

Enzyme therapy for Fabry disease: Neutralizing antibodies toward agalsidase alpha and beta

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Background. Fabry disease is an X-linked inherited disorder that is caused by excessive lysosomal globotriaosylceramide (CTH) storage due to a deficiency in α -galactosidase A (α -Gal A). Two recombinant enzyme preparations have been approved as treatment modality. We studied emergence and properties of α -Gal A antibodies in treated patients.

Methods. During the first 6 to 12 months of intravenous administration of recombinant enzymes (rh- α -Gal A) formation of antibodies was studied in 18 adult Fabry patients (two females).

Results. The female patients did not develop detectable amounts of antibodies following enzyme therapy. After 6 months of treatment with either agalsidase alpha or beta, 11/16 male patients showed high titers of immunoglobulin G (IgG) antibodies that cross-react in vitro similarly with both recombinant enzymes. The anti-rh- α -Gal A IgG neutralizes rh- α -Gal A activity in vitro for 65% to 95%. During infusion with rh- α -Gal A, circulating enzyme-antibody complexes are formed and these complexes are taken up by leukocytes in the peripheral blood. After 6 months of treatment all IgG-negative patients showed a significant ($P < 0.01$) reduction of urinary CTH (1890 ± 797 to 603 ± 291 nmol CTH/24hr urine), compared to IgG-positive patients (mean increase from 2535 ± 988 to 2723 ± 1212), suggesting a negative effect of circulating antibodies on renal tubular CTH clearance.

Conclusion. Emergence of antibodies with in vivo neutralizing capacities is frequently encountered in treated Fabry disease patients. Complete cross-reactivity of these antibodies suggests that it is unlikely that switching from one to the other recombinant protein prevents the immune response and related effects. Further studies on the clinical implications of α -Gal A antibodies are essential.

Gabor Linthorst and Carla Hollak have been involved in the phase III clinical study with agalsidase beta. The authors do not serve as consultants to, hold stock in, or receive money from Genzyme Corp. or TKT, Inc.

Key words: Fabry disease, enzyme replacement, antibodies, targeting.

Received for publication July 23, 2003
and in revised form February 10, 2004, and May 2, 2004
Accepted for publication May 12, 2004

Recombinantly produced biological compounds are increasingly used as treatment modality for diseases in which symptoms or complications are due to the deficiency of an enzyme or protein. Often these products have to be administered life-long, such as administration of rh-Epo for anemia in renal disease or rh-fVIII in hemophilia A. Development of antibodies against administered recombinant proteins can occur and is well documented [1, 2]. Lysosomal storage disorders are characterized by the deficiency of a lysosomal enzyme, which leads to the accumulation of specific substrates, and subsequently causes disease. There are about 60 known different lysosomal storage disorders, each with its specific lysosomal enzyme deficiency and characteristic clinical picture. Gaucher disease, caused by deficiency of the lysosomal enzyme β -glucocerebrosidase, was the first lysosomal storage disorder in which treatment with purified enzyme was shown to be efficacious [3]. This approach has been copied for other lysosomal storage disorders, such as Fabry disease [4, 5], Pompe disease [6], and mucopolysaccharidosis type I [7]. Fabry disease, caused by a deficiency of the lysosomal enzyme α -galactosidase A (α -Gal A), leads to accumulation of mainly globotriaosylceramide (CTH, Gb3) in all cells of the body, but primarily endothelial cells [8]. Other cells that accumulate CTH are myocytes, neuronal cells, and in the kidney, besides endothelial cells, podocytes and mesangial cells also store the lipid [9]. The continuous accumulation of glycosphingolipid leads to several vascular complications, of which, apart from stroke and cardiomyopathy, renal failure is a prominent feature which often results in premature death. In a large cohort of patients diagnosed with Fabry disease, 17% of male patients suffered from end-stage renal failure, requiring dialysis or renal transplantation [10]. In two clinical trials with human recombinant α -galactosidase A, promising improvement in renal pathology and stabilization in renal function during 6 to 12 months of treatment was observed [4, 5].

These studies led to the approval in Europe of two enzyme preparations for the treatment of Fabry disease:

agalsidase alpha (Replagal, TKT Corp., Cambridge, MA, USA) and agalsidase beta (Fabrazyme, Genzyme Corp., Cambridge, MA, USA). In the United States only agalsidase beta has been approved. One of these two studies was performed with agalsidase alpha, which is produced in a continuous human cell line, and has been studied at a dose of 0.2 mg/kg [5]. The other study was performed with agalsidase beta, which is produced by classical cDNA transduction in Chinese Hamster Ovary (CHO) cells, and has been studied at a dose of 1.0 mg/kg [4]. In Europe, physicians may find it difficult to decide what treatment preparation to use because no direct comparison studies have been performed to date. Both enzymes have a terminal mannose-6-phosphate, and targeting is therefore supposed to be at various cell types, including endothelial cells. The formation of antibodies to rh- α -Gal A as determined by enzyme-linked immunosorbent assay (ELISA) after six months of biweekly exposure is reported as 3/14 in patients treated with 0.2 mg/kg agalsidase alpha [5] versus 24/29 in patients treated with 1.0 mg/kg agalsidase beta [4]. The apparent difference in prevalence of antibodies has been interpreted as pointing to differences in composition of the two enzymes based on their different production methods [11, 12].

The occurrence of antibodies results in hypersensitivity reactions in some patients, necessitating lengthening of infusion time and/or the use of premedications such as antihistamines or corticosteroids [4, 5]. The effect of antibodies on rh- α -Gal A activity, cross-reactivity between the two available enzyme preparations, and targeting after complex formation have so far not been investigated. In this study we studied antibody formation in 18 patients receiving either one of the two available enzyme preparations during the first 6 to 12 months.

METHODS

Patients

Patients that were referred to our hospital for treatment with enzyme supplementation therapy and completed at least 6 months of treatment were included in this study. Patients started treatment when they fulfilled the Dutch criteria for initiation of treatment for Fabry disease. These criteria require the presence of chronic acroparesthesias, or cardiomyopathy, cerebral infarctions, hearing loss, creatinine clearance, or a combination of these symptoms. Patients were treated with biweekly agalsidase alpha (Replagal) at a dose of 0.2 mg/kg ($N = 7$) or agalsidase beta (Fabrazyme) at a dose of 0.2 mg/kg ($N = 6$) or 1.0 mg/kg ($N = 5$). Agalsidase beta 0.2 mg/kg was given as part of another ongoing study. Two patients were female. The diagnosis of Fabry disease was confirmed in all patients by demonstrating deficient α -Gal A activity in leukocytes in male patients, or a mutation of the α -Gal A gene in the female patients.

Four patients treated with 1.0 mg/kg agalsidase beta were given premedication (1000 mg acetaminophen and 1 mg clemastine) one hour before infusion. Patients who were given a dose of 0.2 mg/kg agalsidase and one patient receiving 1.0 mg/kg agalsidase beta did not use premedication. Patients who developed infusion-associated reactions were treated with 5 mg dexamethasone orally one hour before start of infusion. All patients gave informed consent. Blood samples were taken pretreatment ($t = 0$) and after 6 months ($t = 6$), and in some patients again after 12 months of treatment ($t = 12$), and were spun at 3000 rpm for 10 minutes. Serum was frozen at -20°C before use.

Purification of serum IgG

Prot A Sepharose (50 μL) was incubated with 50 μL of serum for 60 minutes at room temperature. After spinning, the immunoglobulin G (IgG)-depleted supernatant was transferred and the remaining prot A Sepharose was washed $3\times$ with phosphate-buffered saline (PBS). Agalsidase alpha or beta in 0.1% bovine serum albumin (BSA) was added to both the IgG-depleted supernatant and the IgG-rich Sepharose-PBS mixture and incubated for 60 minutes at room temperature. Samples were spun, and the enzyme activity in supernatant was determined.

Neutralization studies

Different volumes of serum (1 to 5 μL of serum, corresponding with a dilution of 1/25 to 1/5) were incubated with either agalsidase alpha or beta. After 10 minutes of incubation the enzymatic activity was determined.

Enzyme activity assays

The enzymatic activity of α -galactosidase A was measured as described elsewhere [13]. Briefly, 25 μL of sample was incubated at 37°C for 1 hour with 100 μL reaction mixture containing 4-methylumbelliferyl- α -D-galactopyranoside (4-MU-galactopyranoside; Sigma, St. Louis, MO, USA) in a final concentration of 3.5 mmol/L in 100 mmol/L citrate/200 mmol/L phosphate buffer, pH 4.6. *N*-acetylgalactosamine (100 mmol/L) was added to the reaction mixture to inhibit α -N-acetyl-galactosaminidase activity. Reactions were terminated by the addition of 2 mL 300 mmol/L glycine/NaOH buffer, pH 10.6, and fluorescent 4-methyl-umbelliferone was measured with a fluorimeter (Perkin-Elmer Corp., Norwalk, CT, USA) at 445 nm. To determine the enzyme activity in leukocytes, the leukocyte pellet was sonicated twice in 200 μL water for 10 seconds. Protein concentration of the sonicated leukocytes was determined using a Lowry assay with human albumin as reference protein. Results are expressed as nmol/mg protein/hr.

ELISA for the detection of anti-rh- α -Gal A IgG antibodies

Enzyme-linked immunosorbent assay (ELISA) plates (96-well; Nunc, Maxisorp, Denmark) were coated overnight at room temperature with 100 μ L $\sim$ 11 μ g/L agalsidase alpha (Replagal, TKT, Inc., USA) or agalsidase beta (Fabrazyme, Genzyme Corp., USA), which were obtained from the local pharmacy. Plates were washed three times with PBS and blocked with 100 μ L/well 4% gelatin for one hour. Subsequently, plates were washed 5 $\times$ with PBS/Tween 0.05% and incubated with 100 μ L of serial dilutions of serum in PBS for 1 hour under shaking conditions. After washing peroxidase-labeled goat antihuman IgG, antibody in PBS was added and left to incubate for 1 hour. After washing 100 μ L 0.1 mg/mL tetramethyl benzidine (TMB) in 0.11 mol/L, pH 5.5, NaAc and 0.003% H₂O₂ were added and left to incubate for 30 minutes. The colorimetric reaction was stopped by addition of 100 μ L of H₂SO₄, and the absorbance was measured at 450 nm in a Titer-Tek multiscan ELISA reader (Titer-Tek, Huntsville, AL, USA). The titer was determined by the maximum dilution at which the postexposure serum had an absorbance at least twice that of the baseline-sample of that patient at the same dilution.

Detection of IgG-agalsidase complexes

From one patient (patient number 8) who was treated with agalsidase beta, EDTA-anticoagulated blood was taken at several time points during and after infusion 7 (before development of IgG) and 11 (after IgG antibodies became detectable), respectively. Blood was spun at 3000 rpm for 10 minutes, and plasma was stored at -80°C . Red cells were lysed using ammonium chloride, and after washing with PBS, leukocyte pellets were stored at -80°C . Enzymatic activity was determined in plasma and leukocytes as described. In addition, 10 μ L plasma was incubated with Prot A Sepharose or Sepharose-4B beads for 60 minutes at room temperature. The sample was spun and α -Gal A activity was measured in the supernatant and the beads as described.

In vitro uptake of agalsidase alpha or beta

EDTA-anticoagulated blood was drawn from three IgG-negative and one IgG-positive patient. Blood (2 mL, containing between 20% to 35% lymphocytes and 4% to 9% monocytes) was incubated with 5 μ g/mL agalsidase beta or alpha for 120 minutes, shaking at 37°C . After 60 minutes and 120 minutes, blood was spun. Supernatant was harvested and stored at -20°C . Red cells were lysed using ammonium chloride, and after washing with PBS leukocyte-pellets were stored at -20°C . Enzyme activity was performed as described.

Urinary CTH

In 11 patients, 24-hour urine was collected before start and after 6 months of treatment for determination of globotriaosylceramide (CTH). From 6 patients (two IgG-negative, four IgG-positive), 24-hour urine after 12 months of treatment was also available. Samples were stored at -20°C before use. Glycolipid was extracted from urinary sediment obtained after spinning 100 mL urine at 28,000g for 1 hour according to Folch [14]. The chloroform phase was applied to a 500 mg chloroform equilibrated LiChrolut RP18 column (Merck, Whitehouse Station, NJ, USA). Bound glycolipids were eluted with acetone:MetOH (9:1) v/v and dried under nitrogen. CTH was isolated on HPTLC (HP-TLC-silicagel 60) with development solvent of CHCl₃/MetOH/H₂O 50/21/3 v/v/v. Lipids were identified with orcinol staining, and quantitated using reference standards by density scanning (Scanning Quantity One, Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Statistical analysis

The Mann-Whitney *U* test was used to compare urinary CTH levels between IgG-positive and IgG-negative patients. Wilcoxon test was performed to compare urinary CTH levels in individual patients before and after 6 months of treatment.

RESULTS

Patients

Characteristics of the patients are shown in Table 1. Renal function of patients is shown in Table 1. Renal function did not improve or deteriorate during treatment period in all patients. Of the 18 patients treated, 11 developed an IgG antibody. The two female patients (patient 6 and 17) remained IgG-negative. Seven patients had infusion related side effects, 3/5 treated with 1.0 mg/kg agalsidase beta, and 1/6 and 3/7 treated with 0.2 mg/kg agalsidase beta and alpha, respectively. These side effects were chills, fever (followed by acroparesthesias), and in one patient, also nausea and vomiting. All patients with infusion related events were IgG-positive. Two out of seven patients required treatment with corticosteroids (5 mg dexamethasone orally 1 hour before an infusion) to prevent recurrence of chills. In other patients, chills were transient or managed by reduction of infusion rate. There was no correlation with genotype (either nonsense or missense mutation) and the development of IgG antibodies (Table 1). Three patients (numbers 10, 11, and 15) with a residual enzyme activity of 1.5 nmol/mg protein/hr or higher did not develop anti-rh- α -Gal A antibodies.

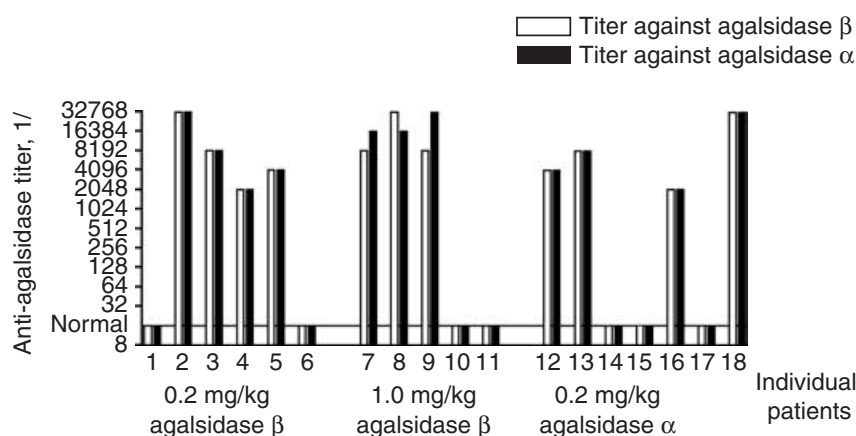
Antibody titers and cross-reactivity

The titers of immunoreactivity toward either agalsidase alpha or beta did not differ significantly for all sera

Table 1. Characteristics of patients treated with agalsidase alpha or beta

Patient	Sex	Dose (mg/kg)	Mutation	α -Gal-A act	24-hr creatinine clearance	Anti -rh- α -Gal A IgG	Side effects
1	M	0.2 beta	F18S	0.3	113	–	None
2	M	0.2 beta	Q386X	0.4	97	+	None
3	M	0.2 beta	R220X	0	58	+	None
4	M	0.2 beta	IVS6-2(a-t)	0	56	+	Chills on 1 occasion
5	M	0.2 beta	R342Q	0.3	151	+	None
6	F	0.2 beta	7299delT	16.1	116	–	None
7	M	1.0 beta	R342Q	0.1	64	+	Chills and fever on a few occasions
8	M	1.0 beta	W226X	0.3	121	+	Chills and fever on a few occasions
9	M	1.0 beta	11051delAG	0	125	+	Recurrent chills and fever, premedication with dexamethasone
10	M	1.0 beta	R112H	1.5	29	–	None
11	M	1.0 beta	N298S	2.4	29	–	None
12	M	0.2 alpha	R220X	0.4	127	+	Recurrent chills and fever, premedication with dexamethasone
13	M	0.2 alpha	R220X	0	68	+	Recurrent chills, stopped treatment after 9 months
14	M	0.2 alpha	R220X	0	41	–	None
15	M	0.2 alpha	D229E	1.5	Dialysis	–	None
16	M	0.2 alpha	W226X	0.2	10	+	Chills on 1 occasion
17	F	0.2 alpha	R220X	11.3	83	–	None
18	M	0.2 alpha	R220X	1.2	130	+	None

F denotes female patients. α -Gal A activity listed as nmol/mg protein/hr; beta refers to agalsidase beta (Fabrazyme); alpha refers to agalsidase alpha (Replagal).

**Fig. 1.** Anti-agalsidase IgG titer in patients treated with agalsidase alpha or beta. Open bars represent titer to agalsidase beta, closed bars to agalsidase alpha (normal <1:16).

(Fig. 1). All antibodies showed complete cross-reactivity; patients who developed antibodies after intravenous treatment with agalsidase alpha had the same titer toward agalsidase beta and vice versa. Titers did not decline (defined as a fourfold reduction or more) in four IgG-positive patients that were monitored until month 12. In some patients there was a tendency in decline (titer reduction between two- and fourfold). Treatment with dexamethasone in two patients showed no impact on titers up to 12 months.

Neutralizing capacity

All IgG-positive sera by ELISA showed a marked in vitro inhibition of enzyme activity, ranging from 65% to 95%. There was no difference in inhibitory effect of raised IgG antibodies between patients on agalsidase alpha or beta (Fig. 2). Again, similar neutralizing activity of IgG

antibodies toward agalsidase beta in patients treated with agalsidase alpha and vice versa was observed (Fig. 2). Depleting sera of IgG by means of Prot A Sepharose beads completely eliminated the inhibitory capacity of serum, implicating that an IgG molecule is responsible for the neutralizing effects of serum.

Effects of antibody formation on pharmacokinetics and targeting in vivo and in vitro

Blood was drawn at various time points during an infusion with agalsidase beta infusion both before (infusion 7) and after development of anti-rh- α -Gal A antibodies (infusion 11). Incubation of the IgG-positive plasma (from infusion 11) with Prot A Sepharose led to a decrease of up to 90% of plasma enzyme activity in the supernatant. Incubation of IgG-negative plasma with Prot A Sepharose did not result in loss of plasma enzyme

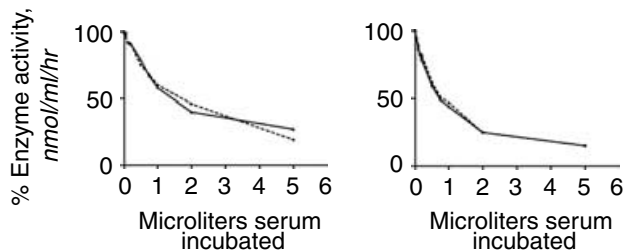


Fig. 2. Neutralization of enzyme activity and cross-reactivity of serum incubated with agalsidase alpha (dotted line) or agalsidase beta (solid line), respectively.

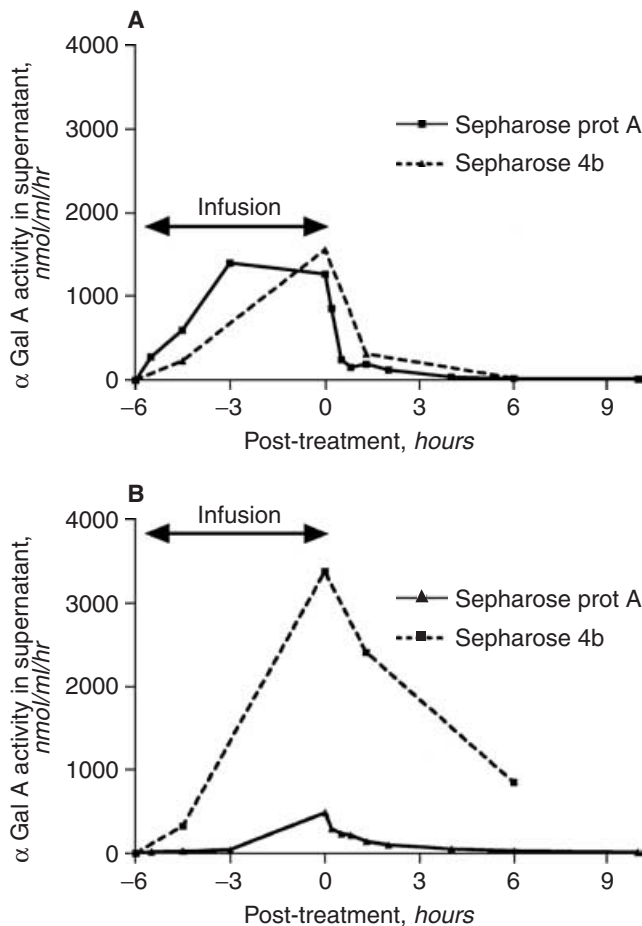


Fig. 3. Demonstration of circulating immune complexes in an IgG-positive patient during an infusion with agalsidase beta in samples taken as part of a pharmacokinetic profile. Before the development of antibodies there is no difference in enzymatic activity in the supernatant of plasma treated with either Sepharose prot A or 4B (A). However, after the development of antibodies enzymatic activity in supernatant is greatly reduced when plasma is incubated with Sepharose prot A, but not with Sepharose 4B, indicating that the majority of circulating enzyme is bound to IgG (B).

activity (Fig. 3A). Enzyme activity was retrieved on the Prot A sepharose beads (data not shown). Incubation of IgG-positive plasma with Sepharose-4B beads did not result in reduction of enzyme activity, excluding nonspe-

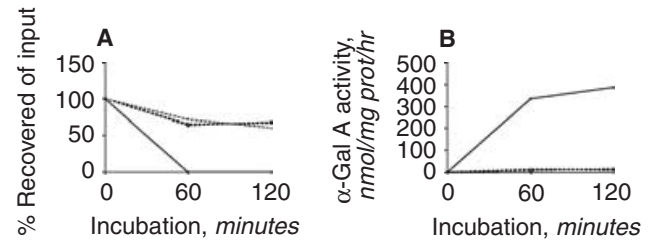


Fig. 4. Enzyme activity in plasma (A) and leukocytes (B) of three IgG-negative (dotted line) and one IgG-positive (solid line) patient after 60 and 120 minutes of in vitro incubation with agalsidase beta.

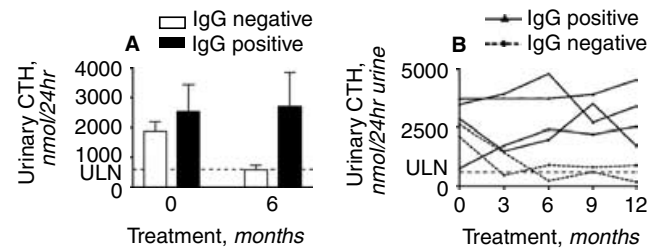


Fig. 5. Urinary CTH in both IgG-positive and IgG-negative patients before and after 6 months of treatment with rh- α Gal A (A). Results expressed as mean $\pm$ SD. * $P < 0.01$. Urinary CTH in two patients without, and four patients with IgG antibodies during a 12-month treatment period, demonstrating persistent urinary CTH clearance in two IgG-negative patients, where no clearance is seen in IgG-positive patients (B). Results expressed as mean $\pm$ SD.

cific binding of enzyme to the beads. This indicates, as examined for agalsidase beta, that a major proportion of circulating enzyme during an infusion is bound to IgG in vivo in anti-rh- α -Gal A IgG-positive patients.

Incubation of 5 μ g/mL agalsidase beta with EDTA-anticoagulated whole blood obtained from an IgG-positive patient before infusion 6 (Table 1, number 18) showed markedly reduced plasma enzyme activity after 60 and 120 minutes, whereas three IgG-negative patients had high plasma enzyme activities (Fig. 4A). In contrast, enzymatic activity in leukocytes taken from the IgG-positive patient was markedly increased compared to the IgG-negative patients (Fig. 4B), suggestive of increased uptake of enzyme in leukocytes because of anti-agalsidase IgG complexes.

Urinary CTH excretion

Mean urinary CTH levels at baseline did not differ significantly between patients with and without IgG antibodies, but were statistically different after 6 months of treatment ($P < 0.01$, Fig. 5A). Mean urinary CTH levels (nmol/24 hr urine) decreased in IgG-negative patients (from 1890 ± 797 to 603 ± 291 , $P < 0.068$) and increased in IgG-positive patients (from 2535 ± 988 to 2723 ± 1212 , $P = 0.49$). The changes in urinary CTH did not differ

between patients treated with agalsidase alpha or beta. After 12 months of treatment, mean urinary CTH in two IgG-negative patients remained low at 525 ± 508 nmol CTH/24hr urine, whereas CTH remained high at 2964 ± 1257 nmol CTH/24hr urine in four IgG-positive patients and showed no significant decline (Fig. 5B).

In the IgG-negative patients no difference was seen in patients treated with agalsidase beta at a dose of 1.0 mg/kg ($N = 1$), or the 0.2 mg/kg agalsidase alpha ($N = 2$) or beta ($N = 1$). Renal function (as determined by 24-hour creatinine clearance) did not show a correlation with CTH levels ($P = 0.52$, $r = 0.22$) before start of therapy. Renal function remained stable in all patients during the study period, excluding the possibility that change in CTH could be attributed to change in renal function.

DISCUSSION

This study shows that Fabry patients often develop antibodies both toward agalsidase alpha and beta, that these antibodies are cross-reactive, and that they have equal neutralizing activity toward agalsidase alpha and beta. Eleven out of 16 male patients (69%) developed IgG antibodies within the first 6 months of treatment. Even though the number of patients studied is limited, there is no clear difference in the prevalence of antibodies between the two enzyme preparations.

Agalsidase alpha and beta are produced by different methods. Agalsidase alpha is produced in a continuous human cell line, whereas agalsidase beta is manufactured by classic DNA recombinant technology in CHO cells. There is currently much debate whether the difference in production system causes different enzymes (e.g., with regard to glycosylation), and subsequently a different response in patients [12]. The present study shows that the IgG antibodies that arise in patients treated with either product are cross-reactive. This indicates that the immunogenicity of rh- α -Gal A in male patients with Fabry disease is probably caused by an epitope that is evenly expressed in both preparations. The absence of a clear difference in prevalence of antibody formation and cross-reactivity supports the supposition that these enzymes are very similar.

The high prevalence of antibodies is probably due to the fact that most male Fabry patients do not express residual enzyme activity. This is in contrast with Gaucher disease, as most Gaucher patients do have some residual β -glucocerebrosidase activity. As a consequence, emergence of antibodies against β -glucocerebrosidase is much lower—of 1122 Gaucher patients treated with placental derived β -glucocerebrosidase, only 142 (12.8%) patients developed antibodies [15]. It is of interest to note that in our study three male Fabry patients with a residual enzyme activity 1.5 nmol/mg prot/hr or higher did not de-

velop antibodies to the administered α -Gal A during the first 6 months, nor did the two female patients. A larger number of patients treated for a longer period of time needs to be studied to see whether absence or presence of significant residual enzyme activity plays an important role in the development of antibodies.

The observation that all antibodies exhibit neutralizing capacities in vitro is in contrast with similar studies on the treatment with recombinant enzyme in Gaucher disease. In the aforementioned study, 10% of the treated Gaucher patients with IgG antibodies toward β -glucocerebrosidase showed inhibition of the enzymatic reaction, with more than 50% [15]. There is one report on enzyme neutralizing IgG antibody in Gaucher disease [16], although neutralizing effect, to some extent, is seen in more patients. In our study, all patients with antibodies showed inhibitory activity toward agalsidase. Apparently there is a single highly immunogenic epitope that has a close relation with the catalytic site, preventing the enzymatic reaction to take place after binding of an antibody on this epitope.

Whether inhibition of rh- α -Gal A activity by the IgG antibodies has a significant clinical effect is unclear (see also [17] for a general discussion on this issue). Prolonged treatment with agalsidase beta in patients who had seroconverted did not show a CTH reaccumulation in biopsied tissue of skin or kidney after 12 months of treatment [4]. In addition, after one year there was no evidence of immune complex depositions in the biopsied kidney tissue. However, the formation and binding of antibodies can have consequences for the targeting of rh- α -Gal A. Intravenously administered rh- α -Gal A is taken up through the mannose-6-phosphate-receptor, which is present on every cell. Antibody-antigen complexes are taken up by mononuclear cells. Induction of antibodies toward human rh- α -Gal A and subsequent formation of circulating enzyme-antibody complex leads to a significant uptake of enzyme by leukocytes. As a consequence, less enzyme may be available for the endothelial cells. We show that an increase of rh- α -Gal A uptake in vitro in leukocytes was observed in a patient who had developed antibodies, compared to three antibody-negative patients. Though this experiment was carried out in a patient treated with agalsidase beta, the complete cross-reactivity of antibodies toward both preparations suggests this should also occur in patients treated with agalsidase alpha. Diminished serum concentrations of rh- α -Gal A after the development of IgG antibodies have indeed been demonstrated in trials with both enzyme preparations [18, 19]. In trials with agalsidase beta, reduced maximum concentrations (C_{max}) and total area under the curve (AUC) up to 26% of baseline values in patients with the highest titers were noted. This might result in a less effective treatment compared to those who are IgG-negative.

This was confirmed in the present study, where IgG-negative patients all showed urinary CTH clearance, in contrast to only 2/7 of the IgG-positive patients. Urinary CTH is believed to originate from shed renal tubular cells. Recently, Thurberg et al confirmed that in Fabry disease these cells, as well as podocytes, contain most CTH storage in the kidney [9]. Interestingly, after 6 and 12 months of treatment with 1.0 mg/kg rh- α -Gal A biweekly, renal biopsies showed a decline in tubular CTH accumulation for the whole group despite the presence of anti-rh- α -Gal A antibodies [9]. Our results demonstrate that development of IgG antibodies apparently results in diminished CTH clearance in these tubular cells. This suggests that targeting of enzyme to the renal tubular cells is decreased following seroconversion. The fact that in the pivotal studies the majority of patients demonstrated a decline in antibody titer after prolonged treatment seen is reassuring but deserves further study [4, 5]. These results emphasize the need for further studies on antibody formation and treatment efficacy in patients treated with the two dosages currently used in daily practice [17].

CONCLUSION

This study shows that a high percentage of Fabry patients develop neutralizing IgG antibodies toward agalsidase alpha and beta. There is no clear difference in immunogenicity of the two enzymes. In addition, antibodies induced with agalsidase alpha or beta have equal affinity for both enzymes in vitro. These findings support an earlier study that suggested that at least the polypeptide moiety of both recombinant proteins is similar [20]. It seems therefore unlikely that switching from one product to the other will evade antibody formation and related side effects. The emergence of antibodies results in a less decrease in urinary CTH compared to IgG-negative patients. No clear effect of antibody formation on creatinine clearance was observed. Whether long-term efficacy of ERT is hampered by these IgG antibodies is currently unknown and should be carefully investigated.

ACKNOWLEDGMENT

This study was financially supported by the Dutch Medical Insurance Board, and was made possible by the cooperation of the Dutch Fabry patients. Mrs. Els Ormel, Fabry study nurse, is gratefully acknowledged for drawing and handling of blood samples.

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