Moxidectin: Metabolic Fate and Blood Pharmacokinetics of ^{14}C-Labeled Moxidectin in Horses

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Serum and whole blood pharmacokinetics of moxidectin and excretion and biotransformation in edible tissues and excreta have been evaluated in the horse at a dose level of 0.4 mg/kg of body weight. Three animals were orally dosed with ^{14}C-labeled moxidectin, formulated as a gel. Total radioactive residues (TRR) were determined in whole blood and in serum collected at selected intervals through 168 h. Sera samples were also assayed for intact moxidectin. At 168 h, animals were sacrificed and TRR determined in edible tissues, namely muscle, liver, kidney, and fat. The mean terminal elimination half-lives for total radioactivity and parent in serum were 154 ± 26 and 82 ± 23 h, respectively. Fecal excretion was the main elimination pathway, accounting for 77% of the administered dose by 168 h. Although minor metabolites were noted, intact parent was the major component in tissues and excreta. Similarly, three animals were intravenously (iv) dosed with ^{14}C-labeled moxidectin, formulated as an aqueous injectable solution. The terminal elimination half-lives for total radioactivity and intact parent in serum were 128 ± 14 and 81 ± 18 h, respectively. On the basis of a comparison of intact parent area under the concentration/time (AUC) values of 4.55 and 11.4 μg·h/g following oral and iv doses, respectively, an oral bioavailability of approximately 40% was estimated.

Keywords: *Moxidectin; blood pharmacokinetics; horses; bioavailability*

INTRODUCTION

Moxidectin is a potent parasiticide against internal and external parasites and is a second-generation macrocyclic lactone (Albers-Schonberg et al., 1981; Takiguchi et al., 1980). Moxidectin has a unique chemical structure as compared to avermectins (ivermectin, abamectin, and doramectin). Moxidectin does not have ivermectin's disaccharide side chain. Instead, it possesses a unique methoxime at the C-23 position and a dimethylbutenyl side chain at the C-25 position. The precursor to moxidectin, nemadectin (Asato and France, 1990), is produced by the *Streptomyces cyaneogriseus* microorganism, a different organism from the one that produces the avermectins. Nemadectin is then synthetically modified to form moxidectin.

Moxidectin is marketed in various formulations for different animal species throughout the world. These formulations include injectable solutions, topical pour-ons, and oral tablets for use in cattle, sheep, swine, and/or dogs. Recently an oral gel formulation has demonstrated broad spectrum activity against parasites of equids, especially unique control of mucosal stages of cyathostomes, which results in prolonged protection of pastures against parasite infestation (DiPietro et al., 1992; French et al., 1992). The gel is readily accepted and easy to administer.

The metabolism of moxidectin in rat (Wu et al., 1993), cattle (Zulalian et al., 1994), and sheep (Afzal et al., 1994) has been reported. In this paper, we report the metabolic fate and blood pharmacokinetics of ^{14}C-labeled moxidectin in horses.

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MATERIALS AND METHODS

Test Substance. The test substance used in this study was ^{14}C-labeled moxidectin (specific activity = 19.5 μCi/mg), formulated as a gel at a nominal concentration of 2% (w/v) for oral administration (radiochemical purity = 96.5%) and as an injectable solution at a nominal concentration of 2% (w/v) for intravenous (iv) administration (radiochemical purity = 96.2%). Moxidectin was prepared by synthetically modifying the corresponding precursor nemadectin isolated from a fermentation using a mixture of ^{14}C-carboxyl-labeled acetate, propionate, and isobutyrate (Ahmed et al., 1993). The chemical structure and the proposed metabolic pathway of moxidectin in horses are shown in Figure 1.

Test System. Seven male crossbred horses (age <12 years; body weight approximately 200−400 kg) were used in this study. The animals were free of external signs of ill health following arrival at the testing site and were acclimatized for approximately 14 days. Animals were housed in a room thermostatically maintained at a temperature of 13−19 °C and a relative humidity of 35−83%. Fluorescent lighting was controlled automatically to give a cycle of 16 h of light and 8 h of darkness. Horses were fed approximately 1 kg of ground concentrate per day (HHF Horse and Pony Nuts, I'Anson Bros Ltd., The Mill, Thorpe Road, Masham, U.K.) and hay *ad libitum* (Leeds University Farms, Wise Warren, Tadcaster, Leeds, U.K.). Water was provided *ad libitum*. Periodic analysis of the drinking water for heavy metals and chlorinated hydrocarbons was performed by the Yorkshire Water Authority, U.K. There were no known contaminants in the feed or the water that would have affected the outcome or the integrity of the study.

Dose Administration. Three horses (group B) were administered a single oral dose of ^{14}C-labeled moxidectin in gel capsules with the aid of a balling gun at a dose level of 0.4

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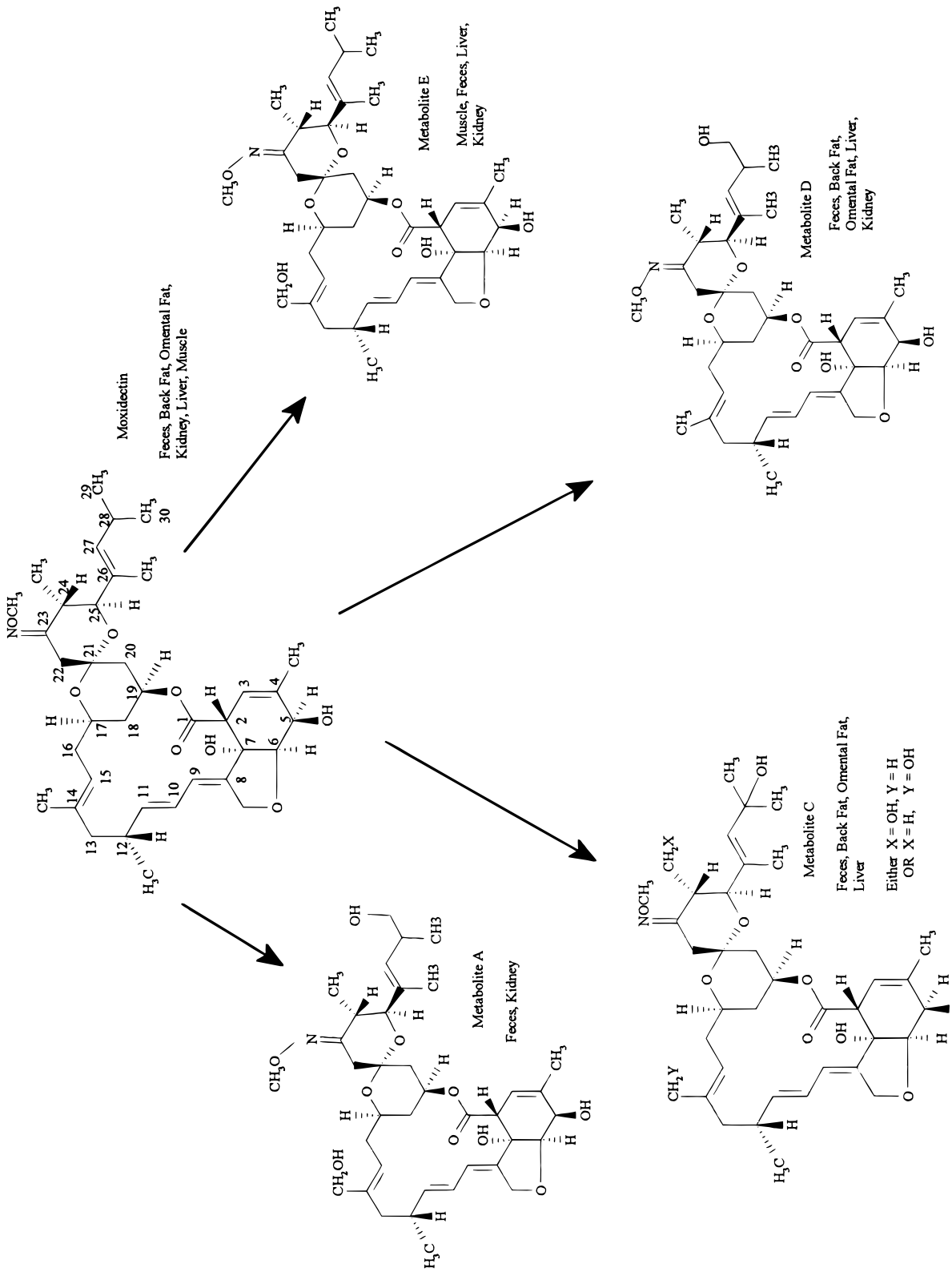

Figure 1. Proposed metabolic pathway of moxidectin in the horse.

mg/kg of body weight. Similarly, three horses (group C) were administered a single intravenous dose of ^{14}C-labeled moxidectin injectable solution at a dose level of 0.4 mg/kg of body weight. A single control animal (group A) was administered a single oral dose of the gel minus ^{14}C-labeled moxidectin in gelatin capsules at a level equivalent to that of the orally treated horses.

Sampling Procedures. Blood samples (~50 mL) were collected from the jugular vein at the following time intervals from dose groups B and C: 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48, 72, 120, and 168 h postdose. Additional blood samples were collected at 0.033 and 0.125 h for dose group C only. Blood samples were collected from the control group A before dosing and 168 h postdose only. An aliquot of blood (~5 mL) from each sample was transferred into tubes containing lithium heparin anticoagulant and stored at approximately 4 °C. These samples were used for the determination of total radioactive residue (TRR) and packed cell volume (PCV). The remainder from each sample was allowed to clot and centrifuged (2000*g* for 10 min at ~20 °C) to produce serum. An aliquot of serum (~5 mL) was used to determine the TRR, and the remainder was used to determine the concentration of unaltered parent moxidectin by a specific high-performance liquid chromatography (HPLC) method. Urine and feces were collected predose and at 24 h intervals until necropsy. Urine and feces were assayed for TRR and the nature of the ^{14}C residue (group B only). Approximately 168 h postdose, animals were stunned by captive bolt and exsanguinated. Samples of muscle (~4 × 500 g of hindquarter), omental fat (~4 × 500 g), back fat (~4 × 500 g), liver (whole), and kidneys (whole) were collected and assayed for the TRR and the nature of the ^{14}C residue. The group A horse was similarly sacrificed and sampled to provide control tissues.

Sample Preparation and Radioanalysis. Tissues and excreta were ground to homogeneity in dry ice. Aliquots of feces and ground tissues (excluding fat) were homogenized in an equivalent volume of water. The homogenates were added to ashless floc in Combusto Cones and combusted in oxygen using a Packard sample oxidizer (Canberaa Packard, Pangbourne, Berks, U.K.). The combusted products were absorbed in Carbo-Sorb and mixed with Permafluor E+ (Canberaa Packard), and the radioactivity was quantified by liquid scintillation counting (LSC). For the radioanalysis of blood and fat, a suitable volume of Soluene-350 solubilizing agent (Canberaa Packard) was added, incubated, and counted by a LSC. Standards of [^{14}C]-Spec-Chec (Amersham International, Aylesbury, Bucks, U.K.) were combusted at the beginning of each day and at regular intervals throughout the day to check the carry-over between samples and to determine the efficiency of the combustion process. Combustion and trapping efficiencies were found to be in excess of 96%, and all reported data are, therefore, uncorrected. All radioassays were performed in duplicate or triplicate except for fractions collected from the HPLC system, for which only a single sample was analyzed. Radioactivity was measured for 10 min or for 2 sigma % (95% confidence level) using LKB (Pharmacia Wallac U.K. Ltd., Milton Keynes, Bucks, U.K.), Beckman (Beckman Instruments, High Wycombe, Bucks, U.K.) or Packard Tri-Carb liquid scintillation counters (Canberra Packard) with the facilities for computing quench-corrected disintegrations per minute (dpm). Efficiency correlation curves were prepared for organic, aqueous, and combusted sample types and were routinely checked by the use of [^{14}C]-*n*-hexadecane standards. The spectrometer was recalibrated when a deviation of >3% was observed in the counting of quality control standards.

The limit of detection for the analysis of each sample type (blood, serum, tissue, and excreta) was taken as twice the background disintegration rate obtained from the measurement of predose or control samples of the same type. The limit of detection was validated by fortifying control matrices with ^{14}C-labeled moxidectin at the rate of 2 ppb.

Extraction of Radiolabeled Residues from Tissues and Feces. All solvents (analytical grade) were purchased from Merck Ltd. (Poole, Dorset, U.K.). Aliquots (50−100 g) of muscle, liver, kidney, and omental and back fat samples were macerated twice or thrice with acetonitrile (100 mL). The organic extracts were pooled and partitioned against hexane (2 × 70−100 mL). The hexane was removed, the acetonitrile extracts were combined, and the excess solvent was removed under reduced pressure using rotary evaporation. The residue was amenable to HPLC characterization in the case of omental and back fat. For other tissues, the residue was partitioned against ethyl acetate (4 × 25 mL), and the ethyl acetate fractions were pooled, concentrated, and reconstituted in mobile phase to facilitate HPLC analysis. Aliquots of fecal homogenate (50 g) were extracted with aqueous methanol (methanol/water, 95:5 v/v; 4 × 100 mL). The extractable fractions were combined, and the excess solvent was removed and partitioned against ethyl acetate as described for tissues. Aliquots of concentrated extracts from tissues and feces were counted by LSC, and extraction efficiencies were determined by comparing the recovered radioactivity versus the TRR found in respective matrices.

Determination of the Concentration of Intact Moxidectin in Horse Serum by HPLC and Fluorescence Detection. A typical serum extraction procedure is described as follows: An aliquot (10 g) of serum sample was admixed with acetonitrile/water (10 mL, 1:1 v/v) and passed through a C_{18} BondElute (Anachem, Luton, U.K.) solid phase extraction cartridge (SPE), which had been preconditioned with methanol and water. The column was washed with acetonitrile/water (5 mL; 1:1 v/v) and water (3 mL). When the cartridges were completely dry, the analyte was eluted with dichloromethane (5 mL), which was dried under nitrogen convection at <45 °C. The residue was reconstituted in a freshly prepared solution of dimethylformamide/acetic anhydride/methylimidazole (0.5 mL, 9:3:2 v/v/v), and the vial was sealed and heated to 95 °C for 1 h. When cool, hexane (1 mL) was added and the mixture shaken vigorously for approximately 1 min. The hexane was removed and loaded onto a Florisil Sep-Pak SPE (Bio-Rad, Hemel Hempstead, U.K.). The hexane was allowed to percolate slowly through the SPE cartridge by gravity and the eluate collected. The reaction mixture was re-extracted with hexane (4 mL) and the solvent added to the Florisil SPE cartridge. The eluate was collected under slight positive pressure. The combined hexane fractions were evaporated to dryness under nitrogen convection at <45 °C. The residue was dissolved in methanol in approximately 2 mL prior to analysis by HPLC. The HPLC system consisted of a Jasco PU-980 pump (Ciba Corning, Analytical, Halstead, Essex, U.K.), with either a Gilson 231XL (Anachem Ltd.) or a Jasco Model AS-950 autosampler and either a Jasco Model 821-FP or 820 F-B spectrofluorometric detector. A Zorbax ODS analytical column (250 × 4.6 mm) was used with an isocratic mobile phase (methanol/water, 98:2 v/v). The flow rate was 1.8 mL/min and the injection volume 20 μL. Fluorescence detection was accomplished with an excitation wavelength at 364 nm and an emission wavelength at 470 nm. From the concentration of moxidectin in calibration samples and the area response for the peak associated with moxidectin, a calibration curve was constructed using linear regression analysis. From the regression equation, the concentration of intact moxidectin in serum samples was estimated as follows:

$$\text{concentration of moxidectin} = (\text{peak area moxidectin} - \text{intercept})/\text{gradient}$$

Prior to analysis of serum samples, the analytical method was validated for specificity, linearity, precision, and accuracy.

Characterization/Identification of Radiolabeled Residues from Tissues and Feces. The nature of the radioactive residues in organic extracts prepared from tissues and feces was analyzed by HPLC. Radiolabeled and nonradiolabeled moxidectin were used as markers for retention time assignments. HPLC analysis was conducted on a Jasco PU-980 system equipped with an injector (Gilson 231 XL sample injector and Gilson 401C diluter) and an Apex ODS (250 × 4 mm; 5 μm) column. A gradient mobile phase consisting of methanol/water (40:60) at time 0 to methanol/water (90:10) over 50 min was used and maintained isocratic for an additional 25 min followed by equilibration to methanol/water (40:60) over 5 min (flow rate = 1 mL/min). The eluent was

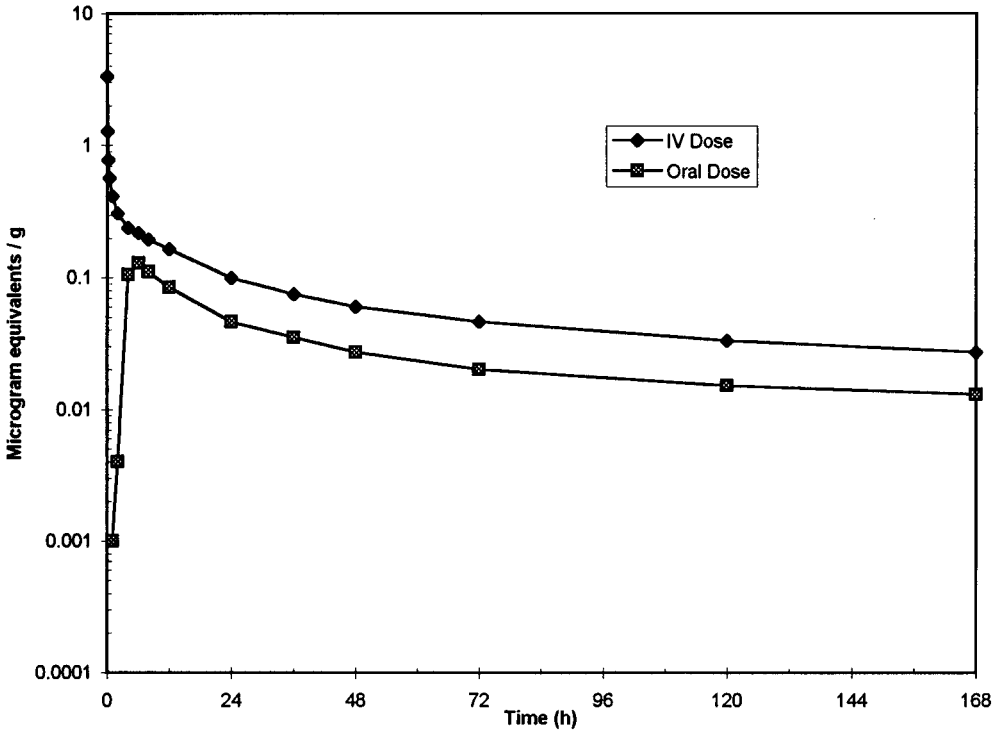

Figure 2. Pharmacokinetics of mean serum total radioactivity following a single oral or intravenous administration of ^{14}C-labeled moxidectin to horses at a dose level of 0.4 mg/kg of body weight.

passed first through a UV detector set at 240 nm and then through a β-ram radiodetector using a liquid mixing cell (Monoflow 3, scintillation fluid, National Diagnostics, Lablogic, Sheffield, U.K.). Due to limited sensitivity, the radioactivity in some samples was assayed by collection of HPLC fractions (0.5 min) followed by LSC.

The concentration (% TRR) of unchanged parent and metabolites determined by HPLC was normalized by multiplying the percent radioactivity in the region by the percent extractability. In instances of extraction efficiencies >100%, a quantitative extractability has been assumed. The TRR in the regions corresponding to parent and metabolites was then determined from the % TRR (HPLC) and the TRR found by combustion analysis (TRR × % TRR/100).

Identification of the Major Radiolabeled Residue by Mass Spectrometry. Organic extracts prepared from tissues and feces from the orally dosed group were analyzed by high-performance liquid chromatography/mass spectrometry (LC/MS) to provide structural confirmation of the major radiolabeled residue. To aid identification, nonradiolabeled moxidectin reference standard was analyzed under identical conditions. The mass spectrometric analysis was carried out on a VG Quattro triple-quadrupole mass spectrometer with electrospray LC/MS interface, coupled to a Jasco ternary gradient HPLC system, and a Hewlett-Packard UV detector and a Lablogic β-ram (Lablogic) radiodetector (liquid or solid cell). The flow rate was 1 mL/min with a split ratio of approximately 20:1 (v/v). The mass spectrometer source temperature was maintained at 130−150 °C with a scan range of either 500−700 or *m/z* 580, 640, and 662 for full scan or selected ion monitoring, respectively. The HPLC conditions and the analytical column used were identical to those described earlier for tissue and feces.

Calculation of Pharmacokinetic Parameters. The pharmacokinetics of serum and whole blood total radioactivity and serum moxidectin concentrations were determined using model-independent methods. All calculations were performed using the commercially available software package TopFit (version 2.0; Gustav Fischer, Stuttgart, Germany). The parameters T_{max} and C_{max} were determined empirically from the concentration versus time for the orally dosed animals. The input included sample time (h), sample concentration and units of concentration, dose administered, and units of dose administered. Nondetected (below the detection limit of the radioassay) values were not used in half-life calculations. The output consisted of the input (as above), time point range used for half-life determination, terminal elimination half-life ($T_{1/2}$), clearance (calculated by dividing the iv dose by the AUC), mean residence time (calculated by dividing the AUMC by the AUC), and volume of distribution (calculated by dividing the clearance by the elimination rate constant, K_{el}). The elimination half-life was calculated by regression analysis of the linear portion of the individual concentration/time curves following C_{max} when plotted semilogarithmically. The elimination rate constant, K_{el}, was calculated as ln $2/T_{1/2}$. The area under the curve (AUC_{0-t}) was determined using the trapezoidal rule and $AUC_{t-\infty}$ was calculated as C_t/K_{el}. The total $AUC_{0-\infty}$ was determined as the sum of the AUC_{0-t} and the $AUC_{t-\infty}$.

RESULTS AND DISCUSSION

Blood Pharmacokinetics. To describe the pharmacokinetics of moxidectin in male horses following oral and iv administration, parameters were determined for total radioactivity in whole blood and serum. Additionally, pharmacokinetic parameters were assessed for intact moxidectin in serum to estimate oral bioavailability (Figures 2 and 3 and Tables 1 and 2).

Following a single iv administration of moxidectin, the mean sera concentration of total radioactivity and intact moxidectin declined in a multiphasic manner, with low levels of total radioactivity (0.027 ± <0.011 μg equiv/g) and intact parent (0.019 ± 0.01 μg/g, mean of two available samples) still detected at 168 h post-dose, respectively. The terminal elimination half-lives for total radioactivity and intact parent were 128 ± 14 and 81 ± 18 h, respectively. The estimated AUCs were 11.4 ± 2.3 μg·h/g for moxidectin and 18.0 ± 3.1 μg equiv·h/g for total radioactivity, indicating that after an iv dose, the main circulating radiolabeled component was intact moxidectin. Moxidectin had a clearance of 0.600 ± 0.130 g/min·kg. The mean volume of distribution was 4.136 ± 0.605 L/kg, suggesting that moxidectin distributes extravascularly into deep tissue compart-

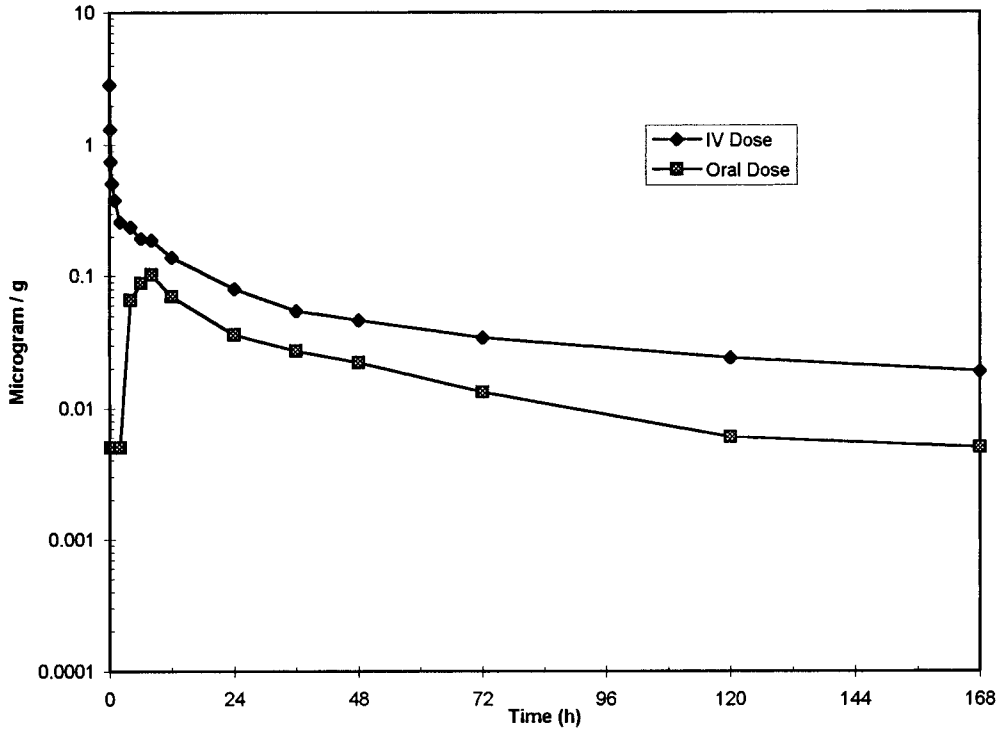

Figure 3. Pharmacokinetics of mean serum moxidectin following a single oral or intravenous administration of ^{14}C-labeled moxidectin to horses at a dose level of 0.4 mg/kg of body weight.

Table 1. Mean Pharmacokinetic (pk) Parameters of Moxidectin following a Single iv Administration of ^{14}C-Labeled Moxidectin to Horses at a Dose Level of 0.4 mg/kg of Body Weight

	total radioactivity		
pk parameters	whole blood	sera	moxidectin sera
$T_{1/2}$ (h)	124 ± 7.4	128 ± 14	81 ± 18
clearance (g/min·kg)	0.544 ± 0.095	0.376 ± 0.062	0.600 ± 0.130
mean residence time (h)	127 ± 30	132 ± 30	92.3 ± 30
volume of distribution (L/kg)	5.862 ± 1.26	4.184 ± 0.944	4.137 ± 0.605
AUC_{0-t} (μg equiv·h/g) or (μg·h/g)	9.07 ± 2.5	12.9 ± 3.4	8.72 ± 0.98
$AUC_{0-\infty}$ (μg equiv·h/g) or (μg·h/g)	12.4 ± 2.3	18.0 ± 3.1	11.4 ± 2.3

Table 2. Mean Pharmacokinetic (pk) Parameters of Moxidectin following a Single Oral Administration of ^{14}C-Labeled Moxidectin to Horses at a Dose Level of 0.4 mg/kg of Body Weight

	total radioactivity		
pk parameters	whole blood	sera	moxidectin sera
C_{max} (μg equiv/g) or (μg/g)	0.091 ± 0.033	0.134 ± 0.045	0.102 ± 0.034
T_{max} (h)	6.0 ± 2.0	6.0 ± 2.0	8.0 ± 0.0
$T_{1/2}$ (h)	138 ± 14	154 ± 26	82 ± 23
AUC_{0-t} (μg equiv·h/g) or (μg·h/g)	3.37 ± 0.68	4.70 ± 0.91	3.51 ± 0.76
$AUC_{0-\infty}$ (μg equiv·h/g) or (μg·h/g)	5.03 ± 1.1	7.67 ± 1.9	4.55 ± 0.68

ments. The moderately low clearance is consistent with a relatively long parent terminal half-life as well as the large volume of distribution.

Following the oral administration of moxidectin, mean peak sera concentrations of total radioactivity (0.134 ± 0.045 μg equiv/g) and intact moxidectin (0.102 ± 0.034 μg/g) were attained within 6−8 h postdose. Thereafter, levels declined in a multiphasic manner, with low levels of radioactivity (0.013 ± 0.003 μg equiv/g) and intact parent (0.005 ± 0.0040 μg/g) still detected at 168 h postdose. Terminal elimination half-lives for total radioactivity and intact moxidectin were 154 ± 26 and 82 ± 23 h, respectively. It is noteworthy that elimination half-lives by the oral route (82 h) was almost identical to the intrinsic value for moxidectin determined by the iv dosing (81 h), suggesting that gastrointestinal absorption is not a rate-limiting process. The AUC values for total radioactivity and intact moxidectin were 7.67 ± 1.9 μg equiv·h/g and 4.55 ± 0.68 μg·h/g, respectively, providing a systemic availability estimate of approximately 40%.

The pharmacokinetic parameters of total radioactivity in whole blood were similar to that observed for serum (Tables 1 and 2). The concentration of radioactivity in serum that would be expected if none of the radioactivity was associated with blood cells was calculated from whole blood concentrations of total radioactivity and packed cell volumes (average values of 28.9 and 31.8% for oral and iv doses, respectively). For each horse, there was a good correlation at each time point between the calculated and observed serum values, demonstrating that the radioactivity was associated with the serum fractions and not bound to or located in red blood cells.

Excretion and Total Radioactive Tissue Residue Distribution. Following oral administration of moxidectin to male horses (group B), radioactivity was

Table 3. Feces: Excretion of Radioactivity in Horses following a Single Oral Administration of ^{14}C-Labeled Moxidectin at a Dose Level of 0.4 mg/kg Body Weight

	% of administered dose			
time (h)	mean daily excretion	SD[a]	mean cumulative excretion	SD
24	17.35	12.743	17.35	12.743
48	41.44	6.959	58.79	13.536
72	8.935	9.006	67.73	8.534
96	2.731	0.880	70.46	8.365
120	1.983	0.503	72.44	8.340
144	2.086	0.772	74.53	8.765
168	2.392	0.874	76.92	7.992
total	76.92	7.992	76.92	7.992

[a] SD, standard deviation.

Table 4. Tissues: Concentration of Total Radioactivity in Horses following a Single Oral Administration of ^{14}C-Labeled Moxidectin at a Dose Level of 0.4 mg/kg of Body Weight

	μg equiv/g of carbon-14 moxidectin				
tissue	002M	003M	004M	mean	SD[a]
back fat	0.912	0.727	0.412	0.684	0.253
omental fat	0.947	0.988	0.717	0.884	0.146
skeletal muscle	0.025	ND[b]	0.037	0.021	0.019
liver	0.308	0.128	0.115	0.184	0.108
kidney	0.047	0.068	0.038	0.051	0.015

[a] SD, standard deviation. [b] ND, not detected. Concentration is assumed to be zero when calculating the mean.

eliminated primarily via the feces (77% of administered dose after 168 h). The radioactivity was eliminated quickly, with >67% of dose being recovered in feces within 72 h postdose, which is consistent with the gastrointestinal tract transition time (Table 3). Renal excretion accounted for only 0.3% of the administered dose. The insignificant renal excretion indicates that biliary excretion is involved in the clearance of moxidectin.

Table 4 summarizes the typical distribution of total radioactive residues (TRR) in edible tissues expressed as micrograms equivalents per gram (ppm). The concentrations of total radioactivity in tissues were highest in the omental fat (0.884 ± 0.146 μg equiv/g) and back fat (0.684 ± 0.253 μg equiv/g). The ^{14}C-residue levels in both fat samples were comparable, indicating a uniform dispersion of [^{14}C]moxidectin and/or its radiolabeled metabolites throughout fat. The lowest ^{14}C-residue levels were observed in the skeletal muscle (0.021 ± 0.019 μg equiv/g). The residue levels in the organs of metabolism and excretion were low, with concentrations of 0.184 ± 0.108 and 0.051 ± 0.015 μg equiv/g in the liver and kidney, respectively. This pattern of distribution in the tissues is similar to that reported in other species (Afzal et al., 1994; Zulalian et al., 1994).

Characterization and Identification of Radiolabeled Residues in Tissues and Feces. The average recoveries of radiolabeled residues from feces and edible tissues were 79 and 96−100%, respectively. Typical HPLC chromatographic properties of the moxidectin reference standard and selected tissue and feces radioprofiles showed that moxidectin had a retention time of approximately 68 min. However, the matrix of some tissue extracts caused the retention characteristics to fluctuate. Thus, retention times of ^{14}C components in the matrices were also recorded relative to moxidectin (RRT). The chromatographic properties of radiolabeled moxidectin and metabolites (both retention times and RRT) were used to tentatively assign their identities as metabolites A, C, D, and E on the basis of a comparison of relative retention times from an earlier cattle metabolism study (Zulalian et al., 1994). These metabolites have also been reported in sheep (Afzal et al., 1994). Unequivocal confirmation of parent in tissue matrices (excluding muscle) and feces was accomplished both by retention time comparison and by electrospray positive ionization mass spectrometry (ESP+MS). The mass spectra of moxidectin showed characteristic ions at *m/z* 662 $(M + Na)^+$ and 640 $(M + H)^+$ and a fragment ion at *m/z* 528 corresponding to the loss of side chain (Stout et al., 1994).

Table 5 summarizes a comparison of the ^{14}C-residue profile in the tissues and feces of horses. Analysis of the extracts from back fat and omental fat confirmed that the major radiolabeled residue was intact moxidectin, accounting for an average of 85% (0.583 μg equiv/g) and 89% (0.790 μg equiv/g) of the TRR, respectively. In both fat samples, two minor metabolites (metabolites C and D) were tentatively identified as the dihydroxylated and monohydroxylated derivatives of moxidectin, respectively. However, the concentration of these metabolites represented <10% of the TRR in either of the fat samples. Analysis of the organic extract of liver from each of the animals confirmed moxidectin was the major radiocomponent, which accounted for an average of 60% (0.112 μg equiv/g) of the TRR. The remaining radioactivity was distributed among at least seven other polar metabolites, including metabolites C−E. The latter is also a hydroxylated metabolite of moxidectin. The only metabolite that exceeded 10% of the TRR was metabolite E, accounting for 11% (0.018 μg equiv/g) of the TRR. Similarly, analysis of the kidney extracts showed moxidectin was the major residue, accounting for an average of 80% (0.040 μg equiv/g) of the TRR. Chromatographic analysis of muscle extracts indicated that moxidectin was the major radiolabeled component, accounting for an average of 48% (0.010 μg equiv/g) of the TRR. All other components were minor with the exception of metabolite E, which averaged 14% (0.003 μg equiv/g) of

Table 5. Mean ^{14}C Residue Profile in the Tissues of Horses, 7 Days Postdose, Expressed as ppm Equivalents and Percentage of the TRR

component[a]	liver ppm (% TRR)	kidney ppm (% TRR)	muscle[c] ppm (% TRR)	omental fat ppm (% TRR)	backfat ppm (% TRR)	feces[d] ppm (% TRR)
moxidectin	0.112 (60)	0.040 (80)	0.010 (48)	0.790 (89)	0.583 (85)	2.72 (69)
metabolite A	ND[e]	0.001 (1)	ND	ND	ND	0.007 (<1)
metabolite C	0.006 (4)	ND	ND	0.015 (2)	0.016 (2)	0.120 (3)
metabolite D	0.019 (9)	0.002 (3)	ND	0.010 (1)	0.002 (<1)	0.081 (2)
metabolite E	0.018 (11)	0.002 (3)	0.003 (14)	ND	ND	0.152 (3)
unidentified[b]	0.027 (15)	0.004 (8)	0.008 (38)	0.059 (7)	0.031 (5)	0.094 (2)

[a] Based on HPLC analysis on an Apex C_{18} reversed-phase column. Mean TRR levels in liver, kidney, muscle, abdominal fat, and back fat were 0.184, 0.051, 0.021, 0.884, and 0.684 ppm, respectively. [b] A mixture of at least two or more components. [c] Based on the HPLC analysis of muscle samples from two animals only, where TRR was above the detection limit of the radioassay (Table 3) [d] Based on the HPLC analysis of feces sampled at 24−48 h postdose. Mean TRR in feces was 4.059 ppm. [e] ND, not detected.

the TRR. Results from the analysis of feces (sampled at 24−48 h postdose) showed that nearly all of the radioactivity extracted was associated with intact moxidectin, which strongly suggested that moxidectin was the main radiolabeled component excreted in bile. Other minor metabolites were noted in feces and were assigned identities as metabolites A, C, D, and E, similar to tissue extracts.

In summary, the results of the chromatographic and spectrometric analysis of each of the matrices discussed above allowed a pathway for the metabolism of moxidectin in horses to be constructed (Figure 1). Moxidectin was the major component of the residue in all matrices. The tentative identification of the hydroxylated metabolites, C and D in the fat, C, D, and E in the liver, A, D, and E in the kidney, E in muscle, and A, C, D, and E in the feces, indicated that phase I oxidative metabolism was the primary biotransformation route for moxidectin in equids.

Conclusion. Following a single oral administration of ^{14}C-labeled moxidectin to horses at a dose level of 0.4 mg/kg of body weight, approximately 40% of the dose was available in the systemic circulation. Once in the systemic circulation, moxidectin is widely distributed into tissues and was the major component of the total radioactive residue. Radiolabeled material was eliminated mainly via biliary mechanisms and subsequently voided with feces. Although minor metabolites were noted, intact moxidectin was the principal excretory component. These data suggest that the absorption, tissue distribution, metabolism, and elimination of moxidectin in the horse were similar to those reported for sheep, cattle, and rat.

ABBREVIATIONS USED

AUC, area under the concentration/time curve; PCV, packed cell volume; RRT, relative retention time; TRR, total radioactive residue.

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