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MECHANISM OF ACTION OF DRUGS WITH ACTIVITY AGAINST MULTICELLULAR PARASITES

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Parasiticides have been the most valuable weapons to combat parasites for almost half a century, constituting more than 50 % of veterinary pharmaceuticals and a good part of the products destined for human health. However, in many parts of the world, parasites develop resistance to these drugs, and it is now a major health problem. One way to avoid this inconvenience is the in-depth knowledge of its mechanisms of action, which will allow a more appropriate use. This work is a review of the different physiological aspects of parasite species and an explanation of the different mechanisms of action of antiparasitic agents.

Once these drugs are ingested or absorbed by parasites the agents come into contact with the parasites structures, and according to the drug's properties it will define the antiparasitic activity either by altering tegument, carbohydrates, protein and lipid metabolism, or motility of the multicellular parasites.

Key words: mechanism of action, pharmacodynamics, antiparasitic agents.

МЕХАНИЗМ ДЕЙСТВИЯ ЛЕКАРСТВ, ОБЛАДАЮЩИХ АКТИВНОСТЬЮ ПРОТИВ МНОГОКЛЕТОЧНЫХ ПАРАЗИТОВ

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Средства, убивающие паразитов (паразитициды), служили наиболее доступным оружием в борьбе с паразитами почти в течение полувека, составляя более половины ветеринарных фармацевтических средств и солидную долю лекарств, предназначенных для защиты здоровья человека. Однако во многих частях света паразиты развили резистентность к этим лекарствам, что сейчас представляет собой главную

проблему здравоохранения. Одним из путей преодоления проблемы является углубленное исследование механизмов действия паразитицидов, что позволит использовать их с наибольшим эффектом. Данная работа посвящена обзору различных физиологических аспектов жизнедеятельности паразитов и объяснению разных механизмов действия противопаразитарных агентов. Будучи переваренными или абсорбированными паразитами агенты вступают в контакт со структурами паразитов и в зависимости от свойств конкретных медикаментов проявляют антипаразитарную активность либо путем изменения свойств тегумента, изменения углеводного, белкового и липидного обмена, либо путем подавления подвижности многоклеточных паразитов.

Ключевые слова: механизм действия, фармакодинамика, антипаразитарные агенты.

POSSIBLE TARGETS OF ANTIPARASITIC DRUGS

The grand revolution on biochemistry during the last half of the twentieth century opened new fields of research for the development of selective parasitic agents (Mansour, 2002). In general, drugs can exert antiparasitic activity by altering tegument, carbohydrates, protein and lipid metabolism, and motility (Di Genova, 2016) (fig.1). The gene-expression machinery has been among the first targets for antiparasitic drugs and is still under investigation as a target for novel compounds. Other targets include cytoskeletal proteins, proteins involved in intracellular signaling, membranes, and enzymes participating in intermediary metabolism. In apicomplexan parasites, the apicoplast is a suitable target for established and novel drugs (Müller, Hemphill, 2013). Some drugs act on multiple sub-cellular targets. Drugs with nitro groups generate free radicals under anaerobic growth conditions, and drugs with peroxide groups generate radicals under aerobic growth conditions, both affecting multiple cellular pathways. Mefloquine and thiazolides are presented as examples for antiprotozoan compounds with multiple side effects (Müller, Hemphill, 2013). Regardless of the mode of

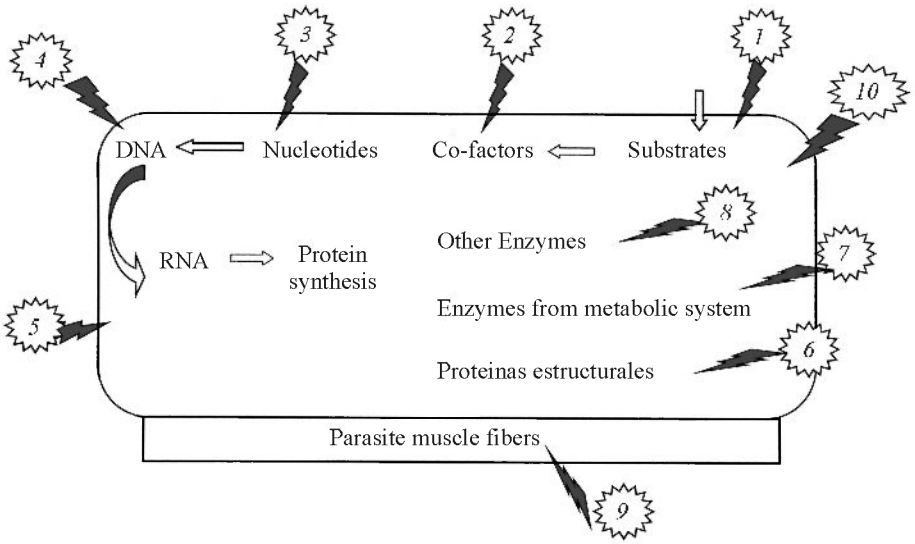

Fig. 1. Physiology of the parasite and potential sites of therapeutic intervention. In this figure we can identify at least 10 site where drugs may potentially exert their effects.

action, antiparasitic drugs must be ingested or absorbed by intestinal worms for expulsion of the parasite from the host. Most intestinal nematodes ingest material by pharyngeal pumping, which is altered by exogenous biological amines in both free and zooparasitic species (Abbott, 2004). Unfortunately, anthelmintics that cause neuromuscular paralysis may inhibit their own uptake by the exposed parasite by depressing this pumping (Martin, 2010).

Though the ability of a drug to cross the parasite's tegument it was always considered a major point to increase its efficacy, Some studies (Shah, 1983) demonstrated that the penetration of carbaryl, dichlorodiphenyltrichloroethane -DDT-, dieldrin and permethrin in insects and parasites is neither rapid nor selective. Thus effectiveness of a drug may depend on its metabolism, storage and transport, more than on its penetration ability. Other authors, however, do not agree since liposolubility of organic chlorides favours penetration and efficacy of organic phosphates (Burgat, Sacaze, 1988). Albendazole reaches higher concentrations than its oxidized metabolites (sulfoxide and sulphone) in *Fasciola hepatica* because the original drug is more liposoluble (Fetter, 1984).

Other studies suggest that penetration and possibility of reaching receptors influence efficacy, although the susceptibility of the parasite should not be ignored. In this way pyrantel would exert little effect on *Trichuris* spp. because of a lack of penetration, although the susceptibility of the parasite should not be ignored (Terada et al., 1983a). It seems almost certain that penetration, distribution, metabolism and elimination are key issues to obtain best effects from an antiparasitic drug. A more selective mechanism of action has been proposed recently for these agents based om the effect on the tegument of cercarias – *Schistosoma mansoni*, tegument changes similar to those produced when larvae penetrate through the skin could be induced. If larvae do not penetrate, they die, because of their osmotic susceptibility to water. This action could be produced by substances like 2-tetradecenoic acid that are only slightly toxic to other aquatic organisms (Haas, 1984).

The induction of abnormal activity in parasites' alimentary and/or reproductive habits could lead to lethal or sublethal effects. Some drugs can induce anorexia, like the thiolic oxidant etilendiamide in *Triatoma infestans*, apparently by sulphydril (SH) groups reacting with abdominal receptors and probably modifying CNS neurotransmission.

Formamidines can produce a similar action in insects, mites and ticks (Holingworth, 1976; Hollingworth, Murdock, 1980; Giordani et al., 1986). Changes in alimentary habits of ticks are induced by drugs altering pheromone production (2, 6 dichlorophenol in the majority of tick genera studied). This pheromone would affect sexual attraction, migration and feeding (Gothe, 1983).

In insects and ticks, ecdisteroids play an important role in growth and development. Arthropods are able to esterify these substances into long chain fatty acids, probably inactivating excessive amounts ingested with the hervibore host's blood (Diehll et al., 1985). A change in ecdisteroids metabolism would be a possible target in the arthropods.

A new group of 1, 5 di-sustituted imidazoles (1 citronelil-5-fenilimidazol) (Kuwano et al., 1984; Kuwano et al., 1985) antagonize synthesis of juvenile hormones in insect larvae (Lepidoptera: *Bombix mori*) favouring precox metamorphosis. Some of the drugs have also anorexic properties perhaps providing a new path to attack insects (Asano et al., 1984).

Regarding carbohydrate metabolism, available information is based on «*in vitro*» helminth research. For a better understanding of differences of drug actions in different species, changes in glucose metabolism in nematodes, trematodes and cestodes must be considered (fig. 1). There are no differences in glucolysis until phosphoenolpyruvate (PEP) is produced (Berg, 2002). Nematodes (*Ascaris suum*) transform most PEP into oxalacetate (OAA). Then, OAA is transformed in mitochondrial membranes into pyruvate and fumarate and this last into succinate (Fairbarn, 1961; Berg, 2002) through fumarate reductase (FR). The formation of adenosine triphosphate is associated with this reaction. Nematodes thus depend largely on their mitochondrial cycle to obtain energy, although there are some differences among them. For example *Litosomoides carinii*, unlike *Ascaris* spp. and some other helminths, can produce ATP in mitochondria in the presence of succinate and pyruvate, plus malate, using also oxygen (Ramp et al., 1985). On the other hand, in *Ascaris* spp., fumarate plays the role of oxygen in an aerobic system (Rew, 1978). *Brugia pahangi* and *Dipetalonema vitae* also use oxygen, as demonstrated by the diminution of ATP production when oxygen is lacking (Mendis et al., 1985).

Cestodes convert PEP 50 % in pyruvate and 50 % in OAA (fig. 1) which is then converted to malate, malate is used as in *Ascaris* spp. to obtain ATP in the mitochondria (Bueding, Saz, 1968).

Trematodes transform PEP into final products like pyruvate and lactate. This would not involve mitochondria, although Isseroff and Walczac (1971) found succinate excretion by *Fasciola hepatica* (fig. 2).

Some larval stages, like oncosferas, appear inactive metabolically and would not be susceptible to drugs that interfere with metabolism (Siracusano, 2009). This is true also for some helminth larvae, like *Ostertagia ostertagii*, although in this case, efficacy of active drugs seems to increase when there is long exposure of larvae to the drug as with reduced drug solubility, passage of low amounts of drugs from rumen to abomasum, and persistence of abomasal concentrations for several days (Armour, 1986).

As in vertebrates (Abou-Donia, Womeir, 1986), differences in susceptibility of different ages parasites to drugs would probably be due to differing activities of enzyme systems.

Protein metabolism and nucleic acid synthesis can be altered by drugs. The antitrypanosomal agents berenyl, antricyde and suramine, block incorporation of aminoacids (AA) into parasite proteins (Smith, 2009). Benznidazol, used to treat Chagas disease is South America, binds covalently to proteins and nucleic acids, altering them (Trochine, 2014). Microtubules, as essential components of almost all eukaryotic cells, are proven drug targets in many helminth diseases and show promise as targets for the development of new antiprotozoal drugs (Fennell et al., 2008).

Little is known about lipid metabolism in parasites and possible ways to modify it. Cholesterol is necessary for development of *Ascaris* larvae (Fleming, Fetterer, 1983), and is obtained by transcuticular transport. This transport is also used by glucose and some anthelmintics (Verhoeven et al., 1976).

Early farmers used seaweed as a natural pesticide to protect plants and livestock but the reason why seaweed was an effective pesticide was unclear. More recently, studies have shown that betaine, an amino acid that functions as an osmolyte and methyl donor, which is present in seaweed, arrests nematode larval

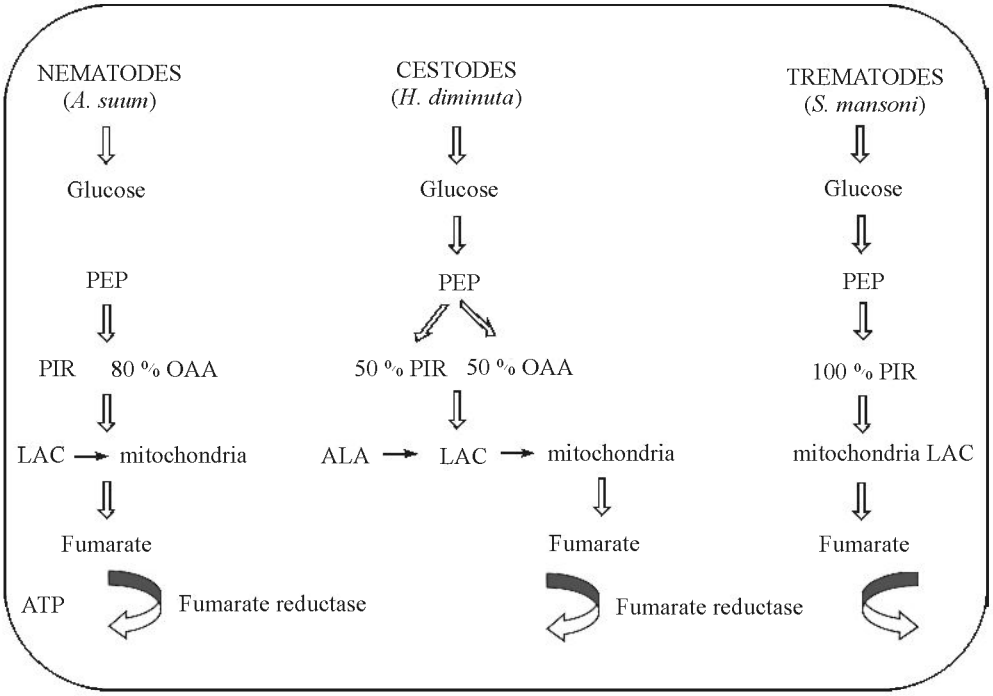

Fig. 2 Metabolic pathways. In this figure we can see the metabolic pathways follow up by the principals helmints.

development, but the molecular target and pathway for its effect still remained unknownBetaine transporter SNF-3 and betaine receptor ACR-23 were found in the nematode *C. elegans* overactivation of the receptor by excess betaine or by the allosteric modulator monepantel resulted in hypercontraction and death of the nematode (Zhen, 2017). Thus, monepantel targets a betaine signaling pathway in nematodes (Peden et al., 2013).

So, as mentioned above, antiparasitic agents use parasite components as site of union to do their jobs. Now, let's take a deeper look at the inside of each group.

1. IMIDAZOL DERIVATIVES

Imidazoles were a breakthrough in antiparasitic treatment. A N substitution of imidazoles created the family of triazoles, which have the same mechanism of action as imidazoles, similar or broader spectrum, but with less effect on the synthesis of human sterols (see table).

1.1. Benzoimidazoles (BIZ)

The BIZ are used to treat nematode and trematode infections in humans and animals. It acts by binding to the microtubules. It also binds to the spindle microtubules and blocks nuclear division (Loukas, Hotez, 2006; Lutz, 2012).

Imidazole derivatives used as veterinary anthelmintics[1]

Group	Drugs
Benzoimidazole (BIZ) derivatives	Thiabendazole (TBZ)
Benzoimidazole carbamates (BIZ-C)	Albendazole (ABZ), Mebendazole (MBZ), Fenbendazole (FBZ), Cambendazole (CBZ), Oxfendazole (OXZ), Oxibendazole (OxiZ), Flubendazole (FluBZ), Lobendazole (LBZ), Triclabendazole (TriBZ), Luxabendazole (LuxBZ), Ricobendazole (RBZ)
Pro-benzoimidazole (pro BIZ)	Febantel, Netobimin, and Tiofanato
Nitroimidazole (NIZ) derivatives	Benznidazole, metronidazole, dimetridazole, tinidazole
Imidazol phenolic complex derivatives	Clotrimazole, miconazole, ketoconazole
Imidazotiazols	Levamisole, Tetramisole

Note. [1] In this table we can observe the differents groups with antiparasitic activity and also the medicines that makes up each group.

The first veterinary BIZ introduced in the 1960's (e. g. Thiabendazole – TBZ, Oxfendazole — OXZ) were highly effective against adults and larvae of most gastrointestinal roundworms (nematodes) of livestock. In the 1970's newer benzimidazoles such as albendazole, fenbendazole, mebendazole and oxfendazole were introduced that are also effective against non-gastrointestinal roundworms (in the lungs, kidneys, skin, etc., depending on compound and dose) as well as against tapeworms (cestodes). Albendazole is also effective against adult liver flukes (*Fasciola hepatica*). TBZ is a special case: it is not effective against roundworms or tapeworms, but controls all larval and adult stages of various parasitic liver flukes (trematodes) (Junquera, 2015).

TBZ represented a major step forward when it became available more than 30 years ago. At that moment; it was a novel, true broad-spectrum and potent anthelmintic with a classic mechanism of action and safe for the animal. Since that time parasite resistance to the BIZ has been discovered in several species (Bowman, 2014). From TBZ, by a series of substitutions, different Benzoimidazole carbamates -BIZ-C were obtained. These have a carbamate group that increases the anthelmintic effect through a second mechanism. Pro-BIZ compounds, like febantel are biotransformed into BIZ, and act similar to this last (Deitsch, 2001).

TBZ and most BIZ-C except Mebendazole -MBZ, inhibit mitochondrial FR «*in vitro*», especially in nematodes (Robetson, 1982). Consequently succinate and ATP, cannot be produced. This is a slow process, and parasite elimination takes some 2—3 days after treatment (Shang, 1981).

Another site of action was suggested to explain the wide spectrum of these drugs including fourth-stage larvae (L4) inhibited stages of *Ostertagia* spp. (Williams, 1991). This also depends on drug concentration and, possibly more importantly, the duration of drug exposure. Steffan et al. (2005) determined the efficacy of Ricobendazole -RBZ against inhibited larvae of *Ostertagia ostertagi* (L4i) at very low for doses of 3.5 and 5.0 mg/kg. However, the drug was highly effective at a dose of 7.5 mg/kg confirming the dose-dependence of BIZ deriva-

tives against trichostrongylideos inhibited stages of nematodes (Duncan, 1976; Stasiuk, 2012).

Whereas the drug or metabolites in plasma provide direct contact with hematophagous species such as *Haemonchus contortus* and *Fasciola hepatica*, helminths are closely associated with gut mucosal epithelium encounter metabolites in digesta as well as those recycling between blood and gut lumen (Hennessy, 1991). On the other hand, FBZ increases the bioavailability of albendazole sulphoxide (ABZSO) and this may potentiate anthelmintic action (Merino et al., 1999; Hunter et al., 2007).

The following mechanism may explain the wide spectrum and activity on diverse stages of parasites (adults, larvae, eggs) of BIZ. The contractile process plays a major role in maintenance of life. These processes depend on the contraction of specialized proteins that appear very similar through animal species. In plathelminths and nematodes, the protein tubulin forms microtubules that are essential for transporting low molecular weight substances that parasites incorporate as food (Fetterer, 1986; Bennet, 2012). Tubulin also forms systems through which parasite cell secretions are transported. BIZ-C bind tubulin by their carbamate group, avoiding its polymerization (Aleyasin, 2015), and interfering microtubule function; this is called the colchicino-like effect (Murdry et al., 1987; Schmit, 2013). This property would suggest that these drugs are mitotic toxins, the difference, however, is that colchicine binds tubulin irreversibly, whereas BIZ-C binds reversibly to both parasite tubulin and mammalian tubulin (Hennessy et al., 1985). This would interfere with normal embryo development of nematodes and trematodes (Coles, Briscoe, 1978).

Despite the structural differences between the external surface of nematodes (the cuticle) and the external surface of cestodes and trematodes (the tegument), the mechanism of drug entrance into both types of helminth depends on the lipophilicity of the anthelmintic and this is the major physicochemical determinant for the drug to reach a therapeutic concentration in the target parasite (Alvarez et al., 2007).

In nematodes, microtubules of absorbent intestinal cells are altered within 24 h of treatment (Armour, 1986). In cestodes, the tegument is autolysed because of lack of transportation of lytic enzymes usually secreted to the media (Roberson, 1982).

In trematodes, BIZ-C with fasciolicidal action do not interfere with energy production but their mechanism is still unclear (Fetterer, 1984). MBZ, is active against cestodes and nematodes like FBZ (Lassegue et al., 1984), both may only interfere with parasite energy metabolism (glucose uptake from the media).

ABZ has little effect on energy production in *in vivo* studies with *F. hepatica* (Fetterer, 1984). This indicates that FR inhibition would not be important. Fetterer (1986) suggested inhibition of microtubules function would explain the letal effect on *F. hepatica* but did not exclude an additional mode of action.

The sulphoxide metabolite of ABZ induces *in vitro* surface and internal tegumental changes in *Fasciola hepatica* (Buchanan, 2003). BIZ-C used exclusively in trematodes, triBZ, neither binds tubulin, nor prevents development of embryos (Fetterer, 1986), although it is fasciolicydal against mature and immature fluke (Richards et al., 1985). There is possible, therefore, that another process in trematodes could be affected by triBZ and other fasciolicydal BIZ-C.

1.2. Nitroimidazoles (NIZ)

Benznidazole is an antitrypanosomic agent, used to treat Chagas disease. *Trypanosoma cruzi* converts this drug to active metabolites, which bind covalently with nuclear material and kinetoplast-DNA of the parasite (Diaz de Toranzo, 1987), inhibiting DNA synthesis and energy metabolism by degrading polysomes. Early activation of the cellular compartment of the immune system by interleukin 12 may favour in vivo benznidazole activity against *T. cruzi* (Michailowsky et al., 1998). A few small observational and randomized studies involving patients with chronic Chagas' disease have shown that benznidazole reduces the circulating parasite load, enhances seroconversion, and may halt the progression of cardiomyopathy (Villar, 2002; Viotti, 2006; Fabbro, 2007).

Metronidazole and dimetridazole were used in bovine trichomoniasis, and also in giardiasis, amoebiasis and balantidiasis in small animals and primates (Roberson, 1982). They are now used to treat protozoal and anaerobic bacterial infections (Waxman, 2004). Tinidazole has been used in equine giardiasis (Elsheihka, Khan, 2011), and a mechanism of action at a ribosomal level has been suggested. This effect could be secondary, as the primary mechanism involves inhibition of DNA replication. With metronidazole it seems that DNA replication is inhibited rapidly in anaerobic bacteria, though protein synthesis continues (Sigeti et al., 1983). Reduction of the nitro group by bacteria or protozoa is necessary to activate the drug (Yeung et al., 1984). Producing toxic metabolites cause DNA strand breakage (Lamp et al., 1999). It should be noted that tinidazole displays much higher mutagenic activity than metronidazole in *Salmonella* spp. (Riviere, Papich, 2013).

1.3. Imidazole complex phenolic derivatives

Miconazole, clotrimazole, ketoconazole and similar drugs are active in superficial and systemic mycosis. Their mechanism of action involves alteration of ergosterol synthesis because of inhibition of acetate incorporation. Ketoconazole may also inhibit P-glycoprotein (Finch, Pillans, 2014). Consequences are increased plasmalemmal thickness, and inhibition of nutrient uptake. TBZ has some antimitotic properties also, but its mechanism probably involves inhibition of transamination and incorporation of aminoacids into proteins.

1.4. Imidazotiazoles and derivatives

Levamisole had a great impact initially because of its anthelmintic efficacy (Chagas et al., 2016) and more recently because of its immunomodulatory activity (Sanda, 2008; Chagas et al., 2016) that is shared, although slightly, by TBZ (Shen, 1981). Its activity over nematodes can be produced by two mechanisms. The less important involves, mitochondrial FR inhibition (Rew, 1978; Webster, 1986) and is attributed to a metabolite lacking sulphur, that blocks sulfhydrile enzyme groups, interfering with ATP synthesis (Ruckebusch, 1981; Martin et al., 2005). Surprisingly, Shen (1981) suggests the immunomodulatory activity is due to a sulphurated metabolite. A second mechanism (Armour, 1986) involves

a sustained nicotine-type overstimulation of ganglia, which can be blocked by mecamilamine (Roberson, 1982). This induces spastic paralysis of nematodes (Taylor et al., 2016). Slight inhibition of acetylcholinesterase is also mentioned, and it acts as a cholinergic agonist in *T. spiralis* (Gimenez Gonzalez et al., 1998). Mammalian toxicity is produced by a similar mechanism.

2. SALYCILANILIDES

2.1. Closantel

Closantel is a wide spectrum, salycilanide endo-ecto-parasiticide, with ability to uncouple «*in vitro*» and «*in vivo*» mitochondrial oxidative phosphorylation. This results in reduced ATP synthesis with consequent reduction in nutrient transport, macromolecular transport and parasite motility (van der Lugt, Venter, 2007).

Some investigations demonstrated closantel had anticholinesterase characteristics, which would explain its ectoparasiticide activity (Brem, Roux, 1984). However, administration of 5 mg/kg to cattle did not produce ectoparasiticidal action, although cholinesterase was reduced by 78 % (Brem, Roux, 1984). Thus anticholinesterase activity is not the most important effect.

As salicylanilide drugs have high membrane activity, a precise definition of its mechanism of action is not yet possible but future research should clarity this.

Rajamuthiah et al. (2014) confirmed that of closantel has an anti-staphylococcal activity against vancomycin-resistant *S. aureus* isolates and other Gram-positive bacteria.

2.2. Diamphenetide

This drug is extremely effective against juvenile flukes, but first needs to be converted to an active trematodicidal metabolite in the host (Rew et al., 1983). This deacetylated metabolite causes severe morphological damage (erosion of the tegument, and breakage of the tegumental osmoregulatory system, blocking of normal development sequence of vitelline cells) to the fluke. Diamphenetide blocks the protein synthesis of the parasite (Naranjo Feliciano, 2009). It does not appear to act on energy metabolism, but may be a potent Na+/K+/ATPase inhibitor. It also induces a rapid spastic (Rew et al., 1983) or flaccid (Fairweather et al., 1984) paralysis.

3. TETRAHYDROPYRIMIDINES: PYRANTEL AND MORANTEL

Studies on the mechanism of action of tetrahydropyrimidines indicate they are similar to levamisole (Andrews, 2004). They act as cholinergic receptor agonists in nematodes (Köhler, 2001), with effects 100 times more potent than acetylcholine (ACH) and not so easily reversible, depending on drug concentration (Terada et al., 1983a). Other author suggested that pyrantel was a nicotinic

agonist in *Ascaris* spp. inducing a spastic paralysis because of persistent ganglionar stimulation (Yan, 2009). Terada et al. (1983b) confirmed this hypothesis working with another nematode, *Angiostrongilus cantonensis*.

4. ETILENDIAMINES: PIPERAZINE

Piperazine is a cheap and readily available anthelmintic agent with very wide therapeutic index (Ghasi et al., 2009). It produces an opposite effect to that of morantel, pyrantel and avermectin (Terada et al., 1983b). Pyrantel would be antagonized by drugs that mimic or stimulate liberation of inhibitory neurotransmiters namely α-adrenergics, and gamma-Aminobutyric acid -GABAergics in mammals. The toxicity could also be due to nicotinic stimulation at a ganglion and neuromuscular junction levels (Terada et al., 1983a). Z350, a synthesized piperazine derivative, posses α-1-adrenoceptor agonic effects and it is also a steroid 5-α-reductase inhibitor (Fukuda et al., 1999). A series of imidazo (1,5-a) quinoxaline piperazine ureas alert-butyl ester side chain at the 3 position all have high affinity for the GABA/benzodiazepine receptor complex an effects ranging from inverse agonists to full agonists. Additionally, several analogues were also effective normalising ciclic guanosin mono phosphate (cGMP) levels after applied stress, also consistent with anxyolytic-like properties (Jacobsen et al., 1999). It was therefore suggested that piperazine could have a quinidine-like action, and therefore could possess antiarrhythmic properties (Ghasi, 2009).

5. FORMAMIDINES: AMITRAZ

The acaricidal and inseticidal activity of amitraz (AMZ) is due to its antagonistic effect on octopamine receptors of the nerve cells in the brain. Parasites become hyperexcited, paralyzed and eventually die. This mode of action is different from those of synthetic pyrethroids, organophosphates and other ectoparasiticides (Junquera, 2015). AMZ actions against ectoparasites can be either pestistatic (sub-lethal) and pesticidal (lethal) depending on the drug concentration (Giordani et al., 1986). The lethal action develops with extreme excitability, incoordination and death. These actions can be explained by its interaction with octopaminegic receptors in affected mites and ticks. Several decades of research support the hypothesis that different isoforms of OPM receptors are localized pre- and postsynaptically, so OPM may act at a peripheral level, by modulating muscular excitability pre- or post-synaptically, depending on the invertebrate species. OPM presence has been demonstrated in the central ganglion of lobsters and some other invertebrates and in the synganglion of ticks (Morton, 1983; Morton, Evans, 1983, 1984). In some of these animals OPM activity is mediated through an OPM dependent adenylcyclase. It is common in insects octopaminergic systems for OPM to be blocked by phentolamine (an α-adrenergic blocker) and not by propranolol (a β-adrenergic blocker) (Evans, 1981; Vehovszky, 2000). Formamidines thus act at two levels as octopaminergic agonists (Essam, 2001), probably producing abnormal responses by a central action (Murdock, Hollingworth, 1980) and the nervous hyperexcitability through central and/or peripheral effect. Its effect is lethal because its binding to OPM receptors is

much more persistent than for OPM itself. Jorens et al. (1997) described AMZ as an α-2-adrenergic agonist.

The AMZ metabolite n-2, 4-demethylphenylmethylformamidine is more potent than AMZ in mites (Morton, 1983) and also in ticks (Schunter, Thompson, 1978).

Prostaglandins have long been associated with a negative effects on metabolism of intestinal helminths but, on the other hand, they are also involved in tick feeding. This suggests that activity of formamidines in general, and of AMZ in particular, could be, at least partly by inhibition of prostaglanding synthesis (Bonsall, Turnbull, 1983).

6. PRAZIQUANTEL

A major characteristic of this drug is its efficacy against cestodes, including those with low susceptibility, like *Echinococcus* spp. and *Diphylidium caninum* (Chai, 2013). Praziquantel is used as a trematodicide in people and is also effective against trematodes of veterinary importance (Armour, 1986; Chai, 2013).

Its antiparasitic effect is a consequence of two actions. One of these produces sustained, violent muscle contractions resulting in spastic paralysis of the parasite (Maule, 2005) and release from the attachment point. In humans, migration from mesenteric veins to the liver was reported. This action is produced by increased parasite membrane permeability to mono- and di-valent cations, specially Ca^{++} (Kohn, 2003; Maule, 2005; Jeziorskia, 2006). Although the rapid and intense contraction of the parasite is attributed to an action on ATPases responsible for calcium homeostasis. Praziquantel concentrations as high as 100 μM did not inhibit Na/K ATPase from tegument, Ca/Mg ATPase from heterogeneous and microsomal fractions of parasites, nor calcium permeability of microsomal vesicles (Cunha, Noel, 1997). These findings in *S. mansoni* do not support the proposed mechanism of praziquantel-induced contraction. The other postulated mechanism is also related to increase Ca^{++} influx, that leads to tegument vacuolisation and vesiculisation and kills the parasite. There is probably some relation between both mechanisms, as paralysis is an ion dependent effect (Bricker et al., 1983).

Doubts have been expressed as to whether it is the parent drug, or a metabolite (Roberson, 1982), that is responsible for the drug's actions. Praziquantel is rapidly taken up but not metabolized by parasites, although is metabolized by host mammals (Jeziorskia, 2006).

In *Schistosoma* spp. praziquantel does not affect immature stages (Matsuda et al., 1983) but favours mature eggs incubation. It has some activity on immature cestodes but lacks effect on hydatid cysts (Roberson, 1982).

7. PHENOLS

Hexylresorcinol (HR) was used in the past against *Ascaris* spp. and *Ancylostome* spp. in dogs and *Ascaris* spp. and *Oxyurus* spp. in humans (Lores Arnaiz et al., 1976), but has been replaced by better tolerated and more efficacious drugs (Krupyanski, 2012).

The mechanism of action was described as involving formation of tegument vesicles that evolved into more complicated lesions that killed the parasite. However, later articles described a more selective mechanism. Flaccid paralysis was found in *A. cantonensis*, *D. immitis* and *A. caninum*, before any tegument lesion occurred (Terada et al., 1985). However, the paralyzed parasites contracted in response to pyrantel, a nicotinic agonist. The paralysis was not antagonized by GABA or α-adrenergic blockers. Thus, HR may impede ACH liberation from cholinergic nerves, an effect that would be augmented by drugs like piperazine and ivermectin. This hypothesis requires experimental confirmation. It has been demonstrated that HR produces a inhibition of lysozyme activity which occurs at hexylresorcinol concentrations lower by an order of magnitude than glycerol inhibiting concentrations (Krupyanskii et al., 2012).

Disophenol, niclosamide and dichlorophen, which are structurally related to HR, could act by a similar mechanism. Phenols are sufficiently membrane active drugs, to make clear definition of their mechanisms of action difficult; there are probably other factors involved as well.

8. ORGANOPHOSPHATES

Organophosphates (OPs) compounds are the organic derivatives of phosphorous containing acids and their effect on neuromuscular junction and autonomic synapses is clinically important (Azazh, 2011). They are wide spectrum drugs that act by interfering with NT of the cholinergic system. They have has been used as internal and external antiparasitic drugs. Among those used to treat ectoparasitosis are propetamphos and diazinon. Dichlorvos and metriophonate (trichlorphon) are the only compounds used today to treat adult gastrointestinal parasites and *S. mansoni*.

OPs act by blocking AChase hydrolysis in sites of cholinergic activity (Chatonnet et al., 1999; Bajgar, 2004). It was suggested that as in mammals, AChase has two binding sites: an anionic site, formed by an ionized carboxylic group that binds to the cationic AChase site complemented by van der Waals forces, and an estearic site, that binds the portion of AChase corresponding to acetic acid (Wilson, 1967; Bajgar, 2004).

Once the substrate has bound reversibly to the enzyme, hydrolysis takes place in two steps: First, choline is liberated leaving the enzyme acetylated, and then acetic acid is liberated and enzyme regenerated. OPS only react with the esteratic cholinesterase site, producing a stable and irreversible union (Eleršek, Filipič, 2011). The under graded AChase accumulates eliciting excitability in cholinergic synapses in ganglia, parasympathetic terminals, neuromuscular junctions and CNS (Burgat-Sacaze et al., 1988). Degradation of the enzyme can be achieved with the combination of AChase and oximes (Gordon et al., 1999).

Arthropods are much more sensitive than mammals to OPs, and differences in sensitivity among different acetylcholinesterases, together with pharmacokinetic characteristics, like penetration into the parasite, could explain the various degrees of resistance found in arthropods.

9. PYRETHRINS AND PYRETHROIDS

Natural pyrethroids (pyrethrins, cinnerins and jasmolins) were obtained from *Crisanthemum cinerariaefolium* flowers. Because of the ester bond that characterizes their structure, natural pyrethrins are easily degraded by environmental factors (light, oxygen and alkalis) or by enzymatic systems of mammals and insects. Because of that the value of natural pyrethrins against insects is limited (Metcalf, 2002).

Synthetic pyrethrins or pyrethroids were developed to increase the potency and width of antiparasitic activity. They can be classified into type I (allethrin, tetramethrin, phenothrin, resmethrin, permethrin), and type II (cypermethrin, flumethrin, cyalothrin, cypothrin) agents. Type II pyrethroids includes a cyano group which increases spectrum and stability.

Pyrethroids' antiparasitic activity involves alteration of CNS function by means of changes in ionic conductance through neuronal membranes (Cagen et al., 1984; Soderlund, 2002). This activity can be mediated through three mechanisms. Firstly, interaction with one or more portions of the GABA receptor leads to synaptic activity (Doherty et al., 1988). GABA inhibits transmission in normal situations, permitting neurones rest (Giordani et al., 1989). This is to accomplished through it opening of Cl- channels and consequent Cl- influx and membrane hyperpolarization (Giordani et al., 1987). Type II pyrethroids binds to the GABA receptor at the Cl- channel level, acting as GABA agonists and producing hyperexcitability and paralysis (Taylor-Wells et al., 2015). Secondly, both classes of pyrethroids interact with nicotinic receptors by binding to ionic channels, depending on the nicotinc ACH receptor, thus altering the ionic flow and inhibiting paralysing the in insects or host.

The third mechanism of action would be mediated through reversible and stereospecific binding to receptors in axonal Na^+ channels, specially α-subunits (Dong et al., 2014). This would retard closing of the channels, with increased Na^+ influx, eliciting repetitive discharges, hyperexcitability and paralysis, especially with type I pyrethroids. For type II agents the process is much slower, giving rise to depolarization and consequent paralysis (Gotoh et al., 1998). Rao and Rao (1997) found permethrin and cypermethrin decreased K^+ uptake across synaptosomes in rat brain. In other way, permethrin and resmethrin consistently stimulated ATP-dependent and ATP-independent uptake of glutamate, without evoking depletion of its vesicular content (Vaccari et al., 1998).

10. ORGANOCHLORINES: DICHLORODIPHENYLTRICHLOROETHANE (DDT)

The efficacy of these drugs against insects and mites and their mammalian toxicity are well known. The mode of action differ between various agents in the group.

DDT can act by contact or by ingestion because of its high liposolubility. It acts as described for type 1 pyrethroids in invertebrates (Lund, 1984) and vertebrates (Herr et al., 1986), altering channels that regulated Na^+ permeability though membranes causing increased excitability (Denholm et al., 2002).

Only a small change in the channels is required to elicit this effect. In vertebrates, tremors affect head and neck first and progress caudally probably causing paralysis, depending on drug concentration and exposure time (Herr et al., 1986).

Cross-resistance had been demonstrated between DDT and pyrethroids in various insects and mites (Nardini et al., 2013). The lethality of DDT is known to correlate with its content in brain. The similarity of the signs of toxicosis with DDT and type I pyrethroids suggests they have a similar mode of action resulting from specific interactions with either GABA or glutamate receptor/ionophore complexes (Doherty et al., 1988).

11. CYCLODIENES: ALDRIN, DIELDRIN, LINDANE

11a. Aldrin and Dieldrin are too toxic to be used safely in animals (U. S. Department of Health and Human Services, 2002). They are included here only as a reference for a better understanding of lindane. They are powerful CNS stimulants in vertebrates and invertebrtates. This action is due to GABA antagonism (Matsumura, 1984).

In mammals, these agents tends to elicit convulsions, even without prodromic signs (Klaassen, 1986). They bind to the picrotoxin (PT) site of the GABA receptor. The degree of binding is well correlated with insecticidal action (Matsumura, 1984). Resistant insects have changes in the spatial structure of the receptor and either do not bind these drugs or bind them in very low amounts. In other cases the number of receptors diminishes (Matsumura, 1984).

11b. Lindane also binds to this same site in mammals namely the complex GABA-benzodiazepines receptor in the zone corresponding to the chlorine ionophore (Benarroch, 2006). This zone binds PT and other convulsant drugs the PT-like convulsants. A potent convulsant, 35-S butylbicyclophosphotionate (TBPS) binds to another site of the ionophore, a site that it shares with lindane in rat CNS (Eldefrawi et al., 1984). This apparent discrepancy in lindane binding sites, is not important, because the sites are adjacent, and the mechanism leading to convulsions is the same in either case involving failure of chlorine ionophores to open, lack of chlorine entry, hyperexcitability and convulsions. A similar mechanism of action probably exists in insects and mites.

The lack of cross resistance between DDT and lindane would be because the drugs act at different sites.

12. AVERMECTINS AND MILBEMICINS

Avermectins (AVM) and milbemycins (MBC) are macrocyclic lactones derived from *Streptomyces avermitilis* and *S. syanogriceous* mycelia respectively (Lumaret et al., 2012). They all are regarded as wide spectrum antiparasitic drugs (Campbell, 1989).

These drugs seem to have similar mechanisms of action, though the mechanisms are not yet completely clear. Activity on GABA receptors has been demonstrated, particularly in mammals, but the process seems very complex (Mendes, 2017). Various theories have been expounded:

1. GABAergic agonism. Ivermectin would bind to GABA receptors exerting a GABA-like action (Estrada-Mondragon, Lynch, 2015).

2. Potentiation of GABA-receptor binding (Campbell et al., 1983; Ménez et al., 2012).

3. Stimulation of presynaptic liberation of GABA. This would be similar to other mechanisms reported in mammalian synaptosomes through which GABA would be liberated from presynaptic endings (Giordani et al., 1988).

4. Increase in the number of GABA receptors (Giordani et al., 1988; Campbell et al., 1993).

5. Stimulation of adrenergic and/or GABAergic, or inhibition of cholinergic mechanisms (Terada et al., 1984). The adrenergic mechanism could be similar to that in mammals, in which GABAeregic neurones' receptors liberate GABA when stimulated by noradrenalin (Giordani et al., 1988; Campbell et al., 1993).

6. Increased Cl^- permeability in muscles (Mehlhorn, Aspock, 2008). Muscles in lobsters (*Schistocerca gregaria*) exposed to B1a dihydroavermectin shown reversible and irreversible increases in Cl- permeability. This could be caused by:

A. Changes in lipids or other membrane components (Haydon et al., 1977).

B. «*in vitro*» changes in GABA receptor selectivity by diverse anions (Cl^-, Br^+, I^-) (Scott et al., 1985).

C. Interaction with glutamate receptors (receptor H), that could hyperpolarize muscle membranes by opening Cl^- channels (Scott et al., 1985). Other authors, however, reported glutamate as an excitatory neurotransmitter (Usherwood, 1984).

D. Modification of chlorine channels not necessarily related to GABA receptors (Smart, 1982).

Binding to parasitic retinol binding protein (PRBP) (Sani et al., 1988; Garofalo, 2002). PRBP binds retinol at the cell surface and the resulting complex migrates through cytoplasm to the nucleus, where has a specific effect on gene expression (Sani et al., 1983). The role of retinoids in helminths is not completely clear, but it could be involved in vital biological functions as in mammals, like growth and reproduction. IVM which has structural similarities to retinol inhibits binding of (3H) retinol to PRBP in very low concentrations (Sani, 1984). Reproductive alterations in parasites could be due to this mechanism instead of GABAergic interaction (Giordani et al., 1988).

All except the last theory involve blockage in postsynaptic transmission by interrupting Cl^- influx entrance; the Cl^- hyperpolarizes cell membranes, so stimuli cannot pass.

Another theory about the mechanism of action could explain the toxicity in different animals. In normal tissues, P-glycoprotein, is found on the luminal surface of cells of renal proximal tubules, small intestine and colon, and bile canalicular face of hepatocytes, as well as in adrenal glands and capillary endothelial cells in brain and testes. P-glycoprotein plays an important role in absorption and distribution of many chemotherapeutic agents across the blood-brain barrier (Didier, Loor, 1995; Benet, 2009).

Lankas et al. (1997) studied a subpopulation of CF-1 mice that showed marked susceptibility to avermectins and lack P-glycoprotein. When IVM was injected into these mice, very high tissue ivermectin concentrations were found, especially in brain. By comparison, CD-1 mice with high P-glycoprotein levels

were relatively resistent to ivermectin toxicosis and tolerated doses more than 50 times greater than the minimum toxic dose for P-glycoprotein deficien mice. When pregnant female CF-1 mice were given ivermectin (Lankas et al., 1998), homozygous P-glycoprotein deficient foetuses all suffered cleft palates, whereas heterozygous littermates were less susceptible, and homozygous P-glycoprotein replete fetuses were unaffected at all doses tested.

The idea that ivermectin was safe for mammalian treatment because of its very complex structure and difficulty in crossing the blood-brain barrier where most GABA inhibitor neurotransmitters act, was re-examined. When considering the use of macrocyclic lactones dogs, veterinarians may take into account the known sensitivity of *Scotch collies* (both rough and smooth) to neurotoxicity when administered these drugs at higher than label doses. But the adage has also been applied to many other herding breeds and has prevented veterinarians from using these drugs in situations where they would have been ideal. The neurotoxicity was attributed to a leaky «blood-brain barrier» in susceptible dogs. Developments in the molecular mechanisms of this phenomenon have opened a new frontier in the area of pharmacogenetics, drug disposition determined by the animal's genotype. Genetic studies have documented the mdr gene deletion in 10 breeds. Dogs that are homozygous for the gene deletion readily show adverse effects from ivermectin and other P-glycoprotein substrate drugs at dosages that cause no adverse effects in normal dogs. Heterozygote dogs may show toxicity at increased doses of substrate drugs, such as daily ivermectin administration for the treatment of demodecosis (Dowling, 2006).

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