

Evolution of anti-filarial therapeutics: An overview

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Introduction and Historical Overview

Lymphatic filariasis (LF) has been documented from ancient times, and its symptoms were first recorded in the 16th century, during early explorations of Asia and Africa. It is a parasitic disease caused by the filarial worms, *Brugia malayi*, *Wuchereria bancrofti* and *Brugia timori*; otherwise known as “elephantiasis” [1]. It is spread by mosquito vectors that mainly afflict people of tropical and subtropical countries. LF is considered the highest amongst the world’s diseases that causes severe disability and disfiguration. It threatens one fifth of the world’s population - almost equal to that affected by malaria involving approximately 73 countries, yet it has been considered a less significant disease. This probably is the reason for its unfortunate tag of ‘neglected tropical disease’. According to World Health Organization (WHO), as of 2012, an estimated 1.4 billion people are at risk of contracting LF [2].

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ABSTRACT

Human Lymphatic Filariasis is a parasitic disease which threatens one fifth of the world’s population. Since the last century, Diethylcarbamazine (DEC) has almost been the sole antifilarial drug. Due to side effects and possible resistance of filarial parasite to DEC, a large number of new molecular targets have been proposed as antifilarial drug candidates - as mandated by WHO,. The discovery of *Wolbachia* endobacteria in filarial species has provided a promising target in chemotherapy. Anti-rickettsial agents like tetracycline, doxycycline, and ciprofloxacin have been shown to deplete *Wolbachia* from the worms. Apart from a direct antibiotic effect, these drugs may generate oxidative stress. This result, along with supported evidence suggests that oxidative impact might provide a formidable prowess against the filarial parasite. However, owing to the reported antioxidant armament of this parasite, a more plausible apoptotic effect might be contemplated that may explain subtle link with oxidative rationale. Herbal medicines have stood the test of time for their safety, efficacy and cultural acceptability. With the possible development of pro-oxidant effects with anti-oxidants like flavonoids, it can be presumed that plants and materials that are rich in flavonoids might be exploited for and used as oxidative stress mediators against filarial parasites. Moreover, exploiting and extrapolating a possible relation of shikimate metabolism, a relatively new role of flavonoid as an apoptosis inducer through anti-folate rationale could be envisaged. Experimental evidence in favour of such apoptotic mechanisms are to be considered for a unique anti-parasitic drug designing concept that has been put forward.

KEY WORDS:

**Lymphatic filariasis
Therapeutics
New targets
Novel rationale**

At present, there are over 120 million infected individuals, and above 40 million of them have been disfigured and severely debilitated by the disease. Therefore, WHO has recognized human lymphatic filariasis as one of the thirteen diseases in its tropical disease research (TDR) scheme highlighting the huge disease burden leading to 5.9 million DALYs and consequently launched the Global

Programme for Elimination of Lymphatic Filariasis (GPELF). Accordingly, lymphatic filariasis has been selected for elimination as a public health problem by the year 2020 [3]. Towards this end, the 'International Task Force for Disease Eradication' has identified filariasis as one of six major infectious diseases and emphasized a serious increase in the development of novel drugs.

In the first decade of the present century, quinine, methylene blue, thymol and atoxyl stood in the foreground as the best anti-parasitic agents of the time. In 1910, antimony preparations were used by Thiroux and antimony tartrate by Bahr. Since that time a large number of substances have been tried with varied level of success. Later, more systematic comparative tests by the British Guiana Filariasis Commission showed that preparations of antimony were more promising than others although it was far from satisfactory. In the later part of the second decade of the last century, several works showed the microfilaricidal action of various preparations like bisnene (urea compound of para-amino-phenyl bismic acid), antimosan, stibosan, neo-stibosan, stiburea, novostiburea, antimony sulphur compound, mercurochrome and plasmochin which are now of historical interest. During World War II, an estimated 15,000 servicemen in South-Pacific acquired acute lymphatic filariasis from exposure to *B. malayi* and *W. bancrofti* [4]. The most significant breakthrough in the form of a non-toxic piperazine molecule, Diethylcarbamazine (DEC) by Hewitt *et al* using the cultured rat filarial parasite *litomosoides-carnii* came in 1947 [5]. DEC was then found to be active against human filariasis, and it rapidly became a popular oral chemotherapeutic agent to combat most forms of human filariasis because of its chemical stability and since then, DEC has been enjoying the status of the almost sole antifilarial drug.

The Current Scenario and its Evolution

DEC (1-diethylcarbamyl-4-methyl piperazine) is the drug of choice available at present in treating human lymphatic filariasis caused by *W. bancrofti* and *B. malayi*. This is used in the form of dihydrogen citrate under the brand names of Hetrazan, Banocide and Notezine. It causes rapid clearance of microfilariae of *W. bancrofti* and *B. malayi* from the blood of humans. The mechanism of action is largely obscure and it is believed that it probably activates muscle cholinergic receptors in the worm, caus-

ing depolarization and muscle paralysis possibly due to the hyper-polarizing effect of the piperazine moiety and causes the dislocation of the parasite from the normal habitats in the host [6]. It has also been reported that the drug produces alterations in the microfilarial surface membranes, thereby rendering them more prone to destruction by host defence mechanisms [7]. However, the most popular postulate as reported earlier considers DEC to mediate *in vivo* microfilaricidal activity in conjunction with the host immune system [8]. Such an effect might trigger inflammatory responses leading to a possible pro-oxidative environment. Therefore, this mechanism appears to be endowed with the possible macrophage mediated oxidative onslaught. Plausibly, the observed apparent lack of effect of DEC *in vitro* provides additional support to this rationale. Interestingly, of late, it is reported that on *in vitro* cultures, filarial parasite can undergo apoptosis on treatment with DEC; however, such apoptotic change alone was not found to be sufficient to induce parasitic killing [9]. The significance of this evidence will be discussed below in detail in the light of the certain experimental evidence. The standard dose of DEC is 6 mg/kg, which is to be given in three divided doses after food over a period of 10-14 days, which reduces microfilaremia levels by approximately 80-90% in several days. Sensitivity to DEC may be expected to be low in endemic areas due to prolonged usage. However, as of now, the reason of such resistance is yet uncertain [10]. A lack of suitable known targets for DEC to facilitate the evaluation for DEC resistance in humans further compounded this predicament. With advances in research and consequent development of our knowledge-base of filarial biochemistry, a large number of new molecular targets are being proposed for antifilarial drug development. The addition of ivermectin and albendazole as newer antifilarial therapeutics as well as the revelation of novel aspects of DEC action contributed towards a major breakthrough. The mechanisms by which ivermectin and albendazole achieve parasite destruction have been well studied in non-filarial organisms. However, the reason for selectivity of ivermectin only against Mf, and not adult worms is yet to be elucidated [11]. Albendazole selectively targets tubulin of the parasite, leading to defects in the fertilization process. Filamentation temperature-sensitive protein Z (Ftsz), which is involved in cell division of the *Wolbachia* para-

site, appears to have functional homology with β -tubulin gene. Therefore albendazole might have dual targets; FtsZ in *Wolbachia* and β -tubulin in *W. bancrofti* [12]. Drug resistance is also observed with both of these antibiotics. Resistance to albendazole appears to be associated with a loss of high affinity receptors, resulting from nucleotide changes, predominantly in the TUB1 gene. Studies of ivermectin on the nematode *Caenorhabditis elegans* show that simultaneous mutation of three genes (*avr-14*, *avr-15*, and *glc-1*) that encode glutamate-gated chloride channel (GluCl) of α -type subunits confers a high-level resistance to the drug [13]. There is insufficient evidence to confirm or refute that albendazole co-administered with DEC or ivermectin is more effective than DEC or ivermectin alone in clearing microfilariae or killing adult worms. There are inconsistent reports regarding the effect of albendazole with ivermectin on microfilaraemia. Now, there is a need of further active research and meta-analysis on the effect of albendazole against the adult and larval stage of filarial parasites, alone and in combination with other antifilarial drugs [14]. Ivermectin kills microfilariae only and can be given as a single dose of 400 mg/kg. Although ivermectin leads to rapid clearance of microfilariae (Mf), sustained reductions in six months or longer after treatment are equivalent or better with single 6 mg/kg dose of DEC. Ivermectin can also be used with DEC as a single dose that gives a more rapid clearance of microfilariae and recurrence is delayed. However, there is limitation in its use in lymphatic filariasis and *Loa loa* co-infection cases [15]. Side effects of ivermectin are similar to that of DEC with additional neurotoxicity. Albendazole in combination with ivermectin is more effective in clearing microfilariae than ivermectin alone. For almost 30 years, it has been known that filarial nematodes contain endosymbiotic bacteria. These endo-bacteria are found in the hypodermis of male and female worms, and in the larval, embryo and oocyte stages. Recently, these endosymbionts were classified to be of the genus *Wolbachia*, a genus of bacteria which are common endosymbionts of arthropods. *Wolbachia* can be used as a drug target and thus holds great promise towards therapeutic options available for filariasis treatment. In general, the discovery of *Wolbachia* endobacteria in filarial species has provided a new promising target for the chemotherapy of human filariasis, as emphasized in recent

WHO/TDR expert meetings [16]. Proof-of-principle of the symbiosis between filarial *Wolbachia* and their host has been demonstrated in animal models by depleting the endosymbionts from filarial parasites using antibiotics [17]. The effects were very similar in models using different filarial species. Since antibiotic treatment had no effect on filarial species devoid of *Wolbachia* endobacteria, such as *Acanthocheilonema viteae*, *Wolbachia* appears to be the target of antibiotic therapy for effective antifilarial effect [18]. Immuno-histochemical studies have shown the depletion of *Wolbachia* from the worms in animals infected with filarial nematodes using anti-rickettsial antibiotics like tetracycline, azithromycin and rifampicin [19]. Partial activity is also seen with ciprofloxacin, erythromycin and chloramphenicol [20]. In several nematode infections, these antibiotics have numerous effects on worm growth and development; worm fertility (particularly female worm embryogenesis) and worm survival, suggesting that prolonged treatment can be detrimental to worms. In animals infected with aposymbiotic *Acanthocheilonema viteae* (*A. viteae*) worms, which do not carry these bacteria, similar long-term antibiotic treatment had no effect on worm biology and development suggesting that this bacteria plays a very important role in the growth and reproduction of the filarial worms that harbour them. Interestingly, in addition to anti-wolbachia properties, tetracycline markedly affected the normal embryogenesis profiles by causing the damage and degeneration of intrauterine embryos of the parasite [21]. Polymerase chain reaction (PCR) assay also confirmed the clearance of *Wolbachia* DNA after prolonged antibiotic therapy. The reduction or clearance of bacterial specific hsp60 and *Wolbachia* surface protein (WSP) as determined by immuno-histochemical staining indicated the absence or clearance of *Wolbachia* in treated worms [22]. These antibiotics were effective in reducing the filarial larval molt (from L3 to L4) and their development *in vitro*. For doxycycline, the first clinical trials were done in people having onchocerciasis infections. A 6 week course of regular doxycycline treatment (100 mg/day) depleted *Wolbachia* in worms and caused extensive degeneration of embryos after 4 months post treatment. The worms became sterile after the loss of *Wolbachia*, with fewer or no microfilaraemia in affected patients. Doxycycline fulfils the role for a

new antifilarial therapy because it produces sterility, kills adult worms in lymphatic filariasis and prevents or lessens adverse reactions due to the rapid killing of Mf by standard microfilaricidal drugs. *Wolbachia*-targeting antibiotics may improve compliance with mass treatment programmes by reducing side-effects that may result from the release of intact bacteria and *Wolbachia* products from dying nematodes. Apart from typical antibiotic effects, these drugs (anti *wolbachia*) like tetracycline, ciprofloxacin and doxycycline, were tested for anti-filarial activity against *Brugia malayi* microfilariae; the results suggested that oxidative damage might be crucial for the antifilarial effect and thereby emphasized exploration of targeted oxidative effect towards the design of novel drug candidates [23]. Therefore, the discovery of endosymbiotic bacteria infecting most species of filarial nematodes that are pathogenic to humans has opened exciting new avenues of research into the pathogenesis of filarial infections. With the advent of bioinformatics along with the public domain access of genomic and proteomic database of the parasite and its endosymbionts, further insight into the *Wolbachia*-nematode relationship would be possible which might open up process for development of new therapeutic approaches to tackle these menacing parasitic diseases.

Recent advances in drug therapy and pharmacological research in the area

The World Health Organization (WHO) has recently defined and stressed on research for exploration and scientific validation of traditional medicine (including herbal drugs) that have been in existence often for several centuries, before the development and spread of modern medicine and are still in use today. Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care. They are well known and acceptable for their efficacy, safety and cultural acceptability. The chemical constituents of them are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body. There are descriptions in ayurvedic literature regarding the medicinal properties of *Acoras calamus*, *Deodar*, *Boerhavia diffusa*, *Plumbago zeylanica / rosea*, and *Terminalia chebula* in elephantiasis [24]. Re-

cently, *Shakotak* (*Streblus asper*) a well-known ayurvedic drug has been considered for clinical trial in lymphatic filariasis; its stem bark is reported to be effective against lymphedema, chyluria and other manifestation of filariasis. It was reported from clinical trial about the tolerability and efficacy of *daflon*, a micronized purified flavonoid fraction (MPFF), in filarial lymphedema cases. As a phlebotonic, *daflon* reduces capillary permeability and has an anti-lipidemic effect. No significant adverse reaction during the entire treatment period in terms of haematological or biochemical parameters was recorded [25]. This demonstrates that *daflon* (500 mg, twice a day for 90 days) is well tolerated, safe and can be used up to 90 days in patients with filarial lymphedema. The drug is efficacious as reflected by a significant reduction in oedema.

Despite the important addition to our knowledge of newer molecules with antifilarial activity, none has developed fruitfully as a macrofilaricidal drug due to their sustainable antifilarial activity and/or high toxicity. This warrants the need for developing an effective and safe drug to kill or permanently sterilize the adult worms. A major issue regarding the traditional herbal drugs is the lack of scientific validity. Of late AYUSH, a governmental initiative to integrate and promote such traditional therapeutics to the scientific area, has provided a significant boost in this area. In our study, extracts of *Butea monosperma* Linn (Palas roots and Leaves), *Vitex negundo* Linn. (Nirgundi roots) and of *Aegle marmelos* Corr (Beal Leaves) were analysed for their antifilarial activity that showed significant pharmacological effect in vitro [26]. Flavonoids/ polyphenols such as *vitexin*, *vitexicarpin* etc. in *Vitex negundo* Linn., coumarin in *Aegle marmelos* Corr., gallic acid in *butea monosperma* Linn, are the major active ingredient of these extracts [27, 28]. Owing to a known plausible paradoxical pro-oxidative prowess posed by the rather conventional antioxidants like flavonoid as a conditional effect [29], a putative oxidative rationale might be surmised in such pharmacological response. Evidence in support of this view has also been recorded by our earlier work [30]. This study, along with our study results, implicating oxidative mechanism with certain antibiotics suggests the feasibility of oxidative rationale. Remarkably, the most popular mechanism of action of DEC entails oxidative assault by the cells recruited to elicit inflammatory response as a key mediator of antimicrobial effect [31].

However, when considering the report of formidable anti-oxidant repertoire of the filarial parasite [32], whether such oxidative mechanisms might be directly implicated in the anti-filarial effect remains questionable. In a major relief of such intriguing dilemma, our earlier work established the significance of possible apoptotic effector mechanisms by an apparent synergistic impact with H₂O₂ + DEC combination [33]. Quite remarkably earlier evidence already showed the way in favour of an apoptotic mechanism by DEC in vitro [34]; although this failed to show filaricidal effect. However, our work managed to demonstrate such impact through synergism with H₂O₂, a recognized oxidant as well as apoptotic inducer. Moreover, our study recorded that this remarkable synergism is not entirely reverted by standard antioxidants suggesting apoptotic response rather than simple oxidative effect [33]. As such, oxidative stress is implicated in apoptosis induction [35]; hence, the observed synergism not only mirrored the effect of two apoptotic inducers working in tandem, but also coupled the apoptotic effector response with the oxidative rationale. Another important revelation comes from the fact that polyphenols / flavonoids are derivatives from shikimate pathways which are also responsible for other important derivatives like folates. Therefore, owing to structural resemblance, an inhibitory role of folate metabolic pathway by the flavonoids or its structural analogues is quite presumable. Certain flavonoids actually showed antiparasitic effects in vitro. A few of them also tested successfully in animal model as well [36]. In fact there is evidence supporting flavonoids from green tea to show significant DHFR inhibition and, more interestingly, that leads to apoptosis [37]. Present work is underway with tea extract in our lab and the results are highly promising (unpublished observation). Our study with such synthetic compounds which are proposed DHFR inhibitors recorded a marked pharmacological impact and also validated the folate metabolic hindrance through reversal experiments [38]. Subsequent work also managed to record evidence of apoptosis [39]. Very recently our work with silver nanoparticles recorded convincing evidence of anti-filarial effect through apoptosis [40].

Concluding remarks

Given the enormous socioeconomic encumber associated with this menacing parasitic infection, inflicting the developing world coupled with its so-called tag of 'neglected tropical disease', human lymphatic filariasis is a perennial problem which perpetually erodes the quality of life and is definitely a formidable challenge to the social development setting in a vicious cycle. This gloom further continues due to poor research initiative and infrastructure in these countries. A challenge is also posed by the relative paucity of parasitic material and the requisite animal model to gather experimental evidence. However, a definite silver lining has come up in the form of remarkable advances in bioinformatics and access to genomic and proteomic databases. This new age scientific revolution will definitely provide an edge to steer the evolution of therapeutics of such parasitic disease towards a finishing line for eliminating this disease.

Conflict of Interest

We declare that we have no conflict of interest.

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