

DR. SIMMONS: Thank you, Dr. Ling.

The final presenter of the subject of animal models will be Dr. George Gunderson. After receiving a D.V.M. degree from Michigan State University in 1960, Dr. Gunderson entered a mixed practice in central Illinois. He later joined Hess & Clark in Ashland, Ohio, in 1965 as a research veterinarian and then joined the field research group. In 1971 he joined the Schering Corporation as Associate Manager of Clinical Medicine. In 1974 he became responsible for their basic research operation. He joined Burroughs Wellcome in North Carolina in 1977 and recently moved to Kansas City where he is now Vice President of Research & Development for the Wellcome Animal Health Division. Dr. Gunderson...

DR. GUNDERSON: I practiced in the same area as Wally Brandt and I'm not at all surprised he was inspected; we always wondered how he managed to stay out of jail down there! Before this session Jerry Ling and I were comparing our notes on these studies; we must have gotten them mixed up because he just delivered my paper. The purpose of this paper is to discuss the value of a streptococcal disease model in dogs and to illustrate drug effect in a model, I'll use the results of a study we conducted with Tribriksen.

DOSE TITRATION AND COMPARATIVE EFFICACY OF TRIBRISSEN 24% INJECTABLE AND INDIVIDUAL COMPONENTS IN A STREPTOCOCCAL INFECTION MODEL IN BEAGLE DOGS

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SUMMARY

The efficacy of Tribrisen, a combination of trimethoprim and sulfadiazine, was compared to the efficacy of its individual components in treatment of induced Streptococcal infection in Beagle dogs. Efficacy was evaluated by observing differences in: (1) Clinical manifestations, (2) white blood cell counts, (3) bacteremia, and (4) bacteriological culture of tissues at necropsy.

The results showed that: (1) All treatments were more effective than no treatment, (2) Tribrisen was more effective than its individual components administered alone, (3) Tribrisen dosed at 30.0 mg/kg was more effective than Tribrisen dosed at 15.0 mg/kg or 7.5 mg/kg.

In addition, statistical analysis of results of the major parameter, changes in clinical manifestation, showed the combination was synergistic¹.

INTRODUCTION

This model of Streptococcal Infection in Beagle Dogs was developed by Dr. T. E. Powers following the discovery that infection with Streptococcus zooepidemicus would consistently result in a joint lameness that was quantifiable. Other quantifiable clinical manifestations are included in the group of observations, but the severity of infection is most heavily based on the degree of lameness. Three other objective measurements, bacteremia, white blood cell counts, and recovery of the infecting organism from tissues at necropsy help illustrate the establishment of disease and the course of recovery, but they are only supportive for the major parameter, total scores of clinical manifestations.

¹Pisearchia, Shah (1976). A Design for the Detection of Synergy in Drug Mixtures. Proc. of Twenty-First Conf. on Design of Experiments. U.S. Army Research Office, Research Triangle Park, N.C.

OBJECTIVES

The objectives of this study were to determine: (1) the efficacy of each individual component, trimethoprim and sulfadiazine, and the combination, Tribriksen, (2) whether the combination was as effective or more effective than the individual components when the combination was given at a lower dose, and (3) which of the three dose levels of Tribriksen 24% Injectable was most effective.

MATERIALS AND METHODS

1. Animals and Husbandry

Six to eight month old purebred Beagle dogs, both males and females, were housed individually in cages. All dogs were acclimated to the laboratory environment for two weeks prior to beginning the study and were fed once daily with normal rations and were allowed water ad libitum. Each dog was identified by a numbered ear tattoo.

2. Experimental Design

The experimental design was a Randomized Complete Block Design. A block consisted of an experiment with nine dogs each randomly assigned to one of the nine treatment groups listed below. The study was conducted in six separate experiments (blocks). Each treatment group consisted of three males and three females.

On Experiment Day -2 (E.D.-2) each dog was infected by an intravenous injection of β -hemolytic Streptococcus zooepidemicus. The organism had an in vitro sensitivity of 0.5 $\mu\text{g/ml}$ to trimethoprim, 9.5 $\mu\text{g/ml}$ to sulfadiazine and 0.05 + 0.95 $\mu\text{g/ml}$ to a combination of one part trimethoprim and 19 parts sulfadiazine.

Clinical observations of each dog were recorded by several observers independently starting on E.D.-2 and continuing through E.D.+5. The observers, veterinarians or senior veterinary students, were unaware of treatment. The parameters and quantification of scoring are shown below.

Measurements for Scoring Clinical Manifestations of Disease Due to S. zooepidemicus Given Intravenously

2.1. Lameness -- Maximum Score = 15

- 2.1.1. If unable to get up and move, has painful inflamed joints of legs = 12 - 15
- 2.1.2. Can get up slightly, pain while moving, has slightly inflamed joints = 10 - 12
- 2.1.3. Can get up easily, moves with hind legs staggering, painful joints on palpation = 7 - 9
- 2.1.4. Shows some lameness and abnormal gait = 4 - 6
- 2.1.5. Almost no lameness, almost normal gait, almost no pain even on palpation of joints = 0 - 3

2.2. Size of Lymph Nodes - (Popliteal and Superficial Cervical) -- Maximum Score = 8

- 2.2.1. Size more than double normal size = 6 - 8
- 2.2.2. Almost double normal size = 3 - 5
- 2.2.3. Normal to slight enlargement = 0 - 2

2.3. Color of Mucous Membranes -- Maximum Score = 5

- 2.3.1. Pale and cyanotic = 4 - 5
- 2.3.2. Pale = 2 - 3
- 2.3.3. Pink or whitish pink = 0 - 1

2.4. Degree of Alertness -- Maximum Score = 4

- 2.4.1. No response to surroundings, lying listless in a prostrate manner = 4
- 2.4.2. Unconcerned to surroundings but has healthier posture = 3
- 2.4.3. No getting up on subjective approach but does move the tail = 2

2.4.4. Active on approach, shows playing behavior, moves tail and shakes the head and ears = 0 - 1

2.5. Condition of Hair Coat -- Maximum Score = 4

2.5.1. Looks dull with falling of hair = 3 - 4

2.5.2. Loss of shine, looking rough but no loss of hair = 2

2.5.3. Shiny compact hair coat with no hair falling off = 0 - 1

2.6. Degree of Dehydration -- Maximum Score = 4

2.6.1. Skin just adherent to s/c tissue = 3 - 4

2.6.2. Can get a skin fold; stays for some time on releasing the fold = 2

2.6.3. Can get a skin fold which disappears immediately as fold is released = 0 - 1

3. Treatment

3.1. Compound Identification

3.1.1. Tribriksen 24% Injectable

One ml contains 240 mg Tribriksen
(200 mg sulfadiazine)
(40 mg trimethoprim)

3.1.2. Trimethoprim Injection - 80 mg/ml

3.1.3. Sulfadiazine Injection - 400 mg/ml

3.2. Dosing

Group	Treatment	Daily Dose (mg/kg)	No. of Dogs
A	NEG Control	0	6
B	SDZ (pos. cont.)	122.0**	5*
C	TMP	5.0	6
D	TMP	2.5	6
E	SDZ	25.0	6
F	SDZ	12.5	6
G	TRI	30.0	6
H	TRI	15.0	6
I	TRI	7.5	6

Note: *Group B not run in first block

**Dose -- 66 mg/kg BID

Drug treatments were started 48 hours after experimental infection (E.D.+0) and continued through E.D.+4, a total of five days.

Treatments were administered by persons not involved in clinical observations. Each day following identification of the dog, its cage number and the drug container, each preparation was injected subcutaneously.

4. Measurements

4.1. Clinical Observations and Scoring

All dogs were observed daily for clinical manifestations. Each dog was given a daily score by each observer from E.D.-1 to E.D.+5. A high score indicated the presence of severe symptoms and a low score indicated the absence of symptoms.

The scores of the measurement were added daily for each dog and became the dog's Total Score for that observer for that day. The sum of all observers' scores divided by the number of observers became the dog's Average Total Score for that day. The sum of the Average Total Scores for all dogs in the group divided by the number of dogs equaled the Group Average Total Score (GATS) for the group on that experiment day.

The GATS on E.D.+0 was the Baseline Average and was used for later evaluation of effectiveness.

When the Negative Control (Group A) GATS reached a maximum, the maximal disease state was present. GATS were calculated for all treatment groups on that day. In this study, the maximal disease state was recorded on E.D.+4.

4.2. Blood Samples

Blood samples were taken daily (E.D.-2 to +5) to determine responses to infection and to treatment. Changes in white blood cell counts (WBC) were evaluated to confirm active infection (E.D.-2 to E.D.+0) and indicate response to treatment (E.D.+1 to E.D.+5).

4.3. Blood Cultures

Blood samples collected aseptically from each dog were cultured for S. zooepidemicus daily. A sample was considered positive if any growth of the infecting organism was seen on the blood plates.

4.4. Bacteriological Culture of Tissues

A necropsy of all dogs was done on E.D.+7 and samples of liver, spleen, kidney, mesenteric lymph nodes, bile, lungs, blood and urine were cultured.

CRITERIA OF EVALUATION

The following measurements were used to evaluate efficacy: (1) reduction in total score of clinical manifestations, (2) reduction of white blood cell counts on E.D.+5 compared to E.D.+0, (3) reduction in number of animals having positive blood cultures on E.D.+5, and (4) reduction in number of tissues having positive cultures at necropsy.

RESULTS

1. Total Score of Clinical Manifestations by Four Observers

A summary of the observation of clinical manifestations is shown on Table 1. Because of the differences in the severity of infection in various groups on E.D.+0 (Baseline), the GATS for E.D.+4 were adjusted for GATS on E.D.+0 by an analysis of covariance.

Table 1

Total Scores of Clinical Manifestations

Gr. Av. Total Score and Adjusted Gr. Av. Total Score on E.D.+4

Group	Treatment	Daily Dose (mg/kg)	No. of Dogs	E.D.+4 GATS	Baseline Average	E.D.+4 Adj. GATS
A	NEG. Cont.	0	6	18.0	12.2	17.9
B	SDZ	122.0 (66.0 b.i.d.)	5	4.9	7.8	6.6
C	TMP	5.0	6	9.3	11.0	9.6
D	TMP	2.5	6	12.0	13.1	11.4
E	SDZ	25.0	6	8.5	16.0	6.7
F	SDZ	12.5	6	8.7	14.2	7.6
G	TRI	30.0	6	2.6	12.3	2.4
H	TRI	15.0	6	3.4	13.0	2.8
I	TRI	7.5	6	1.5	7.7	3.3

Results show that (1) Groups treated with TRI 30.0, TRI 15.0 and TRI 7.5 had lower scores than groups treated with TMP 2.5, TMP 5.0, SDZ 12.5 or SDZ 25.0, (2) the Group treated with TRI 30.0 had a lower score than the group treated with TRI 15.0 and both had lower scores than TRI 7.5, (3) there was no difference in scores for groups treated with either SDZ 25.0 or SDZ 66.0 b.i.d.

Table 2

Comparison of Adjusted Means for Total Score on E.D.+4

TRI 15.0

Group	Treatment	Dose (mg/kg)	Adj. Mean	p-Value
H	TRI	15.0	2.8	
F	SDZ	12.5	7.6	0.05 (H vs F)
H	TRI	15.0	2.8	
D	TMP	2.5	11.4	0.01 (H vs D)

TRI 30.0

Group	Treatment	Dose (mg/kg)	Adj. Mean	p-Value
G	TRI	30.0	2.4	
E	SDZ	25.0	6.7	0.09 (G vs E)
G	TRI	30.0	2.4	
C	TMP	5.0	9.6	0.01 (G vs C)

Table 2 summarizes the comparison of Total Scores on E.D.+4. Statistical analysis of adjusted means on E.D.+4 shows a significant treatment difference between Tribrissen and the individual components.

Table 3

Comparison of Average White Blood Cell Counts
on E.D.-2, E.D.+0, E.D.+5

Treatment Groups	Column A			Column B			Column C		
	E.D. -2	E.D. +0	Diff.	E.D. +0	E.D. +5	Diff.	E.D. -2	E.D. +5	Diff.
Negative Control	15.4	20.1	+4.7	20.1	17.6	-2.5	15.4	17.6	+2.2
SDZ 66.0	11.2	18.2	+7.0	18.2	11.6	-6.6	11.2	11.6	+0.4
TMP 5.0	13.4	19.2	+5.8	19.2	19.9	+0.7	13.4	19.9	+6.5
TMP 2.5	12.3	21.2	+8.9	21.2	18.3	-2.9	12.3	18.3	+6.0
SDZ 25.0	13.1	20.1	+7.0	20.1	15.4	-4.7	13.1	15.4	+2.3
SDZ 12.5	12.7	20.4	+7.7	20.4	17.9	-2.5	12.7	17.9	+5.2
TRI 30.0	15.3	22.7	+7.4	22.7	13.3	-9.4	15.3	13.3	-2.4
TRI 15.0	12.6	20.6	+8.0	20.6	16.0	-4.6	12.6	16.0	+3.4
TRI 7.5	12.7	18.3	+5.6	18.3	17.2	-1.1	12.7	17.2	+4.5

The data in Column A show all groups had increased WBC in response to infection. The range of increase was from 3900 to 8900. In comparing WBC on E.D.+0 to E.D.+5 (Column B) all groups except TMP 5.0 had decreased counts. The group treated with TRI 30.0 had the greatest decrease (9400) during this treatment period. The differences in WBC from E.D.-2 (pre-infection) to E.D.+5 are shown in Column C. The counts of groups treated with TRI 30.0 and SDZ 66.0 had returned to the pre-infection level while WBC in all other groups remained elevated.

2. Blood Culture Results

The summary of blood culture results is shown in Table 4 below.

Table 4
Summary of Daily Blood Culture
(Number Positive/Total)

Treatment Group	Experiment Day							
	-1	0	+1	+2	+3	+4	+5	+7
Negative Control	6/6	5/6	4/6	5/6	4/6	4/6	6/6	6/6
SDZ 66.0	5/5	4/5	1/5	1/5	0/5	0/5	2/5	2/5
TMP 5.0	6/6	4/6	3/6	2/6	3/6	3/6	3/6	3/6
TMP 2.5	6/6	6/6	4/6	5/6	4/6	3/6	3/6	3/6
SDZ 25.0	6/6	6/6	3/6	0/6	0/6	0/6	2/6	2/6
SDZ 12.5	6/6	6/6	2/6	4/6	2/6	1/6	2/6	2/6
TRI 30.0	6/6	6/6	4/6	0/6	0/6	0/6	0/6	0/6
TRI 15.0	6/6	6/6	2/6	1/6	0/6	0/6	1/6	1/6
TRI 7.5	6/6	6/6	1/6	2/6	2/6	0/6	2/6	2/6

Treatment Period

All dogs in all treatment groups showed positive blood cultures indicating a bacteremia on E.D.-1. The majority of untreated control dogs had positive cultures throughout the treatment period and all were positive on E.D.+5 and E.D.+7. None of the dogs receiving TRI 30.0 had positive blood cultures after E.D.+1. In both TRI 15.0 and TRI 7.5

groups, none of the dogs had positive cultures on E.D.+4, but some dogs in both groups showed positive cultures post-treatment. The same results occurred in the SDZ 66.0 and SDZ 25.0 groups. Approximately 50% of the dogs in the TMP 5.0 and TMP 2.5 groups had positive blood cultures throughout the study.

Bacteriological Cultures at Necropsy

Results of bacteriological cultures of tissues at necropsy are shown in Table 5.

Table 5

Summary of Bacteriological Cultures
of Tissues at Necropsy
(Number Positive/Total)

Treatment Group	Tissues								Total
	Liver	Spleen	Kidney	Nodes	Bile	Lungs	Blood	Urine	
Negative Control	4/6	4/6	4/6	1/6	5/6	4/6	6/6	3/6	31/48
SDZ 66.0	1/5	1/5	1/5	2/5	1/5	1/5	2/5	1/5	10/40
TMP 5.0	1/6	5/6	3/6	3/6	1/6	4/6	3/6	2/6	22/48
TMP 2.5	3/6	4/6	1/6	5/6	1/6	4/6	3/6	4/6	25/48
SDZ 25.0	3/6	1/6	1/6	0/6	1/6	2/6	2/6	0/6	10/48
SDZ 12.5	2/6	2/6	4/6	2/6	2/6	2/6	2/6	3/6	19/48
TRI 30.0	1/6	2/6	1/6	1/6	0/6	2/6	0/6	0/6	7/48
TRI 15.0	0/6	0/6	1/6	1/6	0/6	1/6	1/6	0/6	4/48
TRI 7.5	1/6	3/6	1/6	2/6	0/6	2/6	2/6	0/6	11/48
Totals	16/53	22/53	17/53	17/53	11/53	22/53	21/53	13/53	139/424

Six tissues plus urine and blood were cultured from each dog at necropsy. There was a total of 48 samples cultured from all groups except the SDZ-66 group which had only five dogs or a total of 40 samples. The negative controls had 65% (31/48) samples positive at necropsy. Groups treated with TRI 30.0, TRI 15.0 and TRI 7.5 had 14% (7/48), 8% (4/48) and 23% (11/48) positive samples, respectively. The group treated with SDZ 66 had 25% (10/40) positive and the SDZ 12.5 group had 40% (19/48) positive. The groups treated with TMP 5.0 or TMP 2.5 had 46% (22/48) and 52% (25/48) positive tissues, respectively.

DISCUSSION

The three major objectives of this study were met in the major parameter, total scores of observation of clinical signs of disease. Support for all objectives was also shown in the other three parameters.

The effects various treatments had on the total score of clinical observations are best illustrated on Graph 1, Page 15. All scores were adjusted to E.D.+0 scores by an analysis of covariance. Statistical analysis was done on results at E.D.+4 since the untreated controls had the highest score on that day. It is obvious that all treatments were better than no treatment, though treatment with TMP alone was not very effective. It is equally obvious that with doses used in this study, Tribissen at any dose level was more effective than SDZ or TMP at any dose level. And though the differences were slight, on E.D.+4 the dose titration shows TRI 30.0 was more effective than TRI 15.0 and both were more effective than TRI 7.5.

The effect of treatment was reflected in the changes in white blood cell counts (see Table 3). Following treatment the untreated controls and TMP treatment groups had virtually no change in WBCs which parallels the observation on clinical response. The counts of the TRI 30.0 group and positive control group SDZ 66, had returned to the pre-infection levels by E.D.+5 while WBCs of all other treatment groups remained elevated.

The results of daily blood cultures also supports the clinical observations. Table 4 shows that dogs treated with TRI 30.0 had no positive cultures after E.D.+2. The SDZ 25.0 and SDZ 66.0 groups had all negative cultures on E.D.+2 and E.D.+3 respectively, but both had some dogs with bacteremia on E.D.+5 and E.D.+7.

Though there is some variation in the number of dogs positive on each experiment day, the trend again shows that all treatments had some effect; the combination was more effective than the individual components and TRI 30.0 was the most effective dose of Tribriksen.

Examination of the results of bacteriological culture of tissues at necropsy tends to confirm results of clinical observations, WBC counts and bacteremia.

Groups treated with Tribriksen had few infected tissues, groups treated with SDZ had approximately 25% positive tissues and the TMP groups had approximately 50% infected tissues. As in previous observations, all treatments were more effective than no treatment, the Tribriksen combination was more effective than individual components.

SUMMARY AND CONCLUSIONS

In all four parameters measured in this study, Tribriksen, a combination of TMP and SDZ, was more effective than either of the components. In addition, the combination was more effective at dose levels much lower than dose levels of the individual components. Synergy was also shown in a strict statistical model of synergy developed by Shah and Piserchia.

When results of all four parameters were considered, Tribriksen was effective at both 15 mg/kg and 30 mg/kg. The 30 mg/kg dose was slightly more effective than the 15 mg/kg dose. TRI 30.0 was significantly more effective than SDZ at 66 mg/kg/b.i.d.

GRAPH I

Adjusted Group Means for Total Score

