

Phase I study of Solulin, a novel recombinant soluble human thrombomodulin analogue

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Summary

Solulin is a novel recombinant soluble derivative of human thrombomodulin. In this first human study of Solulin, the safety, tolerability, pharmacokinetics and pharmacodynamics of Solulin in 30 healthy volunteers in response to single (0.6–30 mg) and 12 healthy volunteers in response to multiple (1 and 10 mg) ascending intravenous bolus doses compared to placebo are described. Solulin was shown to be well tolerated, and demonstrated linear pharmacokinetics over the clinically relevant dose range, with a plasma elimination half-life of 15–30 hours, indicating that a less than daily dose may be required for therapeutic use. Steady-state plasma levels after multiple dosing were reached after 48 hours. Solulin has shown to be able to inhibit thrombin generation without increasing levels of aPC/PCI complexes. Coagulation parameters INR and PT were not changed, aPTT was elevated to about

10% above the upper limit of normal after the highest single dose only. Thrombin clotting time was prolonged after administration of high dose Solulin (10, 30 mg). No effect on *in vitro* bleeding time has been found. There was no evidence of bleeding risk with Solulin administration. The pharmacodynamic effects correlated with Solulin plasma concentrations. This demonstrates that the antithrombotic effect of Solulin is predictable, suggesting that patient monitoring is not expected. The results of this study provide evidence that Solulin can be expected to be an effective and safe anticoagulant, and further clinical investigation is warranted.

Keywords

Thrombomodulin, sothrombomodulin alpha, Solulin, endogenous thrombin potential, phase I

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Introduction

Solulin (soluble human recombinant thrombomodulin; sothrombomodulin alpha; ZK 158 266) is a recombinant soluble derivative of human thrombomodulin, an endothelial cell membrane protein that binds thrombin and is a cofactor in the protein C anticoagulant pathway (1–3). Thrombomodulin produces anticoagulant and anti-inflammatory effects (4, 5).

Current anticoagulant treatments have a long history of use in thrombotic, thromboembolic, and thrombophilic disorders (6, 7). Many of these anticoagulants, however, are associated with limitations such as bleeding or other complications, e.g. the development of heparin-induced thrombocytopenia (HIT)-II antibodies with subsequent occurrence of HIT (8–12) and the need for once-daily subcutaneous injections (13). Several orally administered anticoagulant medications are registered in single countries or are in advanced stages of development that seek to address the shortcomings of current therapeutic options (14–26).

Recombinant human thrombomodulin analogues are a new class of anticoagulants that have been developed to mimic the effects of human thrombomodulin. Thrombomodulin alpha (ART-123), developed by Asahi Kasei (Tokyo, Japan), is a novel

anticoagulant composed of the active extracellular domain of thrombomodulin (4, 5). Thrombomodulin alpha (ART-123) features a unique mechanism of action in which thrombin generation is suppressed by the activated protein C pathway without direct inhibition of thrombin activity at clinical blood concentrations, thus minimising the risk of bleeding. This was confirmed by two phase I studies in which no changes in bleeding time, coagulation and haemostatic parameters were found after administration of thrombomodulin alpha (ART-123) (27, 28). In a phase III study, thrombomodulin alpha (ART-123) was found to be significantly superior to heparin in treating disseminated intravascular coagulation, with a markedly lower incidence of bleeding-related adverse events (5). Due to this safety profile thrombomodulin analogues are suitable to treat patients with an elevated bleeding risk (29). In addition, the combination of anticoagulant and anti-inflammatory properties could be of advantage in the prevention of the post-thrombotic syndrome (30).

Like ART-123, Solulin consists of the extracellular domains of human thrombomodulin. But the structure of Solulin differs from thrombomodulin alpha (ART-123) in a number of specific mutations that have been incorporated for their favourable properties previously demonstrated in other thrombomodulin analogues

(31–33): Deletion of 3 amino- and 7 carboxyterminal amino-acids were generated to improve stability and cellular export of Solulin; furthermore, four single amino acids exchanges were introduced to mediate oxidation resistance (31, 34), enhance stability of Solulin (35, 36) and prevent the attachment of chondroitin sulphate. While leading to the loss of an additional thrombin binding site and thus reduced activity (37), protein stability and homogeneity are enhanced (38) and the binding site for an interfering protein, Major Basic Protein, is disrupted (39). Solulin is supplied as an aqueous liquid colorless, odorless solution with a pH of 7.

Potential benefits of Solulin as an anticoagulant include its long plasma half-life and high thrombin specificity. In preclinical studies, Solulin was shown to be effective in inhibiting venous and arterial thrombosis in different animal models without excessive bleeding complications (40–42).

We report on the first human study of Solulin. The objectives of this phase I study were to evaluate the safety, tolerability, and pharmacokinetics of Solulin in healthy volunteers in response to single and multiple ascending intravenous (i.v.) bolus doses. The pharmacodynamic characteristics of Solulin were also investigated as a secondary endpoint of the study.

Materials and methods

Eligibility criteria

Healthy Caucasian male volunteers aged between 18 and 45 years were eligible for this study. Eligibility was subject to a screening examination including medical history, physical examination, 12-lead electrocardiogram (ECG), vital signs and clinical laboratory profiles. Coagulation parameters (aPTT, PT, TCT, fibrinogen) were to be within the reference ranges established for Grade 1 events in the Common Terminology Criteria for Adverse Events (CTCAE) (Version 3). The study was approved by the local ethics committee of Stichting Beoordeling Biomedisch Onderzoek (Assen, the Netherlands), and all subjects provided written informed consent prior to study entry.

Overall study design

The study was conducted at a single site in the Netherlands. Subjects were randomised into one of seven sequential cohorts, each consisting of eight patients (six active, two placebo). Solulin was administered as an i.v. bolus injection over 1 minute (min). The cohorts were 0.6, 1, 3, 10 and 30 mg single-dose cohorts, and 1 and 10 mg multiple-dose cohorts. This phase I study followed a single-blind, placebo-controlled, dose-escalation design, with subjects being allocated to a cohort using a centrally generated randomisation list. The placebo consisted of 0.9% NaCl solution administered by the investigator in an identical manner to the active treatment.

The first single dose was chosen based on a no observable adverse effect level (NOAEL) of 1.85 mg/kg in monkeys. This value corresponded to a maximum starting dose in humans of 3.9 mg, or 0.06 mg/kg based on a body weight of 65 kg and after applying a safety factor of 10.

Single-dose cohorts

Each single-dose cohort started with a pilot of two subjects who received treatment on the same day (one Solulin and one placebo). After evaluation of safety and tolerability data from the pilot group, six subjects were then randomised in a 5:1 ratio to receive either Solulin or placebo. For those in the 0.6-mg dosing cohort, dosing of each subject was separated by 24 hours (h). Dosing of subjects in all other single-dose cohorts (1, 3, 10 and 30 mg) proceeded at 30 min intervals. No subject received treatment until safety had been evaluated for at least five of the six Solulin-treated subjects in the preceding single-dose cohort.

Multiple-dose cohorts

Subjects in each multiple-dose cohort (1 and 10 mg) received Solulin or placebo once daily for five consecutive days. No pilot group administration was performed; each subject dose was separated by 30 min intervals. Treatment of the 10-mg multiple-dose cohort occurred only after safety of the 1-mg multiple-dose cohort had been evaluated.

Sample collection and processing

Separate blood samples were collected in citrate Vacutainers® (Becton Dickinson, Oxfordshire, UK) for each pharmacodynamic parameter, and for pharmacokinetic analysis.

Single-dose cohorts

Pharmacokinetic samples were collected prior to drug administration, at 5, 10, 15, and 30 min post-dose and at 1, 2, 4, 6, 12, 24, 36, 48, 72, and 96 h post-dose. Pharmacodynamic samples were collected prior to drug administration, at 5 and 30 min post-dose and at 6, 12, 24, and 48 h post-dose.

Multiple-dose cohorts

Pharmacokinetic samples were collected over five consecutive days prior to each treatment dose. In addition a full profile was measured on day 1 and day 5 each, at 5, 10, 15, and 30 min post-dose and at 1, 2, 4, 6, and 12 h post-dose; Additional samples were taken at 120 h, and 144 h post-dose. Pharmacodynamic samples were collected over five consecutive days prior to each treatment dose. In addition a full profile has been measured on day 1 and day 5 each at 5 min, 30 min, 6 h, and 12 h post-dose; Additional samples were taken at 120 h and 144 h post-dose.

As changes in plasma levels were described for coagulation parameters, depending on the storage time and temperature (43), the samples were immediately put on ice and centrifuged at 1,500 g for 15 min at 4°C. The supernatant plasma was extracted and split into two equal portions, transferred into polypropylene tubes and immediately stored below -20°C in an upright position until bioanalytical analysis.

Pharmacokinetic parameters

The pharmacokinetic profile of Solulin was determined through non-compartmental analysis of single-dose parameters (C_{\max} , t_{\max} , AUC_{0-t} , AUC_{0-8} , $t_{1/2}$, V_z). Steady-state characteristics were also assessed using multiple-dose parameters ($C_{\max,SS}$, $C_{\min,SS}$, $t_{\max,SS}$, AUC_{0-t} , AUC_{0-8} , $t_{1/2}$, V_z , and R). Drug concentration measurements were carried out by an enzyme-linked immunosorbent assay (ELISA) at CRS Clinical Research Services Mannheim GmbH (Grünstadt, Germany) using monoclonal mouse anti-solulin antibodies as capture antibody. Biotinylated monoclonal mouse anti-solulin antibodies were used as detecting antibody. Streptavidin combined with alkaline phosphatase was allowed to bind to biotin and with the addition of 4-nitrophenyl phosphatase solution the color reaction was started. The measurement was conducted at a wavelength of 405 nm, using BIO-TEK Instruments, INC, ELX 800 as reader and BIO-TEK Instruments, INC, KC 4 Signature as software. Quantification of Solulin antibodies was performed by an ELISA previously validated by MDS Pharma Services Switzerland AG (Zürich, Switzerland) using polyclonal rabbit anti-solulin antibodies. Solulin was used as capture antigen and anti-solulin antibodies in the samples were allowed to bind to the capture antigen. Biotinylated Solulin then was used as detection antigen and allowed to bind to the captured anti-solulin antigen. Streptavidin-poly-peroxidase was allowed to bind to biotin and with the addition of 3,3',5,5'-tetramethylbenzidine (TMB) solution, the colour reaction was started, absorbance units at 450 nm were read after stopping the reaction with sulfuric acid by the Molecular Devices SPACTRamax 340PC or VERSAmax Plate Reader controlled by SOPTmax® Pro V4.1.7.1. Lower Level of determination (LLOD) was determined to be approx. 2.5 ng/ml.

Pharmacodynamic parameters

Treatment efficacy in this study was determined by the effect of Solulin on the following pharmacodynamic parameters: INR, aPTT, PT, TAT, TCT, aPC/PCI complex, thrombin generation, and *in vitro* bleeding time (using the PFA-100 test [44]). These parameters were selected for an overview of possible alterations in coagulation. aPTT as a standard coagulation parameter is among others dependent on coagulation factors VIII, IX, XI, XII. Solulin binds to thrombin and forms the thrombomodulin-thrombin complex that is able to activate protein C which in turn inhibits factors Va

and VIIIa. aPTT could theoretically be partially influenced by Solulin administration. PT measures factors I, II, V, VII, and X, and therefore could also be altered. TCT was expected to change after Solulin treatment according to the mechanism of action of the study drug. The thrombin generation assay is described as a sensitive assay system for the overall assessment of the coagulation status (45–54).

With the exception of Solulin activity and thrombin generation, all measurements were performed using validated methods. In the absence of an available direct validated method, the plasma concentration of aPC/PCI complexes was used as an indirect measure of activated protein C. This was determined using a protein C-specific chromogenic peptide substrate, and correlated with the amount of Solulin present in the plasma sample. The Calibrated Automated Thrombogram (CAT) assay was used for assessment of thrombin generation in a sample of clotting plasma (52–55). The plasma was triggered with a low concentration of tissue factor (PPP-reagent LOW, Thrombinoscope, Maastricht, Netherlands) in order to have maximal sensitivity to all influences of the coagulation system (56), including thrombomodulin (57). This method involves the use of a low-affinity fluorogenic thrombin substrate and continuous comparison with a simultaneously run calibrator.

Safety and tolerability parameters

Patient safety and tolerability parameters were derived from physical examinations, vital signs, 12-lead ECG, and clinical laboratory profiles including hematology, coagulation, biochemistry and urinalysis.

Statistical methods

Descriptive statistics calculated for pharmacokinetics included frequency, mean, standard deviation, median, minimum, maximum, geometric mean and coefficient of variation (CV). An analysis of variance (ANOVA) was used to assess the effect of Solulin on dose-normalised pharmacokinetic parameters (C_{\max} , AUC_{0-t} and $AUC_{0-\infty}$). Some steady-state data were subjected to exploratory statistical analysis using Dunnett's t-test. Safety and tolerability analyses were descriptive.

No prospective calculation of statistical power was performed. All statistical results and p-values were interpreted in light of the exploratory character of this study. The sample size was selected as typical in phase I studies to provide data on safety, tolerability, pharmacokinetics and pharmacodynamics. All statistical analyses and reporting were performed using SAS® Version 9.1.3 (Cary, NC, USA). Analysis of pharmacokinetic parameters was performed using WinNonlin® Version 4.1 (Pharsight, St. Louis, MO, USA).

Results

Patient characteristics

The study was conducted between 20 August 2007 and 7 April 2008 at Xendo Drug Development B.V., University Medical Center Groningen, the Netherlands. In the single- and multiple-dose cohorts, 40 of 73 and 16 of 35 subjects screened for eligibility, respectively, were enrolled in the study. All 56 enrolled subjects completed the study and received the planned treatment. Demographic and baseline characteristics are summarised in ► Table 1. All subjects were Caucasian males, and there were no clinically relevant differences between and within the single- and multiple-dose cohorts at baseline.

Pharmacokinetic results for the single-dose cohorts

The pharmacokinetic results of the single-dose cohorts are summarised in ► Table 2. The maximum plasma concentration (C_{\max})

increased with increasing dose (► Fig. 1A), and ranged between 140.7 ng/ml for the 0.6-mg cohort and 5,338 ng/ml for the 30 mg cohort. The highest plasma concentration was observed between 5 and 10 min post-dose, with a calculated t_{\max} between 0.3 and 1.1 h (► Table 2). The area under the curve (AUC) derived from measurements (AUC_{0-t}) was at least 80% of the AUC extrapolated to infinity ($AUC_{0-\infty}$) in all single-dose cohorts, and both the mean AUC_{0-t} and the $AUC_{0-\infty}$ values appeared to increase in a dose-proportional manner (► Fig. 2). The average volume of distribution (V_Z) ranged from 5.0 to 8.8 l, and total clearance (CL) from 0.20 to 0.28 l/h. The mean terminal half-life ($t_{1/2}$) ranged from 14.6 to 30.1 h. The $t_{1/2}$ was only evaluable in two subjects in the 30 mg cohort because Solulin plasma concentrations were higher at 96 h than at 72 h in the remaining four subjects. While there were low subject numbers, and some variability in V_Z and $t_{1/2}$, there was no apparent difference in V_Z , $t_{1/2}$, and CL between the different cohorts, suggesting linear pharmacokinetics.

	Single-dose cohorts						Overall (n = 40)
	Placebo (n = 10)	Solulin					
		0.6 mg (n = 6)	1 mg (n = 6)	3 mg (n = 6)	10 mg (n = 6)	30 mg (n = 6)	
Age (years)							
Median	21.5	22.5	19.5	21.5	23.0	20.5	22.0
Range	18–40	22–44	18–31	20–43	20–33	18–42	18–44
Height (cm)							
Median	185.0	179.0	185.0	185.5	184.5	187.5	185.0
Range	172–193	170–198	171–192	175–189	181–195	177–197	170–198
Weight (kg)							
Median	77.65	75.70	78.85	74.10	80.20	80.30	77.80
Range	63.8–89.9	65.6–108.9	70.8–82.0	64.2–101.0	65.8–99.5	75.2–85.1	63.8–108.9
BMI (kg/m ²)							
Median	23.0	24.0	22.5	21.5	24.0	22.0	23.0
Range	19–26	20–28	21–25	21–28	19–27	21–27	19–28
	Multiple-dose cohorts				Overall (n = 16)		
	Placebo (n = 4)	Solulin					
		1 mg (n = 6)	10 mg (n = 6)				
Age (years)							
Median	26.5	26.0		23.0	25.5		
Range	22–34	21–32		19–38	19–38		
Height (cm)							
Median	183.5	178.5		179.0	180.0		
Range	182–196	174–187		174–180	174–196		
Weight (kg)							
Median	82.50	74.10		74.10	77.95		
Range	80.8–84.8	56.8–91.2		65.3–91.7	56.8–91.7		
BMI (kg/m ²)							
Median	24.5	23.0		24.0	24.0		
Range	22–25	18–27		20–29	18–29		

Table 1: Demographic and baseline characteristics.

Table 2: Pharmacokinetic parameters following single- or multiple-dose exposure to Solulin.

Mean ± SD	Unit	Single-dose cohorts				
		Solulin 0.6 mg (n = 6)	Solulin 1 mg (n = 6)	Solulin 3 mg (n = 6)	Solulin 10 mg (n = 6)	Solulin 30 mg (n = 6)
C_{max}	ng/ml	140.7 ± 26.7	247.9 ± 30.1	768.9 ± 202.9	1,951.9 ± 179.3	5,338.0 ± 623.1
t_{max}	h	0.568 ± 0.789	1.138 ± 1.582	0.612 ± 0.759	0.540 ± 0.744	0.318 ± 0.372
$t_{1/2}$	h	14.59 ± 8.18	25.74 ± 24.06	25.88 ± 10.34	24.81 ± 2.38	30.07 ± 3.80 ^a
AUC_{0-t}	h·ng/ml	2,192.3 ± 990.7	4,625.7 ± 1,639.7	13,998.6 ± 2,498.2	38,236.8 ± 2,292.9	136,499.1 ± 21,955.3
$AUC_{0-∞}$	h·ng/ml	2,556.3 ± 1,194.4	5,664.6 ± 3,092.9	15,079.8 ± 2,600.6	40,598.1 ± 2,531.1	152,074.1 ± 30,634.5 ^a
V_z	l	5.0 ± 1.1	5.9 ± 1.8	7.4 ± 2.6	8.8 ± 1.0	8.8 ± 2.9 ^a
CL	l/h	0.28 ± 0.13	0.21 ± 0.08	0.21 ± 0.05	0.25 ± 0.02	0.20 ± 0.04 ^a
Mean ± SD	Unit	Multiple-dose cohorts				
		Solulin 1 mg (n = 6)		Solulin 10 mg (n = 6)		
$C_{max,SS}$	ng/ml	393.2 ± 88.3		3,207.0 ± 330.2		
$C_{min,SS}$	ng/ml	115.4 ± 30.0		895.9 ± 249.1		
$t_{max,SS}$	h	96.542 ± 0.728		97.097 ± 1.605		
$t_{1/2}$	h	21.20 ± 6.51		24.59 ± 5.82		
AUC_{0-t}	h·ng/ml	5,252.8 ± 1,100.4		41,763.0 ± 8,422.7		
R ^b		1.843 ± 0.375		2.036 ± 0.339		

AUC_{0-t} , area under the plasma concentration-time curve from administration until the last quantifiable sampling point; $AUC_{0-∞}$, AUC extrapolated to infinity; $AUC_{0-τ}$, AUC from administration until time $τ$ (dosing interval); CL, clearance; C_{max} , maximum plasma concentration; $C_{max,SS}$, C_{max} at steady state; $C_{min,SS}$, minimum plasma concentration at steady state; R, accumulation factor; SD, standard deviation; $t_{1/2}$, apparent terminal elimination half-life; t_{max} , time of maximum concentration; $t_{max,SS}$, t_{max} at steady state; V_z , volume of distribution. ^a Estimates are from two subjects. ^b The ratio of AUC_{0-24} from Day 5 to Day 1.

Pharmacokinetic results for the multiple-dose cohorts

The pharmacokinetic results for the multiple-dose cohorts are summarised in ► Table 2. Solulin was not detected in any subject prior to dosing. Steady state was reached after 48 h at both doses, with mean trough concentrations of 115.4 ng/ml for the 1-mg cohort and 895.9 ng/ml for the 10-mg cohort (► Fig. 1B). The mean $t_{1/2}$ of 21.2 h for the 1-mg and 24.6 h for the 10-mg multiple-dose cohorts were comparable to the values observed in the single-dose cohorts. The minimal and maximal concentrations at steady state ($C_{min,SS}$ and $C_{max,SS}$) were 115.4 and 393.2 ng/ml, respectively, for the 1-mg cohort, and were approximately eight-fold higher in the 10-mg cohort. The highest plasma concentrations were observed between 5 and 10 min post-dose, and the measured $t_{max,SS}$ was observed approximately 1 h after the last dose in both the 1-mg and 10-mg cohorts. $AUC_{0-τ}$ [AUC from administration until time $τ$ (dosing interval)] ranged from 3,760 to 6,324 and from 31,020 to 53,435 h·ng/ml after multiple-dose administration of 1 mg and 10 mg, respectively. The accumulation factor (R), which is the ratio of AUC_{0-24} from Day 5 to Day 1, was about 2 for both concentrations.

The pharmacokinetic profile has also been confirmed by measurement of the Solulin activity in the respective samples using a validated assay (based on activation of protein C). These results demonstrate that the measured concentrations refer to soluble thrombomodulin.

Pharmacodynamic results

Solulin administration led to an apparent dose-dependent inhibition of thrombin generation, after both single- and multiple-dose administration. The maximum inhibition in the single-dose cohorts occurred 5 min after Solulin administration, at which time the inhibition of thrombin generation was between 22% (for the 0.6-mg cohort) and 98% (for the 30 mg cohort) (► Fig. 3A). The duration of the inhibitory effect was dose dependent; for Solulin doses between 0.6 and 3 mg the baseline value was approached after 48 h, whereas for Solulin doses of 10 mg and 30 mg the effect lasted for more than 48 h, with the values for thrombin generation still reduced to 55% (30 mg dose) and 22% (10-mg dose) of the baseline values. These effects were consistent with the results of the multiple-dose cohorts (► Fig. 3B). Some accumulation of the pharmacodynamic effect was seen over the 5-day treatment period in the multiple-dose cohorts; in the 10-mg dose cohort, continuous inhibition of thrombin generation by more than 80% was observed over the treatment period.

An effect of Solulin on thrombin clotting time was observed: the thrombin clotting time was prolonged after administration of high Solulin doses in both the single- (10 mg, 30 mg) (► Fig. 4A) and multiple-dose (10 mg) cohorts (► Fig. 4B).

In the single-dose cohorts, a correlation between measured Solulin plasma concentrations and inhibition of endogenous throm-

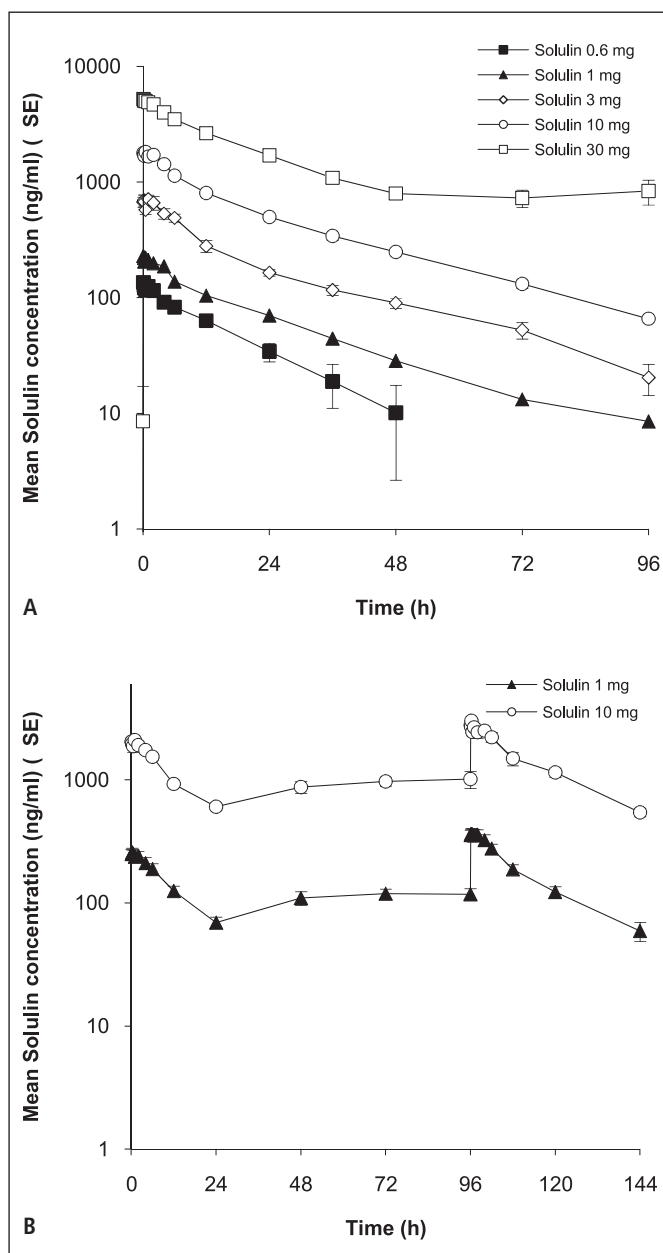


Figure 1: Mean Solulin plasma concentration over time in the single- and multiple-dose cohorts. A) Results obtained for the single-dose cohorts following a single intravenous administration of 0.6, 1, 3, 10 or 30 mg of Solulin. Results are the mean and standard error (SE) for six subjects in each dosing cohort. B) Results obtained for the multiple-dose cohorts following the administration of either 1 or 10 mg of Solulin once daily for five consecutive days. Results are the mean and SE for six subjects in each dosing cohort.

bin potential (ETP) was found (► Fig. 5).

No influence of drug dose on the maximum observed level (E_{max}) was found for prothrombin ratio (international normalized ratio, INR), activated partial thromboplastin time (aPTT), or prothrombin time (PT) in the single-dose cohorts up to a Solulin dose of 10 mg (data not shown). A slight increase in aPTT, to about 10% above the upper limit of normal, was observed in subjects who re-

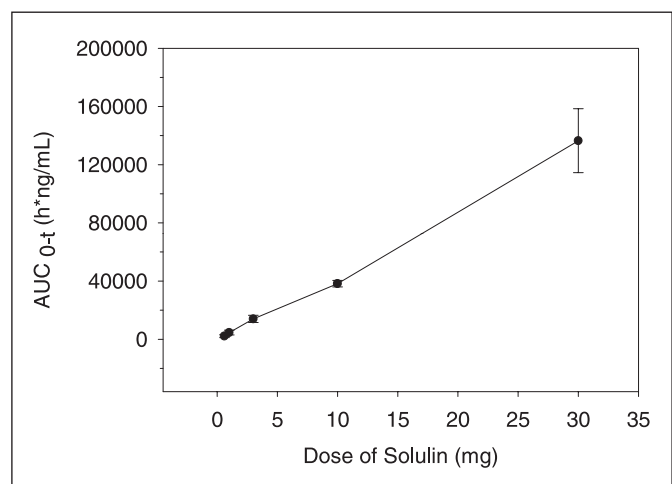


Figure 2: AUC_{0-t}. The area under the plasma concentration-time curve derived from measurements (AUC_{0-t}) appeared to increase in a dose-proportional manner.

ceived 30 mg of Solulin. The results were comparable to those of the multiple-dose cohorts; there was no influence of drug dose on the E_{max} observed for INR and PT, and only a slight increase in aPTT (data not shown).

As an indirect measurement of protein C activation, activated protein C/protein C inhibitor (aPC/PCI) complexes were measured in subjects who received Solulin, and in donor blood samples of 86 healthy volunteers. There was no increase in aPC/PCI complexes in subjects who received Solulin compared to donor samples (data not shown).

No effect of Solulin on thrombin antithrombin III complex (TAT) levels was observed (data not shown). However, the variability of the data was high and the measured TAT levels in several subjects (both pre- and post-dose) were above the reference range of 0.5–5 ng/ml. It is known that traumatic venipuncture, prolonged stasis, or inadequate centrifuging may invalidate the results.

Solulin had no effect on the *in vitro* bleeding time, measured by the platelet function analyzer (PFA)-100 test (data not shown). However, in three subjects from the 10-mg multiple-dose cohort, PFA was prolonged by more than 50%. In one of these subjects, PFA was prolonged to 194% of the baseline value 5 min after the fifth dose and this effect lasted for over 24 h. The other subjects both showed high PFA values once during the observation period.

Safety

In total, 21 subjects experienced at least one treatment-emergent adverse event (AE) during the course of the study: 5/14 subjects (36%) who received placebo, and 16/42 (38%) subjects who received Solulin. There were no serious AEs reported in the study, and all AEs resolved during the trial. All but one AE was mild in intensity; one subject in the 1-mg multiple-dose cohort reported a moderate headache that was not considered treatment related. No bleeding

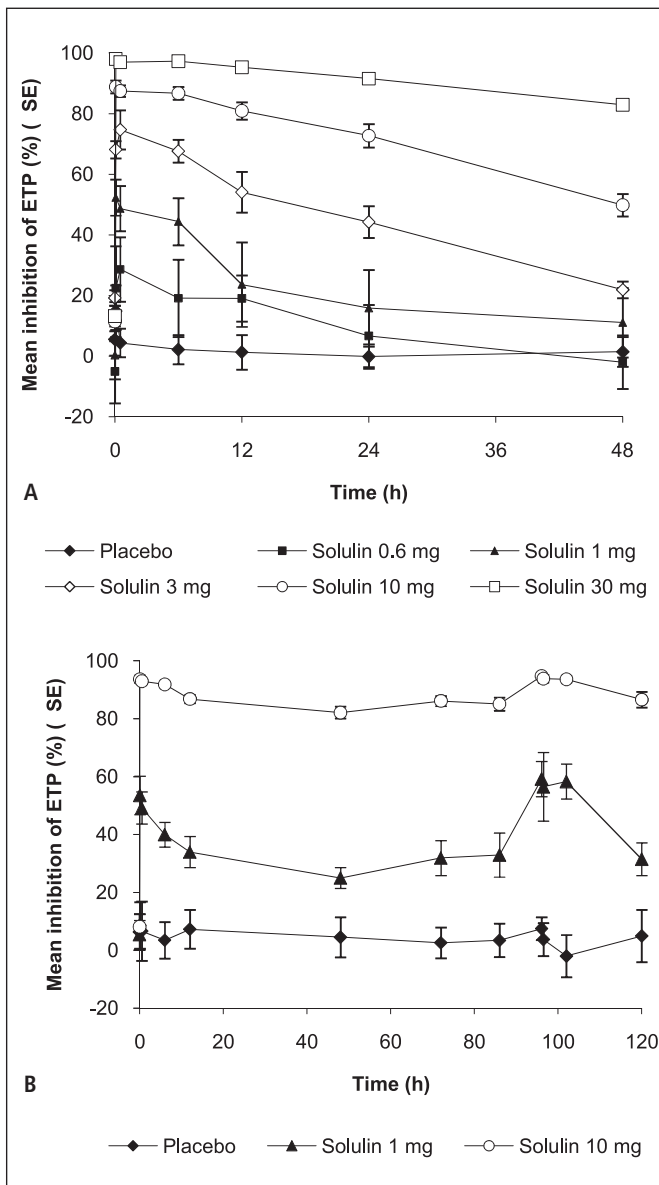


Figure 3: Mean inhibition of thrombin generation. Endogenous thrombin potential (ETP) was assessed using the Calibrated Automated Thrombogram (CAT) assay in plasma samples of healthy subjects. A) The mean inhibition of ETP (%) in the single-dose cohorts was assessed over time. Mean and standard error (SE) values are presented in subjects who received a single intravenous dose of either placebo or Solulin (0.6, 1, 3, 10 or 30 mg). B) The mean inhibition of ETP (%) in the multiple-dose cohorts was assessed over time. Mean and SE values are presented in subjects who received either placebo, or 1 or 10 mg of Solulin once daily over five days.

was observed, also not at the puncture site, and no bruising occurred. No allergic reaction was observed.

There were nine AEs occurring during the trial in the subjects receiving placebo and 37 AEs occurring in the subjects receiving Solulin (22 in the single-dose cohorts; 15 in the multiple-dose cohorts). Two AEs in subjects who received placebo and 10 AEs in subjects who received Solulin were considered by the investigator to be possibly related to treatment. There was no apparent increase

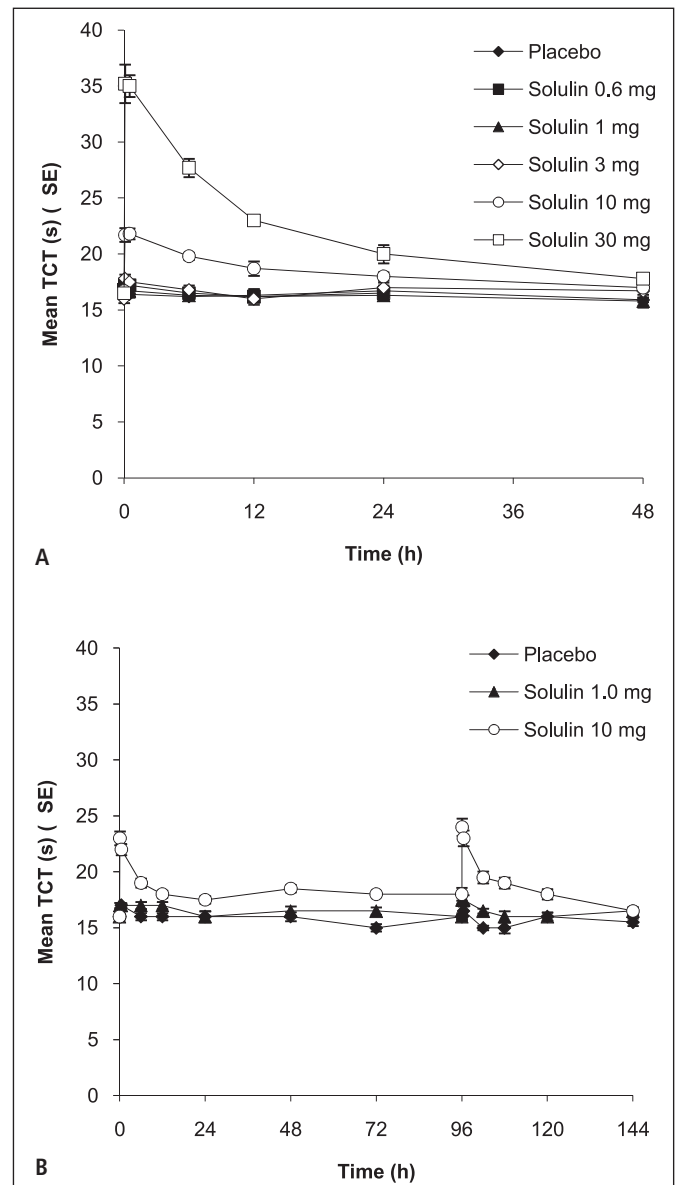


Figure 4: Mean thrombin clotting time. A) The mean thrombin clotting time (TCT) in the single-dose cohorts was assessed over time. Mean and standard error (SE) values are presented in subjects who received a single intravenous dose of either placebo or Solulin (0.6, 1, 3, 10 or 30 mg). B) The mean TCT in the multiple-dose cohorts was assessed over time. Mean and SE values are presented in subjects who received either placebo, or 1 or 10 mg of Solulin once daily over five days.

in the number of AEs with increasing dose.

In the single-dose cohorts, the most commonly reported AEs were headache and nausea (► Table 3), and only five AEs (three cases of headache and two cases of nausea) were assessed as possibly related to treatment, all of which occurred in subjects receiving Solulin. The most commonly reported AEs in the multiple-dose cohorts were catheter site-related reaction, headache, nausea, and somnolence (► Table 3). Seven AEs in subjects receiving multiple doses were considered to be treatment related, two of which oc-

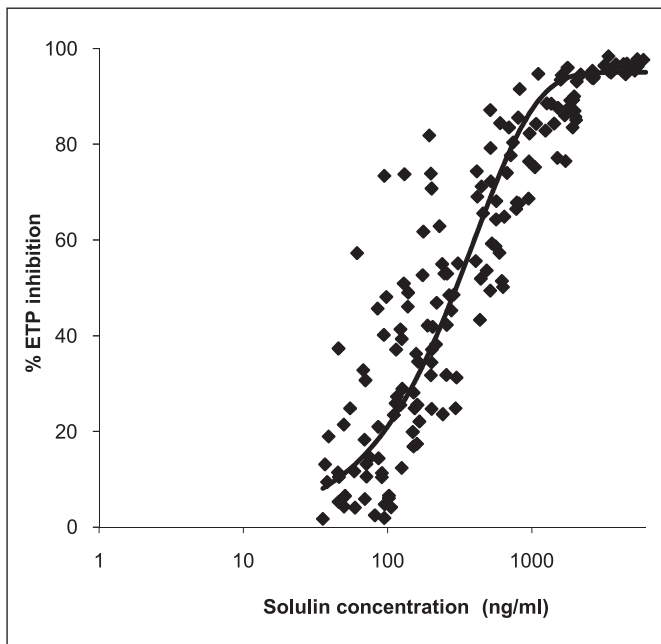


Figure 5: Endogenous thrombin potential (ETP). Correlation between Solulin plasma concentration and endogenous thrombin potential.

curred in subjects receiving placebo. Most of the AEs in the multiple-dose cohorts occurred on Day 1.

Solulin had no clinically significant influence on vital signs, ECG, or clinical chemistry and haematology parameters, and no formation of anti-Solulin antibodies could be detected up to 42 days after single-dose and up to 46 days after multiple-dose administration (data not shown).

Discussion

Solulin is a soluble glycoprotein analogue of human thrombomodulin. It binds to thrombin and forms the thrombomodulin-thrombin complex that is able to activate protein C. Activated protein C inhibits factors Va and VIIIa, which leads to decreased thrombin generation. A direct blockage of thrombin activity requires high Solulin concentrations (1, 31, 58–65). In addition, it exerts anti-inflammatory properties (5, 66–68).

In this first human study of Solulin, the safety, tolerability, pharmacokinetics and pharmacodynamics of single- and multiple-ascending i.v. bolus doses of Solulin in healthy male volunteers was assessed.

Solulin was found to be safe and well tolerated after single (0.6 to 30 mg) and multiple (1 mg or 10 mg once daily over 5 days) i.v. doses. All but one of the AEs that occurred during the study were mild in nature, and there were no serious AEs reported. The most common AEs were headache and nausea. Remarkable differences in the frequency of reported AEs were observed for nausea, which was reported more frequently in subjects receiving Solulin, and catheter/cannula-site reactions, which were reported more fre-

quently in subjects receiving placebo.

Solulin demonstrated linear pharmacokinetics over the clinically relevant dose range, with a plasma elimination half-life of 15 to 30 h, indicating that a less than daily dose is likely required for therapeutic use. Steady-state plasma levels after multiple dosing were reached after 48 h.

Solulin was able to inhibit thrombin generation in a dose-dependent manner; no increase in levels of aPC/PCI complexes was observed in any dose group. This supports the hypothesis that Solulin did not activate protein C in healthy volunteers, which should be expected since it will only be able to act in pathophysiological conditions in which thrombin is generated. There was no influence on the coagulation parameters INR, PT and aPTT after administration of up to a single 10-mg dose of Solulin. A slight increase in aPTT was observed in subjects who received a single Solulin dose of 30 mg, but this finding was not clinically relevant. Thrombin clotting time was prolonged after administration of high Solulin doses (10 mg, 30 mg), with clinical relevance observed after administration of the highest tested dose (30 mg). The effect of Solu-

What is known about this topic?

- Recombinant human thrombomodulin analogues mimic the effects of human thrombomodulin, combining anticoagulant and anti-inflammatory properties. Current anticoagulant treatment options are associated with limitations such as bleeding complications or the need for once-daily subcutaneous injections, and several orally administered anticoagulant medications are in advanced stages of development, addressing these shortcomings.
- Thrombomodulin alpha (ART-123) suppresses thrombin generation in the activated protein C pathway without direct inhibition of thrombin and was found to be significantly superior to heparin in treating disseminated intravascular coagulation, with a markedly lower incidence of bleeding-related adverse events.
- Solulin (sothrombomodulin alpha) is a recombinant thrombomodulin with a long plasma half-life and high thrombin specificity which has shown to be effective in various animal models without excessive bleeding complications.

What does this paper add?

- Data on the safety, tolerability, and pharmacokinetics of single and multiple intravenous bolus doses of Solulin from the first human study of Solulin show good overall tolerability with no evidence of bleeding risk. There were no serious adverse events reported, most frequent adverse events related to Solulin were headache and nausea.
- Solulin demonstrated linear pharmacokinetics, with a plasma elimination half life of 15–30 hours.
- Solulin was able to inhibit thrombin generation in a dose-dependent manner without increasing levels of aPC/PCI complexes, supporting the hypothesis that protein C was not activated in healthy volunteers. Coagulation parameters INR and PT were not changed, aPTT was slightly elevated (not clinically relevant) after the highest single dose only.

Table 3: Safety results in the randomised study population.

n (%)	Subjects with an adverse event							
	Single-dose cohorts				Multiple-dose cohorts			
	Placebo (n = 10)		Solulin (n = 30)		Placebo (n = 4)		Solulin (n = 12)	
	Related	Total	Related	Total	Related	Total	Related	Total
Eye disorders								
Ear discomfort	0	1 (10%)						
Eye pruritis			0	1 (3%)				
Gastrointestinal disorders								
Abdominal discomfort					0	1 (25%)		
Nausea			2 (7%)	3 (10%)			2 (17%)	2 (17%)
Vomiting							1 (8%)	1 (8%)
General disorders and administration site conditions								
Application-site rash			0	1 (3%)				
Catheter site-related reaction	0	1 (10%)	0	1 (3%)	1 (25%)	2 (50%)	0	2 (17%)
Feeling of body temperature change					1 (25%)	1 (25%)		
Infections and infestations								
Nasopharyngitis			0	1 (3%)			0	1 (8%)
Oral herpes			0	1 (3%)				
Musculoskeletal and connective tissue disorders								
Muscular weakness			0	1 (3%)				
Musculoskeletal chest pain							0	1 (8%)
Nervous system disorders								
Disturbance in attention			0	1 (3%)				
Dizziness			0	1 (3%)				
Dizziness, postural							1 (8%)	1 (8%)
Headache			3 (10%)	4 (13%)	1 (25%)	2 (50%)	1 (8%)	2 (17%)
Somnolence								2 (17%)
Psychiatric disorders								
Restlessness			0	1 (3%)				
Respiratory, thoracic and mediastinal disorders								
Epistaxis							0	1 (8%)
Rhinorrhea			0	1 (3%)				
Skin and subcutaneous tissue disorders								
Rash, erythematous			0	1 (3%)				

lin on the intrinsic coagulation system is reflected in the prolongation of the activated partial thromboplastin time rather than the prothrombin time. Solulin had no effect on *in vitro* bleeding time, measured by the PFA-100 test. Furthermore, the pharmacodynamic effects correlated with Solulin plasma concentrations. This demonstrates that the antithrombotic effect of Solulin is predictable, and a necessity for patient monitoring with Solulin treatment is not expected.

Based on these results, it is expected that the clinically effective anticoagulant i.v. dose of Solulin will be between 3 and 10 mg. This dose range is expected to lead to a long-lasting, 60–90% inhibition of thrombin generation without significant influence on aPTT and

INR. Since Solulin needs thrombin before it can exert its activity, it will not influence a parameter related to clotting time because most of the thrombin is formed after the clot appears. The finding that total inhibition of thrombin generation cannot be reached indicates that anticoagulation with Solulin should be safe in the expected therapeutic dose range.

Thrombomodulin acts by a variety of mechanisms to produce anticoagulant and anti-inflammatory effects (2–5). The anticipated benefits of Solulin are based on the assumption that its anticoagulant activity is not associated with relevant alteration of bleeding time, bleeding risk (assessed by aPTT and PT) or other haemorrhagic effects. The pharmacodynamic data obtained from

this study support this hypothesis. These data correspond to the results from studies on thrombomodulin alpha (ART-123) (27, 28). Solulin is expected to only exert its effects when and where endogenous thrombin formation has been activated (such as with an initiated or ongoing clotting process or, if administered post-clotting, at the site of the thrombus).

This first in human study provides supportive data for the anticipated properties of Solulin as an effective and safe anticoagulant, with a pharmacokinetic profile that allows for once daily or less frequent dosing.

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