

## ABSTRACTS

# PHARMACOKINETIC-PHARMACODYNAMIC MODEL FOR FLORFENICOL AGAINST PASTEURELLA HAEMOLYTICA AND PASTEURELLA MULTOCIDA IN CATTLE.

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Antibiotic dosing regimen are often based on empirical experience rather than on rational design. A major goal of clinical pharmacology is to define the dose-effect relationship of therapeutic drugs. For these purposes, pharmacokinetic-pharmacodynamic (PK/PD) models were investigated and this way was recommended by FDA<sup>1</sup> for drug development. The pharmacodynamic effects can be studied *in vitro*. Studies of time-kill curves and post-antibiotic effect provided more useful information for optimising dosage regimens<sup>2</sup> than determination of the minimum inhibitory concentration (MIC) alone. Pharmacodynamic models can be used to describe the time course of antimicrobial activity<sup>3-7</sup> while the relationship between antibiotic and bacterial populations are complex.

The objectives of this study were established the pharmacodynamic parameters describing bactericidal effect of florfenicol against *Pasteurella multocida* and *Pasteurella haemolytica* isolated from bovine respiratory disease and in a second part simulate the behaviour of the different strains during a treatment in the different fluid using a pharmacokinetic/pharmacodynamic model.

*In vitro* effect of florfenicol on *Pasteurella* species was obtained by time kill studies during 24 hours at 37°C with an inoculum of 10<sup>7</sup> bacteria per ml in stationary growth phase and fixed concentrations in Mueller-Hinton broth.

The florfenicol concentrations in serum, bronchial secretion and tissular cage fluid of calves treated by two IM administrations of 20 mg of florfenicol/of body weight at 48 hours intervals were fitted by pharmacokinetics models and results were used to simulate the effect of florfenicol on population strains.

The main results obtained from pharmacodynamic study are :

(i) significant differences of bactericidal rates were observed between the two *Pasteurella* species as it shown in figure 1.

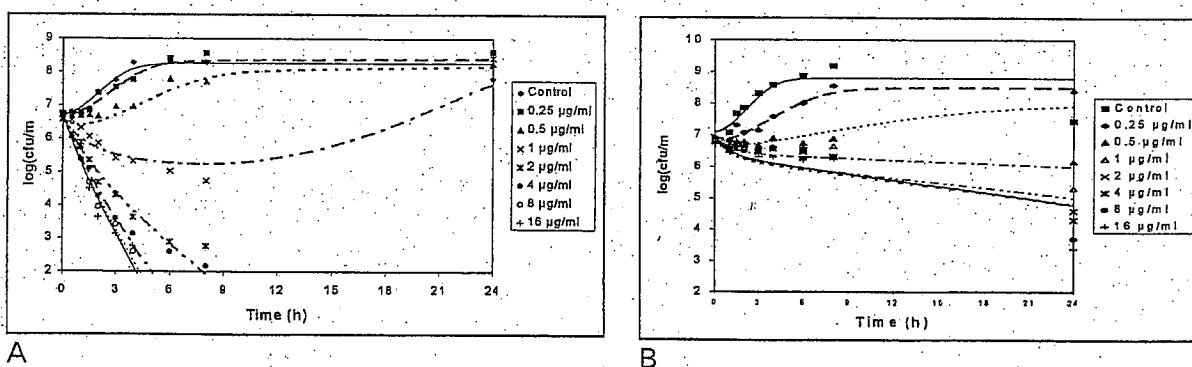
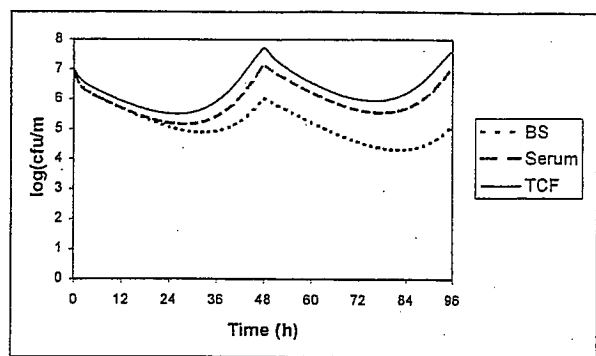
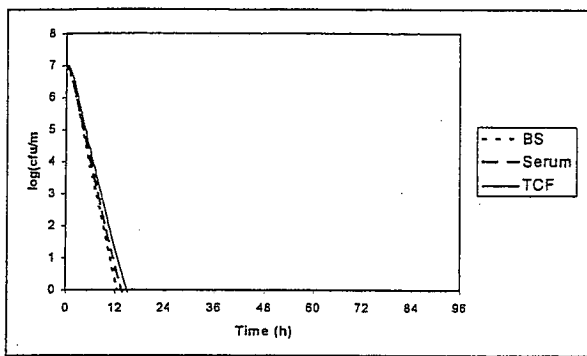


Figure 1 - Logarithm of bacterial population count versus time curve observed and fitted by pharmacodynamic model for a reference (NCTC 10643) *P. haemolytica* strain (A) and a reference (ATCC 43137) *P. multocida* strain (B).

(ii) while florfenicol minimal inhibitory concentration (MIC) are higher for *P. haemolytica* (1 µg/ml) than *P. multocida* (0.5 µg/ml), bactericidal rate was significantly higher for *P. haemolytica* than for *P. multocida* for concentrations above the MIC.

The results of the pharmacodynamic studies were combined with the pharmacokinetic model using to describe the time curve of florfenicol in different fluids. This simulation are presented on figure 2



A

B

Figure 2 - Logarithm of bacterial population count-time curve predicted by PK/PD model in serum, bronchial secretion (BS) and tissular cage fluid (TCF) for a reference (NCTC 10643) *P. haemolytica* strain (A) and a reference (ATCC 43137) *P. multocida* strain (B).

We conclude that florfenicol at a dose rate of 20 mg/kg at 48 hours intervals should be bactericidal against *P. haemolytica* and bacteriostatic against *P. multocida*. Killing curves allow to obtain more information about antimicrobial activity and are better to optimize dosage regimen.

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INFLUENCE OF DIFFERENT PRE AND POST-TREATMENT  
FASTING PERIODS ON THE KINETIC BEHAVIOR OF  
ALBENDAZOLE IN CATTLE.

S. Sánchez, L. Alvarez, J. Sallovitz, C. Lanusse. UNCPBA, Tandil  
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The influence of different factors affecting the disposition kinetics of benzimidazole anthelmintics in ruminants is currently under investigation in our lab. A 48 h-fasting period prior to treatment resulted in a marked enhancement of the plasma and target tissues availability of albendazole (ABZ) and its metabolites in cattle. Further work is required to elucidate how different feed managements may optimize drug-based parasite control. *The influence of different pre and post-treatment fasting periods on the plasma disposition kinetics and availability of ABZ and its metabolites in cattle was investigated.* Twenty five (25) parasite-free Holstein calves were used. Group A (control, animals fed *ad libitum*), Group B (24 h fasting pre-treatment), Group C (24 h fasting post-treatment), Group D (12 h fasting pre-treatment) and Group E (12 h fasting post-treatment). Animals in all groups received ABZ intraruminally at 10 mg/kg. Blood samples were taken over 72 h post-treatment and plasma analyzed by HPLC. Albendazole sulphoxide (ABZSO) and sulphone were the metabolites recovered in plasma. All the fasting intervals studied induced marked changes to the pattern of ABZ absorption; enhanced AUC and delayed Tmax values for both ABZ metabolites were observed. A 24 h-fasting period, including 24 h pre (95%) and 24 h post (117%), resulted in the greatest enhancement of ABZSO AUCs. However, even the 12 h-fasted animals showed increased ABZSO plasma availability (between 36 and 56%) compared to control calves fed *ad libitum*. Longer residence times (40-45%) for ABZ metabolites were obtained in the animals subjected to all fasting periods studied.

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## RUMINAL BIOTRANSFORMATION OF BENZIMIDAZOLE ANTHELMINTICS

G. Virkel, A. Lifschitz, A. Pis, J. Sallovitz and C. Lanusse. UNCPBA, Tandil (7000), ARGENTINA.

Ruminal microflora biotransforms Netobimin (NTB), an inactive pro-benzimidazole anthelmintic, into albendazole (ABZ) by nitroreduction and cyclization. ABZ is oxidized to ABZ sulphoxide (ABZSO) in the liver. Furthermore, in a bacteria-mediated reaction, ABZSO may be reduced back to form its parent thioether (ABZ). Different factors may alter the pattern of ruminal metabolism for these anthelmintic molecules. The current work characterizes **the influence of the type of diet, and monensin-induced changes on microflora composition, on the pattern of NTB and ABZSO biotransformation by sheep ruminal fluid *in vitro*.** Healthy Corriedale sheep were used as a source of ruminal fluid. In Experiment 1 animals were fed on either lucerne hay or a concentrate-based diet; in Experiment 2, ruminal fluid was anaerobically incubated during 24 h either with or without monensin. Aliquots of the collected ruminal fluid were incubated with either NTB or ABZSO at 38 °C under anaerobic conditions for up to 360 min. After a solvent-mediated extraction, samples of the incubation mixture were analyzed by HPLC. Ruminal fluid obtained from sheep fed the concentrate diet showed a greater metabolic capacity for both the nitroreduction-mediated bioactivation of NTB pro-drug, and the sulphoreduction of ABZSO. A dietary-induced change on the bacterial population may explain the most efficient **-nitro** and **-sulphoreduction** observed in ruminal fluid collected from sheep fed the concentrate diet. Ruminal metabolic activity fluid was affected by monensin pretreatment. The rates of NTB nitroreduction and ABZSO sulphoreduction were lower when ruminal fluid was previously incubated with monensin. The lower metabolic reductive rates observed in the presence of monensin, may be based on a selective detrimental effect of the ionophore on the Gram-positive bacterial population. Factors affecting the pattern of drug biotransformation in the rumen should be considered to optimize drug therapy.

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## PHARMACOKINETICS OF MARIMASTAT IN HORSES

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Marimastat (BB-2516) is a matrix metalloproteinase (MMP) inhibitor manufactured by British Biotech Inc. MMPs are important enzymes for regulating the detachment of cells from basement membranes during development and tissue remodelling. MMPs also appear to play a role in the mechanism of hoof separation during the development of laminitis in horses. Using an *in vitro* model of laminitis, we have shown that MMPs are activated during the separation process and the separation can be prevented by MMP inhibitors. There is also evidence for the activation of MMPs during the development of laminitis *in vivo*. As a forerunner to testing the efficacy of MMP inhibitors for preventing laminitis, we have investigated the pharmacokinetics of the MMP inhibitor Marimastat which is undergoing phase III clinical trials in humans. Marimastat is given orally to humans. However, administration of over 3 times the human oral dose to a horse did not give detectable levels of Marimastat in plasma indicating that the drug is poorly absorbed or undergoes extensive first-pass metabolism in this species. The behaviour of the drug when given intravenously to 3 horses fitted a 2-compartment model with a distribution half-life of 3-5 min, an excretion half-life of 41-82 min and an apparent volume of distribution of 8.6-22.5 l/kg.

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Are you a Graduate Student? No

Author Conflict of Interest None

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HYPERIMMUNIZATION INDUCES TOPICALLY ACTIVE  
ANTI-INFLAMMATORY FACTOR(S) IN BOVINE MILK  
D Gingerich, J Fuhrer, J Strobel, J Lange, J Durham  
Stolle Research Corporation, Cincinnati, OH 45242

We previously described the pharmacology of anti-inflammatory factor (AIF) in milk of hyperimmunized cows (Beck, 1997). We recently adapted a topical bioassay to test specific fractions of hyperimmunized milk (HIM) or control milk (CM). Phorbol 12-myristate 13-acetate (TPA) in acetone was applied to the challenge ear of mice to induce swelling; acetone alone to the contralateral ear. HIM and CM fractions were applied topically to assess inhibition of swelling 4 hours after challenge, compared to dexamethasone (DEX). Typical results (mean  $\pm$  sem, n=5):

	Control	CM	HIM	DEX
Swelling (mg)	11.4 $\pm$ 0.6	10.6 $\pm$ 0.9	6.3 $\pm$ 1.5	2.9 $\pm$ 0.7
Inhibition (%)	0.0 $\pm$ 5.7	7.0 $\pm$ 8.0	44.9 $\pm$ 13.0	74.1 $\pm$ 6.4
Prob (vs CM)	ns	--	0.0328	0.0004

Our results confirm that hyperimmunization induces expression of AIF activity in cow's milk and that the AIF is topically active. The findings also support other evidence that AIF is a low molecular weight molecule with potential for transdermal delivery.

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## COMPARATIVE TISSUE DISTRIBUTION OF IVERMECTIN, MOXIDECTIN AND DORAMECTIN IN CATTLE.

A. Lifschitz, G. Virkel, F. Imperiale, and C. Lanusse, UNCPBA, Tandil, ARGENTINA. P. Galtier, M. Alvinerie, INRA, Toulouse, FRANCE.

The relationship between plasma disposition, distribution to target tissues and the antiparasitic efficacy of endectocide compounds is being investigated. The comparative pattern of tissue distribution for ivermectin (IVM), moxidectin (MXD) and doramectin (DRM) in cattle was characterized. The three endectocide compounds were subcutaneously administered to Holstein calves (120-140 kg) at 200 µg/kg using commercially available injectable formulations. Two animals/group were sacrificed at 1, 4, 8, 18, 28, 38 and 48 days post-treatment. Plasma, abomasal mucosa/fluid, intestinal mucosa/fluid, bile, feces, lung and skin samples were obtained, processed and analyzed by HPLC using fluorescence detection. The three molecules were extensively distributed to all target tissues. MXD peak concentrations in abomasal/intestine mucosa, bile, skin and feces were obtained at 1-day post-treatment, which correlates with the rapid MXD absorption from the injection site described previously. IVM and DRM peak concentrations in those tissues were achieved at 4-days post-administration. Tissue availability for the three endectocides was greater than that obtained in plasma. Drug concentrations in abomasal (50-fold) and intestinal (2.5-fold) mucosae were greater than those found in their respective fluids. DRM and MXD concentrations in abomasal/intestinal mucosa and skin resulted >1 ng/g at 28 days post-treatment. Total drug availability (expressed as AUC) for DRM and IVM were greater than that found for MXD in abomasal mucosa, gut mucosa and skin. However, MXD concentrations at 38 and 48 days post-administration were higher than those of DRM and IVM in most of the tissues, particularly in bile and feces, where MXD residence times were significantly longer, reflecting the prolonged plasma persistence of this compound. Unidentified metabolites of the three compounds were detected in plasma and all the tissues analyzed.

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