



Treatment of Fabry disease with different dosing regimens of agalsidase: Effects on antibody formation and GL-3

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ABSTRACT

Two different enzyme preparations are used for the treatment of Fabry disease patients, agalsidase alfa (Replagal, Shire) and agalsidase beta (Fabrazyme, Genzyme). Therapeutic efficacy of both products has been variable probably due to differences in gender, severity, age and other patient characteristics. We studied the occurrence of α -Gal A antibodies and their effect on urinary and plasma globotriaosylceramide (GL-3), plasma chitotriosidase and clinical outcome in 52 patients after 12 months of treatment with either 0.2 mg/kg agalsidase alfa (10 males, 8 females) or beta (8 males, 5 females) or 1.0 mg/kg agalsidase beta (10 males, 11 females). Antibodies were detected in 18/28 male patients after 6 months. None of the females developed antibodies. Following 12 months of 0.2 mg/kg treatment, urinary GL-3 decreased in antibody negative (AB⁻) but increased in antibody positive (AB⁺) patients. Treatment with 1.0 mg/kg gave a reduction in urinary GL-3 in both AB⁻ and AB⁺ patients. Levels of plasma GL-3 and chitotriosidase decreased in all patient groups. Twelve months of 0.2 mg/kg treatment did not change renal function or left ventricular mass. Further, no change in renal function was seen following 1.0 mg/kg treatment and left ventricular mass decreased in both AB⁻ and AB⁺ patients. In summary, α -Gal A antibodies frequently develop in male Fabry disease patients and interfere with urinary GL-3 excretion. Infusion of a dose of 1.0 mg/kg results in a more robust decline in GL-3, less impact, if any of antibodies, stable renal function and reduction of LVMass.

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Fabry disease is an X-linked lysosomal storage disorder in which a deficiency of the lysosomal enzyme α -galactosidase A (α -Gal A, OMIM 301500) [1,2] leads to lysosomal accumulation of globotriaosylceramide (GL-3) in endothelial cells and other cell types in the body. The clinical spectrum of Fabry disease is remarkably heterogeneous, [3]. Complications are mostly of vascular origin and comprise progressive renal insufficiency, cardiac hypertrophy, arrhythmias and cerebral infarctions [4]. Female heterozygotes can also exhibit complications, although the disease usually has a more attenuated course in these patients [5].

Currently 2 different enzyme preparations are used for the treatment of patients with Fabry disease: agalsidase alfa (Replagal[™], Shire) and agalsidase beta (Fabrazyme[™], Genzyme), which are produced by expression of the α -galactosidase A gene in modified human fibroblasts and Chinese hamster ovary (CHO) cells,

respectively. Patients are usually treated with the authorized dose of agalsidase alfa at 0.2 mg/kg or agalsidase beta at 1.0 mg/kg every 2 weeks. The therapeutic efficacy of both treatments has been variable. In patients with (near) normal renal function, renal function is preserved [6], cardiac hypertrophy is reduced and events are rarely seen [7,8]. However, an increasing number of studies over the past years reported further progression of symptoms in patients with more advanced disease, specifically renal insufficiency [6–11]. Factors that may have impact on the therapeutic response are incompletely understood. Irreversible damage to end-organs probably plays a role but differences in uptake of the enzyme [12] or the occurrence of neutralizing antibodies may also influence the outcome. Antibodies towards the enzyme preparations frequently develop in male patients because males do not often express residual enzyme activity. Previous studies have reported the emergence of IgG antibodies in 56% of agalsidase alfa [6] and 88% of agalsidase beta [13] treated male patients. Additional reports have shown these antibodies to have neutralizing capacities with

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an effect on urinary GL-3 excretion [6,14–18] and on chitotriosidase, a marker for storage in macrophages [19]. Whether this translates into a lack of clinical response is difficult to ascertain, given the variability in response also observed in the absence of antibodies. However, studies in other conditions (such as hemophilia A) show that antibodies, in particular neutralizing antibodies, are frequently associated with loss of efficacy of the infused preparation (see [20] for review). A way to overcome this problem is through administration of large(r) amounts of the product, as has been successfully implemented in hemophilia A [21]. To analyze the influence of enzyme dose on the production of antibodies and the levels of GL-3 and chitotriosidase in Fabry patients, we studied different groups of patients, who were treated with either 0.2 mg/kg agalsidase alfa or beta or 1.0 mg/kg of agalsidase beta. In addition we analyzed the impact of a dose increase to 1.0 mg/kg of agalsidase beta in male patients, who experienced treatment failure during the head-to-head study of agalsidase alfa and beta at 0.2 mg/kg every 2 weeks [11].

Methods

Patients

Comparative study

Thirty Fabry patients from the Academic Medical Center (AMC) and 1 patient from the Universitätsklinikum Würzburg (UKW) who were treated with agalsidase alfa ($n = 18$) or beta ($n = 13$) at 0.2 mg/kg for a minimum of 12 months were included in the study. Twenty nine of the AMC patients had been part of the head-to-head study [11]. In addition 5 patients from the AMC and 16 patients from the UKW who had been receiving agalsidase beta at 1.0 mg/kg for at least 12 months were included.

Switch study

Biochemical parameters were analyzed in 7 male patients who had switched from agalsidase 0.2 mg/kg (alfa, $n = 3$; beta, $n = 4$) to agalsidase beta 1.0 mg/kg treatment. These patients were part of our head-to-head study and the dose was increased following treatment failure [11]. All patients had been diagnosed by means of a reduced α -galactosidase A (α -Gal A) activity in leukocytes [22,23] (males) and/or a mutation in the α -Gal A gene (females). Stored blood and urinary samples from Fabry disease patients were analyzed. Informed consent had been obtained as part of previous studies.

Biochemical parameters

Antibodies

Serum samples were evaluated for the presence of α -Gal A antibodies as previously described [14]. To assess neutralizing activity different volumes of serum (0.1–10 μ l of serum, corresponding to a dilution of 1/150–1/1.5) were incubated with a standard amount of recombinant α -galactosidase A (agalsidase beta, 15 μ l of 0.14 μ g/ml = 2.1 ng). After 10 min of incubation at room temperature the enzymatic activity was determined. The serum dilution that resulted in 50% reduction of the enzyme activity was recorded (IC50). Assuming a linear relationship, the amount of enzyme that would be inhibited by 1 μ l of serum was calculated.

Urinary GL-3

Analysis of urinary GL-3 was performed as described before [24,25].

Plasma GL-3

Levels of GL-3 in plasma were measured by high pressure liquid chromatography as recently described [26].

Chitotriosidase

Chitotriosidase activity was measured in plasma using 4-methylumbelliferyl-(4-deoxy)chitobiose as substrate, as described previously [27]. Activity was measured in male patients only, as females tend to show values within the normal range [19]. Chemicals were obtained from Sigma, St Louis, MO, USA. One in every 3 individuals carries a mutation in the chitotriosidase gene resulting in levels of plasma chitotriosidase approximately half that found in individuals with a wild type phenotype [28]. Individuals homozygous for this mutation have no detectable chitotriosidase activity. As genotyping was not performed in a subset of patients (German patients, no cellular material available) and absolute values cannot be compared directly, levels are reported as change from baseline (%). As a result the analysis also included patients with chitotriosidase levels in the normal range.

Clinical evaluation

Clinical evaluation at baseline and after 12 months of treatment had been performed in all patients in the comparative study. Renal function was estimated by calculating the creatinine clearance from plasma creatinine and total urinary creatinine in a representative 24 h urine collection. Echocardiographic analysis at both time points was performed in 20/24 females and 15/28 males. Left ventricular hypertrophy was defined as a left ventricular mass (LVmass) of >259 g in males and >166 g in females [29].

Statistical methods

Results are expressed as median (range). Differences between groups were assessed by the Mann–Whitney *U*-test or Kruskal–Wallis test. The effect of enzyme replacement therapy (ERT) was analyzed by the Wilcoxon signed ranks test. Correlations between variables are described by assessment of Spearman rank correlation coefficients. *p*-Values < 0.05 were considered significant.

Results

Baseline characteristics

The baseline characteristics of the 52 patients included in the study are shown in Table 1. There were no significant differences between the 3 treatment groups with respect to age, sex and levels of biochemical parameters at baseline. Patients in the switch study were 7 males (3, agalsidase alfa 0.2 mg/kg and 4, agalsidase beta 0.2 mg/kg) who switched to agalsidase beta 1.0 mg/kg because of progression of disease (i.e. treatment failure [11]). All patients had significant symptomatology before start of 0.2 mg/kg treatment (i.e. cerebral infarctions, renal impairment and/or cardiac involvement) and switched to 1.0 mg/kg because of progression of vascular lesions on brain MRI ($n = 4$), decline in renal function ($n = 1$) or atrial fibrillation ($n = 2$).

Comparative study

Antibodies

α -Gal A antibodies were detected in 18 male patients after 6 months of therapy (4/10 agalsidase alfa 0.2 mg/kg, 6/8 agalsidase beta 0.2 mg/kg and 8/10 agalsidase beta 1.0 mg/kg). Antibodies were seen more frequently in patients treated with agalsidase beta 1.0 mg/kg than in patients treated with agalsidase alfa 0.2 mg/kg ($p = 0.005$). There was no significant difference in the occurrence of antibodies between the 2 groups treated with 0.2 mg/kg ($p = 0.157$) or between the 2 groups treated with agalsidase beta ($p = 0.453$). None of the female patients developed antibodies. Neu-

Table 1
Baseline characteristics

	0.2 mg/kg alfa		0.2 mg/kg beta		1.0 mg/kg beta		p
N	18		13		21		
Age (y)	47 (19–62)		49 (25–73)		48 (27–70)		ns
Males	10		8		10		
GL-3 urine (mg/mmol Cr)	0.088 (0.014–1.469)	n = 12	0.14 (0.026–0.785)	n = 10	0.347 (0.015–1.61)	n = 17	ns
GL-3 plasma ($\mu\text{mol/l}$)	4.62 (2.14–9.74)	n = 18	3.31 (0.77–9.10)	n = 13	4.39 (1.20–8.74)	n = 21	ns
Chitotriosidase (nmol/ml h)	140 (70–364)	n = 10	133 (60–194)	n = 7	203 (94–529)	n = 8	ns
Antibodies (males)	4/10		6/8		8/10		
Creatinine clearance (ml/min)	101 (22–136)	n = 17	108 (53–154)	n = 13	98 (43–142)	n = 18	ns
LVMass (g)	225 (178–479)	n = 9	265 (192–369)	n = 8	316 (88–686)	n = 18	ns

Median (range).

tralizing activity was present in 17/18 male patients (see Fig. 1A). In 1 patient (Table 2, patient 22), who showed very low antibody titers as measured by ELISA, no neutralizing activity was noticed. IC₅₀ values did not differ between the 3 treatment groups ($p = 0.50$). There was a strong correlation between ELISA antibody titers and IC₅₀ values: $r = 0.80$ $p < 0.001$. No correlation was found between the presence of antibodies and residual α -Gal A activity in leukocytes (Table 2). Nor was there a relation between the presence of a nonsense or missense mutation and the occurrence of antibodies (Table 2).

Urinary GL-3

Urinary GL-3 was measured in 39 patients and was elevated in 37 patients (18 males, 19 females) at baseline, 2 patients (1 male (patient 9, Table 2 and 1 female) presented GL-3 levels within the normal range (0.006–0.017 mg/mmol Cr). Data of patients with elevated levels at baseline were analyzed (Fig. 1B). As changes in urinary GL-3 were similar in both 0.2 mg/kg treatment groups, the data were combined. After 12 months of 0.2 mg/kg treatment there was a reduction of -0.037 (range: -1.377 to 0.012) ($p = 0.002$, $n = 13$) in antibody negative patients (AB[−]), whereas

there was an increase in antibody positive (AB⁺) patients (0.318 (-0.003 to 0.421) $p = 0.05$, $n = 6$). Treatment with 1.0 mg/kg for 12 months resulted in a reduction of -0.05 (-0.319 to 0.008) ($p = 0.07$, $n = 9$) in AB[−] patients and of -0.336 (-1.43 to 0.000) ($p = 0.03$, $n = 7$) in AB⁺ patients. The change in urinary GL-3 after 12 months of treatment in AB⁺ patients was significantly different between the 0.2 mg/kg and 1.0 mg/kg treatment group ($p = 0.002$).

Plasma GL-3

At baseline 22 patients (19 females) showed plasma GL-3 levels within the normal range (<3.18 $\mu\text{mol/l}$). One (patient 9, Table 2) of the 3 male patients with normal plasma GL-3 at baseline also had a normal baseline urinary GL-3 level. (The other 2 patients with normal baseline plasma GL-3 were patient 2 and patient 28, Table 2). The 2 groups treated with 0.2 mg/kg were combined for analysis of the data (Fig. 1C). There was a decrease of plasma GL-3 following 12 months of treatment in all the groups with elevated levels of plasma GL-3 at baseline irrespective of the treatment regimen or presence of antibodies, (0.2 mg/kg AB[−]: -2.32 (-5.81 to 0.32) $p = 0.015$ $n = 9$; 0.2 mg/kg AB⁺: -2.44 (-5.17 to -0.23) $p = 0.008$ $n = 9$; 1.0 mg/kg AB[−]: -2.87 (-3.11 to -2.63) $p = 0.18$ $n = 2$;

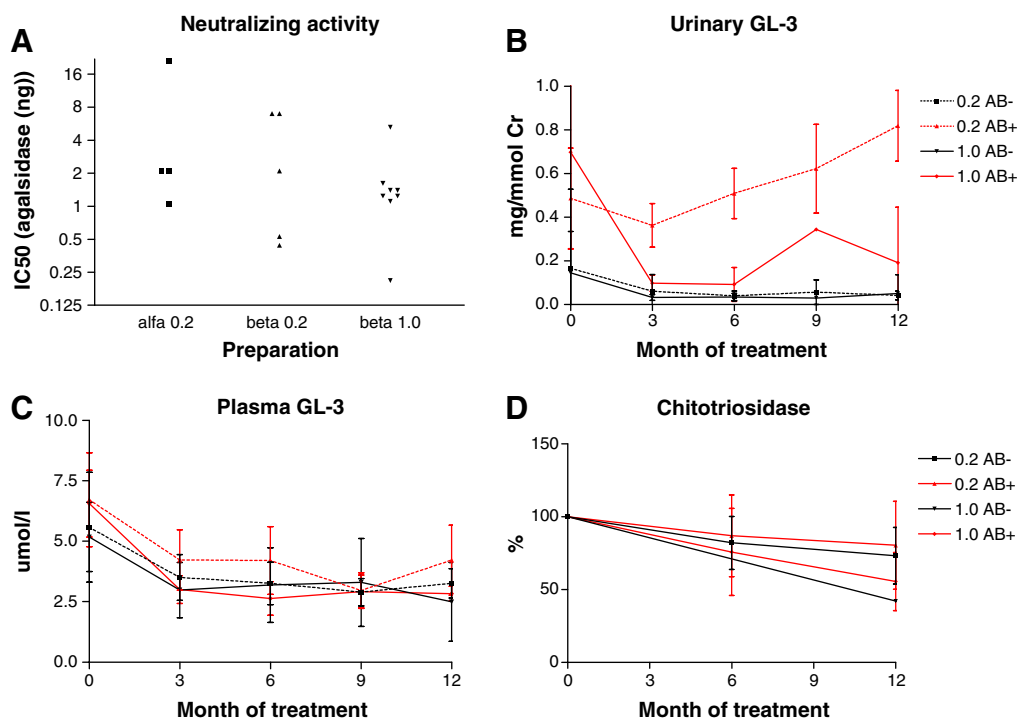


Fig. 1. Antibody formation and GL-3 in patients treated with 0.2 mg/kg or 1.0 mg/kg agalsidase. (A) Neutralizing activity (IC₅₀ in ng) of anti-agalsidase antibodies detected in male Fabry patients treated with agalsidase alfa 0.2 mg/kg, agalsidase beta 0.2 mg/kg or agalsidase beta 1.0 mg/kg at $t = 6$ months. (B) Urinary GL-3 levels in relation to antibodies in patients treated with 0.2 mg/kg or 1.0 mg/kg ERT. Data of all patients (both male and female, $n = 37$) with elevated GL-3 titers at baseline are included. (C) Plasma GL-3 levels in relation to antibodies in patients treated with 0.2 mg/kg or 1.0 mg/kg ERT. Data of all patients (both male and female, $n = 30$) with elevated GL-3 titers at baseline are included. (D) Chitotriosidase levels in relation to antibodies in male patients treated with 0.2 mg/kg or 1.0 mg/kg ERT.

Table 2

Mutations in the α -Gal A gene, residual α -Gal A activity in leukocytes and the presence/absence of antibodies in the male Fabry patients

Patient	Exon	Mutation	Residual α -GalA activity ^a	Antibodies
<i>Missense/nonsense</i>				
1	1	p.Phe18Ser	1.10 ¹	n
2	2	p.Arg112His	2.0 ²	n
3	3	p.Leu129Pro	0.6 ⁵	y
4	3	p.Asp136Tyr	0 ¹	n
5	3	p.Asp136Tyr	0 ¹	n
6	3	p.Asp136Tyr	0 ¹	n
7	3	p.As165Val	1.20 ¹	y
8	4	p.Gly183Asp	1.20 ¹	n
9	5	p.Asn215Ser	0.56 ³	n
10	5	p.Arg220X	0 ¹	n
11	5	p.Arg220X	0.4 ¹	y
12	5	p.Arg220X	0 ¹	y
13	5	p.Arg220X	0 ¹	y
14	5	p.Arg220X	1 ⁴	y
15	5	p.Trp226X	1.2 ⁴	y
16	5	p.Arg227Gln	6.4 ¹	n
17	7	p.Trp339X	1.8 ⁵	y
18	7	p.Glu341Lys	unk	n
19	7	p.Arg342X	unk	y
20	7	p.Arg342Gln	1.50 ¹	y
21	7	p.Arg342Gln	0.3 ⁴	y
22	7	p.Arg342Gln	0.9 ¹	y
23	7	p.Arg342Leu	1.2 ⁵	y
24	7	p.Gln386X	0.1 ⁴	y
<i>Splicing mutations</i>				
25	Intron 6	IVS(–2a > t)	0 ¹	y
26	Intron 3	IVS(–1g > a)	3.60 ¹	y
<i>Coding region deletion</i>				
27	6	c.717–718del	1.8 ⁵	y
28	7	c.1072–1073del	0 ¹	y

^a The ranges of normal leukocyte α -Gal activity in the 5 laboratories making the diagnoses were: (1) 33–77 nmol/mg h; (2) 60–80 nmol/mg h; (3) >2 μ mol/ml h (plasma); (4) 17–55 nmol/mg h; (5) 24–60 nmol/mg h (protein nomenclature according to <http://www.hgvs.org/mutnomen/>; reference sequence NM 000169.1).

1.0 mg/kg AB+: –3.86 (–5.73 to –2.14) $p = 0.012$ $n = 8$). The changes in plasma GL-3 levels were not different between patients treated with 0.2 mg/kg or 1.0 mg/kg (AB– $p = 0.58$, AB+ $p = 0.11$). Further, there was no difference in the decrease of plasma GL-3 between AB– and AB+ patients (0.2 mg/kg: $p = 0.73$, 1.0 mg/kg: $p = 0.53$).

Chitotriosidase

Chitotriosidase levels at baseline were 155 nmol/ml h (25–529) with 29 patients exhibiting levels within the normal range (<190 nmol/ml h). After 12 months of treatment male patients in both the 0.2 mg/kg and in the 1.0 mg/kg treatment groups showed a decrease of chitotriosidase activity (Fig. 1D), irrespective of the presence of antibodies (0.2 mg/kg AB–: –29 (–55 to 9) $p = 0.028$ $n = 7$; 0.2 mg/kg AB+: –21 (–62 to 31) $p = 0.093$ $n = 10$; 1.0 mg/kg AB–: –58 ($n = 1$); 1.0 mg/kg AB+: –40 (–72 to –16) $p = 0.028$ $n = 6$). The decrease in chitotriosidase activity in AB+ patients did not differ significantly between the 0.2 mg/kg and the 1.0 mg/kg treatment group ($p = 0.181$). When the data for AB+ and AB– patients were combined, the percent decline in chitotriosidase levels was greater for patients treated with 1.0 mg/kg (–43 (–72 to –16)) than 0.2 mg/kg (–26 (–62 to 31)) ($p = 0.047$).

Clinical parameters

Renal function

Renal function did not change in the 0.2 mg/kg or 1.0 mg/kg treatment group after 12 months. There was no difference between patients who developed antibodies and those who did not. (0.2 mg/

kg AB–: –2 ml/min (–28 to 25) $p = 0.728$ $n = 21$; 0.2 mg/kg AB+: –4 (–46 to 35) $p = 0.373$ $n = 9$; 1.0 mg/kg AB–: –7 (–64 to 45) $p = 0.130$ $n = 11$; 1.0 mg/kg AB+: 0 (–19 to 11) $p = 0.752$ $n = 7$).

Left ventricular mass

The effect of treatment was studied in patients who had left ventricular hypertrophy at baseline. No decrease in LVMass was found in patients receiving 0.2 mg/kg treatment whether they had antibodies or not (AB–: –23 g (–155 to 136) $p = 0.81$ $n = 13$; AB+: –20 g $n = 1$). In patients treated with 1.0 mg/kg there was a significant decrease in LVMass in both AB– and AB+ patients (AB–: –65 (–234 to 19) $p = 0.036$ $n = 8$; AB+: –77 (–175 to 12) $p = 0.028$ $n = 6$). No difference was found when comparing AB– patients treated with 0.2 mg/kg versus 1.0 mg/kg ($p = 0.161$) or between AB– and AB+ patients treated with 1.0 mg/kg ($p = 0.950$).

Switch study

Antibodies

Two male patients (1 previously treated with agalsidase alfa 0.2 mg/kg and 1 with agalsidase beta 0.2 mg/kg) remained AB– following change of therapy (Fig. 2A and B). Three patients (1 treated with agalsidase alfa and 2 with agalsidase beta) showed an increase in antibody titers and neutralizing activity following switch. All 3 had partly tolerized at the time of switch but antibody titers increased after switching and remained high over the following 24 months. In the patients who showed high titers at the time of switch (1 treated with agalsidase alfa and 1 with agalsidase beta) no change was seen following the switch with both the antibody titers and neutralizing activity remaining high.

Plasma GL-3

Levels of plasma GL-3 remained within the normal range in the 2 AB– patients (Fig. 2C). The levels in 1 AB+ patient were also below the upper limit of normal. The levels of plasma GL-3 normalized in 2 of the 4 patients who were AB+ and had elevated levels of plasma GL-3 at the time of switch. A trend towards a decrease in plasma GL-3 was noted 12 months after switch ($p = 0.066$).

Chitotriosidase

In 1 patient no chitotriosidase activity could be measured because of homozygosity for the null allele. Two patients (1 AB+ and 1 AB–) showed persistent low chitotriosidase levels (Fig. 2D). Values remained high or even increased further in the other 4 AB+ patients following 12–24 months of agalsidase 1.0 mg/kg treatment.

Clinical outcome

Despite the increase in dose, further disease progression was seen in 5/7 patients, all showing worsening of vascular ischemic lesions in the brain. Renal function and LVMass remained stable over the observed 24 months.

Discussion

This study shows that neutralizing antibodies are produced in the majority of male Fabry disease patients undergoing ERT and that they interfere with the excretion of GL-3. Persistent high titers of antibodies occurred in males who had switched from 0.2 mg/kg to 1.0 mg/kg treatment. In line with Linthorst et al [14] we found 64% (18/28) of male patients developing IgG antibodies with neutralizing capacities within the first 6 months of treatment. The occurrence of antibodies was not associated with residual α -Gal A activity in leukocytes or with the presence of a missense or non-

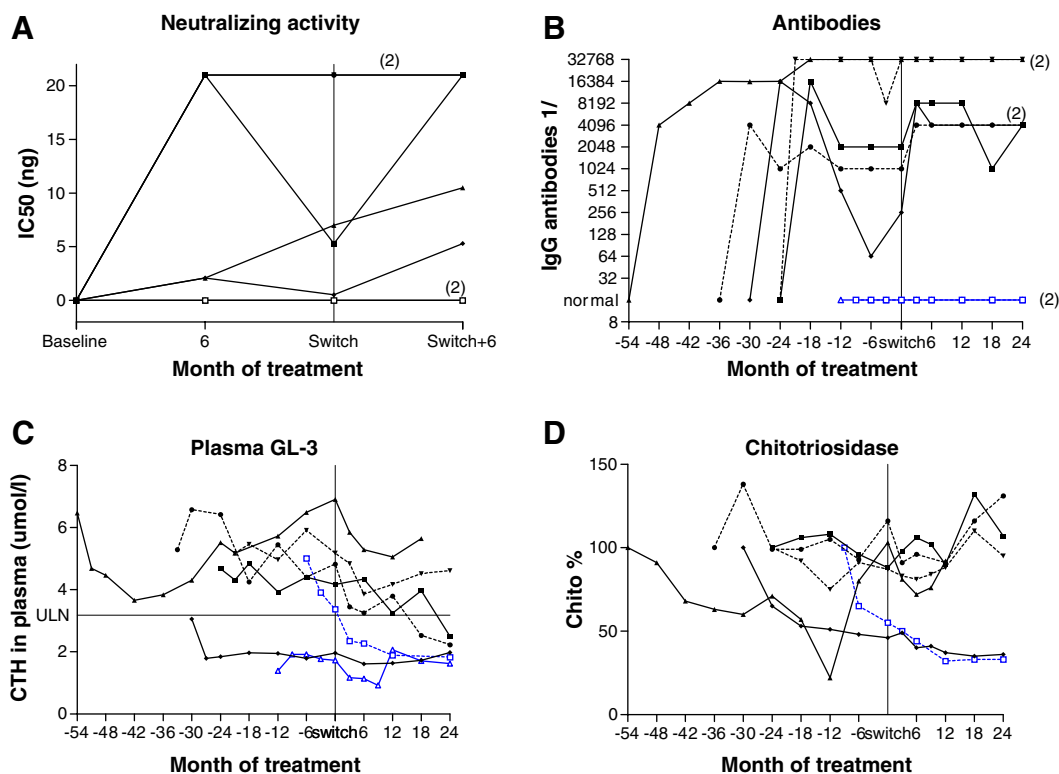


Fig. 2. Antibody formation, chitotriosidase levels and GL-3 in male patients switching from 0.2 mg/kg to 1.0 mg/kg agalsidase. (A) Neutralizing activity of anti-agalsidase antibodies in male Fabry patients who switched from 0.2 mg/kg to 1.0 mg/kg ERT. In case of overlap of graph lines presenting data of 2 separate patients this is indicated in parentheses (e.g. (2)). (B) Antibody titers as measured by ELISA in male patients who switched from 0.2 mg/kg to 1.0 mg/kg ERT. (Dotted lines) patients treated with agalsidase alfa 0.2 mg/kg before switch. (Solid lines) patients treated with agalsidase beta 0.2 mg/kg before switch. Data of antibody negative patients are plotted in blue. In case of overlap of graph lines presenting data of 2 separate patients this is indicated in parentheses (e.g. (2)). (C) Plasma GL-3 levels in relation to antibodies in male patients who switched from 0.2 mg/kg to 1.0 mg/kg ERT. (Dotted lines) patients treated with agalsidase alfa 0.2 mg/kg before switch. (Solid lines) patients treated with agalsidase beta 0.2 mg/kg before switch. Data of antibody negative patients are plotted in blue. Chitotriosidase levels in relation to antibodies in male patients who switched from 0.2 mg/kg to 1.0 mg/kg ERT. (Dotted lines) patients treated with agalsidase alfa 0.2 mg/kg before switch. (Solid lines) patients treated with agalsidase beta 0.2 mg/kg before switch. Data of antibody negative patients are plotted in blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

sense mutation. It is important to note that α -Gal A activity is measured in the presence of *N*-acetylgalactosamine [23] to inhibit α -*N*-acetylgalactosaminidase or α -galactosidase B (α -Gal B), which also hydrolyses the 4-MU substrate. Yet, total inhibition of α -Gal B is not always accomplished and minimal activity may be measured. Absence of an association between mutation type and the occurrence of antibodies may be related to the presence of inactive protein. No residual enzyme activity is measured, but existing inactive protein prevents antibodies from being developed. Thus, information on both residual α -Gal A activity and type of mutation is insufficient to predict antibody development in an individual patient. Immunoquantification of the α -Gal A protein and activity might be a better predictor of an immune response to the replacement enzyme [30].

Antibodies were produced more frequently in patients treated with 1.0 mg/kg of agalsidase beta than in patients treated with 0.2 mg/kg of agalsidase alfa. There was no difference in the occurrence of antibodies between the groups treated with 0.2 mg/kg of agalsidase alfa or agalsidase beta or between the groups treated with 0.2 mg/kg and 1.0 mg/kg of agalsidase beta. The higher prevalence of antibodies in agalsidase beta 1.0 mg/kg treated patients may be due to differences in the cell lines used to produce the enzymes [31,32]. Previous studies have shown that agalsidase alfa and beta have the same amino acid composition, specific activity, stability and uptake by cultured fibroblasts [33–35]. However, minor differences in glycosylation [34] and mannose-6-phosphate receptor-mediated cellular uptake [35] do exist.

Another important factor that may influence antibody formation is the dose, with a higher dose possibly being more immunogenic. The titers of antibodies remained high or increased further in male patients who switched from 0.2 mg/kg to 1.0 mg/kg treatment, whereas patients who did not develop antibodies before the switch remained negative. However, the majority of patients treated with 0.2 mg/kg also have persistent high titers after 24 months (data not shown). Thus, in contrast to previously reported studies [13,9] no decline in antibody titers was seen in patients receiving treatment over a longer period of time, irrespective of the enzyme preparation or dose.

The neutralizing antibodies significantly interfere with urinary GL-3 excretion. In the comparative study, urinary GL-3 was effectively cleared in AB– patients after treatment with 0.2 mg/kg and 1.0 mg/kg. In the AB+ patients, treatment with 0.2 mg/kg resulted in an increase of urinary GL-3 while treatment with 1.0 mg/kg gave a significant reduction. This reduction of urinary GL-3 in 1.0 mg/kg treated AB+ patients has also been reported by Ohashi et al. [18] Plasma GL-3 levels decreased in all treatment groups, irrespective of the applied dose or the presence of antibodies. In the switch patients, who were AB+ and had elevated levels of plasma GL-3 at time of switch, a trend towards a decrease is seen after switch to high dose treatment. The follow up of patients in the comparative study was relatively short. Treatment with 0.2 mg/kg may initially give a decline of plasma GL-3 levels, which may rise again after a period of time. Urinary GL-3 still seems to be a more sensitive parameter than plasma GL-3 as levels are more consistently raised in patients compared with healthy controls.

Chitotriosidase levels decreased in all males in the comparative study, with no difference between AB– and AB+ patients after 12 months of ERT. Patients who exhibited normal chitotriosidase levels at baseline showed equal decline in activity. We reported earlier [19] interference of antibodies with chitotriosidase levels, which became more evident after 24 months of treatment. Thus follow up may not be long enough in this study. A more robust decline in chitotriosidase activity was noted in patients treated with 1.0 mg/kg, though patients in the switch study did not show a reduction in chitotriosidase levels after change from 0.2 mg/kg to 1.0 mg/kg, within this short period of time.

These data suggest that in the presence of antibodies, high dose treatment is more effective in reducing GL-3 levels. By analogy with the treatment of patients with hemophilia A, high dose treatment probably overrules the negative effects associated with antibody formation by saturation of existing antibodies. A translation to clinical efficacy is difficult to make at present. So far no studies have been performed on the clinical outcome of patients with Fabry disease receiving enzyme replacement therapy in relation to antibody formation. Although the data sets are small and there is variation between individuals, we have not detected a change in renal function in any of the treatment groups but there is a more marked decrease in LVMass in both AB+ and AB– patients receiving 1.0 mg/kg treatment. This suggests that high dose treatment is more effective in reducing LVMass, irrespective of the presence of antibodies.

In summary, formation of antibodies is frequently observed in male Fabry disease patients receiving enzyme replacement therapy, with a higher prevalence of antibody formation following treatment with 1.0 mg/kg of agalsidase beta. The excretion of GL-3 seems to be related to the infused dose, with a dose of 1.0 mg/kg resulting in higher excretion than a dose of 0.2 mg/kg. The impact of antibodies on clinical outcome, and its relation to genotype and presence of enzyme protein should be further studied in large groups of patients, preferably in patients with mild disease to avoid lack of response due to advanced disease. Collaborative efforts to undertake such studies will be essential.

Disclosure

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References

- [1] R.O. Brady, Enzymatic abnormalities in diseases of sphingolipid metabolism, *Clin. Chem.* 13 (1967) 565–577.
- [2] J.A. Kint, Fabry's disease: alpha-galactosidase deficiency, *Science* 167 (1970) 1268–1269.
- [3] I. Redonnet-Vernhet, J.K. Ploos van Amstel, R.P. Jansen, R.A. Wevers, R. Salvayre, T. Levade, Uneven X inactivation in a female monozygotic twin pair with Fabry disease and discordant expression of a novel

- mutation in the alpha-galactosidase A gene, *J. Med. Genet.* 33 (1996) 682–688.
- [4] R.J. Desnick, Y.A. Ioannou, M.E. Eng, α -Galactosidase A deficiency: Fabry disease, in: C.R. Scriver, A.L. Beaudet, W.S. Sly, D. Valle (Eds.), *The Metabolic and Molecular Bases of Inherited Disease*, Eighth ed., vol. 3, McGraw-Hill, New York, 2001, pp. 3733–3774.
- [5] K.D. MacDermot, A. Holmes, A.H. Miners, Anderson–Fabry disease: clinical manifestations and impact of disease in a cohort of 60 obligate carrier females, *J. Med. Genet.* 38 (2001) 769–775.
- [6] R. Schiffmann, M. Ries, M. Timmons, J.T. Flaherty, R.O. Brady, Long-term therapy with agalsidase alfa for Fabry disease: safety and effects on renal function in a home infusion setting, *Nephrol. Dial. Transplant.* 21 (2006) 345–354.
- [7] F. Breunig, F. Weidemann, J. Strotmann, A. Knoll, C. Wanner, Clinical benefit of enzyme replacement therapy in Fabry disease, *Kidney Int.* 69 (2006) 1216–1221.
- [8] M. Banikazemi, J. Bultas, S. Waldek, W.R. Wilcox, C.B. Whitley, M. McDonald, R. Finkel, S. Packman, D.G. Bichet, D.G. Warnock, R.J. Desnick, Agalsidase-beta therapy for advanced Fabry disease: a randomized trial, *Ann. Intern. Med.* 146 (2007) 77–86.
- [9] W.R. Wilcox, M. Banikazemi, N. Guffon, S. Waldek, P. Lee, G.E. Linthorst, R.J. Desnick, D.P. Germain, Long-term safety and efficacy of enzyme replacement therapy for Fabry disease, *Am. J. Hum. Genet.* 75 (2004) 65–74.
- [10] D.P. Germain, S. Waldek, M. Banikazemi, D.A. Bushinsky, J. Charrow, R.J. Desnick, P. Lee, T. Loew, A.C. Vedder, R. Abichandani, W.R. Wilcox, N. Guffon, Sustained, long-term renal stabilization after 54 months of agalsidase beta therapy in patients with Fabry disease, *J. Am. Soc. Nephrol.* 18 (2007) 1547–1557.
- [11] A.C. Vedder, G.E. Linthorst, G. Houge, J.E. Groener, E.E. Ormel, B.J. Bouma, J.M. Aerts, A. Hirth, C.E. Hollak, Treatment of Fabry disease: outcome of a comparative trial with agalsidase alfa or beta at a dose of 0.2 mg/kg, *PLoS One* 2 (2007) e598.
- [12] G.J. Murray, M.R. Anver, M.A. Kennedy, J.M. Quirk, R. Schiffmann, Cellular and tissue distribution of intravenously administered agalsidase alfa, *Mol. Genet. Metab.* (2006) 307–312.
- [13] C.M. Eng, N. Guffon, W.R. Wilcox, D.P. Germain, P. Lee, S. Waldek, L. Caplan, G.E. Linthorst, R.J. Desnick, Safety and efficacy of recombinant human α -galactosidase A replacement therapy in Fabry's disease, *N. Engl. J. Med.* 345 (2001) 9–16.
- [14] G.E. Linthorst, C.E. Hollak, W.E. Donker-Koopman, A. Strijland, J.M. Aerts, Enzyme therapy for Fabry disease: neutralizing antibodies toward agalsidase alpha and beta, *Kidney Int.* 66 (2004) 1589–1595.
- [15] P.D. Whitfield, J. Calvin, S. Hogg, E. O'Driscoll, K. Halsall, K. Burling, G. Maguire, N. Wright, T.M. Cox, P.J. Meikle, P.B. Deegan, Monitoring enzyme replacement therapy in Fabry disease—role of urine globotriaosylceramide, *J. Inher. Metab. Dis.* 28 (2005) 21–33.
- [16] FDA briefing document Fabrazyme, 2002. Available from: <http://www.fda.gov/ohrms/dockets/ac/03/briefing/3917B1_03_FDA%20background.pdf>.
- [17] FDA briefing report Replagal, 2002. Available from: <http://www.fda.gov/ohrms/dockets/ac/03/briefing/3917B2_02_FDA-Background.pdf>.
- [18] T. Ohashi, M. Sakuma, T. Kitagawa, K. Suzuki, N. Ishige, Y. Eto, Influence of antibody formation on reduction of globotriaosylceramide (GL-3) in urine from Fabry patients during agalsidase beta therapy, *Mol. Genet. Metab.* (2007) 271–273.
- [19] A.C. Vedder, J. Cox-Brinkman, C.E. Hollak, G.E. Linthorst, J.E. Groener, M.T. Helmond, S. Scheij, J.M. Aerts, Plasma chitotriosidase in male Fabry patients: a marker for monitoring lipid-laden macrophages and their correction by enzyme replacement therapy, *Mol. Genet. Metab.* 89 (2006) 239–244.
- [20] H. Schellekens, Immunogenicity of therapeutic proteins: clinical implications and future prospects, *Clin. Ther.* 24 (2002) 1720–1740.
- [21] J. Wight, S. Paisley, C. Knight, Immune tolerance induction in patients with haemophilia A with inhibitors: a systematic review, *Haemophilia* 9 (2003) 436–463.
- [22] R.J. Desnick, K.Y. Allen, S.J. Desnick, M.K. Raman, R.W. Bernlohr, W. Krivit, Fabry's disease: enzymatic diagnosis of hemizygotes and heterozygotes. Alpha-galactosidase activities in plasma, serum, urine, and leukocytes, *J. Lab. Clin. Med.* 81 (1973) 157–171.
- [23] J.S. Mayes, J.B. Scheerer, R.N. Sifers, M.L. Donaldson, Differential assay for lysosomal α -galactosidases in human tissues and its application to Fabry's disease, *Clin. Chim. Acta* 112 (1981) 247–251.
- [24] K. Mills, P. Morris, P. Lee, A. Vellodi, S. Waldek, E. Young, B. Winchester, Measurement of urinary CDH and CTH by tandem mass spectrometry in patients hemizygous and heterozygous for Fabry disease, *J. Inher. Metab. Dis.* 28 (2005) 35–48.
- [25] E. Young, K. Mills, P. Morris, A. Vellodi, P. Lee, S. Waldek, B. Winchester, Is globotriaosylceramide a useful biomarker in Fabry disease?, *Acta Paediatr. Suppl.* 94 (2005) 51–54.
- [26] J.E. Groener, B.J. Poorthuis, S. Kuiper, M.T. Helmond, C.E. Hollak, J.M. Aerts, HPLC for simultaneous quantification of total ceramide, glucosylceramide, and ceramide trihexoside concentrations in plasma, *Clin. Chem.* 53 (2007) 742–747.
- [27] B. Aguilera, K. Ghauharali-van der Vlugt, M.T. Helmond, J.M. Out, W.E. Donker-Koopman, J.E.M. Groener, R.G. Boot, G.H. Renkema, G.A. van der Marel, J.H. van Boom, H.S. Overkleeft, J.M.F.G. Aerts, Transglycosidase activity of

- chitotriosidase: improved enzymatic assay for the human macrophage chitinase, *J. Biol. Chem.* (2003) 40911–40916.
- [28] A. Schoonhoven, B. Rudensky, D. Elstein, A. Zimran, C.E. Hollak, J.E. Groener, J.M. Aerts, Monitoring of Gaucher patients with a novel chitotriosidase assay, *Clin. Chim. Acta* 381 (2007) 136–139.
- [29] D. Levy, D.D. Savage, R.J. Garrison, K.M. Anderson, W.B. Kannel, W.P. Castelli, Echocardiographic criteria for left ventricular hypertrophy: the Framingham Heart Study, *Am. J. Cardiol.* 59 (1987) 956–960.
- [30] M. Fuller, M. Lovejoy, D.A. Brooks, M.L. Harkin, J.J. Hopwood, P.J. Meikle, Immunoquantification of {alpha}-galactosidase: evaluation for the diagnosis of Fabry disease, *Clin. Chem.* 50 (2004) 1979–1985.
- [31] M. Beck, Agalsidase alfa—a preparation for enzyme replacement therapy in Anderson–Fabry disease, *Expert Opin. Investig. Drugs* 11 (2002) 851–858.
- [32] G.M. Pastores, R. Thadhani, Enzyme-replacement therapy for Anderson–Fabry disease, *Lancet* 358 (2001) 601–603.
- [33] D. Blom, D. Speijer, G.E. Linthorst, W.G. Donker-Koopman, A. Strijland, J.M. Aerts, Recombinant enzyme therapy for Fabry disease. Absence of editing of human α -galactosidase A mRNA, *Am. J. Hum. Genet.* 72 (2003) 23–31.
- [34] K. Lee, X. Jin, K. Zhang, L. Copertino, L. Andrews, J. Baker–Malcolm, L. Geagan, H. Qiu, K. Seiger, D. Barngrover, J.M. McPherson, T. Edmunds, A biochemical and pharmacological comparison of enzyme replacement therapies for the glycolipid storage disorder Fabry disease, *Glycobiology* 13 (2003) 305–313.
- [35] H. Sakuraba, M. Murata–Ohsawa, I. Kawashima, Y. Tajima, M. Kotani, T. Ohshima, Y. Chiba, M. Takashiba, Y. Jigami, T. Fukushima, T. Kanzaki, K. Itoh, Comparison of the effects of agalsidase alfa and agalsidase beta on cultured human Fabry fibroblasts and Fabry mice, *J. Hum. Genet.* 51 (2006) 180–188.