Relative bioavailability of rafoxanide following intraruminal and intra-abomasal administration in sheep

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ABSTRACT
The bioavailability of rafoxanide was compared after intraruminal and intra-abomasal administration in healthy adult sheep (n = 6) in a single dose, 2 parallel group study at 7.5 mg/kg. Rafoxanide concentrations in plasma were measured by means of HPLC analysis. Primary pharmacokinetic parameters for bioavailability and disposition of rafoxanide in plasma for both routes of administration were determined by non-compartmental and non-linear, 1-compartmental pharmacokinetic analysis, respectively. Significantly (P ≤ 0.05) higher peak plasma concentrations (Cmax) of rafoxanide and a more rapid rate of absorption (t0.5) times) was observed in sheep after intra-abomasal (i-a) administration compared to intraruminal (i.r.) administration. A significantly (P ≤ 0.05) longer lag period (tLAG) before absorption (6.8 ± 2.9 h) occurred after i.r. than after i-a treatment (1.9 ± 0.6 h). There was no significant difference (P > 0.05) in AUC, MRT and in the rates of elimination (k10-HL and t1/2) between the i.r. and i-a routes of administration. The results of the study demonstrated the important influence of the rumino-reticulum on absorption of rafoxanide in sheep.

Key words: intra-abomasal, intraruminal, pharmacokinetics, rafoxanide, salicylanilides, sheep.


INTRODUCTION
Rafoxanide (Ranide, Logos Agvet) belongs to the group of halogenated salicylanilide anthelmintic agents used extensively for the control of liver fluke and blood-sucking nematodes in sheep and cattle, and larvae of Oestrus ovis in sheep. Halogenated salicylanilides, in particular rafoxanide and closantel, share similar chemical, pharmacokinetic (extensive plasma protein binding and long elimination half-life) and safety features. The efficacy of rafoxanide and closantel against blood-sucking parasites and the persistent anthelmintic effect of closantel have been directly associated with the pharmacokinetics of these agents. Rafoxanide concentrations in plasma have also been positively correlated with the occurrence of toxicity in lambs, which in addition substantiates the important relationship between the pharmacokinetics of these drugs and their safety. Apart from a few studies, there has been little pharmacokinetic focus on the absorption and disposition of the halogenated salicylanilides. The intravenous disposition of closantel and rafoxanide has only recently been reported.

The absorption of drugs administered orally to ruminants is markedly affected by the anatomical and physiological features of the forestomachs. Absorption from the rumino-reticulum has been reported for several drugs, since the ruminal epithelium does not constitute a barrier for the distribution of liposoluble, non-polar, hydrophilic substances. Absorption of drugs from the rumen, on the other hand, is generally negligible, owing to the slow rate of diffusion across the ruminal epithelium relative to the rate of outflow from the rumino-reticulum to the abomasum. Poor mixing of drugs in the aqueous phase of rumen digesta, relatively low surface area to volume ratio, proportionally lower blood supply to the rumen epithelium and adsorption of drug particles to particulate matter in the rumino-reticulum are the main causes of the slow rate of diffusion. The dilution effect of the large volume of ruminal contents reduces the diffusion gradient between rumen and plasma. Many drugs are rapidly and extensively adsorbed onto the solid phase of rumen digesta, thereby delaying absorption from the rumen and increasing the proportion of drug outflow to the abomasum and lower gastrointestinal tract.

Foreign compounds may be inactivated or activated by reduction, hydrolytic and fission metabolic reactions by microflora or by the reducing conditions within the rumino-reticulum fluid. They are therefore important considerations in the pharmacokinetics and pharmacodynamics as well as the toxicity of drugs administered orally to ruminants. The halogenated salicylanilide cloxanide is deacetylated in the rumen to form an active hydroxyl derivative. Its anthelmintic activity in sheep is reduced if it is passed directly into the abomasum.

Very few studies, other than those on cloxanide, have been conducted to establish the importance of the rumino-reticulum on the absorption of halogenated salicylanilides. Taylor et al. showed that significantly lower peak plasma concentrations and extent of absorption of rafoxanide administered orally occurred in grazing lambs, owing to reduced digesta flow rate, compared to housed lambs that were fed hay. A significantly greater bioavailability of rafoxanide (2.5–3.0 times) administered orally was reported in suckling lambs (5–8 weeks of age) compared to weaned lambs (5 months of age). The increased absorption was ascribed to the presence of an underdeveloped rumen in the suckling lambs compared to the weaned lambs and a more rapid and extensive absorption from the abomasum. Ruminants at the age of 3–8 weeks are regarded as being within the transitional phase of forestomach development. Comparison of the efficacy of rafoxanide at 3.75 mg/kg, administered either intra-abomasally (i-a), orally (p.o.) or intraruminally (i.r.), against 6-week-old Fasciola hepatica, revealed that i-a treatment was more than twice as effective than either p.o. or i.r. treatment. Similar efficacy was shown after p.o. and i.r. administration.

No pharmacokinetic studies have been

reported with rafoxanide administered either i.r. or i-a to demonstrate the difference in bioavailability between the 2 routes. In the current study bioavailability of rafoxanide was compared after i-a and i.r. routes of administration in adult sheep.

MATERIALS AND METHODS

Study design
A randomised balanced, single dose, parallel 2-group, comparative bioavailability study with rafoxanide administered either i-a or i.r., was conducted in 6 healthy adult South African mutton Merino sheep. Equal numbers of ewes (n = 3) and wethers (n = 3) were used. Whole blood counts, haematocrit, serum aspartate transaminase activity and serum creatinine, urea and albumin concentrations of all animals, determined at the start of the study, were within the normal ranges expected for sheep.

An i-a catheter was inserted under general anaesthesia (halothane: Fluothane, ICI) in all sheep 7 days before the start of the trial. A stab incision was made into the abomasum and a feeding catheter inserted through the last intercostal space at the costochondral junction. To prevent leakage of abomasal content, a purse-string was applied at the site of incision. The catheter was stabilised in a channel of abomasal serosa, c. 2 cm in length, by means of an external suture.

Rafoxanide 3 % m/v oral suspension (Ranide®, Logos AgVet) at 7.5 mg/kg was administered either i-a and i.r. Intra-abomasal treatment was administered by means of the i-a catheter and i.r. treatment by injection through the left abdominal wall into the rumen.

Venous blood was collected in 10 ml heparinised vacuum tubes, immediately before treatment and at 0.5, 1, 2, 3, 5, 7, 9, 12, 15, 24 and 48 h, and 3, 5, 7 10, 14, 21, 28 and 35 days after treatment. Timed collection occurred within 30 sec of the scheduled time up to 24 h, and thereafter within 1–5 min.

Blood samples were centrifuged at 3000 r.p.m. for 15 min and the plasma collected. Two equal aliquots of plasma from each animal were transferred into clean polycarbonate tubes and stored at −20 °C until analysed. Recovery and storage of plasma occurred within 12 h of blood collection.

Rafoxanide analyses
Rafoxanide plasma concentrations were determined by high-pressure liquid chromatography (HPLC) according to the method described by M.Müllers and co-workers (Department of Pharmacology and Toxicology, Faculty of Veterinary Science, University of Pretoria, pers. comm., 1998).

Pharmacokinetic analysis
Non-compartmental analysis of the plasma concentration versus time data of rafoxanide for extravascular input was performed by PC Nonlin Version 4.2 (Statistical Consultants, New York) computer programme. The area under the plasma concentration versus time curve (AUC, zero-moment) and the 1st non-normalised moment (AUMC) were calculated according to the trapezoidal method from time zero to the last sampling time.

Extrapolation of AUC to infinity (AUC∞) was performed using the slope (β) of the terminal phase. The slope (β) of the terminal phase of the curve was determined by linear regression analysis of the terminal plasma concentrations of the semilogarithmic plasma concentration versus time curve. Mean residence time (MRT, 1st moment) was derived from AUC/AUMC. Maximum plasma concentration (cmax) of either rafoxanide and time to cmax (tmax) were read directly from the individual plasma concentrations.

Non-linear compartmental analysis of the rafoxanide plasma data was performed using the same pharmacokinetic computer programme using the Nelder-Mead algorithm. Initial pharmacokinetic parameter estimates, used for the non-linear analysis, were derived automatically by initial linear analysis performed by the programme. Akaike’s information criterion, based on the mean values of the final estimates of the associated pharmacokinetic parameters and lack of systematic deviations around the fitted disposition curve, was used to determine the number of exponential terms that best described the data. Primary pharmacokinetic parameters were derived by 1-compartmental analysis with 1st-order input, 1st-order output and lag time of the plasma concentration-time data for each individual animal yielding the microconstants k0 and k∞. Secondary disposition parameters, including k10 half-life (k10-HL) and ks0 half-life (k0-HL) were derived from the primary parameters utilising standard procedures.

Statistical analyses
The descriptive statistics (mean ± SD) for treatments and treatment groups were calculated for all pharmacokinetic parameters within each study. Differences in the mean pharmacokinetic parameters were statistically compared using the Student’s t-test, whereas the nonparametric Wilcoxon rank test was applied to the rate constants (k0 and k∞), rate constant half-lives (k10-HL and k0-HL), tmax and tlag. All statistical procedures were performed using the SAS statistical software programme for Windows.

RESULTS
The mean plasma concentration versus time profiles for rafoxanide following i.r. and i-a administration are illustrated in Fig. 1. The data was best described by a 1-compartmental open model with 1st-order rate constants and lag-time.

Rafoxanide suspension administered i-a reached significantly (P < 0.05) higher peak plasma concentrations compared to i.r. administration in c. 25 % of the time (Table 1). The rate of absorption of rafoxanide after i-a administration was significantly (P < 0.05) more rapid as measured by tmax, tlag and k0-HL than i.r. treatment. There were no significant differences (P > 0.05) in AUC, MRT and rates of elimination (k10-HL and t½) between the 2 routes of administration.

DISCUSSION
The general pharmacokinetic profile of rafoxanide after i.r. and i-a administration observed in this study is consistent with the pharmacokinetics of rafoxanide reported previously in sheep. Very similar AUC, cmax, and t½ results were obtained for rafoxanide after i.r. administration compared with those reported by Mohammed-Ali and Bogan following oral treatment in sheep.

The results of the current study indicate the effect of the rumino-reticulum on the absorption of rafoxanide following i.r. administration in adult sheep. Rafoxanide administered i.r. was absorbed significantly slower than after i-a administration and had an extended lag-time before absorption.

According to Bogan and Marriner, the rumino-reticulum serves mainly as a ‘reservoir’ for drugs and is responsible for comparatively negligible absorption relative to the rest of the gastrointestinal tract. The absorption of drugs administered i.r. is delayed as a result of the dilution effect of the large volume of ruminal contents and adsorption onto rumen digesta resulting in a delay of outflow from the rumino-reticulum.

Unlike the benzimidazole anthelmintics and ivermectin, the bioavailability of rafoxanide was similar for both i.r and i-a route of administration. The higher cmax plasma concentrations of rafoxanide observed in the sheep after i-a treatment is related to the more rapid rate of absorption from the abomasum and not due to an increase in bioavailability. In the case of

the benzimidazoles, although increased plasma concentrations occur due to the increased rate of absorption from the abomasum, the bioavailability as measured by AUC is generally lower due to absence of the reservoir effect of the rumen resulting in a large reduction in residence time of these agents in the body. The long elimination half-life of the halogenated salicylanilides, in contrast, is associated with their extensive plasma binding, and therefore the residence time of rafoxanide in the body is not dependent on the reservoir effect of the rumen. The bioavailability of ivermectin, on the other hand, is significantly reduced after i.r. treatment and is attributed to intraruminal degradation of the agent. Biodegradation of rafoxanide in the rumen has not been reported, although it has been shown to be more than twice as effective against *F. hepatica* when administered i-a as compared to either oral or i.r. treatment.

The study clearly showed the effect of the rumen on the rate of gastrointestinal absorption of rafoxanide. Further studies are required to examine the effect of type of feed, reduction in feed intake and transient feed withdrawal on the pharmacokinetics of the halogenated salicylanilides in sheep and to examine the influence of the rumino-reticulum on the disposition of these agents.

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