

Improved β -Cell Survival and Reduced Insulinitis in a Type 1 Diabetic Rat Model After Treatment With a β -Cell–Selective K_{ATP} Channel Opener

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Treatment with ATP-sensitive K^+ channel openers (KCOs) leads to inhibition of insulin secretion and metabolic “rest” in β -cells. It is hypothesized that in type 1 diabetes this may reduce β -cell death resulting from metabolic stress as well as reduce the immunogenicity of the β -cells during autoimmune β -cell destruction. We have investigated whether the β -cell–selective KCO compound, NN414, can be used to improve β -cell survival in DR-BB rats rendered diabetic by modulation of their immune system. The rats were treated three times daily on days 1–19 with NN414, diazoxide, or vehicle. On day 21, an intravenous glucose tolerance test was conducted to assess β -cell function. Postmortem histological analysis of rats’ pancreata assessed the degree of insulinitis and β -cell volume. Among NN414-treated rats, 46% (16 of 35) were found to have a β -cell mass similar to that of nondiabetic controls and significant glucose-stimulated C-peptide values, whereas only 11% (4 of 36) of vehicle-treated rats possessed a normal β -cell mass and function ($P < 0.002$, by χ^2 test). Furthermore, responsive NN414-treated rats were almost free of insulinitis. Thus, this study demonstrated that treatment with KCO compounds can indeed lead to preservation of β -cell function and reduction of insulinitis in a rat diabetes model. *Diabetes* 53:1089–1095, 2004

Type 1 diabetes is characterized by an immune-mediated destruction of β -cells, leading to clinical diabetes when 80–90% of β -cells have been lost. During this process, the demand on the remaining β -cells for insulin secretion increases as the number of β -cells decreases. In vitro, hyperglycemia induces glucotoxicity (1), a phenomenon that can lead to expression of interleukin (IL)-1 β in β -cells and result in upregulation of Fas and β -cell apoptosis in an auto- and paracrine fashion (2). Normalization of blood glucose through insulin treatment in recent-onset type 1 diabetic

subjects leads to a temporary rest in β -cell destruction and improved β -cell function, with a substantial portion of subjects entering clinical remission (the “honeymoon period”) (3). However, complete normalization of blood glucose is very difficult to obtain through exogenous insulin administration; thus, the remaining β -cells experience periods of hyperglycemia even when patients treat themselves with insulin. These incidences of hyperglycemia might accelerate the loss of the remaining β -cells.

ATP-sensitive K^+ channel (K_{ATP}) openers (KCOs) bind to K_{ATP} channels in the plasma membrane, leading to hyperpolarization and inhibition of Ca^{2+} influx and resulting in inhibition of insulin secretion (4). Thus, treatment with KCOs may lead to metabolic β -cell “rest,” protecting β -cells against exhaustion and making them less prone to apoptosis. Indeed, several lines of evidence suggest that the β -cell “rest” induced by KCOs may protect islets from glucotoxicity and autoimmune destruction. Treatment of rat islets with diazoxide, a nonselective KCO, has been shown to protect against streptozotocin-induced apoptosis in rats in vitro (5) and in vivo (6). Spontaneous development of diabetes was partially prevented in BB rats by the inhibition of endogenous insulin secretion with either insulin or diazoxide (7,8), and 3-month treatment of recent-onset type 1 diabetic subjects with diazoxide led to improved residual C-peptide levels 18 months after treatment was begun (9). Unfortunately, diazoxide is poorly tolerated in vivo, leading to adverse effects including hypotension, edema, and unwanted hair growth (9), effects attributable to the binding of diazoxide to K_{ATP} channels on nonislet cells.

In this study, we explored a new KCO, NN414, which is a Kir6.2/SUR1-selective KCO with improved specificity and selectivity toward β -cells (10). We compared the ability of NN414, diazoxide, and vehicle to preserve β -cell mass and function through a 3-week prevention and intervention trial using DR-BB rats as an accelerated model of type 1 diabetes.

In contrast to DP-BB rats, DR-BB rats do not spontaneously develop diabetes. However, when young DR-BB rats are treated with ART-2⁺–depleting antibody (ART-2 was formerly known as RT6) and polyinosinic:polycytidylic acid [poly(I:C)], β -cell destruction is initiated and diabetes develops within 10–18 days after the start of treatment (11). The poly(I:C) mimics the double-stranded RNA produced during some viral infections; hence, poly(I:C) injection stimulates antigen-presenting cells (APCs) via binding

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APC, antigen-presenting cell; IVGTT, intravenous glucose tolerance test; K_{ATP} , ATP-sensitive K^+ channel; KCO, K_{ATP} channel opener; IL, interleukin; poly(I:C), polyinosinic:polycytidylic acid.

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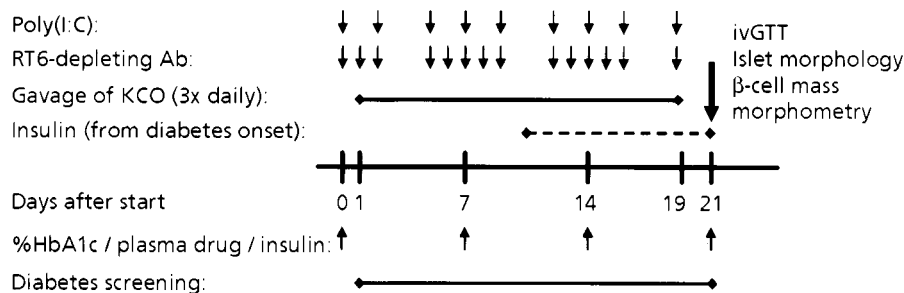


FIG. 1. Research design. DR-BB rats were assigned to an experiment group when they were 24–33 days old (day 0). ART-2⁺-depleting antibody (Ab) and poly(I:C) were given on the indicated days for at least 15 days or until blood glucose was >20 mmol/l. NN414, diazoxide, or vehicle was given by gavage three times daily on days 1–19. Insulin treatment was initiated when blood glucose was >20 mmol/l. Weekly blood samples were taken for measurement of HbA_{1c}, plasma drug, and plasma insulin levels. Daily measurement of body weight and blood glucose was carried out 1 h before afternoon gavage. On day 21, an IVGTT was performed after a 5- to 6-h fast, and pancreata were subsequently removed for histology.

to toll-like receptor 3, leading to activation of APCs and secretion of inflammatory cytokines, thus enabling efficient priming of autoreactive T-cells (12). The ART-2 depletion removes the ART-2⁺ regulatory cells that normally suppress T-cell responses, thus enabling the activation and unhampered expansion of autoreactive T-cells that can mediate β -cell destruction (13). This model therefore uses a systemic modulation of the immune response that allows autoreactive T-cells to become activated combined with the genetic predisposition of the DR-BB rat to develop a β -cell-specific response resembling type 1 diabetes in humans.

RESEARCH DESIGN AND METHODS

DR-BB rats were from our own colony at the Hagedorn Research Institute (11). This study was divided into six independent experiments, with an average of 18 rats (three groups of 6 rats each) per experiment, totaling 108 rats (36 rats per group). One rat was killed because of unrelated disease early in the study. Rats were 24–33 days old at the beginning of each new experiment (day 0). They were weaned 2–3 days before the start of the experiment and randomized into one of the three treatment groups. An outline of the experimental setup is shown in Fig. 1. All experiments were conducted in accordance with Danish Legislation and with approval from the Danish Animal Experimentation Inspectorate.

Diabetes induction. On days 0–14, the rats were injected three times weekly with 2 μ l/g body wt of 2.5 mg/ml poly(I:C) suspension (Sigma) and five times weekly with 300 μ l of concentrated culture supernatant produced from the DS4.23 hybridoma (a gift from Jan Rozing) that secretes the rat anti-rat ART-2⁺-depleting antibody, as previously described (11). For those rats that did not exhibit severe hyperglycemia (blood glucose >20 mmol/l) until after day 14, the treatment was continued until the first day when blood glucose reached >20 mmol/l.

Administration of KCO compounds. The rats were divided into three treatment groups: vehicle, 40 mg/kg diazoxide (Sigma), or 40 mg/kg NN414 [6-chloro-3-(1-methylcyclopropyl)amino-4H-thieno[3,2-e]1,2,4-thiadiazine 1,1-dioxide; Novo Nordisk]. These compounds were administered every 8 h by gavage from the morning of day 1 to the afternoon of day 19. The solvent was an aqueous solution of 40 mg/g glycerol, 4 mg/g gelatin, 6 mg/g sodium carboxymethylcellulose, and 1 mg/g methyl parahydroxybenzoate. Using a rat-feeding needle, 2 ml/kg of solvent alone, diazoxide, or NN414 solution was injected intragastrically.

Observations of rats during experiments. All rats were weighed daily. Blood glucose was measured daily 1 h before the afternoon dosing using a capillary blood sample from the tail obtained with Precision Xtra (Medisense). After the onset of severe hyperglycemia, urine glucose and ketones were measured daily and the animals were treated with insulin.

Insulin treatment of diabetic animals. To avoid complications resulting from hyperglycemia, insulin therapy was initiated at the first incidence of nonfasting blood glucose >20 mmol/l. Insulin (zinc-protracted bovine insulin; Novo Nordisk) was then administered three times daily in conjunction with the gavage. The insulin dosage was adjusted on a daily basis according to weight gain/loss, blood glucose level, glucosuria, and ketonuria; on average, diabetic rats received 12 units \cdot kg⁻¹ \cdot day⁻¹. The last insulin dose was given on day 21 in the morning.

Analysis of plasma insulin, plasma drug level, and HbA_{1c}. On days 0, 7, and 14, a 60- to 80- μ l blood sample was drawn from the tail vein and collected into EDTA-coated tubes. On day 21, plasma was obtained from the blood samples collected in connection with the intravenous glucose tolerance test (IVGTT; see below). Plasma was stored at -20°C until analysis. The samples were analyzed for insulin (day 0, 7, and 14 samples) and drug (days 7, 14, and 21) content.

Insulin analysis was carried out as a sandwich enzyme-linked immunosorbent assay. The mouse anti-insulin monoclonal antibody Hui-18 (Novo Nordisk A/S) was used as catcher, and the guinea pig anti-insulin antibody GP4042D (Novo Nordisk A/S) was used as detector antibody. Plates were blocked with wash buffer (PBS/Tween). The antibody A-TNP (raised against the hapten trinitrophenyl; Novo Nordisk A/S) was included in the buffers to reduce unspecific binding, and peroxidase-conjugated goat anti-guinea pig IgG (Chemicon) was used as the secondary antibody. Finally, the color was developed with tetramethylbenzidine substrate.

NN414 and diazoxide analysis was carried out by high-turbulence liquid chromatography and tandem mass spectrometry detection using a Turboflow 2300 HTLC system for separation and an MDS/Sciex API 3000 mass spectrometer for detection.

HbA_{1c} was measured in 5 μ l whole blood sampled from the tail vein (days 0, 7, and 14) or the periorbital plexus (day 21) in connection with the IVGTT. The blood samples were drawn into a 5- μ l heparin-coated capillary tube and stored at -20°C until analyzed. The blood was mixed with 250 μ l hemolysis buffer. HbA_{1c} was measured on a COBAS Mira by the immunoturbidimetric test, and the hemoglobin was measured by colorimetric test using an HbA_{1c} kit (Roche), according to the manufacturer's instructions. The percentage of HbA_{1c} was then calculated.

IVGTT. On day 21, the rats were fasted from the morning. Diabetic rats were treated with insulin as on the previous day. After a 5- to 6-h fast, the rats were anesthetized with 1.5 μ l/g body wt of a 1:1:2 solution of Dormicum (midazolam 5 mg/ml; Roche), Hypnorm (fentanyl citrate 0.315 mg/ml and fluanisone 10 mg/ml; Janssen), and sterile water. A baseline 600- μ l blood sample was taken from the fully anesthetized rats. A glucose bolus (1 g/kg) was injected intravenously into the tail, and then 2 and 30 min after the glucose challenge, 600- μ l blood samples from the periorbital plexus were collected in EDTA tubes containing 250 KIU Trasylol (aprotinin)/ml whole blood. Plasma was prepared and stored at -20°C for C-peptide analysis. C-peptide was determined using a radioimmunoassay for rat C-peptide (Linco) according to the manufacturer's instructions. In some of the experiments, blood samples from untreated, nondiabetic littermates were included as controls.

Immunohistochemistry. The pancreata were removed en bloc with the intestines and fixed in 4% paraformaldehyde for 24 h, dissected free of surrounding tissue, and weighed. The pancreata were then fractionated by the smooth fractionator method, with $f(1) = 1/4$ (14,15); two paraffin-embedded blocks per pancreas were prepared, each containing 8–11 systematically randomly selected pancreatic tissue cubes. Sections (3 μ m) were stained for insulin by an indirect peroxidase technique and VectorRed, and for glucagon, somatostatin, and pancreatic polypeptide combined, by the streptavidin-biotin-peroxidase technique and diaminobenzidine + Ni, lightly counterstained with Mayer's hematoxylin and mounted with Pertex (see Rolin et al. [16] for a detailed description). Adjacent or consecutive sections were stained with hematoxylin-eosin for evaluation of mononuclear cell infiltration, insulinitis, and general morphology.

Insulinitis. The insulinitis reaction was graded on a 0–4 scale, with 0 = normal islet, 1 = <33% of β -cell area infiltrated, 2 = 33–67% of β -cell area infiltrated, 3 = >67% of β -cell area infiltrated, and 4 = islet with no β -cells at all. All islets

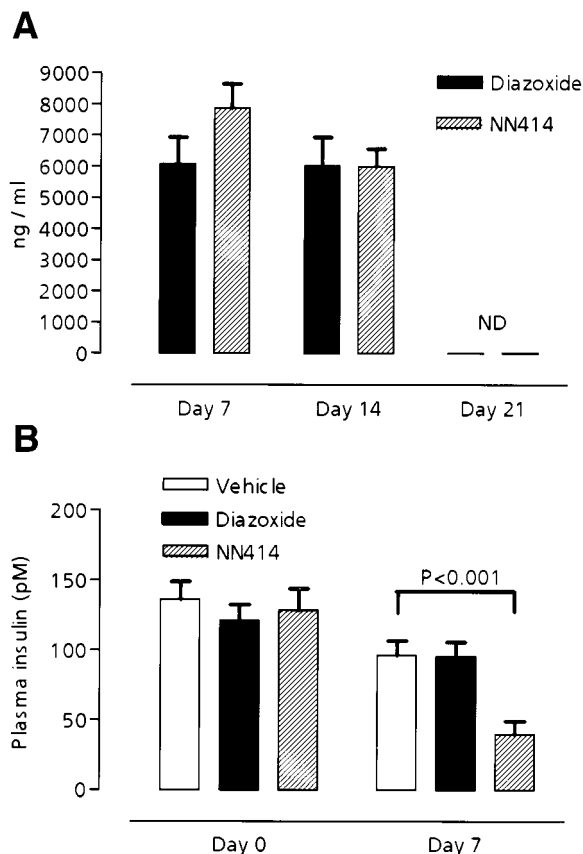


FIG. 2. Plasma drug and insulin levels. **A:** Plasma drug level was measured 7 h after the previous dosing (days 7 and 14) or 48 h after the previous dosing (day 21), and the plasma was analyzed for the relevant drug. The detection limit was 10 ng/ml. ND, not detectable. **B:** Plasma insulin level was measured on days 0 and 7 (i.e., before treatment with exogenous insulin was begun). A 59% decrease in plasma insulin was observed on day 7 in the NN414 group ($P < 0.001$ vs. vehicle by Student's *t* test). There was no difference between diazoxide- and vehicle-treated rats.

in two sections cut 250 μm apart were evaluated by two observers, blinded with respect to the origin of the sections.

Stereology. Insulin-stained sections were used for estimating β -cell volume fraction, as previously described (16). In brief, stereological analyses were performed using an Olympus BX-50 microscope fitted with a video camera and monitor, a PC-controlled motorized stage, and Cast-Grid 2.0 software. The volume fractions of β -cells were estimated by the point-counting stereological technique using a $\times 20$ objective, a grid of 4×64 points, and random systematic scanning of the tissue sections with step lengths of a maximum $600 \times 450 \mu\text{m}$ controlled by the PC. The origin of the sections was blinded to the observer. The estimated β -cell volume was expressed as the percentage of total pancreatic volume.

RESULTS

The study was designed as six individual experiments, each comprising three groups with an average of six rats in each. All data shown below represent the combined results from the six individual experiments. The experimental setup is shown in Fig. 1.

NN414 treatment inhibits plasma insulin level. The same mean plasma drug level was observed in diazoxide- and NN414-treated rats, and there was no change from day 7 to day 14 (Fig. 2A). On day 21, drug levels were below the detection limit. Although the administration of NN414 significantly inhibited plasma insulin levels (average of 59% inhibition 7 h after dosing), diazoxide administration did not ($P < 0.001$) (Fig. 2B). However, inhibition was

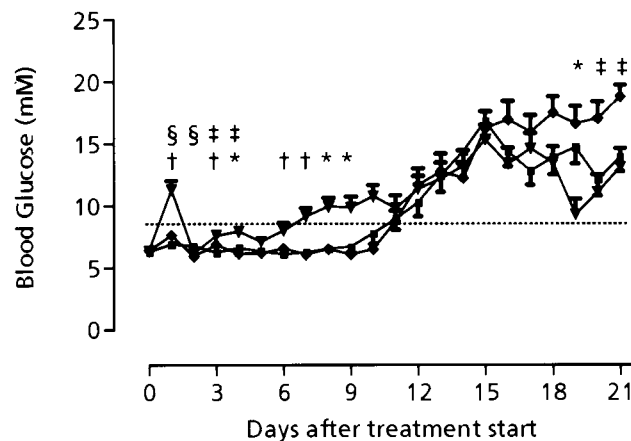


FIG. 3. Development in blood glucose. Moderate hyperglycemia was observed in NN414-treated rats before the onset of diabetes. The dotted line indicates the upper normal range of blood glucose in our study, defined as the mean + 3 SDs of nonfasting blood glucose in untreated rats (8.6 mmol/l). * $P < 0.01$; † $P < 0.0001$ for NN414-treated vs. vehicle-treated rats; ‡ $P < 0.01$; § $P < 0.0001$ for diazoxide-treated vs. vehicle-treated rats using two-tailed Student's *t* test.

higher (average of 80%) during the first 4 h after NN414 administration (K.S., C.F.G., D.L., J.B.H., J.S., H.M., unpublished observations). The fact that this dosage of diazoxide did not inhibit plasma insulin is likely explained by its having a lesser potency than NN414 (17).

Moderate hyperglycemia in NN414-treated rats. As previously described for another KCO, NNC 55-0118 (18), we observed an initial stagnation in body weight development in NN414-treated rats (data not shown) after which the growth rate was similar to that of vehicle-treated rats.

NN414 treatment induced moderate hyperglycemia in rats (nonfasting blood glucose levels 4–17 mmol/l) (Fig. 3). Hyperglycemia was most pronounced on day 1 after the first NN414 dose (coinciding with the stagnation in body weight), with 30 of 35 rats exhibiting blood glucose levels 3 SDs above the mean nonfasting blood glucose of control rats. However, on the following day, only two of the rats were hyperglycemic. This observation suggested that the rats were able to adapt to the drug and compensate for the NN414-mediated inhibition of insulin secretion once the blood glucose level became sufficiently high. A modest, yet significant, increase in blood glucose in diazoxide-treated rats was observed on day 1, and, similar to NN414-treated rats, a modest decrease in blood glucose was seen on day 2 ($P < 0.0001$). Thus, diazoxide might have suppressed insulin secretion early in the study, although we did not see any difference in plasma insulin levels on day 7.

Insulin therapy was initiated after the first instance in which blood glucose was >20 mmol/l. Insulin therapy indirectly induces β -cell “rest” due to a lowering of blood glucose and helps to preserve the remaining β -cell function. By using a combination of insulin therapy and KCO treatment in severely hyperglycemic animals, we attempted to obtain optimal β -cell “rest” and at the same time mimic the clinical situation as much as possible. In the clinic, patients would normally be treated with insulin from the time of diagnosis (i.e., after the clinical manifestation of disease). We chose 20 mmol/l as the threshold for initiation of insulin therapy because NN414 therapy by itself did not induce blood glucose >20 mmol/l. There was

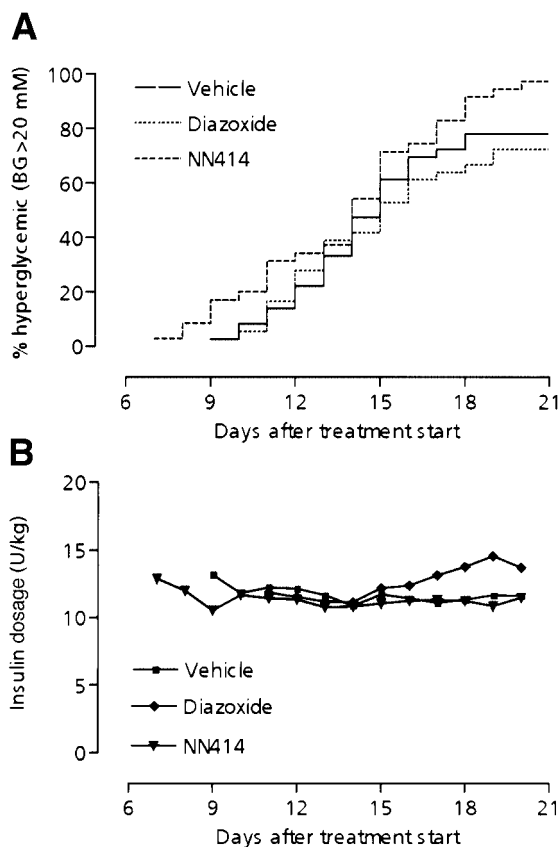


FIG. 4. Diabetes incidence and administered insulin. **A:** Cumulative incidence curves of the first occurrence of blood glucose (BG) >20 mmol/l. This end point was used to initiate insulin treatment. Notice that the presence of hyperglycemia may not truly represent diabetes, as NN414 per se induces hyperglycemia (see Fig. 3). **B:** The actual dosage of insulin administered to the rats is shown. Only rats receiving insulin were included.

no difference among the groups in time to initiation of insulin therapy (blood glucose >20 mmol/l) or in the actual dosages administered (Fig. 4). Overall, 87% of the rats had blood glucose >20 mmol/l at some point, and insulin treatment was initiated on day 14 ± 3 .

Rescue of β -cell function and mass in NN414-treated rats. On day 21, we measured glucose-induced C-peptide levels. Subsequently, all rats were killed for histological assessment of β -cell volume and insulinitis. The β -cell volume as the percentage of total pancreatic volume was measured by stereology. We included five untreated, nondiabetic littermates as controls to obtain the normal β -cell volume and C-peptide levels. These five rats had an average β -cell volume of 0.49% (95% CI 0.29–0.69%). (In this study, rats having a β -cell volume >0.29% are labeled “responders” and rats with a lower β -cell volume are labeled “nonresponders.”)

In the NN414 group, 45.7% (16 of 35) were responders, which was significantly higher than the 11.1% in the vehicle group (4 of 36; $P < 0.002$ by χ^2 test). In the diazoxide group, 13.9% (5 of 36) were responders (Fig. 5A).

As a measure of β -cell function, an IVGTT was carried out under full anesthesia 48 h after the last gavage (in 26 rats per group). The rats were fasted for 5–6 h before the IVGTT. The baseline as well as 2- and 30-min blood glucose levels were measured. The average 30-min glucose-stimulated C-peptide value of the responders was

1,840 pmol/l (range 342–4,600), which was 58% of the average level of the five control rats. There was no difference in C-peptide levels between responders from the three groups. The majority of the nonresponders (77%) had 30-min C-peptide values below the detection limit (25 pmol/l), whereas the remainder all had levels <172 pmol/l. Thus, there was a good correlation between β -cell volume and function. The 30-min C-peptide values of individual rats are shown in Fig. 5B.

Reduced insulinitis in NN414-treated rats. To assess whether NN414 treatment had an effect on insulinitis, the pancreata were scored for the presence of insulinitis on a 0–4 scale, with 0 indicating normal islets and 4 indicating end-stage destruction without any remaining β -cells. All responders consistently had an insulinitis score <2.0, and conversely nonresponders (except one rat) had an insulinitis score >2.0 (Fig. 5C). It is interesting that 15 of the 16 responders among NN414-treated rats had an average insulinitis score <0.5, indicating that they were almost entirely free of insulinitis. By contrast, only two of the responder rats in the vehicle group had an insulinitis score <0.5. Thus, NN414 treatment clearly reduced insulinitis in the responders. There was a good inverse correlation between the degree of insulinitis and the level of residual β -cell function ($r^2 = 0.78$; $P < 0.0001$ for $n = 31$ with C-peptide levels higher than the detection limit) (Fig. 5D).

Dual effect of NN414 on HbA_{1c} level. The finding that NN414 led to moderate hyperglycemia suggested that NN414 might also lead to increased HbA_{1c}. Indeed, we found a small but significant increase in HbA_{1c} on days 7 and 14. Nevertheless, there was no difference in the HbA_{1c} level among any of the groups on day 21 (Fig. 6A). The relative improvement in HbA_{1c} in NN414-treated rats compared with vehicle-treated rats during the last week could clearly be ascribed to the responders, who had a significantly lower HbA_{1c} on day 21 compared with nonresponders (Fig. 6B). Thus, the NN414-induced loss in glycemic control seen during the first 2 weeks of treatment was offset by a higher number of responding rats with significant β -cell function during the last week.

DISCUSSION

In this study, we showed that chronic suppression of insulin secretion obtained by three times daily administration of the KCO compound, NN414, led to preservation of β -cell volume, improvement in β -cell function, and reduction of insulinitis in a type 1 diabetes rat model. We did not observe any effect of the reference compound, diazoxide, a finding most likely attributable to the fact that diazoxide activates the K_{ATP} channel with a much lower affinity than NN414 (10). Thus, it appears that a higher dosage of diazoxide is needed to obtain a beneficial effect on β -cell survival (18). In previous studies in a spontaneously diabetic strain of BB rats, partial protection was obtained with $150 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ diazoxide (7).

At least two pathways leading to β -cell apoptosis exist; apoptosis can occur as a direct consequence of β -cell stress and high metabolic load resulting from hyperglycemia or as a result of insulinitis and the resulting inflammation of the islets. Sturis et al. (19) have clearly shown that NN414 can inhibit glucose-induced apoptosis in cultured human islets. However, we were surprised to also discover

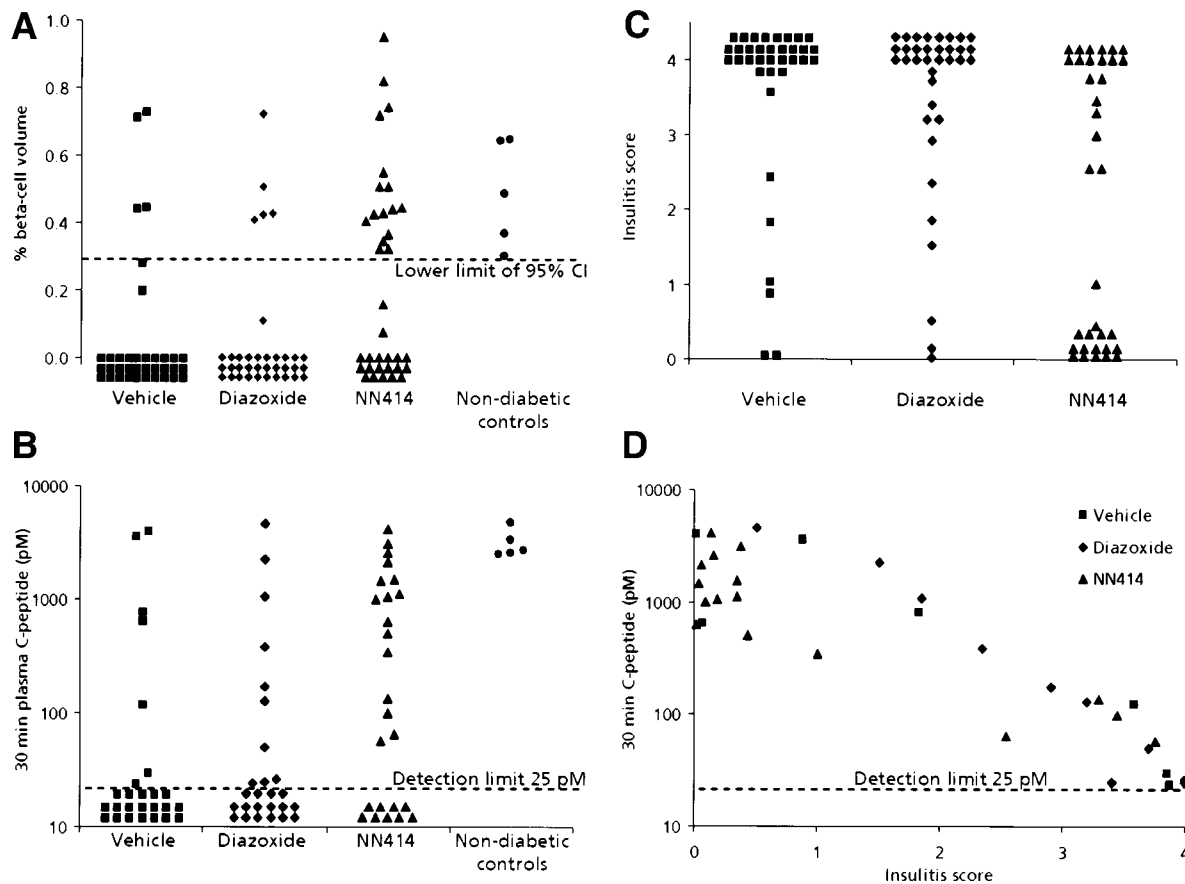


FIG. 5. Rescue of β -cell mass and function and reduced insulinitis in NN414-treated rats. **A:** β -Cell volume on day 21 as percentage of total pancreatic volume. Untreated, nondiabetic littermates ($n = 5$) were included as controls. The dotted line indicates the lower limit of the 95% CI for normal β -cell volume. The number of rats in the NN414 group with a normal β -cell volume (responders) was significantly higher than in the vehicle and diazoxide groups ($P < 0.002$ by χ^2 test). **B:** C-peptide levels 30 min after IVGTT on day 21. All responders had C-peptide levels >300 pmol/l. **C:** Degree of insulinitis in the various groups. Each dot shows the average score of 50–100 islets from each rat. All responders had insulinitis scores <2.0 . **D:** Correlation between 30-min C-peptide levels and insulinitis score ($n = 31$). Rats with C-peptide responses below the detection limit (25 pmol/l) had insulinitis scores >3.9 and were not included.

that responsive rats were almost free of insulinitis. The diabetes model used in this study, DR-BB rats depleted for ART-2⁺ cells and injected with poly(I:C), is a very aggressive diabetes model in which the autoimmune attack on β -cells normally leads to complete β -cell destruction and onset of clinical diabetes within 2–3 weeks (11). In this light, it is remarkable that treatment with NN414 was able to almost completely inhibit insulinitis in responsive rats, with 15 of 16 responders having an insulinitis score <0.5 . Our results suggest a mechanism whereby NN414, in addition to its direct antiapoptotic effect, also inhibits infiltration of islets, probably by reducing secretion of granule content from the islets and thereby reducing immunogenicity and presentation of β -cell antigens. This hypothesis is supported by the finding that islet expression of GAD65, a major type 1 diabetes autoantigen, is increased during exposure to high glucose (20,21). Another explanation could be the absence of glucose-induced apoptosis and possibly necrosis in NN414-treated rats, which would otherwise lead to increased antigen uptake by islet resident APCs and increased presentation of β -cell antigens, thus stimulating the inflammatory process. In this way, treatment with NN414 might also reduce the autoimmune attack on the β -cells.

Almost half of the rats (46%) benefited from the NN414

treatment, whereas the remaining rats exhibited almost complete β -cell destruction, as was also observed in 86–89% of the vehicle- and diazoxide-treated rats. The reason for this heterogeneity is not clear at present; we did not observe any differences between responders and non-responders in drug exposure, plasma insulin levels, or time to onset of blood glucose >20 mmol/l. The responders received slightly lower dosages of insulin during the last 8 days of treatment (data not shown). However, the explanation for this difference is most likely that insulin was administered according to the development in body weight and blood glucose, so that the lower dosage of insulin given to responders reflected their better glycemic control.

In the NN414-treated group, several substantial hyperglycemic episodes (blood glucose >20 mmol/l) were seen in all nonresponding rats, whereas responding rats exhibited only one or at the most two episodes of blood glucose >20 mmol/l (data not shown). We believe that the occurrence of blood glucose >20 mmol/l in the responding rats was due to the combined effect of NN414 (which leads to moderate hyperglycemia) and some decrease in β -cell function. Because insulin therapy was initiated after the first episode when blood glucose exceeded 20 mmol/l, the combined use of insulin and NN414 therapy was sufficient to maintain lower blood glucose. In contrast, nonrespond-

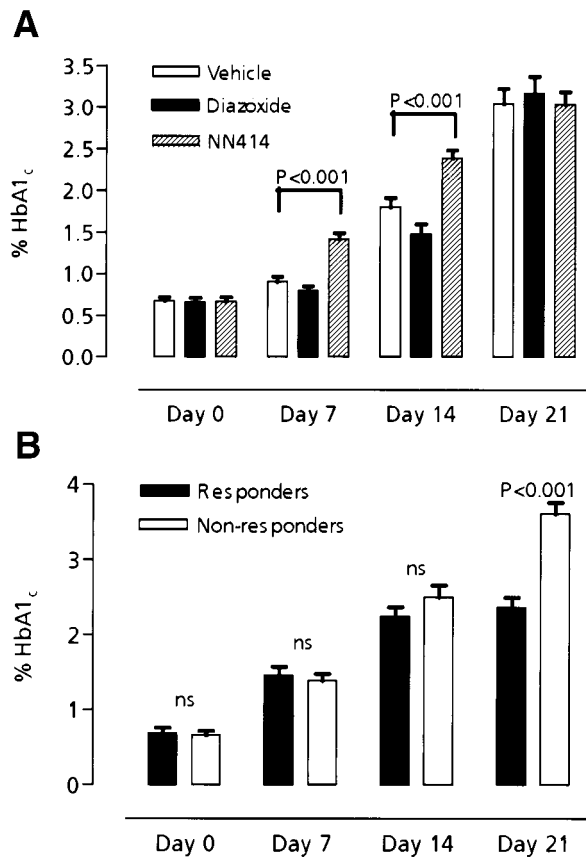


FIG. 6. HbA_{1c} levels. **A:** Weekly HbA_{1c} measurements show an initial increase in HbA_{1c} in the NN414-treated rats, but no difference at the end of the study. **B:** HbA_{1c} level in NN414-treated rats divided into responders and nonresponders (as defined in RESULTS). Student's *t* test was used for statistical analysis.

ing rats continued to exhibit substantial hyperglycemia despite insulin therapy, thus reflecting the more severe loss of β -cell function.

During the first 2 weeks, the NN414-treated rats exhibited a small but significant increase in HbA_{1c} compared with the other groups. This was not surprising considering the fact that NN414 induced moderate hyperglycemia. By contrast, during the last week, most NN414-treated rats received insulin treatment, which correlated with a slight decrease in blood glucose and a smaller increase in HbA_{1c} levels compared with the vehicle group. Both the preservation of β -cell function and the concomitant insulin therapy may have contributed to the smaller increase in HbA_{1c} in the NN414-treated group by lowering the blood glucose level. Thus we believe that the NN414-mediated increase in HbA_{1c} during the first 2 weeks could have been avoided by concomitant insulin therapy from the beginning.

Both in the present study and in an earlier study in DP-BB rats (7), diazoxide could not induce β -cell "rest" and protection similar to insulin. We have shown earlier that high dosages of insulin, involving hours of hypoglycemia, can reduce the incidence of spontaneous diabetes in DP-BB rats by ~50% (22), a finding also demonstrated by Vlahos et al. (7). In the human Diabetes Prevention Trial, subcutaneous insulin treatment did not reduce the incidence of diabetes (23). However, the dosages of insulin used did not induce hypoglycemia and probably did not confer enough β -cell "rest."

A small clinical trial has been conducted where recent-onset type 1 diabetic subjects were treated for 3 months with the nonselective KCO compound diazoxide (9). A significant improvement in C-peptide was seen in up to 18 months follow-up. However, because of the side effects of hypotension, edema formation, and excessive hair growth, diazoxide is not suitable for clinical use. In contrast to diazoxide, NN414 specifically activates β -cell-type K_{ATP} channels and has minimal cardiovascular effects in pre-clinical studies (10). Therefore, we suggest that a β -cell-selective KCO might be used to rescue remaining β -cell function in type 1 diabetic subjects, leading to long-term improvement in C-peptide and glycemic control.

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