Role of Doxycycline in the Management of Dengue Fever

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ABSTRACT

Dengue is the fastest growing mosquito-borne viral infection whose global incidence has drastically risen in the last few years. Currently, there is no direct antiviral therapy available against the dengue viruses; most of the management is aimed at maintaining adequate intravascular volume. Doxycycline, an antibiotic derived from tetracycline with broad antimicrobial and anti-inflammatory activities has been shown to possess antiviral properties. The current article reviews the role of doxycycline in the inhibition of dengue virus and the effect of doxycycline on clinical outcome in the treatment of dengue fever. The anti-dengue activity and anti-inflammatory activity of doxycycline may prove to be helpful in reducing the severity of clinical symptoms such as dengue fever, severe dengue hemorrhagic fever and dengue shock syndrome.

Keywords: Doxycycline, dengue fever, antiviral, anti-inflammatory, cytokines, receptor antagonists

Dengue fever is a rising endemic in many parts of the world. As per World Health Organization (WHO) estimates, there are about 390 million cases of dengue fever worldwide, out of which almost 96 million cases are in need of medical treatment.[1] In spite of several preventive measures taken by the WHO to control the spread of dengue virus (DENV) infection, new outbreaks have been reported in several parts of the world during post monsoon season. A recent map-based study by the University of Oxford estimated that India has the largest number of dengue cases, with about 33 million symptomatic and another 100 million asymptomatic infections occurring every year.[2] In view of this, the DENVs are considered to be important arthropod-borne viruses from a medical and public health perspective.

DENGUE FEVER AND ASSOCIATED COMPLICATIONS

Dengue virus is a member of the family *Flaviviridae*, genus *Flavivirus*. It is an acute infectious disease characterized by biphasic fever, headache, pain in various parts of the body, prostration, rash, lymphadenopathy and leukopenia. Severe or more complicated forms of dengue are associated with a severe febrile illness characterized by abnormalities of hemostasis and increased vascular permeability, which in some instances leads to a hypovolemic shock. Four distinct serotypes of dengue virus including DENV-1, DENV-2, DENV-3 and DENV-4 exist with numerous virus strains found across the world.[3,4]

CURRENT MANAGEMENT OF SEVERE DENGUE INFECTION

The optimal approach towards dengue control and management is prevention of the viral infection. Approach for the prevention of DENV infection and disease in endemic areas includes mosquito control, personal protective measures and vaccination.[5] Since infection with one DENV type provides long-term protection against reinfection with that same type, dengue vaccine is a feasible option. Following infection with one type, there is short lived immunity and cross protection against disease caused but the other three DENV types as well.[6] A number of dengue vaccine candidates are currently under development while one vaccine,[7,8] CYD-TDV (Dengvaxia) has been licensed for use in a few endemic countries in Latin America and Southeast Asia.[9]

There is no direct antiviral therapy available against the DENVs. Management is supportive primarily consisting of maintaining adequate intravascular volume.[5]

Most of the current strategies for anti-dengue drug discovery are targeted towards finding potent inhibitors against virus entry and its serine protease activity, which is crucial for viral replication.

DOXYCYCLINE AND ITS CURRENT CLINICAL USE

Doxycycline is a tetracycline derivative that has broad antimicrobial and anti-inflammatory activity.

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Doxycycline has been shown to possess antiviral activities and is used efficiently to treat tick-borne infections that transmit bacterial, protozoal and viral infections to humans.[10] Its antiviral activity has been reported against herpes simplex virus,[11] retrovirus[12] and DENV.[13] It inhibits DENV replication with effective concentrations (EC_{50}) value estimated to be 55.6 μM.[14]

ROLE OF DOXYCYCLINE IN INHIBITION OF REPLICATION OF DENGUE VIRUS

Inhibitory Effect Against Dengue Virus

Computational docking studies have shown that doxycycline inhibits DENV plaque formation by disrupting the conformational changes in the viral envelope that are necessary for virus entry.[14]

In a study, it has shown that doxycycline exhibits the highest inhibitory effect against dengue NS2B-NS3pro as compared to other tetracycline derivatives and mefenamic acid. Virus replication was observed to be significantly reduced in DENV-infected cells after applying doxycycline at 50% EC_{50}, which were considerably less than 50% cytotoxic concentrations (CC_{50}) values.[15]

DENV possesses positive single-stranded RNA that is translated to polyprotein by the host cells' ribosome. Virus polyprotein is cleaved by viral NS2B-NS3 serine protease and cellular protease to 10 structural and nonstructural proteins.[16] Disruption of viral NS2B-NS3pro would lead to inhibition of viral replication in host cells.[17] Doxycycline has negatively charged moieties and hydrophobic groups and was found to noncompetitively inhibit dengue NS2B-NS3pro with K_i values of 55.6 μM. It is also known that the binding between the dengue protease subunits depends on the interaction between negatively charged amino acids in NS2B and positively charged amino acids in NS3.[18] Hence, doxycycline may inhibit the activity of dengue protease by disturbing the binding between enzyme subunits that lead to significant reduction in its activity.[15]

The value of selectivity index of doxycycline has been shown to be lower than the selectivity index of ribavirin, a nucleoside analog that has shown the inhibition of the DENV methyltransferase.[19] The low side effects associated with doxycycline along with its positive effect on the cytokine levels in patients with dengue infection[20] make it a potential candidate for the treatment of dengue fever.

In an another study where anti-dengue properties of doxycycline were determined against four DENV serotypes *in vitro*, it was demonstrated that doxycycline interfered with DENV protease and impaired virus binding to the host cells, leading to reduced viral replication in infected cells.[21]

Modulation of Cytokine and Cytokine Receptor/Antagonist

Elevated levels of cytokines are considered to be a hallmark of various bacterial and viral infections including dengue.[22-24] Pro-inflammatory cytokines such as interleukin (IL)-6, IL1-β and tumor necrosis factor (TNF) are thought to be the primary factors leading to the majority of symptoms such as fever, malaise and coagulopathies associated with infections. The imbalance between cytokines and their anti-inflammatory counterparts serve as the primary prognostic indicator of disease outcome.[25-27] Agents that can downregulate the levels of cytokines have come up as potential therapeutic agents.[28] Tetracycline administration in patients with tick-borne encephalitis has shown a marked positive shift in the ratio of cytokines to their respective soluble receptors.[29]

A study conducted to investigate the effectiveness of tetracycline and doxycycline to modulate the levels of cytokines and soluble receptor/receptor antagonists in patients with dengue fever or dengue hemorrhagic fever showed that both tetracycline and doxycycline can modulate pro-inflammatory cytokine levels. Similar effect was observed for IL-1RA but not for TNF-R1. The compounds showed rapid downregulation within 3 days of treatment and continuing through Day 7.[20]

In the case of dengue hemorrhagic fever, doxycycline was found to be superior over tetracycline in modulating TNF-R1 concentration. A direct comparison between the two also showed that doxycycline was a much better immunomodulator as compared to tetracycline.[20]

EFFECT OF DOXYCYCLINE ON CLINICAL OUTCOME TREATMENT OF DENGUE FEVER

The anti-dengue activity of doxycycline combined with its anti-inflammatory effects may assist in attenuating dengue clinical symptoms like dengue fever, severe dengue hemorrhagic fever and dengue shock syndrome.[15]

A study was conducted to determine the effect of doxycycline treatment on cytokine levels, including TNF and IL-6, and mortality in dengue patients at high risk of complications. The study results showed that doxycycline can provide a clinical benefit to dengue patients at high

risk of complications. It was suggested that this effect could be mediated by decreasing pro-inflammatory cytokine levels.[30]

CONCLUSION

The results from these studies indicate that doxycycline may prove helpful in providing clinical benefits in the treatment of DENV infection by modulating the cytokine cascade as well as by its ability to interact with the DENV E protein to inhibit a conformational change which is an essential step in the process by which the virus enters the susceptible cells.

The anti-dengue activity and anti-inflammatory activity of doxycycline may prove to be helpful in reducing the severity of clinical symptoms such as dengue fever, severe dengue hemorrhagic fever and dengue shock syndrome. Since, there are no animal models available for dengue infection, clinical studies are required to obtain conclusive evidence of anti-dengue properties of doxycycline.

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