The results from the tube dilution method did not support my hypothesis because PSS and LRS showed increased efficacy of ENRO.

The tube dilution method leaves a large margin for human error because of the many dilutions required for every plate. The points on the graphs that show unexpected results may be due to this complication. The tube dilution method does not directly compare efficacy of the drug as a result of the solutions to a standard, but instead efficacy is inferred from MIC comparisons.

In order to solve the problems that arose from the first method used, the microbiological assay will be attempted. This method should greatly simplify the experiment and give a better indication of how efficacy is affected by each solution. Bioassay utilizes a direct comparison of standard efficacy to ENRO spiked solution efficacy by measurement of zone diameter. The decision to change methods was just introduced recently, so no results have been obtained to report. The final presentation of my data should include this test also.

Figure 1: Microplate model

Time 1 day	Time 0	Concentration (µg/ml) 0.125	0.25	0.5	1	2	4	8	16
Standard	Standard								
PSS	PSS								
LRS	LRS								
D5W	D5W								
Epi-otic®	Epi-otic®								
Vinegar	Vinegar								
PSS									
LRS									
D5W									
Epi-otic®									
Vinegar									

Table 1: MIC of standard and solutions (micrograms/milliliter)

Solution	Time: 0 days	1 day	
Temperature:		25 C	4 C
Standard (not stored)	8	8	
PSS	0.25	0.5	0.25
D5W	4	4	4
LRS	1	2	2
Epi-otic	4	4	8
Vinegar	2	4	4

Figure 2: ENRO Standard

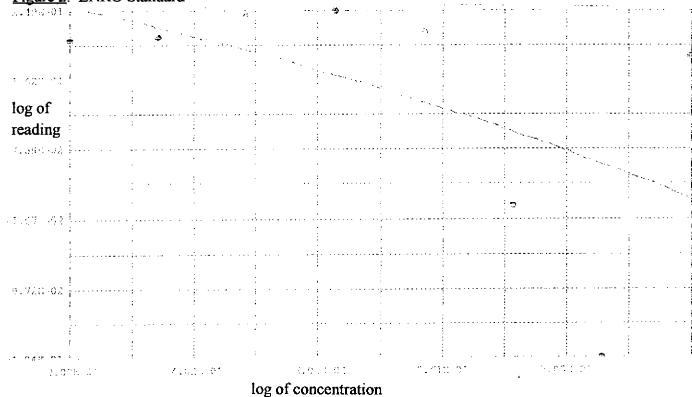


Figure 3: PSS Solution

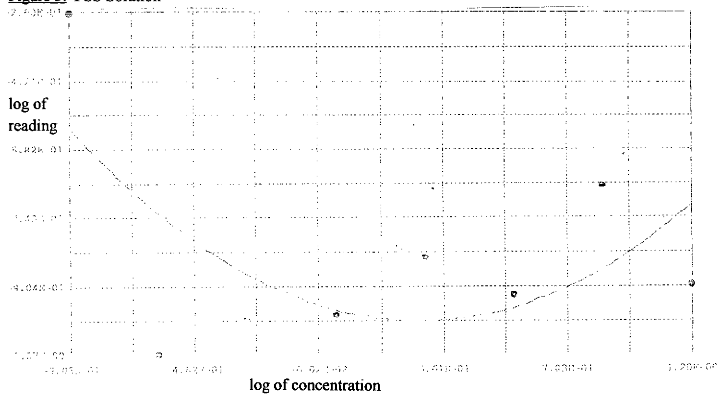


Figure 4: LRS Solution

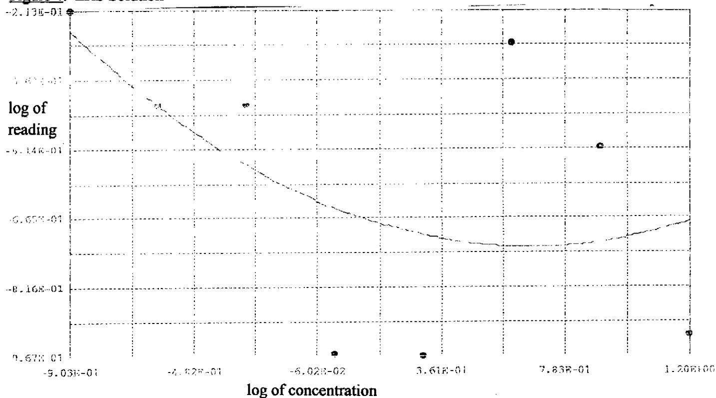


Figure 5: D5W Solution

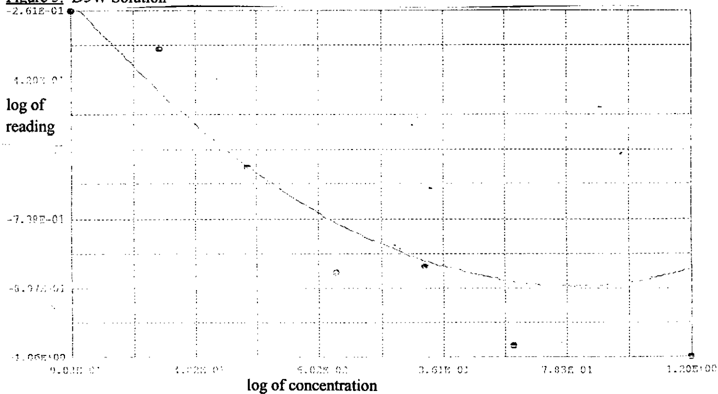


Figure 6: Epi-otic® Solution

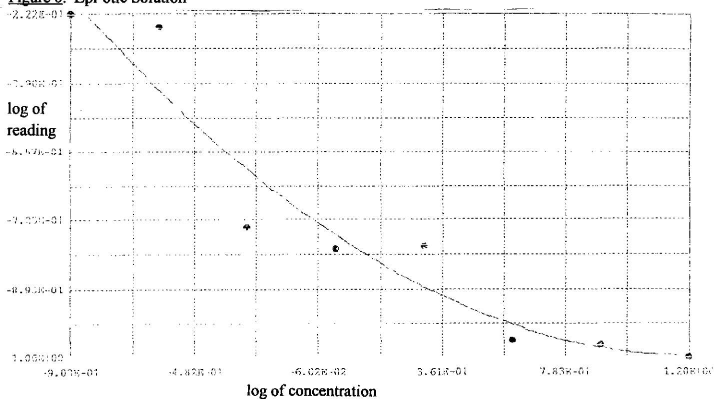
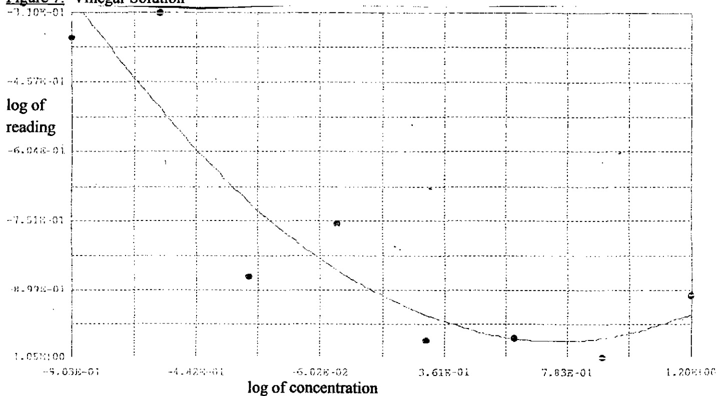


Figure 7: Vinegar Solution



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