Acta Tropica 125 (2013) 330–338

Contents lists available at SciVerse ScienceDirect

Acta Tropica

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

Filamentation temperature-sensitive protein Z (FtsZ) of *Wolbachia*, endosymbiont of *Wuchereria bancrofti*: A potential target for anti-filarial chemotherapy

Rohit Sharma, S.L. Hoti*, V. Vasuki, T. Sankari, R.L. Meena, P.K. Das

Vector Control Research Centre (ICMR), Indira Nagar, Medical Complex, Puducherry 605006, India

ARTICLE INFO

Article history:
Received 14 May 2012
Received in revised form
30 November 2012
Accepted 1 December 2012
Available online 19 December 2012

Keywords:
Wuchereria bancrofti
Wolbachia
FtsZ
Polymorphism
Albendazole sensitivity

ABSTRACT

Lymphatic filariasis (LF) is a leading cause of morbidity in the tropical world. It is caused by the filarial parasites *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori* and transmitted by vector mosquitoes. Currently a programme for the elimination of LF, Global programme for Elimination of Lymphatic Filariasis (GPELF), is underway with the strategy of mass administration of single dose of diethylcarbamazine or ivermectin, in combination with an anthelmintic drug, albendazole. However, antifilarial drugs used in the programme are only microfilaricidal but not or only partially macrofilaricidal. Hence, there is a need to identify new targets for developing antifilarial drugs. Filarial parasites harbor rickettsial endosymbionts, *Wolbachia* sp., which play an important role in their biology and hence are considered as potential targets for antifilarial chemotherapy development. In this study, one of the cell division proteins of *Wolbachia* of the major lymphatic filarial parasite, *W. bancrofti*, viz., filamentation temperature-sensitive protein Z (FtsZ), was explored as a drug target. The gene coding for FtsZ protein was amplified from the genomic DNA of *W. bancrofti*, cloned and sequenced. The derived amino acid sequence of the gene revealed that FtsZ protein is 396 amino acids long and contained the tubulin motif (GGGTGTG) involved in GTP binding and the GTP hydrolyzing motif (NLDFAD). The FtsZ gene of endosymbiont showed limited sequence homology, but exhibited functional homology with β-tubulin of its host, *W. bancrofti*, as it had both the functional motifs and conserved amino acids that are critical for enzymatic activity. β-tubulin is the target for the anti-helminthic activity of albendazole and since FtsZ shares functional homology with, β-tubulin it may also be sensitive to albendazole. Therefore, the effect of albendazole was tested against *Wolbachia* occurring in mosquitoes instead of filarial parasites as the drug has lethal effect on the latter. Third instar larvae of *Culex quinquefasciatus* were treated with 0.25 mg/ml of albendazole (test) or tetracycline (positive control) in the rearing medium for different intervals and tested for the presence of *Wolbachia* by FtsZ PCR. All the treated larvae were negative for the presence of the FtsZ band, whereas all the control larvae were positive. The findings of the study, thus indicated that FtsZ is sensitive to albendazole. In view of this albendazole appears to have dual targets; FtsZ in *Wolbachia* and β-tubulin in *W. bancrofti*. Further, the functional domain of the gene was assessed for polymorphism among recombinant clones representing 120 *W. bancrofti* parasites, prevalent across wide geographic areas of India and found to be highly conserved among them. Since it is highly conserved and plays an important role in *Wolbachia* cell division it appears to be a potential target for anti-filarial chemotherapy development.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Currently, a programme for the elimination of LF has been launched globally, with the strategy of annual single-dose Mass Drug Administration (MDA) of diethylcarbamazine (DEC) or ivermectin, or either of these drugs in combination with an anthelmintic drug, albendazole. However, many countries that have implemented MDA annually for over 5 years are yet to interrupt transmission (Bockarie and Deb, 2010). This is because the drugs used in this programme are only microfilaricidal but not/partially macrofilaricidal (Gyapong et al., 2005). Hence, the adult parasites that survive will continue to remain as source of infection for continued transmission. Hence, there is an urgent need for identifying more targets for macroflaricidal drug development in order to augment the ongoing LF elimination programme. A macrofilaricide will help in achieving the goal of LF elimination more effectively.

Wolbachia are maternally inherited alpha-proteobacteria found in numerous arthropod species as well as filarial nematodes (Taylor et al., 1999). The occurrence of *Wolbachia* in lymphatic filariae was first reported by Sironi et al. (1995), followed by Bandi et al. (1998).

* Corresponding author. Tel.: +91 413 2279073; fax: +91 413 2272 041.
E-mail address: slhoti@yahoo.com (S.L. Hoti).

0001-706X/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.actatropica.2012.12.004

Wolbachia are found both in female and male worms and in all the stages of development. The mode of their transmission is transovarial (Taylor and Hoerauf, 2001). *Wolbachia* play a central role in worm biology, larval and adult worm survival, and embryogenesis (Genchi et al., 1998; Hoerauf et al., 2000; Pfarr and Hoerauf, 2007). Recent human trials indicated that *Wolbachia* endosymbionts of filarial parasites are viable drug targets for treatment of filarial disease (Hoerauf et al., 2003; Supali et al., 2008). Administration of an anti-rickettsial drug, doxycycline was found to block embryogenesis leading to extensive embryo toxicity in female worms which resulted in the significant reduction of number of microfilariae (Bandi et al., 1999; Hoerauf et al., 1999, 2000, 2001, 2003; Langworthy et al., 2000). However, the length of doxycycline treatment, high dose required and medical contra-indications in children and pregnant woman warrant the identification of additional anti-*Wolbachia* drugs (Townson et al., 2006).

Especially, the cell division machinery of *Wolbachia* appears to be an attractive target for drug development (Wang et al., 2003; Jennings et al., 2004; Margalit et al., 2004). Filamentation temperature-sensitive protein Z (FtsZ) of this machinery is of special importance in this regard as it plays an essential role in the cytokinetic ring formation at the site of division (Bi and Lutkenhaus, 1991). It is considered as a target for new generation antibacterial development (Margalit et al., 2004; Vollmer, 2006); in the case of pathogens like multi-drug resistant *Mycobacterium tuberculosis* (Huang et al., 2008) and *Staphylococcus aureus* (Singh and Panda, 2010; Haydon et al., 2008; Andreu et al., 2010). Recently, Li et al. (2011) have reported that FtsZ of *Wolbachia* of *Brugia malayi* can be a good drug target. In the present study, we have characterized the complete ORF of FtsZ gene of *Wolbachia* of *Wuchereria bancrofti*, the major filarial parasite that contributes to 90% of 120 million filarial cases worldwide (Ottesen et al., 2008) and investigated its polymorphism among the parasite populations prevalent in different geographic locations of India. The results of these studies are presented in this article.

2. Materials and methods

2.1. Sample collection

The study was approved by the Institutional Human Ethics Committee of the centre. Blood samples were collected after obtaining written informed consent from microfilaraemic individuals. The staff of National Vector Borne Disease Control Programme (NVBDCP), New Delhi, collected the finger prick blood samples as thick smear from microfilariae carriers residing in locations of Tamil Nadu, Gujarat, Bihar, Pondicherry, Orissa and Karnataka states (Fig. 1). They were collected during night and kindly provided by the Director, NVBDCP.

2.2. Isolation of parasite and purification of DNA

Microfilariae (mf) were isolated from blood smears as described earlier (Bisht et al., 2006). One hundred mf from each area, picked up from 10 to 15 blood smears from different individuals (depending upon the number of mf on each slide and restricting to a maximum of 10 mf from each slide) were pooled and the DNA was extracted from them using Qiagen DNeasy MicroAmp kit (Hilden, Germany) following manufacturer's protocol with minor modifications as follows: mf were suspended in Proteinase K solution and incubated on a thermomixer at 56 °C for 6 h. Final step of elution was done with two volumes (7.5 μl) of TE buffer.

2.3. Polymerase chain reaction and cloning into sequencing vector

For structural characterization, a fragment carrying the complete ORF (1188 bp) of FtsZ gene was amplified using primers designed at flanking regions of a 1350 bp fragment of the whole genome sequence of the *Wolbachia* endosymbiont of *B. malayi* (GenBank accession number AE017321.1), between nucleotide positions 787,475 and 788,824, cloned into a sequencing vector as stated below and sequenced. The primers used are FtsZ full F 5′-CACTTCACCTGACTACTGCTC-3′ (Forward) and FtsZ full R-5′-GGATGAAAAGTGTTTGCATGTT-3′ (Reverse). Amplification was performed using a proof reading enzyme to prevent PCR amplification artifacts and the PCR mix contained 3 μl of pooled mf DNA sample, 10 pM of both forward and reverse primer, 1× PR buffer, 0.2 mM dNTPs and 1.5 units of proof reading enzyme (PR Polymerase, Bangalore Genei, Bangalore, India), in a final volume of 50 μl, The temperature cycles included denaturation at 92 °C for 30 s, annealing at 56 °C for 45 s, and extension at 72 °C for 1 min 30 s. Initial denaturation and final extension was done at 94 °C for 3 min and 72 °C for 10 min, respectively in a thermal cycler (Eppendorf, Hamburg, Germany).

For polymorphism studies the functional domain of FtsZ gene (nt position 209–727) containing GTP binding and GTP hydrolyzing motifs (520 bp) was amplified from DNA of 100 *W. bancrofti* mf from each of the geographical areas stated above, cloned into a sequencing vector and sequenced. The fragment was amplified using the Forward 5′-ACTATTTATGTCTGTAGGGAT-3′ and Reverse 5′-CTGCTCTCTTTTCATCTTTGC-3′ primers. PCR reaction mix was similar to that stated above and the amplification was carried out with 35 cycles of denaturation at 94 °C for 45 s, annealing at 54 °C for 1 min, and extension at 72 °C for 1 min 30 s. The initial denaturation and final extension temperature were 94 °C for 5 min and 72 °C for 10 min, respectively. PCR products were analyzed on 1.5% agarose gel and purified using a gel extraction kit (Qiagen, Hilden, Germany).

The amplified products of the complete ORF (1188 bp) and functional domain (520 bp) were cloned into a sequencing vector, Zero Blunt vector (Invitrogen, Carlsbad, CA, U.S.A) following manufacturer's protocol and sequenced. For polymorphism analysis the insert from 20 clones from each area were sequenced. For this the recombinant clones were picked up randomly and plasmids were purified using the miniprep kit (Nucleospin, ML, Duren, Germany) and sequenced as stated below.

2.4. Sequencing

Sequencing of the amplified fragments and plasmids was performed on an ABI automated Genetic analyzer 3130XL (ABI-Applied Biosystems, CA, USA) by using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit version 3.1 (Applied Biosystems, CA, USA). The clone carrying complete ORF of FtsZ was sequenced in both the directions using M13 forward and reverse primers. Randomly selected clones from different geographical areas were also sequenced in both the directions.

2.5. Sequence alignment

Multiple sequence alignment of 120 nucleotide sequences of *Wolbachia* FtsZ gene from *W. bancrofti* parasites from different geographic regions of India was performed using CLUSTALW (Hall, 1999). Amino acid sequences were derived using BioEdit version 7.0 software and aligned with β-tubulin of *B. malayi* (Gen Bank accession number AAU12501.1) to identify the functional motifs.

Download English Version:

https://daneshyari.com/en/article/6127742

Download Persian Version:

https://daneshyari.com/article/6127742

Daneshyari.com