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Gamithromycin plasma and skin pharmacokinetics in sheep

Michael Kellermann [a,*], Rose A. Huang [b], Andrew B. Forbes [c,1], Steffen Rehbein [a]

[a] Merial GmbH, Kathrinenhof Research Center, Walchenseestr. 8-12, 83101 Rohrdorf, Germany
[b] Merial Limited, PKDM, North Brunswick, NJ 08902, USA
[c] Merial S.A.S., 29 Avenue Tony Garnier, 69007 Lyon, France

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ABSTRACT

This study assessed the plasma kinetics and skin/plasma concentration ratio of the azalide antibiotic gamithromycin (ZACTRAN®, Merial) in sheep after a single subcutaneous administration at 6 mg/kg bodyweight. Gamithromycin concentrations in plasma samples collected at various intervals up to 21 days following treatment and metacarpal skin obtained from animals at two, five and ten days after treatment were determined by liquid chromatography–tandem mass spectrometry methods.

After administration, gamithromycin was rapidly absorbed, and individual maximum plasma concentrations were observed within 6 hours post-dose. Plasma peak concentration was 573 ± 168 ng/ml. The mean area under the plasma concentration versus time curve extrapolated to infinity was 8.00 ± 1.41 μg · hr/ml, and the mean terminal half-life was 34.5 ± 5.4 hours. Gamithromycin skin concentrations were much higher than the plasma concentrations resulting in skin/plasma concentration ratios of approximately 21, 58, and 138 at two, five and ten days post-dose, respectively, demonstrating extensive distribution to skin tissue.

1. Introduction

Gamithromycin is a novel azalide which belongs to the 15-membered semisynthetic macrolide antibiotics of the azalide subclass with a uniquely positioned alkylated nitrogen atom at the 7a-position of the lactone ring. As a characteristic for macrolide antibiotics in general, and even more pronounced in the azalide subclass, high tissue concentrations for extended periods of time including significant accumulation in phagocytic cells are achieved compared to relatively low plasma levels (Amsden, 2001; Bryskier and Bergogne-Berezin, 1999; Jain and Danziger, 2004). Gamithromycin shares the pharmacokinetic and pharmacodynamic properties of the azalide antibiotics (Giguère et al., 2011; Huang et al., 2010) and was therefore developed as a 15% w/v injectable solution (ZACTRAN®, Merial) for the treatment and prevention of bovine respiratory disease associated with a range of bacterial pathogens including *Mannheimia haemolytica*, *Pasteurella multocida*, *Mycoplasma bovis* and/or *Histophilus somni* (Baggott et al., 2011; Forbes et al., 2011; Lechtenberg et al., 2011a, 2011b, 2011c, 2011d; Sgoifo Rossi et al., 2010; Sifferman et al., 2011).

The inherent properties of gamithromycin lead to large volumes of distribution, wide penetration to and rapid accumulation in tissues, and low clearance, causing a prolonged effect following a single dose. This makes gamithromycin a candidate for use in conditions where repeated administration of antibiotics with frequent handling of animals is needed to achieve a desired effect. One of these conditions may be footrot, a contagious disease with worldwide occurrence in small ruminants. In sheep, the disease causes severe, painful lameness frequently affecting a considerable portion of a flock and leads to compromised welfare, loss of production and consequent economic loss. There are several bacterial pathogens implicated in the etiopathogenesis of ovine infectious lameness. *Dichelobacter nodosus* is considered as the main causative agent of footrot (Green and George, 2008; Kennan et al., 2011; Winter, 2008). Currently, there are few antibiotics licensed to treat sheep footrot. Although currently not licensed in sheep, several case reports and studies were published recently from different countries in Europe documenting the therapeutic and metaphylactic efficacy of gamithromycin in sheep suffering from typical or atypical footrot (Angen et al., 2012; Cederlöf and Hansen, 2012; Forbes et al., 2014; Friese, 2012; Sargison and Scott, 2011; Stamphøj, 2012; Strobel et al., 2012, 2014).

The study reported here was conducted to determine the pharmacokinetic profile of gamithromycin following subcutaneous injection to sheep and to compare plasma and metacarpal skin tissue gamithromycin concentrations, as the causative agents of ovine

* Corresponding author. Tel.: +49 8032 70750; fax: +49 8032 707525.
E-mail address: michael.kellermann@merial.com (M. Kellermann).
[1] Dr. A.B. Forbes was a full-time employee of Merial when the study was conducted.

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lameness colonize the skin and soft tissue between and close to the hooves of the sheep.

2. Materials and methods

Seventeen healthy, ruminating Merino sheep (8 intact male, 9 female) were included in the study. The sheep were acclimated to the study facility for eight days and were approximately 5–6 months old and weighed 27.8–38.8 kg the day before treatment (Day −1). No animal had been treated with any antibiotic within four weeks prior to study start.

Animals were handled with consideration for their welfare and in compliance with Merial Institutional Animal Care and Use Committee approvals and applicable local regulations.

2.1. Study design

On Day −1, two animals (one animal per sex) were randomly selected from the 17 animals to form an untreated control group. The remaining 15 animals were treated on Day 0 with a single subcutaneous injection of gamithromycin 15% w/v injection commercially available for cattle (ZACTRAN®, Merial) at a dose of 1 ml/25 kg bodyweight (6 mg gamithromycin/kg bodyweight) at the right site of the neck. Treatment was administered using sterile disposable syringes with 0.1 ml graduations so that the actual amount of gamithromycin delivered ranged from 6.02 to 6.47 mg/kg bodyweight. Blood samples for plasma separation were collected from the jugular vein into lithium heparinized tubes prior to treatment (Day −1) from all animals. After treatment, blood was collected from the treated sheep at approximately 5, 10, 15 and 30 minutes and 1, 2, 3, 6 and 10 hrs post treatment, and on Days 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, and 21. Untreated control animals were sampled only on Days 1 and 2. Plasma was stored in aliquots at ≤−20 °C until assayed for gamithromycin concentration.

Immediately after blood collection, three animals were selected randomly from the treated group and humanely euthanized on each of Days 2, 5 and 10; in addition, untreated control animals were humanely euthanized on Day 2. After humane euthanasia, skin of the metacarpus region was collected with the hair clipped, and stored frozen at <20 °C for later analysis. Before assay, metacarpal skin from both front legs of each animal was combined and ground with the addition of liquid nitrogen using a tissue processor ('freezer mill'), and the ground tissue was thoroughly mixed.

2.2. Analysis of gamithromycin concentrations in plasma and skin tissue

For each matrix, standard curve and quality control samples were fortified with serial concentrations of working standard solutions. Plasma and skin tissue were analyzed using liquid chromatography–tandem mass spectrometry (LC–MS/MS) methods as described previously for the analysis of bovine plasma and tissue (Huang et al., 2010). Deuterated gamithromycin was used as internal standard for all assays. Briefly, samples were mixed with 0.1 M potassium monobasic phosphate buffer followed by centrifugation at approximately 1300 × *g* for five minutes. The resulting supernatant was loaded into a preconditioned 96-well 30 mg solid phase extraction plate for plasma (Oasis® MCX, Waters Corp., Milford, Massachusetts) or 150 mg of solid phase extraction cartridges for skin tissue samples (Oasis® MCX, Waters Corp., Milford, Massachusetts). The eluted samples were analyzed by a Waters Alliance® HT 2795 coupled with a Waters Micromass® Quattro Micro™ (Micromass UK Ltd, Wythenshawe, Manchester, UK) LC–MS/MS system with electrospray ionization in positive ion mode.

The standard curve for plasma analysis ranged from 2.0 to 1000 ng/ml. The lower limit of quantification (LOQ) for gamithromycin in plasma was 2.0 ng/ml, and limit of detection (LOD) was 1.0 ng/ml. The standard curve for skin tissue concentrations ranged from 10 to 3000 ng/ml (equivalent to 10–3000 ng/g). The LOQ for gamithromycin in skin tissue was established as 10 ng/g.

No detectable gamithromycin was found in any sample from untreated control animals, unfortified control plasma samples and zero standard solutions without fortification of deuterated internal standard, demonstrating specificity and absence of interference from the matrices.

2.3. Pharmacokinetic analysis

Plasma pharmacokinetic parameters from the treated animals were determined using a non-compartmental model. The calculations were performed using WINONLIN® software version 5.0.1 (Pharsight Corp, St. Louis, MO, USA). Values below the LOQ were not included in the calculation. The peak concentration (C_{max}), time to peak concentration (T_{max}), last quantifiable concentration (C_{last}), and time to the last quantifiable concentration (T_{last}) were obtained directly from the plasma concentration data. The first order rate constant, λ_z, associated with the terminal log-linear position of the curve were calculated via linear regression of the log plasma concentration versus time curve. The terminal plasma half-life ($t_{½}$) was calculated as $\ln(2)/\lambda_z$. The area under the plasma concentration versus time curve from time 0 to the last quantifiable time point (AUC_{last}) was calculated using the linear up, log down trapezoidal method from the time of dosing to T_{last} and was also extrapolated to infinity (AUC_{inf}). Clearance (CL/F) was calculated as Dose/AUC_{inf} and the volume of distribution (Vd/F) was calculated as the mean residence time to infinity divided by the clearance. The pharmacokinetic parameters were calculated for each animal and then averaged with the standard deviation presented.

The average gamithromycin concentrations of plasma and metacarpal skin tissue were compared on each of Days 2, 5 and 10 to estimate the gamithromycin skin/plasma concentration ratio.

3. Results

Throughout the duration of the study, all sheep were clinically normal and no adverse events were observed.

The analytical methods used for plasma and tissue analysis performed well during the analytical phase of the study. Assay of quality control samples demonstrated an average gamithromycin recovery in plasma of 103% for the fortification levels of 900 ng/ml, 300 ng/ml and 5.0 ng/ml (n = 33; range, 86.6–115%; %RSD, 7.11) and gamithromycin recoveries in skin tissue of 96.1% and 108%, respectively, for the fortification levels of 2500 ng/g and 25 ng/g. For both matrices, the coefficient of determination was always ≥0.99.

The mean plasma concentration versus time curve of gamithromycin following subcutaneous injection is plotted in Fig. 1A, while the absorption phase over the first 10 hours is magnified in Fig. 1B. Gamithromycin concentrations in the plasma of all treated animals at the first sampling time of five minutes post treatment were much higher than the quantifiable level (219 ± 99.3 ng/ml); at Day 9, the lowest quantifiable plasma concentration was only found in one animal while the remaining eight animals had plasma levels below the LOQ.

The pharmacokinetic parameters are summarized in Table 1. Following a single subcutaneous injection of 6 mg/kg bodyweight, using a non-compartmental model, the mean AUC_{inf} was 8.00 ± 1.41 μg · hr/ml, and the mean $t_{½}$ was 34.5 ± 5.4 hours. The AUC_{inf} were captured in this experiment with an average of 2.16% extrapolated (range, 1.13–4.58%). The C_{max} was 573 ± 168 ng/ml ranging from 355 ng/ml to 910 ng/ml for the individual animals. The T_{max} ranged from 10 minutes (0.167 hours) to 6 hours with a median of 15 minutes (0.25 hours) with an average T_{max} of 0.912 hours and a