NITRIC OXIDE RADICAL SUPPRESSES REPLICATION OF WILD-TYPE DENGUE 2 VIRUSES IN VITRO

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Nitric oxide is well accepted as one of the defenses for inhibiting viral dissemination. Macrophages and cells in the macrophage lineage are professional nitric oxide producers which subserve as target for dengue virus. The interaction between nitric oxide and dengue virus in such target cell is unknown. In this report, the impact of nitric oxide on infectious dengue virus serotype 2 production and RNA replication was investigated in vitro. Primary isolates of dengue virus serotype 2 from dengue patients were replicated in mouse neuroblastoma cells in the presence of an exogenous nitric oxide donor, s-nitroso-N-acetyl-penicillamine, SNAP, at the concentration of 50 or 75 or 100 µM. Nitric oxide inhibited viral replication in a dose and a multiplicity of infection dependent manner. Nitric oxide from 50 and 75 µM SNAP delayed and suppressed replication of dengue virus isolates while higher concentration of nitric oxide, 100 µM SNAP, completely inhibited production of infectious particles up to 36 hr study. Twenty-four out of forty tested isolates, 60%, were susceptible to 50 µM SNAP inhibitory effect. The mechanism of inhibition was investigated at the level of RNA synthesis and was found that RNA production was suppressed which correlated to production of the infectious particles. Down-regulation of the RNA synthesis resulted in reduction of protein synthesis which was detected by lower level of NS1 protein synthesis using immunoblotting. In conclusion, nitric oxide from exogenous nitric oxide donor down regulated replication of dengue virus serotype 2 isolates from dengue patients. The suppression was clearly shown at the level of viral RNA and protein synthesis resulting in reduction of viral progenies production. This phenomenon implies that nitric oxide may serve as a defense which diminishes viral load in patients.


A PHASE 1/2 TRIAL OF A TETRAVALENT LIVE-ATTENUATED DENGUE VACCINE IN FLAVIVIRUS-NAIVE THAI INFANTS


Background: The Walter Reed Army Institute of Research (WRAIR) has produced a tetravalent live-attenuated dengue vaccine that has been well tolerated and immunogenic in U.S. adults and Thai children. As infants are considered by many as an important age group for vaccination in dengue-endemic countries, we evaluated the vaccine in Thai flavivirus-naive infants who are at risk for dengue.

Methods: Fifty-one healthy flavivirus-naive infants aged 12-15 months were enrolled and randomly assigned to one of two groups at the Phramongkutklao Hospital of the Royal Thai Army. Group I (N=34) received dengue vaccine at study months 0 and 6; Group II (N=17) received control vaccines (varicella at study month 0; Hemophilus influenza B at study month 6). All received a
licensed inactivated Japanese encephalitis (JE) vaccine at study months 7 and 7.5. Solicited and unsolicited adverse events were collected for 21 and 31 days, respectively, after each vaccination; serious adverse events (SAEs) were collected throughout the study. Safety testing included complete blood count and liver enzymes measured at intervals after each vaccination. Antibody endpoints were determined by 50% plaque reduction neutralization test using each serotype of dengue virus.

Results: Fifty infants completed all study visits; one infant was withdrawn due to re-location remote from Bangkok. All infants tolerated the vaccinations without SAEs attributed to vaccination as reported by an independent data monitoring committee. Two infants experienced on day of grade 3 fever, one with a maximum temperature of 39.2°C occurring 19 days post-dose 1 of dengue/control vaccination and the other with 40.2°C occurring 5 days post-dose 1 vaccination. At the time of this abstract submission, the investigators await unblinding and the release of immunogenicity data.

Conclusion: The WRAIR tetravalent live-attenuated dengue vaccine was well tolerated in this preliminary infant trial.


RANDOMIZED, PLACEBO-CONTROLLED TRIAL OF NONPEGYLATED AND PEGYLATED FORMS OF RECOMBINANT HUMAN ALPHA INTERFERON 2A FOR SUPPRESSION OF DENGUE VIRUS VIREMIA IN RHESUS MONKEYS

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Dengue fever and dengue hemorrhagic fever are caused by infection with any one of the four dengue viruses (DVs) and are significant public health burdens throughout the tropics. Higher viremia levels are associated with greater dengue disease severity. A therapeutic intervention to suppress viremia early in DV infection could potentially ameliorate severe disease. Recombinant alpha interferon 2a (rIFN-α-2a, Roferon-A) suppressed DV replication in human peripheral blood mononuclear cells in vitro. We therefore examined the effects of rIFN-α-2a and pegylated recombinant IFN-α-2a (PEG-rIFN-α-2a, PEGASYS) on DV serotype 2 (DV-2) viremia in rhesus monkeys. Flavivirus-naïve monkeys were inoculated with DV-2 and randomized to receive a single dose of rIFN-α-2a (10 million international units/m²) versus placebo or PEG-rIFN-α-2a (6 µg/kg) versus placebo 1 day after the onset of viremia. Serial daily viremia levels were measured, and convalescent-phase DV-2 neutralizing antibody titers were determined. Compared to placebo, a single injection of rIFN-α-2a temporarily suppressed DV-2 replication and delayed the time to peak viremia by a median of 3 days. However, measures of total viral burden were not different between the two groups. A single injection of PEG-rIFN-α-2a significantly lowered daily viremia levels and improved virus clearance, starting 48 h after administration. There were no significant differences in DV-2 neutralizing antibody titers between the treatment and placebo