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Extended evaluation of the safety and efficacy of GAD treatment of children and adolescents with recent-onset type 1 diabetes: a randomised controlled trial

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Abstract:

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Abstract

AIMS/HYPOTHESIS: The aim of this study was to investigate the safety and efficacy of alum formulated glutamic acid decarboxylase GAD_{65} (GAD-alum) treatment of children and adolescents with Type 1 diabetes after 4 years of follow-up. METHODS: Seventy children and adolescents aged 10-18 years with recent-onset of Type 1 diabetes participated in a Phase II, double-blind, randomized placebo-controlled clinical trial. Participants received a subcutaneous injection of either 20 µg of GAD-alum or placebo at baseline and one month later. At follow-up after 30 months there was a significant preservation of residual insulin secretion as measured by C-peptide, in the group receiving GAD-alum compared to placebo. This was particularly evident in patients with <6 months disease duration at baseline. There were no treatment related serious adverse events. We have now followed these patients for 4 years. 59 patients, 29 who had been treated with GAD-alum and 30 who had received placebo, gave their informed consent. RESULTS: One patient in each treatment group experienced an episode of keto-acidosis between month 30 and 48. There were no treatment related adverse events. In those patients who were treated within 6 months of diabetes diagnosis, fasting C-peptide had decreased significantly less in the GAD-alum group than in the placebo-treated group after 4 years. CONCLUSION/INTERPRETATION: 4 years after treatment with GAD-alum, children and adolescents with recent-onset Type 1 diabetes continue to show no adverse events and possibly preservation of C-peptide of clinical relevance.

Abbreviations:

GAD_{65}, glutamic acid decarboxylase 65

GAD-alum, Alum formulated glutamic acid decarboxylase 65

LADA, Latent autoimmune diabetes in adults

Introduction

In spite of an intensive treatment Type 1 diabetes causes substantial morbidity and mortality [1, 2]. Residual insulin secretion facilitates metabolic control and decreases the risk of keto-acidosis [3], and even modest beta cell function, with stimulated C-peptide above 0.2 pmol/ml, may reduce long-term complications [4]. Type 1 diabetes is an autoimmune disease [5]. However, most attempts with immune intervention to preserve residual beta cell function have achieved limited benefits or have been associated with adverse effects [6-19]. Treatment with anti-CD3 monoclonal antibodies appears so far most promising, but several patients treated in this way, as well as with anti-CD20-monoclonal antibodies, have experienced treatment-related adverse events [20, 21].

Auto-antigens may be used to induce immunological tolerance [22]. Insulin and glutamic acid decarboxylase 65 (GAD_{65}) are major auto-antigens in Type 1 diabetes [23, 24] and have been tested in immunomodulation experiments [25]. Data from studies of the spontaneously diabetic NOD mouse have indicated that GAD_{65} prevents Type 1 diabetes [26, 27]. A dose-finding study in patients with latent autoimmune diabetes in adults (LADA), indicated that a prime and boost injection of 20 µg GAD-alum (recombinant human GAD65 in a standard vaccine formulation with alum) might preserve residual insulin secretion [28]. This treatment was easy to perform and well tolerated by the patients, and at a follow-up after 5 years there were no treatment-related adverse events and potential efficacy in one of the subgroups [29].

We administered GAD-alum or placebo to 70 young patients with recent-onset Type 1 diabetes in order to test whether it would reduce or halt the loss of residual insulin secretion [30] [NCT00435981]. The results of the 15 month study period followed by 15 months of further observation showed significant preservation of residual insulin secretion for at least 30 months. The efficacy was mainly restricted to patients with <6 months duration of diabetes when receiving their first GAD-alum injection. The treatment was very well tolerated by the patients and no treatment related adverse events were seen. We now report the results of an extended follow-up after 4 years to evaluate the long-term clinical effect and safety.

Patients and methods

In total 118 patients were screened, and 70 of these were found to be eligible for inclusion in the treatment phase of the study (Figure 1). The first patient was enrolled in October 2005. The 70 patients were randomized to a double-blind treatment with either 20 µg of recombinant human GAD_{65} formulated in alum (Diamyd®, Diamyd Medical, Stockholm, Sweden; 35 patients) or placebo (the same formulation without $rhGAD_{65}$ 35 patients) [30]. Thus each patient received a subcutaneous primary injection of either GAD-alum or placebo on day 1 followed by a boost one month later. All but one patient received two doses of either GAD-alum or placebo (Figure 1). One patient (a girl in the placebo group) was withdrawn from the study after 1 week; she received only one injection and was lost to follow-up. A total of 69 patients, 35 in the GAD-alum group and 34 in the placebo group, completed the original 30 month study period. Participants had been diagnosed with Type 1 diabetes within the previous 18 months before screening. The inclusion criteria were GAD_{65} auto-antibodies (GADA) and fasting C-peptide levels above 0.1 nmol/l. All patients were treated with multiple daily injections of insulin with a target HbA1c level of less than 6.5% (corresponding to <7.5% DCCT value). At baseline two patients in the placebo group but no patients in the GAD-alum treated group were using an insulin pump. Blinding of subjects and investigators to GAD-alum or placebo was removed at month 30.

After 4 years, all the patients, and for those children <18 years old also their parents, were contacted and asked whether they were willing to participate in a follow-up study. All 69 remaining patients could be reached but only 59 patients agreed to participate, of whom 29 had been treated with GAD-alum and 30 had received placebo. Several of the patients had left their original pediatric clinic and moved to alternative clinics. It took some time to get ethical approval for those new clinics, to recruit and inform physicians and nurses willing to participate in the follow-up, and then finally to organise visits. Therefore, the follow-up was not performed until 49 to 52 months after the first injection of study drug. Baseline Demographic and Clinical Characteristics of Treatment Groups are shown in Table I. Safety data were reported for 56 patients, 28 in each treatment arm. This study was approved by the Research Ethics Committee at the Faculty of Health Sciences, Linköping University, Sweden and the regulatory authorities in Sweden. Written informed consent was obtained from participating individuals in accordance with the Declaration of Helsinki.
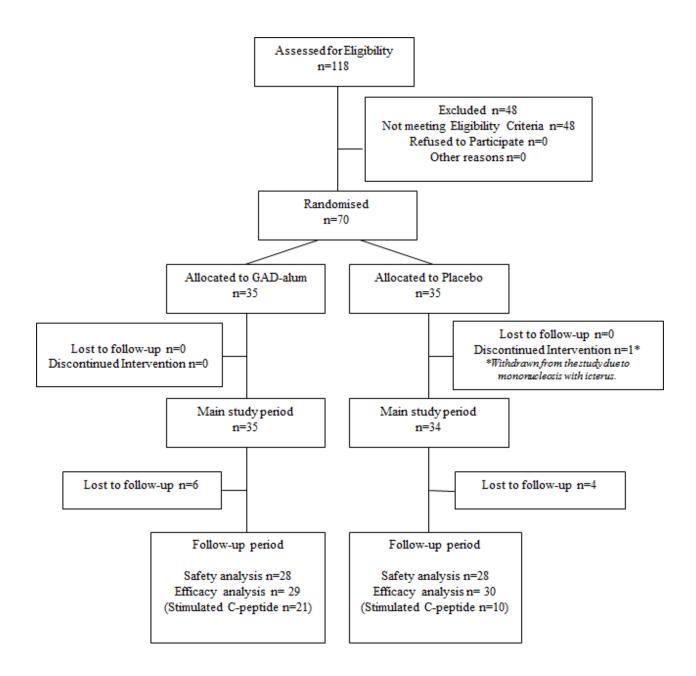
Figure 1. Disposition of Patients.
Enrollment, randomization and allocation of patients to treatment groups during the main study period and follow-up.

A fasting blood sample was received from all 59 patients. We were concerned that excessive demands might cause patients to refuse to participate in the follow-up. We therefore decided to perform a mixed meal tolerance test [MMTT] in accordance with the European study on estimation of beta cell function [31] in only those 31 patients (21 in the GAD-alum group and 10 in the placebo-group) who at the 30 month follow-up had a maximal C-peptide response >0.20 nmol/l. This test includes ingestion of 6 ml Sustacal®/kilogram of body weight with a maximum of 360 ml, to be ingested within 5 minutes. The meal test was performed in the

Table 1. Baseline Demographic and Clinical Characteristics of Treatment Groups

Characteristic	All patients		Included in follow-up		Excluded from follow-up	
	GAD-alum ($n = 35$)	Placebo ($n = 34$)	GAD-alum ($n = 29$)	Placebo ($n = 30$)	GAD-alum ($n = 6$)	Placebo ($n = 4$)
Mean age ± SD, *years*	13.8 ± 2.3	12.8 ± 1.9	13.6 ± 2.4	12.8 ± 1.9	14.7 ± 2.0	12.7 ± 2.0
Mean duration of diabetes ± SD, *months*	9.9 ± 5.3	8.8 ± 5.4	9.4 ± 5.4	8.5 ± 5.4	12.2 ± 4.5	10.8 ± 5.9
Mean BMI at screening ± SD, *kg/m²*	19.5 ± 2.4	20.5 ± 3.2	19.1 ± 2.3	20.3 ± 3.0	20.4 ± 2.7	21.4 ± 4.6
Gender distribution, *n (%)*						
Female	23 (66)	18 (53)	19 (65.5)	15 (50)	4 (66.6)	3 (75)
Male	12 (34)	16 (47)	10 (34.5)	15 (50)	2 (33.3)	1 (25)
Mean fasting C-peptide ± SD, *nmol/l*	0.3 ± 0.2	0.4± 0.2	0.3± 0.2	0.4± 0.2	0.4± 0.2	0.4± 0.3
Mean stimulated C-peptide AUC ± SD, *nmol/l*2hour*	1.2 ± 0.6	1.4± 0.9	1.3± 0.6	1.4± 0.9	1.1± 0.5	1.3 ± 0.9
Mean HbA1c ± SD, *%*	6.3 ± 1.3	6.2 ± 1.0	6.2 ± 1.3	6.2 ± 0.9	6.8± 1.1	6.9± 1.0
Mean insulin dose/kg bodyweight ± SD, *U/kg*	0.7± 0.3	0.7 ± 0.3	0.7± 0.3	0.6 ± 0.3	0.7± 0.2	0.8± 0.4
Mean plasma glucose prior to MMTT ± SD, *mmol/l*	9.4 ± 4.0	8.8 ± 3.3	9.5 ± 4.1	8.7 ± 3.4	8.8 ± 3.5	8.8 ± 1.3
Mean fasting C-peptide/plasma glucose x 10^{-12} ± SD	40 ± 23	45 ± 29	40 ± 23	45 ± 29	41 ± 22	42 ± 37
Median GADA titer, *U/ml*	601	861	539	786	1028	1637

morning (between 7 and 10 A.M.) after an overnight fast, in which no food or drink (with the exception of water) and no smoking occurred after 10 P.M. the preceding day. The patients took no short-acting insulin for at least 6 hours before the test. Patients on continuous subcutaneous insulin infusion continued their normal basal rate, but received no added boluses for at least 6 hours prior to the test.

A general physical examination including vital signs (supine blood pressure, pulse rate, weight and height, Body Mass Index (BMI)) and a neurological examination (Patellar Reflex, Achilles' Reflex, Vibration Sensitivity Big Toe and Muscle Tonus) were conducted.

Blood samples for C-peptide analysis were collected before, 30, 60, 90 and 120 minutes after beginning of the MMTT. Other evaluations included clinical examination, hematology, biochemistry, and blood glucose, insulin requirement (units per kg body weight and 24 hours).

C-peptide levels were measured as study progressed in serum samples with a Time-resolved fluoroimmunoassay (AutoDELFIA^{TM} C-peptide kit, Wallac, Turku, Finland). Results were validated with inclusion of a C-peptide control module containing a high, a medium and a low-level control in each assay (Immulite, DPC, UK). The 1224 MultiCalc® program (Wallac) was used for automatic measurement and result calculation and measurements were expressed in nmol/l.

HbA1c was analyzed by an immunological method, calibrated against the Swedish national standard Mono-S and continuously controlled against the External Quality Assurance in Laboratory medicine in Sweden (EQALIS) reference method.

Statistical analyses

Data management and statistical analyses were performed by the contract research organization Trial Form Support AB, Lund. An analysis of covariance (ANCOVA) model was used for formal analyses where the change from baseline was taken as the response variable, treatment as the explanatory variable and the baseline value as a covariate. Subgroup analyses of patients with <6 months diabetes duration at baseline was done as prespecified in

the analyses plan for the original study [30]. P values <0.05 were regarded as statistically significant.

Results

Baseline demographic and clinical characteristics of all the participants in the Phase II trial are illustrated in Table I. In addition, the same information for patients that later participated or were excluded from the 4 year follow-up is also shown.

As the study was unblinded at the 30 months follow-up, we searched if it was a difference on the 30 months C-peptide levels between the patients who decided to participate in the 4 years follow-up and those who dropped, both for all the patients and for the group who had <6 months duration at baseline. When all patients participating in the trial were compared, no difference between patients who continued in the study and those who dropped out was observed, either for the GAD-alum or the placebo group. Among the patients with <6 months, only one patient from the GAD-alum and one from the placebo group dropped out from the 48 months follow-up (Table II).

Table II. C-peptide levels (nmol/l) at 30 months for patient participating at the 4 year control and for those who dropped out.

		GAD-alum		Placebo	
		Number of patients	C-peptide (nmol/l)	Number of patients	C-peptide (nmol/l)
All patients	Continued in the trial	29	0.13±0.02	30	0.09±0.02
	Dropped out	6	0.11±0.03	4	0.10±0.04
Patients < 6 months	Continued in the trial	10	0.19±0.03	13	0.09±0.03
	Dropped out	1	0.06	1	0.03

Reported adverse events

There were 4 adverse events reported in 4 patients treated with GAD-alum and 6 adverse events reported in 5 patients in the placebo group. One diabetic keto-acidosis in each treatment group led to hospitalization that is to be regarded as a Serious Adverse Event. None of the events were considered to be treatment-related. In addition to these adverse events there were 2 self-reported severe hypoglycaemias in 2 patients in the GAD-treated group and 5 severe hypoglycaemias in 4 patients in the placebo group.
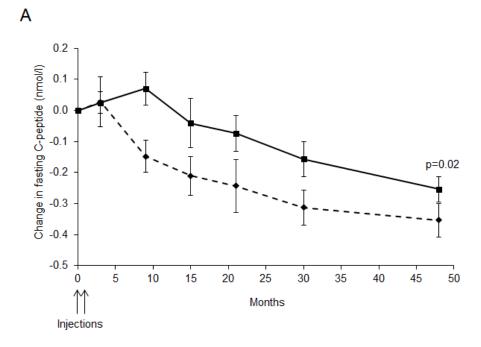
Physical examination

With the exception of one patient with restricted patellar reflex and one patient with low vibration sense in the GAD-treated group, nothing abnormal was found in the neurological examination. The physical examination showed nothing abnormal.

C-peptide

At 4 years there was a continued significant preservation of fasting C-peptide after treatment with GAD-alum compared to placebo in patients who had <6 months diabetes duration at baseline (p=0.02) (Figure 2). The previously observed effect of GAD-alum treatment on the change in fasting C-peptide level for all patients [30] was still apparent at the 4 year follow-up, although it was not statistically significant (Figure 3). There was no difference in fasting C-peptide between GAD-alum treated and placebo treated patients who had a diabetes duration ≥6 months at baseline.

For the follow-up study, the mixed meal tolerance test was performed only in the selected group of patients who had a maximal C-peptide response of >0.20 nmol/l at 30 months follow-up, that is in 21 GAD-alum-treated patients and 10 patients in the placebo group. After 2 hours MMTT, the change of stimulated C-peptide secretion from baseline up to 4 years seemed to decrease less in the individuals who received GAD-alum (mean change -0.990 ± 0.085 nmol/l) than in the placebo group (mean change -1.202 ± 0.0148 nmol/l). The difference in change between the two groups remained constant at 0.212 nmol/l, but was no longer statistically significant, as previously reported for the 30 months follow-up [30].

Exploratory subgroup analyses, was done as prespecified in the analyses plan for the original study [30]. Duration of diabetes at baseline was found to influence the degree C-peptide preservation but no other factor at baseline (age, BMI, sex, fasting C-peptide, stimulated C-peptide at treatment, HbA1c, insulin dose per kg body weight, glucose before MMTT, fasting C-peptide/glucose).
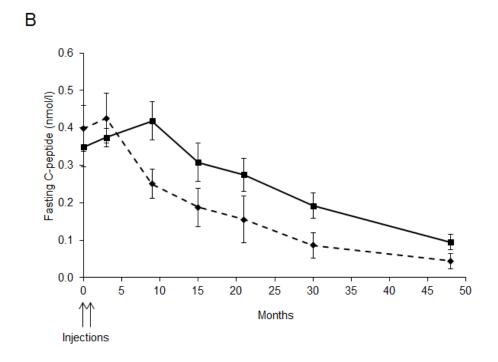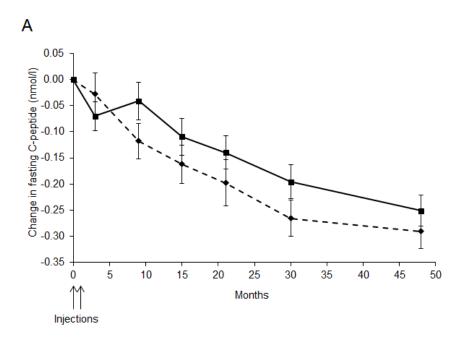
Figure 2. Mean Change in Fasting C-peptide and total C-peptide. (A) Mean change from baseline to 48 months (4 years) in fasting C-peptide and (B) total C-peptide (nmol/l) at each time points are shown for follow-up patients treated within 6 months of diagnosis of Type 1 diabetes (10 patients in the GAD-alum group (☐■☐) and 13 patients in the placebo group (--♦--). I bars indicate standard errors.
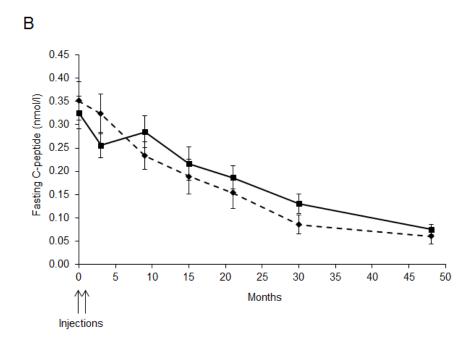
A

B

Figure 3. Mean Change in Fasting C-peptide and total C-peptide. (A) Mean change from baseline to 48 months in fasting C-peptide and (B) total C-peptide at each time (nmol/l) are shown for all follow-up patients (29 patients in the GAD-alum group (☐■☐) and 30 patients in the placebo group (--♦--). I bars indicate standard errors.

Discussion

The initial Phase II trial aimed to study the safety and efficacy of GAD-alum treatment was designed with a study period of 30 months. Several of the adolescents or young adults were not willing to participate in an extended follow-up, which might disrupt their other activities. This explains why we could not follow-up more than 59/69 patients (85.5 %). The small sample limits our chances to show statistically significant differences, but as shown in Table I there is no difference in baseline data prior to treatment between those patients who participated in the follow-up and those who did not, and therefore selection bias should not explain our results. The analyses of the 30 months C-peptide data for the patients remaining in the 4 years study and those who dropped out, both for all the patients and the group with <6 months, shows that the unblinding at the 30 months follow-up did not introduce a bias into the subsequent population participating at the 4 years control. We also believe that the results are complete enough to show that even 4 years after the GAD-alum treatment there were no treatment-related adverse events, either according to history, or according to self-reported numbers of keto-acidosis or severe hypoglycaemia, or according to physical examination.

With respect to the efficacy data we should be cautious about drawing conclusions. The study is no longer double blinded which might influence the treatment and clinical efficacy. Four years after treatment, fasting C-peptide, the primary endpoint in the original study, remained significantly better preserved in patients with <6 months diabetes duration at baseline, a sub-group prespecified in the original analysis plan. No other factor than duration of diabetes at baseline was found to influence the response to GAD treatment.

As the mixed meal tolerance test was difficult to motivate in patients who already at the 30 month follow-up had a very small residual insulin secretion, we performed such tests only in the very selected group of patients with a maximal response of C-peptide >0.20 nmol/l at 30 months. This clearly limits our chances to see an effect of treatment and consequently we can only conclude that the decline in C-peptide Area Under the Curve was not faster in the GAD-alum treated group than in the placebo treated group.

In conclusion, treatment with GAD-alum of children and adolescents with recent-onset Type 1 diabetes has, still 4 years after administration, not lead to any adverse events. Fasting C-peptide remains significantly better preserved relative to placebo in those patients with <6 months diabetes duration at baseline, which suggests a quite long-standing efficacy.

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