TYLOSIN ANTIMICROBIAL ACTIVITY AND PHARMACOKINETICS IN COWS

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Introduction

Tylosin, an antibiotic of the macrolide group, is commonly used in food animal practice. Because it is an organic base (pKa = 7.1), moderately bound by serum proteins (40%), with a high degree of lipid solubility (5), tylosin would be expected to be widely distributed in body fluids and tissues (2).

The purpose of this report is to correlate the pharmacodynamics of tylosin in the cow with its antimicrobial activity *in vitro* and to make some suggestions concerning its clinical application in bovine practice.

Materials and Methods

Microbiological Methods – A survey of the antimicrobial activity of tylosin was conducted using cultures of various micro organisms isolated from bovine, equine and canine patients which were hospitalized at the Ohio State University Veterinary Hospital during 1974. The organisms isolated from cattle included β hemolytic streptococci and *Staphylococcus aureus* cultured from milk of mastitic cows, *Pasteurella* spp. isolated from tracheal aspirations from cattle with pneumonia, and *Corynebacterium pyogenes* cultures from the uterus of cows with metritis.

In the initial phase of the survey, each bacterial isolate was tested for its susceptibility to tylosin by the Kirby-Bauer method (4) using 30 μg discs. Those cultures which had zones of inhibition around 30 μg tylosin discs were then tested by the tube dilution method (3) to determine the minimum inhibitory concentration (MIC).

Pharmacokinetic Methods – The pharmacokinetic characteristics of tylosin in normal cows were determined by conducting several experiments.

The objective of the first experiment was to determine the serum half-life and volume of distribution of tylosin in the cow. Tylosin[1] was administered by a single rapid intravenous injection at a dosage of 12.5 mg/kg of body weight to six mature, clinically healthy Holstein-Friesian cows. Blood samples were collected immediately prior to injection and at precise intervals for 12 hours after injection. Milk samples were collected without prior milk-out at the same times from one of the six cows which was lactating at the time of the experiment. Milk pH was measured with a combination glass electrode and pH meter.[2] Serum was separated and serum and milk samples were frozen for later analysis.

The objective of the second experiment was to determine the intramuscular absorption characteristics of tylosin in the cow. A single dose of tylosin (12.5 mg/kg of body weight) was administered by intramuscular injection to six clinically normal cows. Five of the cows were the same experimental animals used in the first experiment two weeks earlier. None of the six cows was lactating. Blood samples were collected immediately prior to injection and at precise intervals for 24 hours after injection. Serum was separated and frozen for later analysis.

The objective of the third experiment was to determine the serum and milk concentrations of tylosin after repeated intramuscular administration in cattle. These studies were conducted in three lactating, mastitis-free Holstein-Friesian cows. Tylosin was administered intramuscularly at dosages of 12.5 mg/kg of body weight every 12 hours for 48 hours. Blood samples were collected 12 hours after each injection, just prior to the next scheduled treatment. Milk samples were collected from a single quarter of each cow at the same time that blood samples were collected. Milk pH was measured[2] on fresh milk samples. Milking schedules were not inter-

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[1]Tylan 200, Elanco Products Company, Indianapolis, Indiana.

[2]Expandomatic, Beckman Instruments, Inc., Fullerton, California.

rupted so that the cows were milked approximately one hour prior to each 12 hour sampling and injection. Serum was separated and serum and milk samples were frozen for later analysis.

Tylosin activity in serum and milk was asayed microbiologically by the cylinder cup method (1) using *Sarcina lutea* as the reference organism, and expressed as μg/ml. Values for kinetic parameters were calculated (9) for each of the cows and expressed as a mean and standard deviation. The serum half-life ($t_{\frac{1}{2}}$) was determined from the elimination phase of a semilogarithmic plot of the intravenous concentration-time data from each cow. The apparent specific volume of distribution (V'_d) was calculated by an equation that utilizes area under the curve instead of Y intercept.[3]

Results and Discussion

Antimicrobial Activity – Those organisms which showed zones of inhibition around 30 μg Kirby-Bauer discs included β hemolytic streptococci, *Staphyloccus aureus, Pasteurella* spp. and *Corynebacterium pyogenes.* Organisms which were isolated and found to be resistant to tylosin when tested by the Kirby-Bauer method included alpha streptococci, coliforms, and other gram negative organisms. Two resistant strains of *Staphylococcus aureus* of canine origin were also found.

The determination of the MIC of tylosin for each disc-susceptible organism provided more quantitative information on their true susceptibility. All of the disc-susceptible organisms isolated from cattle were susceptible to tylosin at a concentration of <1.0 μg/ml except for *Pasteurella* spp. for which the MIC was 12.5 μg/ml (Figure 1). MIC results for isolates of equine and canine origin were similar, which added support to the above conclusions. *Mycoplasma* spp. were also reported to be susceptible to tylosin at a concentration of <0.09 μg/ml and are included in this discussion for the sake of completeness.

Distribution and Elimination – The mean curve describing the elimination of tylosin from the serum of the six cows after intravenous injection (Figure 2) illustrates that distribution was not complete until two hours after injection. The mean serum half life ($t_{\frac{1}{2}}$), determined independently for each cow, was 1.62 ± 0.17 hours. The apparent specific volume of distribution (V'_d) was 1.10 ± 0.45 L/kg. Calculated values for $t_{\frac{1}{2}}$ and V'_d in the present study were in the same range as previously reported values which were based on data from four cows given a higher intravenous dosage (12). The large V'_d suggests that tylosin, like other lipid soluble organic bases, is widely

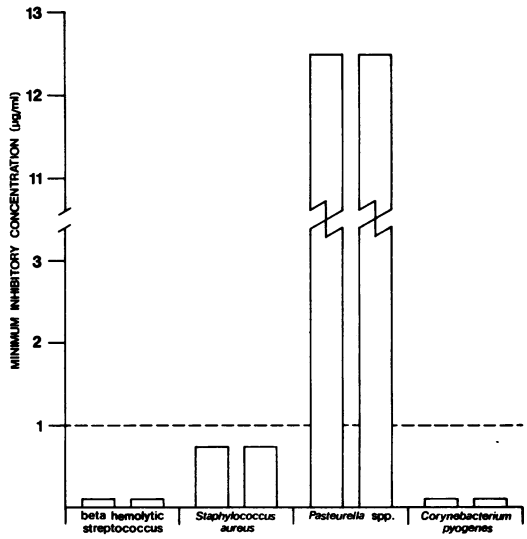

FIGURE 1. Minimum inhibitory concentration of tylosin for representative cultures of various disc susceptible microorganisms isolated from cattle. Dotted line at 1 μg/ml indicates maximum attainable serum concentration of tylosin after intramuscular injection of 12.5 mg/kg in cattle.

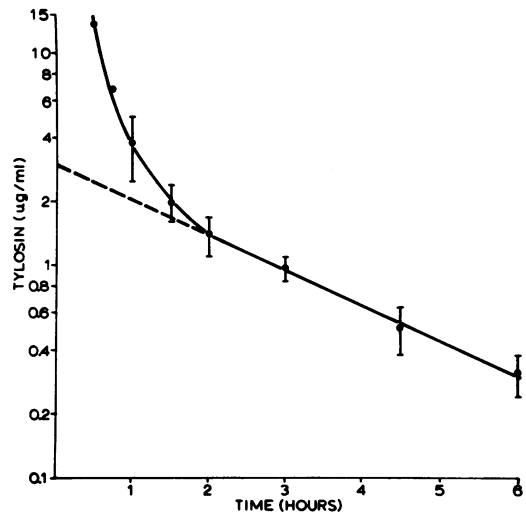

FIGURE 2. Semilogarithmic plot (mean ± standard deviation) of tylosin concentration in serum following intravenous administration at the rate of 12.5 mg/kg to six cows.

[3] $V'_d = \frac{\text{Dosage}}{(\text{area}) \cdot \beta}$ where β is the total elimination rate constant.

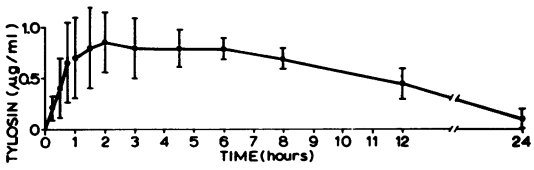

FIGURE 3. Tylosin concentration in serum (mean ± 1 standard deviation) following intramuscular injection at the rate of 12.5 mg/kg to six cows.

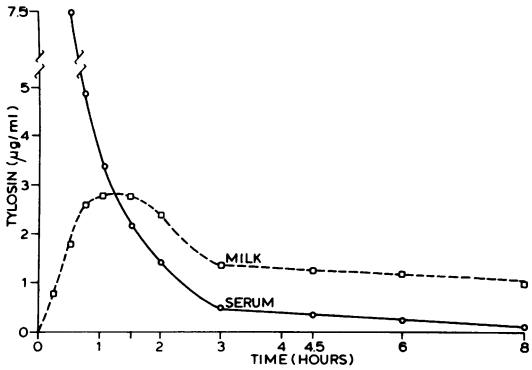

FIGURE 4. Tylosin concentration in serum and milk following intravenous injection at the rate of 12.5 mg/kg to one cow.

distributed in tissues or fluids other than serum (2).

Absorption – The curve depicting serum concentrations of tylosin after intramuscular injection (Figure 3) indicates in this study that the drug was not as well absorbed as previously suggested (10). Serum concentrations peaked at different times (ranging from 90 minutes to four hours) which accounts for the plateau shape of the mean curve and the large standard deviations. It is noteworthy that serum tylosin concentrations of 1 μg/ml were not consistently achieved and definitely not maintained. Concentrations of tylosin were detectable 24 hours after intramuscular injection in each of the six cows. Low but persistent tylosin levels reflect both slow absorption from the muscle and rapid elimination from the serum.

Concentrations in Milk – Tylosin was detected in milk within 15 minutes after injection in the single lactating cow treated intravenously (Figure 4). Drug concentration in milk exceeded its concentration in serum at 90 minutes and remained fivefold higher throughout the sampling period. Milk pH in this cow was stable at 6.6. The detection of tylosin in milk following intravenous injection was not surprising since earlier work with the drug in cows suggested that tylosin passes readily into milk by nonionic passive diffusion (12). Tylosin (pKa = 7.1) is 67% nonionized at serum pH of 7.4 whereas only 20% is nonionized at the normal milk pH of 6.5. Since the nonionized moiety is diffusible, passive diffusion from serum to milk is favored over diffusion from milk to serum. Under equilibrium conditions, a milk:serum ratio of 4.8:1 was predicted for nonmastitic milk of pH 6.5. Correspondingly lower milk:serum ratios are predicted and have been measured for mastitic milk of higher pH (12).

After intramuscular injection in three lactating cows, tylosin was detected in milk within two hours. The concentration of tylosin in milk exceeded its concentration in serum at all sampling times (Figure 5). Milk pH ranged from 6.5 to 6.8. Detectable concentrations of tylosin (mean, 1.49 μg/ml) were found in milk 24 hours after the last injection in the three cows. High concentrations of tylosin in milk following repeated intramuscular injections are generally supportive of the nonionic passive diffusion theory. A definitive statement concerning milk:serum ratios cannot be made from the results of this experiment, however, because milk samples were collected without prior milk-out to simulate clinical conditions. Milk:serum ratios, corrected for differences in protein binding and calculated at various times ranged up to about 20:1.

The laboratory assay method measured biological activity of tylosin. The *in vitro* antimicrobial activity of tylosin has been found to be pH dependent. Tylosin became progressively more active as the pH of the medium was adjusted from 6 to 8 (8). Differences in tylosin concentration between milk and serum may therefore reflect differences in antimicrobial activity due to pH as well as differences in

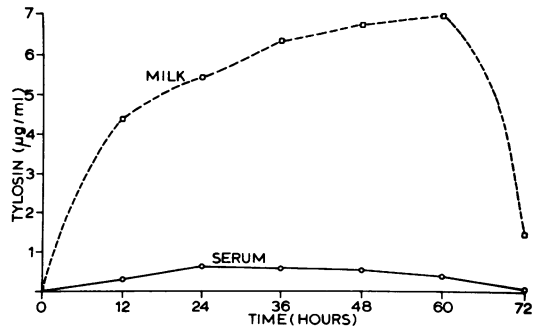

FIGURE 5. Tylosin concentration in serum and milk following intramuscular injection at the rate of 12.5 mg/kg at 12 hour intervals for 48 hours (mean data from three cows).

actual drug concentrations due to different degrees of ionization. In this study, tylosin concentrations were measured in milk with pH of 6.5–6.8, using a milk standard of pH 6.6.

Therapeutic Considerations – The dosage of 12.5 mg/kg of body weight employed throughout these experiments was nearly three times the dosage of 4.4 mg/kg (2 mg/lb[1]) of tylosin recommended for cattle. It is apparent from the results of this study that, even with the higher dosage, tylosin concentrations of 12.5 μg/ml (the MIC for *Pasteurella* spp. Figure 1) are not therapeutically feasible in cattle regardless of the route of administration (Figures 2–5). This finding suggests that *Pasteurella* spp. should be considered resistant to tylosin even though cultures appeared to be susceptible when tested by 30 μg Kirby-Bauer discs. This finding is in agreement with therapeutic trials as well as culture and antibiotic susceptibility studies conducted in a large number of cases of pneumonia in feedlot cattle, in which the MIC of tylosin for the most susceptible *Pasteurella* isolates was 16 μg/ml (6).

Serum concentrations of 1 μg/ml were not consistently achieved after intramuscular injection, despite the high dosage used. Tylosin concentrations in synovial fluid, measured in two cows, paralleled but did not exceed its concentration in serum (Gingerich, D. A., unpublished data, 1974). In milk, tylosin concentrations >1 μg/ml were easily achieved and maintained (Figures 4 and 5). These findings suggest that, with the possible exception of organisms in milk, only those microorganisms which are inhibited by tylosin concentrations < 1 μg/ml should be considered susceptible since concentrations greater than this are not therapeutically feasible.

Because of its limited spectrum of activity, tylosin should not be considered a broad spectrum antibiotic. The results of our antimicrobial survey are in general agreement with previously reported results (8).

The aim of antibiotic therapy is to provide a suitable concentration of drug at the site of infection and to maintain this concentration for a desired time. The microbiological phase of this study represents an effort to estimate the suitable concentration of tylosin required for antimicrobial activity. The pharmacokinetic analysis represents an effort to relate the pharmacological characteristics of tylosin to physiological and clinical reality.

From a chemotherapeutic point of view, it is in the extravascular space where drug-parasite interaction usually takes place. Concentrations of tylosin in milk are emphasized because milk represents an extravascular fluid compartment that is readily accessible for drug assay. The antimicrobial spectrum of tylosin includes some of the major pathogens of the bovine udder: β hemolytic streptococci, *Staphylococcus aureus*, *Corynebacterium pyogenes* and *Mycoplasma* spp. (7, 11). The results of this study suggest that the intramuscular injection of tylosin may be a rational treatment for bovine mastitis due to the above pathogens. If the maintenance of antimicrobial activity in milk can be correlated with clinical efficacy, then a considerably lower dosage than 12.5 mg/kg, BID, might be effective, since the concentrations of tylosin achieved in milk were many fold higher than the MIC for the aforementioned organisms. However, the pH of mastitic milk often approaches the pH of plasma, 7.4. Under these conditions the milk: plasma ratio would be expected to approach 1, a diffusion pattern which is less therapeutically favorable for mastitis therapy.

In body fluids other than milk it would seem highly unlikely that high concentrations of tylosin could be achieved following intramuscular injection. The clinical efficacy of tylosin for other infectious diseases of cattle would likely depend on the pathogenic significance of tylosin-susceptible microorganisms involved.

Due caution must be exercised against the over-interpretation and extrapolation of data obtained from normal cattle to clinically diseased cattle. The only truly significant indication of a therapeutic effect is a favorable clinical response.

Summary

A survey of the antimicrobial activity of tylosin was conducted by 30 μg Kirby-Bauer disc susceptibility testing of various bacterial cultures of animal origin. The *in vitro* sensitivity of Kirby-Bauer susceptible microorganisms was determined by the tube dilution method. The minimum inhibitory concentration of tylosin for β hemolytic streptococci, *Staphylococcus aureus* and *Corynebacterium pyogenes* was <1 μg/ml for most isolates studied, but the minimum inhibitory concentration for *Pasteurella* spp. was 12.5 μg/ml.

Tylosin concentrations were determined in serum and milk of cows following intravenous and intramuscular administration at the rate of 12.5 mg/kg of body weight. Concentrations of <1 μg/ml in serum but >1 μg/ml in milk were achieved.

These findings suggest that β hemolytic streptococci, *S. aureus* and *C. pyogenes* may

be considered susceptible to tylosin, inasmuch as the minimum inhibitory concentration of tylosin for these microorganisms is therapeutically feasible, particularly in milk. *Pasteurella* spp. should be considered resistant to tylosin because therapeutically effective concentrations cannot be achieved in cattle at reasonable dosages.

Résumé

Les auteurs ont effectué une étude sur l'activité antimicrobienne de la tylosine. Ils utilisèrent à cette fin la méthode de Kirby-Bauer, qui consiste à employer des disques contenant 30 μg d'antibiotique, et ils l'appliquèrent à diverses souches de bactéries d'origine animale. Ils utilisèrent ensuite la méthode des dilutions dans des tubes, pour déterminer la sensibilité *in vitro* des microbes qui réagissaient à l'épreuve de Kirby-Bauer. La concentration inhibitrice minimale de tylosine à l'endroit des streptocoques β hémolytiques, de *Staphylococcus aureus* et de *Corynebacterium pyogenes* s'avéra <1 μg/ml, pour la plupart des souches utilisées. Elle fut cependant de 12.5 μg/ml, pour les *Pasteurella* sp.

Ils déterminèrent aussi la teneur en tylosine du sérum et du lait des vaches expérimentales, après leur avoir injecté cet antibiotique, par les voies intra-veineuse et intra-musculaire, à raison de 12.5 mg/kg de poids vif. Ils obtinrent ainsi des concentrations <1 μg/ml dans le sérum, mais >1 μg/ml dans le lait.

Ces résultats permettent de considérer les streptocoques β hémolytiques, *S. aureus* et *C. pyogenes* comme des microbes sensibles à la tylosine, en autant qu'en pratique, particulièrement en ce qui concerne le lait, on puisse obtenir la concentration inhibitrice minimale. Il faut cependant considérer les *Pasteurella* sp. comme des bactéries résistantes à la tylosine, parce que des doses jugées raisonnables ne permettent pas d'obtenir des concentrations thérapeutiques efficaces, chez les bovins.

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