Antibiotics which Target the *Wolbachia* Endosymbionts of Filarial Parasites: A New Strategy for Control of Filariasis and Amelioration of Pathology

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Abstract: *Wolbachia* endosymbionts of filariae are targets for the development of new antifilarial chemotherapy. Doxycycline to deplete *Wolbachia* from the worm has demonstrated the feasibility of this strategy and has provided a new chemotherapeutic tool. Recent research shows that depleting *Wolbachia* will also lessen pathology, and lessen adverse reactions to traditional antifilarial drugs.

Keywords: Lymphatic filariasis, onchocerciasis, *Wolbachia*, tetracycline, doxycycline.

Filarial nematode infections are endemic in more than 80 countries of the tropics. In these countries 200 million individuals are infected and 1 billion people are at risk of infection. The major forms of the human filarial infections are lymphatic filariasis, caused by *Wuchereria bancrofti* and *Brugia* spp., and onchocerciasis or river blindness, caused by *Onchocerca volvulus*. Lymphatic filariasis (LF) leads to recurrent debilitating fevers, lymphangitis and elephantiasis in 44 million individuals [1,2]. The latter pathologies, when they develop, are the result of the host immune response to dead adult worms in the lymphatic vessels. Host inflammatory reactions to dead *O. volvulus* larvae (microfilariae, MF) in the skin and eyes causes skin disease, Sowda or leopard skin, and visual impairment. In Africa, 500,000 cases of visual impairment, of which 270,000 cases resulted in blindness, are the direct result of *O. volvulus*, making onchocerciasis the second most frequent cause of preventable blindness in sub-Saharan Africa [3,4]. It should be noted that the cases of blindness reported here do not reflect the success of the vector control programme for onchocerciasis which concluded in 2002. In the countries participating in the vector control programme, the incidence of blindness due to onchocerciasis was shown to decrease by one third [5]. Previously, it was thought that filarial infections only lead to high morbidity, not mortality. However, three reports have shown an increased mortality rate for persons infected with *O. volvulus* in comparison to uninfected members of the same population [6-8].

Filarial infections have a negative impact on the infected individuals and the community. These negative effects include the health of the individual, the stigmatization of infected individuals, the economic impact due to loss of productivity, and the increased mortality rate [6-9]. Thus, filarial infections are a major public health problem in developing countries.

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BIOLOGY OF FILARIAL INFECTIONS

Adult filarial parasites are sexually dimorphic and reside in the lymphatic vessels (LF) or sub cutaneous nodules (onchocerciasis) where they mate and produce thousands of MF. The adults can survive 15 years in the human host. Insect vectors are required for development of the larvae into the human infective stage, and for transmission to the human hosts. LF is transmitted by several genera of mosquitoes (*Aedes*, *Anopheles*, *Culex*, and *Mansonia*). Onchocerciasis is transmitted by black flies of the genus *Simulium*. The vectors take up MF during blood meals. In the insect, the larvae develop into L3 infective larvae which are deposited on the skin during subsequent blood meals. The larvae enter through the wound made by the insect and develop into adult worms. Current drugs used to control transmission of filariae act primarily against the MF, although there are indications that ivermectin (**6**), (Fig. **1**), the drug used for onchocerciasis infections, may have prophylactic activity against infective stage larvae and there are reports that diethylcarbamazine citrate (**4**), DEC, (Fig. **1**) has macrofilaricidal effects [10-13].

PAST AND CURRENT CONTROL EFFORTS

The WHO has a three step plan to control filarial infections: i) reduce the intensity of infection to levels such that morbidity is below levels where the disease is a public health problem, ii) regional elimination which leads to the prevention of new infections, and iii) eradication of the worldwide incidence of infection. The WHO and individual countries have instituted control programmes that aim to accomplish the first two steps (Table **1**). The Onchocerciasis Control Programme in West Africa (OCP) used insecticides to control the black fly vectors in all participant countries. Togo and Sierra Leone also administered ivermectin. This successful programme ended in 2002 [14].

Currently there are two programmes to control onchocerciasis, the African Programme for Onchocerciasis Control (APOC; http://www.who.int/ocp/apoc), which is administered in hyperendemic areas of participating countries, and the Onchocerciasis Elimination Programme

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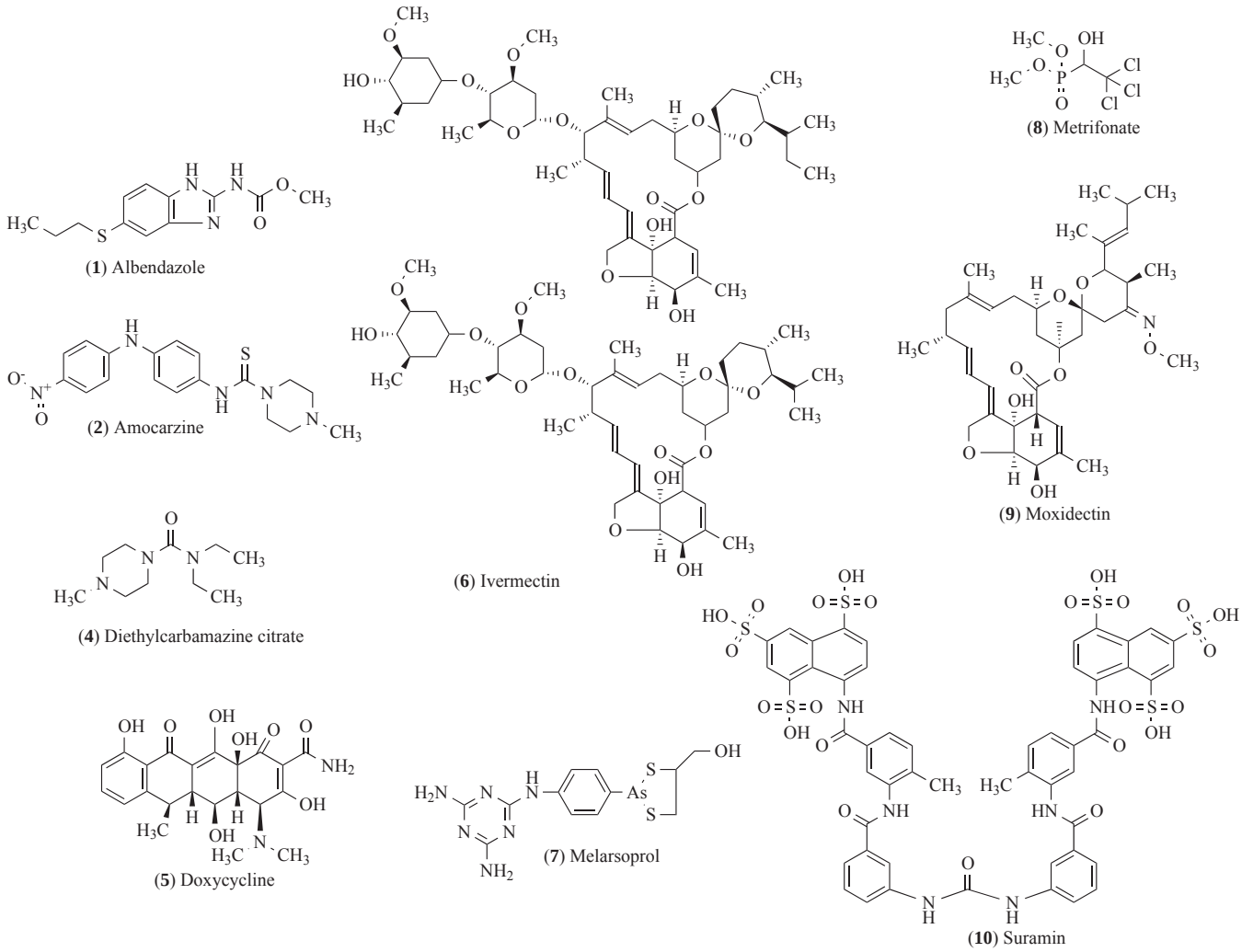

Fig. (1). Chemical structures of antifilarial drugs. Structures were generated using ChemIDPlus on the National Library of Medicine server (http://chem.sis.nlm.nih.gov/chemidplus/).

for the Americas (OEPA; http://www.cartercenter.org) [11,15]. For lymphatic filariasis there is the Global Programme for the Elimination of Lymphatic Filariasis (GPELF; http://www.filariasis.org) [16,17]. All programmes use annual or semi-annual administration of antimicrofilarial drugs to interrupt transmission. APOC and OEPA administer ivermectin (provided free to the WHO by Merck) in regions where onchocerciasis alone is endemic. The GPELF administers ivermectin and albendazole (**1**), (Fig. **1**), (provided free to the WHO by GlaxoSmithKline) in areas where onchocerciasis is co-endemic with LF, and diethylcarbamazine (DEC, can be inexpensively made in endemic countries) and albendazole where LF alone is endemic.

OEPA is administered in Latin America and is expected to be successful as onchocerciasis is hypoendemic in this region. This is mainly due to the fact that the *Simulium* species in Latin American countries are sub-optimal at transmitting the infection. The affected populations are also small enough that it is feasible to administer ivermectin twice a year. Thus, current mathematical models predict that the current levels of coverage, if maintained, will be sufficient to eliminate onchocerciasis from the New World [18].

Onchocerciasis in the rest of the world may prove harder to eliminate. Outside of the Americas, the *Simulium* flies are the optimal vectors. Semi-annual ivermectin treatment is not feasible as the affected regions are much larger than those found in the Americas. *O. volvulus* can live up 15 years, requiring ivermectin administration for many years. Mathematical modeling has predicted that a minimum of 25-35 years is required to break transmission in areas hyperendemic for onchocerciasis when there is a 65% coverage of the infected population [19].

Compounding the problems with treatment of onchocerciasis is that some regions are co-endemic for *O. volvulus* and *Loa loa* (transmitted by *Chrysops* spp.) [20,21]. Ivermectin is also effective against *L. loa*. However, it must be used with caution as patients with high *L. loa* MF loads may develop, due to the rapid killing of the *L. loa* MF, encephalitis that can be potentially fatal [22,23]. Such severe side effects effectively inhibit the current mass drug administration programmes from areas co-endemic for *L. loa*, areas that could be a source for new infections if they

Table 1. Past and Current Filariasis Control Programmes

Programme Name	Time	Countries	Objective and Strategy
Onchocerciasis Control Programme in West Africa (OCP)[a]	1974-2002	Benin, Burkina Faso, Cote d'Ivoire, Ghana, Guinea, Guinea-Bissau, Mali, Niger, Senegal, Sierra Leone, Togo (most countries also had ivermectin in the final years)	Elimination of onchocerciasis as a public health problem. Vector control using insecticides against *Simulium* larvae on a weekly basis.
Onchocerciasis Elimination Programme in the Americas (OEPA)[a, b]	1991-2007	Brazil, Columbia, Ecuador, Guatemala, Mexico, Venezuela	Biannual treatment with ivermectin (Mectizan®) to interrupt transmission.
African Programme for Onchocerciasis Control (APOC)[c]	1995-2009	Angola, Burundi, Cameroon, Central African Republic, Chad, Democratic Republic of Congo, Equatorial Guinea, Ethiopia, Gabon, Kenya, Liberia, Malawi, Mozambique, Nigeria, Rwanda, Uganda, Tanzania, Sudan	Community-directed distribution of ivermectin to interrupt transmission
Global Programme for the Elimination of Lymphatic Filariasis (GPELF)[d, e]	1999-2020	80 countries of Africa, Asia, and South East Asia	Mass drug administration to interrupt transmission. One of two drug regimes to be followed: i) ivermectin and albendazole in areas co-endemic for onchocerciasis; or ii) diethylcarbamazine and albendazole in areas not co-endemic for onchocerciasis. Also providing community training to alleviate morbidity caused by lymphedema.

a is from [14], b is from [15], c is from [11], d is from [17], e is from [16].

border areas where onchocerciasis is eliminated as a public health problem.

There are other indications that ivermectin and DEC treatment alone will not be sufficient. In onchocerciasis, a study in Cameroon of sites that had received ivermectin therapy for 10-12 years found infection rates of 2%-3% at the end of treatment, or when treatment was interrupted. This level of infection is enough to re-establish the infection within a few years [24]. Similar results have been seen with LF. A follow-up study of DEC treatment of *W. bancrofti* infections on a remote island in French Polynesia showed that despite 34 years of drug administration, about 4% of the population is still infected. This includes children as young as 2 years of age, which are clearly new infections [25].

Another potential problem is the development of drug resistance. The follow-up study in French Polynesia speculated that the persistent infection could be due to worms which were resistant to DEC [25]. There have also been reports of "low responders" to ivermectin in foci in Ghana, which could be the presages of ivermectin resistance in onchocerciasis [26,27]. The development of resistance to ivermectin or DEC would be catastrophic to APOC/OEPA and GPELF as these are the only drugs suitable for mass drug administration. Other drugs which have been studied are either less effective than the current ones, or they are too toxic (Table **2**, Fig. **1**). Therefore new macrofilaricidal or faster acting drugs are needed. Ideally these drugs would already be registered for use in humans, and they should not be more expensive to produce than the current drugs available. Current research which has lead to a new antifilarial therapy focuses on the intracellular, endosymbiotic bacteria of the filarial worms.

WOLBACHIA, TARGETS FOR A NOVEL CHEMOTHERAPY AGAINST FILARIASIS

Since the 1970s it has been known that filarial worms contain endosymbiotic bacteria. Morphologically these endobacteria resemble rickettsial endosymbionts [28]. In the worm, the endobacteria are found in the hypodermis, the oocytes, and in all embryonic and larval stages (Fig. **2A**) [29-33]. With the advent of molecular techniques, these endobacteria were identified as *Wolbachia*, closely related to the same genus found in many insects [34]. The endobacteria are transmitted transovarially (vertically) to the next generation. No transmission to other species, as is seen with insect *Wolbachia*, has been described. These two facts suggest a mutualistic symbiotic relationship. Consistent with this, the phylogenies of the *Wolbachia* strains from the various nematodes closely parallel that of the worm host [35,36]. Furthermore, the filarial *Wolbachia* genome is about 85% the size of that from *Wolbachia* in insects [37]. The endobacteria lack the ability to make all but one amino acid, yet retain the ability to make nucleotides, heme, riboflavin and flavin adenine dinucleotide, all of which may be metabolites provided by the endobacteria to the nematode as part of the symbiosis [37].While *Mansonella perstans* and *L. loa* worms do not have *Wolbachia* [38-40], *Brugia* spp., *M. ozzardi*, O. *volvulus*, and *W. bancrofti* contain *Wolbachia* [41,42], thus opening up a new area for discovery of chemotherapeutic drugs against the three major causative agents of filariasis.

WOLBACHIA ARE ESSENTIAL TO THE BIOLOGY OF FILARIAL WORMS

In several different animal filarial infections, both in natural hosts and models for human infections, antiwolbachial treatment with tetracycline has demonstrated that the *Wolbachia* are essential to worm biology [43-45]. In all tests, the reduction in patency, the presence of MF in the blood, can be traced to a block in embryogenesis which is preceded by the depletion of the endobacteria by tetracycline or other anti-rickettsial drugs, i.e. rifampicin [46,47]. In a study with *O. ochengi*, a filarial nematode of cattle, tetracycline treatment even lead to death of the adult worms [48]. The block in embryogenesis seen after antiwolbachial

Table 2. Existing Antifilarial Drugs and Their Mode of Action[a,b]

Drug	Activity	Comment/Contraindication
(1) Albendazole, mebendazole citrate (benzimidazoles)	reduction/interruption of embryogenesis	Weakly active against filarial nematodes. Given in combination with ivermectin or DEC to treat other helminth infections co-endemic with filariasis.
(2) Amocarzine	microfilaricidal	No longer available
(3) *Cassia fikifiki bark*	microfilaricidal	Traditional drug from Liberia. Has not been rigorously tested
(4) Diethylcarbamazine citrate (DEC)	microfilaricidal; potentially macrofilaricidal (40% efficacy)[c]	Used to treat lymphatic filariasis. Due to severe adverse effects, no longer used to treat onchocerciasis.
(5) Doxycycline	interruption of embryogenesis; macrofilaricidal in lymphatic filariasis (80% efficacy)[d]	Complete and permanent inhibition of embryogenesis in onchocerciasis after a 6 week treatment.
(6) Ivermectin	microfilaricidal; partial interruption of embryogenesis after frequent application	Used to treat onchocerciasis. Also used to treat lymphatic filariasis in areas co-endemic for onchocerciasis.
(7) Melarsoprol (Mel W)	potentially macrofilaricidal	Too toxic for mass drug administration
(8) Metrifonate	microfilaricidal	Less effective than DEC; no longer available.
(9) Moxidectin	potentially macrofilaricidal in animals	In trials for lymphatic filariasis and onchocerciasis. Reports of severe adverse side effects in dogs led to voluntary removal at the request of the FDA.[e] Therefore plans for trials in humans must be viewed with caution.
(10) Suramin	reduction/interruption of embryogenesis; macrofilaricidal	Too toxic for mass drug administration

a is from Hoerauf A.; Adjei, O.; Büttner, D., *Curr. Opin. Invest. Drugs*, **2002**, *3*, 533., b is from [14], c is from [70], d is from [13].
e is from http://www.fda.gov/cvm/proheart6091304.html

treatment is a direct effect of the depletion of the *Wolbachia* as treatment of animals infected with *Acanthocheilonema viteae*, a filarial nematode without *Wolbachia*, has no effect on embryogenesis, nor on worm vitality [45]. Treatment of infective larvae with tetracycline also leads to an inhibition in their ability to molt [49]. This may not just be strictly an effect of the depletion of *Wolbachia*, but also a direct toxic effect of tetracycline on the larvae, e.g. by damage to the mitochondria. Support for a different mode of action during larval molting comes from the use of a synthetic tetracycline which lacks antimicrobial activity, but still blocks the larval molt [50].

ROLE OF *WOLBACHIA* AS SOURCES OF PATHOLOGY

Wolbachia appear to have a major role in the development of filarial pathology in *Brugia* spp., *W. bancrofti*, and *O. volvulus* infections. In animal models of filariasis, pathology, including lymphedema in rhesus monkeys, correlates with increased levels of circulating *Wolbachia* DNA or antigen. The appearance of these *Wolbachia* products corresponded with the loss of MF. Presumably killing of MF by the host released *Wolbachia* into the blood [51,52]. A link between the most severe filarial pathology of onchocerciasis and *Wolbachia* has recently been demonstrated in an *in vivo* model for blindness in mice [53]. The development of blindness in mice after injection of worm extract is dependent upon *Wolbachia* as *O. volvulus* extract depleted of *Wolbachia* does not induce blindness. Blindness is also not induced when these mice do not have a functional Toll-like receptor (TLR) 4 molecule. Further work with this model has demonstrated the requirement for the TLR cofactor MyD88 and TLR2, but not TLR9 (I. Gillette-Ferguson and E. Pearlman, personal communication).

Extracts of *O. volvulus* induce inflammatory cytokines by macrophages [54]. Extracts of *Brugia malayi* also induce inflammatory cytokines from macrophage cells [55]. The induction of inflammatory cytokines is dependent on *Wolbachia* as worms depleted of endobacteria by tetracycline or from *A. viteae*, a filaria with no *Wolbachia*, do not lead to significant induction of inflammatory cytokines [53,55]. The lack of significant induction of cytokines by *A. viteae* is not because *A. viteae* is not a source of antigen, but rather due to the lack of *Wolbachia*. Support for *Wolbachia* being powerful inducers of inflammation has also been demonstrated using A23 cells, a *Wolbachia* containing insect cell. Extracts of this cell line also induce an inflammatory response, but A23 cells depleted of *Wolbachia* do not [55]. Furthermore, it has been shown, again with animal models, that neutrophils, innate inflammatory cells, migrate *in vitro* only to worm extracts containing *Wolbachia*. Neutrophils are also only found in extirpated nodules surrounding worms which contain *Wolbachia* [56].

Highly purified *Wolbachia* surface protein (Wsp) elicits strong inflammatory responses *via* TLR2 and TLR4, shown using cells transfected with TLR2 or TLR4 and cells from mice deficient both for TLR2 (*via* a knockout) and TLR4 signaling (*via* a natural mutation of TLR4 which prevents intracellular signaling). This study also showed that human peripheral blood cells isolated from infected and uninfected individuals were strongly induced to have an inflammatory response. Finally, in sections from nodules containing *O. volvulus* MF, macrophages which had engulfed dead MF where positive for *Wolbachia* antigens, including Wsp, indicating the ability of the immune system to present

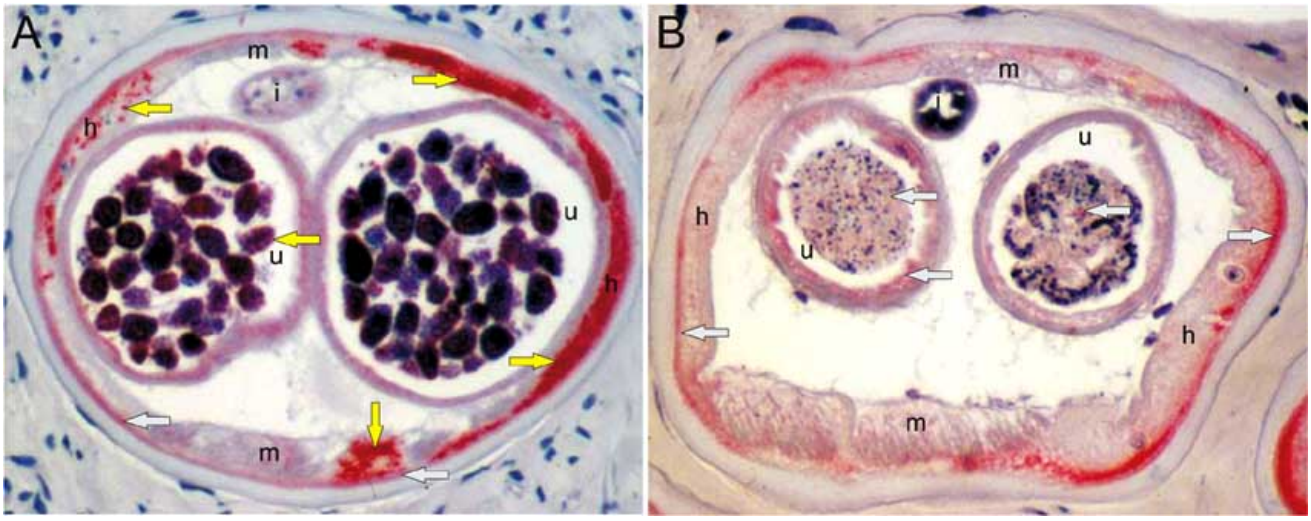

Fig. (2). The *Wolbachia* endosymbionts of filarial nematodes are found in distinct locations of the worm and can be depleted with doxycycline therapy. A) In untreated patients, *Wolbachia* are seen as granular staining in the hypodermis and embryos in the uterine tubes (yellow arrows), but are not seen in the muscles of the body wall, uterine tubes, nor of the intestine. B) Doxycycline treatment depletes *Wolbachia* from the nematodes and their embryos. The light, non-granular staining (grey arrows) of the outer zone of the hypodermis (seen in 1A), the muscles (1B, uterus wall), and embryos represents staining of the nematode mitochondria. Sections are *O. volvulus* females from extirpated nodules stained with anti-sera against *Yersinia* Hsp60 as in [45]. h, hypodermis; i, intestine; m, muscles of the body wall; u, uterine tube. Images are the kind gift of Prof. Dr. D. Büttner, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany.

Wolbachia antigens [57]. Thus, despite their intracellular location, the *Wolbachia* of *Brugia* spp., *W. bancrofti*, and *O. volvulus* are powerful inducers of inflammation when released after MF are killed with ivermectin or DEC. The same would hold true for the natural death of MF, larvae, and adult worms.

ROLE OF *WOLBACHIA* IN ADVERSE REACTIONS TO ANTIMICROFILARICIDAL TREATMENT

It has been described that some patients develop severe systemic inflammatory responses directly after receiving antifilarial drugs [58,59]. The reactions include fever, headache, dizziness, myalgia, arthralgia, and enlargement of the lymph nodes. The severity of adverse reactions has been associated with the microfilarial level before treatment. After DEC therapy, increased serum levels of interleukin-6, -10 and the tumor necrosis factor receptors have been measured [60], all of which are indicative of systemic inflammation. Such adverse reactions are detrimental to mass drug administration programmes as they can reduce participation in the programmes, which will reduce the coverage needed to eliminate filarial diseases.

Several recent reports have linked the increase in *Wolbachia* antigens (Wsp)/DNA after MF death to these adverse reactions to antifilarial drug therapy. The first report to associate *Wolbachia* with adverse reactions examined patients from Indonesia infected with *B. malayi*. Serum was taken from the patients before treatment with DEC and for several time points after. *Wolbachia* DNA was detected in sera from all three patients who experienced severe reactions after DEC and one patient who experienced a moderate adverse reaction [61]. An ongoing study of the effect of treating *B. malayi* patients with doxycycline (**5**), (Fig. **1**) prior to the administration of DEC indicates that patients who received doxycycline had milder adverse reactions than placebo patients. These same doxycycline patients also had statistically lower levels of serum IL-6 (T. Supali, E. Sartono, and M. Yazdanbakhsh, personal communication).

A recent study with *W. bancrofti* patients also showed a significant reduction in moderate adverse reactions in patients who received doxycycline 3 weeks prior to receiving an antifilaricide. The reduction in adverse reactions correlated to a reduction in inflammatory cytokines in the serum of patients. At the end of doxycycline treatment, the levels of MF were reduced in treated patients and these MF had fewer *Wolbachia*. The moderate adverse reactions in the placebo doxycycline group correlated with the levels of *Wolbachia* released into the blood after receiving the antifilaricide. Patients which had doxycycline treatment prior to ivermectin and albendazole had significantly lower inflammatory cytokines which also correlated to *Wolbachia* levels (J. Turner, M. Taylor, K. Pfarr, A. Hoerauf, personal communication).

An association of *Wolbachia* with adverse reactions after antifilarial therapy has also been seen in *O. volvulus* infections [62]. In this study higher levels of *Wolbachia* DNA were detected in the blood of those patients who had moderate to severe adverse reactions after receiving DEC or ivermectin. In those patients who received DEC, the levels of bacterial DNA in the blood were significantly higher. Those patients receiving ivermectin also had significantly higher levels of the inflammatory cytokine tumor necrosis factor-alpha which correlated with bacterial DNA levels.

Although not the focus of this review, two points should be made. The first is that filarial nematodes without *Wolbachia* may also cause pathology/adverse reactions, as is

seen with some *L. loa* infections [22,63,64]. However, pathology is generally seen in *L. loa* patients with high MF levels (greater than 15,000 MF/ml) in the blood or spinal/cerebral fluid. In comparison, pathology may develop in onchocerciasis patients with just 50 MF/skin snip (roughly equivalent to 25 MF/ml). The second point is that *M. ozzardi*, which does contain *Wolbachia* [39-41], does not cause any pathology in most infected patients [65,66]. This may reflect the location of the adult worms in the peritoneal cavity rather than the lymphatic vessels, or the presence of MF in the blood rather than in the skin or the eyes. Additionally, *M. ozzardi* MF are smaller than *Brugia* spp., *W. bancrofti*, and *O. volvulus* and therefore may have fewer *Wolbachia*.

Never the less, these studies show that in patients infected with *Brugia* spp., *W. bancrofti* or *O. volvulus*, *Wolbachia* are the major mediators of adverse reactions seen after antifilarial therapy with DEC or ivermectin. Antiwolbachial treatment prior to antifilarial therapy reduces the severity of the adverse reactions observed in patients infected with these species. Furthermore, the reduction in adverse reactions is seen after a three week regime of doxycycline, which is a shorter regime than that needed to produce a macrofilaricidal effect (i.e., a 6 or 8 week regime), although it leads to amicrofilaremia (A. Hoerauf, M. Taylor, *et al*. unpublished data).

ANTIWOLBACHIAL TREATMENT OF HUMAN FILARIASIS

Based on the promising results from animal experiments, and given that doxycycline is a registered drug, open phase IIa studies have been carried out since 1999 in the rainforest zone of Central Ghana in villages hyperendemic for onchocerciasis. Patients participating in the studies have received 100 or 200 mg/day doxycycline for several weeks. Patients also received ivermectin after doxycycline therapy as part of the implementation of APOC. The antiwolbachial activity has been monitored by evaluating MF in the skin and nodules, and of adult worms in extirpated nodules 2 to 24 months after commencement of therapy. Samples were analyzed by microscopy, immunohistology and PCR.

After a 6 week course of doxycycline treatment (100 mg/day) the endobacteria were eliminated from the worms (Fig. **2B**) [31,67] as was described in animal studies. With loss of the endobacteria, a block in embryogenesis was seen. This block in embryogenesis has been documented 24 months after commencement of therapy [31], making doxycycline the first antifilarial agent which completely blocks embryogenesis without serious side-effects. MF levels in the skin reflect the block in embryogenesis, with 90% of the patients who received ivermectin after doxycycline treatment having no detectable levels of MF, while the remaining patients had very low numbers. This result is in stark contrast to those patients who received ivermectin alone. These patients had a rise of microfilariae in the skin within 4 months after ivermectin administration. Importantly, in nodules from patients who had received doxycycline for 6 weeks at 100mg/day, there was no recrudescence of *Wolbachia* during that time-span (although very low levels of DNA were still detectable in a third of the nodules), as determined by immunohistology but also by semi-quantitative PCR [67]. This suggests that as long as *Wolbachia* are reduced below a threshold necessary for parasite fertility, the levels will not rebound and make the treatment ineffective in the long run.

Treatment regimes of 4, 3, and 2 weeks administered at 200 mg/day have also been tested. Doxycycline given for 4 weeks also blocks embryogenesis and reduces the number of MF in the skin similar to a 6 week regime of 100 mg/day. However, 3 or 2 weeks of doxycycline treatment was not sufficient to block embryogenesis (A. Hoerauf and D. Büttner, unpublished observations). Doxycycline treatment for 2 or 3 weeks given 2 months after the initial treatment of 4 or 6 weeks, similar to the regime followed in the *O. ochengi* study [48], failed to kill the adult worms (A. Hoerauf and D. Büttner, unpublished observations). This may reflect differences between *O. volvulus* and *O. ochengi*, or a difference between mode of administration, i.e. intramuscular injection for *O. ochengi* versus oral administration for *O. volvulus*.

Studies of doxycycline treatment have been extended to *W. bancrofti*, an agent of lymphatic filariasis. The study involved 200 mg/day of doxycycline alone, doxycycline followed by ivermectin 4 months after commencement of doxycycline administration, ivermectin alone or no treatment during the study. All patients received ivermectin at the conclusion of the study. The therapy was evaluated by measuring microfilariae in the blood and the levels of *Wolbachia* in the microfilariae by quantitative PCR.

MF were not detected 12 months after the start of the study in 90% of the patients who received doxycycline and in none of the patients who received ivermectin after doxycycline. In the ivermectin alone group, 9% had MF in the blood after 12 months. Copies of the FtsZ gene of the endobacteria were reduced by 96% after 6 weeks of doxycycline therapy [68]. Because the adult worms reside in the lymphatic vessels, it was not possible to examine the embryogenesis in the worms, but it is likely that the reduced microfilarial levels seen are the result of a block in embryogenesis as seen for onchocerciasis and animal studies. Thus, antiwolbachial treatment has been shown to be an effective therapy in two of the causative agents of human filariasis.

Recent work with *W. bancrofti* in Tanzania has shown that doxycycline depletion of *Wolbachia* leads to adult worm killing [13]. In this study, patients were given 200mg/day for eight weeks. As in other studies, MF levels were reduced to zero after 8 months and remained at this level through the 14 month follow-up of the doxycycline patients in comparison to placebo patients of the same village. Macrofilaricidal activity was measured using two methods. One commercially available method measures antigenaemia, worm antigen levels in the blood. The second makes use of ultrasonography to detect live adult worms at the sites of infection. At 14 months after the start of treatment, antigenaemia was significantly reduced in patients that received doxycycline. Placebo patients showed no reduction in antigenaemia. Ultrasonography is a powerful tool for monitoring macrofilaricidal activity in lymphatic filariasis as it is non-invasive and accurate [69,70]. At the 14 month follow-up, 54 adults were examined by ultrasonography for evidence of adult worms. In the doxycycline group, 75%

fewer patients were positive for filarial dance sign (adult worm movement) in comparison to the placebo group. Thus, doxycycline depletion of *Wolbachia* from *W. bancrofti* resulted in the death of adult worms. An equivalent macrofilaricidal effect after doxycycline has also been seen in an open study in Ghana where patients received doxycycline for just 6 weeks (A. Hoerauf, S. Mand, O. Adjei, personal communication).

In Brazil, Noroes and colleagues also used ultrasonography to demonstrate killing of worms after DEC therapy. However, they only saw worm death in 40% of the patients [70]. In Egypt, another ultrasonography study showed killing of worms following DEC/albendazole similar to that seen with doxycycline [71]. While these results are exciting, the area in Egypt has had many years of antifilarial chemotherapy and has shown a decline in infections. It can not be determined what condition or age the worms were when the patients were treated, therefore natural attrition is likely an additional reason for the high percentage of adult killing reported in the Egyptian study. The study with doxycycline and the one in Brazil were both performed in areas where new infections are still occurring, thus not only old worms are expected to be present in patients. Despite the occurrence of new infections, patients that received doxycycline had fewer live worms after treatment when compared to the placebo patients and the patients in Brazil that received DEC. Thus, doxycycline clearly has a higher efficacy (80% reduction for doxycycline versus 40% for DEC) against adult *W. bancrofti* worms in areas with ongoing transmission.

CONCLUSION

Although not suitable for mass drug administration, doxycycline therapy is a powerful new tool in the battle to eliminate filariasis. Two expert meetings in partnership with the WHO, one in Hamburg [72] and the other in Atlanta (Conference on the Eradicability of Onchocerciasis, Carter Center, January 22-24, 2002) [18] have suggested doxycycline for use in: i) individuals, e.g. when leaving an endemic area for long periods, and ii) regions which have persistent infections either through remnant foci or resistance. A six week course of doxycycline to make adult worms sterile could be more cost-effective than to continue annual ivermectin therapy for another 15 years. Doxycycline is also currently the only alternative for onchocerciasis should the nematodes develop resistance to ivermectin.

Potentially, doxycycline could allow for the introduction of chemotherapy of selected populations in areas co-endemic for loiasis. The RAPLOA programme is a current screening method to rapidly identify communities with high *L. loa* MF levels [73,74]. These communities are then excluded from ivermectin mass administration. Potentially, these communities could be treated with doxycycline, or a future antiwolbachial drug, to eliminate *Wolbachia* from the *O. volvulus* worms, thus making them sterile. Without the production of new *O. volvulus* MF, the level of *O. volvulus* MF would slowly decrease through normal attrition of the larvae. Thus, sources of new *O. volvulus* infections could be eliminated despite loiasis co-infection without administering ivermectin with its associated risk of severe adverse reactions due to the killing of *L. loa* MF.

New drugs against filariasis are clearly still needed. Although the economic feasibility of developing new antifilarial drugs hinders their development, the time required to research, develop, and bring to market new drugs against filarial nematodes is the true hindrance. The results of studies of filarial *Wolbachia*, especially the human trials with doxycycline, have shown that *Wolbachia* are ideal targets for the development of new antifilarial chemotherapies. There is the potential that other drugs already registered for use in humans could be applied to *Wolbachia*. The search for new drugs which act in a shorter time and that are not contraindicated for a large segment of the community, e.g. pregnant women and children under 8 years of age, will be aided by the completion of the sequencing of the *Wolbachia* genome from *B. malayi* [37]. With the publication of this genome, the complete biosynthetic requirements and contributions of the *Wolbachia* are known. Work is being done to see if some of these enzymes have inhibitors which may lead to a faster depletion of the endobacteria from the nematode. Some of these inhibitors may include drugs already registered for use in humans, or novel ones, thus providing a new chemotherapy against filarial nematodes in addition to ivermectin, DEC and doxycycline.

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ABBREVIATIONS

APOC = African Programme for Onchocerciasis Control

DEC = Diethylcarbamazine

GPELF = Global Programme for the Elimination of Lymphatic Filariasis

LF = Lymphatic filariasis

MF = Microfilariae

MyD88 = Myeloid differentiation factor D 88

OCP = Onchocerciasis Control Programme

OEPA = Onchocerciasis Elimination Programme in the Americas

TLR = Toll-like receptor

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