The 15-membered ring macrolide tulathromycin inhibits plasma endotoxin activity in endotoxemic calves

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Introduction

Macrolide antibiotics have not only bacteriostatic effects, but also anti-inflammatory effects via the inhibition of cytokine release. The 15-membered ring macrolide tulathromycin is widely used for cattle with inflammatory pneumonia. However, its anti-inflammatory activity, especially against endotoxin, remains unclear. The purpose of this study was to investigate whether tulathromycin-containing plasma inhibited endotoxin (ETX) activity *in vivo* and *in vitro*.

Materials and Methods

This was an in vitro and *in vivo* study. Ten healthy calves aged 30.9±5.3 days and weighing 90.2 ± 13.0 lb (40.9 ± 5.9 kg) were enrolled in the *in vivo* study. Calves received 1 dose of saline (0.011 mL/lb [0.025 ml/kg], IM, n=5) or tulathromycin (1.13 mg/lb [2.5 mg/kg], IM, Draxxin, Zoetis) 4 times every 3 days to maintain the blood concentrations. For the *in vivo* study, calves received 2.5 μg/kg of O111:B4 lipopolysaccharide (LPS, L2630, SIGMA) on the day when the tulathromycin concentration in the blood stabilized via an indwelling catheter in the jugular vein. The time of ETX challenge was defined as t = 0. At immediately before (pre), and t = 0.5, 1, 2, 3, 4, 8, 12, and 24 hours after ETX challenge, blood samples were taken from the opposite jugular vein. Plasma was harvested by centrifugation for 15 minutes at 3000 rpm at room temperature and stored at -22°F (-30°C) until assay. Plasma samples were diluted 20-times and heated at 176°F (80°C) for 10 minutes as preprocessing. Then, the ETX activity was measured using the limulus amebocyte lysate kinetic turbidimetric assay (LAL-KTA, Endosafe KTA2, Charles River). For the *in vitro* study, plasma was obtained from 6 healthy calves before and after the tulathromycin concentration in the blood stabilized (151.5 ± 43.5 days of age and 275.8 ± 68.1 lb [125.1 ± 30.9 kg]). One hundred microliters of plasma was incubated at 98.6°F (37°C) at 0, 5, 10, 20, and 30 minutes after adding 16.6 μg of O111:B4-LPS. Plasma ETX activity was measured as described above. The data are shown as the means ± SD. Each dependent variable within the groups was compared with the baseline value using Dunnett's test after two-way ANOVA. Measured dependent variables were compared between groups for each sample collection period using the Tukey-Kramer test after two-way ANOVA.

Results

In the *in vivo* study, plasma ETX activity in the control and Draxxin groups peaked at 2.06 ± 0.73 and 0.93 ± 0.65 EU/mL, respectively, at t=30 min, which was significantly higher than 0.10±0.03 and 0.40±0.30 EU/ml at pre, respectively ($P<0.05$). The peak plasma ETX activity in the Draxxin group, in which the blood tulathromycin concentration was steady, was significantly lower than that in the control group ($P<0.05$). In the *in vitro* study, the ETX activity at t = 5 min in the control and Draxxin groups was 1042.3 ± 420.7, and 312.3 ± 38.9 EU/ml, respectively, which was significantly higher than 1.53±3.50 and 0.84±1.02 EU/ml at pre, respectively ($P<0.05$). Furthermore, the endotoxin activity value remained high until 30 minutes before the end of the study in both groups. The peak and AUC0-30 of the ETX activity in the plasma of the Draxxin group were significantly lower than those in the control group ($P<0.05$).

Significance

The mean elimination half-life (t1/2) of tulathromycin in plasma is 75.6 hours (3.15 days). Therefore, to stabilize the blood concentration, 2.5 mg of tulathromycin was intramuscularly administered to calves 4 times in 3-day intervals. In the present study, ETX activity in tulathromycin-containing plasma was significant lower than that in the control group in both the *in vivo* and *in vitro* studies. Our results suggest that 15-membered ring macrolide antibiotics have efficient bacteriostatic activity and can inhibit endotoxin activity in cattle. Therefore, prophylactic administration of tulathromycin may reduce the severity of symptoms even if cattle develop endotoxin-associated inflammation.