

inhibited production of infectious particles up to 36 h study. Using RT-PCR and immunoblotting, the mechanism of inhibition was found to be at the level of RNA replication rather than at the level of protein synthesis. To prove whether inhibitory effect of NO targets at the viral RNA-dependent-RNA polymerase, an *in vitro* RdRp assay of purified NS5 was performed in the present and absent of NO. Results demonstrated that NO suppressed NS5 activity which was detected by significant reduction in viral RNA synthesis. To elaborate the *in vitro* effect of NO into natural dengue infection, the relationship between plasma NO and viral load in dengue patients was investigated. Plasma was obtained from DF and DHF patients on fever day and convalescence phase. From our preliminary data, during the fever day, plasma from secondary DHF patients contained low level of NO when compared to DF patients,  $3.5 \pm 2.42$  and  $8.49 \pm 3.45$   $\mu\text{M/ml}$ , respectively. During convalescence phase, these groups of patients generated a similar level of plasma NO which were  $8.24 \pm 3.12$ , and  $10.33 \pm 5.61$   $\mu\text{M/ml}$ , respectively. The secondary DHF plasma samples were selected for viral RNA copy number quantitation by Real Time RT-PCR. Results indicated that copy numbers of dengue viral RNA in plasma reversely correlated to the level of plasma NO. In conclusion, nitric oxide radical exerted an inhibitory effect to DV replication. The inhibitory target of NO was NS5, viral RNA polymerase. The profound effect of NO on viral load during natural dengue virus infection was detected in secondary DHF patients.

**1<sup>st</sup> Regional Meeting of Pediatric Dengue Vaccine Initiative (PDVI). Bangkok, Thailand. 18-20 October 2004.**

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## **A PHASE I/II TRIAL OF A TETRAVALENT LIVE-ATTENUATED DENGUE VACCINE IN THAI CHILDREN**

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Prevention of dengue through widespread vaccination is an important objective of the WHO and governments of dengue endemic regions. The Walter Reed Army Institute of Research developed live-attenuated monovalent DEN-1, -2, -3, and -4 vaccine candidates and mixed promising candidates to produce tetravalent dengue vaccine (TDV) formulations. Over 174 human subjects have safely received various formulations of the TDV. Formulation 17 (F17) has emerged as a promising candidate. Two doses, 6 months apart, of F17 was administered to 6 and 7 year-old children (6 flavivirus-naive children and 1 flavi-immune child). There were no serious or unexpected adverse events and no alert laboratory values. A measured temperature  $\geq 37.5^\circ\text{C}$  occurred in 4/7 (57.1%) recipients following dose #1 and 3/7 (42.9%) following dose #2. The highest temperature after either dose was  $38.6^\circ\text{C}$  and was observed in one child. Mild headache, muscle and joint aches, and fatigue were also experienced. Percent seroconversion (PRNT<sub>50</sub>  $\geq 1:10$ ) following dose #2 in 7 recipients against DEN-1, -2, -3, and -4 was 85.7%, 100%, 100%, and 100%, respectively. Post dose #1 and #2 GMTs for DEN-1, -2, -3, and -4 were 5, 22, 6, 23 and 39, 583, 290, and 163, respectively. Authors will provide detailed reactogenicity and immunogenicity data.

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