ABSTRACT

Infection with filarial nematodes remains endemic in several countries worldwide and some of these infections are commonly associated with severe disease. The elimination of lymphatic filariasis relies on drug administration using the three drugs currently available for treatment: diethylcarbamazine, albendazole, and ivermectin. However, development of drug resistance is a reported phenomenon. The issue of resistance to antihelminthics used in humans has become increasing importance since the global program to eliminate lymphatic filariasis is implemented in larger population groups and the duration of the program is increasing. Recently, ivermectin resistance has been reported in Ghana, and widespread of resistance to benzimidazole (such as albendazole) is present because specific mutations in the gene encoding $\beta$-tubulin have been associated with drug resistance. Moreover, it is well known that diethylcarbamazine susceptibility is not 100% for lymphatic filariasis treatment. A review of the mechanisms of resistance to these antihelminthics is necessary in order to optimize the treatment for human lymphatic filariasis.

KEY WORDS: Filariasis, resistance, mass drug administration, albendazole, ivermectin, diethylcarbamazine

INTRODUCTION

Lymphatic filariasis is a disease caused by infection with a group of filarial nematodes transmitted by mosquito vectors. The World Health Organization (WHO) considers lymphatic filariasis to be a neglected tropical disease affecting approximately 120 million people in over 73 countries. Wuchereria ban-
Control and treatment of lymphatic filariasis is difficult for several reasons: limitation of effective drugs, vector control programmes unsuccessful and no available vaccines. For that reason, in 2000 WHO launched The Global Programme to Eliminate Lymphatic Filariasis (GPELF) with the objective to eliminate this disease as a public health problem by 2020. The elimination strategy is based on annual treatment of whole communities with combinations of drugs, known as mass drug administration (MDA). Currently MDA use the three drugs available for treatment at different regimens: ivermectin (IVM), albendazole (ALB), and diethylcarbamazine (DEC). The possibility of this global elimination strategy has been established due to the eradication some years ago of lymphatic filariasis in China and other countries using DEC alone.

However, the issue of resistance to antihelminthics using for treating human infections has become increasingly important. Previously, it has been already reported the emergence of high levels of drug resistance in veterinary medicine due to the extensively use of benzimidazoles (BZ) and IVM. In view of this fact, it stands to reason that the MDA in larger population groups for several years may perform a strong selective pressure on parasites, leading also to the emergence of drug-resistant strains. As population groups and the duration of the programme increase, the threat of treatment failure due to the emergence of resistance could be more visible. On the other hand, the genetic variation of filarial parasites can be an important factor that might influence the efficacy of the drug as well as the development and spread of drug resistance.

Until now, it has been demonstrated the evidence of non-susceptibility to DEC in filarial nematodes, although detailed studies of resistance are difficult due to a poor understanding of its mechanism of action. In 2004, resistance to IVM was reported in Onchocerca volvulus, but no confirmed reports of resistance in W. bancrofti have been provided. However, there is growing evidence of resistance to BZ in many nematode parasites.

Despite these findings, the importance and the mechanisms of resistance to these drugs remain still unclear. The objective of this review is to assess the magnitude of anthelmintic resistance in lymphatic filariasis as well as to explore the main determinants of parasite drug resistance in order to better understand the potential treatment failures with MDA and to improve the treatment strategies.

### ANTIFILARIAL DRUGS FOR LYMPHATIC FILARIASIS: MASS DRUG ADMINISTRATION

Because of the findings on the research during the past 30 years, new treatment regimens and strategies to face with lymphatic filariasis were established. Thus, in 1997 WHO classified lymphatic filariasis as a potentially eradicable disease. The main objective of GPELF is to eliminate lymphatic filariasis as a public health problem, decreasing in transmission by reducing the microfilariae load through the supply of a combination of two drugs that should be applied to entire populations at risk by MDA. The elimination strategy is based on stopping the spread of infection and in controlling morbidity. In order to apply this program, the first step consists on mapping the geographical distribution of the disease. Once made, once-yearly administration of single doses of two drugs given together for at least 5 years is the recommended regimen for treatment (table 1). Coverage of at least 65% of the total at-risk population should be established to be effective. Finally, a surveillance phase after MDA should be established with transmission assessment surveys. In areas where onchocerciasis is co-endemic, IVM (150 µg/kg) plus ALB (400 mg) is the recommended treatment, while in areas where onchocerciasis is not endemic, DEC (8 mg/kg) plus ALB (400 mg) is the preferred treatment regimen.

During the period 2000-2011, more than 3.9 billion doses of these drugs were administered to the endemic population. By the end of 2011, 53 of 73 endemic countries were already applying MDA; twelve of these countries had already moved to the surveillance phase. The final objective is to apply MDA for 100% of endemic countries and move to the surveillance phase and for 70% of endemic countries to be verified for no transmission by 2020.

### MECHANISMS OF ACTION OF ANTIFILARIAL AGENTS

#### Diethylcarbamazine (DEC)

DEC is a piperazine derivate used as major chemo-therapeutic agent to treat lymphatic filariasis since 1947. Although the cause by which DEC leads to decrease the concentration of parasites in the peripheral circulation is still unknown, several mechanisms of action have been suggested (table 2). In vitro studies have demonstrated that alterations in parasite eicosanoid metabolism are present. Specifically, the effects on parasite eicosanoid production (block on the

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Recommended treatment strategies for mass drug administration (MDA).</th>
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<tr>
<td><strong>Type of filarias</strong></td>
<td><strong>Region</strong></td>
</tr>
<tr>
<td>Lymphatic filariasis</td>
<td>Africa</td>
</tr>
<tr>
<td>Lymphatic filariasis</td>
<td>Rest of the world</td>
</tr>
<tr>
<td>Onchocerciasis</td>
<td>Africa</td>
</tr>
<tr>
<td>Onchocerciasis</td>
<td>Rest of the world</td>
</tr>
</tbody>
</table>

Source: WHO. IVC: ivermectin; ALB: albendazole; DEC = diethylcarbamazine.
cyclooxygenase pathway) and host endothelium can lead to death of microfilaria. These changes may produce vasoconstriction, amplified endothelial adhesion, and cytotoxic activity and then can lead to immobilization of microfilariae. All these facts could produce activation of the innate and non-specific immune system.

On the other hand, other studies have found evidences that DEC induces microfilarial DNA break with subsequent apoptosis possibly related to an altered function of microtubules, although this process is not enough to eliminate microfilariae, because in in vitro conditions the microfilariae are still alive.

With regard to macrofilaricidal effect, the distinct results in the different studies are most likely due to different level of transmission in the areas where the study was performed. However, available data show that DEC could also eliminate the adult worms causing lymphatic filariasis when administered in low doses in medicated table salt, but this macrofilaricidal effect is limited. Studies that show higher rates of adulticidal effect have often been carried out in areas without new worm infections.

**Albendazole (ALB)**

ALB is a broad-spectrum anthelmintic belonging to benzimidazole group of anthelmintics that has been used widely to treat intestinal helminthic infections since the late 1980s and now also serves as treatment of lymphatic filariasis. Several mechanisms of action were proposed for the BZs in the past, but currently it is well known that the BZ drugs perform their primary action selectively binding to the cytoskeletal protein β-tubulin. This binding inhibits the polymerization and blocks the formation of microtubule matrix which is essential to the functioning of cells. Finally these facts lead to stop cell division. Moreover, ALB blocks glucose uptake in the larval and adults stages, leading to depletion of glycogen and reducing the stores of adenosine triphosphate (ATP) leading to immobilization and death of the parasite. However, the most important effect of ALB on *W. bancrofti* is the prolonged suppression of reproduction by the adult parasites (approximately 9 months). Macrofilaricidal effect has been demonstrated only when high and frequent doses are administrated (400 mg/day/21 days), but it is associated with high side effects and intolerability.

**Ivermectin (IVM)**

IVM was the first commercially available macrocyclic lactone endectocide and until now it is the only macrocyclic lactone used in parasites infecting humans. This drug was discovered in the mid-1970s and it was introduced as antiparasitic agent in 1981, showing activity against a wide variety of nematodes. The target of IVM is aimed at the microfilaria stage of several nematodes (e.g. *O. volvulus*) binding with high affinity to glutamate (GABA)-gated chloride channels causing an increase in the permeability of the cell membrane to chloride ions with hyperpolarization of the nerve or muscle cell. Hyperpolarization results in paralysis and death of the parasite either directly or by lack of feeding in worms. GABA is an important inhibitory neurotransmitter in the nematode neuromuscular system, and some research found that GABA receptors could play a role in the mechanism of action of IVM.

In nematodes, macrocyclic lactones could cause some effects such as paralysis/activation of body muscle, pharyngeal pumping and inhibition of reproduction. Paralysis of the pharyngeal muscle is unlikely to be important in filariae. However, inhibition of reproduction could be the most important effect in these parasites. It is likely that IVM acts on microfilariae in vivo by increasing their sensitivity to the host immune system, although the true mechanism is still unknown.

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**Table 2** Summary of the proposed mechanisms of action and parasite drug resistance for MDA drugs.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mechanism of action</th>
<th>Effects</th>
<th>Mechanism of resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEC</td>
<td>Block on the cyclooxygenase pathway</td>
<td>Immobilization of microfilarias</td>
<td>Single nucleotide polymorphisms Activation of innate and non-specific immune system</td>
</tr>
<tr>
<td></td>
<td>Microfilarial DNA break</td>
<td>Apoptosis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Macrofilaricidal effect</td>
<td>Death of the adult parasite</td>
<td></td>
</tr>
<tr>
<td>ALB</td>
<td>Binding to β-tubulin and blocks the formation of microtubule matrix</td>
<td>Stop cell division</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>Blocks glucose uptake</td>
<td>Immobilization and death of the parasite</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Macrofilaricidal effect</td>
<td>Suppression of reproduction by the adult parasite</td>
<td></td>
</tr>
<tr>
<td>IVM</td>
<td>Affinity to glutamate-gated chloride channel</td>
<td>Paralysis and death of the parasite</td>
<td>Changes in β-tubulin, protein 60 and acidic ribosomal protein P-glycoprotein and ABC transporters dysfunction Polymorphisms of MDR1 and CYP3A genes</td>
</tr>
</tbody>
</table>

MDA: mass drug administration
DEC: diethylcarbamazine; ALB: albendazole; IVM: ivermectin
MECHANISMS OF PARASITE DRUG RESISTANCE

The development of drug resistance in veterinary parasitology suggests that the resistance to the treatment of lymphatic filariasis in human nematode parasites could be also produced. The occurrence of anthelmintic resistance in filarial parasites such as *W. bancrofti* is difficult to demonstrate because these parasites do not have a free-living stage and cannot be cultured in animal models. In this context, parasitological evidence of anthelmintic drug resistance can only be found by demonstrating the persistence of parasites after treatment over a long period of time. However, due to the difficulty to monitor the anthelmintic resistance in lymphatic filariasis, molecular markers for anthelmintic resistance are needed.

Drug resistance for lymphatic filariasis could become a growing risk which may be probably due to the selective pressure on parasite populations. Large scale exposure to antifilarial drugs through MDA programme can lead to the rapid emergence and spread of drug-resistant strains doing useless the efforts to eradicate the infection.

ALB is currently one of the drugs given for MDA in the GPELF programme, so as a component of this treatment it would be necessary to monitor the possibility of emerging of resistance to this medicine in lymphatic filariasis. However, not only drug pressure is responsible for the emergence of resistance, but also untreated populations of *W. bancrofti* might have drug-resistant alleles for ALB. The resistance-associated mutation could be found at a high frequency even before the MDA program had been started, although these mutations may be found at a high frequency in worms from treated patients.

ALB resistance in lymphatic filariasis and in many other nematodes is associated with single nucleotide polymorphisms (SNPs). Molecular markers for anthelmintic resistance have shown that this resistance is known to be produced by substituting tyrosine for phenylalanine in parasite β-tubulin at either position 167 or 200. The mutation at codon 200 appears to be more common in nematodes and has been demonstrated to be recessive. The deletion of phenylalanine and the substitution for tyrosine at codons 167 and 200 is the key factor for the high-affinity binding of ALB and other benzimidazoles to nematode tubulin leading to sensitivity to these anthelmimtics.

A study showed that in worms collected from patients treated with ALB plus IVM there were higher 200-genotype mutation than in untreated patients or those treated only with ALB. The overall allele frequency was 31.6% higher in the treated versus the non-treated worm population, which indicates that this mutation appears after drug treatment. Moreover, the same study shows that in patients who had been submitted to two rounds of treatment with ALB + IVM the allele frequency was an additional 25.9% higher.

Resistance to IVM had been previously found in nematodes infecting animals, although until now no confirmed resistance to IVM in lymphatic filariasis has been reported. However, it was not until 2004 when it was reported resistance to IVM in the human parasite *O. volvulus*. In a study performed in patients living in northern Ghana, a suboptimal response to ivermectin treatment was demonstrated, and more recently, resistance to IVM was found in other population-based study from Ghana. Some studies have been performed by analysing changes in β-tubulin, protein 60 and acidic ribosomal protein genes. However, no clearly relationship has been established between these genes and the apparition of IVM resistance.

On the other hand, research has also focused on the study to other genes such as P glycoprotein and ABC transporter that could be associated with resistance to IVM. Some works have concluded that IVM treatment results in a homozygote deficit of these genes in the treated population and changes in allele frequencies have been found after IVM administration. However, the significance of these findings in the development of resistance to IVM remains unclear.

On the other hand, IVM is a known substrate for both the CYP3A4 and MDR1 genes and several studies have previously identified genetic polymorphisms within the MDR1 gene related with some effects on drug response. In addition, the CYP3A gene and some subfamilies have been involved in the metabolism of some pharmaceutical drugs. It is well known that genetic polymorphisms may cause suboptimal response to some drugs due to a pharmacokinetic variability. Some authors have theorized about the relationship of the polymorphisms of both MDR1 and CYP3A genes and the presence of drug inefficacy.

In 2010, a study exploring the influence of genetic variations within MDR1, CYP3A4, and CYP3A5 genes on response to IVM treatment by examining genotype frequencies in responders and suboptimal responders was performed. The authors found both a significantly higher MDR1 (3435T) variant allele frequency in suboptimal responders than in responders and also than in the control group. With regard to genotypes, CYP3A5*1/*1 and CYP3A5*1/*3 were also found to be significantly different for responders and suboptimal responders. Although this study only included a small population, a relationship between CYP3A4 and MDR1 variants and response to IVM treatment could be established based on host genetic differences. These distinct genetic variants can play an important role in the variable drug response to IVM. However, further studies including larger populations will be necessary in order to confirm these results.

Many studies have reported that drug pressures on parasite populations leads to the development of drug resistance and reduces genetic variation among parasite populations. Eberhard et al. generated evidence of nonsusceptibility to DEC in *W. bancrofti* which indicates the possible threat of widespread resistance to this drug. The genetic variation of microfilarias is an important factor that may influence the efficacy of the medicine as well as the development and spread of drug resistance among parasite populations. DEC quickly reduces
the quantity of microfilariae in the blood, but according with some works this drug does not completely eliminate microfilariae from the blood\textsuperscript{46-49}. Because of the mode of action of DEC is poorly understood, the mechanism of resistance against this drug is currently unknown, so research is needed in order to discover it.

**GENETIC DIVERSITY OF FILARIAL PARASITES RELATED TO DRUG RESISTANCE**

Genetic diversity is mainly produced by mixing genetically different populations, although antifilarial drug pressure on the parasite populations could also play an important role in increasing this gene diversity. The introduction of new drugs for the treatment of filariasis can lead to the selection of alleles encoding resistance to these drugs, affecting the efficacy about the parasite.

Some studies have shown the existence of genetic variability of *W. bancrofti* parasite populations using techniques such as the random amplified polymorphic DNA (RAPD)\textsuperscript{50-51}. The RAPD profile was performed in order to analyse the phylogenetic relationships between the populations. The analysis of the RAPD profiles showed a high degree of genetic variability. In four endemic locations in India, population genetic analysis was carried out based on RAPD profiles of 31 individual populations. The highest number of polymorphic loci was found in the urban parasite populations. A phylogenetic tree was constructed using RAPD profiles and four different clusters were found in a rural area and three different clusters in an urban area. On the other hand, the profiles from the urban area showed that only one locus was common and the percentage of polymorphic loci among individual parasite populations was very high, so a high heterogeneity was found.

Moreover, genetic heterogeneity of microfilaria population within a carrier was analysed. Twenty-one individual microfilarias were investigated and gene diversity was found to be very high. In this case, four different lineages were obtained through the phylogenetic tree constructed, so at least four genotypes of microfilaria exist. Finally, the results of analysis showed that the parasite heterogeneity and number of genotypes increased with age probably due to the acquisition of new infections.

Other studies have used the EPIFIL system which consists of a model that describes some parasites population dynamics\textsuperscript{52-53}. Based on this model, the authors could describe the trend in drug resistance by examining factors affecting the spread of ABZ resistance under several assumptions\textsuperscript{54}. The speed of ABZ resistance spread is higher with treatment with ABZ + DEC than with ABZ + IVM. Moreover, the spread of ABZ resistance is strongly dependent on treatment coverage because this model also indicates that treatment with ABZ + DEC for 10 years at 85% coverage could increase the resistant genotype frequency to approximately 13%. Thus, higher levels of therapeutic coverage can lead to rapid spread of ABZ resistance\textsuperscript{54}.

Using the same EPIFIL system, Schwab et al. attempt to analyse the population genetics of potential multi-drug resistance in *W. bancrofti* due to combination chemotherapy\textsuperscript{55}. This model predicts a decrease of the possibility that IVM resistance will produce a parasite increase, although this decrease may occur in the number of adult worms resistant to both ABZ and IVM. This work also suggests that some changes in the proportion of ABZ and IVM resistant microfilariae could be observed during the treatment period. According to this model, the presence of IVM resistance would increase the rate of ABZ resistance and vice versa.

On the other hand, the model also indicates that children will have a higher proportion of resistance than adults, because acquisition of new infections will be higher in children, whereas could be reduced in adults because of protecting antibodies against filarial infections.

Genetic diversity of the parasite populations may influence the drug response and the clinical outcome, but also it can be a consequence of the drug pressure due to different chemotherapy strategies. With respect to this, Ramasamy et al. described the different genetic structure of *W. bancrofti* populations depending on the chemotherapy strategy\textsuperscript{56}. One hundred and seventy-eight blood samples from microfilaria carriers were analysed using the RAPD technique and genetic profiles were generated. From 74 populations of *W. bancrofti* under MDA treatment with DEC alone, there was 100% of polymorphism percentages, showing high levels of genetic diversity and could be clustered into eight groups. Furthermore, from 60 *W. bancrofti* parasite populations under treatment with DEC + ALB, there were 70% polymorphisms and 18 polymorphic loci, showing 5 clusters. Finally, 34 *W. bancrofti* populations with selective treatment with a standard dose of DEC during a long time were analysed, being the percentage of polymorphisms of 92%, showing 4 groups.

These results demonstrate the influence of the different treatment strategies on the genetic structure of *W. bancrofti*. Genetic diversity of filarial parasites is usually maintained at high levels because of some circumstances, but anti-filarial treatment causes a break in that stable state. Several treatment combinations at different doses may reduce genetic diversity and can lead to the selection of drug resistance alleles\textsuperscript{57}.

The study of cytochrome oxidase subunit 1 (COI) which is a useful marker for taxonomic and population genetic studies, has also been used in order to detect genetically different *W. bancrofti* strains. A study sequenced 22 samples from two Ghana districts\textsuperscript{58}. Genetic analysis shows 11 haplotypes, and 58 polymorphic sites were found between populations, being the southern population more polymorphic than the northern population. Furthermore, the COI gene from 16 patients infected with *W. bancrofti* was sequenced and the authors discovered 109 unique haplotypes with one common haplotype present in 15 of them\textsuperscript{59}. These data confirmed the high levels of genetic diversity in populations of *W. bancrofti*.

Because of the genetic variability may have important
implications in chemotherapy resistance and clinical response, further studies using more genetic markers and comparing the influence of different treatment regimens on the genetic structure of filarias will be necessary in order to identify different parasites strains which can explain in depth the difference in drug response of lymphatic filariasis.

NEED FOR NEW ANTI-FILARIAL DRUGS

Overall, treatment with anti-filarial drugs through the MDA programme had proved in a significant reduction of morbidity caused by microfilarias, although in some areas such as the Polynesian Islands over 50 years of MDA using DEC did not eliminate the infection\(^6\). However, broad use of the three drugs currently available increases the risk for the emergence and spread of drug resistance, which may be a threat to the development of the GPELF. Since there is no available vaccine and the vector control programs have failed, the short-term goal is to identify and develop new classes of drugs and alternative chemotherapeutic strategies.

In the 1970’s a group of intracellular bacteria called Wolbachia was discovered. This microorganism acts as endosymbiont of filarial nematodes except in Loa Loa, and it is well known that filarial parasites depend on these endosymbionts for metabolic and reproductive functions\(^6\). Many antibiotics but, above all doxycycline are known to be effective in the treatment of human filariasis through the action in Wolbachia\(^6\). In LF doxycycline at a dose of 100-200 mg/day for six weeks is microfilaricidal\(^2\), and at a dose of 200 mg/day for 4 weeks cause sterility and death of adult worms\(^4\). However, the macrofilarial effect of doxycycline in onchocerciasis is not 100% and is contraindicated in pregnant or breastfeeding women and children under 9 years, so new drugs will be necessary for these populations.

There have been two recent scientific advances that provide new perspectives for target validation for anti-filarial drug discovery like the development of RNA interference (RNAi) and the large-scale production of gene sequence information. RNAi provides an adequate but also a difficult technique for searching biological functions of genes in parasitic nematodes\(^6\). In this sense, a Scientific Working Group (SWG) recommended strategic guidelines for the research in this field\(^6\). RNAi screening of the filarial genome may found essential genes, some of which may be adequate targets for anti-filarial drug discovery.

On the other hand, the filarias genome sequencing may also serve as identification of drug targets for LF. Genome projects are based on the identification, cloning and sequencing of all genes of the parasites. Currently, about 20 million base pairs of expressed filarial parasite DNA sequence are available in genomic libraries\(^7\). Moreover, the sequencing of the mitochondrial genomes for filarial parasites may provide additional targets for potential useful drugs.

Additional biochemical compounds have been proposed as potential targets for the investigation of new anti-filarial drugs. The targets which have been validated to antifilarials include arachidonic acid, β-tubulin, antioxidant defence system, DNA topoisomerases, GABA receptor channel, glutamate-gated chloride channel and nicotinic acetylcholine receptor\(^8\).

CONCLUDING REMARKS

The goal of the GPELF is to eliminate the parasite infection based on the annual administration of ALB, IVM or DEC in two different regimens according the areas of endemicity. MDA to the general population is currently the main strategy of WHO to make in practice this objective in endemic areas. However, the majority of the medicines have only demonstrated a microfilaricidal effect, but they are ineffective on the elimination of adult worms. Moreover, it is well known that the filarial control programmes based on strong selective pressure on parasite populations could lead to the emergence of drug-resistant strains, and the duration of the programme may increase the probability of treatment failure related to parasite resistance.

Drug pressure may have influence about genetic variation among parasite populations and genetic structure of filarial parasites seems an important factor that may explain the decrease of drugs efficacy in some circumstances. In view of this threat, efforts should be taken on the study of mechanisms of resistance related to parasite genetic structure in order to determine the existence of drug-resistant strains, as well as the finding of new filarial genetic markers for drug resistance. Development of methods to monitor the emergence of drug resistance as well as the discovery of new potential targets for the investigation of new anti-filarial drugs is essential for the future control of the disease.

CONFLICT OF INTEREST

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REFERENCES

5. Gyapong JO, Kumaraswami V, Biswas G, Ottesen EA. Treatment strategies underpinning the global programme to eliminate...