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Gepke Visser · Jan Peter Rake · Philippe Labrune
James V. Leonard · Shimon Moses · Kurt Ullrich
Udo Wendel · Klaas H. Groenier · G. Peter A. Smit

Granulocyte colony-stimulating factor in glycogen storage disease type 1b. Results of the European Study on Glycogen Storage Disease Type 1

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Abstract Patients with glycogen storage disease type 1b (GSD-1b) have neutropenia and neutrophil dysfunction that predispose to frequent infections and inflammatory bowel disease (IBD), for which granulocyte colony-stimulating factor (GCSF) is given. To investigate the use and the value of GCSF treatment in GSD-1b, a retrospective registry of GSD-1 patients born between 1960 and 1995 in 12 European countries was established. Included were 57 GSD-1b patients. Unglycosylated GCSF was given to 18 patients, median age of starting therapy was 8 years, longest duration of therapy 7 years. Dose varied between 2–10 μg/kg, with a frequency from daily to twice per week. Neutropenia (defined as an absolute neutrophil count $<0.5\times10^9$/l) was found in 49 patients. In untreated patients, a significant decrease of haemoglobin, platelet counts and leucocyte counts with increasing age ($P<0.032$, $P<0.04$ and $P<0.001$ respectively) was noted, whereas neutrophil counts remained low but stable with increasing age. In nine patients who were treated longer than 1 year, median neutrophil counts increased significantly and simultaneously median leucocyte counts and platelet counts decreased significantly. In all patients treated, the number and severity of infections decreased and the severity of IBD improved subjectively. The most serious complication of GCSF treatment was marked splenomegaly (four patients). *Conclusion:* in this retrospective study a significant haematological effect was documented and a subjective improvement of infections and inflammatory bowel disease. In view of the uncertainty, prospective controlled trials seem warranted to clarify the indication for the use of granulocyte colony-stimulating factor in this disease.

Keywords Gylcogen storage disease type 1b · Granulocyte colony-stimulating factor · Neutropenia · Treatment

Abbreviations *ESGSD* European study on glycogen storage disease · *GCSF* granulocyte colony stimulating factor · *GSD* glycogen storage disease · *IBD* inflammatory bowel disease

G. Visser (✉) · J.P. Rake · G.P.A. Smit
Beatrix Children's Hospital, University Hospital, Groningen, The Netherlands

P. Labrune
Hospital Antoine-Béclère, Clamart, France

J.V. Leonard
Institute of Child Health, Great Ormond Street Hospital, London, UK

S. Moses
Department of Paediatrics, Soroka Medical Centre, Beersheva, Israel

K. Ullrich
Department of Paediatrics, University Hospital, Hamburg, Germany

U. Wendel
Department of Paediatrics, University Hospital, Düsseldorf, Germany

K.H. Groenier
Department of General Practice, University Hospital Groningen, The Netherlands

Present address: G. Visser
Wilhelmina Children's Hospital, University Hospital Utrecht, PO Box 85090, 3508 AB Utrecht, The Netherlands,
Tel.: +31-30-2504000, Fax: +31-30-2505350,
e-mail: g.visser@oprit.rug.nl

Introduction

Glycogen storage disease type 1 (GSD-1) (McKusick 232200), is caused by inherited defects of the glucose-6-phosphatase complex. This complex has a key role in both glycogenolysis and gluconeogenesis, converting glucose-6-phosphate to glucose. The clinical features are hepatomegaly, growth retardation, osteopenia and kidney enlargement with hypoglycaemia, hyperlactacidaemia, hyperlipidaemia and hyperuricaemia [10]. Based on the most plausible molecular model, glucose-

6-phosphatase is a multicomponent complex with a hydrolytic unit, glucose-6-phosphatase, and one or more membrane transporters [3,4]. Inborn errors of the catalytic unit of glucose-6-phosphatase are called GSD-1a. Defects of the putative transporters were named GSD-1b, GSD-1c and GSD-1d [20], but molecular genetic studies have shown that patients who had been diagnosed as GSD-1b, 1c and the putative 1d, all had mutations in the G6P transporter [2]. Recently a GSD-1c patient without mutations in G6P transporter gene was described suggesting the existence of a distinct GSD-1c locus [18]. In the present study, the term GSD-1b is used to include patients formerly diagnosed as GSD-1b, 1c and 1d. This corresponds to the clinical observations, as GSD-1 divides in two types: GSD-1a patients have 'classical' signs and symptoms as listed above, and GSD-1 non-a patients may also have neutropenia and neutrophil dysfunction that predisposes to severe infections and to inflammatory bowel disease (IBD).

Neupogen (Filgrastim), a recombinant granulocyte colony-stimulating factor (GCSF), has identical biological activity as endogenous GCSF, but contains a N-terminal methionine residue and is not glycosylated. Lenograstim is glycosylated GCSF and in vitro seems to be more potent and stable than Filgrastim. The clinical significance of these differences still has to be established [12,13]. During recent years, this growth factor has been used to accelerate the recovery of neutrophil counts after chemotherapy and also in patients with neutropenia due to other causes. In addition to its enhancing effect on the production of neutrophils, GCSF also modulates several neutrophil functions [15, 16, 24,25].

Patients with GSD-1b and neutropenia have been treated with GCSF since 1989. This resulted in an increase in neutrophil count and regression of the IBD [16, 21,22], but this effect has not been evaluated systematically. This is important because some of the complications of treatment with GCSF could be especially harmful to GSD-1b patients. Significant osteopenia has been described in patients with congenital neutropenia treated with GCSF [11] and there is an increased risk of osteopenia in IBD [7,26]. Osteopenia is a well recognised complication of GSD-1 [17,23] so patients with GSD-Ib on GCSF may be at particularly high risk of this complication. Malignant transformation during long-term treatment with GCSF has been reported in congenital neutropenia, but mainly in those patients with a GCSF receptor mutation [6,8].

The aim of the study was first to evaluate the use of GCSF treatment in GSD-Ib patients and second to investigate the value of GCSF treatment in GSD-Ib. As GSD-Ib is a rare disorder, this has been studied as part of the collaborative European Study on Glycogen Storage Disease Type 1 (ESGSD).

Patients and methods

Patients were identified from hospital records of 16 metabolic centres in 12 European countries. Patients were coded by initials and date of birth to check for duplication. Retrospective patient records were discussed in a multicentre meeting and filled in by either the treating physician or by one of the investigators (JPR). All patients known in the participating centres born after 1960 were included. Clinical details of the patients are described elsewhere [27]. The diagnosis of GSD-1b was established by absent or very low glucose-6-phosphatase-activity in intact microsomes and (sub)normal glucose-6-phosphatase-activity in disrupted microsomes [20], most times in combination with identification of mutations in the glucose-6-phosphate transporter.

Mean haematological values per patient were calculated over each year in all GSD-1b patients. Height measurements were expressed as SD score adjusted for age, sex and ethnic group. Spleen size was documented by ultrasound measurements and related to appropriate standards for age. In two patients treated with GCSF for >1 year bone mineral density of the lumbar spine (L1-L4) was studied longitudinally, using dual-energy X-ray absorptiometry. Z-scores for bone mineral density were obtained by comparing with age-matched (3–16 years) reference values. Although bone mineral density is negatively influenced by skeletal size, so bone mineral density in smaller subjects, such as GSD-1 patients with a stunted height, is underestimated. However, as the difference between the individual measurements was analysed, the patients acted as their own control.

Statistics

All data were analysed using non-parametric tests although some data had a normal distribution. The Mann-Whitney test was used to compare the haematological data of GSD-1b patients with and without GCSF test. The Wilcoxon signed rank was used to compare haematological data of GSD-1b patients before and during >1 year GCSF treatment. Haematological data in different age-groups were analysed using the Jonckheere-Terpstra test which is most appropriate for data with a natural order. Box-and-whisker plots were used as a graphical means of summarising the data. The box indicates the lower and upper quartiles and the centre line is the median. The points at the end of the whiskers are the 2.5% and 97.5% values and outliers points indicate the extreme values [1]. A P value of <0.05 was considered statistically significant.

Results

The ESGSD includes 288 patients with GSD-1, of whom 57 had GSD-1b. The GSD-1b patients were born between 1964–1995; 30 males and 27 females, of whom 49 are still alive. The median age when data were collected was 8.7 years. In most patients (51), the diagnosis was confirmed by liver biopsy. In six patients the diagnosis was based on clinical symptoms and a sibling with a diagnosis in enzyme assay.

Unglycosylated GCSF was used in 18 patients, one patient was given both unglycosylated and glycosylated GCSF. GCSF was always given subcutaneously. The median age of starting therapy was 8 years, with the longest duration of therapy 7 years. The indications for starting GCSF were severe IBD in seven patients (confirmed by colonoscopy and bowel biopsies), frequent or serious infections in seven patients (sepsis two, deep abscess two, respiratory tract infections five, pyogenous skin infections four, gastrointestinal infections four, urinary tract infections three), a combination of both infections and IBD in three other patients and preoperative one gift in one patient. Neutropenia alone was

not a reason to start treatment. The dose used varied from 0.5–3 μg/kg (four patients) to 4–5 μg/kg (11 patients) to 6–10 μg/kg (three patients). The frequency of GCSF administration was daily in eight patients, 2–4/week in seven patients, and intermittent in three patients.

Before any treament, the haematological values of untreated patients ($n=38$) (haemoglobin, platelets, total leucocytes, neutrophils, all non-significant) did not differ significantly from those who were subsequently treated with GCSF for more than 1 year ($n=11$). The haematological data in patients not receiving GCSF showed a significant decrease in haemoglobin, platelet counts and leucocyte counts with increasing age ($P<0.032$, $P<0.04$ and $P<0.001$ respectively), whereas neutrophil counts remained low but stable (not significant) (Fig. 1). By contrast, during treatment median neutrophil counts increased significantly ($p<0.043$), and platelet counts and leucocyte counts also decreased significantly ($P<0.028$ and $P<0.015$ respectively) (Fig. 2).

In all treated patients there was a positive clinical response as both the frequency and the severity of the infections and the IBD improved as subjectively documented by the treating physicians. One patient had still serious relapses of IBD, requiring hospitalisation and parenteral feeding, for which glycosylated GCSF was added to the treatment scheme. Despite this the patient still had recurrent infections and relapses of IBD. Although some individual patients treated with GCSF had catch-up on height, when the whole group was compared with an age and sex matched group of patients not treated with GCSF, there was no difference.

In GSD-1b patients no fractures were reported. Bone mineral density of the lumbar spine in two GSD-1b patients using GCSF >1 year were investigated longitudinally and decreased from SD −2.6 to −2.9 and from SD −2.8 to −2.9 after 4.3 and 1.5 years respectively.

Splenomegaly was reported in 20 of the 57 GSD-1b patients (35.1%), whereas this was only reported in 11 of the 198 GSD-1a patients (5.5%). Progressive splenomegaly was noted in four patients, most marked in two patients with pre-existing splenomegaly. One patient developed hypersplenism and the spleen size regressed when the frequency of GCSF was reduced from daily to twice per week. Transient bone pain was reported in two patients, transient fever and arthralgia in one patient. No side-effects were noted in ten patients.

Discussion

In this study, all GSD-1b patients treated with GCSF responded haematologically to low doses of unglycosylated GCSF (2–10 μg/kg per day). In patients treated for longer than 1 year, median neutrophil counts increased significantly and median platelet counts and total leucocyte counts decreased. Furthermore, there was a subjective by the treating physician reported, but not well documented, reduction in the frequency and severity of the infections and the severity of IBD. Thus the decrease in platelet and leucocyte counts might be a sign of

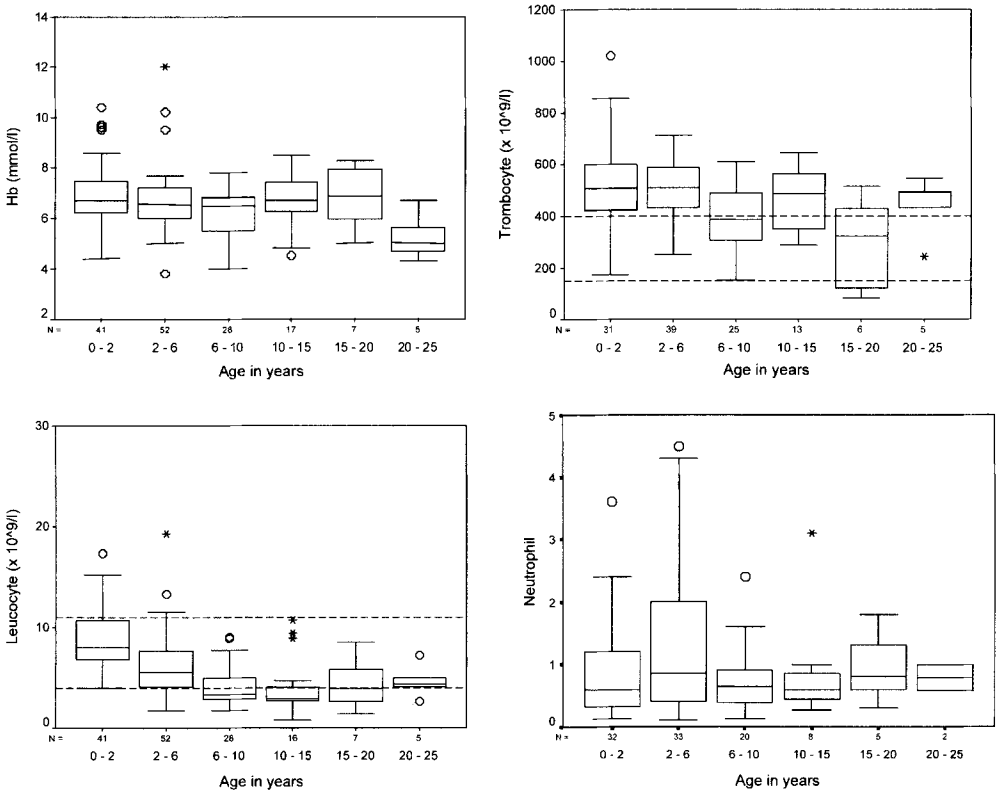

Fig. 1. Haematogical values per age group in GSD-1b patients. The box indicates the lower and upper quartiles and the centre line is the median. The points at the end of the whiskers are the 2.5% and 97.5% values and outliers points indicate the extreme values. In the figures of the platelets and the leucocytes, *dotted lines* represent borders of the normal values

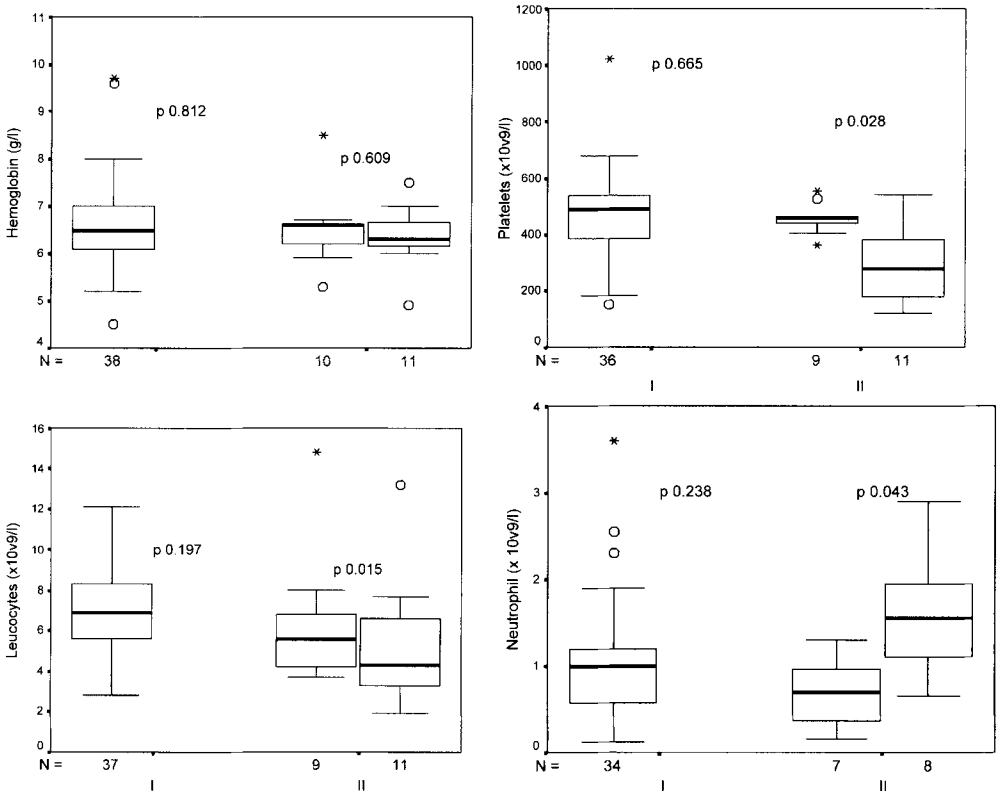

Fig. 2. Haemoglobin, platelet, leucocyte and neutrophil counts in GSD-1b patients without GCSF (*I*) and with GCSF (*II*). Of group II on the left side are values prior to treatment and on the right side are values > 1 year of GCSF treatment. *P* values comparing group I with group II prior to treatment are printed near I, *P* values comparing group II patients before and on treatment are printed above group II. The box indicates the lower and upper quartiles and the centre line is the median. The points at the end of the whiskers are the 2.5% and 97.5% values and outliers points indicate the extreme values

diminished inflammation. Haematological data of untreated patients showed a decrease in haemoglobin, platelets and total leucocyte counts with increasing age, but neutrophil counts which were low remained stable. The decrease in platelet and leucocyte counts in those on GCSF might therefore also represent in part the normal nature course.

Based on the retrospective data, the effect of GCSF on infection rate and IBD could not be quantified. This could, however, in part be due to the fact that retrospective data were used, in which the IBD was underdiagnosed and thus not sufficiently monitored [27]. Two patients with cyclic neutropenia and Crohn ileocolitis were described, who remained in complete remission during GCSF therapy [9]. In GSD-1b no complete remission of the IBD was found, despite the increase in neutrophil counts. However, GCSF does not correct neutrophil function completely [19]. The mechanism by which GCSF might have a beneficial effect on the IBD in GSD-1b is not clear. From studies in rats it is known that an inhibitor of neutrophil activation is effective in reducing colonic injury in both acute and reactivated colitis [28]. In experimental colitis high dose GCSF therapy resulted in a decrease of pro-inflammatory mediators [14].

In the present study, two treated patients studied longitudinally showed slightly progressive osteopenia. However, no untreated patients have been studied longitudinally, so the decrease might be the effect of aging. None have had severe bone complications so far. The nature of osteopenia in GSD-1 is still unknown: both deficient bone matrix formation (osteoporosis) and abnormal bone mineralisation (osteomalacia) have been suggested.

A serious adverse effect of GCSF treatment was splenomegaly, especially if there was pre-existing splenomegaly, which was also found in a recent report of 12 patients on GCSF [5]. One patient (not included in the study) developed splenomegaly on GCSF therapy for which splenectomy was performed but the patient died of postoperative complications. Autopsy showed massive extramedullary haematopoiesis, especially in the spleen. In view of the risk of hypersplenism, careful measurement of spleen size before and during treatment with GCSF is indicated.

Based on this retrospective study, apart from hematological effects no unequivocal improvement in outcome on GCSF in GSD-1b could be established. In view of the uncertainty prospective controlled trials seem warranted to clarify the indication for the use of GCSF in this disease. A proposal for treatment and follow-up of GSD-1b patients is found in this same issue.

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