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Development of assays and assessment of oral bioavailability of the NSAIDs ketoprofen, carprofen and flunixin in sheep

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Abstract

A sensitive and selective analytical method for the determination of ketoprofen, carprofen, flunixin and metabolite 5-hydroxyflunixin in ovine plasma was developed and validated. Sheep were randomised into 4 treatment groups, with a placebo group receiving saline and 3 groups given NSAIDs orally for 6 days. NSAIDs were given at approximately double the dose rate used for non-parenteral administration to cattle and previously used experimentally in sheep every 24 hours: carprofen (8.0 mg/kg), ketoprofen (8.0 mg/kg) and flunixin (4.0 mg/kg). Carprofen, ketoprofen and flunixin were all present in ovine plasma two hours after oral administration, with averages between 4.5 - 8.4µg/mL for ketoprofen, 2.6 - 4.1µg/mL for flunixin, 0.1 - 0.78µg/mL for 5-hydroxyflunixin and 30 - 80µg/mL for carprofen. NSAID concentrations dropped 24 h after administration. The three NSAIDs were bioavailable in sheep two hours after oral administration with carprofen and flunixin reaching inferred therapeutic concentrations in blood by this time.

Project objectives
Objectives:
1. Assess efficacy of ketoprofen, carprofen and flunixin following oral administration in a pain model in sheep.
2. Develop analytical methods for assessing blood concentrations of the 3 NSAIDs.

Success in achieving milestone
The objectives of the project were successfully achieved. Results from this project will assist with future experiments planned for the PhD project self-medication in sheep.

Materials and methods
Fifty Merino hogget ewes (average weight (28.5 kg ± 0.5)) were used in the study. Sheep were randomly allocated to a cohort balanced for weight, within each cohort they were randomly assigned to a pen and treatment group. There were 4 treatment groups: carprofen (8.0 mg/kg, PiaPharma, Chatswood West, NSW), ketoprofen (8.0 mg/kg, PiaPharma, Chatswood West, NSW), flunixin (4.0 mg/kg, PiaPharma, Chatswood West, NSW) and control (saline) group. All of the treatment groups were given their assigned NSAID as an oral solution and control sheep were given an oral saline solution, sheep were given their oral dose daily every morning throughout the six days of the experiment.

Blood was collected for analysis of drug concentrations. Samples were collected 10 minutes prior and 2 hours after each oral dosage of NSAID or saline solution in 10 ml heparin vacutainers using 20 gauge 25 mm needles. Samples were spun at 2000g for 15 minutes and the plasma transferred to tubes for storage at -20 ºC. They were then transported frozen to PiaPharma Pty Ltd, Chatswood West, NSW for analysis Ultra High Pressure Liquid Chromatography.

Results
Carprofen, ketoprofen and flunixin were all present in ovine plasma two hours after oral administration. Control animals receiving saline had less than the detectible limits of each drug at each time point (e.g. ketoprofen < 10ng/mL). The average carprofen concentration reported 2 hours after administration on the 6 days of administration ranged between 30 and 80 µg/mL. Average concentrations for flunixin were between 2.6 and 4.1 µg /mL and between 0.10 and 0.78 µg /mL for 5-hydroxyflunixin. The average ketoprofen concentrations were between 4.5 and 8.4 µg /mL 2 hours after administration. All 3 NSAID concentrations in plasma decreased 24 h after administration. Ketoprofen and flunixin had levels of <.1 µg /mL. The concentrations of carprofen in plasma increased over the testing period, with levels not dropping below the minimum 31.0 µg/mL seen at 0 h.

Discussion

As there is little information on therapeutic concentrations of these NSAIDs in sheep, interpretation and conclusions need to be drawn from comparisons in other species. Therapeutic levels of carprofen in plasma are 10-17µg/mL for dogs (Nolan and Reid, 1993), 7µg/mL for cats (Taylor et al., 1996) and above 1.5µg/mL for horses (Schatzmann et al., 1990; Lees et al., 1994). The average carprofen levels observed in the current study 2 hours after administration were 30-80 µg/mL. Minimum carprofen concentrations 24 h after each administration were between 31.0 - 45.9 µg/mL. These relatively high concentrations align with the reported long half life of carprofen in sheep (Welsh et al., 1992) and are likely to have contributed to the high concentration seen each day at the 2 hour time point after re-administration of the drug. Reported therapeutic levels for flunixin are 0.2-0.9 µg/mL in horses (Toutain et al., 1994). In other studies of oral administration, flunixin was found to have a peak concentration of 0.9 µg/mL at 3.5 hours (Odensvik, 1995). In the current study, plasma concentrations 2 hours after administration were 2.6 - 4.1µg/mL for flunixin and 0.1 - 0.78µg/mL for its bioactive metabolite 5-hydroxyflunixin. Ketoprofen levels were 4.5 - 8.4µg/mL in our sheep 2 hours after administration. In previous studies ketoprofen has been found to have a maximum concentration of 0.77 µg/mL for R(-) and 0.49µg/mL for its S(+) enantiomers (Landoni, 1999). The results suggest that oral administration of these NSAIDs at twice the standard parental dose used in other species led to concentrations that are likely to be within the therapeutic range.

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Appendices

See attached document titled “131222 Analytical report 1309-066_CSIRO NSAID sheep”.