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Comparative *in vitro* characterization of moxidectin and doramectin percutaneous absorption through bovine skin

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Topical formulations have achieved worldwide acceptance in veterinary medicine because their administration is an easy, less labor-intensive and nonstressing form. Any chemical compound that comes in contact with the skin has the potential to be locally and/or systemically absorbed. However, many factors related to the features of animal skin, composition of the topical formulation and to the drug itself can determine marked differences in the percutaneous absorption process. The aim of the current work was to characterize the pattern of *in vitro* percutaneous absorption for moxidectin (MXD) and doramectin (DRM), two of the most worldwide used topical macrocyclic lactone antiparasitic compounds in cattle. The work included the development of a simple and inexpensive *in vitro* assay useful to predict *in vivo* drug percutaneous absorption in cattle. Both drugs were administered as the commercial formulations intended for their topical administration to cattle. The *in vitro* studies were carried out using modified Franz-type vertical diffusion cells. Cattle skin slices of 500 μm thickness were prepared using a dermatome to separate the stratum corneum and upper epidermis from dermis and subcutaneous tissue. The receptor medium was sampled up to 72 h postadministration and drug concentrations were measured by HPLC. The parameters used to estimate the comparative *in vitro* skin permeation showed marked differences between DRM and MXD. A 5.29-fold longer lag time (T_{lag}) was observed for DRM. Similarly, the flux (J) (2.93-fold) and the permeation coefficients (K_p) (2.95-fold) in cattle skin were significantly higher ($P < 0.05$) for DRM compared to those obtained for MXD. Additionally, the data obtained from the *in vitro* permeation studies was correlated with the plasma concentrations of both compounds achieved *in vivo* in cattle treated with the same topical formulations. Correlation coefficients between percentage of drug permeated *in vitro* vs. percentage of drug absorbed *in vivo* (up to 48 h post-treatment) were 0.856–0.887 (MXD) and 0.976–0.990 (DRM). However, the highest *in vitro*–*in vivo* correlations for both molecules were observed up to 24 h post-treatment A rapid screening method for testing different topical formulations can be achieved with the simple *in vitro* cattle skin permeation technique described here, which has been successfully adapted to test the comparative percutaneous absorption of MXD and DRM.

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INTRODUCTION

A great interest for the topical administration of drugs in veterinary medicine has developed worldwide (Magnusson *et al.*, 2001; Riviere & Papich, 2001; Baynes, 2004; Gokbulut *et al.*, 2010). The development of new formulations will need *in vitro* techniques to predict percutaneous drug absorption. These types of experimental systems are necessary for developing

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new/alternative pharmaceutical formulations and for characterizing their percutaneous absorption in a simple manner. Hence, useful information on the diffusion behavior of the drug and effects of the excipients on the barrier function of the skin can be generated (Wagner *et al.*, 2000). Even though this information can be obtained from *in vivo* studies, ethical, economical, and analytical considerations preclude their utilization. Consequently, the development and validation of *in vitro* assays have become of great interest (Wagner *et al.*, 2000; OECD, 2004). Ideally, testing of new formulations should be performed using skin samples of the target species due to significant differences in structure, composition and metabolic capacity (Monteiro-Riviere *et al.*, 1990; Stahl *et al.*, 2009); however, in human medicine this implies great difficulties that have stimulated the search of surrogate membranes (Schmook *et al.*, 2001; Organisation for Economic Co-operation and Development (OECD), 2004; Vallet *et al.*, 2007). Conversely, access to skin of different species, particularly of livestock species, is not a limitation in veterinary medicine. Sufficient amounts of skin samples can be obtained from slaughterhouses and appropriately transported to laboratory facilities. An outstanding advantage of the skin is that it can be relatively easily preserved. Storage at −20 °C for relatively short periods is considered suitable, given the characteristics of the stratum corneum (Organisation for Economic Co-operation and Development (OECD), 2004; Marti-Mestres *et al.*, 2007).

Moxidectin (from the mylbemycin family) and doramectin (avermectins) are among the most worldwide used macrocyclic lactone endo-ectoparasiticide compounds in cattle. The traditional injectable and, the most recently introduced, topical formulations are currently available in the pharmaceutical market to be used in cattle. Although the disposition kinetics and tissue distribution of these highly lipophilic macrocyclic lactones have been studied in topically-treated cattle (Gayrard *et al.*, 1999; Sallovitz *et al.*, 2002, 2003, 2005; Bousquet-Mélou *et al.*, 2004), only limited information is available on the pattern and features of their skin permeation process. The objective of the current work was to assess the comparative percutaneous absorption of moxidectin (MXD) and doramectin (DRM) in bovine skin using a simple diffusion technique. The work included the development of an easy, quick, and inexpensive method adapted to assess drug absorption through bovine skin. Additionally, in order to determine the predictive value of the *in vitro* method, the results were correlated with *in vivo* data (plasma concentration levels) previously obtained in our Laboratory after pour-on administration of both compounds to Holstein calves.

MATERIALS AND METHODS

Experimental units

Diffusion cells. Diffusion cells utilized in the present work were modified Franz-type vertical diffusion cells, adapted to work with bovine skin. Modifications were mainly related to the volumes of receiver and donor chambers (9 and 2 mL, respectively) and to the absorptive area (1.767 cm^2). Assays were performed in batches of five diffusion cells. A number of 10 cells were used per drug.

Receiver medium. Receiver medium was composed of bovine albumin [4.5%; Albumin from bovine serum (BSA), 98%, Sigma Chemical Co., St Louis, MO, USA], ethanol (20%) and buffer phosphate 0.1 M (19% NaH_2PO_4 0.1 M + 81% Na_2HPO_4 0.1 M, pH 7.4; J.T. Baker, Phillipsburg, NJ, USA). Receptor fluid was stirred with a rod at 600 rpm. Temperature of the system was kept at 37 °C by circulating heated water through the outer jackets of the cells (Heating Circulator Model ED; JULABO Labortechnik GmbH, Seelbach, Germany).

Ethanol and BSA were added to the receptor receiver medium to produce more favorable (sink) conditions for lipophilic drugs as are MXD and DRM (log *P* values 6 and 5.6, respectively), determining that the low water solubility of the drug will not limit the permeation (sink conditions) (Scott & Ramsey, 1987; Dick, 1999; Cross *et al.*, 2003; OECD, 2004, 2010). The reason for adding ethanol was to enhance sink conditions in the receptor medium for these lipophilic drugs, since, in previous works in our laboratory, the addition of BSA only to the receptor fluid was not enough to achieve measurable drug fluxes. *In vivo*, sink conditions are provided by the blood flow and the elimination of the drug, that cannot be done in the static diffusion cell system. Addition of ethanol, up to 50%, is suggested in the 2010 guideline issued by OECD (2010). Similarly, stirring of the receiver medium is needed to avoid the influence of unstirred water layers that may be formed with different permeated solute concentrations. The most concentrated layers locate close to the skin sample and will limit solute permeation (Henning *et al.*, 2009).

Skin samples. Bovine skin samples were obtained from the local abattoir from the same animal (Holstein steer). Skin and subcutaneous tissue were cut into 20 × 20 cm square pieces, wrapped with aluminum foil, put into plastic bags with hermetic closing and stored at −18 °C until assay (up to 2 months). For the assays, skin was defrosted; hair was cut with an electrical clipper fitted with a surgical blade (*c.* 0.2 mm height). Stratum corneum and upper epidermis were separated from dermis and subcutaneous tissue with a dermatome, producing slices of 500 μm of thickness. Slices were cut in circles of 3 cm in diameter and mounted on the diffusion cells. All skin used in the assays described here was obtained from the middle back, between the scapular and the lumbar regions.

Drug treatments

After mounting on the diffusion cells, the surface of the skin was hydrated for 1 h with sodium phosphate buffer (0.1 M), allowing the system to equilibrate. After removing by aspiration the buffer solution from the surface of the skin, treatment was carried out with commercial formulations of MXD (Cydectin® 0.5% Pour-on, Fort Dodge) and DRM (Dectomax® 0.5% Pour-on, Pfizer) for cattle. A volume of 850 μL of each formulation was applied onto the skin in the donor chamber, which represented 2.41 mg/cm^2 of skin.

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Sampling times and sample collection

Samples were collected at predetermined times. For MXD at 0, 1, 2, 3, 4, 6, 8, 12, 24, 30, 48, 54, 72 h postadministration, and for DRM at 0, 2, 4, 6, 12, 20, 24, 28, 30, 36, 48, 54, 58 and 72 h postadministration. These differences in sampling times were based on the kinetic behavior observed *in vivo* in previous studies, where MXD showed a faster absorption phase (more sampling times at the beginning) compared to DRM (more sampling at later times) (Sallovitz *et al.*, 2002, 2005).

At each sampling time, 0.1 mL from the receiver chamber of each diffusion cell was collected and an equal volume of drug-free receiver medium was replaced. Samples were stored at −18 °C until analysis.

Drug analysis

For the validation procedure, aliquots (0.1 mL) of receiver medium were spiked with standard solutions of MXD and DRM, achieving concentrations within the range of 0.5–80 ng/mL. Both drugs were utilized as internal standards of each other. Hence, MXD was the internal standard for DRM validation process and vice-versa. The limit of quantification for both drugs was 0.5 ng/mL and linearity ranged between 0.9985 and 0.9994. Recovery percentages were 92.9% and 91% for MXD and DRM, respectively.

Drug extraction was performed in one liquid–liquid phase. After fortification with the internal standard, acetonitrile (1 mL, HPLC grade) was added to each receiver medium aliquot (0.1 mL). Mixtures were agitated for 20 min, sonicated for 10 min and centrifuged at 18 000 ***g*** for 15 min. Supernatants were collected in glass tubes and evaporated to dryness at 60 °C in a water bath under a nitrogen stream. A derivatization process to render endectocide molecules fluorescent was performed according to the technique described by De Montigny *et al.* (1990). Dry residues were dissolved in 100 μL of a 1-methyl-imidazole (Aldrich, St. Louis, MO, USA) solution in acetonitrile (1:1 v/v) and the derivatization reaction was initiated by adding 150 μL of trifluoro-acetic acid anhydride (Aldrich) in acetonitrile (1:2 v/v). After the reaction (<1 min), a 100-μL aliquot was injected directly into the chromatographic system (Shimadzu 10 A HPLC System; Shimadzu, Kyoto, Japan).

The chromatographic conditions included a mobile phase of water–methanol–acetonitrile (3:40:57 v/v/v) at a flow rate of 1.5 mL/min through a reverse phase C_{18} column (5 μm, 4.6 × 250 mm; Phenomenex, Torrance, CA, USA) kept in an oven at 30 °C. Fluorescence detection (Spectrofluorometric detector RF 10; Shimadzu) was at an excitation wavelength of 365 nm and reading at an emission wavelength of 475 nm.

Kinetic and statistical analyses

The volume of formulation applied in the donor chamber allowed to achieve and keep the maximum absorption rate (steady state) (Organisation for Economic Co-operation and Development (OECD), 2004). Estimated parameters to characterize drug appearance in the receiver medium were flux (J), permeability coefficient (K_p), lag time (T_{lag}) and apparent diffusion coefficient (D_{app}) (Pitman *et al.*, 1983; Organisation for Economic Co-operation and Development (OECD), 2004; Niedorf *et al.*, 2008; Henning *et al.*, 2009).

As samples collected were replaced by drug-free medium, the derived concentration values were corrected for progressive dilution using the equation (Khan *et al.*, 2005):

$$M_{t(n)} = V_r \cdot C_n + V_s \cdot \sum_{m=1}^{n-1} C_m$$

where $M_{t(n)}$ is the current cumulative mass of drug transported across the skin at time t, V_r is the volume of the receiver medium, C_n is the current concentration in the receiver medium, V_s is the volume of the sample removed for analysis, and $\sum_{m=1}^{n-1} C_m$: the summed total of the previous measured concentrations from $m = 1$ to $n - 1$

In the linear graph of cumulative drug mass/cm^2 vs. time, least-squares linear regression analysis was used to determine the gradient of the steady-state segment of each permeation experiment. The slopes represent the drug fluxes (J). The linear segment was determined by an iterative procedure described by Niedorf *et al.* (2008). Iteration were stopped when a value of $r^2 > 0.95$ from a fit over, at least, five points was obtained (Niedorf *et al.*, 2008).

The lag time (T_{lag}) is the intercept of the tangent of the linear part of the cumulative permeated drug profile with the x-axis ($y = 0$) (Organisation for Economic Co-operation and Development (OECD), 2004; Niedorf *et al.*, 2008). It was estimated with the parameters of the linear regression.

The permeability coefficient was estimated by using the following equation:

$$K_p = \frac{J}{C_i}$$

where, J is the flux as mentioned above (ng/h) and C_i is drug concentration at the beginning of the experiment in the donor chamber (ng/mL) (Organisation for Economic Co-operation and Development (OECD), 2004).

The apparent diffusion coefficient (D_{app}, cm^2/h) was estimated according to the following equation (Pitman *et al.*, 1983; Henning *et al.*, 2009):

$$D_{app} = \frac{L^2}{6 \cdot T_{lag}}$$

where, L is the thickness of the membrane (stratum corneum and upper epidermis) in cm and T_{lag} is the lag time as previously described.

Drug absorption from *in vivo* data (Sallovitz *et al.*, 2002, 2005) was estimated up to 48 h postadministration. Two methods were used to estimate absorption: (i) cumulative AUC_{0-t} (partial AUC_{0-t}), and (ii) fraction absorbed (FA, Wagner–Nelson method).

Areas under the plasma concentration vs. time curves were calculated by using the linear trapezoidal rule and further

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extrapolated to infinity (Gibaldi and Perrier, 1982). The FA was calculated by using the Wagner–Nelson function: FA = (C_t + ke*$AUC_{0–t}$)/(ke*$AUC_{0–infinity}$) × 100; where ke is the terminal phase rate constant (Wagner, 1974; Akimoto *et al.*, 1995).

The *in vivo–in vitro* correlations were performed by correlating mean pharmacokinetic values ($AUC_{partial}$ and FA) and the mean percentages of drug permeated.

Linear regression, correlation, and statistical analyses were performed by using GraphPad InStat®, version 3.00 software (GraphPad Software Inc., La Jolla, CA, USA). Kolmogorov–Smirnov test was applied to determine normality of data distribution. Statistical significance of the differences was determined using the Student *t*-test (unpaired *t*-test Welch corrected if variances were different) or Mann–Whitney test (if data distribution was not normal). A $P < 0.05$ value was considered significant.

RESULTS

Both MXD and DRM were able to permeate through bovine stratum corneum *in vitro* and were detected in the receiver media from 2 and up to 72 h after their application over the skin sample. However, data from the last sampling times (72 h postadministration) were excluded to avoid possible unreliable data due to loss of barrier capacity of the stratum corneum. Figure 1 shows the cumulative drug mass per cm^2 of MXD and DRM permeated through bovine stratum corneum up to 48 h postadministration. The steady-state portion for MXD was observed between 3.10 h (±0.57 SD; range 2–4) and 20.40 h (± 7.59 SD; range 12–30) postadministration (Fig. 2). DRM steady-state was observed between 12.20 h (±6.14 SD; range 4–24) and 40.20 h (±10.22 SD; range 30–60) postadministration (Fig. 2). Parameters describing the features of MXD and DRM *in vitro* permeation are summarized in Table 1.

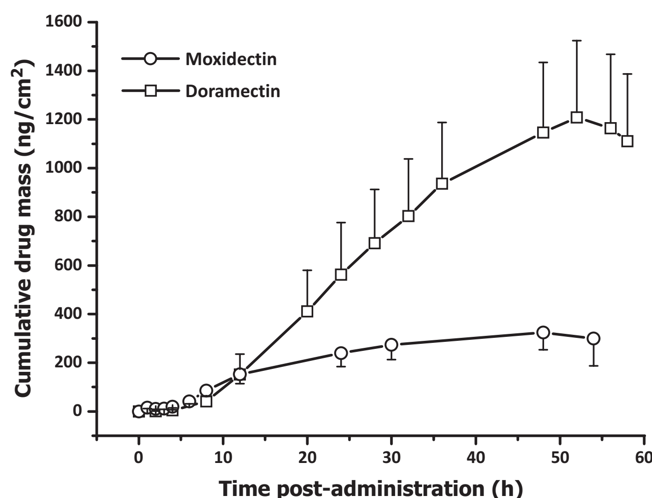

Fig. 1. Comparison of the cumulative moxidectin (MXD) and doramectin (DRM) *in vitro* permeation through bovine stratum corneum. Curves represent mean drug mass permeated per cm^2 (±SEM) (n = 10).

Doramectin presented a longer lag time (T_{lag}) and a higher flux (J) compared to MXD, being DRM parameters 5.29- ($P < 0.001$) and 2.93-fold ($P < 0.05$) higher (Table 1). Statistically significant differences were also observed in the coefficients of permeation (K_p) ($P < 0.05$) and diffusion (D_{app}) ($P < 0.001$). Doramectin permeation coefficient was 2.93-fold higher while its diffusion coefficient was 12.3-fold lower compared to MXD coefficients (Table 1).

In vitro results of cumulative drug mass (ng/cm^2) from 2 and up to 48 h were correlated with *in vivo* plasma concentrations (ng/mL) reported in our previous work after the topical administration of MXD and DRM to Holstein cattle (Fig. 3) (Sallovitz *et al.*, 2002, 2005). *In vivo* data up to 24 and 48 h postadministration were used for the *in vivo–in vitro* correlation.

Linear regression and correlation analyses from 0 to 24 and 48 h postadministration were performed by using the GraphPad InStat® software. Plots of regression relationship between *in vivo* drug absorbed vs. *in vitro* drug permeated up to 24 and 48 h postadministration are shown in Figs 4 and 5, respectively.

DISCUSSION

Interest for topical formulations of antiparasitic drugs intended for use in different animal species has increased worldwide. In companion animals, spot-on formulations of different parasiticide drugs have been used for many years (Riviere & Papich, 2001) and are relevant tools to control different parasitic infections. Topical administration is appealing to food-animal producers because it is of easy administration, less labor-intensive and nonstressing to animals (Baggot & Brown, 1998). Eventually, all chemicals that come in contact with the skin have the potential for absorption either locally or to the systemic circulation. Data on percutaneous absorption of therapeutically relevant drugs is required to determine their systemic exposure, which can be utilized for predicting efficacy and, in the case of food-producing animals, for estimating withdrawal periods. Although useful data on dermal absorption of veterinary drugs can be obtained from *in vivo* trials in animals, these studies present the disadvantage of being time consuming and highly expensive. Hence, when developing new topical formulations, characterization of the percutaneous absorption in a relatively short time is needed to predict systemic availability of the drug and, if necessary, make formulation changes in order to achieve ideal drug absorption patterns. That is why dermal absorption is an area in which *in vitro* approaches have a significant role to play as skin is a relatively easily accessible tissue, particularly skin of livestock animals, and they can be performed under different controlled conditions in a short time without needing a washout period. Much discussion was necessary to agree on and accept the OECD Guidelines on *in vitro* dermal absorption studies (Organisation for Economic

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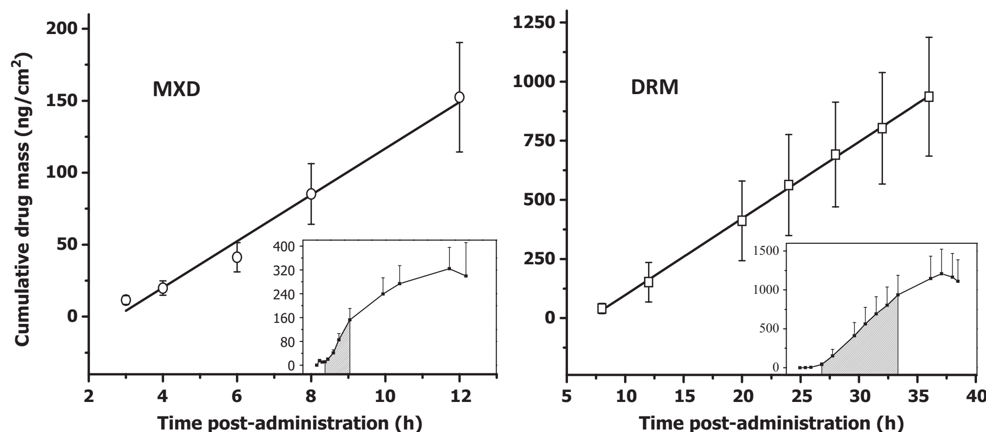

Fig. 2. Linear regression of the steady-state portion of the mean (±SEM) cumulative drug mass (ng/cm^2) vs. time after moxidectin (MXD) and doramectin (DRM) *in vitro* permeation through bovine stratum corneum. The inserts show the steady-state portions in the mean cumulative drug mass vs. time curves shown in Fig. 1.

Table 1. Parameters characterizing moxidectin (MXD) and doramectin (DRM) *in vitro* permeation through bovine stratum corneum after their administration as commercially available pour-on formulations for cattle (n = 10)

Parameter	MXD	DRM
Flux (J) (ng/h/cm^2)	15.56 ± 4.02*	45.58 ± 12.14
Permeability coefficient (K_p) (cm/h)	$0.62 \times 10^{-5} \pm 0.16 \times 10^{-5}$*	$1.8 \times 10^{-5} \pm 0.49 \times 10^{-5}$
Diffusion coefficient (D_{app}) (cm^2/h)	0.049 ± 0.033***	0.004 ± 0.001
Lag time (T_{lag}) (h)	2.44 ± 1.71***	12.91 ± 0.32

Values are presented as mean ± SEM. *Differences statistically significant at $P < 0.05$. ***Differences statistically significant at $P < 0.001$.

Co-operation and Development (OECD), 2004). Although these guidelines are among the most frequently implemented guidelines, their development was intended for experiments that would be extrapolated to humans. However, it is applicable to studies with veterinary drugs. As in human pharmacology (Williams, 2006), the progress in veterinary pharmacology has mainly been hampered by a lack of direct *in vitro–in vivo* comparisons to support the acceptance of the *in vitro* approach for determining systemic availability of topically applied drugs in large animals kept under field conditions. Nevertheless, many *in vitro* studies for characterizing the percutaneous absorption of different drugs in several animal species have been performed (Yazdaniana, 1994; Mills *et al.*, 2003; Mills & Cross, 2006a,b, 2007; Mills, 2007; Ahlstrom *et al.*, 2007, 2009).

In vitro drug absorption through full thickness skin may potentially differ from that achieved *in vivo* due to a lack of microcirculation within the upper dermis. The dermis can, therefore, act as a drug reservoir reducing absorption to the receptor fluid, particularly in static diffusion systems. This can be partially overcome by increasing drug solubility in the receptor fluid with the addition of organic solvents and BSA. Studies

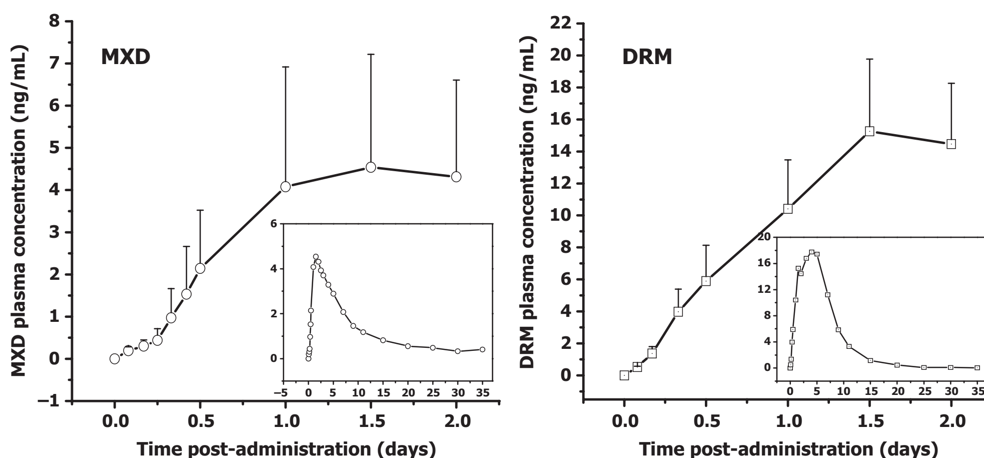

Fig. 3. *In vivo* mean plasma concentration (±SD) versus time curves of moxidectin (MXD) and doramectin (DRM) up to 2 days (48 h) after their topical administration (500 μg/kg) to Hostein cattle. Inserts are the plasma profiles up to 35 days postadministration. Note scale differences in plasma concentrations (adapted from Sallovitz *et al.*, 2002, 2005).

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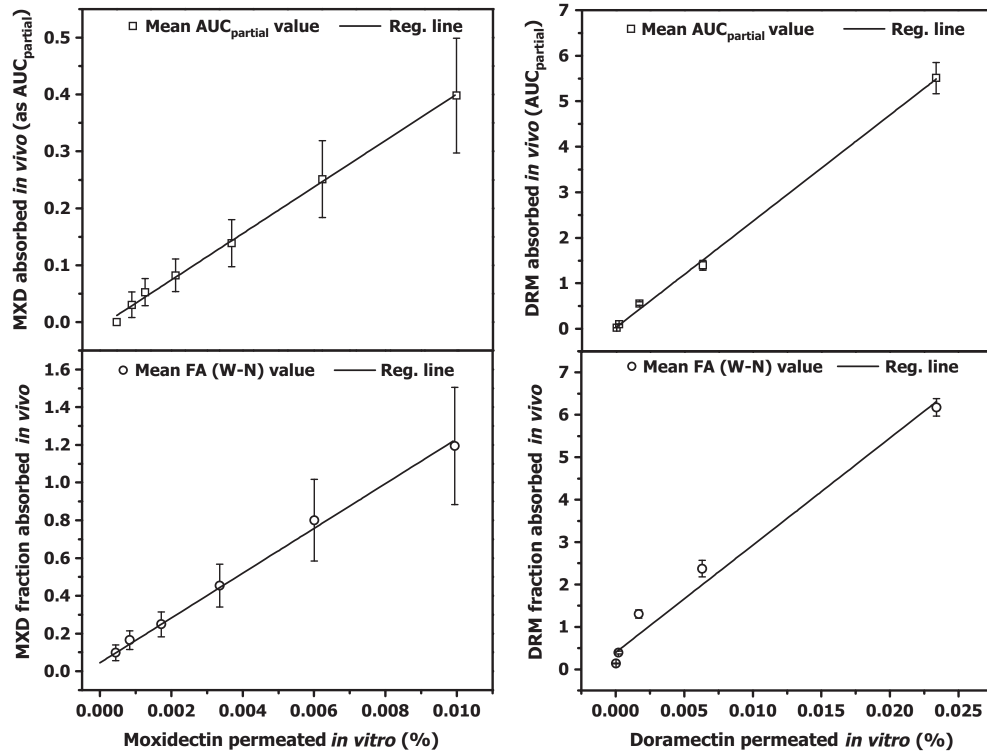

Fig. 4. Moxidectin (MXD) and doramectin (DRM) *in vivo–in vitro* correlation plots from 0 and up to 24 h. *In vivo* drug absorption data (expressed as mean values of the cumulative partial AUC_{0-t} and fraction absorbed estimated by Wagner–Nelson method) (data are from Sallovitz *et al.*, 2002, 2005) are plotted against the mean percentage value of drug permeated *in vitro*.

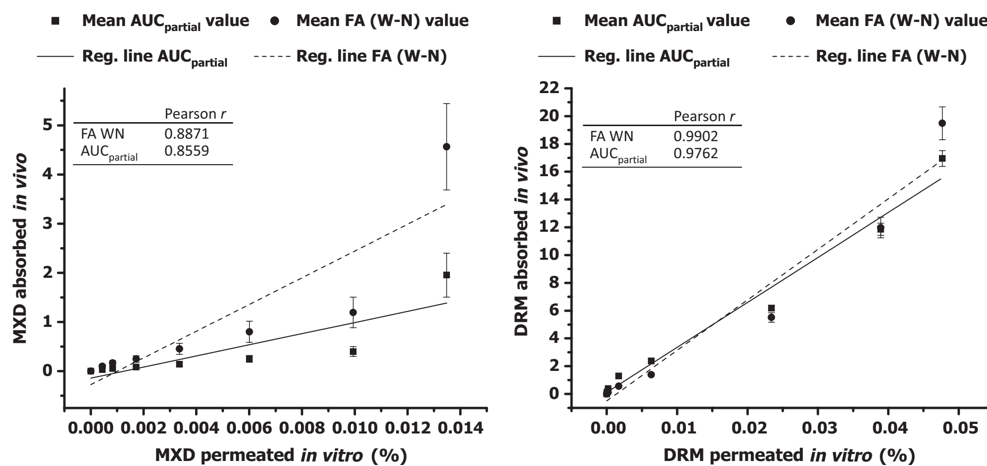

Fig. 5. Moxidectin (MXD) and doramectin (DRM) *in vivo–in vitro* correlation plots from 0 and up to 48h. *In vivo* drug absorption data (expressed as fraction absorbed estimated by Wagner–Nelson method (FA WN) and cumulative partial AUC_{0-t} ($AUC_{partial}$) (mean ± SEM, data are from Sallovitz *et al.*, 2002, 2005) are plotted against the mean percentage values of drug permeated *in vitro*.

reported within EDETOX (Wilkinson *et al.*, 2006) showed that particularly for lipophilic molecules, use of full thickness skin resulted in lower absorption to the receptor fluid than the split thickness skin, and that the total distribution of absorbed material indicated a reservoir in the skin (Riviere, 1999; Williams, 2006). As the stratum corneum is the absorption-limiting barrier (Riviere, 1999; Organisation for Economic Co-operation and Development (OECD), 2004; Wilkinson *et al.*, 2006), current *in vitro* studies have been performed with approximately 300–500 μm thickness skin slices (upper epidermal layers), obtained by using a specially designed dermatome to be used with bovine skin.

The work reported here describes the development of an *in vitro* technique applied to characterize the percutaneous

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absorption of two antiparasitic macrocyclic lactones (MXD and DRM) formulated for their topical administration to cattle. The obtained data support the feasibility of the adapted *in vitro* diffusion model, utilizing bovine stratum corneum, to characterize the dermal absorption of topically administered highly lipophilic drugs in cattle with a good potential to predict *in vivo* absorption. A remarkable advantage of the *in vitro* methodology described here is that the concentration of the permeated drugs (MXD, DRM) can be measured by a commonly used analytical method (HPLC), which is more convenient and accessible than the use of radioactive substances (Baynes, 2004). Although the receiver medium required a high percentage of ethanol (20%) due to the lipophilic nature of the macrocyclic lactones, no effect on permeability was observed since the stratum corneum behaved as an effective drug barrier along the experimental period, as it can be observed in Fig. 1.

Marked differences on permeability through the bovine stratum corneum were observed between MXD and DRM. The mean DRM flux (J) value was significantly higher compared to that obtained for MXD, although DRM required more time to reach it. This is clearly depicted by the longer T_{lag} and smaller diffusion coefficient (D_{app}) observed for DRM (Table 1). This observation can be explained by the higher liposolubility of MXD (log P 6) compared to DRM (log P 5.6) (Lespine *et al.*, 2007). The lipid solubility would allow for a faster penetration/distribution in the skin and an earlier appearance in the receiver medium. However, after slowly diffusing into the skin, a steady flow was established which resulted in a higher cumulative mass per cm^2 of DRM than that observed for MXD. This *in vitro* finding would be in agreement with the available data on the plasma concentration profiles obtained after *in vivo* topical administration of both compounds to cattle (Sallovitz *et al.*, 2002, 2005), where the DRM systemic exposure (*AUC* values) was markedly higher than that reported for MXD (see inserted plots in Fig. 3). This could be explained by the high lipophilicity of MXD, which determines a depot effect in the skin lipids, as it has been described for the skin from different anatomical regions in cattle topically treated with pour-on MXD (Sallovitz *et al.*, 2003, 2005). This depot effect is also observed when comparing MXD skin concentrations after its topical (Sallovitz *et al.*, 2003) and subcutaneous (Lifschitz *et al.*, 1999) administration to cattle, after which, MXD skin concentrations were lower. Recently, J.M. Sallovitz, L.A. Lifshcitz, G.L. Virkel, F.A. Imperiale & C.E. Lanusse (unpublished data) administered MXD to Aberdeen Angus calves by these two routes, i.e. subcutaneously and topically, and marked differences in plasma parameters (C_{max}, T_{max}, and *AUC*) were observed. The lower C_{max} and *AUC*, along with a delayed T_{max}, after the topical compared to the subcutaneous administration, suggest that topical MXD is slowly released and, consequently, absorbed to the systemic circulation due to its retention in the administration site (skin). However, after the subcutaneous injection, this retention capability is hampered since the drug is deposited under the skin.

Additionally, the data obtained from the *in vitro* permeation studies were correlated with the plasma concentrations of both compounds achieved in earlier *in vivo* work in cattle treated with the same topical formulations. The obtained *in vivo–in vitro* correlation results are promising. Good correlation coefficients (Pearson r) were obtained for both MXD and DRM when plotting *in vivo* drug absorption patterns (expressed as either fraction absorbed or cumulative $AUC_{0\text{–}t}$) vs. *in vitro* percentage drug mass permeated (Figs 4 and 5). These coefficients were higher than 0.85 (MXD) and 0.97 (DRM), which may be considered as very good *in vivo–in vitro* correlations for lipophilic compounds (Dressman & Reppas, 2000; Ghosh & Choudhury, 2009).

When comparing *in vitro* cumulative drug mass with *in vivo* concentration data, caution is advised, since the best predictors would be produced when *in vitro* data is compared to the fraction absorbed *in vivo*. However, determining the *in vivo* fraction absorbed after the topical administration of these antiparasitic compounds to cattle may be difficult. As reported by other authors, plasma drug levels after a topical administration may vary due to oral absorption caused by self- and allo-licking (Barber & Alvinerie, 2003; Bousquet-Mélou *et al.*, 2004). Licking behavior is very important in cattle, not only for individual hygiene, but for socializing within the herd (Sato *et al.*, 1991, 1993). Hence, plasma levels of topical administered MXD and DRM in cattle may be determined by two inputs, i.e. oral and percutaneous, which is also reflected in the drug profiles measured in tissues of parasite location (Sallovitz *et al.*, 2003). These dual inputs can be discriminated by different kinetic models, which require more complex mathematical work and intravenous studies (Wagner, 1993; Laffont *et al.*, 2003). However, results reported here showed good correlations with plasma levels measured after pour-on administration to licking cattle. These results can be due to the high *in vivo* individual variability and because, during the first 24 h after topical treatment, dermal absorption is the main source of drug reaching the plasma. After that time, oral drug absorption due to licking becomes the main drug absorption source.

Ultimately, the *in vitro* skin permeation approach described here is an easy, rapid, and inexpensive technique, which can be used as a rapid screening method for testing different topical formulations for cattle. The methodology has been successfully applied to assess the comparative percutaneous absorption of two highly lipophilic antiparasitic compounds (MXD and DRM) through cattle skin. Although promising results were obtained, further studies may be required to establish more accurate *in vivo–in vitro* correlation and with an acceptable predictability.

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