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ORIGINAL ARTICLE

Safety, tolerability, pharmacokinetics and pharmacodynamics of parenterally administered dutogliptin: A prospective dose-escalating trial

Nina Buchtele[1] | Michael Schwameis[2] | Christian Schoergenhofer[1] | Ulla Derhaschnig[1,2] | Christa Firbas[1] | Rudolf Karch[3] | Darrell Nix[4] | Roman Schenk[4] | Bernd Jilma[1]

[1]Department of Clinical Pharmacology, Medical University of Vienna, Austria

[2]Department of Emergency Medicine, Medical University of Vienna, Austria

[3]Section of Biosimulation and Bioinformatics, Center for Medical Statistics, Informatics and Intelligent Systems (CeMSIIS), Medical University of Vienna, Austria

[4]RECARDIO Inc., San Francisco, CA, USA

Correspondence
Bernd Jilma, MD, Department of Clinical Pharmacology, Medical University of Vienna, Waehringer Guertel 18-20, A-1090, Vienna, Austria.
Email: bernd.jilma@meduniwien.ac.at

Aims: Animal studies suggest that inhibition of dipeptidyl peptidase 4 (DPP-IV) may improve heart function and survival after myocardial infarction by increasing cardiac myocytes' regenerative capacity. Parenterally administered dutogliptin may provide continuous strong DPP-IV inhibition to translate these results into humans. This trial investigated the safety and tolerability, as well as pharmacokinetics and pharmacodynamics, of parenterally administered dutogliptin after single and repeated doses.

Methods: In an open-label trial, volunteers received dutogliptin at increasing doses of 30–120 mg subcutaneously or 30 mg intravenously in the single-dose cohorts. Subjects in the multiple-dose cohort received 60, 90 or 120 mg dutogliptin subcutaneously once daily on 7 consecutive days.

Results: Forty healthy males were included in the trial. No related serious adverse events occurred. Mild local injection site reactions with no requirement for intervention comprised 147 of 153 (96%) related adverse events. Subcutaneous bioavailability was approximately 100%. Multiple injections at daily intervals did not lead to the accumulation of the study drug. The accumulation ratios based on $AUC_{0\text{-}24h}$ range from 0.90 to 1.03, supporting this argument. All subjects receiving ≥60 mg dutogliptin yielded a maximum DPP-IV inhibition >90%. The duration of DPP-IV inhibition over time increased in a dose-dependent manner and was highest in the 120-mg multiple-dosing cohort with a maximum $AUEC_{0\text{-}24h}$ of 342 h % (standard deviation: 73), translating into 86% DPP-IV inhibition 24 hours after dosing.

Conclusion: Parenteral injection of dutogliptin was safe and subcutaneous bioavailability is excellent. DPP-IV inhibition increased dose dependently to >86% over 24 hours after multiple doses of 120 mg dutogliptin.

The trial is registered with EudraCT, number 2015-002233-21

The authors confirm that the PI for this paper is Bernd Jilma, MD and that he had direct clinical responsibility for the study participants.

KEYWORDS
parenteral, dutogliptin, healthy, safety, pharmacokinetics/pharmacodynamics

1 | INTRODUCTION

Cardiovascular disease is the leading cause of death worldwide.[1,2] Coronary heart disease accounts for almost 900 000 deaths in Europe annually.[2] In survivors, impaired heart function frequently causes long-term disability. In this context, the process of cardiac remodelling is crucial; this is characterized by structural maladaptation of the heart following neurohumoral activation.[3]

However, despite optimal care in accordance with current guidelines, coronary artery disease is still the most common factor in the development of heart failure as a long-term complication.[4] Every fifth patient develops maladaptive left ventricular remodelling after myocardial infarction.[5,6] Much effort has therefore been put into defining new targets to positively influence postinfarction cardiac remodelling. The stromal cell-derived factor (SDF)-1/CXCR4 axis constitutes a promising target.[7] This humoral axis is crucial for stem cell homing, cardiac myocyte survival and therefore ventricular remodelling.[8] The rationale of reinforcing the key SDF-1/CXCR4 axis has been proven effective in several preclinical studies. However, endogenous molecular signals of stem cell-based repair are short-lived and therefore clinically inefficient.[9] Besides genetic therapies, including engineered cell-based and plasmid-based overexpression of SDF-1,[10,11] dipeptidyl peptidase-IV (DPP-IV) inhibition has been shown to positively affect cardiac remodelling in mice.[12-16] This is not only related to benefits in ventricular remodelling with improved cardiac function, but also to atrial remodelling, preventing subsequent atrial fibrillation.[17]

As DPP-IV is an important cleaving enzyme for SDF-1, reduced DPP-IV activity leads to an increase in SDF-1 levels. The combination of DPP-IV inhibitors with granulocyte-colony stimulating factor (G-CSF) significantly increased homing of circulating stem cells, reduced cardiac remodelling and improved heart function as well as survival.[12,18]

Functions of DPP-IV are various, and include involvement in immunology and inflammation, cancer, neuroendocrinology and glucose metabolism. The exact mechanism, how DPP-IV inhibition may influence cardiac remodelling is still a matter of debate. In this context, DPP-IV inhibition may provide beneficial effects on hyperoxidative stress,[13] restoration of angiogenesis and tissue repair[16] or an improved autophagic response.[15]

Data from clinical use of DPP-IV inhibitors mainly come from its use as therapy in type II diabetes mellitus.[19-21] In this respect, DPP-IV inactivates several peptides involved in glucose metabolism, including glucagon-like peptide 1, glucagon-dependent insulinotropic polypeptide, vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide, but also SDF-1.[22,23]

Oral formulations of gliptines have been shown to effectively improve glycaemic control with a low risk of causing hypoglycaemia,[24] providing DPP-IV inhibition between 60 and 80%.[25] Furthermore, in large clinical trials DPP-IV inhibitors have shown noninferiority against placebo for major adverse cardiovascular events.[19-21] Clinical data on beneficial effects in terms of preserving heart function after myocardial ischaemia are promising, but are only taken from pilot studies.[26]

In preclinical models of heart failure, high dose, but not low dose, administration of DPP-IV inhibitors protected renal and improved cardiac function.[27,28] This leads to the question of whether a parenteral drug formulation providing 100% bioavailability translating into continuously high DPP-IV inhibition is possibly required to provide modulation of the SDF-1/CXCR4 axis including these beneficial effects on cardiac remodelling.

This trial therefore investigated the safety and tolerability, as well as pharmacokinetics and pharmacodynamics (PK/PD), of parenterally administered dutogliptin, a selective DPP-IV inhibitor, in healthy male subjects.

What is already known about this subject

- Animal data suggest dipeptidyl peptidase-IV as a promising target to prevent maladaptive remodelling after myocardial infarction.
- Gliptins inhibiting dipeptidyl peptidase-IV have become of great interest for the treatment of diabetes mellitus type II, but are only available as oral formulations.
- A subcutaneous formulation could overcome the limitation of reduced bioavailability and provide stable dipeptidyl peptidase-IV inhibition over time.

What this study adds

- Escalating doses of subcutaneously administered dutogliptin overall showed a very good safety profile in healthy male subjects.
- Subcutaneous administration of dutogliptin resulted in approximately 100% bioavailability and translated into dipeptidyl peptidase 4 inhibition >86% over 24 hours in subjects receiving 120 mg dutogliptin.
- This study provides promising pharmacokinetics/pharmacodynamics of parenterally administered dutogliptin, warranting further evaluation in a clinical efficacy trial in patients after myocardial infarction to translate results from animal models into humans.

2 | METHODS

2.1 | Study design and participants

In this prospective, dose-escalating phase I trial, healthy male volunteers were recruited from the Department of Clinical Pharmacology, Medical University of Vienna. Inclusion criteria were age between ≥18 and ≤35 years and body weight between ≥60 and ≤95 kg at screening.

Subjects were excluded if they had any history of anaphylaxis, metabolic disease or present human immunodeficiency virus antibodies, as well as hepatitis B or C surface antigen. The complete list of eligibility criteria is available with the supplement. Informed consent was obtained from all subjects before any study-related action was performed.

The intention-to-treat population included all participants who received at least 1 dose of assigned treatment. The per-protocol population included all participants without major protocol deviations.

The trial was approved by the Ethics Committee of the Medical University of Vienna and the National Competent Authority and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines. The trial was registered with EudraCT (2015-002233-21).

2.2 | Study parts and group allocation

This study was conducted in 2 parts (part A+ part B) with dose-escalating groups (Figure 1). In part A, 25 participants were allocated consecutively to 5 dosing cohorts of 5 participants each. Dutogliptin administration was performed as a single-dose application. Participants in group 1 received 30 mg of dutogliptin intravenously. Subjects in groups 2–5 received dutogliptin subcutaneously at doses of 30, 60, 90 and 120 mg, respectively.

In part B, 15 participants were included into 3 dosing cohorts with 5 participants each. Dutogliptin was administered at the same time in the morning on 7 consecutive days at doses of 60, 90 or 120 mg depending on group allocation.

2.3 | Dose rationale

No concentration–effect relationships were derived from available preclinical protocols as the pharmacological basis for the selection of the dose range to be investigated in this study. The starting dose was based on the no observed adverse effects (AEs) level of a 28-day toxicity study in rats. The no observed AEs level observed was 30 mg/kg, translating into 180 mg/m^2. Given a 10-fold safety margin, the calculated dose for an average human with body surface area of 1.8 m^2 was 32.4 mg. Therefore, the 30mg dose was chosen as the primarily administered dose. Typical dose escalation steps were used as appropriate for a first-in-man study design. Once it was established that the bioavailability was nearly 100%, the exposure profiles were simulated and the range of 30–120 mg targeting sustained DPP-IV inhibition (≥80% over 24 h) was set. A stepwise 100, 50 and 33.3% increase was chosen to provide sufficient data for the primary outcome.

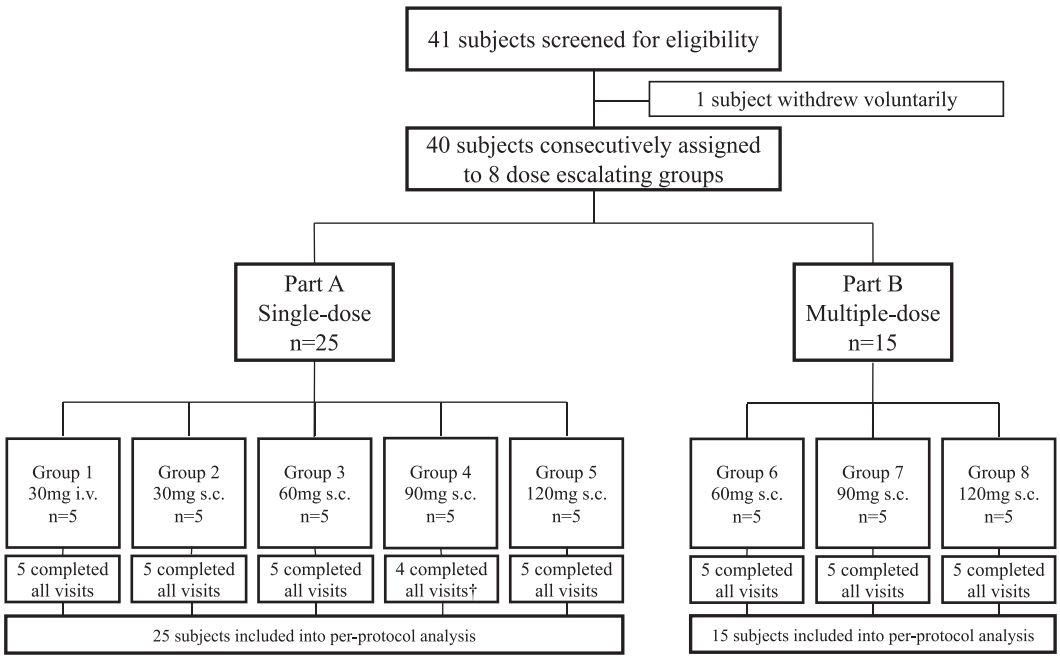

FIGURE 1 Study flowchart. †One subject in Group 4 was lost to follow-up, after completing the visit 24 hours after drug administration. Therefore, all pharmacokinetics and pharmacodynamics analysis could be assessed and the per-protocol analysis still included all 40 subject. i.v., intravenous; s.c., subcutaneous

3 | PROCEDURES

3.1 | Study drug

Oral formulations of dutogliptin have been investigated extensively in large phase III trials involving patients with diabetes mellitus II, and showed excellent safety and tolerability. For this trial, a parenteral formulation was investigated (PHX1149T/dutogliptin tartrate salt) and provided in sterile water for injection.

The study drug was prepared by a pharmacist and handed over to the investigator in ready-to-use syringes for either intravenous or subcutaneous administration. After the first 2 groups in part A, PK variables were analysed to compare differences in bioavailability between intravenous and subcutaneous administration. Based on these results, the future administration route was determined. Recruitment for part B only started after an interim-safety review for all part A subjects.

3.2 | Safety parameters

The interim safety review and final analysis was based on laboratory assessments, physical examination, electrocardiogram changes and monitoring of systemic and local AEs. At the primary study site initiation meeting, all investigators attended a thorough briefing in order to provide consistent reporting of AEs. The severity of AEs was graded according to the Common Terminology Criteria for Adverse Events v4.03. The term *serious AE* was used as defined by the International Conference on Harmonisation Guidelines and Good Clinical Practice guidelines. The relationship between AEs and study drug administration was assessed by the investigators as probable, possible, unlikely or unrelated. Those reported as probably or possibly associated with study drug administration were interpreted as related AEs. Injection-site reactions were assessed and analysed separately from systemic AEs. Injection-site reactions included swelling, haematoma, induration, erythema, itching and pain (with and without touching) at any time point at the site of the subcutaneous injection. Grading of injection site reactions was based on subjective severity as well as size assessed using a standardized measuring tape, where applicable. Local AEs were considered to be moderate or severe if the diameter exceeded 5 or 10 cm, respectively.

Laboratory assessments included blood analysis of haematological variables and clinical chemistry as well as urinalysis. All were performed at the screening visit. In part A, blood safety analysis was additionally done 1 and 7 (±3) days after study drug administration. In part B, blood safety analysis was performed 1, 3, 6 and 14 (±3) days after the first dose. Laboratory values were interpreted in relation to changes from baseline values obtained at the screening visit.

3.3 | Assessment of PK/PD

Blood samples were obtained from indwelling venous lines at predefined time points into 9-mL tubes containing K_3EDTA and immediately put on ice. Samples were centrifuged at 2000 *g* for 10 minutes and plasma was stored at −80°C until being analysed. In part A, group 1, these included 12 time points over 24 hours after end of intravenous infusion. For the remaining groups (2–5), 10 time points for blood sampling were defined over 24 hours after subcutaneous study injection. In part B, blood sampling was performed in all groups at 10 time points over 24 hours after first and last study drug administration, as well as predose and 8 hours after administration on the other days.

Plasma levels of dutogliptin were assessed by liquid chromatography/tandem mass spectrometry. The calibration curve range for liquid chromatography/tandem mass spectrometry was 1.00–1000 ng/mL. Concentrations less than the lower limit of quantitation (1.00 ng/mL) were set to zero. Plasma was extracted using acetonitrile/MeOH/formic acid (90/10/1 v/v/v) spiked with IS (d6-dutogliptin). Samples were centrifuged, the supernatant was removed and then dried under a nitrogen stream. Reconstitution was performed with water/MeOH/1M l-octanesulfonic acid (65/25/10/1) and injected for mass spectrometry analysis.

Analysis of the plasma PK profiles included measurements of C_{max}, T_{max} and T_{last}. With the observed values, area under the plasma concentration *vs* time curve (AUC) up to 24 hours ($AUC_{0\text{-}24h}$) and extrapolated to infinity (AUC_{inf}), elimination half-life ($T_{1/2}$), and the terminal elimination rate constant (λ_z) were calculated. Bioavailability (F), expressed as a percentage, was calculated for each single administration dose group for part 1 only (Table S1). Systemic exposure and accumulation of dutogliptin were assessed by ratios of $AUC_{0\text{-}24h}$ in subjects receiving multiple dose administration.

For PD analysis, the %activity was determined using the DPP IV activity data acquired from a commercially available enzyme-linked immunosorbent assay (Enzo Life Science, Inc., Lausen, Switzerland) in mean fluorescence units (MFU) and converted to %activity with the predose sample = 100% activity of DPP IV [postdose sample %activity = (postdose MFU/predose MFU)*100].

Analyses were carried out according to the manufacturer's instructions.

4 | OUTCOMES

The primary study endpoint was safety and tolerability of parenterally administered dutogliptin in the intention-to-treat population. Secondary outcome measures included PK/PD data in the per-protocol population.

5 | STATISTICAL ANALYSIS

Descriptive analysis was performed for PK/PD variables reported as means with standard deviation (SD). The noncompartmental PK parameters C_{max}, T_{max} and AUC were determined. Plasma DPP-IV inhibition over time was assessed by determining the area under the effect-curve up to 24 hours after administration ($AUEC_{0\text{-}24h}$) using the linear trapezoidal method. With the input from PK/PD assessments from once daily administrations, a 2-compartment model was

established to simulate various doses of twice daily administration to achieve >80% DPP-IV inhibition over 24 hours.

Adverse events were reported descriptively with subjects affected by at least 1 AE, percentages of all subjects with at least 1 AE and total number of recorded events.

No imputation was performed for missing PK/PD data. For missing AEs, assignment to seriousness, severity, outcome or relatedness, worst case imputation would be performed.

Either χ^2 tests or Fisher exact tests were used to determine differences between groups. Intergroup comparisons for continuous variables were performed using either *t* tests or Kruskal–Wallis tests. A 2-sided *P*-value of .05 was defined as significant. SAS version 9.3 (SAS Institute Inc., Cary, NC, USA) was used to calculate the noncompartmental analysis and to summarize the data by dose group for the clinical study report, using a predefined analysis plan for PK/PD data analysis. Phoenix WinNonlin (Certara, St. Louis, MO, USA) was used for interim data analysis and simulations.

6 | RESULTS

Between 24 May and 29 September 2017, 41 volunteers were screened for eligibility, of whom 40 were included. Baseline characteristics were similar between study groups (Table 1). In total, 1 subject was lost to follow-up (Part A), as he did not return for the end-of-study visit. All PK/PD samples were collected and therefore the intention-to-treat and the per-protocol population both included 40 subjects.

6.1 | Safety of dutogliptin

6.1.1 | Systemic adverse events

Laboratory values, including glucose levels, electrocardiogram results and findings on physical examination were compared to baseline values obtained during the screening visit. No changes were observed in these values during the study period.

A total of 6 related systemic AEs were reported. None of these were considered to impair the daily life of study participants, and all were classified as mild (*n* = 5) or moderate (*n* = 1). The single moderate AE was headache that required concomitant medication, which occurred in the 120mg single dose group.

No dose-dependent increase for related systemic AEs was observed (*P* > .999). When pooling in respect to single or multiple-dose administration, there was a nonsignificant trend towards more subjects in the multiple-dose groups with at least 1 related systemic AE (*P* = .06).

Two nonrelated severe AEs were reported, 1 of which was classified as a serious AE because it required hospitalization. The reported serious AE related to a head injury sustained from a physical assault in a public place on day 4 after dutogliptin administration. One day after the head injury, the subject experienced diplopia, which was classified as a severe AE and had not resolved by the end of the study. In addition to the head injury, the subject also suffered fractures to the cervical vertebra C1, mandible and cheekbone, which required surgery. The subject received concomitant medication including metamizol once (500 mg, orally), paracetamol 3 times daily (1 g, intravenously), enoxaparin twice daily (40 mg, subcutaneously) and aspirin (100 mg, orally, ongoing after final examination). A complete listing of nonrelated AEs is given in Table S2.

6.2 | Local tolerability

Mild local injection site reactions requiring no intervention comprised 147 of 153 (96%) related AEs (Table 2). Most frequently occurring local AEs were erythema (*n* = 94) and induration (*n* = 26). None of the local AEs required any intervention or had impact on subjects' daily life. Intravenous bolus injection of the study drug was not associated with any AEs.

Three local AEs (all erythema) were classified as moderate because the diameter exceeded 5 cm, but they did not require further action to be taken.

There was no dose-dependent increase in subjects experiencing at least 1 local AE (*P* > .999). When pooling single-dose administration and multidose administration there were significantly more subjects in the multidose groups experiencing induration (*P* = .001) and pain with touching (*P* = .01).

The number of subjects with at least 1 related local AE was not different between single and multiple subcutaneous drug administration as well as between each dosing cohort. The severity of AEs was similar between all dosing cohorts.

TABLE 1 Demographics of included subjects

	Intravenous single dose (*n* = 5)	Subcutaneous single dose (*n* = 20)	Subcutaneous multidose (*n* = 15)	Total (*n* = 40)
Age (y)	25 (3)	27 (4)	26 (4)	26 (4)
Ethnicity group				
Caucasian	5 (100%)	19 (95%)	14 (93%)	38 (95%)
Asian	0	1 (5%)	1 (7%)	2 (5%)
Weight (kg)	79 (3)	78 (5)	74 (9)	77 (6)
Height (cm)	189 (9)	181 (6)	177 (4)	180 (6)

Results are displayed as means with standard deviation in brackets. Percentages are given within each column. No significant differences in demographic data were observed between groups.

TABLE 2 Listing of related adverse events (AEs)

	Intravenous single dose (n = 5)		Subcutaneous single-dose (n = 20)							
	30 mg (n = 5)		30 mg (n = 5)		60 mg (n = 5)		90 mg (n = 5)		120 mg (n = 5)	
	Subjects with at least 1 AE	Events (n)	Subjects with at least 1 AE	Events (n)	Subjects with at least 1 AE	Events (n)	Subjects with at least 1 AE	Events (n)	Subjects with at least 1 AE	Events (n)
All AEs	0	0	5 (100%)	6	5 (100%)	7	5 (100%)	7	5 (100%)	9
Local AEs	0	0	5 (100%)	6	5 (100%)	7	5 (100%)	7	4 (80%)	8
Erythema	0	0	5 (100%)	5	5 (100%)	6	5 (100%)	5	4 (80%)	4
Induration	0	0	0	0	1 (20%)	1	0	0	2 (40%)	2
Pain with touching	0	0	1 (20%)	1	0	0	0	0	0	0
Pain without touching	0	0	0	0	0	0	0	0	0	0
Swelling	0	0	0	0	0	0	1 (20%)	1	0	0
Hematoma	0	0	0	0	0	0	1 (20%)	1	1 (20%)	1
Other*	0	0	0	0	0	0	0	0	1 (20%)	1
Systemic AEs	0	0	0	0	0	0	0	0	1 (20%)	1
Headache	0	0	0	0	0	0	0	0	1 (20%)	1
Fatigue	0	0	0	0	0	0	0	0	0	0
Other†	0	0	0	0	0	0	0	0	0	0

TABLE 2 Listing of related adverse events (AEs)

	Subcutaneous multidose (*n* = 15)						Total (*n* = 40)	
	60 mg (*n* = 5)		90 mg (*n* = 5)		120 mg (*n* = 5)			
	Subjects with at least 1 AE	Events (n)	Subjects with at least 1 AE	Events (n)	Subjects with at least 1 AE	Events (n)	Subjects with at least 1 AE	Events (n)
All AEs	5 (100%)	40	5 (100%)	47	5 (100%)	37	35 (90%)	153
Local AEs	5 (100%)	39	5 (100%)	43	5 (100%)	37	34 (85%)	147
Erythema	5 (100%)	24	5 (100%)	27	5 (100%)	23	34 (85%)	94
Induration	4 (80%)	9	4 (80%)	7	3 (60%)	7	12	26
Pain with touching	3 (60%)	5	2 (40%)	5	2 (40%)	3	8 (20%)	14
Pain without touching	0	0	1 (20%)	1	2 (40%)	4	3 (8%)	5
Swelling	0	0	1 (20%)	1	0	0	2 (5%)	2
Hematoma	1 (20%)	1	1 (20%)	2	0	0	4 (10%)	5
Other*	0	0	0	0	0	0	1 (3%)	1
Systemic AEs	1 (20%)	1	4 (80%)	4	0	0	6 (15%)	6
Headache	0	0	1 (20%)	1	0	0	2 (5%)	2
Fatigue	1 (20%)	1	1 (20%)	1	0	0	2 (5%)	2
Other†	0	0	2 (40%)	2	0	0	2 (5%)	2

Legend: Numbers reported are numbers of subjects affected by at least 1 AE (% given within subjects) and total number of related events (% given within all related adverse events). *other local AEs comprised related AEs that only occurred once and included burning sensation at the injection site. †other systemic AEs comprised related AEs that only occurred once and included dizziness and nausea.

6.3 | PK/PD

Maximum plasma concentrations increased dose dependently with highest values in subjects receiving 120 mg on day 0 (n = 10; 5093 ng/mL [SD: 873]) and day 6 (n = 5; 6024 ng/mL [SD: 544], P = .05), respectively (Figure S1).

Bioavailability for 30, 60, 90 and 120 mg were 99, 109, 96 and 95%, respectively. The half-life of dutogliptin was 3.68 hours after intravenous injection (SD: 0.79), which was comparable to subcutaneous injection (3.49 h; SD: 0.46). Mean accumulation ratios based on $AUC_{0\text{-}24h}$ were 0.90, 1.02 and 1.03 for 60, 90, and 120 mg dutogliptin, respectively (Table 3). Individual accumulation ratios ranged from 0.86 to 0.95 for 60 mg dutogliptin, 0.92 to 1.16 for 90 mg dutogliptin and 0.92 to 1.09 for 120 mg dutogliptin.

Regarding PD, the time to maximum DPP-IV inhibition was dose and route dependent. Intravenous application of 30 mg dutogliptin resulted in maximum inhibition at 0.5 hours. Lowest DPP-IV activity in patients receiving subcutaneous administration was reached within 2 hours for 30 and 60 mg dutogliptin and within 4 hours for 90 and 120 mg dutogliptin (Figure S2).

DPP-IV activity was reduced to <10% in all participants receiving 60 mg or more subcutaneously. The lowest DPP-IV activity on day 0 of subcutaneous dutogliptin administration was overall 7.19% (SD: 1.60). Subjects receiving 120 mg of dutogliptin had the lowest DPP-IV activity of 5.88% (SD: 1.65). The longest DPP-IV inhibiting effect was seen in the highest dosing cohort receiving multiple administration (120 mg) with a maximum $AUEC_{0\text{-}24h}$ of 342 h % (SD: 73) on day 6 (Figure 2), translating into 86% DPP-IV inhibition over 24 hours.

Results from the PK/PD modelling are available in the supplement. The simulated model for twice daily simulation of subjects receiving 60 mg of dutogliptin are likewise given in the supplement (Figure S3, Table 3).

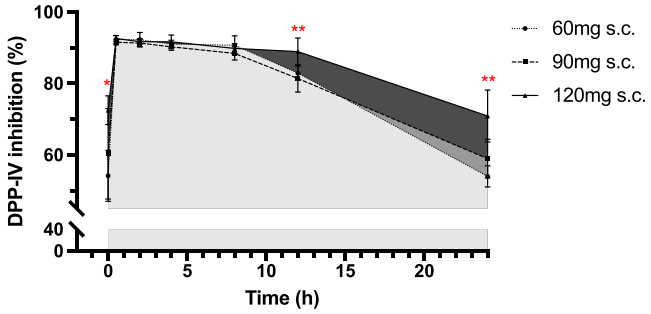

FIGURE 2 Intravenous dipeptidyl peptidase 4 (DPP-IV) inhibition over 24 hours during steady state (day 6). Inhibition of DPP-IV by subcutaneously injected dutogliptin on day 6 in multiple-dosing cohorts. Data are represented as means with standard deviation. All groups include 5 subjects each. * indicates significance at timepoint 0 between highest (120 mg) and lowest (60 mg) dosing cohort; **indicates significance at timepoints 12 and 24 hours between highest (120 mg), medium (90 mg) and lowest (60 mg) dosing cohort. DPP-IV, dipeptidyl peptidase 4; s.c., subcutaneous

7 | DISCUSSION

7.1 | Safety

Parenterally injected dutogliptin showed a very good safety profile overall. No study drug-related severe or serious AEs were observed. While the majority of subjects experienced some injection-site related reaction, none required any action to be taken or caused disruption of daily life.

More subjects with multidose administration of dutogliptin experienced induration and pain with touching, while overall numbers of subjects with 1 local AE were not different. Likewise, subjects with multiple dose administration showed a nonsignificant trend towards experiencing at least 1 systemic AE.

TABLE 3 Pharmacokinetics and pharmacodynamics of dutogliptin

	30 mg i.v. (n = 5)	30 mg s.c. (n = 5)	60 mg s.c. (n = 10)	90 mg s.c. (n = 10)	120 mg s.c. (n = 10)
Pharmacokinetics					
$T_{1/2}$ (h)	3.68 (0.79)	3.37 (0.50)	3.58 (0.41)	3.64 (0.44)	3.32 (0.48)
Peak plasma concentration (ng/mL)	2352 (211)	1232 (97)	2757 (517)	3641 (672)	5093 (873)
T_{max}	0.08 (0)	0.70 (0.25)	0.85 (0.23)	0.85 (0.23)	0.90 (0.2)
$AUC_{0\text{-}24h}$ (h ng/mL)	4080 (249)	4050 (558)	9062 (933)	12170 (1075)	17180 (2326)
AUC_{inf}	4090 (258)	4060 (563)	9110 (930)	12240 (1076)	17260 (2361)
λ_z (1/h)	0.20 (0.05)	0.21 (0.03)	0.20 (0.02)	0.20 (0.02)	0.21 (0.03)
Pharmacodynamics					
Lowest DPP-IV activity (%)	7.63 (0.95)	8.03 (1.76)	7.67 (1.18)	7.58 (1.17)	5.88 (1.65)
$AUEC_{0\text{-}24h}$ (%*h)	908 (149)	849 (74)	538 (44)	491 (86)	431 (93)
Post-dose DPP-IV activity over 24 hours (%)	37.83	35.36	22.42	20.46	17.96

Results are displayed as means with standard deviation in brackets. All values were measured on day 0.
i.v., intravenous; s.c., subcutaneous; $T_{1/2}$, half-life; AUC, area under the plasma concentration *vs* time curve; DPP-IV, dipeptidyl peptidase 4; $AUEC_{0\text{-}24h}$, area under the effect-curve up to 24 hours; λ_z, terminal elimination rate constant.

We interpreted these results as possibly related to the higher number of visits in the multidose group, as subjects were assessed daily for 7 days for any AEs. The increase could furthermore be related to a delayed manifestation of AEs in regard to study drug administration. This could have been missed in single-dose subjects, as follow up for AEs was only performed 1 day after study drug injection, i.e. when delayed AEs might be pending, and on day 7 after study drug injection, when AEs might have resolved and thus were not reported by the subject. To reduce this bias in a future trial, the number of visits and observation periods should be the same for all dosing cohorts, regardless of whether it is a single- or multidrug administration cohort. This could serve to minimize any underreporting of local AEs on days where no visit is performed. Furthermore, a placebo-controlled study could help to assess the relevance of systemic AEs directly related to dutogliptin administration.

Local reactions after subcutaneous injection of drugs are common[29]; these include reactions following the administration of therapeutics used in the treatment of diabetes mellitus type II such as insulin, exenatide and liraglutide. To minimize irritation, rotation of injection site is recommended.[30] It is still being debated whether slow or fast injection rates should be favoured for subcutaneous injections.[31] While the rationale behind slow application is the avoidance of the sudden distension of the subcutis,[30] quick injection provides the advantage of short contact time and minimizes painful lateral movement of the needle within the tissue.[32] Another critical issue causing pain during and after injection is the formulation of the injected drug, particularly with regards to its viscosity and the additional excipients required for adequate resorption, as well as the administered volume.[33] Lowering the volume of parenteral dutogliptin may reduce local irritability, which could easily be accomplished by providing higher concentrated solutions.

7.2 | PK/PD

To date, only oral gliptins with an average bioavailability of about 80% are available.[25] To be more specific, vildagliptin and saxagliptin show oral bioavailability of 85 and 67% respectively, while sitaglitpin's bioavailability is 87%.[25] In comparison, subcutaneous injection of dutogliptin resulted in approximately 100% bioavailability with peak plasma concentrations of 5000 ng/mL after subcutaneous injection of 120 mg. Compared to 500 mg orally administered dutogliptin, this translates into a >6-fold increase in maximal plasma levels.[34]

The terminal half-life of currently available oral gliptines show wide variability, ranging from 3 hours (vildagliptin) to 105 hours (linagliptin). Subcutaneously administered dutogliptin had a half-life of approximately 3.5 hours, which is comparable to that of vildagliptin and saxagliptin. The half-life of oral dutogliptin was 3-fold longer[34] than that of the iv or sc dosing used in the current trial. The apparently longer half-life after oral intake may be due to prolonged (but incomplete) resorption after oral intake.

Results from $AUC_{0\text{-}24h}$ suggest that a steady state exposure was reached in this dose range, with no relevant accumulation upon repeated daily dosing, because mean accumulation ratios did not reach statistical significance. Likewise, dose intervals exceeded 5-times the elimination half-life, which makes accumulation unlikely. Maximum plasma concentrations of dutogliptin were higher on day 6 compared to day 0 in the multiple administration cohort, which, however, does not reflect the systemic exposure over 24 hours.

Subcutaneous injection of 120 mg dutogliptin reduced DPP-IV activity to below 6%, translating into >86% DPP-IV inhibition over 24 hours. Likewise, the 60 mg twice daily administration from the simulated model could be a promising option to achieve an improved continuous DPP-IV inhibition. In comparison, currently available oral doses and formulations of gliptins are capable of reducing DPP-IV activity by between 60 and 80% over 24 hours,[25] and are only approved for treatment of diabetes mellitus type II.

However, besides its well-known function of improving glycaemic control, DPP-IV activity influences several other pathways providing potential cardio- and renoprotective effects.[35] Knock-out mice have shown proof of concept for the crucial involvement of DPP-IV in myocardial infarction, as those animals lacking DPP-IV showed longer survival after the event.[36] Likewise, pharmacological inactivation of DPP-IV by gliptins improved recovery from ischaemic injury in mice.[36]

Another approach to further augment beneficial effects on cardiac remodelling is the combined administration of G-CSF and DPP-IV inhibitors, by specifically targeting the SDF-1 axis. Coadministration significantly improved cardiac function and survival in mice and therefore may be a promising and novel therapeutic approach to prevent heart failure after myocardial infarction.[12,18] The combination of G-CSF and DPP-IV inhibitors is probably crucial to promote the beneficial effects on myocardial remodelling, because G-CSF reduces early cardiomyocyte apoptosis. However, to date, no clinical data are available on the safety of the combined application of G-CSF and DPP-IV inhibitors, which therefore requires further evaluation.

As G-CSF is injected subcutaneously, it could potentially be provided as a 1-syringe combination therapy with dutogliptin. Particularly in patients with myocardial infarction, a subcutaneous formulation of DPP-IV inhibitors could overcome both swallowing difficulties and impaired drug uptake due to pharmacological or critical illness-related impairment of gastric emptying.[37,38] Another advantage of a parenteral formulation is that it is independent of fed/fasting conditions, and therefore reduces intrapatient variability and increases predictable high plasma concentrations and pharmacological activity.

Oral administration of 400 mg dutogliptin inhibited DPP-IV activity by approximately 85%,[34] but led to lower plasma concentrations compared to parenteral application. We can only speculate as to whether local tissue DPP-IV inhibition increases with plasma concentration, which might be warranted for improved cardiomyocyte recruitment, as has been suggested by preclinical models investigating vildagliptin.[27,28] However, it needs to be determined how plasma and tissue concentrations of DPP-IV activity are related. To date, no data are available on regional tissue DPP-IV inhibition after dutogliptin administration. Therefore, no

assumptions can be made on equivalent doses of dutogliptin and vildagliptin to yield beneficial effects regarding cardiac function.

Obviously, local tissue DPP-IV inhibition cannot be measured in humans and therefore large-scale clinical phase II/III trials involving patients after myocardial infarction are warranted to determine whether DPP-IV inhibition achieved by parenterally administered dutogliptin can prevent maladaptive remodelling. For an ongoing phase II proof of concept study (clinicaltrials.gov identifier: NCT03486080), a twice daily regime of dutogliptin administration was chosen based on the simulated 2-compartment model in order to provide continuous high DPP-IV inhibition. While the development of oral dutogliptin has been terminated due to huge market competition in the treatment of diabetes mellitus type II, a new parenteral formulation could pave the way for novel indications, warranting further evaluation in clinical trials.

7.3 | Limitations

Only male volunteers were included in our study, which needs to be considered as a limitation. To date, no sex-related differences in efficacy have been described for approved orally available gliptins.[39] Interestingly, sitagliptin plasma levels were approximately 25% higher in female subjects, which, however, did not translate into differences in efficacy[40] and was thus considered irrelevant in clinical practice, where no sex-specific dose adjustment is suggested.[25]

Approved orally available gliptins show an excellent safety profile, but concerns have been raised regarding an increased risk of pancreatitis associated with DPP-IV inhibitor (sitagliptin, saxagliptin and valdagliptin) intake.[41-44] However, data remain controversial,[45] and it is unclear whether this is an effect related to exposure in terms of maximal concentrations and/or duration of treatment. This phase I trial was too small to detect any rare AEs, and further large-scale clinical trials would be needed to detect these. As the development of oral dutogliptin has been terminated due to large market competition, large long-term safety data are currently not available.

As the starting dose of 30 mg administered dutogliptin was well tolerated, lower doses have not been investigated. In addition, the twice daily simulation has only been performed for the 60-mg dosing cohort. Both need to be considered a limitation of the current investigation.

Another limitation of our study relates to the assay sensitivity and attributed lower limit of detection threshold of the used enzyme-linked immunosorbent assay kit for DPP-IV activity determination. Due to analytical limitations of the DPP-IV assay it cannot be determined whether 100% inhibition of DPP-IV activity can be reached. Therefore, the dose linearity of inhibition can best be seen by a prolongation of the maximum inhibition reached (>90%). Furthermore, downstream effects of DPP-IV inhibition, such as SDF-1 activity, have not been investigated in this trial due to the instability of plasma samples after 1 freeze–thaw cycle.

8 | CONCLUSION

Parenteral injection of dutogliptin was safe and subcutaneous bioavailability is excellent. DPP-IV inhibition increased dose dependently to >86% over 24 hours after multiple doses of 120 mg dutogliptin.

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COMPETING INTERESTS

D.N. and R.S. are employed by RECARDIO, Inc. B.J. received reimbursement for travel expenses and scientific advice related to scientific advisory meetings. N.B., M.S., C.S., U.D., C.F. and R.K. have stated explicitly that there are no conflicts of interest in connection with this article.

CONTRIBUTORS

D.N., R.S. and B.J. designed the study. N.B., C.S., U.D. and C.F. conducted the study and collected data. M.S. did the statistical analysis. M.S., N.B., C.S., R.K. and B.J. analysed and interpreted data. M.S. and N.B. wrote the first draft of the manuscript, drew figures and tables. All authors contributed to drafting of the manuscript. The manuscript has been seen and approved by all authors, has not been previously published and is not under consideration for publication in the same or substantially similar form in any other peer-reviewed media.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Nina Buchtele https://orcid.org/0000-0002-1270-6452
Christian Schoergenhofer https://orcid.org/0000-0002-2286-1077
Bernd Jilma https://orcid.org/0000-0001-5652-7977

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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