Current Prevention and Potential Treatment Options for Dengue Infection

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ABSTRACT - Currently, treatments for dengue infection are only symptomatic as no antiviral agents nor vaccines are available to combat this virus. Despite challenges faced by researchers, many efforts are ongoing to reduce cases of dengue infection either by targeting the vector or the virus. Vector population is monitored and reduced by using mechanical, chemical and biological controls. Chemical control is achieved either by using synthetic or natural insecticides where the latter is more preferable. In biological control, bacteria, fungi and larvivorous fish are utilised to reduce the vector population. Moreover, genes of mosquitoes are also explored to produce progenies which are sterile with low survival ability. Vaccines are among the most effective ways to prevent viral infection. Various approaches have been used and are still being explored towards producing vaccines for dengue. These include live attenuated, inactivated, recombinant subunit, nucleic acid and virus-like particles vaccines. The aim is to produce a vaccine which can target all the four serotypes of the virus. Monoclonal antibodies are widely researched on to equip the host defense mechanism against the dengue virus. Deeper understanding of the virus replication cycle warrants the development of antiviral agents which target viral proteins vital for the replication process. Bioactive compounds are also utilised in the development of antiviral agents. The importance of surveillance and supportive therapy are also discussed.

INTRODUCTION

Dengue virus is a mosquito-borne infection which has become a health threat globally. According to 2009 World Health Organisation (WHO) case classifications, dengue infection is categorised by dengue and severe dengue (1). Severe dengue such as dengue haemorrhagic fever and dengue shock syndrome are responsible for high morbidity and mortality in dengue infections. It is predicted that dengue transmission is ubiquitous throughout the tropics with the highest incidences occurring in South America and Asia. Many of the dengue cases are not reported or classified. Bhatt et al. (2) estimated that 390 million dengue infections occur every year worldwide of which 96 million are with clinical manifestations. Asia contributed 70% of this burden, largely due to the dense population and high suitability for disease transmission. Dengue infection is caused by four serotype viruses namely DENV-1, DENV-2, DENV-3 and DENV-4. The most prevalent serotype is DENV-2 followed by DENV-1 (3, 4) and all serotypes are likely to be associated with dengue haemorrhagic fever (5). The primary vector of dengue virus is *Aedes aegypti* which prefers living indoors in tropical and subtropical regions. *Aedes albopictus* on the other hand is a secondary vector and commonly lives outdoors in the Southeast Asia regions (6).

Each year, large number of dengue cases are reported during monsoon seasons due to the high prevalence of vectors. The rise of dengue cases can be prevented by controlling vectors spread. A number of novel approaches have been employed to control mosquito populations. These include a technique of releasing insects carrying a dominant lethal gene (RIDL) (7), introduction of fungal biopesticides (8) and infecting mosquitoes with *Wolbachia pipientis* (9). Prevention also involves the development of a tetravalent vaccine which was used clinically since 2015 (10). Currently, there is no antiviral agent available to treat dengue and treatment options are only symptomatic. This article primarily discusses prevention alternatives and potential treatment options for dengue.

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PREVENTION OF DENGUE

Given an optimal temperature for vector breeding, and a lack of safe, effective dengue vaccine and urban transmission cycle of dengue virus (human-mosquito-human), dengue preventive measures are therefore emphasised especially in endemic areas such as Southeast Asia (11). A number of preventive strategies have been proposed particularly on reduction of the mosquito vector by using mechanical, chemical and biological approaches. These approaches are discussed in the following parts.

Vector Control

Partnership for Dengue Control (12) is an initiative aiming to promote innovative, integrated and synergistic interventions to achieve sustainable dengue prevention. Ross-Macdonald model outlined that interventions which reduce adult mosquito population density, daily probability of survival and mosquito contact with humans have a significant impact on decreasing virus transmission (13). Interventions offering best sustained controls include indoor and perifocal spraying with residual insecticides to kill adult mosquitoes (14). Water containers are treated with insecticides or biological agents to reduce mosquito larvae (15). On the contrary, highly visible intervention such as aerial and truck mounted ultra-low volume space-spraying has a low impact on mosquito population reduction and is not cost effective (16). Other interventions include the use of personal repellants and insecticides treated materials inside homes (bed nets, curtains and water jar covers) (17, 18). Social mobilisation campaigns (education and public relations) (19), environmental management (20) and legislation (incentives and enforcement) are considered as effective components of sustained mitigation programmes (21). Nevertheless, failure of these strategies has often been associated with the lack of local community involvement and inability to scale-up local, small scale success to mega-cities and large geographical areas (22).

There has been a considerable interest in developing new tools to suppress dengue vector populations as the existing tools are inadequate. New methods and strategies are being explored to reduce the overall mosquito population, manipulate female mosquito behavior and replace wild-type mosquitoes with strains/genotypes that do not transmit dengue virus (22).

Mechanical control

Modern mosquito nets are developed as insecticide-treated bed nets (ITNs) and long-lasting insecticide-nets (LLINs) where pyrethroids insecticides such as permethrin, deltamethrin and alpha-cypermethrin are incorporated into the fabric (Table 1). ITN can be retreated by soaking it in a mixture of insecticides and dried while LLIN can retain the insecticides even after frequent washing (30). A synergist such as piperonyl butoxide is also used in combination with pyrethroid insecticides to enhance their activities (31).

Another approach is the use of a mosquito trap which consists of a carbon dioxide producer and a vacuum fan. Carbon dioxide lures mosquitoes into the trap and they will be sucked by the vacuum fan (32). Besides carbon dioxide, ultraviolet-A in the range of 350-400 nm is also used as an attractant (33). Despite their effectiveness, these traps are expensive and require electricity.

Table1. List of insecticide treated mosquito net products

Product names	Active ingredients	Type of fabric
Duranet© LLIN	Alpha-cypermethrin	High density polyethylene (23)
Interceptor®	Alpha-cypermethrin	Polyester (24)
Interceptor® G2	Alpha-cypermethrin and chlorfenapyr	Polyester (24)
Olyset® Net	Permethrin	Polyethylene (25)
Olyset® Plus	2% Permethrin combined with 1% of the synergist piperonyl butoxide	Polyethylene (25)
PermaNet® 2.0	Deltamethrin	Polyester (26)
PermaNet® 3.0	Deltamethrin combined with the synergist piperonyl butoxide	Polyester or polyethylene (27)
Royal Sentry®	Alpha-cypermethrin	High density polyethylene (27)
Yorkool® LN	Deltamethrin	Polyester (29)

Therefore, their uses are limited to urban areas only. Nevertheless, Gravid *Aedes* Trap (Figure 1) serves as an alternative as it does not require electricity. It mimics an ovipositor of a female mosquito and mosquitoes are attracted to the trap by water or organic lure (34). Once trapped, they will either stick to the sticky surface or be killed by insecticides.

Furthermore, oil and polystyrene beads are also used to control the mosquito population. Oil film and polystyrene beads form a barrier on the water surface preventing mosquito larvae from breathing. This barrier also reduces oxygen concentration dissolved in the water and prevents female mosquitoes from laying eggs in the water (35, 36).

Chemical control

Insecticides are among the most effective ways to control mosquito population but majority of them have negative impacts on health and environment and some are no longer effective due to the emergence of insecticide-resistant mosquitoes (37). Two main strategies have been outlined to address these issues; development of insecticides which can selectively target a specific tissue of the insect (38) and development of new chemical classes of insecticides which are more effective and less harmful (39).

To address the first strategy, endotoxins produced by *Bacillus thuringiensis israelensis (Bti)* were used to kill *Aedes* mosquito larvae (40). Once ingested by the larvae, *Bti* toxins formed pores in larval cell membranes causing gradual cell death (41). However, the emergence of larvae resistant to the toxins renders it ineffective. Adult mosquitoes which survive the treatment might have higher vector competence and this is a matter of concern (42).

Sumimoto Chemical employed the second strategy by developing two products; SumiLarv 2MR (Sumitomo Chemical Australia Pty Ltd, Epping, Australia) and SumiPro EW (Sumitomo Chemical Asia Pte Ltd, Singapore) for dengue vector control (43). SumiLarv 2MR (Sumitomo Chemical Australia Pty Ltd, Epping, Australia) is a resin matrix release formulation containing an insect growth regulator pyriproxyfen. Pyriproxyfen is of low toxicity to humans and are allowed to be used in drinking water (44). This formulation is added into water storage containers (Figure 1) and is able to inhibit the growth of mosquito larvae for at least six months from treatment (43). SumiPro EW (Sumitomo Chemical Asia Pte Ltd, Singapore) on the other hand, is a formulation containing metofluthrin and cyphenothrin. Metofluthrin has a high knockdown effect whereas cyphenothrin has a lethal effect on mosquitoes (45). The formulation is used for spraying and fogging (43).

Moreover, the use of insecticides from plants has also been explored as plant materials are biodegradable and are more environmental friendly. Bioactive compounds in plants for instance terpinoids, saponins, monoterpenes, alkaloids, pyrethrins and antraquinones have shown ovicidal, larvicidal and insecticidal activities (Table 2). Azadiracthin terpinoid acts as an ecdysone agonist which interfere with the growth and development of *Aedes* embryos result in abnormal hatching (55).

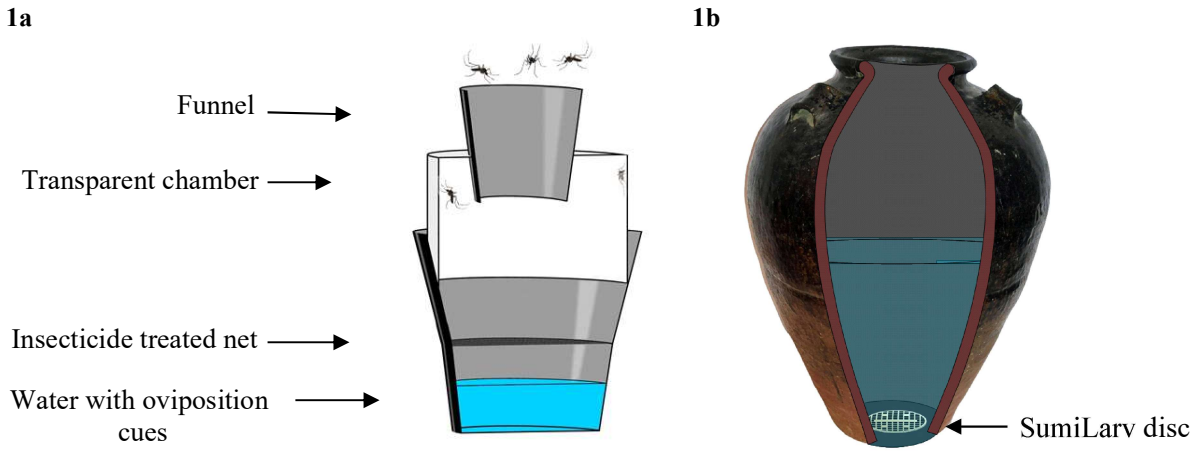

Figure 1. Gravid *Aedes* Trap (**1a**) which mimics an ovipositor of a female mosquito containing water or organic lure. SumiLarv®2MR disc containing an insect growth regulator pyriproxyfen in a water container (**1b**)

Table 2. Plants reported to have ovicidal, larvicidal and insecticidal activities against *Ae. aegypti*

Plant names	Parts used	Activities	Lethal concentrations
Annona crassiflora Mart.	Root	Larvicidal	LC_{50} value of 0.71 µg/mL (46)
A. glabra L.	Seed	Larvicidal	LC_{50} value of 0.06 µg/mL (46)
Cardiospermum halicacabum Linn.	Leaf	Ovicidal	LC_{50} values of 182.51, 200.02, 192.31, 156.80, 164.54 ppm (47)
Cinnamomum impressicostatum Kosterm	Leaf	Larvicidal	LC_{50} value of 13.7 µg/mL (48)
		Insecticidal	LC_{50} value of 167 µg/mL (49)
C. microphyllum Ridl.	Leaf	Larvicidal	LC50 value of 20.6 µg/mL (48)
		Insecticidal	LC50 value of 133 µg/mL (49)
C. pubescens Kochummen	Leaf	Larvicidal	LC_{50} value of 12.8 µg/mL (49)
		Insecticidal	LC_{50} value of 178 µg/mL (49)
Curcuma domestica Valeton	Rhizome	Larvicidal	LC_{50} value of 20.9 µg/mL (48)
Guettarda grazielae M.R.Barbosa	Stem	Larvicidal	LD_{50} value of 51.6 µg/mL (50)
Limonia acidissima (Linn.)	Leaf	Ovicidal	79.2% and 60% activities at 500 ppm (51)
Moringa oleifera Lam.	Seed	Ovicidal	EC_{50} values of 0.32, 0.16 and 0.1 mg/mL (52)
Rourea doniana Baker	Stem	Larvicidal	LD_{50} value of 12.1 µg/mL (50)
Rubia cordifolia L.	Root	Ovicidal	82.40% and 70.40% activities at 500 mg/L (53)
		Larvicidal	LC_{50} and LC_{90} values of 102.23, 350.20 mg/L (53)
Terminalia chebula Retz.	Leaf	Ovicidal	Zero hatchability at 200 and 250 ppm (54)
		Larvicidal	LC_{50} values of 87.13, 93.24 and 111.98 ppm (54)

Saponins extracted from *Balanites aegyptiaca* fruit mesocarps (56), *Quillaja saponaria* barks (57) and *Vitex trifolia* Linn leaves (58) are lethal to instar larvae of *Ae. aegypti* and are able to prevent the emergence of adult mosquitoes. Similar activities are displayed by *Cymbopogon Nardus* (L.) Rendle oil which is rich in myrcene monoterpene (59) and alizarin antraquinone isolated from *Rubia cordifilia* roots (60). Nicotine (61) and *stemona* (62) alkaloids possess acetylcholine modulatory activities while pyrethrins inhibit movements of Na^{2+} through voltage-gated sodium channel of the insect cell membrane (63). Even though these extracts show promising activities as ovicidal, larvicidal and insecticidal agents, their effects on other arthropods should also be investigated.

Furthermore, volatile oil extracted from *C. nardus* (L.) Rendle and *C. citratus* shows good repellent activity against *Ae. aegypti* (64). At present, insect repellant bracelets, patches, lotions, sprays and air fresheners containing these oils are used to repel mosquitoes. Eucalyptus and cinnamon oils are also incorporated into these products (65). Nanoemulsion (66) and encapsulation (67) technologies are used in these products to prolong the protection time.

Biological control

Biological control using bacterial infection such as *Wolbachia* results in the reduction of vector population. Male *Ae. aegypti* infected with the microbe are reproductively incompatible with wild-type females (68) leading to limited numbers or an absence of viable progenies. Infected females, on the other hand carry and transmit the bacteria to their progenies (69). Feeding behavior of infected females is also altered, thus significantly reducing their lifespan (70).

Predatory larvivorous fish have also been utilised to reduce the container index of *Aedes* mosquito larvae. Among the species used are *Gambusa affinis*, *Poecilia reticulate*, *Tilapia mossambica* and *Sarotherodon niloticus* (71). *G. affinis* is tolerant to insecticides making it ideal to be used together with chemical control methods (72). However, due to a broad range of diet, some other arthropod species might be affected too (73). Oviposition of *Aedes* mosquitoes usually occurs in indoor water containers thus efficacy of vector control by these predators is still questionable. Furthermore, introduction of exotic species can affect ecology and biodiversity of aquatic environment (74). Copepod species such as *Mesocyclops* and *Macrocyclops* which feed on first

instar larvae have also been used as a biological control for *Aedes* mosquitoes (75).

The use of mosquitocidal fungi has been explored where *Beauveria bassiana* and *Lagenidium giganteneum* were shown to reduce the survival rate, blood-feeding success and fecundity of *Ae. Aegypti* (76). Generally, fungal growth is temperature-dependent and high mortality of mosquito population is observed within a few days (77). Despite the promising results shown by these zoospores, their stability for large scale manufacturing requires further optimisation for commercial utilisation (78).

Genetic engineering

Other approaches to reduce the vector population include insect genetic engineering. Oxitec engineered a male *Ae. aegypti* strain which can cause conditional lethality (RIDL) where when transgenic males are released into the wild and mate with wild-type females, most of the offspring die in the larval stage (79). However, this approach only works best when the treatment area is small. In addition, regulatory approval is required before the release of transgenic males into the wild (80). Alternatively, genomic modification of mosquitoes which in turn produces an RNA transcript that halts dengue virus replication has been attempted. An RNAi regulated transmission-blocking DENV-2 strain has thus been developed and evaluated (81). This strategy minimises biosafety issues concerning the release of genetic modified organisms to the environment since less transgenic mosquitoes are released compared with the RIDL mosquitoes. In order to yield an optimal outcome, continuous release for about a year might be necessary (82). As the current genetic modified mosquito strain only inhibits transmission of DENV-2, more efforts are made, thereby, to develop strains which also inhibit the activities of the other three serotypes (22). Further, the anti-dengue gene can be potentially linked to a gene-driven survival mechanism. When it is circulating in the mosquito population, based on super-Mendelian inheritance theory, offspring without the anti-dengue gene will eventually die out (83). The recent emergence of CRISPR/Cas9 system exemplifies a more specific gene-directed genome editing in transgenic mosquitoes which in turn permits the building of self-perpetuating and sex-biased *Aedes* strains that eventually concentrate anti-dengue genes in the *Aedes* population (84).

VACCINE

Primary infection with one serotype gives a long-lasting immunity towards that particular serotype but not to other serotypes. Secondary infection with other serotypes increases the risk of dengue haemorrhagic fever and dengue shock syndrome through the antibody dependent enhancement reaction (85). To overcome this phenomenon, research on dengue vaccines emphasises on the development of tetravalent vaccine which gives adequate protection against all four serotypes (86). The main goal of immunisation is to induce a sustained neutralising antibody response where these antibodies are directed against the virus envelope (E) protein, preventing attachment and fusion of viruses with cells (87).

Blaney et al. (88) demonstrated that a live, attenuated tetravalent dengue vaccine developed using reverse genetic engineering was able to confer protection against all four dengue serotypes. However, lack of suitable animal models for virus challenge study has imposed some technical difficulties for validating the vaccine efficacy (89). Infection of human dengue virus isolates in normal mice does not produce significant viraemia. Intracerebral challenge and immunocompromised mouse models have therefore been used to study the protective efficacy of vaccine candidates. Although many studies using immunocompromised mouse models have shown encouraging results, whether the data are able to represent elicitation of immune responses by the vaccine candidates in human recipients still remain debatable (90). Non-human primates were proposed for dengue virus infection and challenge study in dengue vaccine development. Infected/challenged non-human primates succumbed to viraemia, however, failed to show significant clinical signs of infection. This renders hardship in drawing conclusive observation on the protective efficacy of dengue vaccines (91). Of note, extensive research is required in search of suitable animal models for validating the efficacy of dengue vaccines.

Live attenuated vaccine

Several principles which are important and need to be adhered to in the development of live attenuated vaccines include: the live, attenuated virus should be efficient in inducing immune responses and mimics infection of wild-type virus; vaccination with live, attenuated virus should also not lead to significant illnesses. Since live, attenuated virus is able to

survive for an extremely short replicative cycles (92), the presence of relatively low virus particles (10^1 to 10^2 infectious unit/mL of blood) is considered acceptable in live attenuated dengue virus vaccination (93). The low virus titre produced by vaccination should be insufficient for transmission by the mosquito vector. Given the tetravalency of the dengue vaccine, live attenuated dengue serotypes in the dengue vaccine formulation is expected to elicit equal neutralising antibody responses. In case of imbalanced immunity triggered by the tetravalent dengue vaccine, a pathological phenomenon similar to that observed in antibody dependent enhancement might occur in recipients, thereby painstaking and meticulous empirical investigation is required prior to the release of this vaccine. Furthermore, the genetic basis of the four serotypes should be clearly defined so that gene stability can be monitored in all phases of manufacturing and human use (94). This is especially important to nullify possibility of viral reversion to a more virulent phenotype.

Virus serotypes used in dengue vaccine formulation are prepared through serial passaging in primary dog kidney cells with terminal passages in foetal rhesus lung cells (95). By passaging dengue viruses in heterologous host cells, under-attenuated DENV-1 and over-attenuated DENV-4 components are produced (96). Such attenuation compromises immunogenicity of dengue vaccine. In a separate strategy, reverse genetic technology was employed to obtain a desirable balance between the levels of attenuation and immunogenicity of the four serotypes (97). The balance was achieved only for DENV-1 and DENV-4; therefore an alternative chimeric strategy had been used for DENV-2 and DENV-3. A tetravalent vaccine formulated from these serotypes has shown a desirable level of attenuation (peak titres $<10^2$ pfu/mL), broad immunogenicity and protection in rhesus monkeys (98). Phase II clinical trials of the vaccine formulation in Thai schoolchildren (n=4002) showed high efficacy towards DENV-1, -3 and -4 and not DENV-2 which in the region, DENV-2 is the most prevalent serotype (99). After the completion of phase III trial in 2014, Sanofi Pasteur was granted a marketing authorisation for Dengvaxia, the first dengue vaccine to be licensed (10). Surveillance on populations immunised with the vaccine was carried out from time to time and in 2016 WHO cautioned the risk involved with the use of Dengvaxia (100). In November 2017, Sanofi announced that Dengvaxia could worsen the disease outcomes in some patients (101) especially among seronegative children (102). This was followed by the suspension of sale and distribution of the vaccine by the United States Food and Drug Administration (103).

Inactivated vaccine

Inactivated vaccines are, on the other hand, prepared by propagating viruses in Vero cells, concentrated by ultrafiltration and purified on sucrose gradients. The virus titre (approximately 10^9 pfu/mL) is then inactivated with formalin. They contain dengue virus structural proteins and viral RNA which permit the induction of immune responses. The inactivated vaccines are, sometimes more desirable over other alternatives because they are safer. They have low potential for reactogenicity and therefore are more suitable for immunocompromised patients. More importantly, they are not able to revert to a more pathogenic phenotype. They are also expected to induce balanced antibody responses as the four serotypes are equally formulated. Adjuvants and multiple booster doses are commonly recommended in order to increase the vaccine efficacy and hence long-term immunity (104). These additional requirements add to the manufacturing cost and adjuvant-related biosafety issues (105).

Another approach to producing an inactivated vaccine is by using psoralens. Psoralens are photoreactive compounds that cause intercalation of pyrimidine residues when exposed to ultraviolet-A radiation and eventually leads to DNA cross-linking and viral inactivation. Immunogenicity of viral surface epitopes remain intact through this mechanism of inactivation. This vaccine was reported to elicit immunogenic responses to DENV-1 in *Aotus nancymaae* monkeys (106).

Recombinant subunit vaccine

Recombinant subunit vaccine candidates are primarily generated from dengue E antigens. It is believed that these vaccines are safer but unfortunately they share the same challenges as inactivated vaccines. Drawbacks of recombinant subunit vaccines include relatively low yield and improper folding of the antigen subunits (107). These limitations have been resolved using various heterologous expression systems such as of *Drosophila* S2 expression system (108). Gene encoding c-terminally truncated E antigen had been cloned and highly expressed by *Drosophila* S2 cells (109). The recombinant, truncated E antigen was structurally similar to the native E antigen and

therefore highly immunogenic and able to protect both mouse and non-human primate models against viral challenge (120).

Nucleic acid vaccine

In nucleic acid vaccine, antigens are expressed from DNA constructs and introduced directly into recipients. Once in host cells, antigen-coding genes are translated into dengue viral proteins that assemble and form subviral particles (111). They are easier to produce, more stable and are readily manipulated at room temperature. In addition, application of DNA vaccines decreases the likelihood of replication interference and permits vaccination against multiple pathogens concomitantly with a single vaccination compared with conventional vaccines. However, inadequate cellular uptake and expression (112) and the need for multiple dosing and adjuvants and a specialised injection equipment are among the challenges faced by DNA vaccination (94).

Inovio Pharmaceuticals developed a tetravalent DNA vaccine candidate consisting of a DNA plasmid vector expressing envelope domain III (EDIII) of all four dengue serotypes separated by proteolytic cleavage sites (113). Another tetravalent vaccine candidate comprising of a mixture of four plasmid vectors with each expressing prM and E proteins (prM/E) of a dengue virus serotype was developed by the US Centres for Disease Control and Prevention and also by a research laboratory at Kobe University (114, 115). Both vaccine candidates demonstrated reasonably high immunogenicity in mice and non-human primates models (113. 116, 117).

Virus-like particle vaccine

Virus-like particle (VLP) vaccines are commonly made up of viral structural proteins which contain genetic materials and allow presentation of antigenic epitopes to the recipient's immune response (118). Various methods have been employed to generate dengue VLP vaccine candidates. Cytos Biotechnology utilises chemical coupling of recombinant dengue virus EDIII domain to VLP carriers derived from bacteriophage Qβ in which Qβ VLPs are produced economically in *E. coli* (119). Bist et al. (120) developed dengue VLP vaccines by fusing the first 395 amino acids of DENV-2 E protein with Hepatitis B virus surface (HBs) antigens. The fusion protein is expressed in *Pichia pastoris*. Although the E protein is partially truncated, its fusion with HBsAg allows the display of DENV-2 E protein on the surface HBs VLPs. Serological analysis has revealed that Den2E-HBs VLPs are highly immunogenic and able to promote antibody responses against both DENV-2 E and HBs antigens (120). It is noteworthy that such chimeric VLP vaccines are remarkably useful in eliciting immune responses against different infectious agents with only a single vaccine.

MONOCLONAL ANTIBODIES

Functioning as passive immunisation agents, monoclonal antibodies are effective in preventing viral entry into host cells and currently are at various stages of clinical development against dengue virus (121). Studies on antibodies isolated from dengue infected patients have given useful insights on epitopes involved in virus neutralization (122). Among the identified epitopes include a linear epitope located on the domain III of dengue virus E protein and a quaternary E protein dimer epitope. Antibodies bound to these epitopes inhibit conformational changes essentially required for fusion of viral envelope with the endosomal membrane. A monoclonal antibody candidate, namely Ab513 is a promising candidate engineered to bind to linear epitopes (123). Unintentional pathological outcomes such as antibody dependent enhancement should be taken into serious consideration in the case of using dengue virus-specific monoclonal antibody in dengue treatment (124).

ANTIVIRAL AGENTS

Apart from being subjects for antiviral vaccines, viral structural and non-structural proteins are also primary targets for antiviral agents. Replicative components such as NS3 protease and NS5 polymerase are the most focused dengue virus proteins in the development of antiviral agents for dengue (125). By far, none of dengue antiviral agents has entered clinical trials while many are still in the development stages (Table 3). Currently, apart from developing small molecules as potential drugs, research on repurposing current drugs is also being carried out (133).

Table 3. Potential antiviral agents against dengue

Mechanism of actions	Compounds	Serotypes	Lethal concentrations
Inhibition of viral entry	MLH40	DENV-1 to 4	IC_{50} values of 24-31 μM (126)
	ST-148	DENV-2	EC_{50} value of 0.016 μM (127)
	1662G07	DENV-1, 2 & 4	
Inhibition of RdRp enzyme	Sofosbuvir	DENV-2	IC_{50} values of 14.7 ± 2.5 μM (128)
Inhibition of protease enzyme	MB21	DENV-1 to 4	IC_{50} value of 5.95 μM (129)
	SK-12	DENV-1 to 4	EC_{50} values of 0.74-4.92 μM (130)
	ARDP0006	DENV-2	Reduction of viral titer by more than 1 log PFU/mL at 1 μM (131)
Inhibition of helicase enzyme	ST-610	DENV-2	EC_{50} value of 0.272 μM (132)

RNAi

RNA-mediated interference (RNAi) has emerged as a powerful technology especially in limiting viral infections in hosts (134). An RNAi with a length of about 21-23 nucleotides combines with nucleases to form an RNA interference silencing complex which in turn recognises and knocks out target mRNA. The mRNA knockout then interrupts protein translation in cells (135). The same interfering mechanism can be applied to RNAi-mediated antiviral strategy in combating virus diseases. Viral genes responsible for uncoating and replication are the main targets of RNAi-mediated inhibition (136). When treated with RNAi targeting dengue virus envelope proteins, viral entry into host cells is inhibited causing low viral load (137). In order to further inhibit virus replication, RNAi-mediated gene silencing targeting NS4B and NS5 has been carried out (138). Given the high specificity and inhibitory efficacy of RNAi, this technology is a prominent approach in future antiviral development.

Bioactive compounds

Natural products research has been deemed an important avenue in seeking effective antiviral agents as many natural products possess highly active antiviral activities especially in human and animal viral infections (Table 4). Cyclohexenyl chalcone derivatives and panduratin isolated from *Boesenbergia rotunda* display inhibitory activities towards DENV-2 protease (139). Other plant compounds with prominent virucidal and/or inhibitory effects on dengue virus have been extracted from *Momordica charantia* (147), *Andrographis paniculata* (148) and *Azidarachta indica* (149). However, mechanism of action on how these plants extracts inhibit dengue replication vary from one extract to another, and many of them are still not fully explored. Briefly, bioactive compounds in natural products provide a broad range of potential therapeutic applications in antiviral therapy and might be useful in inhibiting different stages of the virus replication cycle.

SURVEILLANCE

Good, perpetual surveillance strategy is essential for effective prevention and control of dengue infections especially in dengue endemic areas. Previously, dengue surveillance scheme for mosquito vector control concentrated on immature forms of the vector such as larvae and pupae (150). This method, however, failed to suppress adult mosquito population and thus the dengue risk remained high (151). Recently, emphasis has been put on measuring adult mosquito populations instead, by using aspirators (152), Biogent Sentinel traps (153) and gravid traps (154). Measurement of adult mosquito population gives a better assessment of the impact of interventions on the risk of dengue infections (150). This is mostly because adult mosquitoes are responsible for urban cycle of dengue transmission. Moreover, the captured mosquitoes can be utilised as test subjects in biological control plans such as infecting them with *Wolbachia* (154). DengueNet, a global epidemiological and virological surveillance system was developed by WHO in 2002 in an effort to establish an updated database for timely dengue control measures and epidemiological research.

Table 4. Plants reported to have anti-dengue activities

Mechanism of actions	Plant names	Parts used	Serotypes	Phytochemicals	Lethal concentrations
Inhibition of DENV protease	*Boesenbergia rotunda* (L.) Mansf.	Root	DENV-2	4-Hydroxypanduratin A	Ki value of 21 μM (139)
				Panduratin A	Ki value of 25 μM (139)
	Byrsonima coccolobifolia Kunth	Leaf	DENV-2, 3	Agathisflavone	$IC_{50,}$ values of 15.1 ± 2.2 μM (DENV-2) (140) $IC_{50,}$ values of 17.5 ± 1.4 μM (DENV-3) (140)
Inhibition of viral replication	*Acorus calamus* L.	Root	DENV-2	Tatanan A	EC_{50} value of 3.9 μM (141)
	Acorus calamus var. angustatus Besser	Rhizome	DENV-2	Diasarone-I	EC_{50} value of 4.5 μM (142)
	Tripterygium wilfordii Hook. f.	Root	DENV-1-4	Celastrol	$EC_{50,}$ values of 0.19 ± 0.09 μM (DENV-1) (143) $EC_{50,}$ values of 0.12 ± 0.11 μM (DENV-2) (143) $EC_{50,}$ values of 0.16 ± 0.14 μM (DENV-3) (143) $EC_{50,}$ values of 0.17 ± 0.08 μM (DENV-4) (143)
	Mammea americana L.	Seed	DENV-2	Coumarin A	50% of viral replication at 9.6 μg/mL (144)
				Coumarin B	50% of viral replication at 2.6 μg/mL (144)
	Garcinia mangostana Linn	Pericarp	DENV-1-4	α-Mangostin	Reduction of infection rate by 47-55% at 20μM (145)
Inhibition of proprotein convertase furin	*Viola yedoensis* Makino		DENV-1-4	Luteolin	Ki value of 58.6 μM (146)

SUPPORTIVE THERAPY

Generally, bed rest and hydration are recommended for patients with mild dengue. In severely affected dengue patients, due to lack of a specific anti-dengue therapy, dengue treatment relies solely on a supportive therapy which attempts to minimise pro-inflammatory responses induced by dengue infections. Analgesics and antipyretics such as paracetamol and acetaminophen are usually given to ease dengue symptoms. Nonsteroidal anti-inflammatory drugs, on the other hand, should be avoided due to the risk of gastrointestinal bleeding and intramuscular haematoma (155). WHO recommends the administration of intravenous crystalloids and colloid solutions for patients experiencing dengue shock syndrome. Crystalloid and colloid solutions contain electrolytes and proteins and polysaccharides, respectively.

Basically, crystalloid solutions are used to treat patients with dengue shock whereas colloids are reserved for patients with profound or refractory shock. Between these two types of solutions, one of the major drawbacks of crystalloid solutions is their limited ability to remain within the plasma. Ringer's lactate is administered along with the solution to retain approximately 20% of the solution in the plasma. In colloid solutions, large insoluble organic contents help to maintain high osmotic pressure in the blood allowing colloid solutions to remain longer in the intravascular space. As a result, less volume is required for the same effect in crystalloid solutions. Furthermore, colloids stand as a good alternative when administration of crystalloids do not improve patient's dengue shock syndrome (156).

CONCLUSION

The quest to find better options for dengue virus vector prevention and treatment is ongoing though many challenges lie ahead. Vector control is once again paramount after the suspension and withdrawal of Dengvaxia from the market. Eradication of oviposition, elimination of immature and mature larvae and adult mosquitoes and protection from mosquito bites by using barriers and repellants are among the methods being used for vector control. Economic condition, awareness on the importance of vector control and the severity of dengue infections in endemic countries, are factors that influence the effectiveness of vector control programmes. Moreover, financial support, improvement in the public health infrastructure, partnerships programmes with non-governmental organisations and active community participations are all important in the successful implementation of vector control programmes. Safety issues concerning Dengvaxia pose new challenges in the development of safer vaccines for all four dengue serotypes. Nevertheless, there are a large number of diverse vaccine candidates in the pipeline which are at various stages of development and clinical trials, which may be approved for clinical use in near future. Efforts to develop antiviral agents utilising synthetic and phytochemical compounds are still ongoing. RNAi is another promising approach in the development of antiviral agents. Supportive therapy remains vital in the absence of antiviral agents.

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