Detection of biomarkers for lysosomal storage disorders using novel technologies
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Chapter Six

Correction in plasma macrophage inflammatory protein (MIP)-1β is dose dependent during the initial phase of enzyme replacement therapy of type I Gaucher disease

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Abstract

Macrophage inflammatory protein (MIP)-1β is overproduced by phagocytes surrounding storage cells in tissue lesions of Gaucher patients. Consequently, levels of MIP-1β are markedly increased in plasma of Gaucher patients and decrease upon enzyme replacement therapy (ERT). Patients with ongoing skeletal disease during treatment, however, do not show a sufficient reduction. Given the debate whether high dose ERT results in a faster and better response in bone we investigated whether a difference in therapeutic enzyme dosing influences the response in plasma MIP-1β concentration. For this purpose we retrospectively determined MIP-1β responses in two comparable patient groups receiving either a relatively low dose (median 15 U/kg/4 weeks (AMC, n=15)) or a relatively high dose (median 120 U/kg/4 weeks (HHU, n=15)) of ERT. Plasma MIP-1β levels improved faster during the first year of treatment in the higher-dosed patient group. This was also observed for responses in chitotriosidase, a storage cell marker and the bone marrow burden score. In conclusion, MIP-1β analysis indicates that not only the initial correction of Gaucher cells but also that of associated phagocytes is enzyme dose-dependent.
Dose-dependent correction of MIP-1β in initial phase of ERT

Introduction

Type I Gaucher disease (GD) is characterized by accumulation of glucosylceramide in lysosomes of macrophages due to deficient activity of glucocerebrosidase (EC 3.2.1.45) [1]. Characteristic glucosylceramide-laden macrophages (Gaucher cells) are present in liver, spleen and bone marrow resulting in severe hepatosplenomegaly, pancytopenia and bone problems, such as avascular necrosis, pathological fractures, bone pain and bone crises [1]. In tissue lesions of Gaucher patients, mature storage cells, which are alternatively activated macrophages secreting chitotriosidase, are surrounded by newly formed, highly inflammatory cells secreting macrophage inflammatory protein (MIP)-1β [2]. MIP-1β, like chitotriosidase, is markedly increased in plasma of Gaucher patients. Enzyme replacement therapy (ERT, Genzyme Corp., Mass. USA), a treatment based on chronic intravenous administration of glucocerebrosidase [3,4], results in clinical improvements in Gaucher patients. Plasma chitotriosidase and MIP-1β are also reduced upon ERT and remaining high levels of MIP-1β during therapy are associated with ongoing skeletal disease [2]. The preferred dosing regimen of ERT still remains topic of debate. Prospective studies that compare different dosing regimens applied to comparable patient groups do not exist. Markedly different dosing regimens have however been used in different treatment centers. For example, patients at the Academic Medical Center (AMC) in the Netherlands were treated with an initial low dose (15-50 U/kg/4 weeks), while patients treated at the Heinrich Heine University (HHU) in Germany, used initial dosages between 60-120 U/kg/4 weeks. De Fost et al. [5] reported on a retrospective analysis of the outcome of ERT in patients treated in both these centers. It was found that the different dosing regimens had not affected outcome of hematologic and visceral parameters, but higher dosing had led to accelerated decrease of chitotriosidase and bone marrow burden score associated with better objective bone response [5]. In the light of these findings, we wished to establish whether also phagocytes surrounding storage cells in tissue lesions of Gaucher patients are ERT-dose dependently corrected. For this purpose we compared the response in plasma MIP-1β levels to different dosing schedules.

Patients, materials, & methods

Patients and dosing regimens

A total of 58 adult Gaucher type I patients who started treatment between 1990 and 2000 in the referral centers for Gaucher disease in AMC, Amsterdam, The Netherlands, and in HHU, Duesseldorf, Germany, and who received ERT with an initial dose of no more than 50 U/kg/4 wks (AMC) or at least 60 U/kg/4 wks (HHU) were included in the study. Patients were matched according to bone marrow burden (BMB) score at baseline (minimum BMB >2, maximum difference for matched pairs of 1 BMB point). This resulted in two comparable groups of 15 patients receiving low and high dose ERT, respectively. Patients at AMC (12 males (mean age 55 years, range 37-71) and 3 females
(mean age 54 years, range 44-61) were treated according to an individualized low-dose protocol as described earlier [6], starting with an initial dose of 15 to 50 U/kg/4 wks. Patients treated in HHU (n=15, 8 males (mean age 53 years, range 28-67) and 7 females (mean age 49 years, range 26-61)) started with a dose of 80 to 120 U/kg/4 wks, given every other week, with the higher initial doses in more severe disease, such as extensive organomegaly or bone disease. The dose was slowly decreased in some patients, who reach stable disease.

Data assessment and analysis

Plasma chitotriosidase activity, plasma MIP-1β levels, and BMB score were determined. Values of MIP-1β and chitotriosidase at start and after 1, 2, 3, 4, and 5 years (± 4 months) of ERT were compared. Samples were not available of every patient at every time point, resulting in the following minimum number of data sets for AMC and HHU; T0: n=15 and 15; T1: n=11 and 7; T2: n=11 and 4; T3: n=5 and 6; T4: n=5 and 4; T5: n=7 and 2, respectively.

In addition, for chitotriosidase and BMB, therapeutic goals were defined and analyzed by life table analysis (Kaplan Meier). For this purpose, all samples during the first 9 years of ERT were used. This resulted in 71 samples for AMC, with a median interval between two consecutive samples of 12 months (n=15) and 53 samples for HHU, with a median interval of 16 months between two consecutive samples (n=15). Approval was obtained from the AMC and HHU Institutional Review Boards for this study. Informed consent was provided in accordance with the Declaration of Helsinki.

MIP-1β

MIP-1β levels in EDTA plasma samples from both centers were measured centrally, exactly as described earlier [2].

Chitotriosidase

Chitotriosidase activity in plasma samples from both centers was measured in one laboratory. The enzyme activity assay with 4-MU-chitotriose (Sigma, St Louis, MO; normal range, 7-124 nmol/mL/h) as a substrate was performed at pH 5.2, as described previously [7]. Genotyping for the chitotriosidase null mutation [8] was performed, and chitotriosidase values of patients who were heterozygous for the chitotriosidase null mutation were multiplied by 2 [9]. For comparison of baseline values and values at yearly intervals of ERT, as well as for Kaplan Meier analysis, patients with a chitotriosidase activity of more than 5000 nmol/mL/h at baseline were selected (n=15 AMC, n=14 HHU). Reduction of chitotriosidase activity to a level of less than 5000 nmol/mL/h was defined as a therapeutic goal for Kaplan Meier analysis.

Bone marrow burden

Scoring of the severity of involvement of the bone marrow was performed as described earlier [5]. Life table analysis was performed for all patients with a baseline BMB in the
range of 2 to 8, and separately for patients with severe bone disease (BMB 6-8). A decrease of 2 points in BMB score was defined as the therapeutic goal.

Statistics
Differences between both dosing regimen groups at baseline and at yearly intervals (± 4 months) during ERT were analyzed by Mann-Whitney U test. Results of life table analysis (Kaplan Meier) were expressed as share of patients reaching the therapeutic goal versus duration of ERT, reflected as median. Differences between the cohorts were determined by the log-rank test.

Results
Patient characteristics
Patient characteristics are presented in Table 1. Patients did not significantly differ with respect to age, gender, number of splenectomies, or overall severity of disease. In both patient groups, less than 10% of the patients were known to be of Ashkenazi-Jewish ancestry. The prevalence of the various mutations in both cohorts was comparable.

Table 1
Patient characteristics of 30 adult Gaucher type I patients.

<table>
<thead>
<tr>
<th></th>
<th>AMC</th>
<th>HHU</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>15</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>Age in 2007</td>
<td>58 (37-71)</td>
<td>53 (26-67)</td>
<td>NS</td>
</tr>
<tr>
<td>Gender (% males)</td>
<td>12 (80%)</td>
<td>8 (53%)</td>
<td>NS</td>
</tr>
<tr>
<td>No. of splenectomies</td>
<td>5 (33%)</td>
<td>6 (40%)</td>
<td>NS</td>
</tr>
<tr>
<td>SSI</td>
<td>7 (4-16)</td>
<td>7 (2-15)</td>
<td>NS</td>
</tr>
<tr>
<td>MIP-1β (pg/mL)</td>
<td>152 (57-383)</td>
<td>171 (10-741)</td>
<td>NS</td>
</tr>
<tr>
<td>BMB</td>
<td>7 (3-8)</td>
<td>7 (3-8)</td>
<td>NS</td>
</tr>
<tr>
<td>Chitotriosidase (nmol/mL/h)</td>
<td>25398 (6889-110754)</td>
<td>15756 (953-66734)</td>
<td>NS</td>
</tr>
<tr>
<td>Start dose (U/kg/4 weeks)</td>
<td>15 (15-50)</td>
<td>120 (80-120)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Table 1. Patient characteristics of 30 adult Gaucher type I patients. The data reflect absolute (and percentual) numbers or median (and range). Abbreviations: AMC, Academic Medical Center, Amsterdam; HHU, hospital of the Heinrich-Heine-University, Duesseldorf; SSI, Severity Score Index [10]; BMB, Bone Marrow Burden [11]; NS, not significant.

MIP-1β
Plasma MIP-1β levels at baseline were not significantly different between both groups (n=15 for AMC and n=15 for HHU, Table 1 & Figure 1), although there were three patients with more extensive elevations in the HHU group. The decrease in plasma MIP-1β after 12 months of ERT was significantly larger in the HHU patient group than in the AMC patients receiving a lower enzyme dose (AMC, n=11 and HHU, n=8, P=0.009) (Figure 2B). After two years of ERT, almost all patients reached plasma MIP-1β levels of less than 85 pg/mL and no significant difference was detected between the two groups with respect to the decrease of plasma MIP-1β (Figure 2A). Sub-analysis of matched patients with BMB ≥ 6 gave very similar results (not shown).
Chitotriosidase

The reduction in chitotriosidase activity was significantly larger in the HHU patient group than in the AMC cohort after 1, 2, 3, 4 and 5 years of ERT (P=0.002, 0.034, 0.052, 0.016 and 0.006, respectively) (Figure 3A). There was a significant difference between both cohorts in time to reach plasma chitotriosidase activity below 5000 nmol/mL/h (P= 0.001) (Figure 3B). After 60 months of ERT 95% of patients from the HHU cohort (n=14) reached a plasma chitotriosidase activity below 5000 nmol/mL/h, vs. 21% of the AMC cohort (n=15).

Bone marrow burden

We compared the time to reach a decrease of 2 points in BMB score of patients with a baseline BMB of 2-8 of both cohorts using life table analysis (n=15 for AMC and n=15 for HHU). HHU patients showed a trend towards a quicker response in reaching the
Dose-dependent correction of MIP-1β in initial phase of ERT

therapeutic goal than AMC patients (P=0.071) (Figure 4A). Sub-analysis of patients with more severe bone marrow involvement (BMB ≥ 6, n=12 for AMC and n=14 for HHU) revealed a more pronounced and statistically significant difference between the two dosing regimens (P=0.021)(Figure 4B).

Figure 3. Impact of ERT on changes in plasma chitotriosidase activity. (A) Plasma chitotriosidase at baseline and after 1, 2, 3, 4 and 5 years of treatment. (B) Time to reach a chitotriosidase of <5000 nmol/mL/hr. Chitotriosidase levels of carriers of the chitotriosidase null mutation were multiplied by two [9]. Abbreviations: AMC, Academic Medical Center, Amsterdam; HHU, hospital of the Heinrich-Heine-University, Düsseldorf; ERT, Enzyme Replacement Therapy.

Figure 4. Impact of ERT on changes in bone marrow burden score. (A) Time to reach a decrease of 2 points in BMB score, as measured by MRI, from baseline of patients with a baseline BMB of 2-8 (B) Time to reach a decrease of 2 points in BMB score from baseline of patients with a baseline BMB of 6-8. Abbreviations: AMC, Academic Medical Center, Amsterdam; HHU, hospital of the Heinrich-Heine-University, Düsseldorf; BMB, bone marrow burden, as determined by MRI [11]; ERT, Enzyme Replacement Therapy.
Discussion

We previously reported that MIP-1α and MIP-1β, both implicated in skeletal complications in multiple myeloma, are significantly elevated in plasma of Gaucher patients and demonstrated that plasma MIP-1β levels correlate with ongoing skeletal disease. We also demonstrated that inflammatory cells surrounding the Gaucher cells are the source of MIP-1β, and not the alternatively activated mature Gaucher cells. Recently de Fost et al. [5] showed that bone marrow involvement as assessed by magnetic resonance imaging, improved more quickly and was more pronounced in patients treated with a higher ERT dosing regimen. A similar observation was made for correction of plasma chitotriosidase, a surrogate marker for total storage burden, which, unlike MIP-1β, is secreted by the mature Gaucher cells. In this retrospective study in a subset of patients that were matched for the degree of marrow involvement (BMB score), we investigated whether a difference in ERT dosing also influences the response in plasma MIP-1β levels. For this purpose, we compared the response in plasma MIP-1β levels in adult Gaucher type I patients that were treated with a relatively low (AMC) and relatively high dosage (HHU) of ERT. We found that plasma MIP-1β, like chitotriosidase, responds faster during the first 12 months of therapy. Prolonged treatment also resulted in a decrease of MIP1-β (< 85 pg/mL) in patients receiving a lower enzyme dose. As has been reported previously, improvement in BMB, a MRI based scoring system of bone marrow involvement, showed a strong trend towards a better response to higher doses, particularly in patients with more extensive bone marrow disease BMB ≥ 6 in whom the difference is statistically significant.

In conclusion, plasma MIP-1β correction in Gaucher patients is ERT dose dependent in the first year of treatment. After prolonged treatment no longer significant differences in MIP-1β response are observed when comparing patients treated with a relatively low or high dosing regimen. Thus, not only the initial correction of Gaucher cells but also that of surrounding phagocytes is enzyme dose-dependent.

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References

enjoy the correction of MIP-1β in initial phase of ERT


