Clinical Study

Modulation of Cytokine and Cytokine Receptor/Antagonist by Treatment with Doxycycline and Tetracycline in Patients with Dengue Fever

J. E. Z. Castro,1 I. Vado-Solis,2 C. Perez-Osorio,2 and T. M. Fredeking3

1 Centro de Investigaciones Regionales “Dr. Hideyo Noguchi”, Universidad Autónoma de Yucatan, 97000 Mérida, YUC, Mexico
2 Facultad de Medicina, Universidad Autónoma de Yucatan, 97000 Mérida, YUC, Mexico
3 Antibody Systems Inc., 1901 Norwood Drive, Hurst, TX 76054, USA

Correspondence should be addressed to T. M. Fredeking, tfredeking@antibodysystems.com

Received 3 August 2010; Revised 11 November 2010; Accepted 17 January 2011

1. Introduction

Dengue fever (DF) is a mosquito borne disease prevalent in tropical and subtropical areas of the world caused by 4 serotypes of Flavivirus [1]. The World Health Organization estimates 50 million cases occur annually in 100 countries with most patients presenting with flu-like symptoms [2]. Approximately 2.5 percent of those infected develop dengue hemorrhagic fever (DHF) characterized by prolonged very high fever, bleeding at mucosal surfaces, and significant hematological abnormalities, which can lead to circulatory collapse and death in 2.5–20% of cases [1–3]. Unfortunately, the last 2 decades have seen a marked increase in geographical distribution and significant outbreaks of both DF and DHF [2]. There is no vaccine against, or specific treatment for, either disease entity other than general supportive care.

What determines if infection leads to DF or DHF is not well understood, but prior exposure to dengue virus may play a critical role [2]. Cytokines are known to play a role in several viral hemorrhagic fevers including dengue [4, 5]. Previous studies have shown a correlation between increased levels of several cytokines and disease severity which may have prognostic value [5–8]. In general, these studies have shown that levels of cytokines adversely affecting coagulation tended to be higher in DHF versus DF [9, 10].

Given the critical role of cytokines in the inflammatory process and coagulopathies, there have been numerous attempts to suppress their levels in an attempt to control various diseases [11–13]. Various classes of antibiotics have been shown to possess immunomodulating properties [14]. Of these, drugs belonging to the tetracycline family were found to benefit patients with multiple sclerosis, Huntington’s disease and rheumatoid arthritis presumably by suppressing microglia activity [14–18]. This, in turn, lowered levels of several proinflammatory cytokines including tissue necrosis factor (TNF) and interleukin 1 beta (IL-1β) [14, 18].
Recently, the levels of several proinflammatory cytokines in patients with tick-borne encephalitis (TBE) treated with tetracycline were found to be significantly lower than in untreated controls [5].

The present study was conducted to evaluate the ability of tetracycline and doxycycline to modulate cytokines and cytokine soluble receptors and receptor antagonists in patients with DF or DHF.

2. Materials and Methods

2.1. Subjects. The study protocol was approved by the Independent University of Yucatan, Merida, Yucatan, Mexico. Informed consent was obtained from all adult patients and from the parents of children. Patients (34 untreated controls, 45 which received Doxycycline, and 35 which received tetracycline, see below) were recruited between June and December 2009 from hospitals in rural Latin America.

Blood samples were obtained from hospitalized patients 8–55 years of age presenting with symptoms characteristic of DF or DHF. For a presumptive diagnosis of DF or DHF (Day 0), a fever for more than 2 days accompanied by two or more of the following were present severe headache, retro-orbital pain, myalgia, arthralgia, rash, leucopenia, and hemorrhage. Serum was collected, and dengue virus infection was confirmed by PCR testing. DHF was defined as being PCR-positive for dengue virus accompanied by fever with one or more of the following being present: severe headache, retro-orbital pain, myalgia, arthralgia, rash, leucopenia, and hemorrhage. Serum was collected, and dengue virus infection was confirmed by PCR testing.

Blood samples were obtained from hospitalized patients 8–55 years of age presenting with symptoms characteristic of DF or DHF. For a presumptive diagnosis of DF or DHF (Day 0), a fever for more than 2 days accompanied by two or more of the following were present: severe headache, retro-orbital pain, myalgia, arthralgia, rash, leucopenia, and hemorrhage. Serum was collected, and dengue virus infection was confirmed by PCR testing. DHF was defined as being PCR-positive for dengue virus accompanied by fever with one or more of the following being present: severe headache, retro-orbital pain, myalgia, arthralgia, rash, leucopenia, and hemorrhage. Serum was collected, and dengue virus infection was confirmed by PCR testing.

For patients 18–55 years of age, an initial oral dose of 200 mg was followed by 100 mg administered at 12 hour intervals for 10 days. The same dosing regimen was used for patients 15–17 years of age, but only for 7 days. For those 8–14 years old, a single day 1 loading dose of 4 mg/kg/day was followed by 4 mg/kg/day divided between two doses given 12 hours apart for 7 days. Tetracycline (Tetrex, Hormona SA de C.V., Edo de Mexico, Mexico) was given as follows. In patients 18–55 years of age, 500 mg were administered orally at 8 hour intervals for 10 days. For those 15–17 years old, 500 mg were administered at 12 hour intervals for 7 days. Patients 8–14 years of age received 50 mg/kg/day divided between 3 doses given 8 hours apart for 7 days.

2.2. Diagnostic Tests. Infection with dengue virus was confirmed by PCR using multiple primers.

2.3. Cytokine Assays. Blood samples were processed to obtain serum which was stored at −70°C. Assays were performed in a blinded manner after all samples were collected. Serum cytokine and cytokine receptor levels were quantified using commercial ELISA tests kits (R & D Systems, Minneapolis, MN, USA) per the manufacturer’s instructions. The limits of detection are as follows: IL (interleukin)-6, 0.7 pg/mL; IL-1β, <1 pg/mL; IL-1 receptor antagonist (IL-1RA), 14 pg/mL; TNF, 0.6 pg/mL; and soluble TNF receptor (sTNF-R1), 3 pg/mL.

2.4. Statistical Analysis. Statistical analysis comparing cytokine and cytokine receptor/antagonist levels between controls (untreated) and treatment groups (intergroup analysis) was performed using the unpaired Student t-test. Data was adjusted for day zero values for each patient. Intragroup analysis (comparing day 0 versus day 3 and day 7 within the same group) was done using a paired Students t-test. A P value < .05 was considered statistically significant.

3. Results

3.1. Effect of Doxycycline and Tetracycline Treatment on Serum Cytokine and Cytokine Receptor/Antagonist Levels in Patients with DF. All patients demonstrated elevated cytokine and cytokine receptor/antagonist levels at day 0 evidenced by higher mean levels in all study groups (Table 1). Over the 7-day observation period, proinflammatory cytokine (IL-6, IL-1β, and TNF) remained elevated in the control group. Similarly, IL1-RA and sTNF-R1 concentrations also remained elevated over the observation period. Treatment with doxycycline resulted in statistically (P < .01) lower levels of proinflammatory cytokines by day 3, both when compared to that seen at day 0 (intragroup analysis) and when compared to day 0 values in the control group. This decline continued with day 7 values significantly (P < .01) lower than those seen on day 3 (both inter- and intragroup). Treatment with tetracycline resulted in a similar trend, but generally it was not as rapid or pronounced. IL1-RA and TNF-R1 (molecules which downregulate cytokine activities) were found to be well above that seen in healthy individuals at time 0 in all patients. Increases between day 0 and day 7 values were modest in the control group. Treatment with doxycycline or tetracycline resulted in IL1-RA levels being significantly higher by day 3 and day 7, respectively. TNF-R1 levels were not significantly different between untreated patients and those receiving either tetracycline or doxycycline. Intragroup analysis comparing day 0 with day 3 and day 7 values demonstrated that both IL1-RA and TNF-R1 levels were significantly higher in all groups.

3.2. Effect of Doxycycline and Tetracycline Treatment on Serum Cytokine and Cytokine Receptor/Antagonist Levels in Patients with DHF. Doxycycline and tetracycline were also found to be effective at modulating cytokine and cytokine receptor/antagonist levels in patients with DHF (Table 2). Cytokine (IL-6, IL-1β, and TNF) levels were elevated in all groups at day 0 and remained so in untreated patients through day 7 (Table 2). Treatment with either doxycycline or tetracycline resulted in a significant (P < .01) reduction in cytokine levels by day 3 posttreatment when compared to either day 0 values in the control group or intragroup day 0 levels. This decline continued through day 7. Control patients with DHF also displayed a modest (15–30%) but
and DHF. However, in some instances, doxycycline appeared to be more effective. We, therefore, compared cytokine levels in patients with DF and DHF after 3 and 7 days of treatment (Table 3). Day 3 levels for proinflammatory cytokines IL-1β and TNF-α and were significantly lower in patients with DF or DHF who received doxycycline versus tetracycline. IL-6 levels at day 3 were comparable (P > .05) in patients treated with either drug. By day 7, IL-6 concentrations were significantly lower in the IL-1RA and TNF-R1 above those observed with tetracycline both at day 3 and day 7.

**4. Discussion**

Elevated cytokine levels are a hallmark of numerous bacterial and viral infectious diseases including dengue [5, 7–9].
Proinflammatory cytokines, such as IL-6, IL-1-β and TNF, are believed to cause the majority of symptoms, such as fever, malaise, and coagulopathies associated with infections. Indeed, the degree of imbalance between such cytokines and their anti-inflammatory counterparts may be the primary prognostic indicator of disease outcome [19–21]. These findings have led to the development of a broad spectrum of potential therapeutic agents, including monoclonal antibodies and antibiotics, which act to downregulate various cytokines [22–25]. Drugs belonging to the tetracycline class of antibiotics possess several advantages including a long history of safe use and low cost. Additionally, their ability to cross the blood-brain barrier with relative ease may provide a clinical benefit to patients with DF or DHF, a much more severe disease syndrome.

An additional potential benefit to using doxycycline in the treatment of DF or DHF is its recently discovered ability to inhibit dengue virus multiplication in tissue culture [28]. Doxycycline, but not tetracycline, was able to interact with the dengue virus E protein to inhibit a conformational change which is an essential step in the process by which the virus enters susceptible cells.

The present study indicates that doxycycline may provide a clinical benefit in the treatment of dengue virus infection by modulating the cytokine cascade. Unfortunately, the study design did not allow for us to determine this. We hope to initiate a study in the near future to determine if doxycycline can provide a clinical benefit to patients with DF or DHF by monitoring disease severity, mortality rates, and time of hospital discharge postenrollment.

### References

[1] D. J. Gubler, “Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century,” *Trends in Microbiology*, vol. 10, no. 2, pp. 100–103, 2002.

[2] T. Pang, M. J. Cardosa, and M. G. Guzman, “Of cascades and perfect storms: the immunopathogenesis of dengue hemorrhagic fever-dengue shock syndrome (DHF/DSS),” *Immunology and Cell Biology*, vol. 85, no. 1, pp. 43–45, 2007.

[3] M. G. Guzman and G. Kouri, “Dengue and dengue hemorrhagic fever in the Americas: lessons and challenges,” *Journal of Clinical Virology*, vol. 27, no. 1, pp. 1–13, 2003.
