Half-life prolongation of therapeutic proteins by conjugation to ATIII-binding pentasaccharides: a first-in-human study of CarboCarrier[®] insulin

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- A novel CarboCarrier[®] technology has been developed for enhancing PK–PD profiles of small proteins and polypeptides at sub-anticoagulant concentrations.
- Previously, data have been published describing how binding of insulin, via coupling to a pentasaccharide, to the plasma protein antithrombin III (ATIII) can be used to enhance the half-life in preclinical species.

WHAT THIS STUDY ADDS

- Here we describe the first-in-human clinical study, in which safety, tolerability, pharmacokinetics and relative bioactivity of CarboCarrier[®] insulin, the first therapeutic protein conjugated to an AT III-binding pentasaccharide, have been assessed.
- The current study validates the CarboCarrier[®] technology concept in humans making use of CarboCarrier[®] insulin as the first example.

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AIM

Conjugation to antithrombin III ATIII-binding pentasaccharides has been proposed as a novel method to extend the half-life of therapeutic proteins. We aim to validate this technological concept in man by performing a first-in-human study using CarboCarrier[®] insulin (SCH 900948) as an example. A rising single dose phase 1 study was performed assessing safety, tolerability, pharmacokinetics and relative bioactivity of CarboCarrier[®] insulin. Safety, tolerability and pharmacokinetics (PK) of single doses of CarboCarrier[®] insulin in healthy volunteers were explored, and the dose–response relationship and relative bioactivity of CarboCarrier[®] insulin in subjects with type 2 diabetes were investigated.

METHODS

After an overnight fast, subjects were randomized to a treatment sequence. PK and pharmacodynamic (glucose, insulin and C-peptide) samples were obtained for up to 72 h post-dose. Effects of CarboCarrier[®] insulin were compared with those of NPH-insulin.

RESULTS

CarboCarrier[®] insulin was safe and well-tolerated and no consistent pattern of adverse events occurred. CarboCarrier[®] insulin exposure ($C_{\max}$ and AUC) increased proportionally with dose. The mean terminal elimination half-life ranged between 3.11 and 5.28 h. All CarboCarrier[®] insulin dose groups showed decreases in the mean change from baseline of plasma glucose concentrations compared with the placebo group.

CONCLUSIONS

CarboCarrier[®] insulin is pharmacologically active showing features of insulin action in man. The elimination half-life of the molecule was clearly extended compared with endogenous insulin, indicating that conjugation to ATIII-binding pentasaccharides is a viable approach to extend the half-life of therapeutic proteins in humans. This is an important step towards validation of the CarboCarrier[®] technology by making use of CarboCarrier[®] insulin as an example.

Introduction

A variety of strategies has been employed to enhance the exposure of polypeptide or protein therapeutics, and thus to improve the PK–PD profiles of such compounds enabling lower frequencies of administration, important for convenient clinical use [1]. Theoretically, the binding of a polypeptide drug to long-lived plasma proteins in the bloodstream enables tight control over targeted drug concentrations with improved exposure and distribution in the circulation that may potentially lead to lower doses, decreased side effects and enhanced predictability. In this context, not all plasma proteins may have been explored to the full extent. Earlier, preclinical data have been published describing how binding of insulin to the plasma protein antithrombin III (ATIII) can be used to enhance the half-life of such a compound, without compromising the coagulation system [2]. We now provide the results of the first study in humans with an ATIII-binding pentasaccharide coupled protein, CarboCarrier® insulin (SCH 900948).

In type 1, insulin-dependent, diabetes mellitus (T1DM) patients, tight control of plasma glucose concentrations is being achieved by the exogenous administration of insulin. It is generally believed that tight glucose control can also prevent complications in people with type 2 diabetes (T2DM). The best results are being obtained by mimicking the overall physiological profile. This profile is being approached by 1) the administration of short- and intermediate-acting insulin products (analogues) to control post-meal glucose excursions and 2) dosing of longer acting insulin products to maintain basal concentrations of insulin [3, 4].

The very short plasma half-life of insulin poses a challenge for creating sufficiently long-acting formulations or analogues with optimal pharmacokinetic–dynamic (PK–PD) properties that provide consistent glycaemic control. Approved long acting insulin analogues for basal insulin therapy form a subcutaneous depot at the injection site (insulin glargine [5], insulin detemir [6]) and/or exhibit an extended half-life through hydrophobic interaction of fatty acid groups with serum albumin (insulin detemir). Insulin detemir (indicated for once daily or twice daily use) and insulin glargine (indicated for once daily use) require once to twice daily administration [7]. However, both detemir and glargine demonstrate substantial within subject variability which complicates achieving reliable day to day glycaemic control. Recently, an ultra long acting basal insulin (insulin degludec) has been described that seems to provide glycaemic control comparable with insulin glargine combined with reduced dosing frequency (three times weekly) [8].

It was the intent to develop a long acting basal insulin preparation for the treatment of both T1DM and T2DM with a duration of action of around 24 h, a flat action profile, and a low within subject variability. Ideally, coupling of insulin to a carrier pentasaccharide would yield a

long-acting insulin with a peakless basal insulin concentration, thereby providing optimal quality of glucose control, monitored by measuring changes in glycated haemoglobin (HbA1c), fasting and post-prandial glucose responses, hypoglycaemia frequency and severity and patients' satisfaction. It was hypothesized that a CarboCarrier® insulin conjugate, fully soluble, binding to ATIII in the circulation forming an intravascular depot, could potentially result in a more predictable day to day control of blood glucose concentrations and thus in a reduced risk of hypoglycaemia. In addition, we aimed to validate the CarboCarrier® technology concept of half-life prolongation in man by performing a first-in-human study using CarboCarrier® insulin as an example.

Here we describe the first-in-human clinical study, in which safety, tolerability, pharmacokinetics and relative bioactivity of CarboCarrier® insulin, the first therapeutic protein conjugated to an ATIII-binding pentasaccharide, have been assessed.

Methods

Subjects

The trial protocol and informed consent form were approved by the appropriate authorities and by the Independent Ethics Committee (Medisch Ethische Toetsings-Commissie) of the Foundation 'Evaluation of Ethics in Biomedical Research' (Stichting BEBO), Assen, the Netherlands. All patients had given written consent prior to entering the study, including for publication of results.

In part 1, healthy volunteers of adult age (30 to 55 years (inclusive)), with a body mass index between 22 and 32 kg m⁻² and normal coagulation screen were included. In part 2, insulin-naïve subjects with T2DM of adult age (18 to 65 years (inclusive)), with a disease duration >12 months, a body mass index between 25 and 40 kg m⁻² and a normal coagulation screen were included. Patients were required to have a HbA1c between 6.5 and 10.0% and were allowed to use one or two oral anti-diabetic agents (metformin, insulinsecretagogues, DPPIV inhibitors, α -glucosidase inhibitors). Female subjects were postmenopausal (defined as 12 months with no menses and with a FSH concentration between 23.0 and 116.3 U ml⁻¹) and/or surgically sterilized (e.g. documented hysterectomy or tubal ligation). The criteria were such that the young, slim, highly trained healthy volunteers were to be excluded as this population was expected to have a high sensitivity to insulin resulting in an enhanced risk for hypoglycaemic episodes.

Trial design

In part 1, three groups of eight subjects were randomized to a treatment sequence of single-doses of subcutaneously injected CarboCarrier® insulin or placebo 6:2 (group A: period 1 (0.5 IU or placebo), period 4 (12 IU or placebo);

group B: period 2 (2 IU or placebo), period 5 (18 IU or placebo); group C: period 3 (6 IU or placebo), period 6 (24 IU or placebo) in a rotating panel design.

Subjects were admitted to the study centre 1 day (day –1) before the first dose for baseline assessments to confirm eligibility. Subjects received standardized meals throughout the study, starting at day –1. At baseline and in the morning of each treatment day blood was drawn for the determination of aPTT and PT(INR), to exclude an influence of study drug on coagulation parameters. In addition, subjects were checked for bruises, haematomas and injection site reactions. After an overnight fast (starting at 22.00 h) subjects were randomized to a treatment sequence with two dosing periods, received the first dose at approximately 08.00 h and continued their fast for 6 h thereafter. Pharmacokinetic (PK) and pharmacodynamic (PD; glucose, insulin and C-peptide) samples were obtained pre-dose (0 h), at 30 min and at 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 36, 48 and 72 h after dosing. Safety assessments were performed throughout the study. Vital signs and adverse events were recorded. Close monitoring of hypoglycaemia was done by testing of glucose concentrations with bedside glucometers. On day 4, safety assessments were again performed along with final PK–PD sampling. The decision to progress to the next dose level was made based on review of the laboratory, ECG and adverse event data by the sponsor's physician and with agreement of the study site and the Independent Ethics Committee. The next dose was administered during the second period. PK and PD sampling and safety assessments were performed as described above. When final safety assessments and PK–PD sampling had been performed, subjects were discharged from the study.

In part 2, one group of eight subjects with T2DM was randomized to four sequences to receive a subcutaneous injection of 20 IU (A) and 36 IU (B) of CarboCarrier® insulin, a dose of 20 IU of NPH-insulin (Protaphane, Novo Nordisk) (C) and placebo (D) according to the crossover William's design. Subjects were admitted to the study centre 2 days (day –2) before the first dose. This was 1 day earlier than the healthy volunteers in part 1, providing additional time for metabolic stabilization. Subjects received standardized meals throughout the study, starting at day –2. At baseline and in the morning of each treatment day blood was drawn for the determination of aPTT and PT (INR), to exclude an influence of study drug on coagulation parameters. In addition, subjects were checked for bruises, haematomas and injection site reactions. After an overnight fast (starting at 22.00 h) subjects were randomized to a treatment sequence with four dosing periods, received the first dose at approximately 08.00 h and continued their fast for 6 h thereafter. Subjects did not receive their usual oral anti-diabetic medication the day before dosing and the morning of dosing. PK and PD samples were obtained pre-dose (0 h), at 30 min and at 1, 2, 3, 4, 5, 6, 10, 12, 24 and 48 h after dosing. Safety assessments were performed throughout

the study. Vital signs and adverse events were recorded. Close monitoring of hypoglycaemia was done by testing of glucose concentrations with bedside glucometers. The decision to progress to the next dose was made based on review of the laboratory, ECG and adverse event data by the sponsor's physician and with agreement of the study site. The next dose was administered after 7–14 days. PK and PD sampling and safety assessments were performed as described above. This schedule, starting with day –2, was repeated for each subsequent dose. When final safety assessments and PK–PD sampling had been performed, subjects were discharged from the study. The subjects underwent close monitoring of glucose concentrations (PD) and symptoms of hypoglycaemia to evaluate pharmacodynamics of CarboCarrier® insulin in comparison with pharmacodynamics of NPH-insulin, and to establish a PK–PD relationship for both treatments.

Investigational drug

SCH 900948 (CarboCarrier® insulin) is a pentasaccharide-GMB-insulin conjugate. Insulin consists of a covalently linked A and B chain. The A chain contains a disulfide bridge between the cysteines 6 and 11. The cysteines 7 and 20 of the A chain form disulfide bridges with the cysteines 7 and 19, respectively, of the B chain. The pentasaccharide moiety is covalently coupled to the 29th amino acid of the B chain of insulin via a gamma-maleimidobutyric acid (GMB) linker. The molecular formula of SCH 900948 Org 211559-1 in the current formulation is $C_{320}H_{478}N_{67}O_{137}S_{14}Na_9$. The drug product is a white, freeze dried cake containing 0.5 mg SCH 900948 per vial. After reconstitution with 0.5 ml water for injections, the drug product is used as a single dose subcutaneous injection. SCH 900948 was primarily in a monomeric form and only minor amounts of aggregates were present (<2%). It has been assumed that 6 nmol SCH 900948 is equivalent to 1 IU of insulin activity, as is the case for regular insulin.

Bioanalysis

The PK assay that was performed to detect SCH 900948 was a validated sandwich-type ELISA employing an anti-insulin antibody as capture and horseradish peroxidase-conjugated anti-ATIII antibodies as detector, measuring intact CarboCarrier® insulin (SCH 900948)–ATIII complex [2].

Study assessments

Safety Safety was assessed by AEs, vital signs, ECG, routine laboratory tests, coagulation parameters (aPTT, PT (INR)), skin check for bruises and haematomas, physical examination, local injection site tolerability.

Pharmacokinetics The main plasma PK parameters calculated were (dn= dose-normalized) peak concentration ((dn)-C_{max}) and its time of occurrence (*t*_{max}), terminal half-life (*t*_{1/2}), (dose-normalized) area under the curve

((dn-)AUC), apparent clearance (CL/F) and apparent volume of distribution (V_z/F). In addition the peak : trough ratio (P : T ratio: C_{max}/C_{24} (concentration at 24 h)) was calculated.

Pharmacodynamics Plasma glucose concentrations, serum insulin concentrations, and serum C-peptide concentrations were determined. Concentration vs. time curves were generated to evaluate the action profile of CarboCarrier® insulin.

Statistical analysis

Before transitioning to part 2 of the study, an interpart interim analysis using unblinded data was conducted. This was to allow the study team to determine if 'effective' plasma concentrations had been reached, in order to perform dose setting for part 2. After completion of the study, both study parts were analyzed separately.

Safety The number of subjects reporting any AEs, the occurrence of specific AEs and discontinuation due to AEs were tabulated (data on file). Adverse event tabulations included all treatment emergent and treatment related adverse events, which were also further classified by severity and relationship to treatment. Safety parameters were summarized descriptively for each CarboCarrier® insulin treatment group, placebo and NPH-insulin (part 2 only).

Pharmacokinetics Mean concentration vs. time plots and descriptive statistics of pharmacokinetic parameters by dose were presented.

Pharmacodynamics Plasma glucose concentrations, mean change from baseline of plasma glucose concentrations, serum insulin concentrations, and serum C-peptide concentrations were used to evaluate treatment (dose) groups. Parameters were listed, graphically presented and summarized by treatment (dose) for each part separately.

Results

Demographics and baseline characteristics

In part 1, a total of 24 adult subjects (20 men and four women) between the ages of 33 and 55 years (mean 44.6 years) were treated. In part 2, a total of eight adult subjects (seven men and one woman) with T2DM between the ages of 51 and 65 years (mean 60.3 years) were treated. Overall, the average age ranges for both females ($n = 5$, 42–59 years, median 52 years, average 50.8 years) and males ($n = 27$, 30–64 years, median 46 years, average 48.0 years) were quite similar.

Safety

In part 1 of the study, a total of 17 subjects (71%) reported at least one AE during the study, three (50%) in the placebo

group, three (50%) in the 0.5 IU group, five (83%) in the 2 IU group, four (67%) in the 6 IU group, two (33%) in the 12 IU group, five (83%) in the 18 IU group and five (83%) in the 24 IU group. The most common AEs (dizziness, headache and somnolence) were related to hypoglycaemia and occurred in the higher dose cohorts. AEs in the placebo group almost exclusively consisted of AEs that are commonly seen in healthy volunteers who are being exposed to placebo in the setting of a clinical phase 1 unit, such as headache, somnolence and light nausea. Also back pain was reported by four (17%) subjects. There were no deaths or serious AEs. Isolated laboratory values out of normal ranges in haematology, serum chemistry and urinalysis were noted pre- and post-dose in both placebo and active-treated subjects. There were no clinically significant changes or trends in clinical safety laboratory values following administration of any dose of CarboCarrier® insulin (except for lowering of glucose, which relates to the pharmacological action of this insulin preparation). There were no clinically significant changes in vital signs or ECGs. In part 2 of the study, six subjects (75%) reported at least one AE during the study, three (38%) in the placebo group, four (50%) in the 20 IU SCH 900948 group, six (75%) in the 36 IU CarboCarrier® insulin group and three (38%) in the 20 IU insulin control group. The AEs were almost exclusively related to hypoglycaemia (headache, disturbance in attention, hunger, somnolence) and each AE was seen in only one or two subjects. There were no deaths or serious AEs. Isolated laboratory values out of the normal range in haematology, serum chemistry and urinalysis were noted pre- and post-dose in both placebo and active-treated subjects. There were no clinically significant changes or trends (except for lowering of glucose) in clinical safety laboratory values following administration of any dose of CarboCarrier® insulin. There were no clinically significant changes in vital signs.

Pharmacokinetics

The all subjects pharmacokinetically evaluable (ASPE) group consisted of a total of 25 subjects (three groups each of $n = 6$ healthy volunteers in part 1 and one group of $n = 8$ patients with T2DM in part 2). Data from one subject (part 1) were excluded from the ASPE group because of unexplained background in the CarboCarrier® insulin PK assay. The arithmetic mean CarboCarrier® insulin concentration vs. time curves by dose with linear (main figure) and logarithmic (insert) concentration scales are shown in Figure 1. Mean CarboCarrier® insulin plasma concentrations increased proportionally with dose in both healthy subjects and in subjects with T2DM (Figure 1). A summary of the CarboCarrier® insulin pharmacokinetic parameters by dose is presented in Table 1. Please note that the AUC_{dn} value for the 0.5 IU dose was rather low as for the 6 to 12 h post-dose period at the 0.5 IU dose all concentrations were below the detection limit (1 ng mL^{-1}) of the assay.

Figure 1

Mean CarboCarrier® insulin plasma concentration in healthy volunteers and type 2 diabetes patients. y axis: CarboCarrier® insulin (ng ml^{-1}); x axis: time (h); Main figure: linear concentration scale; Insert: logarithmic concentration scale. Data of healthy volunteers are given as solid lines, data of type 2 diabetes patients as dashed lines. Open squares: 0.5 IU; closed squares: 2.0 IU; open circles: 6.0 IU; closed circles: 12 IU; open triangles: 18 IU; closed triangles: 24 IU; open diamonds: 20 IU; closed diamonds: 36 IU

Pharmacodynamics

The all subjects treated (AST) group consisted in total of 32 subjects and no subjects were excluded. Twenty-four healthy subjects were treated in part 1 and eight patients with T2DM were treated in part 2. The PD parameters glucose, insulin and C-peptide were measured.

Glucose Figures 2A and 2B show the arithmetic mean change from baseline of plasma glucose levels for part 1 and for part 2 by dose. Tables 2A and 2B show the summary statistics of the mean change from baseline of plasma glucose concentrations by time point. In part 1, during the first 6 h, all dose groups (0.5 IU up to and including 24 IU) showed a larger decrease (after 6 h ranging from -0.57 to $-1.75 \text{ mmol l}^{-1}$) in the mean change from baseline of plasma glucose compared with the placebo group (after 6 h $-0.25 \text{ mmol l}^{-1}$). The mean change from baseline of the AUEC from 0–6 h showed a decrease (ranging between -0.29 to $-1.17 \text{ mmol l}^{-1}$) for all dose groups compared with the placebo group ($+0.01 \text{ mmol l}^{-1}$) in healthy subjects (Figure 2A). In part 2, both dose groups (20 IU and 36 IU)

showed evident decreases (after 6 h -3.9 mmol l^{-1} for the 20 IU dose group and -3.6 mmol l^{-1} for the 36 IU dose group) in mean change from baseline of plasma glucose concentrations compared with the placebo group (after 6 h -2.0 mmol l^{-1}). These decreases were larger than those in the NPH-insulin group (after 6 h -2.6 mmol l^{-1}) for subjects with T2DM. The mean change from baseline of the AUEC from 0–6 h showed a larger decrease (-2.3 mmol l^{-1} for the 20 IU dose group and -2.0 mmol l^{-1} for the 36 IU dose group) for both dose groups compared with the placebo group (-1.0 mmol l^{-1}) and the NPH-insulin group (-1.3 mmol l^{-1}) in subjects with T2DM (Figure 2B).

Insulin The arithmetic mean serum insulin concentrations (as measured with a generic insulin assay) were determined by dose. In healthy volunteers, there was a clear dose–response effect on the mean serum insulin concentrations after dosing of CarboCarrier® insulin (data on file). In patients with T2DM, the 20 IU and 36 IU dose group showed a mean increase of at least $1200 \mu\text{U ml}^{-1}$ from 3 h up to 10 h after dosing. Surprisingly, for unknown reasons,

Table 1

Summary of PK parameters of CarboCarrier® insulin

Parameter (unit)		Part 1 Healthy volunteers						Part 2 Patients with T2DM	
		0.5 IU (n = 6)	2 IU (n = 6)	6 IU (n = 5)	12 IU (n = 6)	18 IU (n = 6)	24 IU (n = 5)	20 IU (n = 8)	36 IU (n = 8)
$t_{\max}$ (h)	Median	3.01	3.00	5.00	4.50	5.50	6.00	6.00	6.00
$t_{\max}$ (h)	Min-max	2.0–4.0	3.0–5.0	3.0–6.0	3.0–6.0	3.0–8.0	5.0–10.0	5.0–6.1	5.0–10.0
$C_{\max}$ (ng ml ⁻¹)	Mean	2.35	12.6	33.7	62.1	97.3	124	105	174
$C_{\max}$ (ng ml ⁻¹)	dn-Mean†	4.69	6.28	5.62	5.17	5.40	5.16	5.27	4.83
$C_{\max}$ (ng ml ⁻¹)	CV (%)	11.2	21.9	15.3	32.8	19.0	18.1	47.6	43.4
AUC(0, t_{last}) (ng ml ⁻¹ h)	Mean	12.7	84.5	281	568	1030	1380	1180	2380
AUC(0, t_{last}) (ng ml ⁻¹ h)	dn-Mean†	25.4	42.3	46.9	47.4	57.3	57.3	59.0	66.1
AUC(0, t_{last}) (ng ml ⁻¹ h)	CV (%)	26.4	30.2	8.31	18.6	35.1	11.4	39.9	35.0
AUC(0, ∞) (ng ml ⁻¹ h)‡	Mean	19.9	92.3	290	579	1040	1380	1210	2430
AUC(0, ∞) (ng ml ⁻¹ h)‡	dn-Mean†	39.8	46.2	48.4	48.3	57.8	57.7	60.4	67.5
AUC(0, ∞) (ng ml ⁻¹ h)‡	CV (%)	25.3	30.6	8.39	18.5	34.9	11.3	38.4	33.2
$t_{1/2}$ (h)	Mean	4.11	3.25	3.11	3.25	4.81	4.47	3.80	5.28
$t_{1/2}$ (h)	CV (%)	25.5	23.4	22.6	47.7	27.6	15.5	15.0	14.1
CL/F (ml h ⁻¹)§	Mean	1290	1110	987	1020	905	831	748	750
CL/F (ml h ⁻¹)§	CV (%)	32.5	27.7	8.46	20.2	33.2	10.8	40.9	21.9
V_z/F (ml)§	Mean	7190	4950	4360	4610	5950	5320	4190	5680
V_z/F (ml)§	CV (%)	9.35	18.0	15.7	41.3	27.4	13.2	47.1	25.3
P : T ratio	n	0	0	2	4	6	5	8	8
P : T ratio	Mean	–	–	21.1	18.5	25.7	24.6	12.5	30.6
P : T ratio	CV (%)	–	–	44.7	22.1	72.6	82.8	41.2	110

Mean and CV (%) refer to arithmetic mean and arithmetic CV (%); dn-: dose-normalized. Not calculable; C_{24} was below LLOQ for all the subjects. †Unit of dn-Mean is ng ml⁻¹/IU for $C_{\max}$ and ng ml⁻¹ h/IU for AUC. ‡For dose of 0.5 IU the percentage extrapolated was above 25%. §For calculation of CL/F and V_z/F dose was recalculated to µg from IU (with 1IU CarboCarrier® insulin corresponding to 47.5 µg).

the NPH-insulin did not show detectable serum concentrations of insulin in the insulin assay that was used. CarboCarrier® Insulin was detected by the serum insulin assay that was deployed (data on file).

C-peptide The arithmetic mean C-peptide concentrations were determined by dose. As expected, for all dose groups there was an increase in serum C-peptide concentrations after 6 h related to food intake (data on file). The rise in serum C-peptide concentrations after food intake differed between subjects included in part 1 and part 2 (with a smaller increase in serum C-peptide concentrations in part 2), illustrative of the fact that in part 2 patients with T2DM were included (data on file).

Discussion

A novel CarboCarrier® technology has been developed for enhancing PK–PD profiles of small proteins and polypeptides at sub-anticoagulant concentrations [2]. It has been previously described that such compounds display attractive pharmaceutical properties in animals, with a PK–PD profile that is based on a well-defined mechanism of action involving ATIII as a plasma protein carrier [1]. We here report the results of a phase 1, rising single dose study in humans in order to assess safety, tolerability, pharmacoki-

netics and relative bioactivity of CarboCarrier® insulin. The data obtained in this study provide important evidence to validate the CarboCarrier® technology concept in humans, using CarboCarrier® insulin as an example.

CarboCarrier® insulin, has been engineered to explore whether coupling of insulin to a pentasaccharide followed by binding of this complex to the plasma protein ATIII increases insulin's half-life in man. In this phase 1 study, CarboCarrier® insulin was safe and well-tolerated and no consistent pattern of AEs occurred besides the AEs that were associated with hypoglycaemia (the pharmacological effect of the compound). No clinically significant changes or trends in clinical safety laboratory measurements were seen after administration of CarboCarrier® insulin (except for lowering of blood glucose). No clinically significant changes in vital signs or ECGs were seen after administration of CarboCarrier® insulin. Of importance, evaluation of coagulation parameters did not indicate any change in coagulation in subjects treated with CarboCarrier® insulin. This was expected as all tested CarboCarrier® Insulin doses that were evaluated are below the 50 nM level, which is the concentration threshold for avoiding any undesired clinically significant ATIII-mediated anticoagulant activity [2].

From a pharmacokinetic perspective, CarboCarrier® insulin exposure ($C_{\max}$ and AUC) increased proportionally with dose. The $t_{\max}$ seemed to increase slightly with the dose with a median $t_{\max}$ ranging from 3 to 6 h. The mean

Figure 2

(A) Mean change from baseline glucose concentrations in healthy volunteers. y axis: glucose concentrations, change from baseline (mmol l⁻¹); x axis: time (h); Main figure: 0–6 h data; Insert: 0–24 h data. Open squares: placebo; closed squares: 0.5 IU; open circles: 2.0 IU; closed circles: 6.0 IU; open triangles: 12 IU; closed triangles: 18 IU; open diamonds: 24 IU. (B) Mean change from baseline glucose concentrations in type 2 diabetes patients. y axis: glucose concentrations, change from baseline (mmol l⁻¹); x axis: time (h); Main figure: 0–6 h data; Insert: 0–24 h data. Open squares: placebo; closed squares: 20 IU CarboCarrier® insulin; open circles: 36 IU CarboCarrier® insulin; closed circles: 20 IU insulin

Table 2A

Summary statistics of change from baseline of plasma glucose concentrations (mmol l⁻¹) by time point following SCH 900948 dosing to healthy volunteers

Relative time	Statistics	Placebo	CarboCarrier® insulin					
			0.5 IU	2 IU	6 IU	12 IU	18 IU	24 IU
0.5 h post-dose	<i>n</i>	12	6	5	6	6	6	6
	Mean	0.12	-0.33	0.06	-0.13	0.07	-0.27	-0.1
	SD	0.48	0.53	0.15	0.36	0.20	0.66	0.27
1 h post-dose	<i>n</i>	12	6	6	6	6	6	6
	Mean	0.1	-0.15	0.02	-0.17	-0.18	-0.52	-0.32
	SD	0.52	0.43	0.10	0.24	0.17	0.57	0.24
1.5 h post-dose	<i>n</i>	12	6	6	6	6	6	6
	Mean	0.12	-0.27	-0.13	-0.37	-0.33	-0.65	-0.48
	SD	0.47	0.38	0.10	0.36	0.12	0.33	0.34
2 h post-dose	<i>n</i>	12	6	5	6	6	6	6
	Mean	0.13	-0.18	-0.24	-0.40	-0.48	-0.98	-0.88
	SD	0.62	0.35	0.17	0.39	0.19	0.59	0.34
3 h post-dose	<i>n</i>	12	6	5	6	6	6	6
	Mean	0.10	-0.53	-0.42	-0.63	-0.82	-1.22	-0.53
	SD	0.53	0.30	0.20	0.29	0.26	0.37	1.81
4 h post-dose	<i>n</i>	12	6	6	6	6	6	6
	Mean	-0.08	-0.68	-0.37	-0.67	-0.87	-1.85	-0.98
	SD	0.73	0.53	0.29	0.32	0.30	0.50	0.73
5 h post-dose	<i>n</i>	12	6	6	6	6	6	6
	Mean	-0.08	-0.28	-0.47	-1.05	-0.97	-1.62	-1.58
	SD	0.66	0.30	0.22	0.54	0.39	0.48	0.28
6 h post-dose	<i>n</i>	12	6	6	6	6	6	6
	Mean	-0.25	-0.57	-0.60	-0.92	-1.10	-1.75	-0.88
	SD	0.60	0.39	0.30	0.39	0.39	0.42	1.28
AUEC 0–6 h	<i>n</i>	12	6	6	6	6	6	6
	Mean	0.01	-0.38	-0.29	-0.57	-0.63	-1.17	-0.78
	SD	0.50	0.28	0.14	0.31	0.24	0.42	0.47

Mean and SD refer to the arithmetic mean and standard deviation.

terminal elimination half-life was increased dramatically when compared with (unconjugated) insulin (minutes) and ranged between 3.11 and 5.28 h. This limited increase in half-life was unexpected as the elimination half-life of the insulin-pentasaccharide complex was shorter than the anticipated half-life (of ~120 h in humans) of the pentasaccharide, being idraparinux, alone [9]. This could potentially be explained by an active and substantial clearance of the insulin-pentasaccharide complex from the body, presumably, but not necessarily exclusively, taking place in the liver, as a result of interaction of the insulin-pentasaccharide complex with insulin receptors that are abundantly present in this organ [10]. The P:T ratio was highly variable with an average ratio ranging from 12.1 to 30.6. Pharmacodynamics were in line with pharmacokinetics data. All CarboCarrier® insulin dose groups showed decreases in the mean change from baseline of plasma glucose concentrations compared with the placebo group. Furthermore, the data indicated that the relative bioactivity of CarboCarrier® insulin was not inferior to the bioactivity of NPH-insulin (Figure 2B). As expected, for all CarboCarrier® insulin dose groups there was an increase in serum C-peptide concentrations after 6 h related to food intake. The rise after food intake differed for the subjects

with T2DM of part 2 compared with the healthy subjects in part 1 as a result of the underlying pathogenesis of T2DM.

In healthy individuals, insulin produced in the pancreas is delivered into the portal circulation, resulting in the liver being exposed to higher insulin concentrations than peripheral tissues. As such, subcutaneous injections of the currently available insulin preparations do not mimic the normal physiology of insulin delivery. In principle, subcutaneously delivered insulin that preferentially targets the liver could further optimize the treatment of insulin-dependent diabetes mellitus patients. If indeed the faster than expected clearance of the insulin-pentasaccharide complex is being caused by insulin receptor mediated uptake in the liver, CarboCarrier® insulin could have features of a liver-targeted insulin that would be of benefit to patients. Obtaining formal evidence for this would require further detailed mechanistic studies. Of interest, faster than expected clearance of the insulin-pentasaccharide complex was also seen in rats [2] and as such the current data may in fact represent the clinical validation of earlier preclinical observations.

It is of importance to note that the described study design was suited to assess the pharmacokinetics as well as the safety and tolerability of single subcutaneous doses

Table 2B

Summary statistics of change from baseline of plasma glucose concentrations (mmol l⁻¹) by time point following CarboCarrier® insulin dosing to type 2 diabetes patients

Relative time	Statistics	Placebo	CarboCarrier® insulin		Insulin
			20 IU	36 IU	20 IU
0.5 h post-dose	<i>n</i>	8	8	8	8
	Mean	-0.06	-0.16	0.03	-0.24
	SD	0.22	0.33	0.24	0.50
1 h post-dose	<i>n</i>	8	8	8	8
	Mean	-0.16	-0.33	-0.34	-0.23
	SD	0.26	0.35	0.49	0.28
2 h post-dose	<i>n</i>	8	8	8	8
	Mean	-0.48	-1.24	-1.40	-0.61
	SD	0.46	0.63	0.43	0.39
3 h post-dose	<i>n</i>	8	8	8	8
	Mean	-1.20	-2.38	-2.68	-1.36
	SD	1.19	1.09	0.85	0.78
4 h post-dose	<i>n</i>	7	8	8	8
	Mean	-1.51	-3.08	-2.79	-1.70
	SD	0.66	1.13	1.86	0.54
5 h post-dose	<i>n</i>	8	8	8	8
	Mean	-1.71	-3.54	-3.28	-2.24
	SD	0.80	1.22	2.09	0.87
6 h post-dose	<i>n</i>	8	7	8	8
	Mean	-1.98	-3.86	-3.59	-2.61
	SD	0.88	1.25	1.82	0.88
AUEC(0,6 h)	<i>n</i>	8	8	8	8
	Mean	-0.98	-2.31	-2.03	-1.25
	SD	0.61	0.93	0.93	0.50

Mean and SD refer to the arithmetic mean and standard deviation.

of CarboCarrier® insulin, but not so much the glucose lowering effects as they were, in the current design, confounded by counter-regulation and by food. As a PK assay was available that is specific for CarboCarrier® insulin, reliable concentration–time curves could be generated that were not influenced by the endogenous release of insulin as a result of, for example, intake of a meal. Thus, the current study was not designed to comply with recommendations for the pharmacodynamic characterization of new long acting insulin products, which ask for hyperinsulinaemic euglycaemic clamps in people with T1DM. Such a study design was to follow the current design in case CarboCarrier® insulin would have yielded a PK profile that would be suitable for once daily administration. The current study was also not ideal for the evaluation of potential antibody responses to CarboCarrier® insulin as subjects received different doses (or different study drugs) during various phases of the protocol. Of interest, immunogenicity of CarboCarrier® insulin in preclinical species was low. The evaluation of the putative immunogenicity in humans was hampered by the fact that technical difficulties in designing a suitable assay precluded the exploratory use of the samples to obtain a first impression of potential immunogenicity.

In conclusion, the data obtained in this phase 1 study indicate that CarboCarrier® insulin (SCH 900948) is phar-

macologically active and shows features of insulin action. Although the elimination half-life of the molecule was clearly extended compared with endogenous insulin (hours vs. minutes), it was insufficient for once daily treatment. Our results provide the first evidence that the half-life of therapeutic proteins can be engineered by conjugation to ATIII-binding pentasaccharides without compromising the pharmacological activity of the protein in humans. As such, the current study is an important milestone in validating the CarboCarrier® technology concept in humans making use of CarboCarrier® insulin as the first example. In principle, the CarboCarrier® technology is applicable to potent peptides and proteins for which the target receptor is readily accessible from the circulation. It is expected that pharmacokinetic profiles similar or identical to the profile of uncoupled long-acting pentasaccharide can be achieved for peptides and proteins that do not show faster than expected clearance and that would be cleared exactly as the uncoupled pentasaccharide. Preliminary data have revealed that the CarboCarrier® technology indeed can be extended to other polypeptide drugs and peptide hormones in which the characteristic PK profile of the carrier pentasaccharide appears to be fully transferred to the polypeptide conjugate [11]. Further validation of the CarboCarrier® technology concept in humans is warranted.

Author contributions

AM designed the study, analyzed data and wrote the manuscript. MP analyzed data. JvK analyzed data and performed statistical evaluations. RT was the Principal Investigator conducting the study. MdK designed CarboCarrier® insulin (SCH 900948). RB designed the study, analyzed data and was the study physician. Dr Miltenburg is the guarantor of this work, had full access to all the data and takes full responsibility for the integrity of the data and the accuracy of data analysis.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author). André MM Miltenburg, Rob JW Berg, Marita Prohn, Jacqueline HM van Kuijk and Martin de Kort declare that they are or were employees of, at that time, NV Organon, Oss, the Netherlands. NV Organon, now MSD, sponsored this clinical trial. The other authors declare that they have no competing interests.

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