COMPARATIVE PHARMACOKINETICS OF ENDECTOCIDE COMPOUNDS IN CATTLE

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Although endectocide compounds (avermectins and milbemycins) do not exhibit activity against cestode and trematode parasites, they are highly effective against the adult, developing and hypobiotic larvae of most gastrointestinal (GI) nematodes, lungworms and many arthropod external parasites. High lipophilicity and the prolonged persistence of their potent broad-spectrum activity are distinctive features among antiparasitic drugs. Their antiparasitic spectrum and efficacy pattern are similar; however, each compound has its own dosage limiting species and differences in physicochemical properties among them, may account for differences in formulation flexibility, kinetic behaviour and in the potency/persistence of their endectocide activity. Thus, even slight modifications to the disposition kinetics or to the pattern of plasma/tissue exchange, may notably affect the persistence of their antiparasitic effect. In fact, different factors such as animal species (Alvinerie & Galtier, 1997), level of feed intake (Ali & Hennessy, 1996), nutritional status/body composition (Lifschitz et al.,1997), formulation of dosage form (Lo et al., 1985; Wicks et al., 1993), route of administration (Lifschitz et al. 1999; Alvinerie et al., 1998), have been shown to substantially affect the systemic availability of different endectocides in sheep and cattle.

The avermectins and milbemycins are closely related 16-membered macrocyclic lactones; both chemical groups are produced through fermentation by soil dwelling actinomycetes (*Streptomyces*) and have similar biological activities. They share some structural and physicochemical properties, and their broad-spectrum antiparasitic activity against nematodes and arthropods at extremely low dosage rates. A high affinity binding to glutamate-gated chloride channels, producing a slow and irreversible increase in membrane conductance (Shoop et al., 1995) which paralyses the parasite somatic musculature, particularly the pharyngeal pump (Geary et al., 1993), is now proposed as a main mode of action. The avermectin's family includes a series of natural and semisynthetic molecules, such as abamectin, ivermectin (IVM), doramectin (DRM) and eprinomectin (EPM). Nemadectin, moxidectin (MXD) and milbemycin 5-oxime belong to the milbemycin's family. The molecular structures of the two groups of endectocide compounds are superimposable; however, milbemycins do not have the C_{13} disaccharide substituent in the macrolide ring. IVM, a semisynthetic derivative of the avermectin family, was the first marketed endectocide; DRM was obtained by mutational biosynthesis . EPM is the most recently introduced semisynthetic avermectin compound, developed for topical use in cattle (Shoop et al., 1996). MXD is a milbemycin compound obtained by chemical modification of the natural compound, nemadectin. IVM, DRM and MXD, currently marketed as injectable, pour-on (cattle) and oral (sheep, goats) formulations, are the most commonly used endectocides worldwide to control endo- and ectoparasites in livestock. Altogether, earlier work on IVM plasma kinetics and the more recently published data on the comparative plasma (Lanusse et al.,1997) and target tissues (Lifschitz et al., 1999, 2000) disposition kinetics of IVM, DRM and IVM in cattle, the disposition of MXD (Alvinerie et al.,1998) and DRM (Hennessy et al., 1999) in sheep and the characterization of plasma/milk profiles of EPM in cattle (Shoop et al. 1996; Alvinerie et al., 1999) and goats (Alvinerie et al. 1999b), have contributed greatly to the understanding of the overall pharmacokinetic properties of the endectocide molecules and to correlate them to their claimed antiparasitic persistence.

Avermectins and milbemycins are highly lipophilic substances that dissolve in most organic solvents. They are large molecules and despite possessing two sugar rings and two hydroxyl groups (avermectins) are relatively insoluble in water. MXD solubility in water is greater than that of IVM and DRM. These and other physicochemical differences between the endectocide molecules may account for differences in formulation flexibility and in their resultant kinetic behaviour. The aqueous solubility of an active ingredient and the features of its pharmacotechnical preparation may influence the systemic availability, which relies on the rate and extent of absorption of a drug from the site of injection to the bloodstream. The vehicle in which the endectocide compounds are formulated may play a relevant role in their absorption kinetics and resultant plasma availability. The plasma profiles of IVM (Lo et al., 1985) and DRM (Wicks et al., 1993) in cattle have been shown to be substantially affected by the composition of the administered formulation. Following parenteral administration, the low solubility of IVM and DRM in water and its deposition in subcutaneous tissue favour a slow absorption from the injection site and provide prolonged duration in the bloodstream. Lanusse and co-workers (1997) have recently characterized the comparative plasma disposition kinetics of IVM, MXD and DRM after their subcutaneous administration of the formulations commercially available

for administration to cattle (**Figure 1**). While IVM was administered as the non-aqueous (60% propylene glycol/40% glycerol formal) preparation, a oil-based formulation of DRM containing sesame oil/ethyl oleate (90:10), was administered to the experimental animals. The absorption of MXD, administered as an aqueous-based solution, from the site of subcutaneous injection was significantly faster than those of IVM and DRM. MXD peak plasma concentrations were achieved significantly earlier (8 h) than those of IVM (4 d) and DRM (6 d post-treatment). It seems likely that MXD administered as an aqueous solution was more rapidly available for absorption from the subcutaneous tissue than both IVM and DRM formulated in non-aqueous formulations, which agrees with the values of absorption half-lives obtained for MXD (1.32 h), IVM (39.2 h) and DRM (56.4 h). The low solubility of IVM and DRM in water, their formulation in non-aqueous preparations, and their deposition in the subcutaneous tissue favour a slow absorption from the site of injection.

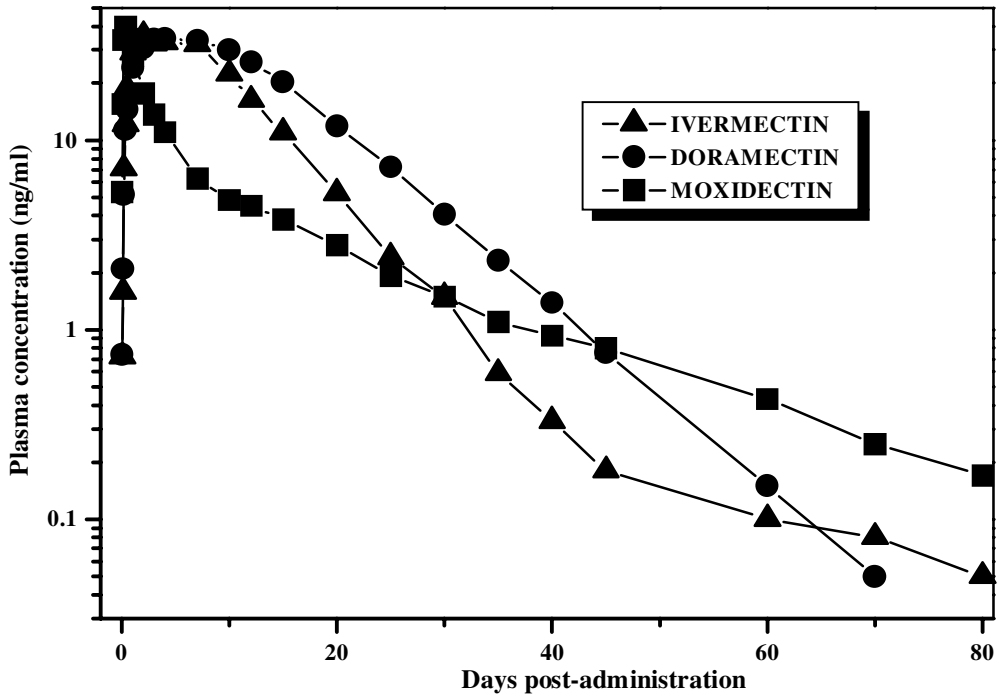

Figure 1: Mean comparative plasma concentration of ivermectin, doramectin and moxidectin obtained after their subcutaneous administration (200 µg/kg) to cattle. Adapted from Lanusse et al. (1997), . *J.Vet. Pharmacol. Ther.* **20**, 91-99.

Large amounts of unchanged endectocide compounds are excreted by bile and faeces in sheep and cattle. Unchanged IVM (Bogan & Mckellar 1988; Lifschitz et al., 2000) and DRM (Lifschitz et al., 2000) are excreted in high concentration in the bile of cattle; IVM (Chiu et al., 1990) and DRM (Henessey et al., 1999) are primarily eliminated in faeces, with less than 2-3% of the total administered dose being excreted in urine. Consistently, high concentrations of unchanged MXD are excreted by bile and faeces (Zulalian et al., 1994), with concentrations greater than 2 ng/ml (ng/g) being recovered both in bile and faeces up to 48 days post-treatment in cattle (Lifschitz et al., 1999) (**Figure 2**); interestingly, in this study was clearly shown how MXD concentration profiles found in bile and faeces reflected that observed in plasma, although the availability of the drug in both

bile and faeces was significantly greater than that measured in the bloodstream. Different pharmacological approaches to delay the bile-faecal elimination and to extend the plasma-intestine recycling time to achieve increased systemic availability for endectocide molecules, are currently being investigated in our laboratory.

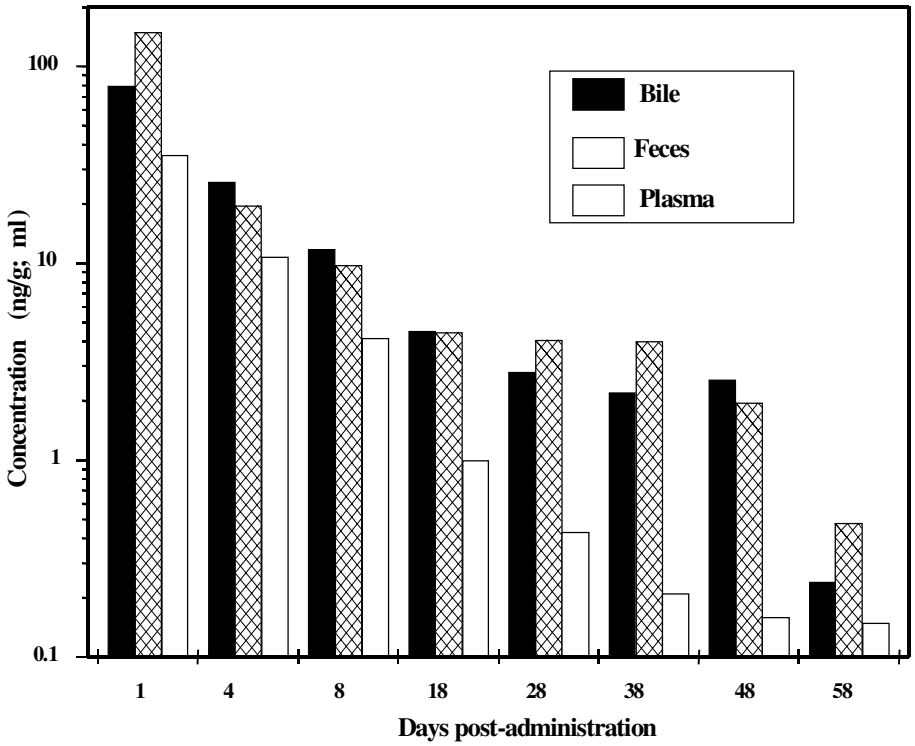

Figure 2: Bile and faecal elimination of moxidectin. Comparative concentration profiles in plasma, bile and feces obtained after its subcutaneous administration to cattle (200 µg/kg).

Drug concentrations at the site of parasite location and the time of parasite exposure to them, are relevant to achieve optimal activity. The time of parasite exposure to active drug concentrations determines the persistence of the antiparasitic activity of endectocide compounds. Data on the plasma kinetic profiles can help to explain the comparative efficacy and persistence of the antiparasite activity of these molecules. However, the characterization of drug concentration profiles at the site of parasite location permits a more direct interpretation and provides a basis for understanding the differences in therapeutic and preventive efficacies observed for these drugs. The characterization of drug concentration profiles at the sites of parasite infection for MXD (Lifschitz et al., 1999), IVM and DRM (Lifschitz et al., 2000) in cattle, and the characterization of the kinetic disposition of DRM in fluid and particulate digesta throughout the gastrointestinal tract in sheep (Hennessy et al., 1999), represent a considerable contribution to understand the comparative and prolonged persistence of activity of these compounds. The high lipophilic endectocide molecules are extensively distributed from the bloodstream to different tissues. Their extensive tissue distribution agrees with the large availability of these drugs in different parasite location tissues such as the GI mucosal tissues, lungs and skin in cattle, where concentrations markedly greater than those observed in plasma were measured during 50-60 days post-treatment. A strong correlation between plasma and tissue concentration profiles for each compound was observed.

The early peak plasma concentration (Tmax= 8 h) observed for MXD in cattle (Lanusse et al., 1997) agrees with a rapid absorption process from the site of subcutaneous injection, which was reflected in the early Tmax value observed for MXD in all the target tissues and fluids analyzed (1 day post-treatment) (Lifschitz et al., 1999). Conversely, the slow release of DRM from the subcutaneous depot of the oily vehicle is consistent with the

observation that peak concentrations in different tissues and fluids were attained at 4 days post-treatment. The MXD peak concentrations (Cmax) attained in the sites of parasites location were between 49 % (intestinal mucosa) and 148 % (abomasal mucosa) higher than that achieved in plasma. Despite of the high correlation observed between concentrations in plasma and in tissues and fluids, concentrations of the three compounds were greater in all target tissues compared to plasma, with tissue/plasma ratios ranging from 1.45 to 3.44 (IVM), 1.20 to 2.47 (DRM) and from 1.45 to 3.72 (MXD).

MXD is the most lipophilic endectocide substance; its high lipophilicity accounts for a wide tissue distribution (volume of distribution=13.6 l/kg) and long residence in plasma, which is clearly reflected in the tissue pharmacokinetic results obtained in cattle. Distribution of the drug into adipose tissue accounts for the large distribution obtained for this compound compared to other antiparasitic drugs. The concentration of MXD in fat after 28 days of treatment in cattle has been shown to be ninety-fold higher than that detected in plasma (Zulalian et al., 1994). In addition, a prolonged half-life of MXD in fat (14 days) (Zulalian et al., 1994) compared to that of IVM (7 days) (Chiu et al., 1987) has been reported after subcutaneous treatment in cattle. The large tissue distribution of MXD in cattle agrees with the high availability of MXD in the GI mucosal tissues, lungs and skin with concentrations ranging between 1 and 2 ng/g at 28 days post-treament, and with the extended detection of MXD concentrations > 0.1 ng/g up to 58 days post-treatment in those tissues (**Figure 3)** The high Cmax values and total drug availability obtained in tissues where target parasites are located, are in agreement with the extensive tissue distribution of MXD and may be relevant in terms of antiparasitic activity against internal and external parasites. Long residence times for MXD (between 6.8 and 11.3 days) were obtained in the different sites of parasite location, which agrees with the extended residence of MXD previously reported in fat (Zulalian et al., 1994) and in the bloodstream (Alvinerie et al., 1996; Lanusse et al., 1997). Agreeably, MXD depletion half-lives in target tissues ranged from 7.73 (skin) to 11.8 (intestinal mucosa) days. The deposit of MXD in adipose tissue may act as a drug reservoir that contributes to the long persistence of this molecule in the bloodstream and in different target tissues.

MXD has a broad-spectrum of activity against adult and larval forms of many GI nematodes in cattle. However, this pattern of efficacy varies among different nematode parasites; for instance, *Cooperia spp*. is not fully susceptible to MXD, and dose rates higher than the recommended one, may be required to achieve 100% efficacy against all trichostrongyle species (Scholl et al., 1992). While the reason for this differential susceptibility among different nematodes remains unknown, the evaluation of MXD concentrations, as well as other endectocide molecules, in different sections of the GI tract may provide some useful information.

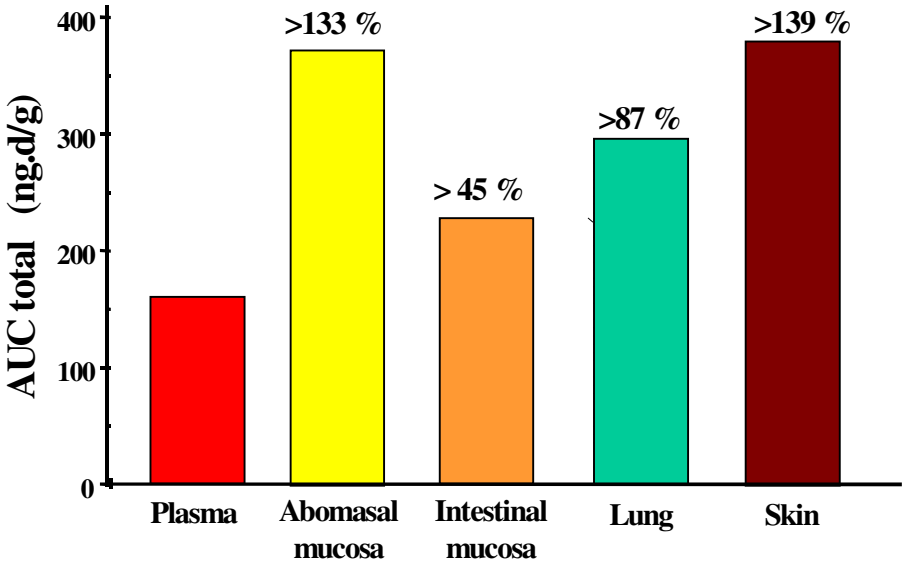

Figure 3: Comparative area under the concentration versus time curve (AUC) for moxidectin obtained in plasma and in different target tissues. Values on the top of each bar indicate percentage of enhancement in AUC compared to plasma.
Adapted from Lifschitz et al. (1999), *J. Vet. Pharmacol. Ther.,* **22**, 266-273.

High MXD concentrations were detected in both abomasal and small intestinal mucosal tissues up to 58 days post-treatment, which accounted for the large AUC values (371 and 231 ng.d/g, respectively) observed in both target sites. These concentration profiles obtained in the mucosal tissue were greater than those obtained in abomasal (AUC: 23.4 ng.d/ml.) and intestinal (AUC: 88.2 ng.d/ml) fluids. Only low MXD concentrations (below 1 ng/ml) were found in abomasal fluid following its subcutaneous administration to cattle, which is in agreement with the data previously reported for IVM (Bogan & McKellar, 1988), where the drug was not detected in abomasal fluid, even when administered at ten times the therapeutic dose in sheep. The greater MXD concentrations observed in the small intestinal fluid collected distal of the bile duct, compared with the abomasal fluid, may be associated to the important biliary excretion of the drug. The higher lipid composition of the GI mucosa compared to the fluids, considered as a more polar medium, may account for the greater MXD availability found in the mucosal tissues. The high availability and extended residence of MXD in the abomasal and intestinal mucosal tissues agree with the reported persistence of the anthelminitc effects of the drug against *Ostertagia ostertagi,* and other nematodes, for at least 35 days post-treatment (Vercruysse et al., 1997). Although differences in feeding mechanisms and site of location in the digestive tract among different nematodes should be considered to understand the overall action of MXD, the persistence of the broad spectrum anthelmintic activity of the drug against adult and inmature GI parasites is facilitated by its pattern of distribution and prolonged residence in the digestive mucosa.

Endectocides are highly effective in eliminating mites and suckling lice species. The pattern of IVM and DRM disposition in skin tissue showed that high concentrations of both molecules (>27 ng/g) are attained during the first eight days post-treatment (Lifschitz et al., 2000). The sustained presence of high concentrations of IVM and DRM in skin were reflected in the prolonged MRT values (6.8 and 9.3 days, respectively), which may also account for the efficacy of these drugs against single host ticks. The duration of effective levels of endectocide compounds in plasma may be important in the treatment of tick infestations, since this parasite can accumulate lethal drug concentrations through its feeding activity over a period of several days. Drug uptake and efficacy against arthropod ectoparasites can be markedly influenced by parasite feeding habits. Thus, the lower efficacy of endectocides against biting lice can be attributed to the lower exposure of this ectoparasite to body fluids containing drug. MXD concentrations greater than 9 ng/g were detected during the first 8 days post-treatment in the skin of treated cattle (Lifschitz et al., 1999), with a peak concentration of 84.2 ng/g achieved at day 1 post-administration. Drug concentrations in skin declined gradually with time post-treatment, as shown for other tissues, being detectable up to 58 days (>0.2 ng/g). These high MXD concentrations and its sustained presence in the skin account for the excellent efficacy of the drug against different ectoparasites in ruminants. Interestingly, the MXD concentration profiles in the skin (dermis and epidermis) were greater than those found in the hypodermal tissue; MXD total availability in skin was 6 times greater than that observed in the subcutaneous tissue. Blood vessels in the skin are limited to the dermis which receives the largest vascular supply. The dermis participates in the exchange of compounds

between blood and tissues and as a fat reservoir (Monteiro-Riviere, 1991); this physiological role of the dermis and the high lipophilicity of MXD, may explain the differential distribution pattern of MXD in the skin, accounting for the higher availability of the drug in the dermis compared to the subcutaneous tissue. The arthropod ectoparasites are exposed to systemic agents during feeding; the nature of their food source, frequency and duration of feeding can have a marked influence in drug uptake and efficacy (Jackson, 1989). The specific distribution of MXD in the different skin layers and its prolonged residence, agree with its pattern of ectoparasiticide activity. The lower efficacy of avermectins and milbemycins subcutaneously administered against biting compared to sucking lices (McKellar & Benchaoui, 1996), may be associated with the superficial feeding of these parasites, particularly on sloughed epidermal cells, which may contain low drug concentrations.

The time of parasite exposure to active drug concentrations determines the persistence of the potent and broad-spectrum antiparasitic activity of endectocide compounds. These molecules are extensively distributed to the most important sites of parasite location. The progress achieved in the comprehension of the disposition kinetics and on the characterization of drug concentration profiles in target tissues is a first great step to optimize their use in livestock avoiding the development of parasite resistance.

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