LDL receptor-related protein: Molecular analysis and identification of new ligands
Neels, J.G.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Chapter 6

Soluble Low Density Lipoprotein Receptor-related Protein as a Novel Therapeutic Marker for Gaucher Disease

Jaap G. Neels, Sonja van Weely, Birgit M.M. van den Berg, Carla E.M. Hollak,* Anton-Jan van Zonneveld, Hans Pannekoek, Johannes M.F.G. Aerts

From the Department of Biochemistry and †Department of Hematology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands.

Submitted for publication.
Summary

Gaucher disease is one of the most frequently encountered inherited lysosomal storage disorders in man. The disease is due to a primary deficiency in lysosomal glucocerebrosidase activity and is characterized by an excessive accumulation of macrophages throughout the body. In the present study, we measured the level of a soluble form of the low density lipoprotein receptor-related protein (LRP) in sera of a total of 61 Gaucher patients. The mean soluble LRP (sLRP) level was increased by 2.7-fold in sera of these patients as compared to the mean sLRP serum-level measured in 50 normal individuals. When 38 patients were treated with enzyme supplementation therapy the sLRP serum-levels rapidly decreased to normal levels. Preliminary analysis of patients receiving the novel oral substrate reduction therapy, OGT 918, also resulted in normalization of sLRP serum-levels.

This is the first study reporting increased serum-levels of sLRP in an inborn error of metabolism and our findings suggest that sLRP can be used to monitor efficacy of treatment of Gaucher patients.

Introduction

The low density lipoprotein receptor-related protein (LRP) is a member of the low density lipoprotein (LDL) receptor family of endocytic receptors (reviewed in Refs. 1 and 2). LRP can bind a diverse spectrum of structurally unrelated ligands in a calcium-dependent manner including apolipoproteins, lipases, proteinases, proteinase-inhibitor complexes, Kunitz-type inhibitors, matrix proteins and several others (1,2). The receptor-associated protein (RAP) inhibits the binding of all ligands to LRP and functions intracellularly as a chaperone for LRP (3). LRP mediates the binding and transport of ligands from the cell surface to the endosomal/lysosomal degradation pathway. Recently, LRP was shown to interact with cytosolic signaling proteins like Disabled-1 and FE65 and can therefore also act as a transducer of extracellular signals (4). The broad range of ligands together with the endocytic and signal transducing properties of LRP suggest a role for the receptor in distinct physiological and pathophysiological processes, ranging from lipoprotein metabolism, cell growth and cell migration, fibrinolysis and thrombosis to atherosclerosis and Alzheimer’s disease. LRP is abundantly present in various tissues like liver, placenta, lung and brain, and is expressed in an array of cell types, including parenchymal cells, Kupffer cells, neurons, astrocytes, smooth muscle cells, monocytes, adipocytes and fibroblasts (1,2). In the mature form, LRP consists of an α- and β-chain that are tightly associated in a non-covalent fashion (5). The β-chain contains a transmembrane domain and a short cytoplasmic tail that is essential for endocytosis and signal transduction (6,7). The α-chain functions as a large ectodomain that interacts with the ligands of the receptor (5,8).

Recently, a soluble form of LRP (sLRP) was identified in plasma (9,10). This sLRP consists of a truncated extracellular portion of the β-chain associated with an intact α-chain (11). The mechanism by which sLRP is released from the cell-surface seems to involve the endoproteolytic action of a so far unidentified metalloproteinase (11). However, the precise origin of sLRP in plasma is still unclear. After shedding from the
cell-surface, sLRP retains its ligand binding properties. Previously, it was reported that the concentration of plasma sLRP appears to alter in some patients with impaired liver function and there is preliminary evidence suggesting the presence of elevated levels in patients with atherosclerosis in whom coronary and/or peripheral vascular disease had been evaluated (9,10).

The monocyte is the only cell-type in the circulation that expresses LRP (12) and LRP has been defined as a monocyte differentiation antigen (CD91) (13). During the differentiation of monocytes to macrophages there is a major increase in the expression of the receptor (14). This is also true for the expression of metalloproteinases; expression of multiple metalloproteinases increases during monocyte to macrophage differentiation (15). This led to our hypothesis that an accumulation of macrophages in the body may lead to a higher sLRP level in serum. To test our hypothesis we decided to measure the sLRP levels in sera from Gaucher patients.

Gaucher disease, also known as glucosylceramidosis, is one of the most frequently encountered inherited lysosomal storage disorders in man and is characterized by an excessive accumulation of macrophages throughout the body. The disease is due to an autosomal recessively inherited deficiency in lysosomal glucocerebrosidase activity (EC 3.2.1.45), resulting in accumulation of its substrate glucocerebroside, also known as glucosylceramide, in macrophages (16). The characteristic lipid-laden macrophages, Gaucher cells, may be present in all organs, but usually there is a preferential accumulation in spleen, liver and bone marrow. The presence of Gaucher cells underlies the common symptoms in Gaucher patients, such as hepatomegaly, splenomegaly, pancytopenia and skeletal deterioration (16,17). The clinical manifestations of Gaucher disease are highly variable with respect to age of onset, progression, severity and neurological involvement. Three phenotypes are distinguished on the basis of onset of neurological symptoms: type 1, the 'adult' or non-neuronopathic form; type 2, the 'infantile' or acute neuronopathic form, and type 3, the 'juvenile' or sub-acute neuronopathic form (16,17). Type 1 Gaucher disease is the most prevalent phenotype. Presently, Gaucher patients are treated successfully by chronic intravenous administration of recombinant glucocerebrosidase (18). Recently, the outcome of a clinical trial with type 1 Gaucher patients based on inhibition of glucocerebroside biosynthesis has been reported (19). The iminosugar, N-butyldeoxynojirimycin (OGT 918, Vevesca™) is a reversible inhibitor of the ceramide-specific glucosyltransferase that catalyzes the formation of glucocerebroside - a treatment approach which is termed substrate reduction therapy. Since enzyme supplementation therapy is extremely costly and the monitoring of the clinical response of Gaucher patients to both types of therapy may be quite difficult, secondary biochemical abnormalities as possible early indicators of response to treatment are very useful.

In this study we show that the serum sLRP level is increased in Gaucher patients and rapidly decreases during both enzyme supplementation and substrate reduction therapy. Therefore, serum soluble LRP might be useful as a sensitive harbinger of efficacy of treatment in Gaucher patients.
Experimental procedures

Patient materials- A total of 61 Gaucher patients, diagnosed on the basis of clinical signs, demonstration of deficient glucocerebrosidase activity and genotyping, were examined. They included 57 patients with type 1 and 4 patients with type 3 Gaucher disease as classified according to the criteria described (17) and classification was confirmed by determining the glucocerebrosidase genotype (20). The patients were known to us by referral to the Academic Medical Center. The clinical manifestations of the type 1 patients were classified using the modified severity scoring index (SSI), which is based on an assessment of the extent of liver, spleen and bone involvement and the severity of pancytopenia (21). Disease state was considered mild when SSI was between 1 to 6, moderate when between 7 to 14, and severe when between 15 to 19. Using the SSI, mild disease was present in 25 patients, moderate disease in 25 patients and severe disease in 7 patients. The control population consisted of 50 healthy volunteers, all of whom were found to have normal glucocerebrosidase activity. Thirty-six of the type 1 Gaucher patients (14 mild, 15 moderate and 7 severe) and 2 of the type 3 patients were studied during therapy with intravenous enzyme supplementation therapy. Most type 1 patients started with placenta-derived glucocerebrosidase (Ceredase, alglucerase injection, Genzyme, MA) at a dose of 1.15 U/kg three times a week (15 U/kg per month) and type 3 patients with 25 U/kg twice a week. The dose was adjusted in time according to the individual responses based on previous described criteria (22). During therapy, all patients have switched over to recombinant glucocerebrosidase (Cerezyme, imiglucerase injection, Genzyme, MA). Six type 1 Gaucher patients were studied before and after one year of substrate reduction therapy with oral administration of OGT 918 (N-butyldeoxynojirimycin, Oxford GlycoSciences, Oxon, UK) at a starting dose of 100 mg three times daily. The dose was adjusted during the study as described (19).

Serum samples were usually prepared immediately after collection of blood and were stored at -20 °C. We noted that the sLRP measurements in serum, citrate- or heparin-plasma gave similar results using the assay described below. However, EDTA plasma was not suitable for this calcium-dependent assay.

Proteins and reagents- Recombinant RAP was synthesized as a glutathione S-transferase fusion protein (GST-RAP) in Escherichia coli and purified as described previously (23). The RAP-GST/pGex plasmid was a generous gift from Dr. J. Kuiper (Sylvius Laboratory, University of Leiden, Leiden, The Netherlands). Purified human placental LRP was kindly provided by Dr. S.K. Moestrup (Institute of Medical Biochemistry, University of Aarhus, Aarhus, Denmark) and was confirmed to be free of contaminating RAP by SDS-PAGE analysis and silver staining. Isolation of the monoclonal Fab fragment Fab A2 was performed as described (24). Proteins were biotinylated using an EZ-Link™ sulfo-NHS-LC-biotinylation kit, following the instructions of the supplier (Pierce, Rockford, IL). Protein concentrations were determined using a microBCA protein assay reagent kit (Pierce, Rockford, IL). All other chemicals used were reagent grade from Sigma (St.Louis, MO) or Merck (Darmstadt, Germany).

Soluble LRP assay- The sLRP assay was conducted essentially as described by Quinn et al (9). Briefly, this assay detects (s)LRP, captured on a RAP-coated plate, using the
anti-LRP α-chain monoclonal Fab fragment, Fab A2. This Fab fragment was previously selected for LRP binding from a phage display library (24). Microtiter plates (Maxisorp, Nunc, Denmark) were coated with 1 μg/well GST-RAP (50 μl) diluted in sodium carbonate buffer, pH 8.6 (16 hr, 4 °C). After blocking with 3% BSA in PBS for 30 min at 37 °C, 60 μl of a 6 times diluted serum sample in assay buffer (20 mM HEPES, 0.15 M NaCl, 5 mM CaCl₂, 0.05% Tween 20, 1% BSA, pH 7.4) was added to the well and incubated 2 h at 37 °C. A standard curve was prepared by incubating 50 μl/well of affinity-purified placental LRP dilutions in the concentration range 0.1-0.5 μg/ml. After washing, the plates were incubated with 50 μl/well of a biotinylated anti-LRP Fab fragment (Fab A2, 250 ng/ml) in assay buffer, 1 h at 37 °C. After washing, 50 ul of 1:5000 dilution streptavidin-horseradish peroxidase (Amersham Pharmacia Biotech, Roosendaal, The Netherlands) was added (30 min, 37 °C), and bound proteins were quantified with 200 μl/well chromogenic substrate (0.1 mg/ml tetra-methyl-benzidin (Merck, Darmstadt, Germany), 0.03% (v/v) H₂O₂ diluted in citrate buffer, pH 5.5). Color development was stopped by addition of 50 μl/well 1M H₂SO₄, and the optical density at 450 nm (A₄₅₀) was determined. Soluble LRP concentrations were calculated from the standard curve and was corrected for mass difference between full-length LRP (600 kD) and sLRP (570 kD). Experiments were performed in duplicate. As a control binding to immobilized BSA was measured.

Enzyme assays- Chitotriosidase activity was measured as described before (25). The assay mixture contained 0.027 mM 4-methylumbelliferyl-tri-N-acetylglucosaminide (Sigma, St. Louis, MO), 0.1% BSA and 0.1/0.2 M citrate/phosphate buffer (pH 5.2). β-Hexosaminidase activity was measured with 1.6 mM 4-methylumbelliferyl-β-N-acetylglucosaminide (Sigma, St. Louis, MO) as substrate in 0.05/0.1 M citrate/phosphate buffer (pH 4.0). Tartrate-resistant acid phosphatase activity was measured using 4-methyl-umbelliferyl phosphate as substrate in the presence of 3 M mercaptoethanol as described by Chambers et al (26). Angiotensin-converting enzyme activity was measured using hippuryl-L-histidyl-L-leucine as substrate.

Statistics- Data are expressed either as median with range or means plus or minus SD. All statistic analyses were performed using the SPSS version 10.0.5 software. Significance of differences between the experimental groups was evaluated using non-parametric tests. The sLRP data from the control group were compared with the sLRP data from Gaucher patients that had not received therapy using the Mann-Whitney test. The Wilcoxon signed ranks test was used to determine the significance of the difference in sLRP level in Gaucher patients before and during enzyme supplementation therapy. P < 0.05 was considered to represent a significant difference. Non-parametric Spearman’s (rho) test was performed to determine the correlation between sLRP and other parameters.

Results

Soluble LRP serum-levels in Gaucher patients
To measure the sLRP levels in serum we used an ELISA assay similar to the one Quinn et al used previously (9). This assay is relatively easy to perform and consists of coating the ligand RAP onto a microtiter plate, incubate with a serum dilution and, after washing, detect the sLRP bound to immobilized RAP using a LRP-specific antibody. It
is of interest to mention that we could confirm the observation of Quinn et al (11) that sLRP serum-levels are increased in cord blood, which validates our assay (data not shown). Furthermore, our analysis revealed a marked elevation of sLRP levels in the serum of a total of 61 Gaucher patients tested, compared to the levels detected in 50 normal individuals (Figure 1) (P<0.0001). Of the 57 tested Gaucher disease type 1 patients, the mean serum sLRP level was 1.12 μg/ml and ranged from 0.52 to 2.15 μg/ml whereas the normal mean serum sLRP level was 0.43 μg/ml and ranged from 0.07 to 1.01 μg/ml (Table 1). The 4 Gaucher disease type 3 patients (indicated with the open circles in Figure 1) had a higher serum sLRP level compared to type 1 patients. Their mean sLRP level was 1.96 μg/ml and ranged from 1.69 to 2.28 μg/ml.

In general the Gaucher patients tested had, on average, a 2.7-fold higher serum sLRP level compared to normal individuals.

![Graph](image)

**Fig. 1:** Comparison of serum sLRP levels in controls (n= 50) versus type 1 (●) and type 3 (○) Gaucher patients before (●, n=57; ○, n= 4) and upon (●, n=36; ○, n= 2) 2 to 9 years of enzyme supplementation therapy. — indicates mean level. Data are means of duplicate experiments and are represented as percentages with 100% = control mean. The non-parametric Mann-Whitney test was used to determine the significance of the difference in serum sLRP levels between the controls and untreated Gaucher patients. The significance of the difference in serum sLRP levels in Gaucher patients before and during enzyme supplementation therapy was analyzed using the non-parametric Wilcoxon signed ranks test.

**Soluble LRP serum-levels upon enzyme supplementation therapy**

Next, the serum sLRP levels in thirty-six type 1 and two type 3 patients that received between 2 to 9 years of enzyme supplementation therapy was measured. As is depicted in Figure 1 and Table 1, the treated Gaucher patients had serum sLRP levels similar to the control group. The mean sLRP level in the thirty-six type 1 sera was 0.42 μg/ml and ranged from 0.15 to 0.91 μg/ml during treatment. Similar, the sera of the two treated type 3 patients contained 0.13 and 0.46 μg/ml sLRP, respectively.
Table 1: Serum sLRP levels in Gaucher disease patients

<table>
<thead>
<tr>
<th>Patients</th>
<th>sLRP level before therapy (\bar{X} \pm SD) (^b)</th>
<th>sLRP level during treatment (\bar{X} \pm SD) (^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gaucher disease type 1</td>
<td>1.12 ± 0.40 (n=57) (range: 0.52-2.15)</td>
<td>0.42 ± 0.16 (n=36) (range: 0.15-0.91)</td>
</tr>
<tr>
<td>Gaucher disease type 3</td>
<td>1.96 ± 0.25 (n=4) (range: 1.69-2.28)</td>
<td>0.13 and 0.46 (n=2)</td>
</tr>
<tr>
<td>Normal individuals</td>
<td>0.43 ± 0.22 (n=50) (range: 0.07-1.01)</td>
<td>ND</td>
</tr>
</tbody>
</table>

\(^a\) sLRP levels were measured in sera taken from patients before they underwent enzyme supplementation therapy.

\(^b\) \(\mu g/ml\).

\(^c\) Patients received between 2 to 9 years of enzyme supplementation therapy.

In addition, the decrease in sLRP serum-levels, in time, in a set of 14 Gaucher patients treated with enzyme supplementation therapy was measured. Some typical results representative for the set of patients tested are shown in Figure 2 and Table 2. Patient nr.1 and patient nr.2 (Figure 2A and 2B, respectively) are examples of Gaucher type 1 patients of whom clinical manifestations were classified as being moderate by using the severity scoring index (SSI, see Table 2), which is based on an assessment of the extent of liver, spleen and bone involvement and the severity of pancytopenia. Based on clinical improvement with respect to hematological markers and reduction in organomegaly these patients were classified as good responders to enzyme supplementation therapy. The initial sLRP serum-levels in these patients before the start of therapy (1.48 and 1.82 \(\mu g/ml\), respectively) were in the higher range of levels measured in this study and decreased during therapy to ultimately reach normal levels. In contrast, patient nr.3 is an example of a Gaucher type 1 patient with severe clinical manifestations and was less responsive to enzyme supplementation therapy. This patient also started therapy with a high serum-level of sLRP (1.25 \(\mu g/ml\)) but, although the level decreased in time, the level stayed in the upper part of the normal range. Finally, patient nr.4 is an example of a mild disease Gaucher patient on whom enzyme supplementation therapy was successfully applied. The low sLRP level at the initiation of therapy (0.66 \(\mu g/ml\)) seems to reflect the mild disease-state of the patient. During therapy the sLRP level nonetheless decreased and dropped even below normal mean levels. Overall, these results indicate that the sLRP serum-levels decrease to normal levels during enzyme supplementation therapy.

**Comparison of sLRP serum-levels with other Gaucher disease markers**
In Gaucher disease a number of plasma abnormalities can be found (27). The most important abnormalities that have been identified today are increased levels of chitotriosidase, \(\beta\)-hexosaminidase, tartrate-resistant acid phosphatase (TRAP), and angiotensin-converting enzyme (ACE). Intra-individual comparisons between the different biochemical markers in time during therapy can prove useful for monitoring the effect of therapy. Therefore, next to serum sLRP levels we measured the activities of chitotriosidase, \(\beta\)-hexosaminidase, TRAP and ACE in time during enzyme supplementation therapy (see Table 2).
Patient nr. 1 showed a gradual decrease in serum level of each parameter during seven years of enzyme therapy but, except for sLRP, most markers did not return to control range levels. A slight increase in the levels of β-hexosaminidase, ACE and sLRP occurred during the seventh year that might indicate a progression in disease state during this last year of therapy.

Except for TRAP, there was a significant decrease of all parameters in patient nr. 2 during therapy. However, most markers, except for sLRP and β-hexosaminidase, did not reach control range levels.

In patient nr. 3, all markers, except for chitotriosidase, fluctuated throughout the entire period of therapy around the level measured before starting therapy. This is in agreement with a minor response to therapy.

The mild disease patient nr. 4 showed a fast decrease to a steady-state serum level of each parameter. Only sLRP and β-hexosaminidase reached control range levels, but then again these levels were already within control range before start of therapy.

In conclusion, in most cases of the subset of Gaucher patients analyzed (*n* = 14) a gradual decrease in serum level of each parameter was observed during enzyme supplementation therapy but, except for sLRP and β-hexosaminidase, no complete correction in serum enzyme levels was reached.

Fig. 2: Decrease in serum sLRP level in time during enzyme supplementation therapy in four type 1 Gaucher patients (A–D). Control mean (—) plus or minus SD (...) is indicated. Data are means of duplicate experiments and are represented as percentage reduction from initial value (=100%).
## Table 2: Severity scoring index, genotype and serum levels of chitotriosidase, β-hexosaminidase, TRAP, ACE, and sLRP at different timepoints of enzyme supplementation therapy in Gaucher disease type 1 cases

<table>
<thead>
<tr>
<th>Patients</th>
<th>Time (yr)</th>
<th>SSI</th>
<th>Genotype</th>
<th>Chitotriosidase$^a$</th>
<th>β-hexosaminidase$^a$</th>
<th>TRAP$^a$</th>
<th>ACE$^b$</th>
<th>sLRP$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>9</td>
<td>N370S/RecNci</td>
<td>25446</td>
<td>4111</td>
<td>4316</td>
<td>n.d.</td>
<td>1.48</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td>21964</td>
<td>2562</td>
<td>3046</td>
<td>255</td>
<td>0.72</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>14813</td>
<td>2244</td>
<td>2610</td>
<td>233</td>
<td>0.87</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td>9757</td>
<td>2318</td>
<td>2236</td>
<td>185</td>
<td>0.46</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td>7274</td>
<td>1961</td>
<td>1920</td>
<td>172</td>
<td>0.39</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td>6179</td>
<td>2076</td>
<td>1872</td>
<td>183</td>
<td>0.75</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>7</td>
<td>N370S/L444P</td>
<td>29562</td>
<td>2224</td>
<td>617</td>
<td>183</td>
<td>1.82</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>16091</td>
<td>1407</td>
<td>464</td>
<td>118</td>
<td>1.57</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>10118</td>
<td>1216</td>
<td>706</td>
<td>80</td>
<td>0.99</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td>5083</td>
<td>1036</td>
<td>456</td>
<td>62</td>
<td>0.45</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td>2704</td>
<td>1132</td>
<td>363</td>
<td>52</td>
<td>0.81</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td>2550</td>
<td>1155</td>
<td>551</td>
<td>66</td>
<td>0.35</td>
</tr>
<tr>
<td>3$^d$</td>
<td>0</td>
<td>15</td>
<td>N370S/N370S</td>
<td>14822</td>
<td>3276</td>
<td>2784</td>
<td>198</td>
<td>1.25</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>12715</td>
<td>2966</td>
<td>808</td>
<td>183</td>
<td>0.97</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>10795</td>
<td>2330</td>
<td>964</td>
<td>150</td>
<td>0.83</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td>8590</td>
<td>2866</td>
<td>1158</td>
<td>191</td>
<td>0.79</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td>8249</td>
<td>2838</td>
<td>1327</td>
<td>146</td>
<td>0.59</td>
</tr>
<tr>
<td>7.5</td>
<td></td>
<td></td>
<td></td>
<td>7214</td>
<td>3400</td>
<td>1170</td>
<td>190</td>
<td>0.78</td>
</tr>
<tr>
<td>4$^d$</td>
<td>0</td>
<td>4</td>
<td>N370S/?</td>
<td>14563</td>
<td>1311</td>
<td>1291</td>
<td>156</td>
<td>0.66</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>2554</td>
<td>612</td>
<td>347</td>
<td>72</td>
<td>0.45</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>1876</td>
<td>820</td>
<td>415</td>
<td>76</td>
<td>0.41</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td>2015</td>
<td>1082</td>
<td>459</td>
<td>72</td>
<td>0.23</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td>1847</td>
<td>1045</td>
<td>603</td>
<td>85</td>
<td>0.37</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td>1482</td>
<td>954</td>
<td>607</td>
<td>68</td>
<td>0.34</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Control mean</th>
<th>Control range</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>863</td>
</tr>
<tr>
<td>206</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

$^a$ nmol/ml per h $^b$ U/l $^c$ Second allele is unknown. $^d$ Carrier of an inherited defect in the chitotriosidase gene (28).

### Preliminary analysis of soluble LRP serum-levels upon substrate reduction therapy

In addition to enzyme supplementation therapy promising results were recently reported for a clinical trial with substrate reduction therapy (19). Therefore, a preliminary analysis of serum sLRP levels was performed in a subset of six Gaucher type 1 patients before and after one year of oral treatment with OGT 918. Four out of these six patients showed a decrease of serum sLRP levels reaching normal levels after one year of therapy. Figure 3 shows the response of one of these four patients. The other two patients had serum sLRP levels that were already in the normal range before start of therapy and remained the same after one year of treatment. From this we conclude that a correction in sLRP serum-levels is observed during both enzyme supplementation and substrate reduction therapy.
Fig. 3: Comparison of serum sLRP level in a type 1 Gaucher patient before and after one year of oral treatment with OGT 918. This is representative for a group of four out of six patients tested. Data are means of duplicate experiments and are represented as percentages with 100 % = control mean.

Discussion

This is the first study reporting increased serum-levels of sLRP in an inborn error of metabolism. Our results indicate an increase of, on average, 2.7-fold in sLRP level in sera of Gaucher patients compared to healthy individuals (Figure 1, Table 1). This raises the question whether serum sLRP measurement is of value for the clinical management of Gaucher disease, a disorder for which a costly therapeutic intervention exists. It is generally accepted that the diagnosis of Gaucher disease always has to be primarily established on the basis of the demonstration of deficient glucocerebrosidase activity. Nevertheless, abnormalities in serum levels of biochemical markers such as chitotriosidase, β-hexosaminidase, TRAP and ACE in Gaucher disease are often employed to further confirm the diagnosis. Except for chitotriosidase, these abnormalities have in common that they are neither universal nor very pronounced (27). The latter holds true for the increase in sLRP level, as can be deduced from the overlap in sLRP levels measured in the control group with the levels measured in Gaucher patients (Figure 1, Table 1). In this respect, the abnormality in serum chitotriosidase is far more striking than that in the other parameters. However, about 6% of all Gaucher patients, as noted for control subjects, completely lack serum chitotriosidase activity as a result of a recessively inherited defect in the chitotriosidase gene (28). The abnormalities in β-hexosaminidase, TRAP, ACE, and chitotriosidase are not unique for Gaucher disease. For example, modest elevations in plasma chitotriosidase activity are also encountered in some other pathologies, e.g. some distinct lysosomal lipid storage disorders, leishmaniasis, beta-thalassemia, sarcoidosis and chronic granulomatous disease (CGD) (25,29,30). We tested whether the sLRP level was increased in two sets of nine patients diagnosed for sarcoidosis or CGD, respectively, but did not detect increased sLRP levels (data not shown). This suggests that, as a marker, sLRP is more specific for Gaucher disease as compared to the other markers.

Although it has to be concluded that serum sLRP measurement is not extremely valuable for further confirmation of diagnosis of Gaucher it might still be a very useful
tool for monitoring disease progression and the response to both enzyme supplementation therapy and substrate reduction approaches. It is evident from Figures 1 to 3, and Table 2 that despite the inter-individual variation in the levels of all markers, a gradual decrease in serum level of each parameter was observed in most cases. Only for sLRP and β-hexosaminidase complete correction in serum enzyme levels was reached during enzyme supplementation therapy (Table 2). From this we might conclude that serum enzymes like TRAP and ACE, and particularly chitotriosidase, may rather reflect the mature Gaucher cell mass that is present in the body. In contrast, sLRP and β-hexosaminidase might rather be markers for the newly developing Gaucher cells and may thus serve as more specific indicators of disease progression rather than of the total body number of mature Gaucher cells. In this respect it is interesting to note that we observed that in general both the excess serum sLRP level and β-hexosaminidase activity show a faster response to enzyme therapy compared to the other parameters. This is even more pronounced with substrate reduction, an approach that primarily aims to reduce the formation of novel lipid laden cells. Because Gaucher disease is intrinsically more progressive in type 3 patients, the high sLRP levels in sera of these patients are also in agreement with serum sLRP being a potential disease progression marker. In addition, these patients show relatively low chitotriosidase levels suggesting that compared to type 1 patients there are relatively more newly formed storage cells than mature Gaucher cells. A larger number of (the rare) type 3 patients will have to be analyzed to substantiate the significance of these observations.

A sensitive marker for monitoring the rate of novel storage cells formation in Gaucher patients, as a measure for actual disease progression, is presently highly warranted for optimal clinical decision making. Monitoring of serum sLRP may prove to be of value in this respect both in connection with initiation of therapy and optimization of therapeutic intervention.

The precise relationship of serum sLRP with the presence of Gaucher cells appears to be complex. We could not detect sLRP in conditioned media from in vitro cultures of primary macrophages, although LRP mRNA derived from these cultures increased in time based on analysis of Northern blots (data not shown). In this respect, it should be noted that Quinn et al could detect sLRP in conditioned media from primary hepatocytes but not from the human hepatoma cell line HepG2, nor from cultured normal human fibroblasts, suggesting that the release of sLRP is not a constitutive property of all cultured cells that express LRP (9). Therefore, we can not exclude that in vivo sLRP can be shed from (Gaucher) macrophages by either the action of a (metallo)proteinase that is expressed by a different cell type or by a (metallo)proteinase that is only expressed by macrophages under certain in vivo conditions. The increase of sLRP could also simply reflect the general high degree of proteinase activity in Gaucher serum (31) and, in that respect, might still be a marker of disease progression. To enhance our insight into the relationship of sLRP with Gaucher cells, more specific research as to the source of this factor is needed.

Due to marked inter-individual variation in the levels of the biochemical markers analyzed there are only weak, though significant, positive correlations between sLRP and the other markers (data not shown). Soluble LRP levels correlated best with β-hexosaminidase activity (rho=0.5, P<0.0001) as compared to the other parameters, in agreement with both being potential indicators of disease progression. Severity scoring
indexes are mainly based on the extent of organ involvement in Gaucher disease and are not fully accurate in classifying the severity of the disease. As a result the positive correlation between a certain marker and SSI value is also significant but weak (data not shown). This is also true for sLRP and SSI. These observations are illustrated by the data presented in Table 2. We also analyzed whether there was a correlation between sLRP and parameters such as weight, hemoglobin levels, platelet count, and spleen and liver volume. No significant and/or strong correlations were observed (data not shown). LRP binds multiple classes of ligands and has been implicated in a broad range of normal and disease processes ranging from lipoprotein metabolism, cell growth and cell migration, fibrinolysis and thrombosis to atherosclerosis and Alzheimer’s disease. The soluble form of this receptor might influence the activity of the membrane-bound counterpart by affecting its interaction with ligands. However, although we can not exclude that local concentrations of sLRP might be high, the low circulating soluble receptor concentration (nanomolar range) is probably insufficient to effectively compete ligand binding to the cell-bound molecule. In contrast, the large masses of macrophages in Gaucher patients that highly express LRP might have an impact on the catabolism of ligands and therefore on certain processes in which cell-bound LRP is involved. In this respect, LRP might be linked to Gaucher disease in several ways. For example, abnormalities in lipoprotein metabolism reported in Gaucher patients (32) may in part be explained by increased catabolism of VLDL and chylomicron remnants through endocytosis by LRP on macrophages. Secondly, we recently identified both coagulation factor IXa as well as its cofactor VIII as ligands of LRP and suggested a modulatory role of LRP in coagulation (33-35). This might be relevant in the context of reported deficiencies of certain coagulation factors and abnormalities in coagulation in Gaucher patients (36). Finally, it is of interest to note that LRP can mediate cellular uptake and lysosomal delivery of sphingolipid activator protein (SAP) precursor (37). Proteolytic processing of this precursor generates SAP-A, -B, -C and -D, the so-called saposins and these function as the obligatory activators of lysosomal enzymes involved in glycosphingolipid metabolism including glucocerebrosidase (38).

In conclusion, we have identified sLRP as an additional marker in Gaucher disease that can be used for monitoring the efficacy of therapeutical intervention. Future studies should resolve the precise relationship of this marker with the presence of storage cells and the practical value of regular measurements of sLRP levels in Gaucher patients. Further research on the physiological consequences of chronically elevated serum sLRP levels as exist in Gaucher patients is also warranted.

Acknowledgements

We gratefully acknowledge the help of Drs. H.D. Bakker and D.K. Bosman and research nurses M. Ek and M. Wiersma in collecting serum samples. In addition, we like to thank Profs. T.M. Cox and A. Zimran for supplying some of the substrate reduction therapy serum-samples. We are very grateful to the patient members of the Dutch Gaucher Society and their relatives, and to all other Gaucher patients for their cooperation. This work was supported by Grant 902-26-175 from the Netherlands Organization for Scientific Research (J.G.N.).


