Lymphatic Filariasis: An Overview

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Abstract: Wuchereria bancrofti, Brugia malayi, Brugia timori are the main parasites which causes lymphatic filariasis. Lymphatic filariasis is a neglected topical disease with more than 1.3 billion people worldwide. These are spread from by blood-feeding black flies and mosquitoes. These worms occupy the lymphatic system, including the lymph nodes; in chronic cases, these worms lead to the disease elephantiasis. Filariasis is diagnosed in microfilaraemic cases primarily through direct observation of microfilariae in the peripheral blood. Occult filariasis is diagnosed in amicrofilaraemic cases based on clinical observations and, in some cases, by finding a circulating antigen in the blood.

Key words: Lymph • Hematuria • Proteinuria • Doxycycline

INTRODUCTION

Lymphatic filariasis is a parasitic disease caused by roundworms Wuchereria bancrofti, Brugia malayi, Brugia timori. According to WHO, it is a neglected topical disease with more than 1.3 billion people in 72 countries worldwide affected and a current infection of 120 million with disfigurement in 40 million people [1]. Inflammatory reactions initiated by host against adult stage worm residing in the lymphatic vessels are considered to be major contributing factor for the disease occurrence and its subsequent progression to more complex pathology [2, 3]. Typical symptoms of filariasis are broadly categorised into asymptomatic, acute and chronic conditions [1]. Asymptomatic stage is the most commonly prevailing condition among filarial infected patients with sub-clinical manifestations like microscopic hematuria, proteinuria and dilated lymphatics [4]. This condition is believed to be driven by immunoregulatory mechanisms initiated by worms to lengthen their survival period [3]. Acute conditions begins as a result of hosts inflammatory response to the death or damage caused to the adult worm where the patients suffers from various conditions like high fever, painful inflammation, local transient oedema and pulmonary eosinophilia [3]. Chronic condition is majorly characterised by pathological alterations in lymphatics like hydrocele, lymphoedema resulting in enlargement of body parts with associated pain which may progress to more serious condition like elephantiasis [2]. The life cycle of worm consists of five stages involving human as definitive host and mosquito as intermediate host and transmitting vector. The adult stage worms residing in the lymphatic vessels exhibit sexual dimorphism and male worms are shorter in length compared to female worm. Their location in the body varies from species to species with bancrofti worms predominantly residing in genital regions and other species in upper and lower extremities [4]. The worms have an average life span of 5 years and copulate to produce first stage larva, microfilaria [4]. These circulate in the blood and exhibit periodicity between peripheral and deep vascular circulation in accordance with the timing of mosquito bite. They are taken up by blood feeding mosquito and further development of microfilariae larvae into infective third stage larvae occurs in the thoracic muscles of mosquito over a span of 10-15 days. Transmission of these infective stage larvae occurs when they are deposited on the surface of skin near punctures caused by blood feeding mosquitoes, which subsequently migrates into the body through these punctures. Infective stage larvae undergo molting to transform into fourth stage larvae inside the human lymphatics. Over a period of 6-9 months, development of adult worm from this larvae stage occurs in the central lymphatics and life cycle repeats. The life cycle of filarial worms is depicted in the figure 1.

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Chemotherapy in Filariasis: Drugs currently used in the treatment of lymphatic filariasis in various mass level drug administration level programmes recommended by WHO are diethylcarbazine (DEC), albendazole (ALB), ivermectin (IVM) [1]. But these drugs had shown action mainly on infectious stage microfilaria larvae (microfilaricidal) thereby halting the transmission and shown poor affect on adult worm (macrofilaricidal) which is the major cause of disease [5].

Filarial worms contain an intracellular bacteria wolbachia which was genomically related to endosymbiotic wolbachia of arthropod and this made their further consideration for investigation in the involvement in disease progression and its potential as a targeting for chemotherapy [6-9]. Further their symbiotic association with filarial worm was supported by study in which tetracycline therapy in murine model of filariasis using *L. sigmodontis* infected rodents has terminated wolbachia and halted the growth and fertility of worm [10]. In this study treatment with antibiotic drugs that will not affect rickettsial bacteria (wolbachia) did not affected the worm development in *L. sigmodontis* and further tetracycline was found to be insensitive to filaricidal worm, *Acanthocheilonema viteae* which don’t have wolbachia. Doxycycline (DOX), a tetracycline anti-biotic tested along with other anti-bacterial drugs on wolbachia of insect cell lines which are closely related to wolbachia of worms has shown potent activity in reducing the wolbachia levels [8] Later DOX targeting wolbachia in an invitro study employing *Brugia malayi*, was found showing potent anti-filaricidal affect by inhibiting embryogenesis and killing adult worm and microfilariae larvae [7]. In this study doxycycline was more potent in anti-wolbachial activity compared to other investigated antibacterial drugs. Similar results were obtained from other animal studies [11-13]. The above mentioned animal studies along with further genetic studies elucidating wolbachia genome has revealed support to bacterium as essential for fertility and survival of worm [14]. Proven more potent in anti-wolbachial action DOX mechanism of action is based on inhibition of bacterial protein synthesis by binding to the 30S bacterial ribosome and preventing access of amino acyl tRNA to the acceptor (A) site on the mRNA-ribosome complex. Their preferential action on bacteria is due to their low affinity for mammalian ribosomes and their inability to achieve sufficient concentration inside mammalian cells for action [6]. Evaluation of DOX in clinical trials has demonstrated macrofilaricidal affect evidenced by reduction in number of adult worm, their antigenemia and
microfilaraemia larvae[15-17]. An 8 week course of 200mg/day doxycycline in human trials has shown significant reduction in microfilaria larvae and macrofilaricidal affects with 51% reduction of pre-treatment antigenemia in blood and 75% less people having adult worms in scrotal sacs compared to the placebo at the end of 14 months observation period [15]. Similar macrofilaricidal results were observed with reduction in filarial antigenemia levels by 94% and absence of adult worms from the scrotal region in 89% of tested people at 24 months after six week regimen of 200 mg/day with four months follow up administration of 150–200 µg/kg ivermectin and 400 mg albendazole [16]. Further similar dose studies for 4 and 3 weeks were done with the former study involving administration of ivermectin150 µg/kg after four months of starting doxycycline therapy which showed macrofilaricidal affect approximately similar to that of above 6 week study and later study with administration of 150–200 µg/kg ivermectin and 400 mg albendazole after four months of starting therapy showed reduction in microfilaraemia with no significant macrofilaricidal activity[17,18]. These all above studies were done in people infected with bancroftian filariasis (caused by *Wuchereria bancrofti*). Recently trials in humans infected by *brugia filariasis* were done using 100mg/day for 6 weeks. In this study macrofilaricidal affect were found by reduction in microfilariae larvae levels and macrofilaricidal affect was observed by reduction in wolbachia levels at end of 1 year observation [19]. Moreover doxycycline has shown no severe adverse effects which are associated with conventional anti-filariasis drugs [20, 21]. However it needs prolonged therapy for showing anti-wolbachia action as observed in above 3 week study [18] which lacks significant macrofilaricidal affect and in a study comprising bovine model of onchocerciasis, an extra-lymphatic filarial disease in which short regimen of Doxycycline (10 mg/kg daily for 14 days) has eliminated >60% adult female worms compared to >80% observed with long-term treatment (20 mg/kg monthly for 6 months) [20]. As DOX cannot be recommended for use in mass drug administration programmes due to the cost factor in long term treatment and its contradiction in children (<9 years) and women [17], a shorter and safer regimen for DOX should be developed for reducing the duration of therapy and improving the clinical efficacy. A liposomal delivery system for DOX along with other anti-filarial drugs was evaluated for its potential in treatment of filariasis [22, 23]. The study was concluded with macrofilarial affect observed with the combined therapy.

**Drug Delivery for Lymphatic Filariasis**: As DOX was proved more effective in treating the lymphatic filariasis on long term administration, efficient delivery strategies should be developed for reducing its duration of therapy to make it more patience compliant. Delivery systems which release the drugs at sustained rate maintaining the required concentration of drug for prolonged time in body can reduce its frequency of administration. In addition as filarial worms are present inside lymphatic vessels, delivery approaches for selective accumulation of DOX at this target site can improve its efficacy and may reduce the both dose and adverse affects associated with therapy. Nanoparticulates as drug carriers as proven from various studies can have the advantage of both prolonging the life of drug inside the body through sustaining the drug release for and increasing drug accumulation at specific sites inside the body [24-31]. Therefore, targeting of DOX loaded nanoparticulates to lymphatic tissues in filariasis may improve the disease condition.

Lymphatic targeting for nanoparticulates by Sub-cutaneous route (SC) involves two factors: firstly it’s draining from the site of administration and secondly, their preferential uptake into the lymph nodes [24, 25]. The most investigated route of a nanoparticulates for lymphatic targeting was SC route it has shown good targeting ability and less chance for drug degradation compared to other routes [25,26]. The nanoparticulate intended for lymphatic targeting initially must be uptake by the lymphatic capillary from interstitial space when injected S.C.. Its uptake depends on both the physiological structure of interstitial space and lymphatic system and upon the physiochemical properties of the nanoparticulates [25]. The ideal size for uptake of colloids by S.C administration was between 1-100 nm as the ground substance in interstitial space form narrow aqueous channels of 100 nm diameters through which they have to passed [32]. However sterically stabilized polymeric nanoparticles of 200 nm poly (lactide-co-glycolide) (PLGA) coated with poly (lactide)-poly (ethylene glycol) (PLA: PEG) has also shown uptake although to a lesser extent into lymphatics [32]. Similarly, negatively charged surface favours prefential uptake due to presence of negatively charged polysaccharide glycosaminoglycan in the interstitial fluid which repels colloids, forcing their uptake [25]. Finally, hydrophobicity which governs the aggregation and opsonization of nanoparticles may cause aggregation or interaction of colloids with interstitial space and reduce their uptake [25]. This may be the reason for the lesser
uptakes of liposomes into lymphatics as these are made of hydrophobic lipids and would have possibly interacted with interstitial space [32]. Lymphatic targeting studies has shown increased uptake for surface modified polymeric nanoparticles compared to liposomes when administered by SC route [24, 27]. In this studies surface modified polymeric nanoparticles of poly (lactide-co-glycolide) (PLGA) and polystyrene (PS) coated with poly (lactide)-poly (ethylene glycol) (PLA: PEG) [25] and PS nanoparticles surface modified with poloxamines and polaxomers [28] have shown higher uptake into lymphatics. Thus developed nanoparticulates should be sufficiently small, hydrophilic, negatively charged and be satirically stabilised so that they can selectively targeted to lymphatic tissues [28].

Polymeric of poly (D, L-lactide-co-glycolide) (PLGA) and polycaprolactone (PCL) were selected for the preparation of DOX loaded polymeric nanoparticles. These polymers are chosen due to their anionic nature, biodegradability in the body, approval by food and drug administration (FDA), USA [29, 33]. Further studies were reported for use of polymers in the preparation of nanoparticles for drug delivery [34, 35]. They are surface modified for steric stabilization with adsorption of pluronic F-68 on their surface during their preparation. They are prepared by solvent displacement method reported earlier with minor modification [36]. Nanoparticles were optimized for their size, charge by varying the parameters. Optimized nanoparticles was evaluated for their in-vitro and in-vivo release pattern.

Fig 1.2: Pictorial representation of sub-cutaneous route of administration for nanoparticles (Modified from [25])
**Recent Advancement on Lymphatic Filariasis:**

Hawley et al.,[25] reported that targeting of colloids by to lymphatic system involves two stages, firstly its uptake from injection site and secondly its accumulation in the lymph nodes. The initial uptake of colloids from interstitial space into lymphatic capillaries upon sub-cutaneous injection depends upon physiology of lymphatic system, interstitial space and the physiochemical characteristics of colloids. The targeted colloids by SC route due to limitations imposed by physiology of interstitial space and lymphatics should have specified criteria in their physiochemical properties like size, surface charge, concentration, molecular weight, hydrophobicty for their uptake into lymphatics. Size of the colloids was the one of the important factors affecting the lymphatic uptake with lowest size in tens of nanometre showing faster accumulation than large size particles. Concentration of injected colloids has shown inverse relationship with rate of uptake with higher concentration of colloids showing low rate of uptake. Surface charge evaluation showed negatively charged colloids showing faster uptake compared to positively charged. Molecular weight effect on colloids were seen with preferential lymphatic absorption for MW of > 16000 daltons. Hydrophobic colloids have shown reduced uptake taken into lymphatic system compared to similar colloids which are hydrophilic due to their surface coating with polaxomers and poloxamines. This affect was due to prevention of opsonization of surface modified colloids which will prevent phagocytosis. They also emphasized that subcutaneous route is the preferred route for lymphatic targeting in which size and hydrophobicity are the important factors. Based on earlier reported studies, they also mentioned that polymeric nanoparticles may have a better chance for targeting compared to liposomes.

Melody [37] described the anatomy, physiology and biology of the lymphatic system in accordance with lymphatic drug delivery. Lymphatic system in body helps in transporting lymph which is the excess interstitial fluid back to blood circulation, absorption of essential fats from intestine and provides immunity to body. It is organized into different types of vessels with lymphatic capillaries initially take up lymphatic fluid subsequently open into lymph vessels which transport lymph through lymph nodes, trunks and ducts to the circulation. Lymph is formed when interstitial fluid is up taken into lymphatic capillaries and it mediated by interstitial fluid pressure and support of the extracellular matrix. Lymph is propelled in unidirectional way from its vessels by its contraction supported by valves along their length. Solute transport through lymphatic system depends on properties of interstitial space for its uptake and later in lymphatic system it will be affected by filtration through lymph nodes and phagocytosis. Lymphatic function assessment was mentioned using various parameters like tissue clearance rate, amount of radioactivity per unit time per unit tissue volume, measurement of solute concentration ratios between plasma and lymph and local lymphatic capillary pressures. Finally lymphatic tissue role in cancer metastasis and oedema are explained.

Anil Dangi et al. [23] evaluated liposomes containing doxycycline (DOX) and rifampicin (RFN) administered by sub-cutaneous route along with free oral administration of diethylcarbazine (DEC) for treatment for filariasis. The liposomes are formulated for this drug in order to reduce their duration of therapy. The efficacy of the formulation was evaluated in filariasis infected mastomys with a dose for every 3 days of 10mg/kg for DOX and RFN 50 mg/kg for DEC. The activity of the formulation was evaluated by determining the microfilaricidal (microfilariae larvae killing activity) and macrofilaricidal activity (adult worm killing activity) in mastomys. Maximal microfilaricidal affect 88.1 % on day 20 was observed when liposomal DOX, RFN along with oral diethylcarbazine was combine administered. Similarly the maximum macrofilaricidal affect of 74.8 % at the end of observation on day 120 was observed with combine administration of liposomal DOX, RFN and oral DEC. In vivo release of formulation along with free drug for DOX was evaluated in mastomys. The peak concentrations for DOX and RFN were found at the end of 12 h post administration. The formulations showed sustained release till the end of observation for 48h maintaining the concentration of DOX and RFN above their minimum inhibitory concentrations (MIC). The free form of these drugs when administered maintained drug above their MICs for 18 and 6 hours for DOX and RFN.

Oussoren et al. [32] explained about the liposomes for delivering of diagnostic and therapeutic agents to lymphatic tissues by S.C. route of administration. The information on lymphatic absorption and lymph node uptake reported by various studies for lymphatic targeting of liposomes were mentioned. Factors affecting the liposomes after sub-cutaneous injection such as injection site, liposomal size, lipid composition and lipid dose were discussed. Influence of surface modification of liposomes using polyethylene (glycol) (PEG), ligands was reported. Finally the review was concluded with explanation of the mechanism of localization of liposomes in regional nodes and therapeutic and diagnostic applications of liposomes.
Moghimi et al. [27] applied the concept of steric stabilization for examining the lymphatic uptake and distribution of sub-cutaneously administered model polystyrene nanospheres. Block co-polymers of poloxamines and poloxamers series are used to produce the satirically stabilized nanospheres. Their results showed an existence correlation between the lengths of stabilizing polyoxyethylene (POE) chains of block-copolymers and their rate of transport into lymphatic capillaries. Faster particle uptake into lymphatic capillaries was reported for nanospheres with longer POE chains and effective opsonization inside lymph nodes was found for nanospheres with POE chains of 5-15 ethylene oxide units. They observed failure of opsonization if the dimensions of the stabilizing POE chains exceed the vanderwaals force of attraction and these are rapidly drained into lymphatic capillaries and escaped uptake by macrophages of the regional lymph nodes and even circulated for long time in blood. Finally based on their observations they suggested that sterically stabilized polymeric nanoparticles may be advantageous for lymphatic delivery.

Hawley et al. [24] assessed the nanospheres of polystyrene and poly (lactide-co-glycolide) with their surface modified using poly (lactide)-poly (ethylene glycol) (PLA-PEG) for lymphatic targeting. Parameters like the length of PEG chains are varied which affected the size and charge of colloids to determine their affect on the uptake into lymphatic system. Their bio-distribution in the body after S.C. administration was evaluated in rats. The results showed the faster uptakes of surface modified nanospheres into lymphatics compared to unmodified nanospheres. The faster uptake was believed to be caused by mechanism of steric stabilization due to the reduction of interaction of hydrophilic POE chains with the interstitial space forcing the particle uptake. Electron microscopy study of excised organs revealed the major route of particle uptake was by intercellular spaces between adjacent endothelial cells of lymphatic capillary.

Rao et al. [28] evaluated the lymphatic uptake and lymph node retention of biodegradable nanoparticles of poly (lactide-co-glycolide) (PLGA) by varying their parameters like sizes, hydrophobicity and surface charge densities by in-vivo studies using rats. Size parameter was evaluated by surface modification with polyethylene glycol-co-polyactic acid (PEG-PLA) by co-precipitation which resulted in nanoparticles of different sizes. Hydrophobicity affect was evaluated by comparative study using polystyrene (PS) nanoparticles which are more hydrophobic compared to the PLGA nanoparticles. Charge density varied by co-precipitating the nanoparticles with different ratios of PLGA terminated with carboxylic group was used for studying charge affect. The results of size affect study showed that particles of 50 nm had shown higher concentration of uptake into initial lymphatic node at 6 h and 48 hrs observation followed by the 100 nm and 200 nm. Similarly the serum concentration profile followed similar trend with higher concentration for 50nm. The cumulative accumulation of nodes also showed similar profile. Hydrophobicity study using PS nanoparticles showed no significance difference in the uptake between size 60 nm and 112 nm nanospheres which was believed due to their aggregation of 60 nm particles occurred by hydrophobicity of particles. Their serum level was found to be very low compared to PLGA. Charge study showed increased uptake of most anionic particles compared to particles with less anionic charge. This trend was observed in both lymphatic node and serum uptake.

Zhang et al. [31] explained about the application of nanotechnology generally of with particle size between 1-100 nm in medicine, known as nanomedicine. Nanomaterial owe their advantage in medicine mainly due their unique physiochemical properties like small size, large surface area and high reactivity in comparison to the bulk materials of similar composition. These nanoscale systems in medicine can provide more convenient route of administration, lower therapeutic toxicity, extended product life and can reduce the health care costs. They can be used in therapeutics for controlling the drug release, selective targeting and in diagnosis for detection at molecular scale. Advantages of nanoparticles in drug delivery are improvement in solubility of poorly soluble drugs, extension of half-life of drug, minimization of side effects and timely release of drug inside the body. Nanoparticles particle based therapies approved for clinical use with most of them coming from liposomes and drug conjugates were discussed. Nanoparticles therapeutics currently undergoing clinical trials were also mentioned. These include pegylated, ligand mediated liposomes, drug conjugates, nanoemulsions, dendrimers and inorganic nanoparticles. Further nanoparticles which are in preclinical development consisting of polymeric nanoparticles, micelles, nanoshells, dendrimers, engineered viral nanoparticles, albumin based nanoparticles, polysaccharide based nanoparticles, metallic nanoparticles and ceramic nanoparticles are covered.
Nutman et al. [4] had briefly discussed about the history, causative agent, epidemiology, symptoms, pathology, immunity, diagnosis, treatment along with preventive measures for lymphatic filariasis. The history of filariasis mentioned in the ancient literature along with the discovery of adult worm larval stages and their life cycle were mentioned. The five stages in the life-cycle of filarial forms in relation to their hosts along with their way of transmission were discussed. The role of wolkachial, endosymbiotic bacteria of filarial worm in disease progression and worm development are mentioned. Their epidemiology of filariasis in the world accordance with different species of filarial worm, their vectors and symptoms observed were detailed. The manifestations of the disease in form of various symptoms like sub-clinical patent infection, acute adenolymphangitis, lymphedema of extremities and genetalia, hydroceles and tropical pulmonary eosinophilia. The pathologi and immunological mechanisms involved in appearance of symptoms were explained. Diagnosis based on epidemiological history like appearance of lymphedema of the extremities or genetalia, physical findings like detection of parasite antigen, DNA, adult worms and laboratory tests used for detection of microfilarae on blood smears. Antibodies are used. Treatment based on chemotherapy and pathology based therapy were mentioned. Finally, the preventive measures for filariasis using drugs, pest control and bed nets are mentioned.

Dreyer et al. [2] explained pathogenesis of brancoftian filariasis by proposing a model based on the bridging of data from various clinical, parasitological, surgical, therapeutic, ultrasonographic and histopathological studies. This model is based on the explanation of disease condition lymphangiectasia was caused by non-obstructive mechanisms based mainly on the ultrasonic data obtained during lymphangiectasia condition. This model was contrary to traditional theory which was based on the obstructive mechanisms and immune cell infiltration caused by adult worms responsible for lymphangiectasia condition. This was supported by a study conducted in filariasis infected nude mice having no immune components showed lymphangiectasia caused by adult worms. This was further supported by the inflammation present in acute filarial lymphangitis, a condition followed after lymphangiectasia caused due to death of adult worms. This was further supported by studies assessing hydrocele and lymphodema of extremeties.
REFERENCES


