From macrocyclic lactones back to tetracyclines: new targets for the antiparasitic treatment in animals and humans

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Università degli Studi di Milano

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Antiparasitic drugs

The “ideal” antiparasitic drug should have a broad spectrum of activity against the different developmental stages of the parasite, have a wide margin of safety, be compatible with other compounds, and in veterinary medicine be easy to administer to a large number of animals, not require a long withholding periods because of residues, and be economical.
**Anthelmintics:**

- **Cholinesterase inhibitors:** organophosphates
- **Cholinesterase receptors:** imidazothiazoles and pyrimidines
- **Inhibitors of tubulin polymerization:** benzimidazoles and pro-BZD
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- **Inhibitors of enzymes in the glycolytic pathway:** clorsulon

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- **Cell membrane depolarizers:**
  - muscle hyperpolarization: piperazine
  - acting in nematodes primarily at the interneuron-motoneuron interface: macrocyclic lactones (MLs)
Human and animal filarial infections

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IVM is a mixture of AVM B\(_{1a}\) and AVM B\(_{1b}\).

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Other MLs used for treatment and control of endo- and ectoparasites in animals are doramectin, eprinomectin and selamectin, all belonging to the family of AVMs, and milbemycin oxime and moxidectin, belonging to the family of milbemycins (MBMs).
IVM efficacy against nematodes and arthropods took parasite control to a new level. For the first time, a single product, both safe and efficacious against the majority of economically-important internal and external parasites of all food-producing and companion animals, was made available. The amount of product required for activity was 10 to 100 times less than that of previous used products. Ivermectin showed an unprecedented high efficacy (often up to 100%) against inhibited, larval and adult stages of the major nematodes and larval and adult arthropods.

Since their discovery, MLs have exhibited selective toxicity, which defines an ideal anti-infective agent as one that has a chemical target in the microorganism causing infection, but either has no target or has no access to a target in the infected host making these compounds very safe and possible to use in potentially all mammalian hosts, reptiles, birds and fish.
It is likely that the entire family of AVMs and MBMs shares a common mode of action. In target organisms, the action is receptor mediated, and ligand-ligated chloride channels are the target proteins for this class of compounds. AVMs potentiate and/or directly activate arthropod and nematode glutamate-gated chloride channels. Modulation of other ligand-gated chloride channels, such as those gated by neurotransmitter \( \gamma \)-aminobutyric acid (GABA) may also be involved.

The consequence of the AVM-receptor interaction is an increased membrane permeability to chloride ions. In nematodes and arthropods, AVMs potentiate the ability of neurotransmitters such as glutamate and GABA to stimulate an influx of chloride ions into nerve cells resulting in loss of cell function.
Wolbachia endosymbionts: basic information

The presence of intracellular endosymbionts were microscopically observed in filarial nematodes at the beginning of the seventies (McLare et al., 1975; Kozek, 1977)

These bacteria were subsequently identified as *Wolbachia*.

... at the beginning of *Wolbachia* story in the filarial worms
The first report of endosymbiont bacteria in filarial nematodes was in 1995, when the presence Wolbachia was demonstrated by molecular methods in *Dirofilaria immitis*.

Afterwords, *Wolbachia* was found in many species of human and animal filarial parasites (*Onchocerca volvulus*, *Wuchereria bancrofti*, *Brugia malayi* and *D. repens*).

This bacteria are philogenetically close to rickettsiae and are transovarial transmitted to microfilariae. Large amounts of bacteria are found in the cuticle of male and female adult nematodes, in female gonads and in embryos.

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**WAM, Dirofilaria and host immune response**

WB of serum from cats with *D. immitis*

- A control protein
- B rWSP

Healthy cats  *D. immitis*+ cats
How does Wolbachia interact with the immune system?

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LPS-like (B. malayi, O. volvulus)

Wolbachia associated molecules (WAM)

Wolbachia surface protein (WSP) (D. immitis, B. malayi)

hsp60 (B. malayi, D. immitis)

PAMPs: pathogen-associated molecular patterns

• production of reactive oxygen intermediates (NO)
• production of pro-inflammatory cytokines
• up-regulation of co-stimulatory molecules (adaptive immunity)
Since TLR2 was involved in the recognition of WSP, we next used bone marrow-derived mouse Mphi of the genotype TLR2⁻/⁻ and compared their response to WSP with the reactivities of Mphi from wild-type mice. Stimulation of Mphi from wild-type mice with WSP in three separate experiments resulted in the release of high amounts of TNF-α while significantly reduced levels (41%) were found in supernatants of Mphi from TLR2⁻/⁻ mice.

This result not only further suggests an important role of TLR2 in immune responses towards WSP, but also implies the involvement of TLR2-independent pathways...

**ELISA with human sera on a recombinant protein of Wolbachia**

(Wolbachia surface protein, WSP)

A 10 sera from patients with pulmonary nodules due to *D.immitis*
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Effects of tetracyclines and derivates (oxytetracycline, doxycycline) on filarial nematodes (*in vivo*)
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**CONCLUSIONS**

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Brugia pahangi control (female)  Brugia pahangi treated (female)

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