Research Article

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Temperature rise after peginterferon alfa-2a injection in patients with chronic hepatitis C is associated with virological response and is modulated by *IL28B* genotype

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Background & Aims: Interferon treatment for chronic hepatitis C is associated with non-specific symptoms including fever. We aimed to determine the association of temperature changes with interferon antiviral activity.

Methods: 60 treatment-naïve patients with chronic hepatitis C (67% genotype 1/4/6, 33% genotype 2/3) were admitted to start peginterferon alfa-2a and ribavirin in a clinical trial. Temperature was measured at baseline and 3 times daily for the first 24 h and the maximal increase from baseline during that time (ΔT_{max}) was determined. Serum HCV-RNA, interferon-gamma-inducible protein-10 (IP-10) and expression of interferon-stimulated genes (ISGs – *CD274*, *ISG15*, *RSAD2*, *IRF7*, *CXCL10*) in peripheral blood mononuclear cells (PBMCs) were measured at very early time points, and response kinetics calculated. The *IL28B* single nucleotide polymorphism, rs12979860, was genotyped.

Results: Temperatures rose by 1.2 ± 0.8 °C, peaking after 12.5 h. ΔT_{max} was strongly associated with 1st phase virological decline (r = 0.59, p <0.0001) and was independent of gender, cirrhosis, viral genotype or baseline HCV-RNA. The association with 1st phase decline was seen in patients with rs12989760CC genotype (r = 0.65, p <0.0001) but not in CC/CT (r = 0.13, p = 0.53) and patients with CC genotype had a higher ΔT_{max} (1.4 ± 0.8 °C *vs.* 0.8 ± 0.6 °C, p = 0.001). ΔT_{max} was associated with 6- and 24-h induction of serum IP-10 and of PBMC ISG expression, but only in patients with rs12989760CC. ΔT_{max} weakly predicted early virological response (AUC = 0.68, CI 0.49–0.88).

Conclusions: Temperature rise following peginterferon injection is closely associated with virological response and is modulated by *IL28B* polymorphism, reflecting host interferon-responsiveness. Published by Elsevier B.V. on behalf of the European Association for the Study of the Liver.

Introduction

Interferon (IFN) alpha, and in the past decade, pegylated interferon (PegIFN), have been the backbone of antiviral therapy for patients with chronic hepatitis C [1]. Treatment with PegIFN is commonly associated with side effects and especially influenza-like symptoms of pyrexia, myalgia, and rigors [2–4]. Several hours after a PegIFN injection, a rapid rise in body temperature is commonly noted, thought to be mediated by IFN activity on the hypothalamus and initiation of the febrile pathway. However, the factors that predict the magnitude of this febrile response and its association with the antiviral efficacy of PegIFN are yet unknown.

We hypothesized that the spike of fever after the initial PegIFN injection will reflect interferon responsiveness and be correlated with the antiviral efficacy, as determined by the first phase virological decline [5] and sought to determine which baseline and on-treatment factors affect it.

Materials and methods

Study design and population

A retrospective analysis of data from a prospective treatment trial [6]. Sixty treatment-naïve adult patients with chronic HCV infection were admitted as inpatients to the NIH Clinical Center for 5-6 days to undergo a liver biopsy and begin combination therapy with PegIFNalfa-2a and ribavirin (RBV) using standard doses and duration. All patients received their first PegIFN injection at 8:00 AM. Patients were allowed a single dose of acetaminophen (up to 650 mg) if needed for fever or pain. The study protocol was approved by the Institutional Review Board and all patients gave written informed consent (ClinicalTrials.gov registration NCT00718172).

Keywords: Fever; Temperature; Interferon alfa; *IL28B*; Hepatitis C; Treatment.
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Abbreviations: HCV, hepatitis C virus; PegIFN, peginterferon alfa 2a; IP-10, interferon-gamma-inducible protein-10; IFN, interferon; PGE2, prostaglandin E2; ISG, interferon-stimulated gene; RVR, rapid virological response; EVR, early virological response; SVR, sustained virological response.

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Oral temperatures

Temperatures were measured by a Genius 2, an infrared tympanic electronic thermometer (Kendall, Mansfield, MA) set in oral mode prior to the first injection of PegIFN, and once per 8 h shift for the first 24 h. Maximum oral temperature change (ΔT_{max}) was defined as the difference between the baseline temperature and the maximal recorded temperature within the first 24 h.

Viral kinetics

Quantitative HCV RNA levels were measured at baseline, 6, 24, 48, 72 h, and weekly from week 1 until week 4, and every 4–8 weeks thereafter, using Cobas TaqMan real-time polymerase chain reaction (Roche Diagnostics, Palo Alto, CA), with a lower limit of detection of 15 IU/ml. The first-phase virological decline was defined as the logarithmic decline in serum HCV RNA levels from baseline to the nadir of the first 72 h. The second-phase slope was defined as the log-linear slope of decline from week 1 to week 4, or to the last quantifiable measurement.

A fast response was defined as $\geqslant 2$ $\log_{10}$ drop in HCV RNA levels by week 4. Rapid virological response (RVR) was defined as undetectable virus at week 4. Patients who were HCV RNA negative or had a 2 $\log_{10}$ drop in HCV RNA by week 12 were categorized as having a complete or partial early virological response (EVR), respectively. Patients who remained HCV RNA negative 6 months after stopping treatment were determined to have a sustained virological response (SVR).

Cytokine measurements

Serum levels of interferon-gamma-inducible-protein-10 (IP10), a serum protein which is the product of *CXCL10*, an interferon-stimulated gene (ISG), were measured by cytometric bead array (BD Biosciences, San Jose, CA) in a subset of 39 patients with samples drawn at baseline, 6 h, 24 h, 1, 2, 7, and 28 days after the first dose of PegIFN. Peripheral blood mononuclear cells (PBMCs) were obtained at the same time points and expression levels of *CXCL10*, *CD274*, *ISG15*, *RSAD2*, and *IRF7* were measured by quantitative PCR.

Genotyping

The *IL28B* single nucleotide polymorphism (SNP), rs12979860, was genotyped using TaqMan assay (Applied Biosystems Inc., Foster City, CA).

Statistical analysis

Statistical analyses were performed using GraphPad Prism 5.0 (La Jolla, CA) and SPSS v.19 (IBM Statistics). Non-parametric correlations were used, unless a linear relationship was evident.

Results

Patient characteristics

The patient characteristics are detailed in Table 1. Of the 60 patients, the majority (57%) were infected with genotype 1, were Caucasian (58%) and the average age was 52 ± 9 years. There was no gender predominance.

Temperature changes after initial PegIFN injection

After an initial PegIFN injection, oral temperature rose by 1.2 ± 0.8 °C, reaching an average peak of 37.9 °C (range 36.7–39.6 °C). In 20 patients (33%), oral temperature rose above 38.0 °C. Peak temperature was reached mostly during the evening shift, at a median of 12.5 h (range 6.4–20.4) after injection.

To compare the effect of PegIFN to the normal diurnal temperature variation, we analyzed another set of temperature measurements in 15 of the 60 patients, obtained when they were admitted to the NIH Clinical Center 6 months after stopping antiviral treatment for an additional liver biopsy. ΔT_{max} without PegIFN was significantly lower than when the patients received PegIFN (0.4 ± 0.5 *vs.* 1.2 ± 0.8 °C, $p = 0.005$, paired *t*-test).

Association with early viral kinetics

There was a strong linear correlation between ΔT_{max} and the 1st phase virological decline (r = 0.59, $p < 0.0001$, Fig. 1A) with a rise of 0.49 °C (95% CI 0.31–0.67) for each 1 $\log_{10}$ decline in viral levels. A similar correlation was seen with the 2nd phase slope of virological decline (r = 0.39, $p = 0.007$, n = 45, Fig. 1B), but the latter association did not remain significant when adjusted for 1st phase decline ($p = 0.63$).

Use of antipyretics

Antipyretics were requested by 29 (48%) patients during the first 24-h period after PegIFN injection. Since oral administration of acetaminophen reduces temperature within 1 to 3 h of ingestion by 1 °C on average, we performed a sensitivity analysis by adding 1 °C to measurements that were performed 1–3 h after a dose of acetaminophen. The associations of the adjusted ΔT_{max} were similar to those of the unadjusted analyses (1st phase r = 0.59, $p < 0.0001$; 2nd phase r = 0.33, $p = 0.026$, Supplementary Fig. 1). Patients who requested antipyretics were more likely to be infected with genotypes 2/3 (45% *vs.* 19% of those not needing antipyretics, $p = 0.034$), were more likely to have rs12979860 CC genotype (71% *vs.* 36%, $p = 0.007$), and were more likely to become fast responders (62% *vs.* 27%, $p = 0.006$). Age, gender, BMI, race and baseline viral levels were not associated with antipyretic use.

Association with demographic factors

ΔT_{max} did not differ between males and females (1.3 ± 0.8 °C *vs.* 1.2 ± 0.84 °C, $p = 0.46$) and the association of ΔT_{max} with 1st phase decline was similar for males (r = 0.69, $p < 0.001$) and females (r = 0.45, $p = 0.013$). There was also no association between ΔT_{max} and the presence of cirrhosis ($p = 0.61$). ΔT_{max} differed between races ($p = 0.016$, Kruskal-Wallis test), with a statistically significant difference between Asians and Caucasians (1.8 ± 0.7 °C, *vs.* 0.9 ± 0.7 °C, $p = 0.009$). In African-Americans, ΔT_{max} was not significantly different from Caucasians (1.2 °C, $p = 0.20$). The magnitude of association between ΔT_{max} and 1st phase virological response was remarkably similar across races; every 1 $\log_{10}$ decline in viral levels was associated with a temperature increase of 0.40 °C in Caucasians, 0.41 °C in Asians, and 0.56 °C in African Americans.

Association with viral genotypes

Patients infected with genotype 2 or 3 virus had a similar rise in temperature (1.3 ± 0.9 °C) compared to genotype 1 patients (1.1 ± 0.7 °C, $p = 0.17$, Fig. 2A), despite a marked difference in 1st phase virological decline between the two groups (2.3 ± 0.8 *vs.* 1.2 ± 0.8 $\log_{10}$, $p < 0.0001$). The slopes of linear regression of ΔT_{max} *vs.* 1st phase decline were similar for genotype 1 (slope = 0.55 [CI 0.28–0.81], r = 0.59, $p = 0.0002$, Fig. 2B) and for genotype 2/3 (slope = 0.75 [CI 0.33–1.16], r = 0.68, $p = 0.001$, Fig. 2C), suggesting that the association is independent of viral