

The pharmacokinetics, pharmacodynamics, safety and tolerability of NN2211, a new long-acting GLP-1 derivative, in healthy men

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Abstract

Aims/hypothesis. Glucagon-like peptide-1 (GLP-1), a polypeptide hormone secreted by the L-cells in the gastrointestinal tract, has shown promising effects as a new treatment modality for patients with Type II (non-insulin-dependent) diabetes mellitus. However, the pharmacokinetic profile of native GLP-1 with a rapid elimination has limited its therapeutic potential. NN2211 is a fatty acid derivative of GLP-1, which pre-clinically has shown a protracted pharmacokinetic profile, while maintaining its biological activity. This study aimed to investigate the safety, tolerability, pharmacokinetics and pharmacodynamics of NN2211 in healthy male subjects following seven days treatment.

Methods. In a double-blind, randomized, dose escalation, placebo controlled study, healthy male subjects were enrolled at five consecutive dose levels of NN2211 (1.25, 5.0, 7.5, 10.0, 12.5 µg/kg). Six subjects were allocated at random at each dose level to active or placebo treatment with a ratio of 2:1. Dosing with NN2211 was performed on day 1, and days 5–11. The 84-h pharmacokinetics and 24-h glucose and insulin profiles were assessed on day 1 and day 11.

Results. Following s.c. administration the half-life of NN2211 was found to be 12.6 ± 1.1 h, with a subsequent accumulation index after a daily dose for seven

days of 1.4–1.5. There were dose-proportional increases in exposure (AUC and C_{\max}) with increasing doses. Overall, there were no statistically significant differences from placebo in the 24-h glucose and insulin profiles. In subjects treated with NN2211 rather than placebo, there was a higher incidence of adverse events, most notably dizziness and adverse events related to the gastrointestinal system. There were no serious adverse events but three subjects were nonetheless withdrawn because of dizziness, fever and nausea. There were no clinically relevant changes in vital signs, ECG parameters, physical examination or safety laboratory parameters. A significantly lower diuresis was observed in the actively treated subjects, without a clinically relevant change in packed cell volume.

Conclusions/interpretation. This study shows NN2211 has a pharmacokinetic profile supporting a daily dose in human beings, but also that subjects treated with NN2211 rather than placebo, had a higher incidence of adverse events, most notably dizziness and adverse events related to the gastrointestinal system. [Diabetologia (2002) 45: 195–202]

Keywords GLP-1, NN2211, Type II diabetes, pharmacokinetics, controlled clinical trial, pharmacokinetics, pharmacodynamics, safety.

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Abbreviations: AUC, Area under the curve from time 0 to infinity; AUC_{0-24} , area under the curve from 0 to 24 h; C_{\max} , maximal plasma concentration; CL/f , oral clearance; DPP-IV,

dipeptidyl peptidase IV; GLP-1, glucagon-like peptide-1; LLOQ, lower limit of quantification; PCV, packed cell volume; Rac, accumulation ratio; t_{\max} , time to maximal plasma concentration; $t_{1/2}$, half-life; V_z/f , the oral volume of distribution during the elimination phase

Glucagon-like peptide 1 (GLP-1) is a polypeptide hormone secreted from the L-cells in the gastrointestinal tract [1]. The antidiabetogenic potential of GLP-1 has been demonstrated in both animal models and in patients with Type II diabetes [2–7]. The effect of GLP-1 in lowering glucose is mediated through a number of mechanisms – notably, a glucose-dependent stimulation of insulin secretion [8], reduction in plasma glucagon concentrations [9], delay in gastric emptying [10], appetite decrease [11, 12], and a direct stimulation of growth and proliferation of the beta cells [13]. The blood glucose lowering effect, as well as the glucose dependency, (i.e., stimulation of insulin secretion only when plasma glucose levels are above normal) of this hormone has made it a promising candidate for the treatment of Type II diabetes [5; 7; 14–17]. In addition, data from patients with Type I (insulin-dependent) diabetes mellitus suggests that GLP-1 could have beneficial effects in this population [18, 19].

However, the pharmacokinetic profile of native GLP-1 has severely limited its therapeutic potential as a treatment modality for patients with Type II diabetes. Native GLP-1 is very rapidly degraded by dipeptidyl peptidase IV (DPP-IV) [20], and after s.c. administration the plasma half-life has been assessed to be around 1 h in pigs and human beings [2, 21] and 3 h in dogs [22]. It has been shown that improved control in Type II diabetes patients with sulphonylurea failure can be obtained by 24-h GLP-1 exposure as compared to 16-h, suggesting that a long-acting compound is required to realise full therapeutic potential [23].

To this end it has previously been shown, that fatty acid derivatization provides a protracted action of insulin [24, 25], and has also been reported to prolong the half-life of GLP-1 after s.c. administration to dogs [26]. NN2211 is a fatty acid derivative of GLP-1, providing a compound that has shown prolonged pharmacokinetic properties as compared to native GLP-1 in human beings following a single dose [27], while maintaining its biological action both in vitro [21] and in experimental animal models [28–30]. This study was undertaken in order to further investigate the effect of NN2211 in human beings following repeated doses; especially the pharmacokinetic, pharmacodynamic and safety profile after seven days with a daily dose administered.

Subjects and methods

Study overview. The protocol was approved by the Independent Ethics Committee in Manchester, UK, and conducted in accordance with the Helsinki Declaration and Good Clinical Practice. Subjects consented to participate in the study in writing after a full explanation of the study had been given. The study was designed as a single-centre, randomised, double-blind, placebo-controlled, parallel-group dose-escalation trial,

with initial single, and subsequent multiple s.c. doses of NN2211 to healthy men. A parenteral formulation of NN2211 and matching placebo was used. Sterility and endotoxin concentrations complied with the European Pharmacopoeia. Five dose levels were studied, (1.25, 5.0, 7.5, 10 and 12.5 µg/kg) and six subjects were allocated at random at each dose level to active or placebo treatment with a ratio of 2:1. Each subject was allocated to one dose level only. Subcutaneous doses were administered as a single dose on day 1, and followed by once-daily multiple dosing on days 5–11 in the morning between 8:00h–10:00 h. All meals served were standardised, breakfast, lunch and dinner were served 1, 4, and 11 h after the dose was administered, respectively.

Subjects. Altogether 30 healthy men (age 18–45 years; BMI 19–30 kg/m²) were enrolled in the study. All subjects were in good general health based on medical history, physical examination, vital signs, electrocardiogram (ECG), laboratory measurements (serum biochemistry, haematology, hepatitis, HIV and urinalysis).

Safety assessments. Adverse events were monitored throughout the study period and a physical examination was performed at days 0 and 14. The ECG (12-lead) and vital signs (blood pressure, pulse rate, respiratory rate and temperature) were recorded the day before dosing, and before, during and after t_{max} of NN2211 (9 h) on all dosing days. Clinical laboratory samples (biochemistry, haematology, urinalysis) were taken the day before drug administration, on day 4 and 14. The 24-h urine volumes were collected on day 1–2 (single dose) and day 11–12 (7 days of doses).

Pharmacokinetic assessments. Plasma samples (EDTA) for determination of NN2211 were collected at the following time points after the first dose and last dose administration on days 1 and 11: predose and 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 24, 36, 48, 60, 72 and 84 h after a dose was given. In addition trough samples were collected before doses were given on day 5, 6, 7, 8, 9, 10 and 11. The concentration of NN2211 was determined by a validated two-site immuno-assay using a capturing antibody (GLPB1F1) and a detection antibody (biotin-labelled Mab26.1) both raised against native GLP-1 as described previously [31]. In each setup a calibration curve was included consisting of NN2211 in 0-plasma ranging from 18 to 4500 pmol/l. The samples were obtained as EDTA plasma, if dilution of samples was needed it was done in normal human plasma.

In short, the assays were performed as follows: samples and calibrators were incubated at 37°C for 4 h in order to remove endogenous GLP-1 reactivity. Thereafter, 20 µl sample or calibrator was transferred to the coated plate with 100 µl buffer and incubation at 4°C overnight. After a washing, the biotin-labelled Mab26.1 was applied, the plates were incubated, washed and a streptavidin-labelled peroxidase solution added followed by incubation. Finally, after washing, the enzyme substrate containing H₂O₂ and TMB was added and the colour reaction stopped after incubation by addition of 3 N phosphoric acid. The plates were read at 450 nm with 620 nm as reference and the concentrations calculated by extrapolation from the calibration curve.

The validation of the assay showed the following parameters. Assay repeatability was calculated at three concentrations (low, medium and high), and ranged from 2.4 to 6.5%. The day-to-day assay variation at the same three concentrations ranged from 3.7–10.1%. The linearity was tested with dilution up to 16-fold. The mean linearity, expressed, as diluted sample result in per cent of undiluted sample result, was 106% (range: 93–118%). As the variation was acceptable, and the linearity

was good at least up to 16-fold dilution, samples could reliably be measured with concentrations at least up to 72 000 pmol/l of NN2211. The lower limit of quantification (LLOQ) was determined to be 18 pmol/l. The limit of detection (LOD) was 3 pmol/l. The recovery was examined in six different plasma samples. The mean recovery was 102 % (range 93–113 %). Interference from a number of peptides was investigated. The NN2211 (7–37) isomer interfered positively but less than 4 %, NN2211 (9–37) isomer had negative interference less than 12 %, GLP-1 (15–37) had negative interference less than 3 %, MPGF (Major Pro Glucagon Fragment) had negative interference less than 4 %. Because all these interferences were low, and the concentrations of the peptides in the majority of the samples were low compared to the NN2211 concentrations, interference was considered irrelevant.

Derived pharmacokinetic parameters from the concentration data were calculated for each individual subject in each dosing group, by use of non-compartmental methods, using the commercially available software WinNonlin Version 2.1 (Pharsight, Cary, N.C., USA). The maximum serum concentration (C_{\max}) and the corresponding time (t_{\max}) were read directly from the concentration-time data. Area under the concentration-time curve was calculated by the trapezoidal rule from the time zero to 24 h (AUC_{0-24}), and the last measurable serum concentration (AUC_{∞}). The terminal elimination rate constant was estimated by log-linear regression on the terminal log-linear part of the concentration-time curve, and used for calculating the residual area from last measurable concentration to infinity (AUC_{∞}), and the elimination half-life ($t_{1/2}$). The AUC (to infinity) was calculated as the sum of AUC_{in} and AUC_{∞} . Oral clearance (CL/f) and the oral volume of distribution during the elimination phase (V_z/f) were calculated. The accumulation index was calculated as $AUC_{\text{day 7}}/AUC_{\text{day 1}}$ (R_{ac}).

Pharmacodynamic assessments. Plasma samples for insulin and glucose measurements were taken at the following time points after doses were given on day 1 and 11: predose and 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, and 24 h. In accordance with the manufacturers instructions glucose was analysed by the enzymatic oxidation (am Trinder) method on a Hitachi 171 (Boehringer Mannheim, Copenhagen, Denmark), whereas insulin was analysed by ELISA (DAKO, code No. K6219). The two assays had an imprecision of 3 and 8 %, respectively. The area under the curve from time 0 to 24 h (AUC_{0-24}) was calculated by the trapezoidal rule.

Statistical methods. Only data from subjects who had been given a dose and exposed to trial product on all study days 1 and 5–11 were included in the statistical analysis (due to low injection volumes and air in cartridges several subjects had exposure below LLOQ at the two lowest dose levels). Thus, three subjects had exposure below LLOQ at 1.25, two at 5.0 $\mu\text{g}/\text{kg}$, and in addition three subjects were withdrawn due to adverse events one at 1.25, 7.5, and 10 $\mu\text{g}/\text{kg}$. Normal distribution of the data was assumed.

Safety. Non-transformed average volume of urine excreted over the 24-h sampling periods was analysed by ANOVA and 95 %-CI was calculated.

Pharmacokinetics. AUC, C_{\max} , and R_{ac} were logarithmically transformed before analysis and then subjected to ANOVA. Dose proportionality was assessed for AUC and C_{\max} and an estimate of the slope of the regression line and corresponding 95 %-CI were calculated. Dose proportionality was seen if the slope was not statistically significantly different from unity.

Pharmacodynamics. The 24-h profiles of glucose and insulin on days 1 and 11 were subjected to non-compartmental analysis. The AUC_{0-24} values on days 1 and 11 were included in the statistical analysis following log-transformation and analysed by ANOVA.

Results

Adverse events following NN2211 or placebo administration are presented in Table 1. Overall, there was a higher frequency of adverse events in the actively than in the placebo treated subjects. Three subjects were withdrawn due to adverse events, notably dizziness (1.25 $\mu\text{g}/\text{kg}$), fever and pharyngitis or both (7.5 $\mu\text{g}/\text{kg}$), and nausea and diarrhoea or both (10 $\mu\text{g}/\text{kg}$). Of these dizziness and nausea/diarrhoea were considered to be possibly related to NN2211 administration, whereas fever (seven days duration) was not considered to be related. There were no serious adverse events during the study. The most frequently reported adverse events were headache, dizziness and events related to the gastrointestinal system. In subjects treated with NN2211 compared to placebo, there was a higher frequency of dizziness and adverse events related to the gastrointestinal system. There were no clinically relevant changes in vital signs, ECG parameters, physical examination or safety laboratory parameters (haematology, biochemistry and urinalysis). Combined urine volume data (day 1 and 11) showed a statistically significant reduction following a dose of NN2211 compared to placebo treatment (95 %-CI [-885, -318]). The difference in packed cell volume (PCV) from day 0 to 14 was 0.0067 ± 0.023 in the placebo group compared with -0.023 ± 0.024 in the actively treated subjects.

Figure 1 shows the concentration time profiles of NN2211 following s.c. administration, and the derived pharmacokinetic parameters are given in Table 2. NN2211 was slowly absorbed; with maximum plasma concentrations obtained approximately 10–14 h after a dose was given, and subsequent mean elimination half-life in the range of 11–13 h (Table 2). The mean accumulation ratio (R_{ac}) was calculated to be in the range of 1.4 to 1.5. The pharmacokinetic parameters calculated after one week of treatment was in accordance with those calculated on day 1 (Table 2). The statistical analysis showed a dose-proportional increase in C_{\max} , and AUC following both single and repeated daily doses between 5–12.5 $\mu\text{g}/\text{kg}$ administered s.c. to healthy subjects (95 %-CI for day 1: C_{\max} [0.056, 1.31], AUC [0.099, 1.078], and for day 11: C_{\max} [0.48, 1.36], and AUC [0.28, 1.27]). Plots of AUC and C_{\max} against the dose of NN2211 are shown in Figure 2. There was a statistically significant, accumulation ($R_{\text{ac}} = 1.4\text{--}1.5$) of NN2211 following repeated daily administration, except for the 5.0 $\mu\text{g}/\text{kg}$ dose level (95 %-CI [0.83, 1.7]).

Table 1. Adverse events following NN2211 administration in healthy men. Subjects that had exposure below LLOQ were excluded, corresponding to 3 subjects in the 1.25 µg/kg and 2 in the 5.0 µg/kg groups, respectively (*n* Number of subjects, *e* number of adverse events, % proportion of subjects having the event)

	NNC90-1170		NNC90-1170		NNC90-1170		NNC90-1170		Total		Placebo	
	1.25 µg/kg		5 µg/kg		7.5 µg/kg		10 µg/kg		12.5 µg/kg		NNC90-1170	
	<i>n</i>	<i>e</i>	<i>n</i>	<i>e</i>	<i>n</i>	<i>e</i>	<i>n</i>	<i>e</i>	<i>n</i>	<i>e</i>	<i>n</i>	<i>e</i>
Subjects exposed (<i>n</i>)	1		2		4		4		4		15	
Adverse events	1	(100%)	2	(100%)	4	(100%)	4	(100%)	4	(75%)	12	(93%)
Skin infection												
Arthralgia					1	(25%)	1	(25%)	1	(25%)	1	(7%)
Myalgia					1	(25%)	1	(25%)	1	(25%)	1	(7%)
Dizziness	1	(100%)	1	(50%)	2	(50%)	4	(100%)	1	(25%)	1	(7%)
Headache			1	(50%)	3	(75%)	11	(75%)	7	(75%)	2	(53%)
Eurache					1	(25%)	1	(25%)	1	(25%)	1	(7%)
Taste perversion					1	(25%)	1	(25%)	1	(25%)	1	(7%)
Mental dullness					2	(50%)	3	(75%)	1	(25%)	1	(7%)
Somnolence					1	(25%)	1	(25%)	1	(25%)	1	(7%)
Abdomen enlarged					1	(25%)	2	(50%)	2	(50%)	2	(13%)
Abdominal pain					2	(50%)	2	(50%)	2	(50%)	2	(13%)
Appetite decreased					2	(50%)	2	(50%)	2	(50%)	1	(7%)
Constipation					1	(25%)	1	(25%)	1	(25%)	1	(7%)
Diarrhoea					1	(25%)	4	(100%)	1	(25%)	1	(7%)
Dyspepsia					2	(50%)	6	(150%)	3	(75%)	4	(27%)
Flatulence					1	(25%)	2	(50%)	2	(50%)	1	(7%)
Haemorrhage rectum					1	(25%)	1	(25%)	1	(25%)	1	(7%)
Mouth dry					1	(25%)	1	(25%)	1	(25%)	1	(7%)
Nausea					1	(25%)	2	(50%)	4	(100%)	1	(7%)
Tooth disorder					1	(25%)	1	(25%)	1	(25%)	1	(7%)
Vomiting					1	(25%)	1	(25%)	1	(25%)	1	(7%)
Flushing					1	(25%)	3	(75%)	1	(25%)	1	(7%)
Dyspnoea					1	(25%)	3	(75%)	4	(100%)	1	(7%)
Pharyngitis					1	(25%)	3	(75%)	1	(25%)	1	(7%)
Oliguria					1	(25%)	3	(75%)	1	(25%)	1	(7%)
Eye infection					1	(25%)	1	(25%)	1	(25%)	1	(7%)
Fatigue					2	(50%)	4	(100%)	3	(75%)	5	(33%)
Fever					1	(25%)	1	(25%)	1	(25%)	1	(7%)
Injection site inflammation									2	(50%)	2	(13%)

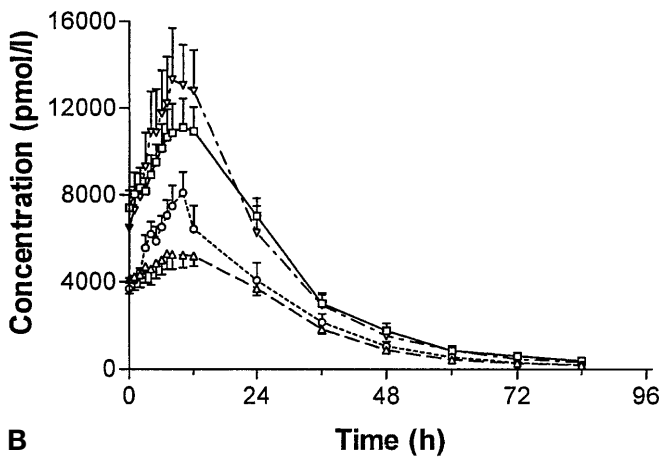
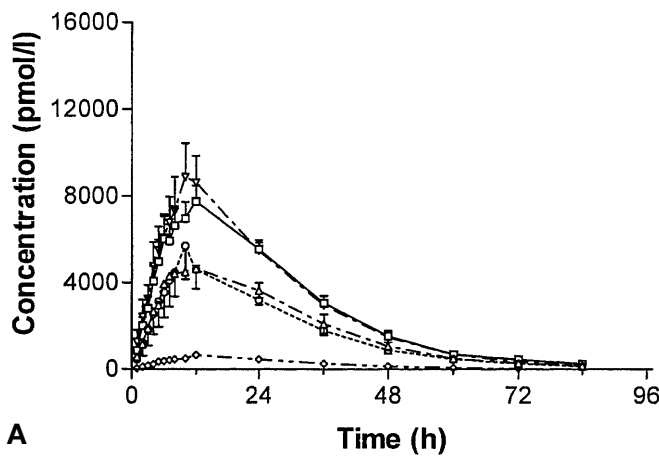


Fig. 1. Mean NN2211 plasma concentration (pmol/l) (\pm SEM) versus time (h) profiles after s.c. administration of NN2211 to healthy men following a single dose ---- \diamond ---- 1.25 μ g/kg, ---- \triangle ---- 5 μ g/kg, --- \circ --- 7.5 μ g/kg, — \square — 10 μ g/kg, --- ∇ --- 12.5 μ g/kg (**A**), and 7 days of daily s.c. dosing ---- \triangle ---- 5 μ g/kg, --- \circ --- 7.5 μ g/kg, --- \square --- 10 μ g/kg, --- ∇ --- 12.5 μ g/kg (**B**)

The mean insulin and glucose time profiles are shown in Figure 3 and 4, respectively. Overall, for the 24-h profiles, there was no statistically significant

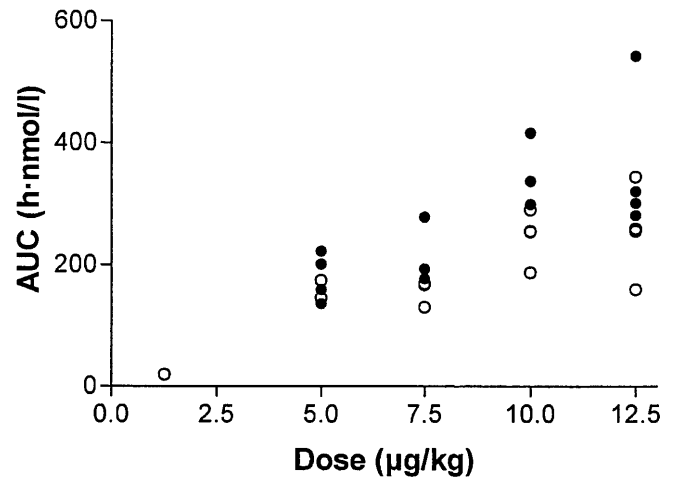


Fig. 2. Mean NN2211 plasma concentration AUC (h · nmol/l) versus dose (μ g/kg) after s.c. administration of NN2211 to healthy volunteers at 5 different dose levels; following a single dose on day 1 (\circ), and following 7 days of consecutive dosing on day 11 (\bullet)

difference between active and placebo treatment (glucose 95 %-CI [0.89, 1.04], insulin 95 %-CI [0.84, 1.22]). However, corresponding to t_{max} of NN2211 (and meals) a reduction of insulin concentrations was observed in the actively treated subjects. Furthermore, higher glucose concentrations in the placebo treated subjects were found, most pronounced in connection to meals giving lower post-prandial glucose concentrations (corresponding to 2, 5, and 12 h) in the actively treated subjects as compared to the placebo group (Fig. 5).

Discussion

The findings in this study of an increase in gastrointestinal side effects following NN2211 is in accordance with previous reports on GLP-1 [3, 23]. The effect is probably a consequence of GLP-1's effect on reducing gastric emptying [11, 12, 32, 33]. In addition

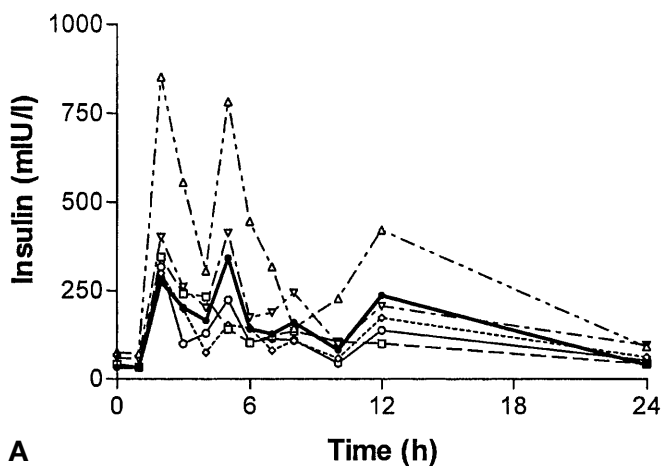
Table 2. Mean pharmacokinetic parameters of NN2211 on day 1 and day 11

Parameter	Unit of measure	Dose 7.5 μ g/kg		Dose 10 μ g/kg		Dose 12.5 μ g/kg	
		Day 1	Day 11 ^b	Day 1	Day 11 ^b	Day 1	Day 11
AUC	h · nmol/l	149 \pm 22	216 \pm 54	246 \pm 43	351 \pm 60	254 \pm 75	361 \pm 122
AUC ₀₋₂₄	h · nmol/l	86 \pm 32	138 \pm 32	138 \pm 26	225 \pm 33	152 \pm 46	247 \pm 80
R _{ac}	NA	—	1.40 \pm 0.26	—	1.45 \pm 0.14	—	1.44 \pm 0.24
C _{max}	nmol/l	6 \pm 3	8 \pm 2	8 \pm 1	11 \pm 2	9 \pm 3	14 \pm 4
t _{max}	h	14 \pm 7	10 \pm 0.01	12 \pm 0.1	11 \pm 1	11 \pm 1	10 \pm 2
Cl/f	ml/min/kg	0.20 \pm 0.03	—	0.16 \pm 0.03	—	0.19 \pm 0.06	—
Vz/f	l/kg	0.22 \pm 0.06	—	0.17 \pm 0.03	—	0.19 \pm 0.05	—
t _{1/2} ^a	h	12.4	12.4	12.8	12.8	11.6	12.9

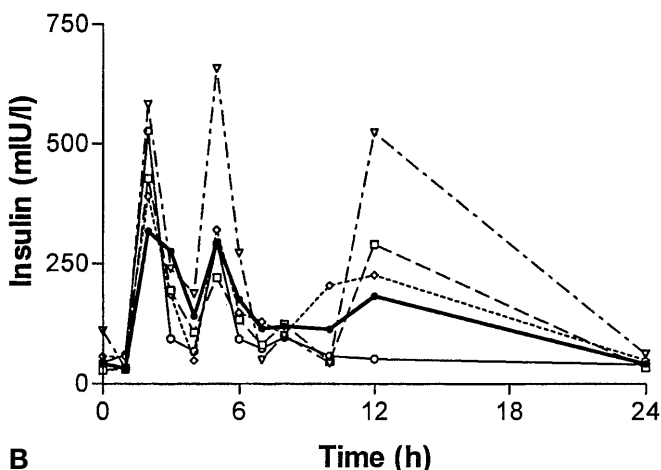
n equals 4 unless otherwise indicated

^aHarmonic mean

^bMean of 3 subjects



A Time (h)

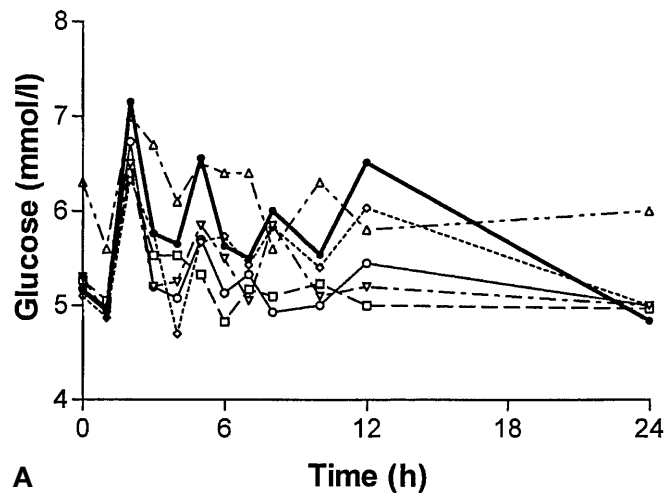


B Time (h)

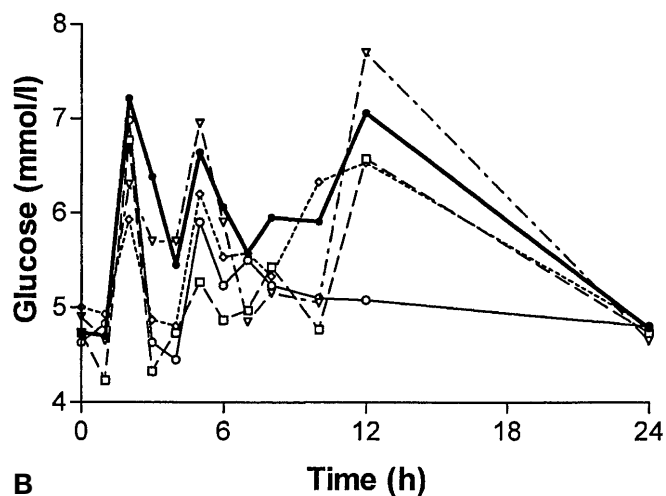
Fig. 3. Mean insulin versus time concentration (mIU/l) versus time (h) profiles following a single dose —●— placebo, ---△--- 1.25 µg/kg, ---▽--- 5 µg/kg, ---□--- 7.5 µg/kg, ---◇--- 10 µg/kg, —○— 12.5 µg/kg (**A**), and 7 days of daily s.c. dosing —●— placebo, ---▽--- 5 µg/kg, ---□--- 7.5 µg/kg, ---◇--- 10 µg/kg, —○— 12.5 µg/kg (**B**). Breakfast, lunch and dinner were served at 1, 4 and 11 h after doses were given

there was a higher frequency of dizziness reported after NN2211 compared with placebo administration, which is in accordance with a previous report following 7 days GLP-1 infusion [23]. Finally, a significant reduction in urine volume (0–24 h) was observed in this study following NN2211 treatment. As GLP-1 administration has been shown to reduce water intake [1], the reduced urine volume could be secondary to this, even though water intake was not assessed in this study. The reduced urine volume was not followed by a clinically relevant change in packed cell volume from day 0 to 14, suggesting that it was not followed by dehydration.

Native GLP-1 has a half-life following i.v. or s.c. administration in human beings of approximately 5 min [1] and 1 h [2], respectively. The pharmacokinetic profile of NN2211 demonstrated in this study with a mean elimination half life of 12.6 ± 1.1 h is



A Time (h)



B Time (h)

Fig. 4. Mean glucose versus time concentration (mmol/l) versus time (h) profiles following a single dose —●— placebo, ---△--- 1.25 µg/kg, ---▽--- 5 µg/kg, ---□--- 7.5 µg/kg, ---◇--- 10 µg/kg, —○— 12.5 µg/kg (**A**) and seven days of daily s.c. dosing —●— placebo, ---▽--- 5 µg/kg, ---□--- 7.5 µg/kg, ---◇--- 10 µg/kg, —○— 12.5 µg/kg (**B**). Breakfast, lunch and dinner were served at 1, 4 and 11 h after the dose

consistent with a previous single dose study in human beings [27]. The steady-state pharmacokinetic parameters were similar to those following a single dose. There was a slight but statistically significant accumulation of NN2211 following a daily s.c. administration to healthy subjects, as indicated by the mean accumulation ratio (Table 1). The protraction mechanism of NN2211 compared with native GLP-1 probably relies on a combination of albumin binding, as has been shown for other fatty acid derivatives [24, 25], lower susceptibility to metabolism by DPP-IV [34], and a slower absorption profile. Taken together these data indicate that a daily dose of NN2211 can be beneficial to human beings.

In this study, there was no conclusive indication that NN2211 stimulated insulin concentrations above placebo. This could be because the effects on gastric

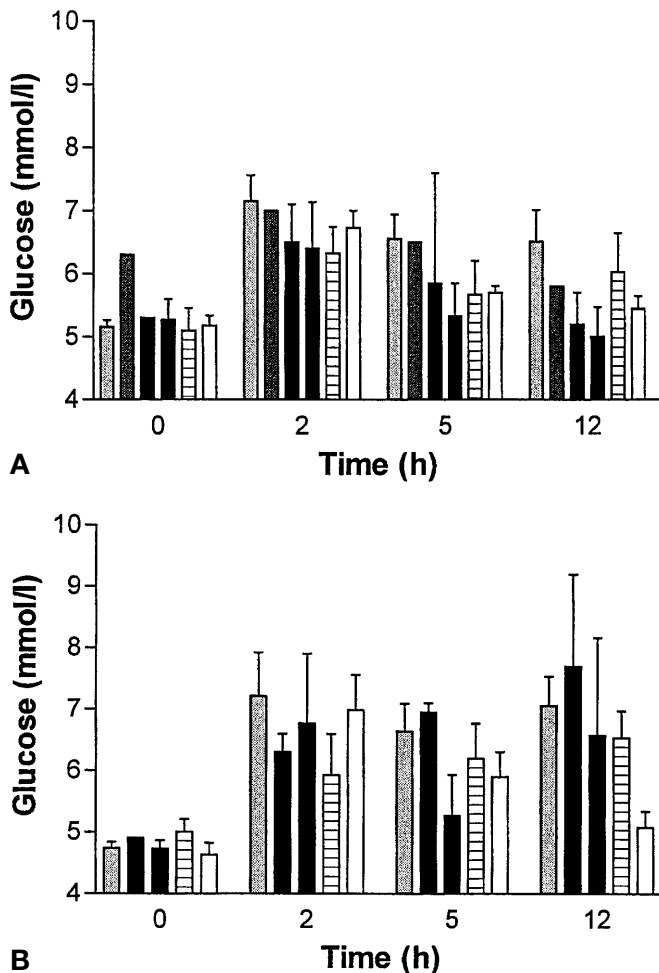


Fig. 5. Mean (\pm SEM) rise in plasma glucose concentrations (mmol/l) at time of dosing (tasting) after breakfast, lunch and dinner a single dose (lightly shaded bars) placebo, (moderately shaded bars) 1.25 μ g/kg, (heavily shaded bars) 5 μ g/kg, (solid bars) 7.5 μ g/kg, (bars with line through) 10 μ g/kg, (empty bars) 12.5 μ g/kg (**A**) and seven days of daily s.c. dosing (lightly shaded bars) placebo, (darkly shaded bars) 5 μ g/kg, (solid bars) 7.5 μ g/kg, (bars with line through) 10 μ g/kg, (empty bars) 12.5 μ g/kg (**B**). Breakfast, lunch and dinner were served at 1, 4 and 11 h after a dose

emptying by GLP-1 could outweigh the effects on the insulin secretion in healthy volunteers, as described previously [34]. The latter study reported insulin concentrations following a meal to be significantly lower in the active compared to the placebo treated group [34]. Thus, although insulin secretion was stimulated by GLP-1, the glucose-mediated stimulation was less profound because of the inhibition of gastric emptying, and in sum this gave lower insulin concentrations (i.e. the gastric effect outweighed the insulin secretion effect). This is consistent with the lower insulin levels observed following meals and maximal plasma concentrations of NN2211 in this study. In line with this, it has been speculated that the primary effect of native GLP-1 in healthy subjects could be to inhibit gastric empty-

ing rate and mediate satiety, rather than acting as an incretin hormone [35] (in this study decreased appetite was reported at a higher frequency in actively compared placebo treated subjects). However, even though the effects on the insulin secretion could have been scattered by the concomitant effect on gastric emptying time, the glucose concentrations indicate a combined effect. In this study, there was a general trend towards lower mean glucose concentrations following active treatment as compared to placebo, i.e. the mean glucose concentrations at approximately 2, 5 and 12 h following breakfast, lunch and dinner at 1, 4 and 11 h after a dose was given. Similar findings were reported previously in healthy human beings [33]. Furthermore, the relatively modest changes in glucose concentrations observed here and previously, are consistent with a GLP-1 glucose dependent mechanism of action [8]. In this study, glucagon was not measured, however, following a single dose with NN2211 in healthy men no significant effect on glucagon was observed [27]. The data presented suggest that a therapeutic dose range for NN2211 could be from 1–10 μ g/kg in patients with Type II diabetes. However this will depend on a combination of efficacy and side effect profile of NN2211 in this cohort.

In summary, subjects treated with NN2211, had a higher incidence of adverse events than those given a placebo, most notably dizziness and adverse events related to the gastrointestinal system. While there were no serious adverse events, three subjects were withdrawn due to adverse events. Significantly lower 24-h diuresis was observed in the actively treated subjects, without a clinically relevant change in PCV. No statistically significant changes in insulin or glucose were found but a reduction in glucose concentrations after meals was observed in NN2211 treated subjects, supporting the notion that the effects of GLP-1 on glucose is not only mediated by insulin but, more importantly, through inhibition of gastric emptying. Furthermore, the pharmacokinetic data showed that NN2211 has a markedly protracted profile in human beings. In contrast to other known GLP-1 like compounds, notably LY307161 [36, 37] and exendin-4 (AC2993) [38], the findings presented here suggest that NN2211 has sufficient protraction to cover a daily dose administration in human beings. This is important not only for improving compliance and convenience for the patients but also in realising full therapeutic potential, as it was recently shown that in order to achieve acceptable control in patients with Type II diabetes who were poorly controlled on sulphonylurea treatment 24-h GLP-1 infusion had to be maintained [23].

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