Pharmacokinetics of oxytetracycline and tulathromycin in plasma and semen of beef bulls

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Abstract

Objectives were to evaluate the pharmacokinetic parameters of tulathromycin and long-acting oxytetracycline in bulls' plasma and semen. In the first experiment, long-acting oxytetracycline (10 or 20 mg/kg) were given to bulls either subcutaneously or intramuscularly. In the second experiment, tulathromycin (2.5 mg/kg) was given subcutaneously. Liquid chromatography coupled with tandem mass spectrometry was used to measure antibiotic concentrations in plasma and semen samples. Plasma maximum concentration (C_{max}) for 10 mg/kg oxytetracycline was 2,841 ng/ml at 12 hours (T_{max}) with a half-life of 20.1 ± 5.9 hours. Semen C_{max} was 11,515 ng/ml at 24 hours (T_{max}) with a half-life of 23.7 ± 4.1 hours. For 20 mg/kg, the plasma C_{max} was 5,269 ng/ml at 12 hours (T_{max}) with a half-life of 18.1 ± 0.4 hours. Semen C_{max} was 55,040 ng/ml at 24 hours (T_{max}) with a half-life of 15.7 ± 1.2 hours. Plasma C_{max} of tulathromycin was 160 ng/ml at 21 ± 6 hours (T_{max}). Semen C_{max} was 1,539 ng/ml at 33.0 ± 18.0 hours (T_{max}). The C_{max} between plasma and semen was different ($p = 0.008$). Plasma terminal half-life (81.4 ± 27.6 hours) of tulathromycin had a tendency ($p = 0.10$) to be shorter than semen (114.7 ± 21.7). It was concluded that for genital infections of bulls either tulathromycin or long-acting oxytetracycline are adequate antibiotics based on their pharmacokinetic properties.

Keywords: Ruminants, abortion, diseases, pregnancy loss

Introduction

In bulls, antibiotics are used for numerous reproductive clinical disorders.[1-3] Seminal adenitis syndrome represents 1 of the most frequent reproductive diseases in young and old bulls[3,4] and is usually treated with antibiotics given locally or systemically.[3,5,6] Antibiotic selection for this clinical condition and other genital infections (e.g. orchitis) is based on personal experience, anecdotal evidence, extrapolation from other species, or based on the results of microbiological culture and sensitivity tests. The chosen antibiotic has to be used at the correct dose, route, and frequency for an acceptable period.[7]

Most of the information regarding pharmacokinetics of antibiotics in the male genital tract is derived from human and dog models.[8-10] However, the anatomy and physiology of these 2 species are different from ruminants.[11]

All tetracyclines are equally active and typically have similar broad spectrum for both aerobic and anaerobic gram-positive and gram-negative bacteria, mycoplasmas, *Rickettsia*, *Chlamydia*, and even some protozoa, e.g. amoeba.[12] Oxytetracycline povidone is a broad-spectrum antibiotic with a long half-life and is used for wide range of diseases. Tetracyclines inhibits binding of the bacterial 30S ribosomal subunit, specifically at the aminoacyl-tRNA acceptor («A») site on the mRNA ribosomal complex, thus preventing ribosomal translation.[12]

Tulathromycin is a macrolide triamilide antibiotic approved for the treatment and prevention of respiratory diseases in cattle, pigs, and other animals.[13,14] Like other macrolides, it binds to the 50S subunit of bacterial ribosomes and consequently inhibits protein synthesis, leading to inhibition of cell division and cell death. Tulathromycin's spectrum of activity includes gram-negative,

gram-positive, and Mycoplasma microorganisms.[13,15] In cattle, tulathromycin presents unique pharmacokinetic characteristics such as rapid absorption from the injection site, extensive tissue and high-volume distribution, elevated and sustained drug concentration in lungs, and slow elimination.[13]

Although oxytetracycline and tulathromycin are used in bulls for genital infections, their pharmacokinetics in bulls' semen has not been investigated. The objective was to investigate the pharmacokinetics of oxytetracycline and tulathromycin in plasma and semen after a single parenteral route treatment in beef bulls.

Materials and methods

The study was approved by the University of Bologna animal welfare committee and conducted at the university national institute of artificial insemination.

Oxytetracycline

Animals

Four Simmental bulls were randomly selected and subjected to physical and breeding soundness examinations.[16] Average age was 15.3 ± 0.3 months (range; 15 - 16 months), weight was 648.9 ± 25.9 kg (range; 645 - 680 kg), and body condition score17 was 6.0 ± 0.4 (range; 5.5 - 6.5). Bulls were maintained in individual pens and received a ration of corn silage, mixed hay, and alfalfa with water ad libitum. Additionally, each bull received once a day 2.5 kg of concentrate (pellets with 14% crude protein).

Experimental design

Bulls had no history of oxytetracycline treatment. A single dose (10 or 20 mg/kg) of long-acting oxytetracycline (Terramicina, Zoetis Italy, Rome) was given. Two bulls received 10 mg/kg and 2 bulls received 20 mg/kg, either subcutaneously or intramuscularly on the right-side neck. Each bull received < 10 ml per injection site. Plasma and semen samples were collected at 0, 12, 24, 36, 48, 72, and 96 hours after treatment. Blood (10 ml) was collected from caudal vessels in vacuum tubes containing lithium heparin. Semen was collected via electroejaculation using an automatic electroejaculator (60 mm 2 electrode; Pulsator V, Lane Manufacturing, Denver, CO). Samples were immediately refrigerated, centrifuged (600 × g for 30 minutes) within 1 hour after collection, and stored at - 80°C.

Oxytetracycline analysis

Oxytetracycline concentrations in plasma and semen were measured[18] using liquid chromatography coupled with tandem mass spectrometry.

Tulathromycin

Animals

Four Simmental bulls were randomly selected and subjected to a physical examination and a breeding soundness examination.16 Average age was 15 months ± 0.2 months (range; 15 - 16 months), average weight was 639.3 ± 32.9 kg (604 - 681 kg), and average body condition score17 was 6.1 ± 0.5 (5.5 - 6.5). Bulls were maintained in individual pens and received a ration of corn silage, mixed hay, and alfalfa with water ad libitum. Additionally, each bull received once a day 2.5 kg of concentrate (pellets with 14% crude protein).

Experimental design

Bulls had no history of tulathromycin treatment. A single dose (2.5 mg/kg) of tulathromycin (Draxxin, Zoetis Italy, Rome) was given subcutaneously either at left ear base (n = 2) or on the left side neck (n = 2). Plasma and semen were collected at 0, 12, 24, 48, 72, 96, 144, 192, and 240 hours after treatment, as described above for oxytetracycline treatment.

Tulathromycin analysis

Liquid chromatography coupled with tandem mass spectrometry was used to measure[19] tulathromycin concentrations in plasma and semen.

Pharmacokinetic parameters

Noncompartmental analysis was performed to estimate the pharmacokinetic parameters in plasma and semen for each individual animal. A standard software, PK-Solver add-in for Excel,[20] was used to estimate the pharmacokinetic parameters. Following variables were calculated for plasma and semen of each animal: time of peak drug concentration (T_{max}), peak drug concentration (C_{max}), apparent elimination half-life (t1/2), calculated as ln (2)/λz, λz being the first order rate constant associated with the terminal portion of the time-concentration curve as estimated by linear regression of time versus log concentration, and area under the time-concentration curve from time zero to the last observed concentration ($AUC_{0\text{-last}}$), calculated by the linear trapezoidal rule.

Data analyses

Statistical software[21] was used to determine parameters (mean, standard deviation, and range). A paired Student's t-test and a software program (PK-Solver) for pharmacokinetics parameters[20] were used. An alpha error of 5% was used to accept the alternative hypothesis.

Results

Oxytetracycline

All bulls had a mild swelling at the injection site. Plasma

oxytetracycline concentrations in bulls treated with 10 mg/kg of oxytetracycline either subcutaneously (1,470 ± 1,090 ng/ml) or intramuscularly (1,330 ± 990 ng/ml) were not different (p = 0.82). Similarly, mean semen concentrations (5,710 ± 4,640 ng/ml and 5,390 ± 3,160 ng/ml) were not different (p = 0.88).

Plasma oxytetracycline concentrations in bulls treated with 20 mg/kg of oxytetracycline either subcutaneously (2,540 ± 1,970 ng/ml) or intramuscularly (2,590 ± 2,030 ng/ml) were not different (p = 0.96). Similarly, mean semen concentrations (25,600 ± 22,900 ng/ml and 19,400 ± 17,200 ng/ml) were not different (p = 0.58). Because there were no significant differences for the 2 sites of treatment, the means for plasma and semen were analyzed together.

Plasma and semen concentrations for the 10 mg/kg dose (1,400 ± 990 and 6,480 ± 3,520 ng/ml) and 20 mg/kg dose (2,570 ± 1,910 and 26,200 ± 18,700 ng/ml) were different (p = 0.001 and p = 0.001).

Oxytetracycline doses of 10 mg/kg and 20 mg/kg resulted in different (p = 0.07) mean plasma concentrations (1,400 ± 990 and 2,570 ± 1,910 ng/ml) and in different (p = 0.004) mean semen concentrations (6,480 ± 3,520 ng/ml and 26,200 ± 18,700 ng/ml).

Oxytetracycline plasma concentrations above 1,000 ng/ml persisted (p = 0.001) for 48 hours for the 10 mg/kg dose compared to 66 hours for the 20 mg/kg dose. Oxytetracycline semen concentrations above 1,000 ng/ml remained elevated for over 96 hours for both the 10 and 20 mg/kg dose and remained above that threshold longer (p = 0.0001) compared with plasma levels (96 versus 57 hours). At 96 hours, the oxytetracycline mean semen concentrations for 10 mg/kg dose were 1,700 ± 260 ng/ml and for the 20 mg/kg dose were 3,340 ± 260 ng/ml.

For the 10 mg/kg dose, the plasma C_{max} was 2,841 ± 401 ng/ml at 12 hours (T_{max}) with a half-life of 20.1 ± 5.9 hours. The plasma AUC0-last was 112,560 ± 8,067 ng/ml/hour. The semen C_{max} was 11,515 ± 2,445 ng/ml at 24 hours (T_{max}) with a half-life of 23.7 ± 4.1 hours. The semen AUC0-last was 550,387 ± 13,081 ng/ml/hour.

For the 20 mg/ml dose, the plasma C_{max} of 5,269 ± 111 ng/ml was achieved at 12 hours (T_{max}) with a half-life of 18.1 ± 0.4 hours. The semen Cmax was 55,040 ± 10,605 ng/ml at 24 hours (T_{max}) with a half-life of 15.7 ± 1.2 hours. The semen AUC0-last was 2,153,942 ± 384,669 ng/ml/hour.

Tulathromycin

Mean plasma concentrations of tulathromycin for 2 subcutaneous injection sites (77.9 ± 43.3 and 73.7 ± 39.7 ng/ml) were not different (p = 0.84). Mean semen concentrations of tulathromycin (608 ± 374 ng/ml and 867 ± 599 ng/ml) were also not different (p = 0.29). In the absence of significant differences between the 2 injection sites, means for plasma and semen were analyzed together. Mean plasma tulathromycin was lower (p = 0.001) than semen (75.8 ± 40.2 and 781 ± 482 ng/ml).

The plasma C_{max} was 160 ± 26 ng/ml at 21.0 ± 6.0 hours (T_{max}). The semen C_{max} was 1,539 ± 444 ng/ml at 33.00 ± 18.00 hours (T_{max}). The C_{max} between plasma and semen was different (p = 0.008) with no differences (p = 0.35) in T_{max} between plasma and semen. The terminal half-life for plasma (81.4 ± 27.6 hours) had a tendency (p = 0.10) to be shorter than in semen (114.7 ± 21.7 hours). The plasma AUC_{0-last} (15,440 ± 1,717 ng/ml/hour) was lower (p = 0.01) compared to semen (171,071 ± 58,556 ng/ml/hour).

Discussion

Oxytetracycline

Oxytetracycline treatment has been reported to produce local irritation and extensive tissue damage.[22] Therefore, subcutaneous treatment may be a better option than intramuscular. Nevertheless, pharmacokinetics of the drug should be considered before an alternative route of treatment is recommended. Similar to reports[23-27] for 5, 10, or 20% oxytetracycline formulations, mean plasma or semen concentrations for both doses of oxytetracycline were not different for 2 routes of treatment. The area around the injection site was larger in the subcutaneous route than in the intramuscular route. Inflammation after subcutaneous treatment could have contributed to more absorption of oxytetracycline, resulting in similar pharmacokinetics.

Plasma C_{max} at 12 hours (T_{max}) for 10 mg/kg was lower than reported.[28] First blood sample collected at 12 hours (plasma oxytetracycline concentration was already in the descending phase) in the present study could explain the difference. Plasma C_{max} for 20 mg/kg was lower than reported[25,29,30] that also had different T_{max} (4.7 - 6.2,[25] 6,[29] and 3.9 hours[30]). First blood sample collected at 12 hours in the present study could explain the difference. Plasma $AUC_{0\text{-last}}$ for the 20 mg/kg dose was in agreement with a previous report.[25]

To the authors' knowledge, this is the first study that determined pharmacokinetics of long-acting oxytetracycline in bull semen for 2 doses. Successful treatment requires selection of an antibiotic based on the microbiological results and pharmacokinetic properties. Higher concentrations in semen than plasma were attributed to a lipophilic drug with a high volume of distribution and high tissue concentrations.[30] When bacteriostatic antibiotics such as long-acting oxytetracycline are used, the plasma or semen concentrations should not decrease below the effective minimum inhibitory concentrations during treatment. Therefore, the selection of dosing intervals and the desired minimum semen concentrations require basic pharmacokinetic information as a guideline. In general, a serum concentration between 500 and 1,000 ng/ml has been suggested as the effective therapeutic level. The minimum inhibitory concentrations for

tetracyclines against most susceptible pathogenic microorganisms in cattle (*Bacillus anthracis*, *Mycoplasma* spp., *Pasteurella* spp., *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Streptococcus pneumoniae*) ranged from 120 - 1,000 ng/ml.[31]

Tulathromycin

Only a mild swelling at the site of injection was detected, especially at the base of the ear, that had disappeared at 5 days after treatment.

The average plasma C_{max} (160 ng/ml) was lower than reported (277 ng/ml,[32] 300 ng/ml,[33] and 500 ng/ml).[13,15] One possible explanation for this difference could be due to the timing (12 hours after treatment) of first blood sample, a time the plasma concentrations of tulathromycin must have been already in a descending phase. This is supported by 2 reasons. First, in those studies, it was determined at 0.25 hour,[34] < 1 hour,[13] and 0.7 hour;[33] second, 24 - 240 hours concentrations were similar to reported.[13,15]

The terminal half-life of 81.4 ± 27. 6 hours (range; 71 - 96 hours) was in agreement with earlier reports.[13,15,32,33]

The plasma $AUC_{0\text{-last}}$ in the present study (18,382 ng/ml/hour) was comparable to earlier reports[13,34] (17,885 and 16,700 ng/ml/hour) but higher than others.[15,32,33,35]

The efficacy of any antimicrobial is determined by both its pharmacokinetic and pharmacodynamic properties. Antibiotics have been classified into 2 major groups: those with bacteriostatic antimicrobial action that exhibit time-dependent killing action or those with bactericidal antimicrobial action that behave with either time-dependent or concentration-dependent killing, or both.[15] Tulathromycin has both bacteriostatic antimicrobial and bactericidal antimicrobial time-dependent action.[13]

To the authors' knowledge, this is the first study that determined pharmacokinetics of tulathromycin in bull semen using a standard recommended dose for cattle. Semen C_{max} of tulathromycin was higher than plasma with a tendency of longer half-life compared to plasma. Apparently, tulathromycin elimination from male's genital tract was slower, probably because of delayed exposure in the organs of elimination, and this can be considered an advantage for male reproductive treatments. Furthermore, the semen $AUC_{0\text{-last}}$ was higher than plasma.

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Conflict of interest

All authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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