Available online at www.sciencedirect.com

ScienceDirect

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

Contemporary Issue

Wolbachia: The selfish Trojan Horse in dengue control

Lt Col M.S. Mustafa [a,*], *Brig V. Rastogi* [b], *Col R.K. Gupta* [c], *Col S. Jain* [d], *Lt Col P.M.P. Singh* [e], *Lt Col A. Gupta* [f]

[a] *Officer Commanding, Station Health Organisation (Large), Allahabad 908406, India*
[b] *Brig IC Adm & Cdr Tps, Command Hospital (Southern Command), Pune 411040, India*
[c] *Director, AFMS (Health), O/o DGAFMS, New Delhi, India*
[d] *ADH & Senior Adviser (PSM), HQ 11 Corps, C/O 56 APO, India*
[e] *Officer Commanding, Station Health Organisation (Large), Bengaluru, India*
[f] *Officer Commanding, Station Health Organisation (Large), Dehradun, India*

ARTICLE INFO

Article history:
Received 26 September 2014
Accepted 3 July 2015
Available online 31 August 2015

Keywords:
Dengue fever
Wolbachia
Aedes
Strain
Biological control

ABSTRACT

Dengue fever has re-emerged as a major public health challenge. Of late, several promising attempts have been made to control the disease with limited success. An innovative method of biological control of dengue is the use of the bacterium *Wolbachia*. Selected strains of *Wolbachia* have been introduced into *Aedes aegypti* to prevent transmission of dengue viruses by the vector. *Wolbachia* prevents dengue transmission by either directly blocking the virus or by decreasing the lifespan of the vector. The mechanism by which it causes these effects is not clearly understood. The main concern of this technique is the emergence of a new dengue virus serotype which may evade the protection offered by *Wolbachia*. The technique is environment friendly and holds promise for control of other vector borne diseases.

Introduction

Dengue fever has re-emerged as a major public health challenge worldwide; with 2.5 billion people at risk of infection, more than 100 million cases and 25,000 deaths being reported annually.[1] As there is no licensed vaccine or specific treatment against dengue, preventive measures are the best strategy, which consist mainly of environmental management, chemical control, and personal protective measures. However, such measures have met with limited success due to poor/improper implementation.

Besides, as the *Aedes* mosquito is a day biter, individual protection using bednets is easier to preach than bringing into practice, thus accentuating the need for alternate options in dengue control.

Recently, there have been several promising new attempts to control dengue. However, the much awaited vaccine trial in

* *Corresponding author*. Tel.: +91 9410430079.
E-mail address: msmustafa2001@yahoo.co.in (M.S. Mustafa).
http://dx.doi.org/10.1016/j.mjafi.2015.07.002

Thailand did not meet the expectations; providing only 30% overall effectiveness, though it did provide higher coverage for three of the four dengue virus serotypes.[2]

Biological methods have been used recently with some success. An innovative promising method of biological control of dengue is the use of the bacterium *Wolbachia*.

The novel technique

The new technique is based on the premise that the symbiotic bacterium *Wolbachia* easily infects a wide range of invertebrate hosts including crabs, mites, insects and filarial nematodes. The infection is so common that almost 75% of all arthropod species are infected. Meta-analysis of earlier studies based on a variety of sampling techniques has revealed that out of 3500 mosquito species, 85 have been screened for *Wolbachia*. Of these 85 species, 31.4% have been found to be infected.[3] *Wolbachia* does not infect *Aedes aegypti* in the native form, though the bacterium infects other species of mosquitoes such as *Culex pipiens* and *Aedes albopictus* naturally.

In this technique, selected strains of *Wolbachia pipientis* have been introduced into *A. aegypti* to prevent transmission of dengue viruses by the vector. These strains were initially detected in a laboratory population of the common fruitfly, *Drosophila melanogaster*; wherein it protected the vector from certain RNA viruses.[4] Since the dengue virus also has an RNA genome, the possibility of using *Wolbachia* for protection of *Aedes* mosquitoes from infection with dengue viruses has been explored. The infection is passed vertically from the female mosquito to the offspring transovarially. *Wolbachia* thus introduced are capable of spreading rapidly in wild *A. aegypti* populations.

Why *Wolbachia*?

As a symbiont, *Wolbachia* has several useful effects; thereby making it an ideal candidate for dengue control. The bacterium increases the vigour of the female *Aedes* mosquito thereby enabling the infection to spread to virtual fixation. This is the reason why *Wolbachia* is often referred to as the selfish bacterium. Besides, as *Wolbachia* infects a wide variety of hosts, one strain of the bacterium isolated from a particular species may be introduced into another species. In addition, *Wolbachia* produces a range of effects that can be exploited for disease control.

Mechanism of action

Meta-analysis of studies conducted earlier[4–6] have shown that *Wolbachia* negates the capability of the vector to transmit disease through biological action by either directly blocking transmission of the virus or by decreasing the life span of the vector; thereby nipping the viral infection prematurely. As *Wolbachia* cannot be cultured in vitro, the mechanism by which it causes these effects is poorly understood.

A. albopictus inherits *Wolbachia* infection maternally. In male *A. albopictus* mosquitoes naturally infected with *Wolbachia*, the bacterium modifies the sperm, thereby resulting in failure of karyogamy after fertilisation, leading to death of the embryo. If the *Wolbachia* infected male and infected female mate together, *Wolbachia* retrieves this modification, resulting in normal growth and development of the embryo. Hence, mating between *Wolbachia* infected males and uninfected females will be incompatible and the eggs would not be able to hatch. However, reciprocal mating is compatible, as *Wolbachia* infected female *Aedes* mosquitoes can mate successfully with both *Wolbachia* infected and uninfected males, with the result that all the offspring will have *Wolbachia*. This mechanism of unidirectional cytoplasmic incompatibility[7] provides a disproportionate mating advantage to *Wolbachia* infected females vis-A -vis the uninfected females, thereby promoting maternally inherited *Wolbachia* infection into virgin host populations.

When *A. aegypti* mosquitoes infected with the *wMel* strain of *Wolbachia* were fed with dengue virus contaminated blood along with non-infected mosquitoes, and the degree of dengue infection was analysed after a period of two weeks by quantifying the amount of dengue viral nucleic acid in both groups of mosquitoes. It was observed that the dengue viral nucleic acid in *wMel* – infected mosquitoes was 1500 fold less than in the *Wolbachia* – uninfected mosquitoes; thereby affording protection to the *wMel* – infected mosquitoes against subsequent infection by dengue virus.[8]

Similarly, the mosquito saliva was also analysed for the amount of dengue infection. It was found that dengue was present in only 4.2% of saliva samples taken from the *wMel* – infected mosquitoes vis-A -vis 80.2% of saliva samples taken from non-infected mosquitoes.[8]

The modality through which *wMel* stops the virus from replicating is not very clear. However, there is substantive evidence which suggests that the *Wolbachia* competes with the dengue virus for the limited sub-cellular fatty acid resources required for viral replication.[9]

The blocking achieved by *Wolbachia* is not absolute. *Wolbachia* naturally infects *A. albopictus*, which is a vector for arboviruses including dengue and chikungunya virus. Studies of virus dynamics in *A. albopictus* have shown a decline in *Wolbachia* density as the viral life cycle enters the transmission stage,[10] suggesting the reversal of interference caused by *Wolbachia* with the passage of time.

Protection afforded by *Wolbachia* against the dengue virus depends on the magnitude of *Wolbachia* infection in the mosquito. For example, the *w*AlbB strain of *Wolbachia* does not provide any protection to *A. albopictus* against dengue. However, when *A. aegypti* is infected with the same *w*AlbB strain, it prevents dengue viral infection in *A. aegypti*. The reason for the same is *w*AlbB is capable of surviving in greater numbers in *A. aegypti*, than in *A. albopictus*. This points to the fact that *Wolbachia* infection must reach a minimum critical level before dengue infection can be prevented.

The *wMelPop-CLA* strain of *Wolbachia* shortens the life of the *A. aegypti* mosquitoes. By infecting female mosquitoes with the *Wolbachia* strain *wMelPop-CLA* their lifespan could be reduced to half, thereby eliminating the infected mosquitoes before the virus could reach maturity.

Life-shortening effects of *wMelpop-CLA* were investigated by keeping *wMelpop* infected and uninfected pupae in a small plastic cups containing 170 ml water inside a plastic container with mesh sides. The longevity of the infected mosquitoes thus hatched from the pupae was then compared with the uninfected controls. It was observed that the controls died much slower than their infected counterparts; the average decrease in longevity in the infected group being 43–50%.[11] This life-shortening effect *wMelPop-CLA* is not affected by environmental factors such as low nutrition levels and high densities.

The life shortening approach has the disadvantage that as it also reduces the rate of reproduction of mosquitoes by half, it limits their ability to pass on the bacterium. In contrast, mosquitoes infected with the virus blocking *wMel* strain had lifespans, reproductive rates and offspring viability similar to that of uninfected controls.

Issues and concerns

Dengue viruses, being RNA viruses, have high mutation frequencies with mutation rates being more than 100 times greater than the mutation rates of DNA genomes. The accumulation of mutations coupled with the possibility of intramolecular recombination due to simultaneous infections with different dengue virus serotypes, could lead to the emergence of a novel dengue virus serotype[12] which may rapidly circumvent the protection offered by *Wolbachia*. A precedence has been set by *Drosophila* which acts as a vector for the sigma-virus, against which *Wolbachia* offers no protection.

Another concern is that the introduction of *Wolbachia* in *A. aegypti* may possibly lead to increased virulence of the dengue virus. Besides, as each strain of *Wolbachia* has a unique effect on the vector, it is difficult to predict how it will manipulate the native mosquito once it is released.

Wolbachia infection must reach a critical, minimum level in order to guard against dengue infection. The *Wolbachia* density needs to be maintained so that the same is not overcome by evolution of the dengue virus. As different strains of *Wolbachia* have varying ability to block the dengue virus, strains which have an effective and durable blocking effect on the dengue virus need to be identified.

Wolbachia is introduced into new hosts by microinjection; which in itself is a daunting technical task and requires expert supervision. Notwithstanding, microinjection being a physical delivery procedure, has more reliability and efficiency as compared to other manipulation techniques like endocytosis, electroporation, the use viral vectors or gene guns. Maternal microinjection is always preferred as the eggs are incubated inside the female until oviposition which may increase the survival rate. Improvised embryonic microinjection protocols such as dechorionation of eggs[13] during microinjection have been developed recently with higher survivability rates. Another positive note for the use of *Wolbachia* by microinjection is that of the three major vector mosquitoes, the embryos of the dengue vector *Aedes* can be microinjected more easily, followed by *Culex* embryos. *Anopheles* embryos are not easily amenable to microinjection, and have the least survival rates.

Latest progress

Field trials have been conducted in Australia, wherein Wolbachia infected *A. aegypti* mosquitoes were released across two locations over a period of ten weeks. One month after the release, more than 90% the native *A. aegypti* mosquitoes were found to be carrying *Wolbachia*, thereby corroborating the fact that *Wolbachia* could be transmitted easily in the wild.[14] Similar tests are being conducted in the dengue endemic hotbeds of Indonesia, Vietnam and Brazil; the final goal being the release of *Wolbachia* – infected *Aedes* mosquitoes in areas with a high incidence of dengue.

Conclusion

The technique is environment friendly and has the potential for wide area application at low cost. Being a biological method of vector control, it requires only an initial one-time effort, following which the symbiont remains in place with the vector mosquito. *Wolbachia* may be used in combination with the existing control approaches and could also augment the dengue vaccine once it is developed hopefully.

As *Wolbachia* also blocks other arboviruses such as yellow fever and chikungunya virus, it holds promise for control of these vector borne diseases in the near future.

The community has to be taken into confidence through active participation as the release of *Wolbachia* infected mosquitoes in newer habitats may lead to serious human concerns.

Conflicts of interest

The authors have none to declare.

REFERENCES

1. Mustafa MS, Bansal AS, Rastogi V. Flightless Aedes mosquitoes in dengue control. *MJAFI*. 2011;67:192–193.
2. Sabchareon A, Wallace D, Sirivichayakul C, Limkittikul K, Chanthavanich. Suvannadabba S. Protective efficacy of the recombinant, live-attenuated, CYD tetravalent dengue vaccine in Thai schoolchildren: a randomised, controlled phase 2b trial. *Lancet*. 2012;380:1559–1567.
3. Bourtzis K, Dobson SL, Xi Z, et al. Harnessing mosquito – Wolbachia symbiosis for vector and disease control. *Acta Trop*. 2014;132:S150–S163.
4. Min KT, Benzer S. Wolbachia, normally a symbiont of Drosophila, can be virulent, causing degeneration and early death. *Proc Natl Acad Sci U S A*. 1997;94:10792–10796.
5. Fleury F, Vavre F, Ris N, Fouillet P, Bouletreau M. Physiological cost induced by the maternally-transmitted endosymbiont Wolbachia in the Drosophila parasitoid *Leptopilina heterotoma*. *Parasitology*. 2000;121:493–500.
6. Reynolds KT, Thomson LJ, Hoffmann AA. The effects of host age, host nuclear background and temperature on phenotypic effects of the virulent Wolbachia strain popcorn in *Drosophila melanogaster*. *Genetics*. 2003;164: 1027–1034.

7. Blagrove MSC, Camilo A, Failloux AB, Sinkins SP. Wolbachia strain wMel induces cytoplasmic incompatibility and blocks dengue transmission in *Aedes albopictus*. *Proc Natl Acad Sci U S A*. 2012;109:255–260.
8. Walker T, Johnson PH, Moreira LA, et al. The wMel Wolbachia strain blocks dengue and invades caged *Aedes aegypti* populations. *Nature*. 2011;476:450–453.
9. Hoffmann AA, Montgomery BL, Popovici J, et al. Successful establishment of Wolbachia in Aedes populations to suppress dengue transmission. *Nature*. 2011;476:454–457.
10. Mousson L, Martin E, Zouache K. Wolbachia modulates Chikungunya replication in *Aedes albopictus*. *Mol Ecol*. 2010;19:1953–1964.
11. Yeap HL, Mee P, Walker T, et al. Dynamics of the "Popcorn" Wolbachia infection in outbred *Aedes aegypti* informs prospects for mosquito vector control. *Genetics*. 2011;187:583–595.
12. Monath TP. Dengue: the risk to developed and developing countries. *Proc Natl Acad Sci U S A*. 1994;91:2395–2400.
13. Sampath KS, Puttaraju HP. Improvised microinjection technique for mosquito vectors. *Indian J Med Res*. 2012;136:971–978.
14. Ritchie S. Rear and release: a new paradigm for dengue control. *Austral Entomol*. 2014;53:363–367.