British Journal of Clinical Pharmacology

Br J Clin Pharmacol (2018) **84** 2270–2279 2270

ORIGINAL ARTICLE

A first-in-man safety and pharmacokinetics study of nangibotide, a new modulator of innate immune response through TREM-1 receptor inhibition

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Received 14 August 2017; **Revised** 25 May 2018; **Accepted** 29 May 2018

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Keywords drug safety, pharmacokinetics, Phase I, randomized controlled trial

AIMS

The peptide nangibotide is the first clinical-stage agent targeting the immunoreceptor TREM-1 (triggering receptor expressed on myeloid cells-1) and is being investigated as a novel therapy for acute inflammatory disorders such as septic shock. This first-in-man, randomized, double-blind, ascending dose, placebo-controlled Phase I study evaluated the safety, tolerability and pharmacokinetics of nangibotide.

METHODS

Twenty-seven healthy subjects (aged 18–45 years) were randomized into eight groups. Nangibotide was administered as a single continuous intravenous infusion. The first two groups received a single i.v. dose of 1 and 10 mg, respectively, over 15 min. Subsequent groups were randomized in a product : placebo ratio of 3:1 at doses ranging from 0.03 to 6 mg kg^{-1} h^{-1} over 7 h 45 min, preceded by a 15-minute loading dose of up to 5 mg kg^{-1}.

RESULTS

Nangibotide was safe and well tolerated up to the highest dose tested. There were only few adverse events and they were mild in severity and considered unrelated to treatment. Nangibotide displayed dose-proportional PK properties, with a clearance of 6.6 l kg^{-1} h^{-1} for a subject of 70 kg and a 3 min effective half-life, which are compatible with extensive enzymatic metabolism in blood. Central and peripheral volumes of distribution were 16.7 l and 15.9 l respectively, indicating limited distribution of the drug mainly in blood and interstitial fluid. No circulating anti-drug antibodies were detectable up to 28 days after administration.

CONCLUSIONS

The novel immunomodulator nangibotide displayed favourable safety and PK profiles at all doses, including expected pharmacologically active doses, and warrants further clinical development.

DOI:10.1111/bcp.13668

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- TREM-1 is an amplifier of the innate immune response by synergizing with toll-like receptors and is a crucial mediator of septic shock.
- Pharmacologic modulation of TREM-1 showed protective effects in preclinical models of acute and chronic inflammatory disorders.
- The novel immunomodulator nangibotide is the first clinical stage drug candidate to address TREM-1 and this first-in-human study evaluates its safety and pharmacokinetics.

WHAT THIS STUDY ADDS

- Nangibotide was safe, well tolerated and displayed dose-proportional pharmacokinetics at doses of up to 6 mg kg^{-1} h^{-1} for 7 h 45 min preceded by a 15-minute loading dose of 5 mg kg^{-1}.
- Further clinical evaluation of nangibotide as a novel treatment for septic shock is warranted.

Introduction

The innate immune response involves the coordinated action of several effectors such as epithelial and endothelial cells, as well as blood mononuclear and polynuclear cells, and relies on the activation of a large family of pattern recognition receptors (PRR) by pathogen motifs and danger signals [1]. PRRs orchestrate the early host response to infection and injury as well as the subsequent activation and shaping of adaptive immunity [2]. The extent of the inflammatory response mediated by these receptors is influenced by their synergistic interaction with triggering receptors expressed on myeloid cells (TREMs) such as TREM-1 [3–6].

The TREM-1 immunoreceptor is expressed on innate immune cells such as mature monocytes ($CD14^{high}$), macrophages, and neutrophils [7–10], as well as hypoxic dendritic cells [11] and epithelial cells [12–14]. TREM-1 was initially characterized for its pathophysiological role during septic shock [7, 8], and since, in other acute diseases such as ischaemia/reperfusion injury after myocardial infarction [15, 16], haemorrhagic shock [17], ischaemia-reperfusion [18, 19], pancreatitis [20, 21] and acute kidney injury [22, 23].

TREM-1 is one of the most upregulated pathways during the genomic storm observed in septic shock patients [24, 25]. Co-engagement of TREM-1 and toll-like receptors (TLRs) leads to a hyperactivated and exuberant inflammatory response which is responsible for the onset and progression from sepsis to septic shock. Currently, there is no specific causal treatment for septic shock, and previous attempts to develop treatments have failed, including therapies targeting endotoxins and TLRs [26]. Considering the role of TREM-1 as an amplifier of the immune response, modulating TREM-1 activation to prevent excessive inflammation would seem to be a logical approach.

Nangibotide is the first drug candidate addressing TREM-1 to reach clinical stage development; it inhibits TREM-1 activation by acting as a decoy receptor [27]. It is a chemically synthesized peptide, derived from the protein TREM-like transcript-1 (TLT-1), and is composed of 12 L-amino-acids and has a molecular weight of 1342.5 g mol^{-1}. In animal models of septic shock, the administration of LR12, a species-specific orthologous peptide, was linked to protective effects. In rodent models of polymicrobial sepsis, LR12 was associated with a substantial reduction (but not total inhibition) of both pro- and anti-inflammatory circulating and tissue markers as well as sepsis-induced tissue abnormalities, concomitantly with increased bacterial clearance and survival rate [27]. Similarly, Weber *et al*. showed that TREM-1 deficiency in various infections reduced excessive inflammation while preserving the capacity for microbial control [28]. The administration of LR12 in a pig model of peritonitis resulted in a decreased need for noradrenaline, which reveals a substantial protective effect on the cardiovascular system. In this model, the peptide also prevented sepsis-induced tissue abnormalities and dysfunctions [29]. Nangibotide was also able to mitigate endotoxin-associated clinical and biological alterations with no obvious side effects in non-human primates [30]. In rodent models of myocardial ischaemia-reperfusion, treatment with LR12 controlled leucocyte trafficking, thus reducing systemic and *in situ* inflammatory reaction. This led to reduced infarct size and ventricular dilation, and several weeks later to improved systolic and diastolic ventricular functions [15]. In a pig model of cardiogenic shock, LR12 was associated with substantial protection of the cardiovascular system and decreased infarct size [16].

We describe the first-in-human Phase I study conducted to evaluate the safety, tolerability, and pharmacokinetic (PK) profile of the novel immunomodulator drug candidate, nangibotide, in healthy subjects.

Methods

This randomized, double-blind, ascending dose, placebo-controlled, first-in-man Phase I study was conducted at Richmond Pharmacology (St George's University of London, NCT03463044) between 2 March 2016 and 25 August 2016. The study was performed in accordance with the Declaration of Helsinki and International Council on Harmonization Good Clinical Practice, and approved by the South Central – Berkshire B Research Ethics Committee, UK. All subjects freely gave written informed consent before enrolment.

This study allowed sequential adaptation of study design within protocol-defined boundaries, including adaptation of dose levels and dosing regimens, the split of groups into sub-groups, adjustment of group, addition of optional groups, and adjustment of frequency and timing of samples and assessments, on the basis of emerging safety and PK data [31].

Nangibotide has been shown to dose-dependently dampen TREM-1 activation, whereas it has not displayed any pharmacological effect in physiological conditions when TREM-1 was not activated [3, 5, 8, 15, 27, 32]. Therefore, TREM-1 pathway is not expected to be activated in healthy subjects and thus no specific pharmacodynamic effects were monitored upon administration of nangibotide in this study, except usual inflammatory parameters such as blood leucocyte count and C-reactive protein.

Study population

Twenty-seven healthy males who had volunteered as study subjects, aged between 18 and 45 years, and with a body mass index ranging 18–30 kg m^{-2} were included in this study. The subjects were considered to be healthy based on their medical history, physical examination, electrocardiogram (ECG), vital signs, and laboratory tests; and free from any significant illness which could potentially confound the study results. They also were unlikely to require concomitant treatments, which could interfere with the study drug. As discussed and agreed with Medicines and Healthcare products Regulatory Agency, female subjects were not included in this study because data from investigations on teratogenicity were not yet available.

Treatment

The study drug was a stable powder containing 400 mg of free base lyophilized nangibotide peptide (H-Leu-Gln-Glu-Glu-Asp-Ala-Gly-Glu-Tyr-Gly-Cys-Met-NH_2, 1342.5 g mol^{-1}, CAS number 2014384-91-7) in sodium citrate and arginine buffer at pH 5.5, reconstituted in sodium chloride solution to a final concentration of 10 mg ml^{-1} for intravenous (i.v.) infusion. Placebo was the same sodium chloride solution used for reconstitution.

Study design

This study included eight groups and two sequential Parts (A and B). PK and safety data were analysed between each group. Progression between groups was allowed after safety, tolerability and PK review by a blinded safety review committee.

The starting dose was selected with a very conservative safety margin in relation to non-observable adverse effect level (NOAEL) (140 mg kg^{-1} day^{-1}), given the fact that this product was first-in-class and this pathway had never been targeted previously in humans. The escalating dose strategy was selected to document a range of concentrations covering the predicted pharmacologically active dose observed in preclinical pharmacology models which is 1 mg kg^{-1} h^{-1} across species in disease relevant models [29, 30]. Since the efficacy of a broader range of doses may be assessed in patients in later clinical trials, both lower and higher doses were administered to healthy volunteers to document the tolerability of these doses (see Supporting Information Data S1).

Within each group, a mandatory sentinel dosing strategy was adopted in order to avoid simultaneous exposure of all subjects. This sentinel approach consisted of only one subject on Investigational Medicinal Product (IMP) the first day for each group. The remaining subjects were dosed after a minimum interval of 24 h, and after safety and tolerability of the first subject had been evaluated as acceptable.

Nangibotide was administered as a continuous intravenous infusion (CIV). For the first two dose levels (Part A, groups 1 and 2), there was no placebo and only one subject was to be dosed. One subject each received a single i.v. dose of nangibotide of 1 or 10 mg, respectively, over a period of 15 min. One additional and not initially planned subject was included in group 2 for further evaluation of the pharmacokinetic profile at the selected dose regimen.

In Part B (groups 3–8), eligible healthy subjects were randomized to receive nangibotide or matching placebo in a product:placebo 3:1 ratio at doses ranging from 0.03 to 6 mg kg^{-1} h^{-1} over a period of 7 h 45 min, necessary to characterize the pharmacokinetics and the safety of the product, preceded by a 15-minute loading dose ranging from 0.5 to 5 mg kg^{-1}, as described in Table 1.

Safety evaluations

All observed or subject-reported adverse events (AEs) were assessed for intensity and relationship to the drug treatment. The investigator was to follow up any AE until it resolved or until the medical condition of the subject stabilized. Other safety assessments included an evaluation of physical examination, vital signs (supine systolic and diastolic blood pressure, heart rate and body temperature), ECG parameters (12-lead ECG, telemetry and Holter), laboratory tests (haematology, chemistry, coagulation parameters, and urinalysis), local tolerability at the infusion site, and immunogenicity (presence of anti-nangibotide antibodies). No specific potential safety risks were identified during pivotal preclinical toxicity studies since no toxicities were observed at the highest dose tested. However, considering that the IMP is a peptide, healthy volunteers were monitored for the appearance of anti-drug antibodies (ADA) (see immunogenicity

Table 1

Dose escalation scheme

Group	No. of subjects	Loading dose 15 min	Maintenance dose 7 h 45 min
Part A (no placebo)			
		(mg)	
1	1	1	-
2	1 + 1	10	-
Part B (nangibotide 3:1 placebo)			
		(mg kg^{-1})	(mg kg^{-1} h^{-1})
3	4	0.5	0.03
4	4	1.0	0.10
5	4	2.0	0.30
6	4	5.0	1.00
7	4	5.0	3.00
8	4	5.0	6.00

For Part A (groups 1 and 2), each subject received nangibotide. For Part B (groups 3–8, double-blinded) three subjects received nangibotide and one subject received placebo (3:1 ratio). Among a total of 27 randomized subjects, 21 received nangibotide and six received placebo

evaluations below) and were closely followed up for appearance of any allergic, anaphylactic or local reactions (infusion site).

PK analytical method

Blood nangibotide concentrations were determined by a validated LC–MS/MS method according to European Medicines Agency (EMA) guidelines on bioanalytical method validation EMEA/CHMP/EWP/192217/2009, version EMA/275542/2014. The peptide was stabilized in the blood samples by treatment with a 10% trichloroacetic acid solution (1500 mg ml^{-1}, 4°C) and centrifugation (3500 g, 10 min, 4°C) to collect and freeze the supernatant before analysis. The lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) were 5 ng ml^{-1} and 1000 ng ml^{-1} respectively. Standard curve is linear over the range 5 ng ml^{-1} to 1000 ng ml^{-1} with a correlation coefficient greater than 0.995. Within-run and between-run precision and accuracy within ±20% LLOQ and ±15% (other levels up to ULOQ) were achieved during a three-run validation for quality controls (QCs) for nangibotide. Within-run and between-run precision and accuracy data for quality control samples are available in Table S1.

PK evaluations and modelling

Blood samples for PK evaluations were collected during Part A at pre-dose, 15 min and 60 min after start of infusion. During Part B, blood samples were collected at pre-dose, then 5 min, 15 min, 18 min, 30 min, 45 min, 1 h, 2 h, 4 h, 6 h after the start of infusion, 15 min before the end of infusion (7 h 45 min) and 2 min, 4 min, 7 min, 15 min, 30 min, and 2 h after the end of infusion. Samples were immediately processed according to the validated bioanalytical method and were stored at −80°C until analysis. Data were analysed by a non-compartmental analysis (NCA) using WinNonlin® Professional software (Version Phoenix 6.4, Pharsight Corporation, Mountain View, CA, USA). The following PK parameters were determined for each group of Part B: the maximum observed concentration following the loading dose (C_{max}); the average steady-state concentration during the maintenance infusion from 30 to 465 min ($C_{avg\ 30–465}$); and the area under the blood concentration *vs*. time curve from time 0 to the last observation time ($AUC_{0\text{-}t}$). In addition, all concentration–time data were analysed by non-linear mixed effects modelling applying an open two-compartment model with first order elimination rate from the central compartment implemented in NONMEM software version 7.3.0 [33]. This analysis allowed the estimation of the central (CL) and intercompartmental (Q) clearances, and the central (V_1) and peripheral (V_2) volumes of distribution. Based on these parameters the distribution ($t_{1/2\alpha}$), terminal ($t_{1/2\beta}$), and effective $t_{1/2}$, also called overall half-life [34], were calculated.

Immunogenicity evaluations

Anti-nangibotide antibodies in serum were evaluated at baseline, Day 8, and Day 28, by an indirect ELISA method validated according to methods described by Shankar *et al*. [35] and EMA guidance on immunogenicity assessment of biotechnology-derived therapeutic proteins (EMEA/CHMP/BMWP/14327/2006 issued in April 2008). Screening samples determined as positive were further assessed using the confirmatory assay where the cut point was established to have 0.1% of false positive samples [35]. Validation of the method showed no drug interference. The sensitivity of the assay was established to be 0.3125 μg ml^{-1}.

Statistical methods

AEs were coded using the MedDRA dictionary version 18.1. They were classified by System Organ Class (SOC) and Preferred Term (PT). Treatment-emergent AEs (TEAEs) were summarized and listed.

The statistical analysis was carried out using the SAS® package (release 9.4) or using Phoenix WinNonlin. Nangibotide concentrations *vs*. time were plotted on linear and semi-logarithmic scales. Blood concentrations and PK parameters were listed for nangibotide. Summary statistics of PK parameters including means, geometric means and coefficients of variation were presented for each dose. PK dose-proportionality was tested on dose normalized log transformed C_{max} and $AUC_{0\text{-}t}$ by analysis of variance (ANOVA). The first-order conditional estimation (FOCE) method within NONMEM was used to estimate the population PK parameters. Log-normal random distribution (i.e., inter-individual variability) of the parameters was assumed and estimated for CL and V_1. Additive residual error model (i.e., intra-individual variability) was applied to the log-transformed concentrations. Once the structural model was established, the influence of available covariates, i.e., dose and body weight, were graphically explored on nangibotide PK model parameters; if judged relevant, they were further tested for their significance using a *P*-value of <0.01 ($\chi^2_{P=0.01,v=1}$ = 6.63 difference in the objective function), the covariate entering the model in a multiplicative way as a power function (e.g. $(COV/COVpop)^{power}$ with COVpop = reference value). A visual predictive check (VPC) method was applied to evaluate the final model comparing the observations with their posterior predictive distribution according to the model. For this model, 500 simulations of the present study were performed using final model estimates and the 90% confidence interval of model-simulated nangibotide concentrations were calculated for groups 3–8.

Results

Baseline characteristics

A total of 27 subjects were included and randomized to receive treatment; 26 subjects completed the study and only one subject withdrew prematurely. Indeed, one subject from group 7 was lost to follow-up after the visit at Day 8 and was thus prematurely withdrawn. Twenty-seven subjects were included in the safety data set, the PK and immunogenicity evaluations. Age ranged between 19 and 40 years, with a mean ± SD of 24.9 ± 5.4 years. There were 21 (77.8%) Caucasian subjects, 3 (11.1%) Black subjects, and 3 (11.1%) Asian subjects. Weight ranged between 57.2 and 100 kg, with a mean ± SD of 74.7 ± 8.9 kg; and BMI was between 18.7 and 29.2 kg m^{-2}, with a mean ± SD of 23.2 ± 2.8 kg m^{-2}. Other characteristics were similar across all groups (Table 2). No prior medication was reported. Baseline 24 h-Holter ECG

Table 2

Demographic and baseline characteristics

		Group 1 (N = 1)	Group 2 (N = 2)	Group 3 (N = 4)	Group 4 (N = 4)	Group 5 (N = 4)	Group 6 (N = 4)	Group 7 (N = 4)	Group 8 (N = 4)	Total (N = 27)
Age (years)	N	1	2	4	4	4	4	4	4	27
	Mean (SD)	30.0 (–)	31.0 (12.7)	25.3 (2.9)	25.0 (8.0)	24.5 (7.0)	23.0 (2.9)	23.5 (3.0)	23.5 (4.4)	24.9 (5.4)
	Median	30.0	31.0	25.0	22.5	21.0	23.5	24.0	22.5	24.0
	Min; Max	30; 30	22; 40	22; 29	19; 36	21; 35	19; 26	20; 26	20; 29	19; 40
Race	N	1	2	4	4	4	4	4	4	27
	Caucasian	1 (100.0%)	2 (100.0%)	4 (100.0%)	1 (25.0%)	3 (75.0%)	4 (100.0%)	3 (75.0%)	3 (75.0%)	21 (77.8%)
	Black	-	-	-	2 (50.0%)	1 (25.0%)	-	-	-	3 (11.1%)
	Asian	-	-	-	1 (25.0%)	-	-	1 (25.0%)	1 (25.0%)	3 (11.1%)
Weight (kg)	N	1	2	4	4	4	4	4	4	27
	Mean (SD)	81.2 (–)	68.6 (16.1)	71.0 (4.6)	76.7 (3.3)	76.9 (18.7)	71.4 (8.7)	77.4 (6.3)	76.0 (1.7)	74.7 (8.9)
	Median	81.2	68.6	71.7	77.1	73.1	74.4	77.1	76.7	75.7
	Min; Max	81.2; 81.2	57.2; 80	65.3; 75.5	72.4; 80.4	61.4; 100	58.7; 78.2	70.6; 84.9	73.5; 77.1	57.2; 100

was normal in all subjects. At screening and visit at Day −1, there were no clinically significant findings in all subjects for haematology, biochemistry, coagulation and serology.

Safety evaluations

Nangibotide was well tolerated at doses of up to 6 mg kg^{-1} h^{-1} during CIVs of 7 h 45 min that were preceded by a 15-min loading dose up to 5 mg kg^{-1}. At least one treatment emergent adverse event (TEAE) was experienced by 5 out of 21 (23.8%) subjects who received nangibotide and one out of six subjects who received placebo (16.7%) (Table 3). All TEAEs were mild in severity and considered unrelated to treatment. They were resolved by the end of the study, except the haemangioma in one subject in group 2, which had an unknown outcome at the end of the study.

No clinically significant abnormalities were observed for laboratory tests (haematology, biochemistry, coagulation and urinalysis) (see Supporting Information Tables). Vital signs, physical examination, infusion site reaction, telemetry and ECG values were unremarkable, with no safety issues identified and with no clinically meaningful differences among all groups.

PK evaluations and modelling

For all subjects in all groups, the pre-dose sample did not contain quantifiable nangibotide. The blood concentration of nangibotide for the subject in group 1 at 15 min was below the limit of quantification (LOQ) of 5 ng ml^{-1}. Following a single i.v. dose of 10 mg in the two subjects from group 2, the average blood concentration of nangibotide at the end of the 15-min CIV was 59.6 ng ml^{-1} (4.7% CV).

Nangibotide blood concentrations *vs*. time obtained from groups 3–8 are shown in Figure 1A and associated descriptive statistics of nangibotide PK parameters calculated by NCA are presented in Table 4. From groups 3–8, nangibotide blood concentrations increased up to C_{max} which was observed at the end of the loading infusion (i.e., 15 min) in 14 out of 18 subjects. In the four remaining subjects, all from the highest dose groups (7 and 8), C_{max} was apparently already achieved 5 min after the start of the CIV. After the loading infusion, nangibotide blood concentrations rapidly decreased to reach steady state within 15 min, which remained stable (i.e. plateaued) until the end of the maintenance dose. For groups 3–6, a rapid elimination phase was observed that reached non-quantifiable concentrations (below the LOQ) within 10 min after the end of infusion of nangibotide. For groups at the highest doses (7 and 8), there was an apparent two-step elimination phase composed of a rapid phase followed by a slower elimination phase (Figure 1A).

As a new drug candidate, it was important to document the dose-proportionality of nangibotide disposition. The dose-proportionality is illustrated in Figure 1B for C_{max} versus loading dose and in Figure 1C for $AUC_{0\text{-}t}$ *vs*. total administered dose (i.e., 0.73–51.5 mg kg^{-1}). According to ANOVA, there was a dose-proportional increase in C_{max} and $AUC_{0\text{-}t}$ with the dose ($P < 0.0001$), with a slope of 1.18 (90% CI 1.04–1.33) for C_{max} and 1.04 (90% CI 0.99–1.09) for $AUC_{0\text{-}t}$.

The graphical analysis for the two highest doses detected a biphasic decay of nangibotide concentrations with a slow elimination phase occurring at very low concentrations (i.e., about 1% of steady-state concentrations). This slow phase was not observable at all other doses, probably because nangibotide concentrations were below the LOQ. Nevertheless, an open two-compartment model was found best suitable to describe the general PK properties of nangibotide. Concentrations below the LOQ were ignored. Covariate analysis on clearance showed that body weight was a statistically significant covariate, whereas dose was not statistically significant (power = −0.00258 ± 0.0239) and therefore was not included in the final model. The estimates of the model parameters are outlined in Table 5. Structural model parameters were estimated with good precision (5–18% SE). Inter-individual variabilities of <14% and residual error (33%)

Table 3

Table TEAEs by preferred term – nangibotide *vs*. placebo – safety set

MedDRA preferred terms	Placebo (N = 6)	Nangibotide (N = 21)	Groups								Onset
			1 (N = 2)	2 (N = 2)	3 (N = 4)	4 (N = 4)	5 (N = 4)	6 (N = 4)	7 (N = 4)	8 (N = 4)	
All TEAEs	2	9	-	4	2	1	-	2	1	2	-
Administration site pain	1		-	-	-	-	-	1	-	-	Day 1
Administration site rash	1		-	-	-	-	-	1	-	-	Day 1
Fatigue		1	-	-	-	-	-	-	-	1	Day 1
Tooth development disorder		1	-	-	-	-	-	-	1	-	Day 28
Infusion site reaction		1	-	-	1	-	-	-	-	-	Day 2
Viral pharyngitis		1	-	-	1	-	-	-	-	-	Day 6
Dizziness		1	-	1	-	-	-	-	-	-	Day 1
Headache		1	-	1	-	-	-	-	-	-	Day 1
Rash		1	-	1	-	-	-	-	-	-	Day 2
Haemangioma		1	-	1	-	-	-	-	-	-	Day 3
Cerumen removal		1	-	-	-	1	-	-	-	-	Day 23

N, number of subjects total per group

were acceptable. The central and peripheral volumes of distribution of nangibotide were estimated at 16.7 l and 15.9 l, respectively. Clearance was estimated at 463 l h^{-1} for a typical 70 kg healthy subject and effective $t_{1/2}$ at about 3 min. Covariate analysis indicated that nangibotide clearance increased with body weight with an exponent estimate of 0.725 (35% SE).

The VPC presented in Figure S1 showed that almost all the observed concentrations fell within the 90% confidence interval of the simulated data indicating that the developed model could reliably predict the individual nangibotide concentration–time profiles in healthy subjects.

Immunogenicity evaluations

Serum samples for the detection of ADA were collected for all subjects at baseline, Day 8 and Day 28, with the exception of one subject from whom a serum sample could not be collected at Day 28. All subjects were negative for the presence of ADA.

Discussion

This was a first-in-man Phase I study of nangibotide, conducted at a single centre, to evaluate its safety, tolerability and PK profile in healthy subjects. Results demonstrated that nangibotide was safe and well tolerated at doses of up to 6 mg kg^{-1} h^{-1} given over 7 h 45 min, preceded by an up to 5 mg kg^{-1} 15-min loading dose. There were no AEs related to the drug treatment, and no clinically relevant and significant abnormalities were observed for all the safety parameters evaluated. Nangibotide did not trigger any ADA responses at the doses tested. The dose administered to group 6 (1 mg kg^{-1} h^{-1} maintenance dose) corresponds to the pharmacologically active dose used in previous non-clinical pharmacological studies in pigs and cynomolgus monkey models of septic shock [29, 30].

Within the dose range tested in our Phase I study, blood nangibotide concentrations increased in a dose-proportional manner. The level of exposure correlated to the entire dose range investigated in this study (0.03–6 mg kg^{-1} h^{-1}), and the dose-proportional PK enabled accurate prediction of blood nangibotide concentration according to dose. Nangibotide displayed a short effective half-life of approximately 3 min, which justifies the CIV dosing schedule. Comparable data had previously been reported in cynomolgus monkeys, in whom nangibotide concentrations decreased very rapidly after the end of infusion [30]. At the highest dose levels in the present study (groups 7 and 8), a slow elimination phase was detected, which represented 1% of steady-state concentration of nangibotide. The reasons for this slow elimination phase are not yet clear; however, there was no evidence of accumulation of nangibotide in blood, and a half-life of 3 min would allow rapid clearance after the end of infusion.

Nangibotide kinetics at all doses administered in the study could be adequately modelled by an open two-compartment model with a linear elimination pathway. The central volume of distribution of about 17 l approximated the extracellular fluid volume, and the main clearance estimated at 6.6 l h^{-1} kg^{-1} for a subject of 70 kg was indicative

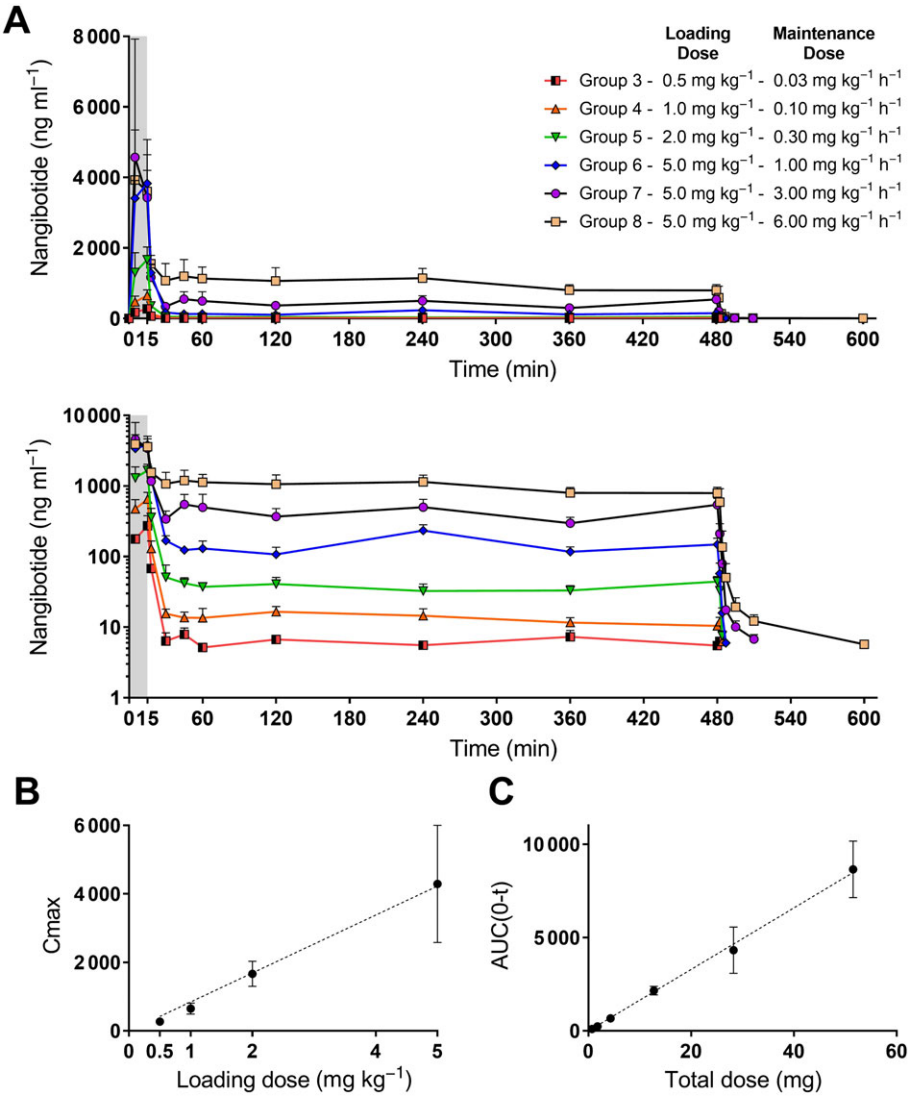

Figure 1

Pharmacokinetic properties of nangibotide. A: Mean and standard deviation (SD) blood concentration of nangibotide in a linear and $\log_{10}$ scale versus time. All subjects had no detectable nangibotide before treatment. B, C: Mean (+SD) maximum observed concentration following the loading dose (C_{max}) and area under the blood concentration *vs.* time curve from time 0 to the last observation time ($AUC_{0\text{-}t}$) versus dose

Table 4

Non-compartmental analysis (NCA) of nangibotide pharmacokinetic parameters – groups 3 to 8

	Dose			C_{max}, ng ml^{-1}		$C_{avg\ 30–465}$, ng ml^{-1}		$AUC_{0\text{-}t}$, h.ng ml^{-1}	
Group	**Loading (15 min)**	**Maintenance(7 h 45 min)**	***n***	**Mean (CV%)**	**GM**	**Mean (CV%)**	**GM**	**Mean (CV%)**	**GM**
3	0.50 mg kg^{-1}	0.03 mg kg^{-1} h^{-1}	3	273 (39)	256	NA	NA	102 (11)	102
4	1.00 mg kg^{-1}	0.10 mg kg^{-1} h^{-1}	3	651 (25)	638	13.6 (18)	13.5	246 (14)	244
5	2.00 mg kg^{-1}	0.30 mg kg^{-1} h^{-1}	3	1668 (22)	1643	36.9 (14)	36.6	673 (23)	662
6	5.00 mg kg^{-1}	1.00 mg kg^{-1} h^{-1}	3	3831 (10)	3819	153 (12)	152	2162 (11)	2153
7	5.00 mg kg^{-1}	3.00 mg kg^{-1} h^{-1}	3	4783 (66)	4185	425 (22)	418	4320 (29)	4209
8	5.00 mg kg^{-1}	6.00 mg kg^{-1} h^{-1}	3	4264 (21)	4200	997 (20)	983	8651 (17)	8557

CV, coefficient of variation; GM, geometric mean; NA, not assessable

Table 5

Results for the nangibotide population final model

Parameter (unit)	Model estimate (SE%)	IIV CV% (SE%)
V_1 (l)	16.7 (7.5)	13.9 (87)
CL (l h^{-1})[a]	463 (4.0)	7.83 (47)
BW on CL[b]	0.725 (35.2)	-
V_2 (l)	15.9 (15.0)	-
Q (l h^{-1})	9.4 (17.3)	-
Alpha (h^{-1})	28.3	
Beta (h^{-1})	0.579	
$t_{1/2\alpha}$ (min)	1.47	
$t_{1/2\beta}$ (min)	71.8	
Effective $t_{1/2}$ (min)	2.91	
Residual error (CV%)	33.2 (5.45)	

CV, coefficient of variation; IIV, inter-individual variability; SE, standard error

[a]for 70 kg

[b]$CL_i = CL \times (BW_i/70)^{0.725}$

of extensive metabolism in blood. The central and peripheral volumes of distribution of nangibotide were estimated at 16.7 l and 15.9 l, respectively. This could indicate that the drug is not eliminated by the liver or the kidneys but mainly metabolized in the blood or in the tissues. Indeed, nangibotide is a short peptide of 1342.5 g mol^{-1} composed of 12 natural L-amino-acids, so elimination is most likely mediated by plasma proteases. We have observed that the use of proteases inhibitors increases the half-life of the peptide *in vitro* (unpublished results). The clearance increased with body weight, interestingly, with an exponent estimate close to the three-quarters allometric exponent generally used to scale drug clearance to body weight [36]. This supports the use of a weight-based dosing for further clinical development studies.

This study was designed to support further clinical studies and assessed the safety and pharmacokinetics of a CIV of nangibotide in humans. The choice of CIV was based on preclinical studies. Indeed, a CIV was necessary to maintain a constant blood concentration of nangibotide over time in an experimental cynomolgus monkey endotoxemia model [30]. This CIV of nangibotide was associated with beneficial pharmacodynamic effects and reduction of endotoxin-associated early and late clinical and biological alterations, such as cytokines, haematologic and haemodynamic changes. A rapid clearance of nangibotide was observed after the end of infusion. Likewise, in a pig model of peritonitis, CIV of nangibotide was associated with an attenuation of cardiovascular failure, coagulation disorders, and organ failure. The noradrenaline infusion rate needed to maintain blood pressure over 18 h after peritonitis was significantly lower in the nangibotide-treated animals than in controls [29].

Nangibotide therapy is currently being developed for the treatment of patients with acute inflammatory disorders such as septic shock. Nangibotide acts by scavenging the ligand of TREM-1, the circulating levels of which having been associated with worse outcome in septic shock patients [27]. An intravenous loading dose followed by a CIV administration of nangibotide during the first 4 or 5 days after onset of septic shock may be foreseen, with the objective of blunting an already established hyperactivated state of the immune system and high circulating concentration of TREM-1 ligand at treatment initiation, and maintaining a constant blood concentration of nangibotide and counteracting a dysregulated host response and sustained inflammation. Indeed, septic shock patients with a complicated outcome such as secondary infections are known to display a more dysregulated pro-inflammatory and vascular host response during the first 4 days after ICU admission as compared to patients with a less complicated outcome [37]. These data are correlated to a median time of continuous renal replacement therapy (RRT), inotropes support and vasopressor support of 4, 3 and 4 days respectively, with interquartile ranges of 2–6 days [38]. Changes in TREM-1 expression on monocytes have been observed at time of admission to ICU and lasted over several days until ICU discharge or death [39]. This was also true for circulating levels of sTREM-1, a soluble form of TREM-1 generated by proteolytic cleavage of the membrane-bound form of TREM-1 [39–44]. The pharmacologically active dose of nangibotide used in previous preclinical pharmacological studies in pig and monkey models of septic shock was 1 mg kg^{-1} h^{-1} [29, 30], which was associated with an average constant blood concentration of 91.4 ± 5.1 ng ml^{-1} [30]. We expect a similar pharmacologically active dose in patients based on body weight conversion. Nevertheless, even if this dose and blood concentration was pharmacologically active in preclinical models, higher exposure in patients may be needed and exploration of optimal dose/exposure will be part of future clinical studies. In that study, nangibotide was well tolerated in healthy volunteers up to a dose of 6 mg kg^{-1} h^{-1}, corresponding to an average blood concentration of 997.0 ng ml^{-1} (20.0%CV).

Although this study presents important data pertaining to the clinical PK and safety profiles of nangibotide, some limitations deserve discussion. Nangibotide is being developed as a specific pharmacological inhibitor of TREM-1, an immunoreceptor implicated in the amplification of the innate immune response [7–9] and in the pathogenesis of acute inflammatory disorders such as septic shock [27, 45] as well as acute myocardial infarction [15, 16, 46]. TREM-1 is only expressed at low levels in resting situations, and an initial priming through TLRs is needed to induce up-regulation and subsequent activation of TREM-1 [3, 5, 8, 15, 32]. Nangibotide has been shown to dose-dependently dampen the TREM-1-induced amplification of innate immune cells, whereas it has not displayed any pharmacological effect in physiological conditions when TREM-1 is normally not activated [27].

All data in this study were obtained from healthy subjects who received nangibotide in the absence of inflammation and therefore in the absence of a TREM-1 activated pathway. Therefore, this study does not assess the safety of nangibotide during an acute inflammatory disorder. In addition, pathophysiological changes associated with critical illness, such as increased volume of distribution as well as changes in cardiac and renal output may have an impact on nangibotide PK. Further nangibotide safety and PK

studies in critically ill patients with a TREM-1 activated pathway will be necessary. Nevertheless, the data generated here are essential to build a model of nangibotide kinetics after administration to patients.

Conclusions

Nangibotide CIV was safe and well tolerated at doses of up to 6 mg kg^{-1} h^{-1} for 7 h 45 min preceded by a 15-min loading dose of up to 5 mg kg^{-1}. Nangibotide displayed dose-proportional PK and a half-life of around 3 min. Its PK profile was compatible with extensive metabolic clearance in blood. Based on these findings, nangibotide is expected to be a therapy suitable for the intensive care environment, in particular for the treatment of patients with septic shock. Before initiating large clinical trials to assess the efficacy of nangibotide in that indication, a safety and PK assessment of the nangibotide therapy during such an acute inflammatory disorder will be necessary. Such investigation of nangibotide in patients with septic shock is underway (Drug Product name: MOTREM, NCT03158948, EudraCT Number: 2016-005032-14).

Competing Interests

M.D., S.G. and J.J.G. are co-founders of Inotrem SA, a French Company which develops nangibotide peptide. M.D., J.J.G., V.C. and M.S.M. are employees of Inotrem. S.W. is a consultant contracted to Inotrem. U.L. is an employee of Richmond Pharmacology Ltd, contracted to Inotrem to conduct the study. I.D. is an employee of MnS Modeling and Simulation, contracted to Inotrem to establish the PK model.

The study was supported and funded by Inotrem SA. Inotrem SA received funding from the Agence Nationale de la Recherche (ANR-15-RHU-0004).

Contributors

V.C., U.L., S.W., S.G., J.J.G., M.D. and M.S.M. designed and prepared the study protocol. U.L. was the Principal Investigator of the study and was responsible for the study initiation and conduct. V.C., U.L., S.W. and M.S.M. analysed and interpreted the safety data. A.O., I.D., J.J.G., M.D. and M.S.M. analysed and interpreted the PK and immunogenicity data. I.D. established the PK model. All authors prepared the manuscript.

Editorial support was provided by Wendy Wilkinson and Mike Leedham of Micron Research Limited, Ely, UK, and funded by Inotrem.

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Supporting Information

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

http://onlinelibrary.wiley.com/doi/10.1111/bcp.13668/suppinfo.

Data S1 Dose rational
Figure S1 Visual predictive check for the nangibotide population model
Table S1 Acuracy and precision on QCs for all analytical runs for nangibotide
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