Sustained Effects of Interleukin-1-Receptor Antagonist Treatment in Type 2 Diabetes Mellitus

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**Objective:** Interleukin (IL)-1 impairs insulin secretion and induces beta-cell apoptosis. Pancreatic beta-cell IL-1 expression is increased and interleukin-1-receptor antagonist (IL-1Ra) expression reduced in patients with type 2 diabetes mellitus. Treatment with recombinant IL-1Ra improves glycemia and beta-cell function and reduces inflammatory markers in patients with type 2 diabetes mellitus. Here we investigated the durability of these responses.

**Research Design and Methods:** Among 70 ambulatory patients with type 2 diabetes and A1C and body mass index higher than 7.5% and 27, respectively, randomly assigned to receive 13 weeks of anakinra, a recombinant human IL-1Ra, or placebo, 67 completed treatment and were included in this double-blinded 39 week follow-up study. Primary outcome was change in beta-cell function following anakinra withdrawal. Analysis was done by intention-to-treat.

**Results:** Thirty-nine weeks following anakinra withdrawal the proinsulin to insulin (PI/I) ratio but not stimulated C-peptide remained improved by -0.07 (95% CI -0.14 to -0.02, P=0.011) compared to placebo treated patients. Interestingly, a subgroup characterized by genetically determined low baseline IL-1Ra serum levels, maintained the improved stimulated C-peptide obtained by 13 weeks of IL-1Ra treatment. Reductions of C-reactive protein (-3.2 mg/l [95% CI -6.2 to -1.1, P=0.014]) and of IL-6 (-1.4 ng/l [95% CI -2.6 to -0.3, P=0.036]) were maintained until end of study.

**Conclusions:** IL-1 blockade with anakinra induces improvement of the PI/I ratio and in markers of systemic inflammation lasting 39 weeks following treatment withdrawal.
Type 2 diabetes mellitus is caused by inability of the functional beta-cell mass to compensate for increased insulin needs due to insulin resistance (1). During the course of the disease beta-cell function progressively declines irrespective of treatment with glucose-lowering drugs (2-4). Beta-cell mass is reduced through apoptosis (5) and type 2 diabetes is associated with a low-grade systemic inflammation (6), but the mechanisms underlying beta-cell failure and destruction in type 2 diabetes remain elusive.

In vitro, long-term exposure to high glucose and the peptide hormone leptin secreted by adipose tissue, induce beta-cell apoptosis and production of the proinflammatory cytokine interleukin (IL)-1 in beta-cells and pancreatic islets, respectively (7,8). IL-1 inhibits the function and induces apoptosis of beta-cells (9) and has been implicated as a mediator of the beta-cell destruction leading to type 1 diabetes mellitus (10). Exogenous addition of interleukin-1-receptor antagonist (IL-1Ra), a naturally occurring competitive inhibitor of IL-1 signaling protects the beta-cells from the deleterious effects of high glucose and leptin exposure (7,8).

Both beta-cell expression and serum levels of IL-1Ra are reduced in patients with type 2 diabetes (8,11). This inadequate IL-1 antagonism seems to be a genetic trait, since genetic polymorphisms in the gene encoding IL-1Ra are associated with altered serum levels of IL-1Ra (12-15).

We previously showed that 13 weeks of IL-1Ra treatment improved beta-cell function, reduced A1C and markers of systemic inflammation in patients with type 2 diabetes (16). The aim of this 39-week follow-up study was to investigate the durability of these effects.

**RESEARCH DESIGN AND METHODS**

**Study Design and Patients:** This study was a 52-week investigator-initiated, placebo-controlled, double-blind, parallel group randomized proof of concept clinical trial conducted in Switzerland (University Hospital Zurich) and Denmark (Steno Diabetes Center) between January 2004 and March 2006. The protocol for the study was in accordance with the declaration of Helsinki and approved by the local ethical committees. Written informed consent was provided by all patients before entering the study.

The study was designed *a priori* in two parts (figure 1). The first part was a 13-week intervention study to test the efficacy and safety of recombinant human IL-1Ra (anakinra, [Kineret]) in patients with type 2 diabetes, with metabolic control as primary outcome and beta-cell function, insulin sensitivity and inflammatory markers as secondary outcomes, as reported previously (16). The second part of the study reported here (Consort information in Online Appendix available at [http://care.diabetesjournals.org](http://care.diabetesjournals.org)) was a 39-week follow-up study commencing at the time of withdrawal of study drug to test the durability of the intervention on beta-cell function, inflammatory markers, insulin requirement and insulin-sensitivity. In the follow-up study glucose-lowering therapy was intensified if indicated, and insulin treatment was initiated if A1C was higher than 8.0% or fasting plasma glucose exceeded 8 mmol per liter. Other medications were added or increased in dose at the discretion of the investigator. The study was unblinded after the last patient’s final visit (week 52).

Inclusion and exclusion criteria have been described previously (16). Inclusion criteria were: Age of 20 years or more, type 2 diabetes according to criteria from the American Diabetes Association (17) for more than 3 months, BMI > 27 kg/m², A1C > 7.5%, and no change in either type or doses of medications in 3 months preceding the study.
Briefly, exclusion criteria were: Autoantibodies to GAD 65 or islet cell antibody 512, A1C > 12%, fasting c-peptide < 400 pmol/liter, and current treatment with anti-inflammatory drugs (low-dose aspirin was allowed).

**Study outcomes:** Primary outcome of the follow-up study was change between baseline and 52 weeks in beta-cell secretory function measured by the fasting ratio of proinsulin to insulin (PI/I) and the area under the concentration–time curve (AUC) for stimulated C-peptide during an oral glucose-tolerance test, an intravenous stimulation test with glucose, glucagon and arginine and the two tests combined. Secondary outcomes included change between baseline and 52 weeks in IL-6, C-reactive protein (CRP), insulin requirements, A1C and the homeostasis model assessment insulin-sensitivity index (HOMA-SI) (18). Furthermore IL-1Ra genotypes were determined.

**Study Procedures:** In the follow-up study patients were seen at week 13 on the last day of anakinra or placebo treatment and at weeks 26, 39 and 52. A1C was measured at every visit and glucose-lowering therapy was intensified if indicated. At baseline, and at 13 and 52 weeks, beta-cell secretory function was assessed by the fasting PI/I ratio and by a 2-hour oral glucose-tolerance test directly followed by a 12-minute intravenous glucose, glucagon and arginine stimulation test as described previously (16). Physical examination, funduscopy, routine safety blood tests and analysis of urinary albumin excretion were performed at baseline and after 13 and 52 weeks. At baseline whole blood was sampled for DNA analysis of the IL-1Ra gene (*IL-1RN*).

**Laboratory values:** C-peptide, insulin, and proinsulin were determined at the Steno Diabetes Center. Insulin and proinsulin were assessed by enzyme-linked immunosorbent assays, and C-peptide levels were determined by a time-resolved fluoroimmunoassay. IL-1Ra, IL-6 and CRP were measured at the University Hospital Zurich by enzyme-linked immunosorbent assays. Measurements of A1C levels and routine clinical laboratory tests were performed locally in the central laboratory units of the two participating centers. DNA was extracted from whole blood buffy coats by the Maxwell® 16 System (Promega). Genotyping the variable number tandem repeat (VNTR) polymorphism in intron 2 of *IL-1RN* was analyzed as described previously (19) with use of the (Forward) 5′-CTC AGC AAC ACT CCTAT-3′ and (Reverse) 5′-TCC TGG TCT GCA GGTAA-3′ primers and separation of the PCR products on 2% agarose gel. Genotyping of the single nucleotide polymorphism (SNP) tagged by rs4251961 near 5′ of *IL-1RN* (13) was performed using TaqMan® SNP Genotyping Assays (c-32060323-10, Applied Biosystems) according to the manufacturer's description.

**Statistical Analysis:** All end points were analyzed in the intention-to-treat population, defined as patients completing 13 week of anakinra or placebo treatment. Missing data were imputed by last observation carried forward. Differences in continuous variables were compared by two-sample t-test. The Mann-Whitney test was used in the case of non-normal distribution. For categorical variables, Fisher’s Exact test was used. Correlations were analyzed by regression analysis. A P value of less than 0.05 was considered to indicate statistical significance.

**RESULTS**

Thirty-three of 34, who finished anakinra treatment, and 31 of 33 patients, who finished placebo treatment, completed the study (figure 1).

At the end of the study, 39 weeks following withdrawal of anakinra or placebo treatment, PI/I ratio was lower in the former
anakinra treated patients compared to the former placebo treated patients with a baseline adjusted between-group difference of -0.07 (95% CI -0.14 to -0.02, P=0.011) (figure 2A). No differences in AUC for C-peptide during the oral glucose-tolerance test, the intravenous stimulation test and the combined test at study end between former anakinra and placebo treated patients were observed with baseline adjusted between-group differences of 11.0 nmol/liter x min (95% CI -13.4 to 35.3, P=0.224), 2.9 nmol/liter x min (95% CI -3.1 to 9.0, P=0.333) and 11.1 nmol/liter x min (95% CI -18.3 to 40.6, P=0.282) for the oral, intravenous and combined test, respectively.

Following 39 weeks of anakinra withdrawal CRP (-3.2 mg/l [95% CI -6.2 to -1.1, P=0.014]) (figure 2C) and IL-6 (-1.4 ng/l [95% CI -2.6 to -0.3, P=0.036]) (figure 2D) were reduced compared to placebo-group patients. No correlation between the reduction in PI/I ratio and the reductions in CRP \( r^2=0.007, \ P=0.670 \) or IL-6 \( r^2=0.012, \ P=0.556 \) was observed in the anakinra treated patients.

At 52 weeks the baseline adjusted mean difference in A1C from baseline between the former anakinra and placebo treated patients was -0.05% (95% CI -0.62 – 0.52, P=0.867) (figure 2B). The absolute mean reduction in A1C was from 8.7 (SE 0.2) % to 7.8 (0.2) % and from 8.2 (0.2) % to 7.4 (0.1) % in the anakinra and placebo groups, respectively. Insulin sensitivity at study end assessed by HOMA-SI was unchanged with a baseline adjusted between-group difference (anakinra vs. placebo) of -0.04 l^2/mM x mU (95% CI -0.20 to 0.13, P=0.485). The average daily insulin dose from withdrawal of anakinra or placebo treatment until week 52 increased by 18.9 (4.0) IU and 28.5 (7.9) IU (P=0.670) in the anakinra and placebo groups, respectively. Daily metformin dose was increased by 69 (50) mg and 308 (115) mg (P=0.257) in the anakinra and placebo groups, respectively. Other oral glucose lowering medications were unchanged during the study.

Statins were initiated in 7 and 8 patients during the follow-up of anakinra and placebo treated patients, respectively. Three anakinra and 4 placebo group patients started aspirin during the follow-up. The average weight gain during the study for anakinra and placebo treated patients was 1.3 (SE 0.6) kg and 1.7 (0.8) kg (P=0.678), respectively. Three symptomatic self-reported hypoglycemic episodes were noted; two in the placebo group and 1 in the anakinra treated group. No change in blood pressure, lipids, 24-hour urinary albumin excretion, or retinal fundus photo was observed during the study.

As reported previously (16) 21 of 34 patients responded to treatment defined as any reduction in A1C following 13 weeks of anakinra treatment (responders to anakinra), whereas 10 of 33 patients on placebo treatment experienced reductions in A1C (P<0.0001). To characterize the responders to anakinra treatment a subgroup analysis was performed. At baseline patients responding to anakinra (n=21) were older (P=0.015) and had a higher rate of cardiovascular disease (P=0.009) compared to non-responders to anakinra (n=13) (Online Appendix Table A1).

Furthermore baseline IL-1Ra serum level was lower in responders compared to non-responders to anakinra (519 [SE 104] pg/liter vs 1299 [314] pg/liter, P=0.009) and remained unchanged at the end of the study (figure 3A). As polymorphisms of the \( IL1RN \) gene encoding IL-1Ra have been associated to serum levels of the protein, two \( IL1RN \) gene polymorphisms were investigated. Allele 2 of the VNTR polymorphism in intron 2 of the gene has been associated with an increased serum IL-1Ra level (12,15). An overall correlation between allele 2 carrier status and baseline serum IL-1Ra levels was found \( r^2=0.18, \ P=0.021 \); however, there was no difference between responders and non-
responder with respect to allele 2 frequency
(P=1.0) (figure 3B). Allele C of the SNP
rs4251961 tagging near 5’ of IL1RN has
been associated with a lower serum IL-1Ra
level and higher serum CRP and IL-6 levels
(13,14). In this study the correlation between
allele C carrier status and baseline serum IL-
1ra levels was confirmed (r²=0.46, P<0.001),
however no correlation to baseline CRP
(r²=0.11, P=0.124) or IL-6 (r²=0.06, P=0.254)
was found. Allele C frequency was higher
(58%) in the responders to anakinra treatment
than in the non-responders (21%, P=0.007)
(figure 3B).

Identical target A1C at 52 weeks
(figure 3C) was reached with lower increases
in insulin dose (10.8 [SE 2.6] IU/day) in the
patients responding to anakinra compared to
anakinra unresponsive patients (31.4 [6.0]
IU/day, P=0.006) (figure 3D).

At the end of anakinra treatment
(week 13) responders to anakinra showed
improved beta-cell function as assessed by the
PI/I ratio (P=0.041) and AUC for C-peptide
during the stimulation tests (oral test: P=0.006,
intravenous test: P=0.048, and
combined tests: P=0.025) compared to
patients not responding to anakinra treatment
(figure 4). The improved beta-cell function
in responders to anakinra treatment was
maintained 39 weeks after anakinra
withdrawal, whereas a reduction in beta-cell
function in non-responders to anakinra
treatment was seen 39 weeks after anakinra
withdrawal (figure 4), with the exception of
the AUC for C-peptide of the intravenous
stimulation test, which was unchanged
(P=0.793) (figure 4C). The inflammatory
markers CRP and IL-6 were reduced
compared to baseline both at the time of
anakinra withdrawal and at the end of the
study irrespectively of whether the patients
were responsive (reduction in A1C) or
unresponsive to anakinra treatment. No
correlation between the changes in CRP and
IL-6 and any measurement of beta-cell
function in either of the two subgroups were
found (data not shown). No subgroup
differences in insulin-sensitivity measured by
HOMA-SI were observed.

CONCLUSIONS
In the first part of this clinical trial we
reported that 13 weeks of IL-1Ra treatment
with anakinra reduced A1C, improved beta-
cell function and reduced inflammatory
markers (16). In the present protocolled
follow-up study we show that the reduced PI/I
ratio and the CRP and IL-6 serum levels were
maintained 39 weeks after anakinra
withdrawal, indicating that at least 39 weeks
of remission of these parameters were caused
by 13 weeks of anakinra treatment.

Furthermore a subgroup analysis
showed that the 21 (62%) patients, who
experienced any reduction in A1C following
13 weeks of IL-1Ra treatment with anakinra
(responders), maintained their anakinra-
induced improved beta-cell function assessed
by both the PI/I ratio and stimulatory testing
39 weeks after anakinra withdrawal, whereas
the beta-cell function of the patients treated
with placebo and those unresponsive to
anakinra treatment further deteriorated
following cessation of placebo and anakinra
therapy, respectively. The superior beta-cell
function of the anakinra responsive patients
was reflected by a lower insulin requirement
to obtain target glycemia in this group.

The preserved beta-cell function 39
weeks following anakinra withdrawal
indicates that modulation of beta-cell function
or increased beta-cell mass occurred during
IL-1Ra therapy. Apart from one study using
short term intensive insulin treatment (20)
where the 45-51% who responded had
maintained acute insulin response to
intravenous glucose testing, no other therapy
has been shown to maintain beta-cell function
in type 2 diabetes following treatment withdrawal.
Both leptin and hyperglycemia induce IL-1 production in the pancreatic islets (7,8). Reduction of hyperglycemia improves beta-cell function (20). However the improvement of beta-cell function observed in this study does not seem to be a consequence of improved glycemia per se since declining beta-cell function was observed in patients not responsive to anakinra and placebo group patients, irrespective of the improved glycemic control obtained during the follow-up phase. This suggests that the inhibition of IL-1 signaling was the mechanism behind the improved beta-cell function.

Elevated IL-1Ra serum levels are found in non-diabetic individuals prone to develop type 2 diabetes (21), whereas patients with overt type 2 diabetes have reduced beta-cell expression and serum levels of IL-1Ra (8,11), indicating that a down-regulation of IL-1Ra expression is a consequence of the diabetic state. In this study low baseline IL-1Ra serum levels predicted the glycemic and beta-cell secretory responses to anakinra treatment. The increased frequency of the C allele of SNP rs4251961, associated with lower IL-1Ra and higher IL-1 serum levels (13,14) in the responders to anakinra treatment indicates that the observed 62% response-rate to anakinra treatment may be genetically determined. In accordance with this notion we have previously shown that beta-cells from 6 out of 10 patients with type 2 diabetes expressed IL-1 mRNA (22). The observed frequencies of the T and C alleles of SNP rs4251961 in this study did not differ from the frequencies in populations without type 2 diabetes (13,14), indicating that the C allele is not predisposing to diabetes development per se, but is a pharmacogenetic bio-marker for a glycemic response to anakinra treatment.

The lack of association between changes in CRP and IL-6, surrogate markers of systemic IL-1 activity, and the PI/I ratio or any reduction in A1C following anakinra therapy indicates that systemic inflammation is not likely to affect beta-cell function, and that the improved beta-cell function caused by anakinra treatment is due to inhibition of IL-1 produced in the vicinity of the beta-cell. IL-1 production could thus originate from the beta-cell (7) or from macrophages infiltrating the islets (23) as the IL-1B gene is overexpressed in blood monocytes from patients with type 2 diabetes (24).

The sustained reduction of CRP and IL-6 levels at 39 weeks after anakinra withdrawal is notable, and indicates that transient IL-1 blockade is capable of inducing not only a metabolic but also a 39-week long inflammatory remission. In this study the inflammatory remission was not related to insulin sensitivity. This is in line with recent findings from the Whitehall II study (25).

Limitations to this follow-up study include that multiple hypotheses were tested and that a subgroup analysis was performed.

In summary, this study suggests that 13 weeks of anakinra treatment is capable of inducing a 39-week long preservation of beta-cell function and a similar inflammatory remission in patients with type 2 diabetes. Low baseline serum IL-1Ra levels predicted the metabolic response, and this phenotypic trait was associated with the C-allele of an IL-1RN SNP. Future studies will have to confirm these results and to test higher doses and longer treatment and follow-up periods in patients with type 2 diabetes selected based on their IL-1RN SNP genotype. If confirmed the perspective of these findings is that anakinra treatment could be used as substitution therapy in patients with type 2 diabetes with genetically determined IL-1Ra deficiency and as induction therapy during flares of hyperglycemia during the course of the disease.

DISCLOSURE

Dr. Donath is listed as the inventor of a patent filed in 2003 for the use of an
interleukin-1-receptor antagonist for the treatment or prophylaxis against type 2 diabetes; otherwise the authors have no relevant conflict of interest to disclosure.
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IL-1Ra and sustained beta-cell function

Fig. 1: Enrollment and outcome.
Of the 70 patients who underwent randomization, 67 completed 13 weeks of anakinra or placebo treatment and were included in the present 39 week follow-up study. Thirty-three and 31 of the former anakinra and placebo treated patients, respectively, completed the study.

Fig. 2: Change in glycemic and inflammatory markers at study end.
Data of proinsulin to insulin ratio (A), A1C (B), C-reactive protein (C) and interleukin-6 at baseline and end of study (week 52) in patients treated with anakinra (former anakinra = black bars) or placebo (former placebo = white bars) from baseline until week 13. Data are mean (SE).

Fig. 3: Interleukin-1-receptor-antagonist serum levels and genotypes, and A1C and insulin requirements.
(A) Serum interleukin-1-receptor antagonist levels at baseline and end of study (week 52) in patients with (responders = white bars) or without (non-responders = black bars) any reduction in A1C following 13 weeks of anakinra treatment. (B) Allele frequencies of allele 2 and C of the VNTR tandem repeat polymorphism in intron 2 and the SNP tagged by rs4251961, respectively, of the interleukin-1-receptor antagonist gene in responders (white bars) and non-responders (black bars) to anakinra treatment. (C) A1C at baseline (week 0), and 13, 26, 39 and 52 weeks in responders (white squares) and non-responders (black triangles) to anakinra treatment. Data are mean (SE) or frequencies where indicated.

Fig. 4: Beta-cell function following anakinra withdrawal.
Beta-cell function assessed by the ratio of proinsulin to insulin (A), and AUC for C-peptide during an oral glucose-tolerance test (B), an intravenous stimulation with glucose, glucagon and arginine (C) and the oral and intravenous test combined (D) at baseline, anakinra withdrawal (week 13) and end of study (week 52) in patients with (responders = white bars) or without (non-responders = black bars) any reduction in A1C following 13 weeks of anakinra treatment. (A) *P=0.041 vs non-responders at week 13, †P=0.435 vs responders week 52, ‡P=0.016 vs non-responders week 52 and §P=0.005 vs non-responders week 52. (B) *P=0.006 vs non-responders at week 13, †P=0.750 vs responders week 52, ‡P=0.021 vs non-responders week 52 and §P=0.008 vs non-responders week 52. (C) *P=0.048 vs non-responders at week 13, †P=0.039 vs responders week 52, ‡P=0.793 vs non-responders week 52 and §P=0.092 vs non-responders week 52. (D) *P=0.025 vs non-responders at week 13, †P=0.947 vs responders week 52, ‡P=0.021 vs non-responders week 52 and §P=0.001 vs non-responders week 52. Data are mean (SE).
Fig. 1
Fig. 2
Fig. 3
**IL-1Ra and sustained beta-cell function**

Fig. 4

**A Ratio of Proinsulin to Insulin**
- Responders
- Non-responders

**B AUC for C-Peptide after Oral Glucose**

**C AUC for C-Peptide after IV Stimulation**

**D Combined AUC for C-Peptide after oral Glucose and IV Stimulation**