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Anti-*Wolbachia* therapy for onchocerciasis & lymphatic filariasis: Current perspectives

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Onchocerciasis and lymphatic filariasis (LF) are human filarial diseases belonging to the group of neglected tropical diseases, leading to permanent and long-term disability in infected individuals in the endemic countries such as Africa and India. Microfilaricidal drugs such as ivermectin and albendazole have been used as the standard therapy in filariasis, although their efficacy in eliminating the diseases is not fully established. Anti-*Wolbachia* therapy employs antibiotics and is a promising approach showing potent macrofilaricidal activity and also prevents embryogenesis. This has translated to clinical benefits resulting in successful eradication of microfilarial burden, thus averting the risk of adverse events from target species as well as those due to co-infection with loiasis. Doxycycline shows potential as an anti-*Wolbachia* treatment, leading to the death of adult parasitic worms. It is readily available, cheap and safe to use in adult non-pregnant patients. Besides doxycycline, several other potential antibiotics are also being investigated for the treatment of LF and onchocerciasis. This review aims to discuss and summarise recent developments in the use of anti-*Wolbachia* drugs to treat onchocerciasis and LF.

Key words Black flies - doxycycline - filariasis - lymphatic filariasis - macrofilaricides - minocycline onchocerciasis - *Wolbachia*

Introduction

Onchocerciasis (also known as river blindness disease) and lymphatic filariasis (LF) are human filarial diseases caused by parasitic worms. These cause a major health burden mainly in African and Indian subcontinent with small foci in southern and central America[1]. *Onchocerca volvulus,* the filarial nematode species that causes onchocerciasis, is transmitted to humans via infected *Simulium* (black flies) bites. Patients with onchocerciasis exhibit skin lesions but in advanced untreated cases, the infection may lead to loss of vision due to the inflammatory response towards microfilariae in the eye[2]. LF caused by *Wuchereria bancrofti, Brugia malayi* and *B. timori* are transmitted to humans through mosquito bites. In patients with LF, lymphatic complications such as elephantiasis, hydrocoele and lymphoedema cause significant morbidity[3].

To date, the treatment and control of onchocerciasis and LF depend on microfilaricides such as ivermectin and albendazole. Given under mass drug administration (MDA) programme to all adults (except pregnant women) and children >15 kg either once or twice a year, it is effective for decreasing microfilarial load, but are of limited potential against microfilariae. As a result, continuous delivery for at least 15-17 yr is required to break the transmission cycle[1,4]. The issue of resistance to these drugs has also been raised as persistent microfilaridermias despite multiple treatments of ivermectin observed among patients in a few endemic areas of onchocerciasis[5,6]. Consequently, the transmission of the filarial nematode is not interrupted, since children in the MDA area still have microfilariae in their skin or blood.

Because of the disadvantages of the current standard therapy for onchocerciasis and LF, the search for new potential treatment targets continues. Chemotherapeutic agents targeting *Wolbachia*, a bacterial endosymbiont present in the nematodes causing onchocerciasis and LF, have been extensively studied[7], and are discussed in this review.

Wolbachia: A bacterial endosymbiont

Wolbachia is an endosymbiont bacteria found in numerous arthropod species, first identified by Hertig and Wolbach in 1924 in the mosquito *Culex pipiens*[8]. It is a Gram-negative α-proteobacteria, a member of the Rickettsiales order[9]. Many filarial nematodes are recognized as host to *Wolbachia*[10], except a few species such as *Loa loa, Acanthocheilonema viteae, Onchocerca flexuosa* and *Setaria equina*[11,12]. The main species for LF and onchocerciasis such as *Wuchereria bancrofti, B. malayi* and *O. volvulus* were also found to contain an intracellular bacterium which showed similarities with *Wolbachia* according to DNA sequencing data[13]. Moreover, filarial nematodes are found to be infected with *Wolbachia* at all stages of their life cycles[14].

An important mutualistic symbiosis exists between *Wolbachia* and their nematode hosts. This interaction has contributed to their survival. *Wolbachia* is necessary for growth, fertility and viability of the nematode host, while the host supplies amino acids needed for *Wolbachia*'s development[15]. Furthermore, *Wolbachia* is transmitted vertically via oocytes in the filarial worms[14]. Therefore, sterilization of the worms will decrease the presence of the intracellular endosymbiont. Without *Wolbachia*, the viability of the filarial worms will be affected[16]. All these features make *Wolbachia* an interesting target for filarial drug treatment[1].

Role of *Wolbachia* in the pathogenesis of filariasis

The knowledge on the role of *Wolbachia* in the pathogenesis of human filarial infections is mostly derived from studies of the molecular pathogenesis of inflammation triggered by filarial nematodes[17]. The involvement of *Wolbachia* from the acute phase of infection to the development of chronic complications includes the induction of pro-inflammatory and immunomodulatory mechanisms in the host[17]. The release of *Wolbachia* lipoprotein could cause activation of innate inflammatory responses as well as Th1-adaptive immune responses[18]. The degranulation of eosinophils needed for filarial eradication is inhibited by *Wolbachia*, resulting in an incompetent neutrophil response of the host[19]. This manipulation of the local inflammatory response by *Wolbachia* provides longevity to the host through a defensive mutualism by conferring immunity against the lethal effector cell response[19].

The disease manifestations are correlated with both circulating *Wolbachia*-related antigen and antibodies. The presence of circulating *Wolbachia* antigens might result from the natural excretion of *Wolbachia* products by the nematodes or the release of these products from the dying worms[20,21]. Initially, it was thought that the development of pathology seen in filarial diseases occurred as a result of the immune response alone. However, a study[22] has shown that *O. volvulus* does not induce corneal inflammation when depleted of *Wolbachia* using antibiotics[22]. Thus, the presence of *Wolbachia* was also implicated in the pathogenesis of *O. volvulus* keratitis. In an experimental study, soluble extract of *O. volvulus* containing *Wolbachia* was injected into the corneal stroma of mice. The study showed that *Wolbachia* led to the Toll-like receptors (TLR)-2 activation in macrophages and local stromal cells resulting in the production of pro-inflammatory cytokines[23]. Expressed in the cornea tissues, TLR are endosomal and surface receptors that respond to the bacterial antigen[24]. Subsequently, neutrophil-mediated corneal inflammation ensues while repeated infections will exacerbate the inflammatory response causing permanent corneal destruction[25]. As a result, the visual acuity will be affected, and chronically infected patients will become blind[26].

Similarly, in LF, *Wolbachia* has also been implicated in the pathogenesis of the disease. Production of lymphangiogenic factors such as angiopoietin-1 and vascular endothelial growth factor is triggered by activation of TLR-2 by extracts of *B. malayi*[27]. High levels of these lymphangiogenic factors are associated with the severity of the disease[27,28]. On the contrary, depletion of *Wolbachia* in patients infected with *W. Bancrofti* using doxycycline caused an improvement in symptoms as shown by a reduction of the dilatation of the scrotal lymphatic vessels[29].

Anti-*Wolbachia* therapy

***Overview*:** Current filariasis treatment and public health control programmes depend on the utilization of microfilaricides which target the microfilariae instead of the adult worms[3]. In the MDA programmes led by the WHO, albendazole combined with either ivermectin or diethylcarbamazine are given as treatment of LF[3], while only ivermectin is given for onchocerciasis in all endemic areas[2]. Although these microfilaricidal drugs have been shown to reduce the number of cases, the disadvantage lies in the long duration of treatment; approximately 17 years for onchocerciasis[1] and five years for LF [1]. As the adult worms also have a long lifespan, any disruption of the anti-microfilariae treatment process will hamper the prevention of transmission[1]. The emergence of resistance to ivermectin in some endemic regions represents an issue of concern[6].

Initial pre-clinical studies demonstrated that antibiotic therapy targeting the endosymbiont bacteria could cause embryotoxicity, developmental retardation and death of adult filarial nematodes[30,31]. It is known that anti-*Wolbachia* therapy has potent macrofilaricidal activity and also prevents embryogenesis resulting in successful eradication of microfilarial burden, thus averting the risk of adverse events resulting from the destruction of the targeted parasite or the co-infection with *L. loa*[1]. How the antibiotics affected *Wolbachia* was not fully understood until a transcriptomic and proteomic study demonstrated that there were genome-wide responses of *Wolbachia* to doxycycline causing impairment of the bacterial metabolism[32]. The subsequent depletion of *Wolbachia* causes significant apoptosis of both the adult germline and the somatic cells in the embryos and microfilariae that leads to sterilization of the filarial nematodes[16]. From these observations, *Wolbachia* appeared to be the right target for the treatment of human filarial diseases[33].

Since the first human clinical trial showing the effectiveness of doxycycline in eradicating *Wolbachia* and leading to the death of adult worms[33], doxycycline has been established as an effective anti-*Wolbachia* treatment with good macrofilaricidal activity[28,34]. Doxycycline is readily available, cheap and safe to use in adults patients. Though macrofilaricidal drugs (like doxycycline) have been shown to be effective[35], several factors have restrained their use in the public MDA programmes.

The anti-*Wolbachia* consortium (A-WOL) was founded in 2007 with the objective to identify new anti-*Wolbachia* drugs that are safe to the contraindicated groups and could be administered on a large scale with a shorter course of treatment[36]. From a total of 2773 compounds screened from the registered drugs, 121 had anti-*Wolbachia* activity wherein only four compounds were orally available and superior to doxycycline with minocycline showing the greatest potential[37].

***Doxycycline*:** The first human trial of anti-*Wolbachia* drugs was done by Hoerauf *et al*[33] in Ghana. In this prospective trial to investigate the effectiveness of targeting *Wolbachia* in human onchocerciasis, doxycycline was administered at 100 mg daily dose for six weeks. One of the outcomes was the depletion of more than 80 per cent *Wolbachia* four months after the treatment. Furthermore, the embryogenesis of the treated worms was interrupted and viability affected compared to the non-treated worms. In the subsequent extension of the study[38], the patients who received doxycycline and ivermectin were compared with patients who had received ivermectin alone. When examined after 19 months, amicrofilaridermia was observed in the doxycycline and ivermectin group, suggesting that doxycycline significantly enhances the microfilaricidal effect of ivermectin. This study established that the worm sterilization was long term, up to 1.5 years after treatment and was probably irreversible[38].

Besides the synergistic microfilaricidal effect with ivermectin, macrofilaricidal effect as well as sterilization of the female *Onchocerca* worms after a course of doxycycline treatment was also discovered. Hoerauf *et al*[39], in their double-blind randomized placebo-controlled trial in Ghana, showed that a six-week course of doxycycline had a higher macrofilaricidal effect of up to 60 per cent,

as compared to 50 per cent for the shorter four-week course. Furthermore, the residual live female worms analyzed from the onchocercomata nodules were sterile. A similar randomized controlled study in Cameroon[40] and another open study by Hoerauf *et al*[41] using doxycycline at 100 mg/day for five weeks confirmed that both macrofilaricidal and embryotoxic activities were attributable to the monotherapy with doxycycline without administration of ivermectin. A good safety profile was observed as no serious adverse events were reported from the doxycycline-treated group. Besides, the utilization of antibiotics in areas of co-endemicity with loiasis is shown to be safe and effective for onchocerciasis[40].

A Cochrane systematic review analyzed three randomized controlled trials comparing the treatment of doxycycline added to ivermectin versus ivermectin only on 466 onchocerciasis patients in West Africa[42]. In the two previous studies mentioned[39,40], microfilarial loads in skin snips were quantified. The results showed sustained effects of six-week doxycycline treatment of *Wolbachia* depletion, microfilaricidal and macrofilaricidal as well as sterilization effect for up to two years[39,40]. In the study that measured visual outcomes at six-month follow up, they found that although the onchocerciasis clinical symptoms improved, no significant visual improvement was found among the patients treated with doxycycline plus ivermectin when compared to those who received ivermectin alone[43]. Better designed trials of doxycycline with standardized clinical outcomes such as the efficacy of the interventions in preventing visual acuity and visual field loss would provide more condusive evidence.

Doxycycline has also demonstrated its efficacy as anti-*Wolbachia* therapy to reduce the burden of LF. In a randomized clinical study, doxycycline therapy for three weeks significantly reduced the microfilarial load and was more effective in inducing a microfilaraemia for up to two years when compared to monotherapy with either albendazole or ivermectin[44]. The severity of lymphoedema also improved after treatment with doxycycline[45]. These results were translated into the use of doxycycline to improve clinical symptoms. In another randomized controlled trial which compared doxycycline with amoxicillin on patients with and without active LF infection, though significant improvement of the severity of lymphoedema was noted for both drugs at one and two years of follow up, higher progression of disease was observed in 29 per cent of patients in the amoxicillin group and 56 per cent of patients in the placebo group, compared to a lower five per cent in the doxycycline arm[27]. The treatment time of LF with doxycycline is also shorter compared to that in onchocerciasis[37], possibly due to the presence of the filarial nematodes in the lymphatic vessels rather than the skin where drug penetration is more difficult. Taking into account the benefits shown even in patients without active infection and the fact that the efficacy of albendazole or ivermectin as microfilaricides is still undetermined for LF[46], the addition of doxycycline as part of the standard treatment for LF should be considered.

Regarding the treatment duration, intermittent short multiple courses over a longer period are found to be better than a continuous intensive once-off shorter duration course[47-49]. For example, an *in vivo* study using *O. ochengi* in cattle discovered a significant post-treatment macrofilaricidal activity in the group receiving intermittent antibiotics (20 mg/kg monthly for 6 months) compared to a continuous course (10 mg/kg daily for 2 wk)[47]. This superiority of the prolonged intermittent regimen was explained by the findings from an *in vitro* study using the *Wolbachia*-infected *Aedes albopictus* mosquito, which showed that significant *Wolbachia* depletion occurred after antibiotics withdrawal[48]. A later animal study also proved that a six-month regimen of oxytetracycline with once a month administration was more efficacious than a shorter but continuous course, either at three or six weeks duration[49].

Although doxycycline is the best available treatment for onchocerciasis or LF in patients attending clinical settings, there are barriers that restricted doxycycline usage in the public health MDA programmes[1]. A study done in Cameroon showed that by the end of six weeks, successful adherence was achieved in 97.5 per cent of the 13,000 community members who started the treatment at the health district levels[50]. The cost of the six-week doxycycline treatment was found to be US$ 2.77 which was relatively economical compared to a single dose of ivermectin at US$ 1.77[51]. This has proved that a large-scale distribution of doxycycline for the treatment of filariasis using a community-directed approach is feasible and economical. Evidence from a meta-analytical modelling framework suggests that a shorter course of four-week doxycycline is sufficient for an effective anti-*Wolbachia* therapy, enabling a better compliance[35].

Doxycycline resistance in filariasis has not been reported. In general, the risk of drug resistance with doxycycline is much lower compared to penicillin or fluoroquinolones[52,53]. However, another limitation of doxycycline usage is its contraindication in pregnancy and in case of young children remains unresolved. The main mechanism of action of this antibiotic from the tetracycline class is on the inhibition of the bacterial RNA polymerases (RNAPs) and protein synthesis[54]. Hence, in the foetus and young children, doxycycline has the potential to bind to calcium in the developing bones and teeth, thus forming deposits and/or resulting in dental staining and hypoplasia[54]. This disadvantage motivated an active search for other potential anti-*Wolbachia* treatment for filariasis. Because of the potential shown in earlier studies and better safety profile, rifampicin, azithromycin and minocycline have also been assessed in clinical trials as anti-*Wolbachia* chemotherapy[55,56].

***Azithromycin*:** The initial *in vitro* study assessing the effect of azithromycin on *B. malayi* resulted in a partial inhibition of molting of the parasite[57]. This was not reflected in the subsequent clinical trial of six-week azithromycin monotherapy administered at either 250 mg/day or 1.2 g/per week. The treatment showed no significant reduction of the worm load after 12 months[58]. In another open-label randomized study in Guatemala[59], onchocerciasis patients were given either rifampicin or azithromycin or both drugs for a short course of five days. Neither *Wolbachia* depletion nor anti-filarial effects were observed in either group. These studies confirmed the futility of azithromycin in the treatment of human filariasis.

***Rifampicin*:** Rifampicin is a broad-spectrum antibiotic that is safe for children and pregnant women[60]. However, it had a false start when an animal study showed insignificant results with rifampicin. In the study, *O. Ochengi* infected cattle were treated with either rifampicin alone or in combination with oxytetracycline for three weeks. Both regimens proved to cause no significant macrofilaricidal or macrofilaricidal effect as compared to oxytetracycline monotherapy[49]. This negative result may be explained by the small sample size which was only six cattle for each group. On the other hand, a human study of rifampicin[61] showed positive results. Rifampicin monotherapy was given to 26 onchocerciasis patients for two or four weeks. The study showed significant reduction of *Wolbachia* at 18 months post-treatment compared to the untreated group, with a higher proportion of reduction observed in the four-week rifampicin regimen[61]. Later on, a preclinical study proved a synergistic effect between albendazole and rifampicin that significantly shortened the treatment duration[62]. After treatment with a seven-day combination of albendazole and rifampicin, *Wolbachia* was almost totally eradicated, and as a result, the macrofilaricidal effect was fast.

The potential use of rifampicin in filariasis may be limited by its role as the backbone treatment for tuberculosis, since a widespread use of rifampicin poses a substantial risk of cross-resistance with *Mycobacterium* spp[63]. To minimize this risk, a study tested a short course (1-2 wk), high dose rifampicin (35 mg/kg) in preclinical onchocerciasis and LF mouse models and it accelerated the treatment effect on both filarial diseases[64]. Since rifampicin had the advantage of safer use in children and during pregnancy compared to doxycycline, this regimen should be further investigated in clinical trials to determine its safety and clinical efficacy as well as the suitability to be utilized in a MDA programme.

***Minocycline*:** Minocycline is a semi-synthetic tetracycline which is bacteriostatic on a wide range of bacterial species[65]. In the A-WOL cell culture screening assay, minocycline was identified as the most potent compound[37] more effective than doxycycline and rifampicin in an initial *in vitro* screen[66]. In a LF murine infection model, a 28-day treatment of minocycline with a dosage of 25 mg/kg twice daily resulted in a greater reduction of *Wolbachia* than obtained with doxycycline (99.51 vs. 90.35%) with a higher rate of worm sterilization[67]. In a pilot randomized, open-label, clinical trial in Ghana, a three-week course of minocycline resulted in 73 per cent depletion of *Wolbachia* compared to 64 per cent with a three-week course of doxycycline[68]. This suggested that minocycline was more potent than doxycycline and more randomized controlled trials will be needed to further validate minocycline efficacy. Although minocycline is a potent and well-tolerated antibiotic without severe adverse events[68], it is also contraindicated for children and pregnant women.

***Corallopyronin A*:** Corallopyronin A, originally isolated from the myxobacterial strain *Corallococcus coralloides*, is a natural compound and a non-competitive RNAP inhibitor[69]. It has many bioactivities including microfilarial activity. The genome of *Wolbachia* found in filarial nematodes encodes a complete RNAP which is a suitable target for the

Table. Summary of anti-*Wolbachia* chemotherapeutic agents

Drug compound	Target	Mechanism of action	Contraindication	Current stage
Tetracyclines (doxycycline/ minocycline)	16S part of the 30S ribosomal subunit	Protein synthesis inhibitor by blocking the aminoacyl-tRNA binding to ribosome	Pregnancy and children <9 yr	Human clinical study[28,33,38-40,44,68]
Rifamycins (rifampicin/rifapentin)	RNA polymerase	Inhibition of RNA polymerase	Not known	Human clinical study[72]
Moxifloxacin	DNA gyrase	DNA gyrase inhibitor and inhibiting cell replication	Pregnancy and children	Human clinical study[72]
Corallopyronin A	RNA polymerase	RNA synthesis blocker	Not known	Pre-clinical study[70,71]
Berberin	FtsZ	Bacterial cytokinesis blocker	Not known	Pre-clinical study[74]
Rapamycin	bmTOR	bmTOR inhibitor, which controls autophagy	Pregnancy	Pre-clinical study[75]
Globomycin	LspA	Pro-lipoprotein accumulation in the cytoplasmic membrane	Not known	Pre-clinical study[76]
Succinyl acetone	ALAD	Haem pathway blocker	Not known	Pre-clinical study[77]

tRNA, transfer RNA; bmTOR, *B. malayi* target of rapamycin; LspA, lipoprotein signal peptidase; ALAD, amino levulinic acid dehydratase

antibiotic. *In vitro* and *in vivo* studies in an animal model have shown the potential of corallopyronin A against *Wolbachia* with a good safety profile[70,71]. However further clinical trials will be required to examine the efficacy of corallopyronin as a treatment regimen in patients with onchocerciasis and LF.

***Other anti-Wolbachia therapy*:** Other potential antibiotics that have progressed into clinical trials are moxifloxacin and rifapentine. Both of these antibiotics are currently investigated in phase 2 randomized open-label clinical trials, with the following dosages: rifapentine 900 mg/day plus moxifloxacin 400 mg/day for 14 or for seven days[72]. Previous *in vitro* and *in vivo* experiments using moxifloxacin and rifapentine showed that this combination administered for only 4-7 days also significantly depleted *Wolbachia* load in adult worms[73].

Berberine[74], rapamycin[75], globomycin[76] and succinyl acetone[77] are currently in the initial phase of experimental research. The current anti-infective compounds being used as anti-*Wolbachia* treatment are summarized in the Table. Besides antibiotics, several non-antimicrobial compounds such as anti-oxidants and anti-histamines have demonstrated activity against *Wolbachia in vitro* which opens up other potential anti-*Wolbachia* therapeutic combinations to be explored and exploited in future research[73].

Conclusion

Despite doxycycline being the best available treatment for onchocerciasis and there are barriers in its usage and implementation in the public health MDA programmes. Drug adherence, toxicity, resistance, and financial cost will have to be taken into consideration before the final decision is made by health authorities. Meanwhile, other potential alternatives for depleting *Wolbachia* in adult filarial worms may be identified within the A-WOL Consortium product portfolio. Also more clinical trials will have to be carried out to determine their efficacy and suggest better strategies to manage onchocerciasis and LF in the future.

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