Acta Tropica 125 (2013) 150–156

Contents lists available at SciVerse ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

A double-blind controlled field trial of doxycycline and albendazole in combination for the treatment of bancroftian filariasis in India

Prajna Gayen[a], Ananya Nayak[a], Prasanta Saini[a], Niladri Mukherjee[a], Sudipta Maitra[b], Prasanta Sarkar[c], Santi P. Sinha Babu[a,*]

[a] Parasitology Laboratory, Department of Zoology, Visva-Bharati University, Santiniketan 731 235, West Bengal, India
[b] Physiology and Biochemistry Laboratory, Department of Zoology, Visva-Bharati University, Santiniketan 731 235, West Bengal, India
[c] Physician, Ex-Medical Officer, Bolpur Sub-Divisional Hospital, Bolpur 731 204, West Bengal, India

ARTICLE INFO

Article history:
Received 9 March 2012
Received in revised form 16 October 2012
Accepted 23 October 2012
Available online 30 October 2012

Keywords:
Lymphatic filariasis
Wuchereria bancrofti
Wolbachia
Microfilaria
Macrofilaricide

ABSTRACT

In a placebo controlled field trial, the effects of doxycycline (200 mg/day) for 23 days followed by doxycycline (200 mg/day) in combination with albendazole (ABZ) (400 mg/day) for 7 days on depletion of *Wolbachia* endobacteria from *Wuchereria bancrofti* and microfilaricidal activity were studied in 68 patients (34 males and 34 females) from West Bengal, India. The drugs in combination (i.e., doxycycline + ABZ) provided the best efficacy by totally eliminating the circulating microfilaria (mf) (in 42% cases) on day 365 with (99.8%, $P < 0.05$) suppression even on day 365 post-treatment compared to both exclusive doxycycline (69%, $P < 0.05$) and ABZ (89%, $P < 0.05$) groups. Thus, our results have established that a 30-day course of doxycycline in combination with a 7-day course of ABZ is sufficient to ensure long-term reduction in mf level by depleting *Wolbachia* from worm tissues. Doxycycline combined with ABZ led to a greater reduction in mf density in blood at 4 months (post-treatment) in comparison to doxycycline or ABZ alone. There were significant differences between the three treatments after 12 months (post-treatment). Further, the impact of a 7-day regimen of ABZ was surprisingly good in reducing mf compared to doxycycline-alone group. Adverse reactions were mild. A 30-day course of doxycycline and ABZ in combination is a safe and well-tolerated treatment for lymphatic filariasis with significant activity against microfilaremia.

1. Introduction

Lymphatic filariasis is a major tropical disease and one of the major causes of global disability. More than 120 million people are infected with filarial worm and about one billion people are at the risk of infection (WHO, 2005). According to 1995 estimates, in India, 533 million people were exposed to filarial infection of which 21 million people with symptomatic filariasis like lymphangitis, hydrocoel, lymphoedema, or elephantiasis and 27 million microfilaria (mf) carriers (Rao, 2005). We have reports of a prevalent status (10.9%) of bancroftian filariasis in the rural areas endemic for filaria in two districts, Bankura and Birbhum, in West Bengal, India (Gayen et al., 2010). Present-day antimicrofilarial or macrofilaricidal treatment regimens have certain well-documented limitations. Diethylcarbamazine (DEC) and ivermectin are effective at killing mf but are associated with systemic and inflammatory adverse reactions (Carme et al., 1991; Ottesen et al., 1999). Albendazole (ABZ) increases the efficacy of diethylcarbamazine and ivermectin and is used in combination with either of the drugs as the basis of long-term intervention programme (Molyneux et al., 2003). However, DEC makes any multidrug regimen unsafe for community-wide use in Africa because of the coendemicity of onchocerciasis; the region being such a one where DEC can induce severe reactions in patients with co-infections (Carme et al., 1991; Ottesen et al., 1999). Jayakody et al. (1993) for the first time have shown ABZ to be effective against bancroftian filariasis in humans. Albendazole (400 mg twice daily) was given for three weeks to 15 microfilaraemic men, and the results were compared with those of 12 other microfilaraemic men treated for three weeks with DEC (6 mg/kg/day). Whilst the microfilaricidal activity of the ABZ regimen was impressive, 11 of these 15 men experienced a syndrome of acute pain, fever and inflammation of the scrotal sac and adjacent tissue, probably induced by dying parasites. However, the frequency and severe reactions in these long-term, high-dose ABZ-treated individuals discouraged further study of this treatment regimen, although the efficacy of ABZ against *W. bancrofti* infections had been clearly established. Albendazole, an effective, safe and endurable, has significant 'beyond filariasis' improvements when compared to other antifilarial drugs. It has a broad-spectrum effect on intestinal helminthes; and as a result of which, it augments

* Corresponding author. Tel.: +91 3463 261268; fax: +91 3463 261176.
E-mail address: spsinhababu@gmail.com (S.P. Sinha Babu).

0001-706X/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.actatropica.2012.10.011

cognitive functions and nutritional status, especially in children. This will be of particular importance in countries, such as India, where the National Government has launched a specific nutritional supplement programme, and where the success of such programme is expected to be low due to the prevalence of infected patients in the population. Additionally, GlaxoSmithKline has committed itself to the donation of ABZ to all countries endemic for lymphatic filariasis as long as it is required (Ottesen et al., 1997). Thus, as far as ABZ is concerned, the cost for its inclusion in the MDA programme is not significant. Therefore, the advantages of using ABZ + doxycycline in place of DEC or ivermectin include fewer adverse reactions, less expenses and greater acceptance by the treated populations.

Thus, the present-day requirement for filarial chemotherapy is a cheap, nontoxic and novel antifilarial drug with long-term antimicrofilarial or macrofilaricidal activity.

One potential target is to use antirickettsial antibiotics to deplete *Wolbachia* endosymbionts that exist in the lateral cords of adult female of most filarial nematodes including *Dirofilaria immitis*, *Litomosoides sigmodontis*, *Onchocerca volvulus*, *W. bancrofti*, and *Brugia malayi*. Doxycycline has been introduced as a novel chemotherapeutic agent, targeting *Wolbachia* endobacteria in the filariae. Eight-, 6-, 4-, and 3-week courses of doxycycline are superior to standard antifilarial treatment (Hoerauf et al., 2000, 2001, 2003a; Tuner et al., 2006; Taylor et al., 2005; Debrah et al., 2007; Turner et al., 2010). Moreover, 8-week regimens produce considerable macrofilaricidal activity (Taylor et al., 2005). Treatment with doxycycline for 3 weeks is equally effective in inducing a long-term amicrofilaraemia, but it is not effective in killing the adult parasites. For the first time, we conducted a double-blind, placebo controlled field trial of doxycycline (200 mg/day) for 23 days followed by doxycycline (200 mg/day) + ABZ (400 mg/day) for 7 days, doxycycline (200 mg/day) for 30 days and ABZ (400 mg/day) for 7 days for *W. bancrofti* infected persons from the eastern part of India. Our primary aim has been to assess the impact of doxycycline on *Wolbachia* loads within mf and of the combination of doxycycline and ABZ on mf level during the observation period.

2. Material and methods

2.1. Study area and study design

The trial was conducted in two rural areas endemic for filaria in the two districts of Bankura and Birbhum, West Bengal, India from 2006 to 2008. A total of 68 (34 males and 34 females) asymptomatic mf carriers were included in this study.

The protocol for this study was approved by the Human Ethical Committee of Sub Divisional hospital, Bolpur, West Bengal, India and the Institutional Ethics Committee, Visva-Bharati University, Santiniketan - 731 235, West Bengal, India. Individuals eligible for selection in the survey were residents of the villages namely, Dhabal, Moukura and Baramasia in the district of Bankura and Binuria, Bandanga and Majhipara in the district of Birbhum. The study site was selected on the basis of prevalence of lymphatic filariasis which had been assessed before by microscopic observation of mf in the peripheral blood. We collected blood samples at random from apparently healthy people irrespective of sex and age. Prior to survey, a social worker explained the villagers about the purpose of the survey to them. The blood collection team visited the selected villages between 21:00 and 23:00 h. A 20 mm^3 peripheral blood smear was collected for subsequent laboratory assessment. Occurrence of mf was recorded by observing the Giemsa-stained blood smear under a microscope. After a detailed explanation of the objectives of the study in the local language to villagers, individuals were asked for informed consent to participate. Individuals (both male and female) between the ages of 18 and 65 who had a minimum body weight of 40 kg and were in good health and females who were not pregnant and were not breast feeding, were eligible for this study. Exclusion criteria included abnormal hepatic and renal function (SGPT > 60 I.U./L, SGOT > 40 I.U./L, Creatinine >1.4 mg/100 ml), intolerance to doxycycline and ABZ and history of alcohol abuse. The patients included in our study were naive because they did not take any anti-filarial drug prior to our trial.

Persons who gave informed consent were assigned randomly to receive 200 mg doxycycline (2 capsules of 100 mg each, Dr. Reddy's Laboratory) for 30 days or 200 mg doxycycline for 23 days followed by 600 mg doxycycline (200 mg) in combination with ABZ (1 tablet of 400 mg each, Smithkline Beecham) for 7 days or ABZ (1 tablet of 400 mg each, Smithkline Beecham) for 7 days or matching placebo for 30 days (Fig. 1). All participants received the standard treatment with DEC (6 mg/kg, Glaxo SmithKline) combined with ABZ (1 tablet of 400 mg each, Smithkline Beecham) 12 months after the drug and placebo treatment courses had commenced.

After their pretreatment evaluation the study patients were randomly assigned to one of four groups by a trial monitor who was not associated in the study. The study was double-blind: neither the patient nor the evaluating physician was aware of the kind of medication that was given. Blinding and coding of drugs was done by an independent monitor (a scientist who was not an investigator) after repacking in identical capsules provided by a pharmaceutical company (Dey's Medical) in Kolkata.

2.2. Isolation of parasite from blood

Surveys to monitor filarial infection status (mf load) were performed by finger prick night samples immediately before the beginning of the treatment. While undergoing the treatment, on fifteenth and forty-fifth days (day numbers 15 and 45) all patients (treated and placebo) donated 8 ml of venous blood for exact mf quantification using membrane filtration method (Ottesen et al., 1990), and isolation of total RNA from mf (pooled samples from each group) using TRI Reagent (Sigma, USA). Additional blood samples (2–3 ml) were taken from patients on days 30, 60, 90, 120, and last sampling was done at day 365 post-treatment.

2.3. Synthesis of cDNA

Total RNA was extracted using TRI Reagent (Sigma, USA). After RNA isolation, residual DNA contamination was removed by digestion with RNase-free DNase (Promega, USA). First-strand cDNA was prepared from 3 μg total RNA using the first strand cDNA synthesis kit (Fermentas, USA). The synthesized cDNA was used for PCR reactions with gene specific primers.

2.4. RT-PCR

Conventional RT-PCR was performed using a thermal cycler (Master Cycler Eppendorf, Germany). The reaction mixture contained PCR buffer (1×) with $(NH_4)_2SO_4$, dNTP mix (2 mM), $MgCl_2$ (2 mM), 1 μM each of forward and reverse primers, 1.5 units of *Taq* polymerase (Fermentas, USA) and RT product. During thermal cycling, denaturation was done at 95 °C for 3 min followed by 35 cycles at 94 °C for 45 s, 51 °C for 1 min and 72 °C for 1 min. Final extension was done at 72 °C for 7 min. For amplification (control and treated samples) filarial-specific 28 S rRNA, the primer pairs BD1A F and BD1A R (Smith and Rajan, 2000) were used and β-actin served as a control. To amplify the expression of *Wolbachia*-specific *wsp* gene, the primers WSPintF and WSPintR (Bazzocchi et al., 2000) were used and 16S rRNA served as a control. PCR products were resolved in 2% agarose gel and stained with ethidium bromide to