

SECTION III

EXPERIMENTAL DESIGN OF DOSE DETERMINATION STUDIES WITH ANTIMICROBIAL DRUGS

Chairpersons

Dr. Carl Aronson
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DR. PAUL: Good morning and welcome to the third section of the Symposium on Dose Determination with Animal Drugs. The chairpersons this morning are: For the second half of this section, Dr. Bob Simmons who is the Director of Professional Relations at Beecham Laboratories. Handling the first part of this third section is the President of the American Academy of Veterinary Pharmacology and Therapeutics and Associate Professor of Pharmacology and Toxicology at the University of Pennsylvania -- Dr. Carl Aronson. Carl...

DR. ARONSON: Good morning, ladies and gentlemen. Our session this morning is going to deal with the experimental design of dose determination studies with antimicrobial drugs.

Our first speaker this morning on the topic of clinical trials for dose determination is Dr. Thomas Keefe. Dr. Keefe received his D.V.M. degree from the University of Missouri in 1963. He served as the Director of Clinical Research at Bristol Laboratories for 5 years; he is currently the Veterinary Medical Director of Beecham Laboratories and has held this position for 10 years. In this position he has participated in a number of NADA approvals. It is with pleasure that I introduce our first speaker, Dr. Tom Keefe.

DR. KEEFE: Thank you very much. It is certainly a pleasure to be here. Today I have the pleasure of discussing the subject of mastitis. I think from a clinical point of view in dose determination, it is probably the easiest type of clinical study to do as far as dose determination and approval of new drugs. I feel very confident that, at least up until the past month or so, I could tell our management that for "x"-hundred thousand dollars they could get a new mastitis product approved assuming we had the basic toxicology done. So I really think it is a reasonable and easy type of dose titration study from the field point of view.

DOSE DETERMINATION STUDIES WITH
ANTIMICROBIAL DRUGS - BOVINE MASTITIS

Thomas J. Keefe, D.V.M.

Since the time antibiotics became widely used in the treatment of bovine mastitis in the late 1940's, researchers, clinicians and regulators have been interested in optimizing the best dose of the drug. The three primary factors concerning these groups are: human safety, animal safety and an efficacious dose. Most veterinarians would rank these three factors in essentially the same order as above. Human safety must be our first consideration. The second should be animal safety (above all - do no harm) and the third to provide an efficacious treatment.

The purpose of this paper is to discuss how we arrive at an efficacious dose in this modern day of "government regulations." One of the primary obstacles in achieving this objective is defining an efficacious treatment. The Food and Drug Administration prefers the term "optimum dose" while most veterinarians prefer "efficacious dose." The Agency defines an optimum effective dose as "most sufficient dose level of the antimicrobial drug product needed to accomplish the intended effect."¹

Now that this objective is defined, how do we go about determining the optimum dose of an antimicrobial and still keep the project within the scope of practical reality.

My task with the subject of bovine mastitis is relatively easy compared to those trying to determine the optimum dose for canine cystitis or pneumonia. Fortunately, the nature of dairy business, management and bovine mastitis is such that the pharmaceutical companies can in fact generate data that satisfies the Agency's demands.

Before we specifically discuss these details, let's review the basis for the FDA's authority to regulate the pharmaceutical industry and indirectly the practice of veterinary medicine.

Regulations/Guidelines:

In August, 1981 the Food and Drug Administration published a 13-page revised draft of guidelines for the evaluation of antimicrobial drugs for intramammary infusion. These

guidelines were assembled to inform the drug industry of 1) the basis for certain regulatory requirements concerning new animal drugs, 2) the type of data which must be collected to demonstrate that a product is safe and effective, and 3) requirements concerning the manufacture and control of the drug product.¹

These guidelines specifically refer to the Code of Federal Regulations which gives the FDA authority to require data on the safety and efficacy of a new proposed drug. This authority is found in section 21CFR512 and 514.1(a) and (b). Effectiveness studies supporting a new drug application must be adequate and well controlled as defined in 21CFR514.111. Good Laboratory Practice Regulations (21CFR58) also apply to bovine mastitis products. All aspects of the clinical studies are subject to the Biosearch Monitoring Program (21CFR511.1). Manufacture of a mastitis product is subject to the Current Good Manufacturing Practices Regulations (21CFR211).

In these guidelines, dose titration or optimal dosage is referred to on pages 6, 8, 9 and 10.

Page 6: Another objective of clinical studies is the collection of information on the drug's effectiveness, optimal dosage, and adverse effects on cows with infectious mastitis due to susceptible microbe(s) which are identified and characterized. Therefore, data should be collected for each species of mastitis-causing microorganisms for which a label claim is made.

Page 8: Adequate and well controlled studies (titration and field) are needed to qualify a drug product for FDA approval and consideration.

A control and three dose points should be examined.

It is necessary to determine the optimum effective dose. The number and dose intervals required for optimum effectiveness should also be determined.

For combination drugs, each active ingredient should be shown to contribute to the effect claimed by the appropriate scientific methodology, including dosage titration studies, to establish the most effective dose of the combination (see BVM's Combination Drug Guidelines).

Stage II Clinical Investigations (titration studies)

A. Lactating Cows:

1. Two independent investigators should collect titration data on all the titration points, including the control point, in each of the herds studies.
2. The infected quarters should be treated no later than 72 hours after collection of milk samples for bacteriological culture.

In clinical cases, treatment can be administered immediately after milk sampling. One half of the total number of clinical cases should be from control animals. An approved active drug control may be used in these animals if necessary.

3. Post-treatment quarter milk samples for culture should be taken during the third week after treatment.

B. Nonlactating Cows:

1. Same as #1 under A of the Lactating Cows.
2. For treatment claims (clinical cases)
 - a. Treat only infected QUARTERS.
 - b. Samples for milk culture should be taken a week later.
 - c. A placebo (vehicle) should be used for controls.
3. For treatment and prevention claims only
 - a. Treat only infected COWS (all four quarters).
 - b. Post calving sampling interval -- consecutive duplicate samples taken one, two, and three weeks apart.
 - c. A placebo (vehicle) may be used for controls.

Page 10: Stage III Clinical Trial (field studies)

A. Lactating Cows:

1. Use three different geographical locations and two independent investigators per location.
2. The optimum effective dose selected from Stage II data is tested along with dose frequency.
3. The procedure for drug treatment and post-treatment milk sampling of infected quarters in Stage III is the same as Stage II (lactating cows).

B. Nonlactating Cows:

1. Same as #1 under Lactating Cows.
2. Same as #2 under Lactating Cows.
3. Post calving sampling interval -- consecutive duplicate samples taken one, two, and three weeks apart.
4. A placebo (vehicle) should be used for controls.

With the foregoing guidelines in mind, let's discuss what is involved in conducting a dose determination clinical field study in bovine mastitis. Much of the data presented here is drawn from personal experience of the author in conducting clinical mastitis studies with the following antibiotics: potassium hetacillin, sodium cephalixin, benzathine cloxacillin, sodium cloxacillin and amoxicillin trihydrate. This discussion is limited to single agent mastitis products. For a discussion of combination mastitis products, the reader should review the published work of C. Miller (Upjohn).²

EXPERIMENTAL DESIGN - CLINICAL FIELD STUDY:

Once the necessary pre-clinical studies (process development, analytical development, formulation, stability, pharmacokinetics, microbiology, toxicity, milk-out and tissue residue) have been completed, a clinical development program can be put together. Generally, an INAD along with a protocol is submitted to the Bureau of Veterinary Medicine requesting permission to undertake a field study. After the Agency has reviewed the milk-out/tissue residue data, toxicity and the clinical protocol, a conference is held with the Agency to discuss the specific details of the protocol and study design.

Three dosage levels of the test drug are selected. The selection of these three dosage levels is based upon data from the following informational sources: intuitive, historical, pharmaceutical/microbiological, experimentally induced infection, clinical trials and most commonly, a combination of the above. The objective is to select the "optimum" level and then choose a level below and above with the latter being no more effective.

Based upon recent communications with the Agency, there seems to be a preference for a no-treatment group rather than an active control. Assuming we are dealing with subclinical mastitis, a no-treatment group has not caused any serious problems. However, if we were trying to generate clinical mastitis data, a no-treatment group would be unacceptable on ethical grounds. An active control is a must if the study involves clinical mastitis.

All dose titration studies should be conducted on a blind basis to eliminate any bias on the part of the investigator, microbiologist and/or dairyman. Blinding is relatively easy in bovine mastitis due to opaque syringes in common use.

We generally select an active control with the same directions for use as the test product. The test drug is filled in identically appearing syringes. The syringes are then labeled and packed in boxes of twelve.

A computerized randomization schedule is generated for the four treatment groups. From this randomization table, a treatment schedule is constructed. Each box as well as each of the 12 syringes contained in the box are labeled with the appropriate code (1, 2, 3, 4, etc.). If a no-treatment group is being used instead of an active control, this group is simply not treated until after completion of the study.

Clinical investigators are then contacted, with at least 2 or 3 in each of three geographical areas. A thorough review of the product introductory brochure, protocol and case report form is conducted during the site visit. A detailed discussion of the microbiology is necessary since this is the real basis of approval of the NADA. It is best to have the microbiology conducted by an outside laboratory to further blind the study. The laboratory should be requested to conduct their procedures according to the booklet published by the National Mastitis Council.³

At the present time, duplicate or consecutive milk samples are required. Duplicate milk samples are defined as "two milk samples collected at a single milking," while consecutive milk samples are "milk samples collected at different milkings." The use of duplicate samples means that if a 100 cow herd is being cultured, the laboratory will receive 800 milk samples, a mammoth undertaking for any laboratory. This is a very costly and time consuming effort.

Identification of the pathogen as to genus and species is currently required. In light of the variation in MIC's of *Streptococcus non-agalactiae* and other organisms, this requirement is justified. However, the frequency of occurrence of these less common pathogens is too infrequent to generate adequate data for label claims. Therefore, the number of these less common pathogens required for label approval should be reduced.

The guidelines require that an infected quarter must be treated no later than 72 hours after collection of the pre-treatment milk samples. Since the Agency only permits treatment of infected quarters, culture results must be known prior to treatment. Considering the number of milk samples (8/cow) required, this places a tremendous burden on the

microbiology laboratory. Post-treatment milk samples are to be taken the third week after treatment.

No concurrent therapy or changes in management of the herd are acceptable during the course of the study. CMT and strip cup readings are necessary. These should be taken at the time of the pre-treatment and post-treatment samplings.

Following post-treatment sampling of the 4 groups, these data are analyzed. The primary factors evaluated are bacteriological elimination rate and clinical response. Table #1 lists the factors that may influence the bacteriological elimination which should be evaluated.

Table #1

Factors That May Influence Bacteriological Elimination

1. Organism
2. Investigator
3. Production
4. Age
5. Stage of Lactation
6. Quarter Involved
7. Management Ability
8. Type of Facility
9. Leukocytic Response
(CMT and Strip Cup)

The most important factor listed in Table #1 is the individual organism. Because of the variation in susceptibility of the pathogen to the test antibiotic, the organism response (elimination) to the drug is the primary consideration upon which market approval is based. The method of expression of this response is "% eliminated or cured." Obviously, only "acceptable cases" may be included in this overall elimination (cure) rate.

The Agency is also interested in looking at the bacteriological cure rate by investigator. The primary purpose of this analysis is to detect any extreme variation between investigators. In the 5 NADA projects conducted by the author, no significant variation was seen among investigators, if an adequate number of cases were done by each investigator.

Likewise, the other 7 parameters with the exception of CMT, rarely ever significantly influenced the bacteriological cure

rates in a dose titration study. With the advent of computers, the additional analysis of these data does not create a burden. If the Agency and industry want to save review time, these parameters could be deleted without obscuring the efficacy of the product.

The CMT and/or presence of abnormal milk influences the bacteriological cure rates in dose titration studies. As one would expect, as the degree of inflammation present in the udder increases, the efficacy of the higher dose generally increases. CMT or somatic cell counts are an important variable in evaluating the efficacy of a mastitis product for lactating cows. This is not the case where the product is intended for dry cows.

Clinical evaluation of the animal response is not really significant to the overall evaluation of the test drug. The various parameters (bacteriology, CMT, strip cup, etc.) are more specific and certainly more objective. Accordingly, this parameter could be omitted without impinging upon the reliability of the results obtained.

NEEDED CHANGES IN GUIDELINES:

Based upon my experience with 5 major NADA's during the past 15 years, I believe there are at least 5 areas that need clarification or revision. Some of these proposed changes may not be popular with all of my industrial colleagues and many of these suggestions will meet with resistance from the Bureau of Veterinary Medicine. However, to meet the economical demands facing the pharmaceutical and dairy industries, these changes must be given serious consideration:

1. Frequency of Dose

It is necessary for the Agency to establish a "standard" number and frequency of dosing intramammary products in both lactating and dry cow therapy. A standard dose concept would save industry from reinventing the wheel everytime a product is introduced. Considering the fact that the current standard treatment regimen for lactating cows is 1 dose after each milking (8 or 12 hours) for 3 consecutive treatments and 1 dose per quarter after the last milking for dry cows, why not adopt this regimen as "standard." If a company wishes to vary from this standard, it could do the necessary studies to show that

once every 6 hours or once every 48 hours for example is more efficacious or for that matter, the company could demonstrate that 1 dose is equal to 3, or 6 is better than 3 doses. In other words the burden of proof is on the individual who wants or believes that their product has some beneficial advantage, not on everyone. At the present time, the guidelines require the establishment of the optimum effective dose using a method which includes a number of doses at various intervals.

The Agency seems to accept the suggested standard referred to above in their review process to date, however, some day a "reviewer" is going to interpret the guidelines differently after a company has spent 3-4 years generating data under an assumed frequency/number of doses as standard. Let's avoid this "moving target" by having the Agency formalize a standard number of doses and intervals.

Clinical Versus Subclinical

Currently, the FDA requires that 10% or so of the total number of infected cows in the pooled data have clinical mastitis and that these cows will be used to evaluate the treatment claim. Again, to prevent a "moving target" let's define "clinical mastitis" from a reasonable clinical research point of view. This definition cannot be the one that is used in textbooks since it will be literally impossible to generate the necessary data for label approval if that definition is employed. Therefore, an alternate, more reasonable definition must be developed.

In my view, a reasonable definition of clinical mastitis for regulatory purposes would be as follows: CMT of 3, positive strip cup and the isolation of a pathogen. According to Schalm, a CMT of 3 represents a somatic cell count of $>5,000,000$.⁴ A positive strip cup would indicate grossly abnormal milk. The necessity for demonstration of the presence of a pathogen on pre-treatment culture should be obvious to everyone, i.e. how else could you demonstrate the efficacy of an antibiotic, if you could not show the elimination of the offending organism?

In the past, NADA approvals were essentially based upon subclinical mastitis. This was undoubtedly due to the ease in which subclinical cases could be standardized for

statistical purposes. It now appears that the FDA reviewers are beginning to recognize the need for data in cows with clinical mastitis. As a veterinarian, I agree with the Agency that the evaluation of a drug in cases of clinical mastitis is a necessity. Talk to any dairy practitioner and he or she will tell you the current lactating products do not work in clinical mastitis cases. Why should they? They have not been evaluated in clinical mastitis!

But, rather than over-react and go from one extreme to another, it is essential that we - industry and Bureau of Veterinary Medicine - work together to establish reasonable guidelines for evaluating products intended for the treatment of clinical mastitis. If guidelines are issued which are too burdensome, it will prevent the development of any new mastitis products.

What the Agency must NOT do is over-emphasize the clinical mastitis aspect of future products and leave existing "approved" products with broad clinical claims. Basic fairness would dictate that all manufacturers of products for the treatment of bovine mastitis be notified that the Agency's policy with respect to clinical mastitis has been revised, and that the company must generate label claims for clinical mastitis (CMT 3, positive strip cup, pathogen) by a specified time (say 3 years) or their labels will be modified to reflect subclinical claim only.

A side advantage of products labeled for clinical mastitis would be the reduction in use of many of the so-called "bathtub remedies" that cause the USDA, FDA, industry and academia problems.

3. Maximum Efficacy

From a philosophical point of view, the Agency needs to start thinking maximum efficacy, not minimum efficacy. If there is a question as to efficacy of the dose of 2 antibiotics, by all means choose the higher of the 2, assuming there is adequate safety. Industry is not motivated to encourage use of higher levels of drug in order to sell more, but to enhance efficacy or improve performance of their drug. Titrating antibiotics to the minimum effective levels increases resistance, undertreats marginally sensitive organisms, results in more relapses,

and may result in more bacteriostatic action rather than bactericidal.

4. E. coli Mastitis + Supportive Therapy

The veterinary profession needs more effective antibiotics to treat E. coli and other coliform type mastitis. However, before the pharmaceutical industry can meet this need, the Agency must change their attitude towards supportive therapy. There is no way a company can generate an adequate number of cases by administering a 10 ml syringe of antibiotic to a cow with acute E. coli mastitis and walk away. In the case of E. coli mastitis, supportive therapy (oxytocin, corticosteroids, fluids, etc.) is a must. No antibiotic is going to correct this disease without supportive therapy.

Why can't the Agency approve a "treatment regimen" for a disease condition such as E. coli mastitis? The label could state Drug X is effective for the treatment of acute E. coli mastitis when it is administered in conjunction with oxytocin, corticosteroids and fluids when these supportive treatments are given at their recommended dosage.

If a product was labeled in such a manner, the practitioner would have a legal treatment procedure to use with a certain degree of confidence in its efficacy and the Agency would have the opportunity to establish proper withdrawal times for such a treatment.

5. Single Cultures

The Agency currently requires duplicate or consecutive milk sampling to establish the presence of infection and prove bacteriological cure. Dr. Don Jasper conducted a study in over 3000 quarters and has shown that this duplication of work merely decreases the error rate by 2%.⁵ Considering the cost of this duplication, is the reduction in error rate worth the expenditure? By reducing this cost, companies could generate more cases which would surely be of more value to the Agency than a 2% increase in the accuracy of duplicative samples. Duplicate culturing is a waste of resources and should be changed.

CONCLUSIONS:

In conclusion, dose determination studies under clinical field conditions with mastitis products are relatively easy to conduct under current guidelines. The products involve themselves to blinding and the nature of the disease permits the investigator to obtain a large number of similar cases. The primary parameter of efficacy is bacteriological elimination rate which is an objective measurement of efficacy.

However, for the pharmaceutical industry to serve the needs of the profession, there are 5 changes or points that the Bureau of Veterinary Medicine and industry must consider. These points are 1) standardizing the number and interval of dosing in lactating and dry cow therapy, 2) require clinical cases for a clinical claim, 3) emphasis on maximum efficacy, not minimum, 4) modification of their current thinking on concurrent/supportive therapy in acute E. coli mastitis and 5) single cultures rather than duplicate or consecutive.

By industry and the Bureau of Veterinary Medicine working together using common sense, the veterinary profession and the dairy industry will have the very best pharmaceuticals available anywhere in the world.

REFERENCES

1. Guidelines for Evaluation of Antimicrobial Drugs for Intramammary Infusion Products, August, 1981, FDA.
2. C.C. Miller, JAVMA, Vol. 170, No. 10.
3. Microbiological Procedures for the Diagnosis of Bovine Mastitis, National Mastitis Council, 910 Seventeenth St., N.W., Washington, DC 20006.
4. BOVINE MASTITIS, O.W. Schalm, et.al., Lea & Febiger, Philadelphia, PA, 1971.
5. Don Jasper, AJVR, Vol. 35, No. 10.