MERIT RESEARCH JOURNALS
www.meritresearchjournals.org

Merit Research Journal of Medicine and Medical Sciences (ISSN: 2354-323X) Vol. 12(2) pp. 026-032, February, 2024
Available online http://www.meritresearchjournals.org/mms/index.htm

DOI: 10.5281/zenodo.10717326

Review

Research Update on the Impact of Milbemycin Oxime in the Context of Expanding the use of Macrocyclic Lactones

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Abstract

Milbemycin oxime is a macrocyclic lactone (ML), chemically similar to avermectin. Discovered, a decade later, by Japanese researchers from the Kitasato Institute, milbemycin is characterized by small structural differences from avermectins, which are given by the lack of substitution at the level of the C-13 group of the macrolide ring, for the avermectin molecule being specific the substitution of the bis-oleandroxyl group (saccharide). The purpose of this bibliographic study is to synthesize and review a set of research focused on the veterinary impact of macrocyclic lactones, updating the use of Milbemycin oximine in the therapy of canine helminthiasis. In carrying out the study, extensive documentation was carried out based on the analysis of current research accessed on the Pubmed/Medline and Google Scholar sites, using as search terms "macrocyclic lactones, milbemycin oxime, avermectins, helminthiasis, and dogs". The synthesis of the analyzed information revealed that the milbemycin molecule presents certain superior pharma-cotherapeutic characteristics to macrocyclic lactones in general, the discovery of this active molecule contributes to the achievement of important progress in agro-biological research and practice, as well as in ensuring the well-being of animals. Thus, it is now well known that the MLs pharmacological group includes the avermectin and milbemycin subfamilies and that among them milbemycin oxime is particularly active, which also inhibits the growth of mycobacteria. The acaricidal activity of milbemycin can also be mentioned, and it has been confirmed as one of the most important effects of this active substance.

Keywords: Avermectins, Dog, Helminthiasis, Macrocyclic lactones, Milbemycin oxime

Abbreviations: AB- Abamectin; **AVM**- Avermectin; **DI**- Dirofilariae immitis; **DRM**- Doramectin; **EPM**- Eprinomectin; **HM**- Human medicine; **IVM**- Ivermectin; **ML**- Macrocyclic Lactones; **MbA3A4**- Milbemycin A3/A4; **MB**- Milbemycin; **MBO**- Milbemycin oxime; **MIC**- Minimum inhibitory concentration; **MXD**- Moxidectin; **PZQ**- Praziquantel; **SEM**- Selamectin; $T_{1/2}$ - Half-life time; T_{max}- maximum concentration time; **VM**- Veterinary medicine

INTRODUCTION

Following the discovery of macrocyclic lactones (MLs), many advances have been made over the years in agricultural, biological, and animal health research, adding to this progress the control of some of the most common human parasites; most results due to collaboration between Merck and Co., Inc. and the World Health Organization. All this collaboration proposed in 1975 an innovative strategy for accelerating technological processes in favor of discovering new treatments for tropical diseases (Campbell, 2012).

In addition to their antiparasitic and pesticidal effects, MLs also have other properties such as antifungal,

anticancer, antidiabetic, and antiviral activity and is used for the treatment of several metabolic diseases (Batiha et al., 2020). The US, China, Japan, and Europe have used milbemycin oxime (MO) (which is the semi-synthetic analog of milbemycin A3/A4) to control mites, worms, and insects in pets. Another milbemycin A3/A4 (MbA3A4) derivative is lepimectin, which is widely used in agriculture (Zhang et al., 2016)

This group of antiparasitic agents was awarded the Nobel Prize in 2015 in the Medicine and Physiology category (Wang et al, 2020). The prize was awarded to Dr. William C. Campbell, Professor Youyou Tu, and Professor Satoshi Ŏmura for their contribution to the development of effective chemotherapy drugs against major and devastating diseases such as lymphatic filariasis, malaria, and river blindness. Dr William C. Campbell (Ireland, USA) and Professor Satoshi Ŏmura (Japan) contributed to the discovery of avermectin (AVM) and Professor Youyou Tu (China) contributed to the discovery of artemisinin. Both drugs have a strong antiparasitic effect so AVM and its derivatives contributed to the decrease in the incidence of river blindness as well as lymphatic filariasis and artemisinin reduced the percentage of mortality in malaria (Dlugónska, 2015).

General data regarding the history and evolution of MLs

Macrocyclic lactones are found in several pharmaceutical products with particularly frequent antiparasitic applications in veterinary medicine (VM) in recent decades (Nolan and Lok, 2012). Milbemycins were first isolated from Streptomyces hygroscopicus subsp. aureolacrimosus in 1967 in Japan (Zhang et al., 2016). In 1970, Japanese researchers at the Kitasato Institute discovered a microorganism that had never been reported before. American researchers from Merck later contributed by observing the antiparasitic capacity against some arthropods and some roundworms (Campbell, 2012). AVMs are produced by the fermentation of an actinomycete, Streptomyces avermitilis isolated from soil (Batiha et al., 2020). The main strain, Streptomyces bingchengensis, which contributes to the synthesis process of milbemycin has been investigated and sequenced for decades (Yan et al., 2021). The use of MbA3A4 on a large scale has been achieved since 1990 in developed countries due to its very potent acaricidal activity as well as to combat some insect species such as *Aphidoidea*, *Liriomyza*, and *Aleyrodidae* that have become resistant to organophosphorus and avermectin pesticides used on many plant species such as strawberries, tea, citrus and apple (Zhang et al., 2016).

Milbemycins are part of a group of 16 macrocyclic lactones whose members show excellent insecticidal, anthelmintic, and acaricidal activities, which can be generated following the fermentation process by several species of *Streptomyces*. The low toxicity, active on mammals of MB molecules as well as their derivatives make it possible to use them on a large scale in the large pharmaceutical veterinary, medical, and agricultural industries. (Yan et al.; 2021). A study was recently conducted on a coral reef system located at the Steinhart Aquarium in San Francisco, CA, USA, which had experienced a population explosion of pycnogonid sea spiders (*Arthropoda*: *Class Pycnogonida*). Thus, for the eradication of spiders with a minimal harmful effect on 16 coral colonies of 3 species (*Pocillopora damicornis*, *Acropora tenuis*, and *Stylophora pistillata*) immersion therapy with MO was used. This treatment had a beneficial effect on the eradication of spider populations and through biopsies it was demonstrated that the dosing and treatment with MO was safe and effective, not altering the health of the 3 coral species (Krol et al., 2023).

Abamectin (ABM) as well as ivermectin (IVM) were the first MLs used in VM to combat parasitic effects; later MO, selamectin (SEM), doramectin (DRM), and eprinomectin (EPM) were also discovered. Each of these derivatives is differentiated from one another by various attributes. At that time, only IVM was the product of choice used in the treatment of parasitic diseases in human medicine (HM) (Campbell, 2012).

Currently, under the MLs class, there are 4 derivatives used worldwide in dogs and cats, namely SEM, MO, moxidectin (MXD), and IVM. They were first used as prophylactic products to combat Dirofilaria immitis (DI). Their application strategy targeted third and fourth-stage larvae, following a monthly use of ML products (Nolan and Lok, 2012).

Milbemycins were first isolated from *Streptomyces hygroscopicus* subsp. *aureolacrimosus* in 1967 in Japan followed by the isolation of an innovative microorganism represented by *Streptomyces bingchengensis*, this was an essential role on the scale of the industrial production of milbemycins. This latter bacterial agent contributes to the production of MbA3A4, C5-O-methylmilbemycins of the β-class (B2, B3, β1, and β2) of four α-classes, the nanchangmycin polyether and traces of novel MB analogues (Zhang et al.; 2016). The MLs family is thus divided into 2 large subfamilies, namely: avermectins and milbemycins. It was later proven that they also show activity against mycobacteria such as *Mycobacterium ulcerans*, *Mycobacterium tuberculosis*, *Mycobacterium marinum*, and many others. Following a study on MLs activity in vitro, low inhibition concentration values were demonstrated compared to 80 strains of species belonging to non-tuberculous mycobacteria. Thus, MLs showed intervariable anti-bacterial activity depending on species and compounds. Following these results, it was proven that milbemycin oxime presented the activity with the widest spectrum presenting a minimum inhibition concentration of 8mg/L (Muñoz-Muñoz et al.; 2021).

Following an applied study on the use of MLs aimed at the use in VM as well as in HM, the in vitro activity on strains of *Mycobacterium ulcerans* was analyzed. This research demonstrated that both MO and SEM had the strongest effects against *M. ulcerans* species with MIC values that lie from 2 to 8 µg/mL and from 2 to 4 µg/mL, respectively. IVM and MXD did not present a significant activity so the inhibition concentration values had an index lower than 32 µg/mL. By testing the kinetic bactericidal activity of SEM, it was demonstrated that its activity is dependent on exposure. Thus, the pharmacokinetic information published together with the supported data highlighted the fact that SEM is the ML with the most effective activity for the treatment of *Buruli ulcer* (Scherr et al., 2015).

S. hygroscopicus subsp. aureolacrimos, contributed to the isolation and synthesis of 13 types of milbemycins with α1–α10 and β1–β3 representations as well as D–H milbemycins. Thus, in 1975, the structures of MB β1–β3 molecules were elucidated, being represented by ring structures with 6.6 members represented by spirocheted rings. In 1980, the synthesis of D-K Milbemycins obtained by UV and nitrosoguanidine treatment of a high-yield strain was completed. MB D has a strong nematocidal activity, being used very often in the prophylactic and therapeutic veterinary medical field. MBO was isolated following the semi-synthetic derivation of the 5-oxoimino group from an 80:20 ratio of milbemycin A3(α1) and A4 (α3), representing a very potent nematocide, frequently used in basic treatments and prophylaxis for livestock company (Shiomi, 2021).

Recently, following the genetic modification procedures of *Streptomyces bingchengensis* strain BCJ60, two derivatives with strong nematicidal and acaricidal activity were obtained as represented: 27-methoxymilbemycin a31 and 27-oxomilbemycin a31 (Li et al., 2017).

Following the genetic modification of *Streptomyces avermitilis* AVE-H39, two new MB derivatives (5,27-epoxy-13α-hydroxy-25-ethyl and 5,27-epoxy-13α-hydroxy milbemycin β11) were obtained, elucidated by the technique HR-ESIMS and extended NMR spectroscopic effects. The nematocidal and acaricidal activity of these two compounds is moderate (Qi et al., 2020).

Structure and chemical properties of MLs

The structure of MBO molecules is similar to that of AVM (Batiha et al., 2020). So about a decade after the discovery of AVM, Sankyo discovered and synthesized the MB molecule, a new highly potent ML against mites (Jung et al., 2002). Its name reflects both its parasitic target and its actinomycete origin, derived from the German (milbe) (Campbell, 2012). Unlike AVM, MB is not substituted at the level of the C-13 group of the macrolide ring, where the bisoleandroxyl (saccharide) group is substituted, a group-specific to AVM molecules. Other structural changes are represented by the alkyl group at the level of the C-25 group in both classes. By deleting the hydroxyl group from the twelfth position, the product 13-deoxy avermectin results, which is an aglycone of AVM that is almost similar to MBei, called milbemycin glycosylate (Zhang et al., 2015).

Regarding the properties of AVM, they are slightly volatile and easily soluble in water, so that in less than 6 hours, 50% of IVM dissolves in water, compared to other drugs that require 16.8 days to dissolve 90% of the drug. AVM molecules are soluble in organic solvents such as ethyl acetate, ethanol, chloroform, and diethyl ether. Their vapors have high atmospheric pressure, so it is very difficult to spread them in the atmosphere (Batiha et al., 2020). Another important property of ivermectin is its ability to bioaccumulate in the animal body, a fact due to its lipophilicity. The high molecular weight of the drug thus constitutes an impediment that counteracts its lipophilic nature and prevents its passage through the cell membrane (Beshbishy et al., 2019).

Current affairs regarding the mechanisms of action of macrocyclic lactones

After the chemical structure of the AVM molecule was discovered, due to the major interest in it, the foundations were laid for a postulate on the mechanism of action that contributes to the process of blocking acetylcholine at the synapses of the parasite's vegetative nervous system (Dowd and Frank, 2017; Campbell, 2012). The action of AVM on nematodes is due to stopping the transmission of electrical impulses in the muscles by amplifying the effect of glutamate, which causes the opening of chloride channels (Bloomquist, 2003; Subbanna et al., 2020).

Glutamate-gated chloride channels are the key components on which IVM and other related compounds act on nematodes. A small family of genes contributes to the coding of the subunits of these channels, thus achieving the assembly of several channel subtypes. The subunit composition of most native receptors is unknown (Wolstenholme, 2022).

This process causes the intracellular penetration of chlorine ions, generating hyperpolarization and culminating in neuromuscular paralysis. The administered dosage does not cause toxic damage to mammals because they do not have glutamate-dependent chloride channels (Srivastava et al., 2020).

Although the MB molecule has been discovered for more than two decades, its mode of action has not been precisely understood (Cobb and Boeckh, 2009). The most potent MB is nemadectin, which has a long unsaturated chain at the C-25 position. The present molecule represents a substrate for the synthesis of moxidectin (MOX), which is also a good insecticide and nematicide (Gao et al., 2010). Although MO has a similar

mechanism of action to that of AVM, the half-life is much longer compared to them. They act similarly by mediating the opening of glutamate-sensitive chloride channels in invertebrate myocytes and neurons, thus leading to hyperpolarization and blocking signal transfer (Toranmal et al., 2019). Similar to other AVMs, it shows an increased affinity for the channels ionic glutamate-dependent, specific to vertebrates (Batiha et al., 2020).

Pharmacokinetic features of MLs in dogs

For the MO molecule, t_{max} is approximately 2-4 hours and $t_{1/2}$ is approximately 1-4 days. Bioavailability is 80% (Milbemax Chewable Tablets for Dogs, n.d.). Following a study by Letendre et al., a comparison of the pharmacokinetic, safety, and efficacy results of Milbemax with those observed when the active substances were administered alone was carried out. Jung et al. (2002) described the pharmacokinetics of MO after the administration of the products Milbemax and Interceptor (a product containing only MO from Novartis). Following the administration of higher doses of MO and PZQ in the Milbemax formula, $t_{1/2}$ of MB was reached in 1.6 and 3.9 days for the forms of A3 and A4, and $_{Tmax}$, the representative value for them was 2.4 and 2.5 hours; similar values for Milbemax, although its bioavailability is lower (54% for A4) compared to Nexgard Spectra product (65.1% for A4) determined in fasted dogs, following comparison of AUC and Cmax values (Letendre et al., 2017).

Veterinary medical products containing macrocyclic lactones

The enormous commercial success achieved by the use of IVM in VM attracted the attention of major pharmaceutical companies to synthesize second-generation products in the following years (Taylor, 2004). Currently, for the prophylaxis of DI in dogs and cats, ML can be administered in oral (MBO, IVM), injectable (IVM, MXD), sustained-release, and topical (MXD, SEM) forms (Nolan and Lok, 2012). The products intended for otic application have a concentration of 0.01% of IVM or MBO. Besides these, there are other MLs such as EPM and DRM, which are used strictly because they do not conform to the approved labeling but meet the conditions set by the Animal Drug Use Clarification Act of 1994 (AMDUCA) and the US Government for Food and Drug Administration (FDA). These products are generally used for the treatment of equines and ruminants and are formulated for subcutaneous, topical, or oral applications (Table 1).

In veterinary parasitology, there are enough studies focused on testing the effects of AVM on parasitic nematodes in various animal species, from which several relevant aspects can be derived. Among these, we mention the use of AVM in the production of extremely active antiparasitics, the efficiency of their parenteral, oral, and even external administration to various intestinal nematodes, their high level of effectiveness in combating parasitosis with intestinal nematodes, their good efficiency in the treatment of extraintestinal parasitosis, including DI, their efficacy against nematodes resistant to benzimidazoles, and their high level of pharmaceutical safety, which makes them well tolerated by most animal species (Campbell, 2012).

In the Southern region of Europe, the DI population is very high, and due to this, chemoprophylaxis must be very well developed. Thus, the prophylactic and therapeutic effect against Thelazia callipaeda infestation of a new MO molecule formulation represented by the Interceptor1 product, Novartis, Animal Health, was evaluated. Due to the periodic administration of MBO under southern European conditions, increased protection of dogs against heartworm caused by DI was thus demonstrated throughout the season (Ferroglio et al., 2008).

MBO can be administered both in chewable form for the prevention of DI and in the form of otic drops with a concentration of 0.1% for the treatment of scabies. The low concentration of the dose is an advantage of the product, so drug overdose is difficult to achieve. Its dosage is between 0.5-2 mg/kg in small animals for DI prophylaxis. Mild symptoms such as hypersalivation, lethargy, ataxia, and mydriasis occurred in susceptible dogs given a higher dose of 5–10 mg/kg (Merola and Eubig, 2012). The results of a study led by researcher W.J. Tranquilli on the effects of MBO and IVM affecting the Collie breed concluded that their commercial formulations have similar safety margins and that the toxic effect of MB is dose-dependent in this IVM-sensitive breed. Thus, the Collie breed is sensitive to approximately 120 µg/kg of IVM, equivalent to a dosage 20 times higher than a normal dose, as well as to 10 µg/kg of MB, also having a dosage 20 times higher than the normal one (Tranquilli et al., 1991).

Following some scientific research on the most effective AP treatments in dogs infected with sarcoptic mange, it was concluded that the administration of MXD 2.5% and local imidacloprid 10% or SEM 12%, or of afoxolaner and afoxolaner plus MBO, oral Sarolaner, topical or oral fluralaner, it is possible to improve itching and clinical signs as well as parasitological healing (Dumitrache and Cadiergues, 2023).

Following the synergistic effects of endectocide and anthelmintic AP, an extremely beneficial effect can be obtained both for the owner of the pet and for the veterinarian, following the wide spectrum of action that allows preventing and treating dogs infested with several types of parasitic agents. Such an example is given by a recent study by Snyder, Daniel E., et al. (2021), which supports the fact that the combination of MO with other

Table 1. Indications for the use of macrocyclic lactones in the canine clinic, including helminth species sensitive to pharmaceutical products from this group (Nolan and Lok, 2012).

Commercial products with MLs	Indications for susceptible helminthic species	
	Label uses	**Off label uses**
Ivermectin	*Otodectes cynotis*	*Ancylostoma caninum* *Toxocara canis* *Ancylostoma braziliense* *Trichuris vulpis* *Toxascaris leonina* *Strongyloides stercoralis* *Thelazia callipaeda* *Spirocerca lupi* *Filaroides (Oslerus) osleri* *Pelodera Strongyloides* *Capillaria plica and C. bohemi* *Dipetalonema reconditum* *Sarcoptes scabiei* *Demodex canis* *Cheyletiella sp.* *Dirofilaria immitis (adults)*
Milbemicyn oxime	*Ancylostoma caninum* *Trichuris vulpis* *Toxascaris leonina* *Toxocara canis* *Demodex canis*	*Spirocerca lupi* *Angiostrongylus vasorum* *Thelazia callipaeda* *Crenosoma vulpis* *Baylisascaris procyonis* *Dirofilaria repens* *Sarcoptes scabiei* *Cheyletiella sp.*
Moxidectin	*Ancylostoma caninum* *Ancylostoma braziliense* *Uncinaria stenocephala* *Toxascaris leonina* *Toxocara canis* *Otodectes cynotis* *Trichuris vulpis*	*Thelazia callipaeda* *Crenosoma vulpis* *Dirofilaria repens* *Demodex canis*
Selamectin	*Otodectes cynotis* *Ctenocephalides felis* *Sarcoptes scabiei* *Dermacentor variabilis*	*Toxascaris leonina* *Toxocara canis* *Trichodectes canis* *Heterodoxus spiniger* *Linognathus setosus* *Rhipicephalus sanguineus* *Notoedres canis* *Cheyletiella sp.*

active substances intended for dogs was highlighted after testing their effectiveness through natural infestations with different species of adult intestinal nematodes through laboratory studies that had a dosage confirmation purpose (Schnitzler et al., 2012). It was thus demonstrated that even the smallest dose of MO (0.75 mg/kg) prevents the development of the adult stage of Angiostrongylus vasorum, the French heartworm, as well as the larval and immature stages of A. caninum and T. canis (Böhm et al., 2014; Bowman et al., 2014).

A recent study carried out on a batch of dogs naturally infested with sarcoptic mange treated with several APs, of which MO and MO combined with afloxoranel showed remarkable results. Thus, MO as a single treatment showed excellent results in reducing itching after the third weekly dose without presenting adverse effects observed in dogs considered potentially sensitive to IVM. No allergic reaction was observed during the study (Romero-Núñez et al., 2020). Another study based on the efficacy of oral chewable tablets based on afoxolaner (NexGard®) and afoxolaner together with MO (NexGard Spectra®) demonstrated increased efficacy against canine sarcoptic mange, as previously shown in the evaluation of the two products against infestation with Dermacentor reticulatus ticks (Rehbein, 2016).

Following a recent study on a population of red pandas Ailurus fulgens, it was demonstrated that they were successfully treated, following the infestation with Angiostrongylus vasorum by administering a dose of MO (12.5 mg/subject) and praziquantel (PZQ) (125 mg/subject) for 3 consecutive weeks, followed by a 20-day break, thus receiving an oral tablet (Milbemax,

Novartis, Italy). The mode and route of infestation are unknown, the most possible hypothesis would be following the ingestion of dog feces infested with the intermediate or paratenic host located outside the zoo, placed through food or materials or following active movement, Denmark being immune by this parasite (Bagardi; 2019).

CONCLUSIONS

The studies and practical observations analyzed in this documentation argue the current trend of expanding the use of macrocyclic lactones in veterinary clinics and other fields of agor-biological practice. It is also confirmed that the products based on milbemycin oxime are particularly effective in the prophylaxis and therapy of canine helminthiasis, these can be administered both alone and in association with other anthelmintics, to extend the anthelmintic spectrum.

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