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The Pharmacokinetics of Rafoxanide following Single Dose Intravenous and Oral Administration in Goats

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ABSTRACT

The study was conducted to determine the plasma concentrations and pharmacokinetic parameters of rafoxanide after a single intravenous (10 mg/kg) and oral (22.5 mg/kg) administrations in black Bengal female goats. Maximum (87.63 ± 11.71 μg/mL) and minimum (1.8 ± 0.24 μg/mL) plasma concentrations of rafoxanide were recorded at 0.08 and 12 h respectively after i.v. administration. The elimination half life ($t_{1/2}\beta$), total body clearance (Cl_B) and volume of distribution (Vd_{area}) values were 2.89 ± 0.26 h, 0.05 ± 0.002 L/kg/h and 0.19 ± 0.02 L/kg respectively. An adequate plasma level of rafoxanide (14.63 ± 2.12 μg/mL) was detected at 1h followed by gradual increase and peak concentration level (30.88 ± 4.30 μg/mL) was recorded at 36 h after oral administration. The rate of absorption (K_a), elimination half life ($t_{1/2}\beta$), total body clearance (Cl_B) and volume of distribution (Vd_{area}) values were 0.07 ± 0.009 h^{-1}, 138.02 ± 13.99 h, 0.003 ± 0.0003 L/kg/h, and 0.57 ± 0.06 L/kg respectively. Plasma protein binding percentages of rafoxanide varied from 81.06 to 92.28 and its association and dissociation constants were $1.26 \times 10^7 \pm 0.18 \times 10^7$ L/mol and $0.83 \times 10^{-7} \pm 0.12 \times 10^{-7}$ mol/L respectively. On conclusion, rafoxanide is slowly absorbed from the gastrointestinal tract, slowly eliminated; highly protein bound and persists for long time in blood of goat.

Keywords: *Rafoxanide, Pharmacokinetics, Goats, Oral, Intravenous*

Anthelmintic rafoxanide belongs to the group of halogenated salicylanilide and is found to be highly effective against mature and immature stages of fluke in sheep, goat and cattle [1]. It acts by uncoupling of oxidative phosphorylation of parasites [2]. Common pharmacokinetic parameter suggests that it undergoes extensive plasma protein binding and has long elimination half life [3-5] in sheep. Sheep infected with 6 – week – old *Fasciola hepatica* retained high concentration of rafoxanide in plasma compared to non-infected sheep [6]. Plasma concentrations of rafoxanide have been reported to be positively correlated to the toxicity in lambs [7], though three times of therapeutic dose level did not produce any toxic symptom in sheep [8]. Both intravascular and oral disposition kinetics of rafoxanide have been carried out in sheep and lamb respectively [8-9].

Black Bengal goat is a recognized breed of goat and its population is much greater than that of sheep in the state of West Bengal, India. It is a prolific breeder and its meat is popularly consumed by human beings. Mortality of goat and kid due to fascioliasis is very common during rainy and autumn seasons in West Bengal. It has been observed that clinical efficacy of rafoxanide against fascioliasis was more if it was administered orally at 15 mg/kg instead of conventional dose of 7.5 mg/kg and at this dose level, goat did not exhibit any sign of toxicity (unpublished data). Report on the pharmacokinetic study of rafoxanide on goat is not available in literature. Considering the above, the paper discusses the plasma concentration at different time interval and pharmacokinetic behavior of rafoxanide following intravascular and oral administrations in black Bengal goat.

MATERIALS AND METHODS

Animals

Six clinically healthy adult black Bengal nulliparous female goats weighing between 10-12 kg (1-1½ yr. age)

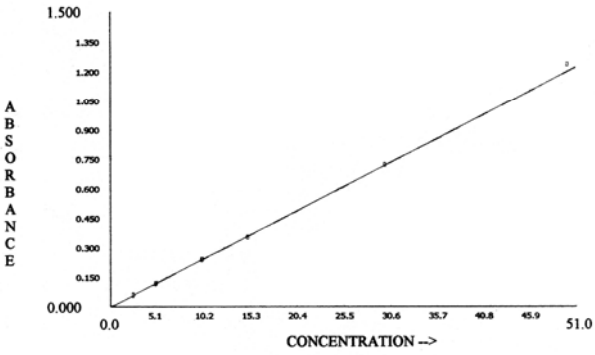

Fig 1. Standard curve of rafoxanide showing absorbance against several concentration.

were utilized in this experiment. The animals were kept in individual custom-made stainless steel cage (size – 48" × 48" × 36") at temperature (22 ± 2°C) controlled animal room having provision of artificial light during the experiment. The animals were acclimatized with the laboratory environment for 7 days. As the study was conducted on healthy goats, all animals were dewormed with albendazole (5 mg/kg), 30 days prior to the onset of the study. The animals were fasted overnight prior to the start of experiment and provided balanced feed and *ad libitum* water during study period. The objective and design of the study of rafoxanide in goat has been scrutinized and approved by the Institutional "Animal Ethics Committee".

Intravenous administration

A single dose (10 mg/kg, body weight after overnight fasting) of rafoxanide (Technical grade, 99.9 % purity, supplied by M/S Gharda Chemicals Ltd., Mumbai, India) dissolved in 4 mL of dimethyl sulphoxide was administered via left jugular vein of each goat. Blood samples (4 mL each) were collected from the right jugular vein in heparinized tube before and at 0.08, 0.16, 0.33, 0.5, 0.75, 1, 2, 4, 6, 8, 12, 24, 36 and 48 h of drug administration except 0.5, 1, 4 and 12 h when 10 mL of blood samples were collected for plasma protein binding study.

Oral administration

After an interval of 3 months, the same six goats were used for oral kinetic study. A single dose of rafoxanide (22.5 mg/kg) dissolved in dimethyl sulphoxide was administered by oral intubation to each animal. The blood samples (4 mL each) were collected from jugular vein before and at 1, 3, 6, 9, 12, 24, 36, 48, 72, 96, 120, 144 and 168 h of drug administration.

Analytical method

From the collected blood samples, plasma was separated by centrifugation at 3000 rpm for 30 min and stored at 4°C in refrigerator for subsequent drug analysis. To determine rafoxanide concentration in plasma, 2 mL of saturated ammonium sulphate in 2.5% sulfuric acid was added to a centrifuge tube containing 2 mL of plasma and shaken for 1 min. Then, 11 mL of ethyl acetate was added and shaken vigorously for 5 min. This was centrifuged at 2500 rpm for 30 min. The supernatant was collected and analyzed in the UV 2600 double beam UV-VIS spectrophotometer at 284.8 nm wave length against the blank prepared with plasma separated from blood collected before administration of drug. Concentration of rafoxanide present in each blood sample was then calculated from standard curve prepared earlier and expressed as μg/mL.

Plasma Protein Binding Assay

The plasma protein binding of rafoxanide was determined [10] in plasma samples collected at 0.5, 1.0, 4.0 and 12.0 h post intravenous dosing. Dialysis bag (M/S Sigma Chemicals Co., USA) was cut into suitable pieces, washed with distilled water and kept immersed overnight in phosphate buffer (pH 7.4) at 37°C. A small part of each piece of dialysis bag was inserted around one end of a glass tube (2-3") having both ends open and then secured tightly with thread. A tight knot was put on the other end of the extended dialysis bag so as to make a closed bag sufficiently voluminous to hold 2.5 mL of plasma sample. The dialysis tube containing 2.5 mL of plasma was then suspended in a test tube of large size containing 2.5 mL of phosphate buffer and then placed into an incubator in standing position for 24 h at 37°C. The protein content of plasma of each sample was also estimated [11]. The protein binding activity was then expressed as percentage. The binding capacity, dissociation and association constants were also calculated by the method of least square regression technique [12].

Recovery

Recovery of rafoxanide from goat plasma was carried out *in vitro* to ascertain the reliability of analytical method after fortifying with 2.5, 5, 10, 15, 30, 50, 75 and 100 μg/mL of rafoxanide. The maximum absorbance of rafoxanide was found to be at 284.8 nm. A standard carve was drawn by the internal program of computer attached with UV–VIS spectrophotometer using the absorbance against several concentration of rafoxanide at 284.8 nm and the linearity was found to be maintained up to the concentration of 50 μg/mL (Fig 1). The recovery of the assay was 85-97%. The minimum limit of detection of rafoxanide was 1.5 μg/mL. The limit of sensitivity for rafoxanide was 1.75 μg/mL of plasma.

Kinetics

Plasma drug concentration versus time for each goat was analyzed using computerized curve fitting program 'PHARMKIT' (Supplied by Dept. of Pharmacology, JIPMER, Pondicherry, India). Pharmacokinetic parameters were calculated from the computerized curve [13]. The value of AUC was calculated by trapezoid rule [14].

Statistical analysis

Statistical analysis of data was conducted using standard formula [15]. The data were analyzed sepa-

rately for each individual animal and the average drug plasma concentrations at various time intervals have been drawn in semilogarithmic graph paper.

RESULTS

Intravenous

Mean plasma concentration of rafoxanide at different time intervals in goats is shown in Fig 2, which reveals "two compartment open model" kinetics of rafoxanide following i.v. administration. Maximum plasma concentration of rafoxanide (87.63 ± 11.71 µg/mL) was recorded at 0.08 h followed by rapid decline initially, thereafter the concentration decreased slowly with a minimum plasma concentration of 1.80 ± 0.24 µg/mL at 12 h. The drug could not be detected in plasma samples collected beyond 12 h post i.v. dosing in goats.

The zero time plasma concentration of rafoxanide was recorded to be 96.98 ± 5.59 µg/mL following i.v. administration. The values of hybrid rate constants, α and β were 6.38 ± 0.91 h^{-1} and 0.25 ± 0.02 h^{-1} respectively. The distribution (α) and elimination (β) half lives of rafoxanide were 0.12 ± 0.02 h and 2.89 ± 0.26 h respectively. The values for volume of distribution based on area (Vd_{area}), area under curve (AUC), volume of distribution in central compartment (Vd_c) and total body clearance (Cl_B) were 0.19 ± 0.02 L/kg, 90.16 ± 8.25 µg . h/L, 0.10 ± 0.01 L/kg and 0.05 ± 0.002 L/kg/h respectively while the values of hybrid rate constant for transfer of drug from central to peripheral compartment (K_{12}) and vice-versa (K_{21}) were 2.71 ± 0.50 h^{-1} and 3.47 ± 0.55 h^{-1} respectively.

Oral

The semilogarithmic plot of mean plasma concentration of rafoxanide monitored at different time intervals after single dose oral administration is presented in Fig 2. The maximum and minimum plasma concentrations of rafoxanide were 30.88 ± 4.30 and 13.40 ± 3.08 µg/mL at 36 and 168 h respectively.

The data depicted in Table 1 shows that hybrid rate constant related to the slope of absorption phase (K_a) was 0.07 ± 0.009 h^{-1} while this value with respect to the elimination phase (β) was 0.005 ± 0.0007 h^{-1} following oral administration. The absorption (K_a) and elimination (β) phase half life of rafoxanide were 11.21 ± 2.07 and 138.02 ± 13.99 h respectively. The average respective values for volume of distribution by area method (Vd_{area}), area under curve (AUC), volume of distribution in central compartment (Vd_c) and total body clearance (Cl_B) were 0.57 ± 0.06 L/kg, 96.20 ± 6.72 µg. h/L, 0.35 ± 0.04 L/kg and 0.003 ± 0.0003 L/kg/h while the values for transfer from peripheral to central compartment (K_{21}) and vice-versa (K_{12}) were found to be 0.04 ± 0.005 h^{-1} and 0.02 ± 0.003 h^{-1} respectively.

Protein binding

The percentages of plasma protein binding of rafoxanide were 81.06 ± 0.39, 85.14 ± 1.21, 87.92 ± 0.57 and 92.28 ± 0.23 at the total drug concentration of 42.73 ± 3.87, 33.33 ± 3.34, 13.00 ± 1.73 and 1.90 ± 0.31 µg/mL in plasma collected at 0.5, 1, 4 and 12 h post dosing in goats and its dissociation (K_β) and association rate constant (K'_a) were found to be 0.83 × 10^{-7} ± 0.12 × 10^{-7} mol/L and 1.26 × 10^7 ± 0.18 × 10^7 L/mol respectively.

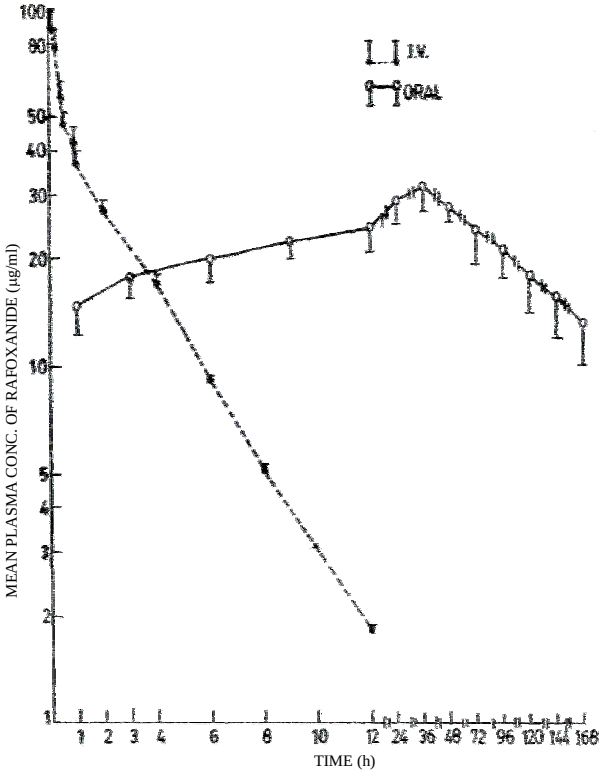

Fig 2. Semilogarithmic plot of mean plasma rafoxanide concentration (µg/mL) against time following single oral and intravenous administration to goats.

DISCUSSION

Pharmacokinetics of rafoxanide following a single intravenous administration showed a "2-compartmental open model" with first order rate constants, which corroborated the findings of Swan *et al.* [8] in sheep. Further, rapid and limited distribution coupled with slow elimination and small total body clearance led to long persistence of rafoxanide in blood of goat. Similar pharmacokinetic behavior of rafoxanide has been reported by Swan *et al.* [8] in sheep following intravenous administration.

The absorption rate constant (K_a) and absorption half life ($t_{1/2}K_a$) values denote slow absorption of the drug from the gastrointestinal tract of goat after oral administration. Lipid soluble non-ionized drug molecules diffuse freely across the biological membrane till equilibrium is reached, and that ionized molecules are virtually excluded from transmembrane diffusion [16]. The systemic availability of rafoxanide after oral dosage was 47%. Rafoxanide is a weak organic acid and most of it is expected to remain as ionized fraction at alkaline pH of gastrointestinal tract of ruminants resulting in slow diffusion through biological membrane leading to lower bioavailability after oral administration. Further, gastro-

intestinal absorption of rafoxanide depends upon the digesta flow rate and Hennessy *et al*. [17] suggested that goat has reduced digesta flow rate.

Maximum concentration of rafoxanide was recorded in plasma of goat at 36 h after a single oral administration. Swan and Mulders [9], and Swan *et al*. [18] reported that the maximum concentration of rafoxanide in the plasma samples of sheep varied from 24-36 h following a single oral administration of graded dose levels. Elimination half-life is long in goat and comparable to that observed in weaned lamb [9]. Extensive binding of rafoxanide and closantel to plasma albumin (> 97%) has been reported in sheep and goat respectively [5]. Binding of rafoxanide was above 81% mainly due to higher association constant compared to dissociation constant, while the same was found to be bound more than 99% in the plasma of sheep [6]. The numerical difference in respect of plasma protein binding of rafoxanide between sheep and goat might be attributed to species variation. Area under curve (AUC) value in goat was higher than that of sheep [6] after oral administration of rafoxanide (7.5 mg/kg). In this study, rafoxanide was administered at much higher dose (22.5 mg/kg) and therefore the difference in AUC value between sheep and goat is expected. Further, transfer of compound from peripheral tissue to blood (K_{21}) was two times greater than that of the transfer of compound from central to periphery indicating that rafoxanide has the least possibility of being retained in tissue.

Table 1. Pharmacokinetic parameters of rafoxanide following single dose intravenous (10 mg/kg) and oral (22.5 mg/kg) administration to goats. (n = 6; mean ± SE)

Kinetic parameters	Intravenous	Oral
C^0_p (µg/mL)	96.98 ± 5.59	66.00 ± 7.26
α (h^{-1})/ K_a (h^{-1})	6.38 ± 0.91	0.07 ± 0.009
$t_{1/2}$ α (h)/ $t_{1/2}$ K_a (h)	0.12 ± 0.02	11.21 ± 2.07
β (h^{-1})	0.25 ± 0.02	0.005 ± 0.0007
$t_{1/2}$ β (h)	2.89 ± 0.26	138.02 ± 13.99
AUC (µg. h/L)	90.16 ± 8.25	96.20 ± 6.72
Vd_c (L/kg)	0.10 ± 0.01	0.35 ± 0.04
Vd_B (L/kg)	0.20 ± 0.02	0.61 ± 0.07
Vd_{ss} (L/kg)	0.14 ± 0.05	0.54 ± 0.06
Vd_{area} (L/kg)	0.19 ± 0.02	0.57 ± 0.06
Cl_B (L/kg/h)	0.05 ± 0.002	0.003 ± 0.0003
Kel (h^{-1})	0.45 ± 0.03	0.01 ± 0.001
K_{12} (h^{-1})	2.71 ± 0.50	0.02 ± 0.003
K_{21} (h^{-1})	3.47 ± 0.55	0.04 ± 0.005
fc	0.55 ± 0.05	0.63 ± 0.02
T ~ P	0.87 ± 0.17	0.61 ± 0.012
F (%)		47.0

On conclusion, it may be suggested that the administration of rafoxanide via the oral route would be more beneficial for the clinical purpose as the drug is absorbed and eliminated from the body slowly yielding effective concentration for a prolonged period. It has lesser affinity to get accumulated in tissues.

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