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Effect of tulathromycin on abomasal emptying rate in healthy lactating goats

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ABSTRACT

Tulathromycin is a long-acting semi-synthetic macrolide antibiotic that is synthesized from erythromycin. Macrolides have pharmacodynamic properties beyond their antimicrobial effects, including anti-inflammatory and immunomodulatory properties that are perceived to be clinically beneficial. An additional pharmacodynamic property of macrolides is a prokinetic effect, which is marked in adult cattle and calves administered erythromycin and less prominent in calves administered spiramycin, tilmicosin, and tylosin. Based on structural similarities to erythromycin, the hypothesis was that parenteral administration of tulathromycin would increase abomasal emptying rate in healthy adult goats. Accordingly, five adult lactating goats (30–36 months of age) received each of the following 3 treatments: IM injection of 2 mL of 0.9% NaCl (control); IM injection of tulathromycin (2.5 mg/kg body weight); IV injection of tulathromycin (2.5 mg/kg body weight). Abomasal emptying rate was assessed by acetaminophen absorption, which was injected into the abomasum through a surgically placed abomasal cannula at 50 mg/kg BW, 15 min after each treatment. Jugular venous blood samples were obtained periodically after injection and plasma acetaminophen concentrations determined using a colorimetric nitration assay. The maximum observed plasma acetaminophen concentration (Actual C_{max}) and time of actual C_{max} (Actual T_{max}) were determined, and pharmacokinetic modeling was used to calculate model C_{max} and model T_{max} and abomasal emptying half-time (T50). Results showed that tulathromycin (IM and IV) increased abomasal emptying rate, as indicated by a shorter time to actual T_{max} and model T_{max}, and shorter T50, than control. The clinical relevance of these findings remains to be determined.

1. Introduction

Macrolides are a group of chemically related compounds whose antimicrobial activity stems from the presence of a large macrocyclic lactone ring to which one or more deoxy sugars, usually desosamine and cladinose, are attached (Giguère et al., 2006). The original member of the group, erythromycin was isolated from the soil borne bacteria *Streptomyces erythreus* in 1952. Other macrolides are derived from related bacteria (e.g., tylosin) or by chemical modification of the original compounds (e.g., tulathromycin) using fermentation under optimized conditions followed by organic synthesis. Macrolides are categorized according to the number of macrocyclic lactone ring components as 12-membered, 13-membered,

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14-membered, 15-membered, and 16-membered groups (Giguère et al., 2006). Tulathromycin is a novel long-acting semi-synthetic macrolide antibiotic of the triamilide group that has been available since 2004 for use in veterinary medicine. Tulathromycin is an equilibrated mixture of a 15-membered (90%, isomer A) and 13-membered (10%, isomer B) macrocyclic ring with a unique chemical structure consisting of 3 polar amine groups (European Medicines Agency, 2002; Evans, 2005). Tulathromycin is a broad-spectrum antimicrobial, with in vitro activity against certain gram-negative and gram-positive bacteria, including the bacterial pathogens most commonly associated with bovine and swine respiratory disease (Norcia et al., 2004).

Macrolides have pharmacodynamic properties beyond their antimicrobial effects, including anti-inflammatory and immunomodulatory properties that are perceived to be clinically beneficial (Giguère et al., 2006; Buret, 2010; Fischer et al., 2011). An additional pharmacodynamic property of macrolides is a prokinetic effect, which is marked in adult cattle and calves administered erythromycin (Wittek and Constable, 2005; Nouri and Constable, 2007; Wittek et al., 2008a,b) and less prominent in calves administered spiramycin (Rashnavadi et al., in press), tilmicosin (Nouri and Constable, 2007), and tylosin (Nouri and Constable, 2007). Based on structural similarities to erythromycin, particularly the presence of an amino-sugar at C-5 of the macrocyclic lactone ring, we hypothesized that parenteral administration of tulathromycin would increase abomasal emptying rate in healthy adult goats. Preliminary support for this hypothesis was provided by a recent study in milk-fed calves that demonstrated tulathromycin exerted a weak prokinetic effect (Rashnavadi et al., in press). We investigated our hypothesis using healthy adult goats that had a surgically placed abomasal cannula.

2. Materials and methods

2.1. Animals

Five adult lactating Zaraibi (Egyptian Nubian) goats weighing 20–22 kg and aged from 30 to 36 months were obtained from local farms. Goats were housed together at Cairo University in one large indoor stall and fed fresh cut Berseem clover (*Trifolium alexandrinum*, also known as Egyptian clover) and ad libitum concentrate mixture of corn (75%), barley (15%), and molasses (10%) with free access to food and water. Goats were milked by hand once a day. The health of all goats was monitored before and throughout the experimental period by periodic physical examination and monitoring of feed consumption and fecal characteristics.

2.2. Drugs

Tulathromycin (DRAXXIN®, Pfizer, Animal Health Division, Cairo, Egypt) was obtained as a ready to use, sterile, aqueous, colorless solution in 20 mL vials. Each milliliter of tulathromycin contained 100 mg of tulathromycin as free base in 50% propylene glycol vehicle, monothioglycesol (5 mg/mL), and citric acids and hydrochloric acids added to adjust pH.

Acetaminophen (paracetamol; Zhejiang Kangle Pharmaceutical Co., Ltd., Zhejiang China) was obtained as a white, fine powder that was sparingly soluble in water.

2.3. Experimental design

Goats had been used in a study to characterize the pharmacokinetics of tulathromycin (Amer et al., 2012) and subsequently had an abomasal cannula surgically placed as described (Ahmed et al., 2005). Goats were housed for four weeks after surgery to permit recovery. Food, but not water, was removed overnight for 12 h and injections were administered in the morning. Each goat received a single IM injection of 1 mL 0.9% NaCl (negative control). One week later, goats received an IV injection of tulathromycin (2.5 mg/kg BW) into the right jugular vein using a needle and syringe after clipping the venipuncture site and scrubbing with tincture of iodine (2.5%). Six weeks later goats received an IM injection of tulathromycin (2.5 mg/kg BW) into the semimembranosus muscle; the duration between tulathromycin injections was to ensure adequate clearance of tulathromycin, based on an estimated mean half-life after IV injection of 4.1 days in these goats (Amer et al., 2012) and 2.5–4.6 days after SC injection in juvenile and market aged goats (Clothier et al., 2011; Young et al., 2011; Romanet et al., 2012). A wash out period of a week was considered adequate for complete clearance of acetaminophen, based on pharmacokinetic studies in cattle (Grochowina and Janus, 2007; Ehsani-Kheradgerdi et al., 2011) and sheep (Sharifi et al., 2009).

Acetaminophen (50 mg/kg body weight in 10 mL of 0.9% NaCl solution containing 1 drop of 99% absolute methanol to enhance solubility) was injected into the abomasum through the abomasal cannula at a dose of 15 min after injection of tulathromycin or negative control. Plasma tulathromycin concentrations were near their maximal value at this time after IM administration (Amer et al., 2012).

Venous blood samples (5 mL) were collected from the jugular vein of each goat immediately before treatment was administered (time = 0 min) and at 5, 10, 15, 20, 25, 30, 40, 50, 60, 90, 120, 150, 180, and 240 min after the start of injection into the abomasum. These sampling times were selected in an attempt to have at least 6 data points before and after the time of maximal acetaminophen concentration in order to facilitate nonlinear regression analysis for pharmacokinetic modeling. Blood samples were collected into 6 mL tubes containing sodium fluoride and potassium oxalate, centrifuged at 3000 × *g* for 15 min, and 2 mL of plasma harvested and stored at −20 °C until analysis for determination of acetaminophen concentration.

2.4. Sample analysis and pharmacokinetic modeling

Plasma was thawed at approximately at 25 °C and analyzed spectrophotometrically by use of a colorimetric nitration assay as described (Marshall et al., 2005). The maximum observed plasma concentration (Actual C_{max}) and time of maximum observed plasma concentration (Actual T_{max}) were obtained from a plot of the plasma acetaminophen concentration – time data. Changes in plasma acetaminophen concentrations over time were analyzed using commercially available software (SAS 9.2, SAS Inc., Cary NC; WinNonLin, Pharsight Corp., Cary NC). A 1-compartment model with first order elimination could not be fit to the plasma acetaminophen concentration–time data.

Noncompartmental analysis of an extravascular input was therefore applied using commercially available software (Model 200; WinNonLin, Pharsight Corp., Cary NC); this approach calculates the area under the curve using a linear trapezoid method to the last sample time and extrapolation to infinity as the ratio of last measured plasma concentration and the terminal slope of the plasma concentration versus time curve. Mean residence time was calculated from the noncompartmental analysis using standard equations.

Pharmacokinetic analysis was also performed using the first derivative of Siegel's modified power exponential formula, as previously described (Marshall et al., 2005). The equation was derived from the fact that the acetaminophen concentration-versus-time curve represented as a cumulative dose curve is an inverse analog of the scintigraphic curve with the following equation: $C(t) = m \times k \times \beta \times e^{-k \times t} \times (1 - e^{-k \times t})^{\beta - 1}$, where $C(t)$ is the acetaminophen concentration in plasma at a specified time point, t is time, m (units of {μg/mL} × min) is the area under acetaminophen concentration–time curve when time is infinite, k (units of min^{-1}) is an estimate of the rate constant for abomasal emptying, β is a constant that provides an estimate of the duration of the lag phase before an exponential rate of emptying is reached, and e is the natural logarithm. Nonlinear regression was used to estimate values for m, k, and β as described (Marshall et al., 2005). Values for model C_{max} and model T_{max} were obtained by fitting the estimated values for k, β, and m in the nonlinear equation to the cumulative dose curve equation for acetaminophen. The abomasal half emptying time (T50) was calculated by fitting the estimated values for k and β to the following equation: $T50 = (-1/k) \times \ln(1 - 2^{-1/\beta})$ (Marshall et al., 2005).