Novel controlled release intramammary delivery system for treating bovine mastitis during lactation period

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ABSTRACT SUMMARY

A novel controlled release delivery system was developed for intramammary applications in dairy cows for the lactation period. In-vitro and in-vivo data show that cloxacillin release from the new intramammary vehicle can be tuned which opens the opportunity to optimize drug concentrations in milk during the treatment period. On the other hand this has a direct impact on the milk withholding period.

INTRODUCTION

The International Dairy Federation (IDF) stated that the clinical mastitis rate is about 40 cases per 100 cows per year1. The cure rate of bovine mastitis varies between 20% and 80% depending on the mastitis causing organisms. In fact, bovine mastitis remains a global problem being responsible for annual losses of over US$35 billion2.

On the other hand, antibiotic resistance of certain bacterial infections in humans is a concern internationally and in part, this resistance is believed to be due to use of antibiotics in food-producing animals3. Consequently, the use of antibiotics for treating bovine mastitis is mainly restricted to beta-lactams (penicillins and 1st and 2nd generation cephalosporins). Thus new innovative antibiotic treatments for mastitis will arise from novel controlled release delivery systems.

Within the Mastitis Research Centre (University of Otago, Massey University and former Bomac now Bayer Animal Health) a new intramammary controlled release delivery system for the treatment of bovine mastitis during the lactation period was developed.

EXPERIMENTAL METHODS

Oily cloxacillin (CLX) suspensions with tunable release were manufactured using ultra turrax equipment. Micronized sodium cloxacillin was supplied by Bayer Animal Health. Each formulation contained 400mg CLX free form as the sodium salt. The drug was suspended in a medium chain triglyceride among other excipients. Formulations were prepared aseptically for animal trials. Viscosities of oily suspensions were determined with a Haake rheometer using cap cylinder technique at 100rpm and 20 °C.

A modified USP 2 apparatus was used for drug release studies using 150ml buffered release media pH=6.8, paddle stirrer, stirring speed 100rpm, temperature 37 °C and 1g formulation. HPLC analysis (Column C18,150x 4.6 mm, 5μ; λ=230nm; mobile phase: phosphate buffer pH 6.4 : Methanol: ACN (55/36/9, v/v/v); sample solvent 20% ACN, 80% milliQ water) was carried out to determine CLX concentration in release media up to 3 hours. Each drug release experiment was carried out twice.

An approved (Kaiwhina Animal Ethics Committee) in-vivo pilot milk residue study was carried out using 6 healthy lactating quarters per formulation. The impact of the new controlled release mechanism on drug concentrations in milk was studied after intramammary infusion in healthy cows. The treatment regime was three doses at 24 hour intervals (3x24h). Six quarters per formulation (three front and three back quarters) were each administered with a 5g syringe containing 400mg CLX free form as sodium salt. Quarter milking was executed to collect samples at normal milking times (12/12). Inhibitory

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substance concentrations in milk were determined by a commercial analytical laboratory and reported as IS/ml penicillin G equivalents and then converted to CLX concentrations.

All animals underwent a general health inspection by a qualified veterinarian and were confirmed as clinically healthy prior to the commencement of the study. Further inclusion criteria were: producing at least 10 L milk per day, four functional quarters, not treated with an anti-microbial within 14 days of the commencement of the study and somatic cell counts (SCC) at cow-level not greater than 250,000 cells/mL at pre-screening on farm.

RESULTS AND DISCUSSION
Mean CLX release from a novel intramammary delivery system are shown in Figure 1. Drug releases after 3 hours were between ~5% and ~50%. CR3 showed almost identical release as the existing product Orbenin® LA (200mg CLX free form as sodium) although the viscosity is about 10-fold lower. The viscosities of CR1 to CR 3 vary only slightly but the release profiles were very different.

Figure 1: Mean CLX release for novel controlled release formulations (CR1, CR2 & CR3) and Orbenin® LA. (n=2)

Figure 2 shows mean CLX concentrations in raw milk for two formulations (CR1 and CR2) after three intramammary infusions at -48h, -24hours and 0 hours. Peak drug concentrations were similar for both formulations and on average about 100mg/L. Milk levels for both formulations were above the minimum inhibitory concentration MIC₉₀ (Staph aureus and Strep uberis both 0.5mg/L) during the treatment interval. While the CLX concentration for CR1 decreased relatively quickly to about 3mg/L at 24hours after infusion, CR2 formulation maintained CLX concentration at higher level of about 40mg/L. Consequently, the withholding period (WHP) of both formulations differed: WHPₐ₋₆0h and WHPₐ₋₁₂₀h (WHP was determined when concentration regression curve cuts the maximum residue limit (MRL) for CLX (MRL-EU=0.03mg/L).

CONCLUSION
Traditionally, drug release from intramammary infusions is prolonged by increasing the viscosity of the product. A new controlled release mechanism has been developed to manipulate drug release in milk without significantly increasing the viscosity of the formulation. Thus delivery of antibiotic can be tuned to achieve optimal efficacy and reasonable withholding times.

REFERENCES

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