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HAIRY-NOSED WOMBAT (*LASIORHINUS LATIFRONS*)

Clare E. Death,[1,5,6] David A. Taggart,[2,3] Desmond B. Williams,[4] Robert Milne,[4] David J. Schultz,[2] Carlysle Holyoake,[1] and Kristen S. Warren[1]

[1] Conservation Medicine Program, School of Veterinary and Biomedical Sciences, Murdoch University, 90 South Street, Murdoch, Western Australia, 6150, Australia
[2] Royal Zoological Society of South Australia, Adelaide Zoo, Frome Rd., Adelaide, South Australia, 5000, Australia
[3] School of Earth and Environmental Science, University of Adelaide, South Australia, 5005, Australia
[4] School of Pharmacy and Medical Sciences, Sansom Institute for Health Research, University of South Australia, North Terrace, Adelaide, 5001, Australia
[5] Current address: Faculty of Veterinary Science, The University of Melbourne, 250 Princess Highway, Werribee, Victoria, 3030, Australia
[6] Corresponding author (email: dclare@unimelb.edu.au)

ABSTRACT: Sarcoptic mange, caused by *Sarcoptes scabiei* var. *wombati*, could be a significant threat to populations of southern hairy-nosed wombats (*Lasiorhinus latifrons*; SHNW) in Australia. Treatment is currently based on the off-label use of various parasiticidal drugs, with limited clinical efficacy trials. Our primary aim was to determine the pharmacokinetic parameters of a macrocyclic lactone, moxidectin, to assist in the development of effective treatment protocols. Pharmacokinetic parameters were determined in four female SHNW following a single subcutaneous injection of 0.2 mg/kg moxidectin. Blood samples were collected for 38 days following injection (August–September 2008), for analysis using liquid chromatography and tandem mass spectrometry. The mean peak plasma concentration occurred at 13.6 hr, with a mean peak plasma level of 98.6 ng/ml. The mean elimination half-life was 5.03 days, resulting in a mean area under the curve of 377 ng.day/ml. The peak plasma moxidectin concentration was higher than that seen in livestock species but the plasma elimination half-life was shorter. This study suggests that a single injection of 0.2 mg/kg moxidectin may not be sufficient to clear a mange infection in this species.

Key words: *Lasiorhinus latifrons*, macrocyclic lactone, mange, marsupial, moxidectin, pharmacokinetics, *Sarcoptes scabiei*, wombat.

INTRODUCTION

The earliest report of an epizootic of sarcoptic mange in wombats, caused by *Sarcoptes scabiei* var. *wombati* (Acari: Sarcoptidae), was in free-living common wombats (*Vombatus ursinus*) in New South Wales, Australia (Gray, 1937). Outbreaks have been reported more recently in the southern hairy-nosed wombat (*Lasiorhinus latifrons*; SHNW; Ruykys et al., 2009), but not in the critically endangered northern hairy-nosed wombat (*Lasiorhinus krefftii*; Bryant and Reiss, 2008). *Sarcoptes scabiei* burrows in the lower stratum corneum of the skin (Arlian, 1989) and may cause mild localized lesions or progress to a generalized severe crusting hyperkeratotic dermatosis with alopecia, excoriations, deep fissures, and secondary pyoderma (Bryant and Reiss, 2008).

Despite the obligate parasitic nature of *S. scabiei*, transmission probably occurs after indirect contact between wombats, which are known to share burrows (Skerratt et al., 1998). The *S. scabiei* life cycle takes approximately 2 wk to complete on the host; however, all life stages can be found in the environment and some survive off the host for up to 3 wk under ideal conditions of low temperature and high relative humidity (Arlian, 1989). Burrows are likely to enhance mite survival off the host by providing such conditions (Skerratt et al., 1998).

Sarcoptic mange is the major infectious disease of the Vombatidae (Hartley and English, 2005). In common wombats, epizootics can dramatically reduce local abundance and threaten the survival of small, isolated populations (Skerratt, 2003). The SHNW has a limited, patchy distribution, and sarcoptic mange can

cause significant morbidity and mortality, with substantial effects on local abundance (Taggart and Temple-Smith, 2008; Ruykys et al., 2009). There are also significant welfare implications for severely affected wombats. Treatment of mange can be important in wildlife conservation programs (Wobeser, 2002); however, dosages are often based on anecdotal experience, extrapolations, and allometric scaling (Mayer et al., 2006). Macrocyclic lactones, such as ivermectin and moxidectin, can effectively treat wombats with sarcoptic mange (Skerratt, 2003; Skerratt et al., 2004; Ruykys, 2005; Bryant and Reiss, 2008), but pharmacokinetic studies have not been performed. Although the chemical structure and apparent pharmacokinetic behavior of these compounds may show differences, the overall pharmacokinetic and efficacy patterns show similarities (Lanusse and Prichard, 1993). Clinical trials using moxidectin have not been performed; however, Ruykys (2005) found that one subcutaneous (SC) injection of ivermectin at 0.2 mg/kg cleared mild to moderate mange in *L. latifrons*. Skerratt et al. (2004) also found that mild mange in *V. ursinus* was cured by one injection of ivermectin at a higher dose (0.4 mg/kg), though retreatment was required in moderate mange. In another study, Skerratt (2003) used 0.3 mg/kg of ivermectin to treat cases of differing severity in *V. ursinus* and found that three treatments 10 days apart were generally effective, with a second course required in severe cases. Reinfection may occur from the environment (Arlian, 1989) or when eggs hatch up to 7 days posttreatment, as macrocyclic lactones are not effective against mite eggs (Arends et al., 1999; Papadopolous et al., 2000).

Pharmacokinetics refers to the study of the bodily absorption, distribution, metabolism, and elimination of drugs (Lanusse and Prichard, 1993). The role of pharmacokinetic studies in assessing the efficacy of parasiticidal drugs relies on the assumption that plasma concentration profiles reflect the active concentration profiles at the site of parasite location (Lifschitz et al., 1999). In general, tissue and plasma concentrations of moxidectin exhibit a parallel time-profile disposition, with levels in tissue (including skin and hair) being higher and persisting for longer than levels in plasma (Lifschitz et al., 1999).

Data relating to the minimum inhibitory drug concentrations required for effective treatment of microorganisms does not exist for parasites, presenting difficulties when determining dosages for antiparasitic drugs (Lifschitz et al., 1999). We rely on clinical trials of efficacy, in combination with pharmacokinetic data, to develop drug dosage protocols. Therefore, the primary aim of this study was to determine the pharmacokinetic parameters and the plasma drug disposition profile of moxidectin in the SHNW following SC administration. Because SHNW have a metabolic rate of only 42% of the predicted eutherian level (Wells, 1978), we hypothesized that the elimination half-life of moxidectin from SHNW plasma would be longer than livestock species.

MATERIALS AND METHODS

Animal handling and sample collection

Four female SHNW were acquired from the Murraylands region of South Australia (34°55′S, 139°28′E). They were captured at night using a "stunning" technique (Taggart et al., 2003) and acclimatized to captive conditions for 3 mo, until displaying stable food consumption and bodyweight. To enable handling and blood sample collection, wombats were anesthetized with zolazepam/tiletamine (3–6 mg/kg intramuscular, Zoletil 100®; Virbac Animal Health, Milperra, New South Wales, Australia; Taggart et al., 2005). During the first 24 hr of the blood collection regimen all wombats were also anesthetized using isoflurane (3.5%) delivered in oxygen (2 l/min) via face mask for approximately 2 min prior to each venipuncture. Blood was collected from the cephalic vein using 22-gauge needles, placed into heparinized tubes (BD lithium heparin Vacutainer®, Franklin Lakes, New Jersey, USA), and then centrifuged at approximately 1200 × G for 15 min. The

recovered plasma was stored frozen at −20 C in plain plastic tubes and analyzed within 3 wk of collection.

On day 0 each wombat was given 0.2 mg/kg moxidectin (Cydectin® injectable, Virbac Animal Health, Milperra, New South Wales, Australia) injected subcutaneously in the groin region. Blood samples were taken before and at 2, 4, 6, 8, 12, and 24 hr posttreatment, and then on day 2, 4, 7, 10, 21, 30, and 38 (August 2008 to September 2008). Preliminary experiments determined that beyond this time, the levels of moxidectin in the plasma would be below the limit of quantification of this analytical method for measuring moxidectin.

Liquid chromatography–tandem mass spectrometry (LC-MS-MS)

The solvents used during the analysis, acetonitrile (ACN; J.T. Baker, Mallinckrodt Baker Inc., Phillipsburg, New Jersey, USA) and formic acid (98%, Fluka, Sigma-Aldrich Corp., St. Louis, Missouri, USA), were of high performance liquid chromatography grade. Water was purified with a Milli-Q system (Millipore, Billerica, Massachusetts, USA). A 10 mg/ml commercial solution of moxidectin (Cydectin® injectable, Virbac Animal Health, New South Wales, Australia) was utilized as the primary analyte and a 120 mg/ml commercial solution of selamectin (Revolution®, Pfizer Animal Health, West Ryde, New South Wales, Australia) as the internal standard. The chromatographic system consisted of an SCL-10AVP controller, LC-10ADVP pump, and SIL-HTc AutoSampler (all from Shimadzu Corporation, Columbia, Maryland, USA), connected to a triple quadrupole API™ 3000 LC-MS-MS mass spectrometer (Applied Biosystems Inc., Foster City, California, USA). The mass spectrometer was operated in positive electrospray ionization mode with the temperature of the nebulizer set at 250 C. A mobile phase consisting of ACN:H_2O:formic acid (90:10:0.1) was pumped through an Alltech Alltima HP C_{18} HL column (50 mm × 2.1 mm, 3-μm particle size, Grace, Deerfield, Ilinois, USA) at 0.2 ml/min at ambient temperature. Peak area ratios (area of analyte/area of internal standard) were used to estimate the moxidectin concentration in spiked and experimental samples.

Calibration standards were prepared fresh in drug-free plasma at nominal concentrations of 1, 2, 5, 10, 20, 50, 80, and 100 ng/ml; quality controls (QCs) were prepared using a separate working standard at 2.5, 15, and 75 ng/ml, plus blank and internal standard only samples. Unknown samples, standards, and quality controls (duplicate 100 μl of each) were prepared by combining with 50 μl of internal standard stock solution and 200 μl of ACN, mixing on a vortex mixer and centrifuging at approximately 2,500 × G (Varifuge 3.0, Heraeus-Sepatech, Osterode, Germany) for 10 min at 4 C. The supernatant was transferred to a new tube and mixed with 100 μl H_2O, and 50 μl was injected into the LC-MS-MS system. The retention times were approximately 3 min for the analyte and 4.7 min for the internal standard and the overall run time was 10 min.

The linear regression lines for moxidectin in the range between 1 ng/ml and 100 ng/ml showed an average correlation coefficient (r) of 0.9983 over three analytical runs. The intraday accuracy and precision using low, medium, and high QC samples in replicates of six were within acceptable limits (Shah et al., 2000). The QC samples assayed in duplicate for each analytical run were found to be within 10% of their nominal values when back-calculated from the calibration curve. The validated lower limit of quantification was 1 ng/ml. One freeze–thaw cycle caused changes in measured concentrations of less than 5%.

Pharmacokinetic and statistical analyses

Concentrations of moxidectin in the plasma of individual animals were evaluated by noncompartmental analysis using WinNonlin (Version 5.2., Pharsight Corporation, Mountain View, California, USA). The peak concentration (C_{max}) and the time to peak concentration (T_{max}) were read from the plotted concentration-time curve for each animal. The terminal rate constant (k) was calculated from the last three data points of the concentration-time curves. The terminal (elimination) half-life ($T_{1/2(el)}$) was calculated as

$$T_{1/2(el)} = \frac{\ln(2)}{k_{el}}$$

The areas under the concentration-time curves (AUC) were calculated using the trapezoidal rule (Gibaldi and Perrier, 1982) and further extrapolated to infinity by dividing the last experimental concentration by the terminal rate constant (k). The SC mean residence time (MRT) for moxidectin was calculated as

$$MRT_{SC} = \frac{AUMC}{AUC}$$

where AUC is as defined previously, and the area under the first moment curve (AUMC) is defined as the AUC of the product of time and

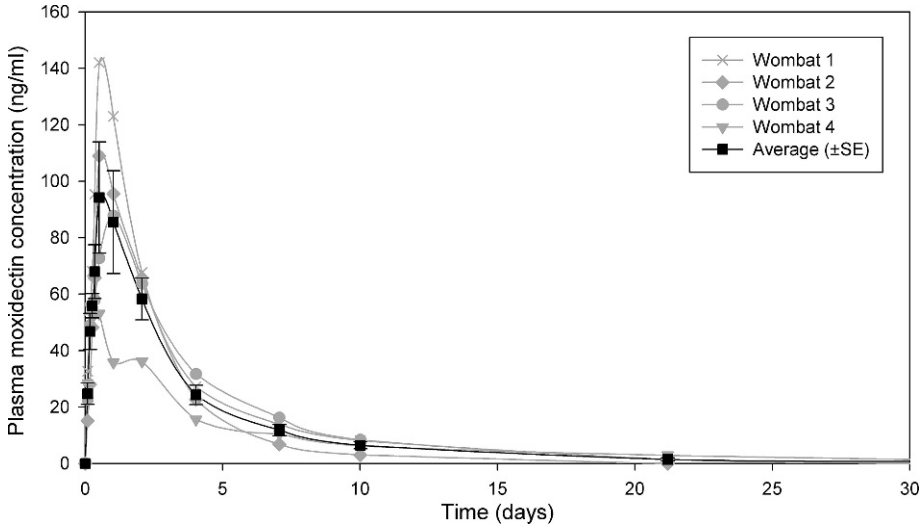

FIGURE 1. Individual (gray symbols) and mean (black symbols) plasma concentrations of moxidectin obtained after subcutaneous administration of 0.2 mg/kg moxidectin to four southern hairy-nosed wombats (*Lasiorhinus latifrons*), August 2008 to September 2008, Adelaide, South Australia. Error bars represent standard error.

plasma concentration (calculated by the method of Riegelman and Collier, 1980).

RESULTS

The individual and mean profiles of concentration of moxidectin in plasma against time are shown in Fig. 1. Moxidectin was detected in plasma between 2 hr and 30 days postadministration. Individual and mean pharmacokinetic parameters are given in Table 1. The peak plasma concentration occurred at a mean of 13.6 hr, with a mean peak plasma level of 98.6 ng/ml, and the mean AUC was 377 ng·day/ml. Thereafter, the plasma concentration decreased progressively with a mean elimination half-life of 5.03 days. Half-lives in the four SHNW studied ranged from 2 days to 9.5 days.

DISCUSSION

Our primary aim was to determine the concentration of moxidectin in plasma over time, as reflected in the AUC, and the half-life for its elimination from plasma following a single SC dose of 0.2 mg/kg moxidectin. To maintain concentrations over an approximate twofold range (from maximum to minimum values), it is generally required that dosing intervals be similar to the half-life of a drug (Birkett, 1998). Thus, our data validate a dosing protocol recommended by Bryant and Reiss (2008; i.e., 0.2–0.3 mg/kg weekly, until 2 wk after negative skin scrapings are obtained). Based on a plasma half-life of approximately 5 days in the SHNW, this is the regimen most likely to maintain reasonably consistent plasma levels and achieve reliable elimination of sarcoptic mange infections in situations where it is possible to repeatedly treat individuals (i.e., captivity). When these results are assessed in the context of the life cycle and off-host survival of *S. scabiei*, it is unlikely one injection of 0.2 mg/kg moxidectin would reliably clear a mange infection, due to the pressure of immediate reinfection by mites in the environment and eggs hatching on the animal. Clinical trials will be required in order to verify these assumptions.

The plasma elimination half-life of moxidectin in the SHNW was shorter and the peak concentration was higher than values cited for cattle, goats, and

TABLE 1. Individual and mean pharmacokinetic parameters obtained after subcutaneous administration of 0.2 mg/kg moxidectin to four southern hairy-nosed wombats (*Lasiorhinus latifrons*), during August 2008 to September 2008 in Adelaide, South Australia.

Kinetic parameters[a]	Wombat 1	Wombat 2	Wombat 3	Wombat 4	Mean±SE
C_{max} (ng/ml)	142	109	87.7	55.7	98.6±18.2
T_{max} (hr)	12.4	12.6	25.0	4.50	13.6±4.30
$T_{1/2(el)}$ (day)	4.40	2.06	4.14	9.52	5.03±1.59
AUC total (ng·day/ml)	461	334	419	295	377±38.2
$MRT_{(SC)}$ (day)	4.52	3.09	5.13	9.58	5.58±1.40

[a] C_{max} = maximum plasma concentration; T_{max} = time to maximum plasma concentration; $T_{1/2(el)}$ = elimination half-life; AUC total = area under the curve; MRT = mean retention time; SC = subcutaneous.

sheep receiving the same treatment (Lanusse et al., 1997; Escudero et al., 1999; Barber et al., 2003). The hypothesis that the elimination half-life of moxidectin from SHNW plasma would be longer than livestock due to the lower metabolic rate of the wombat was therefore rejected. In addition to metabolic clearance of moxidectin, other factors such as absorption from the injection site (dependent on the tissue characteristics and degree of perfusion of the site by blood) and pattern of distribution throughout the body may all contribute in variable degrees to half-life. The SHNW also demonstrate significant digestive and renal physiologic adaptations to their semiarid lifestyle (Hume and Barboza, 1998; Wells and Green, 1998; Taggart and Temple-Smith, 2008) that may contribute to pharmacokinetic parameters assessed in this species. Lipophilic compounds such as moxidectin will be preferentially sequestered into adipose tissue and this contributes to their persistence. The levels of body fat that would be considered normal in livestock are unlikely to be encountered in wild wombats, thus it cannot be assumed that lipophilic compounds will persist to a similar degree. In sheep and pigs, moxidectin is less persistent in thin compared to fat animals (Craven et al., 2002a). This study supports the notion that the ratio of fat to body weight influences the pharmacokinetics of lipophilic macrocyclic lactones (Craven et al., 2002a, b; Barber et al., 2003) more than metabolic rate. Body condition indices correlate poorly with body fat levels in wombats (Woolnough et al., 1997), which would make this difficult to investigate in clinical trials.

To control mange in wild wombat populations, it is important to know whether a single treatment is likely to eradicate *S. scabiei* infection, because retreatment is difficult. If one treatment does not eliminate mites, then the welfare of the individual may not be addressed adequately. Ineffective treatments could extend the period of infectivity of diseased individuals within populations and encourage development of resistant parasites, while excessive dosing may waste resources and cause toxicity in compromised individuals. Although reinfection of a successfully treated individual in the wild could occur, it has been shown that dogs medically cured of a sarcoptic mange infection are less susceptible to infection when rechallenged (Arlian et al., 1996). Skerratt et al. (2004) proposed the possibility of halting the transmission of *S. scabiei* within a wombat population by reducing the average intensity of infection to a low level, via removal of moderate to severely parasitized animals, and treatment of the remainder with an effective long-acting acaricide. Our results do not suggest that a single injection of 0.2 mg/kg moxidectin in wild wombats could achieve such an outcome.

Treatment of disease in fragmented wildlife populations may become important as continuing threats negatively impact habitat and fauna. To this end,

effective management of sarcoptic mange in wombats may play a crucial role in their conservation. This study is the first pharmacokinetic trial in a wombat species and these results add objectivity to decisions about mange treatment in wombats. Further studies should compare the pharmacokinetic parameters of oral, injectable, topical, short-acting, and long-acting formulations of macrocyclic lactones in the wombat and utilize clinical trials to determine the effectiveness of various treatment protocols.

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