Limited Efficacy of Thalidomide in the Treatment of Febrile Attacks of the Hyper-IgD and Periodic Fever Syndrome: A Randomized, Double-Blind, Placebo-Controlled Trial

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ABSTRACT
Hyper-IgD and periodic fever syndrome (HIDS) is an autosomal recessive disorder featured by recurrent febrile attacks. Previous unpublished experience (J. van der Meer and R. Powell) suggested that thalidomide may prevent febrile attacks. Six HIDS patients (5 male and 1 female) who had at least one febrile attack every 4 to 8 weeks, entered a randomized, double-blind, placebo-controlled crossover trial to explore the efficacy of a daily 200-mg thalidomide dose in the treatment of recurrent febrile attacks of HIDS. The patients received either thalidomide, 200-mg daily, or placebo for 16 weeks, followed by a 4-week washout period and another 16-week treatment (cross-over) with either thalidomide or placebo. Patients completed a weekly diary card noting attacks and side effects. During the study, C-reactive protein (CRP), serum amyloid A (SAA), interleukin (IL)-6, tumor necrosis factor (TNF)-α, IL-1 receptor antagonist, soluble TNF receptor p55 and p75, and lipopolysaccharide-stimulated IL-1β and TNF-α production were measured at six different points, whereas urine neopterin levels were measured weekly. During the active treatment with thalidomide, there were 10 attacks compared with 13 attacks with placebo. Thalidomide resulted in a nonsignificant decrease of CRP and SAA, but the concentrations of other inflammatory mediators, including urine neopterin, remained unchanged. One patient developed sensory polyneuropathy, but this resolved when thalidomide administration was stopped. The effect of thalidomide in HIDS is limited to a decrease in acute phase protein synthesis without an effect on the attack rate.

In 1984, van der Meer and colleagues described six patients with periodic fever and a constantly elevated serum polyclonal IgD and called the syndrome hyperimmunoglobulinemia D and periodic fever syndrome (HIDS). HIDS is an autosomal recessive disorder, and to date more than 150 familial and isolated cases are known (Drenth et al., 1994). It should be distinguished from other periodic fever syndromes, such as the autosomal recessive familial Mediterranean fever and autosomal dominant tumor necrosis factor receptor-associated periodic fever syndrome (http://www.hids.net; Drenth and van der Meer, 2000). HIDS patients have a long history of recurrent attacks of fever, frequently preceded by chills and accompanied by headache, bilateral cervical lymphadenopathy, and occasionally by oral and vaginal aphthous ulcers, abdominal pain, and diarrhea. Laboratory analyses invariably reveal an acute phase response during attacks and a constantly elevated serum level of polyclonal IgD (Drenth et al., 1994). Symptoms commence at an early age and persist throughout life, and the febrile attacks occur every 4 to 8 weeks, lasting 3 to 7 days. Patients are asymptomatic between attacks, although the acute phase response may persist. Symptomatic episodes are associated with increased concentrations of inflammatory cytokines, such as TNF-α, interleukin (IL)-6, and interferon (IFN)-γ, and of the anti-inflammatory compounds IL-1ra and soluble TNF receptor p55 and p75 (Drenth et al., 1995b). HIDS is caused by missense mutations in the mevalonate kinase gene, which leads to reduced enzyme activity of mevalonate kinase and results in small amounts of mevalonic acid in the urine (Drenth et al., 1999; Houten et al., 1999; Cuisset et al., 2001). Despite the progress in understanding the pathogenesis of the disorder, treatment remains largely supportive. Although initial reports suggested benefits from colchicine, subsequent experience with the drug failed to substantiate this (van der Meer et al., 1984; Ostuni et al., 1988). Similarly, isolated case reports have mentioned success with various
dosages of steroids, immunoglobulin infusions, and cyclosporine, but application in a larger group did not confirm this.

Thalidomide, infamous for its severe teratogenicity, is able to inhibit TNF-α production by human mononuclear cells and to normalize elevated plasma TNF-α concentrations (Sampaio et al., 1991; Moreira et al., 1993). In addition, thalidomide inhibits IFN-γ synthesis and constrains leukocyte chemotaxis (Zwingenberger and Wnendt, 1995). The drug has been shown to have a consistent benefit in a wide variety of inflammatory disorders, such as erythema nodosum leprosum (Powell, 1999; Calabrese and Fleischer, 2000) and Behçet’s syndrome (Rigby et al., 1995). A large randomized trial showed that thalidomide is very effective for treating oral and genital ulcers and follicular lesions of Behçet syndrome (Hamuryudan et al., 1998). Preliminary data obtained in two HIDS patients showed that thalidomide resulted in a dramatic relief of symptoms (J. van der Meer and R. Powell, unpublished observations). Therefore, we initiated a 36-week randomized, double-blind, placebo-controlled trial to assess the effect of a daily 200-mg thalidomide dose on the frequency, intensity, and duration of attacks in HIDS patients.

Materials and Methods

Patients. Patients were selected from the Nijmegen HIDS registry. This database carries the pertinent clinical and laboratory data of patients with HIDS. Because all patients were required to visit the University Medical Center St. Radboud regularly throughout this trial, we recruited only Dutch patients. The patients were informed about the trial by a nation-wide patient meeting in Nijmegen, August 1999. Only male HIDS patients and women who had had a surgical sterilization procedure were invited to take part in the study. Additionally, female patients were required to either use condoms or refrain from sexual intercourse during the study. Only patients over 18 years old and having frequent febrile attacks (more than one attack every 6 weeks) were eligible for the study. All patients who enrolled in this study were screened for mutations in the mevalonate kinase gene. The numbering of the patients in this study refers to the original patient number in the Nijmegen HIDS registry (Drenth et al., 1994). Given the average attack frequency in our patients (at least one every 6 weeks), we defined effective thalidomide treatment as treatment that was able to decrease the number of attacks by 50%. Our study was designed to have an 80% power of detection and a decrease of 45% in the frequency of attacks.

Trial Design. The trial was a 36-week randomized, placebo-controlled crossover trial evaluating the efficacy of thalidomide taken at a dosage of 200 mg daily in the treatment of HIDS patients suffering from recurrent febrile attacks. The study consisted of two periods of 16-week treatments with thalidomide or placebo separated by a 4-week washout. The trial was conducted at the outpatient clinic of the Division of General Internal Medicine of the University Medical Center St. Radboud in Nijmegen, The Netherlands. Permission of the local Medical Ethical Committee had been obtained. A clinical pharmacist who was not directly involved in the trial prepared a simple, computer-generated, random-number list. The code was kept at the Department of Clinical Pharmacy and was opened only after all data had been entered into a computer for analysis. Before study entry, the prospective candidates were informed about the design, purpose, and duration of the study and received oral and written information concerning adverse effects of thalidomide. Patients gave written informed consent before enrolling in the trial. Subjects were screened at the beginning of the trial, and baseline demographic and relevant clinical information was obtained. They all had normal results on clinical neurological evaluations. Physical examinations and laboratory measurements were performed at baseline and at weeks 8, 16, 20, 28, and 36.

Drugs. At baseline and at the 20-week visit, the patients received two bottles containing either 100-mg thalidomide tablets (Grüenthal GmbH, Aachen, Germany) or placebo tablets identical in appearance to the thalidomide tablets. Patients were instructed to take two tablets each evening. At week 16 and week 36, unused tablets were collected and counted to determine compliance. Patients were allowed to use antipyretic drugs, such as acetaminophen or nonsteroidal anti-inflammatory drugs, throughout the study period. One patient (patient 4) used a fixed low-dose steroid (prednisone 10 mg/day) throughout the study period.

Outcome Measures: Attacks. The primary outcome measure was defined as the number of attacks. To register the number and severity of attack, all patients were asked to complete a weekly diary card to register the presence or absence of symptoms and the severity using a visual analog scale. The following characteristics were listed: lymphadenopathy, abdominal pain, nausea, diarrhea, vomiting, arthralgia, skin lesions, aphthous ulcers, and headache. The patients could indicate the severity of the symptoms on a scale ranging from 0 to 7. Attacks were defined as fever (>38.5°C) together with one or a combination of the following symptoms: abdominal distress (pain, vomiting, diarrhea), joint involvement (arthralgia, arthritis), skin lesions, and/or lymphadenopathy. Each week this symptom card was mailed to the principal investigator by the patient.

Acute Phase Response and Cytokine Measurements. Serum IgD was measured at the onset of the trial using an enzyme-linked immunosorbent assay (ELISA), as described elsewhere (Drenth et al., 1996). Blood samples were drawn six times throughout the whole study period: at baseline and at weeks 8, 16, 20, 28, and 36, and the serum/plasma concentrations of the following inflammatory mediators were measured: C-reactive protein (CRP) and serum amyloid A (SAA) were measured using sensitive ELISAs (Hazenberg et al., 1990). Measurements of serum TNF-α, IL-1ra, and IL-1β were performed using fluid phase radioimmunoassays, whereas IL-6 was measured with an ELISA (Drenth et al., 1995c). The concentrations of sTNFRI p55 and sTNFRII p75 were measured in serum using an ELISA developed by Hoffman-La Roche (Basle, Switzerland) (Drenth et al., 1995b). Cytokine production was measured using a whole blood culture system developed in our laboratory’s assay (Drenth et al., 1995b). Briefly, two 2-ml tubes containing 24 µl of EDTA-K3 (10,000 kallikrein-inactivating units per milliliter; Bayer, Leverkusen, Germany) were drawn. One tube was incubated immediately, and the other tube was incubated after addition of 25 µl of lipopolysaccharide (Escherichia coli serotype 055:B5; Sigma, St. Louis, MO; final concentration 10 µg/ml of blood). After 24 h of incubation at 37°C, both tubes were centrifuged at 2250g for 10 min and then at 15,000g for 5 min to obtain platelet-poor plasma. Aliquots were stored at −70°C until assay.

Characterization of HIDS by Mutation Analysis. RNA was extracted from Epstein-Barr immortalized cell lines, and cDNA was produced using standard techniques. The complete coding region of the mevalonate kinase gene was amplified by polymerase chain reactions, and we determined the nucleotide sequences of the amplified fragments by standard semiautomated methods on an ABI PRISM 377 (PerkinElmer Life Science, Boston, MA) (Drenth et al., 1999).

Neopterin Measurement. Previous data indicated that the excretion of neopterin in urine conveniently correlated with the febrile attacks. We therefore asked the patients to collect weekly urine samples, which were stored at −20°C until assay. Neopterin was determined by reversed-phase high-performance liquid chromatography (Lim et al., 1993a). Briefly, urine samples were centrifuged to remove debris, diluted in a 1 to 10 ratio with water containing dimethylterpine as an internal standard, and injected directly onto a Techsphere 5 ODS column (HPLC Technology, Ltd., Welwyn Garden City, Hertfordshire, UK). A binary gradient elution was used with an initial mobile phase of 2% methanol in 15 mM phosphate buffer, pH 7.
6.4, increasing to 25% methanol after 12 min, and creatinine was detected separately using a kinetic alkaline picrate (Jaffe) method. The ratio of neopterin to creatinine was calculated to compensate for variations of urinary density. The median urine neopterin value for a group of 65 healthy controls was 149 μmol/mol creatinine (range 62–273).

**Monitoring of Side Effects.** Because thalidomide can cause peripheral neuropathy, a careful neurological examination was performed at each visit. Patients who developed any sign of neuropathy were excluded from continuation of the trial. The patients mailed a weekly diary card, which contained a list of potential side effects. In total, they could choose from a list of 16 precoded side effects.

**Statistical Analysis.** Continuous variables were compared by using the Wilcoxon rank-sum test. Probability (P) values were calculated on the basis of two-tailed t tests. A P value of less than 0.05 was considered to be the lowest level of significance.

**Results**

**Patients.** Six patients (5 male and 1 female) enrolled in the study between August and September 1999. The demographic data are depicted in Table 1. The mean age at the start of the study was 32.5 (standard deviation 13.7) years, and all patients had had their first attacks before the end of their first year of life. Patients reported an attack frequency once every 2 to 9 weeks. The length of the febrile episodes varied between 2 to 7 days in these HIDS patients. All patients had elevated serum IgD concentrations, which ranged from 154 to 4224 IU/ml.

**Mutation Analysis.** Four patients carried the V377I missense mutation (valine replacing isoleucine at codon 377 of the mevalonate kinase protein). This mutation is considered to be the prototype mutation in HIDS. One patient was homozygous for this mutation, and three were compound heterozygote, most commonly in combination with I268T (isoleucine replacing a threonine at codon 268). In patient 26, we were unable to detect a mutation on one of the chromosomes.

**Treatment Outcomes.** All patients were able to complete the trial. However, one patient (patient 102) receiving thalidomide discontinued treatment after 9 weeks because he developed numbness and paraesthesias of the extremities. Four days after discontinuation of the trial medication, the signs of sensorial neuropathy had disappeared, and a physical examination performed at the end of the trial 6 weeks later showed normal discriminative and vibration sensations and physiological muscle stretch reflexes. The data from this patient were analyzed on an intention-to-treat analysis. Compliance rates for thalidomide or placebo use, as calculated from the returned pill counts, were similar in both groups (95% versus 96%). Three patients used antibiotics at some point during the trial because of suspected upper airway infections. During thalidomide treatment, patients 31 and 89 were treated with a combination of amoxicillin/clavulanic acid, and patient 34 was treated with erythromycin.

In the complete study period, including the washout period, the six patients registered a total of 30 attacks (one attack every 7.2 weeks). During active treatment with thalidomide, there were 10 attacks compared with 13 attacks with placebo (Table 2). The number of symptomatic days was similar with thalidomide (65 days) or placebo (87 days). The length of attacks was similar during both treatment blocks (thalidomide 6.3 days versus placebo 6.2 days). With thalidomide, the number of symptoms was 2.9 per attack compared with 3.5 with placebo. The severity of the separate symptoms during the febrile episodes, as indicated on a scale from 0 to 7, was 3.8 (standard deviation 2.2) with thalidomide and 4.2 (standard deviation 2.8) with placebo.

**Circulating Mediators.** Thalidomide treatment resulted in a moderate decrease of CRP and SAA when compared with placebo. At start of the active treatment, mean CRP concentration was 40.1 mg/l, and this decreased to 12.2 mg/l at the end of the trial. A similar effect was seen on SAA concentrations, which decreased from 30.8 mg/l to 3.9 mg/l (Fig. 1, upper panel). These differences were not significant. Thalidomide treatment did not affect plasma IL-1RA or IL-6 concentrations. With placebo, both these parameters rose during the last 6 weeks of the trial (Fig. 1, lower panel). Likewise, active treatment did not influence the sTNF-α concentrations in plasma when compared with placebo.

**Ex Vivo Production.** Figure 2 shows the LPS-stimulated production of the various cytokines tested. The production of IL-1β in patients with HIDS during thalidomide treatment was similar to that of placebo. Given the specific TNF-α inhibiting properties of thalidomide, we anticipated a decrease of the TNF-α production. However, we failed to note an effect of the drug on the ex vivo LPS-stimulated production of TNF-α.

**Neopterin.** Patients collected 92 weekly urine samples in the placebo period and 88 urine samples with thalidomide treatment. Figure 3 shows box and whisker plots of the values throughout the trial. With placebo the median value was 270.5 μmol/mol creatinine, which did not differ from those obtained during thalidomide (281 μmol/mol creatinine) or during the washout period (264.5 μmol/mol creatinine). In all 204 samples submitted, 51% had values above the upper limit of those found in healthy individuals (273 μmol/mol creatinine). Febrile attacks clearly influenced neopterin lev-

<table>
<thead>
<tr>
<th>No. in HIDS Registry</th>
<th>Sex</th>
<th>Age</th>
<th>Age at Onset</th>
<th>Average Length of Attack</th>
<th>Average Frequency per Week</th>
<th>Maximal IgD</th>
<th>Mutations in MVR Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>days</td>
<td>IU/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>51</td>
<td>0</td>
<td>3–4</td>
<td>1/4</td>
<td>991</td>
<td>V377I/I268T</td>
</tr>
<tr>
<td>26</td>
<td>M</td>
<td>20</td>
<td>3</td>
<td>2–3</td>
<td>1/3–4</td>
<td>4224</td>
<td>V377I</td>
</tr>
<tr>
<td>27</td>
<td>M</td>
<td>28</td>
<td>18</td>
<td>2–3</td>
<td>1/2</td>
<td>1731</td>
<td>P167L/I268T</td>
</tr>
<tr>
<td>31</td>
<td>M</td>
<td>19</td>
<td>0</td>
<td>7</td>
<td>1/4</td>
<td>656</td>
<td>V377I/I268T</td>
</tr>
<tr>
<td>89</td>
<td>F</td>
<td>35</td>
<td>3</td>
<td>4</td>
<td>1/2–3</td>
<td>376</td>
<td>V377I/V377I</td>
</tr>
<tr>
<td>102</td>
<td>M</td>
<td>48</td>
<td>12</td>
<td>4–5</td>
<td>1/6–9</td>
<td>154</td>
<td>I268T/I268T</td>
</tr>
</tbody>
</table>

M, male; F, female.
els, and higher values were seen during periods with disease activity. During the 30 attacks, the average neopterin value was 657 μmol/mol creatinine, clearly higher than with remission (mean values 329 μmol/mol creatinine). There was no significant difference between thalidomide and placebo with respect to the neopterin excretion during attacks (data not shown). Most notably, we detected very high values (>1000 μmol/mol creatinine) in 5 of 167 samples of patients who reported to have a remission. One patient had a value of 2598 μmol/mol creatinine but denied any clinical disease activity before or at the point of sampling.

**Side Effects.** Three patients did not note any side effects. The other three patients recorded a total of 165 symptoms, as indicated by their diaries. During thalidomide, 115 symptoms were recorded compared with 50 during placebo. As depicted in Table 3, patients most commonly complained about a dry mouth and fatigue. As previously mentioned, one patient reported symptoms of polyneuropathy necessitating termination of thalidomide treatment. No patient developed neuropathy after the trial (Table 3).

**Discussion**

In this article, we describe the results from the first randomized placebo-controlled therapeutic trial in HIDS. Given the propensity of the HIDS symptoms to wax and wane over time, we developed a thorough randomized trial to assess the therapeutic efficacy of thalidomide. The assessment period of 36 weeks was long enough because all patients had repetitive attacks at either phase of the trial. Intervention with thalidomide did result in a minor, nonsignificant decrease of febrile attacks in HIDS patients, and four of six patients had fewer attacks with thalidomide. However, this effect is clinically insignificant. One reason for disappointing results might be that the dosage of thalidomide was too low. On the other hand, if increasing the dosage would lead to a greater benefit, this would probably come at the cost of a higher incidence of unwanted side effects. In light of the results of this randomized trial and given the small balance between efficacy and side effects, thalidomide is not the drug of choice in the treatment of HIDS attacks. However, we need to consider other (biochemical) effects of treatment.

Biochemically, treatment with thalidomide was associated with a small decrease of serum CRP, SAA, and IL-1RA. Decrease of acute phase proteins such as SAA may be a desired goal in the treatment of a periodic fever syndrome. SAA is thought to be the precursor of AA-type amyloidosis, a much feared complication of periodic fever syndromes. For example, in familial Mediterranean fever colchicine treatment, results not only in a decrease of the frequency of attacks but also a decrease in the incidence of amyloidosis (Ben-Chetrit and Levy, 1998). In our patients, high CRP and SAA values were detected throughout the study period. In all samples collected during the complete study period, the av-

**TABLE 2**

The distribution of the number of attacks in the six individual HIDS patients during the crossover trial among placebo and thalidomide (200 mg/day)

<table>
<thead>
<tr>
<th>No. in HIDS Registry</th>
<th>Placebo</th>
<th>Washout</th>
<th>Thalidomide</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>26</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>27</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>31</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>89</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>102</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>7</td>
<td>10</td>
</tr>
</tbody>
</table>
Thalidomide in Hyper-IgD and Periodic Fever Syndrome

A comparative study of adverse events among patients treated with thalidomide and placebo during the 36 weeks of the randomized trial. Values are given as number of weeks as indicated on diary card returned by the patients and by the number of patients who experienced adverse symptoms at any time during the study.

<table>
<thead>
<tr>
<th>Event</th>
<th>Placebo</th>
<th>Thalidomide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Weeks</td>
<td>No. of Patients</td>
</tr>
<tr>
<td>Fatigue</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>Numbness</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Paresthesias</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Dry mouth</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Drowsiness</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Sleepiness</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

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