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Pharmacokinetics and *ex vivo* whole blood clot formation of a new recombinant FVIII (N8) in haemophilia A dogs

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Summary

N8, a new recombinant factor VIII (rFVIII) compound developed for the treatment of haemophilia A, is produced in Chinese hamster ovary (CHO) cells and formulated without human- or animal-derived materials. The aim of the present study was to compare the pharmacokinetics (PK) and the procoagulant effect, measured by *ex vivo* whole blood clot formation, of N8 and a commercial rFVIII in a cross-over study in haemophilia A dogs. N8 and Advate® (100 IU kg^{-1}) were administered intravenously to three haemophilia A dogs. Blood was sampled between 0 and 120 h postdose and FVIII:C analysed. PK parameters maximum plasma concentration, area under the curve, half-life ($t_{½}$), clearance, mean residence time (MRT) and volume of distribution and incremental recovery were calculated. Whole blood clotting time (WBCT) and thromboelastography (TEG®) were used to determine the haemostatic potential. No adverse reactions were observed with N8 or Advate®. N8 and Advate® exhibited similar PK parameters, with $t_{½}$ 7.7–11 h and MRT 11–14 h. Both rFVIII compounds corrected the prolonged WBCT (>48 min) to the range of normal dogs (8–12 min), i.e. N8 to 7.5–10.5 min and Advate® to 7.5–11.5 min. N8 and Advate® also normalized the whole blood clot formation according to TEG®. The native whole blood clotting assays (WBCT, TEG®) appeared to be more sensitive to low concentrations of FVIII than assays in citrated plasma samples. In conclusion, comparison of N8 and Advate® in haemophilia A dogs revealed similar safety, similar PK and similar effects in whole blood clot formation assays.

Keywords

factor VIII; haemophilia A; N8; pharmacodynamics; pharmacokinetics; thromboelastography

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Author contribution

D. M. Karpf analysed the data and wrote the paper. M. Kjalke designed the research study, performed the research, analysed the data and wrote the paper. L. Thim contributed essential reagents tools. H. Agersø analysed the data. E. P. Merricks and N. Defriess performed the research and analysed the data. T. C. Nichols and M. Ezban designed the research study, analysed the data and wrote the paper. All authors have reviewed drafts of the paper and approved the final submitted version.

Disclosures

E. P. Merricks, N. Defriess and T. C. Nichols have no competing interests. D. M. Karpf, M. Kjalke, L. Thim, H. Agersø and M. Ezban are employees of Novo Nordisk A/S. Financial support for the study and for writing support by Sharon Rayner (PAREXEL) was provided by Novo Nordisk A/S.

Introduction

Haemophilia A is the most common severe inherited bleeding disorder, with an estimated incidence of only one in every 5000 live male births [1]. It results from a deficiency or defect in coagulation Factor VIII (FVIII), leading to impaired thrombin generation on activated platelets [2]. The bleeding tendency of haemophilia A correlates well with the level of FVIII activity in plasma and without treatment, patients with severe haemophilia A (FVIII plasma levels <1% of normal) experience frequent spontaneous bleeding episodes [3]. For an optimal outcome, prophylactic treatment is important. In young boys, prophylaxis with recombinant FVIII (rFVIII) concentrate decreases the frequency of haemorrhages and prevents joint damage compared with episodic treatment at the time of haemarthrosis [4]. To improve haemophilia care, treatment for all patients must be available. However, there is disparity in the availability of treatment worldwide, with more than 75% of patients with haemophilia receiving either inadequate or no treatment [5–7].

N8 is a new rFVIII compound developed for the treatment of haemophilia A [8]. N8 is produced in Chinese hamster ovary (CHO) cells and formulated without the use of animal- or human-derived materials, thereby eliminating the risk of transmission of infections. N8 comprises an 88-kDa heavy chain (A1-*a1*-A2-*a2*) followed by a 21 amino acid sequence of the natural B domain (amino acids 741–750 fused with 1638–1648) and a 79-kDa light chain containing the *a3*-A3-C1-C2 domains [8]. Characterization of the N8 protein has confirmed its primary structure, including the presence of all six known tyrosine sulphations of FVIII such as tyrosine sulphation of Y1680 required for binding to von Willebrand factor (VWF) [8]. Detailed functional *in vitro* characterization has shown that N8 is fully active in a variety of assays measuring FVIII activity, with no functional difference with the comparator licensed recombinant or plasma-derived FVIII products, Advate® (Baxter, Vienna, Austria) and Haemate® (CSL Behring, Marburg, Germany) [9]. Binding of N8 to VWF was confirmed, suggesting normal clearance in the presence of VWF. A global Phase III clinical trial programme is assessing the efficacy and safety of N8 in patients with severe haemophilia A. A Phase I pharmacokinetic study in patients with severe haemophilia showed bioequivalence of N8 to Advate(R) [10] and a global Phase III clinical trial programme is currently assessing the efficacy and safety of N8 in patients with severe haemophilia A.

Dogs with congenital haemophilia A and B are used extensively to evaluate the pharmacokinetics (PK) and efficacy of human coagulation factors [11–13]. There are close similarities in gene and protein structure between human and canine FVIII [14]. In addition, several studies have shown similarities between haemophilia dogs and human patients with regard to severe bleeding phenotype, as well as the PK of haemostatic proteins [13]. Thus, haemophilia dog models are established as highly predictive of the efficacy and safety of replacement therapy in humans [13].

The aim of this study was to determine the PK and the *ex vivo* effect on whole blood clot formation of N8 after intravenous (i.v.) administration to haemophilia A dogs. For comparison, Advate® [15] was used in a cross-over design. The ability of N8 and Advate® to induce clot formation in haemophiliaAdogs was evaluated by whole blood clotting time (WBCT) and thromboelastography (TEG®) assays. As it was not feasible to evaluate *in vivo* efficacy in the dogs at multiple time points, the whole blood clot formation parameters were used as surrogate markers for pharmacodynamic (PD) effects.

Materials and methods

Haemophilia A dogs

The study was conducted using three haemophilia A dogs [one male (K18) and two female (M37 and M51)] from the haemophilia dog colony at the Francis Owen Blood Research Laboratory University of North Carolina, Chapel Hill, NC, USA [13]. The dogs had not previously been dosed with human FVIII. The protocol was approved by the Institutional Animal Care and Use Committee at the University of North Carolina as well as by Novo Nordisk's animal ethical committee.

Study design

N8 and Advate® (100 IU kg^{-1}) were administered to the haemophiliaAdogs intravenously. In a cross-over design, dogs K18 andM51received Advate® first followed byN8 and dog M37 received N8 first followed by Advate®. A washout period of 48 h between the two infusions was designed to limit the risk of interference from the previous infusion. The dogs were infused with a bolus dose in a cephalic vein via a 22G Butterfly at 0.7 mL min^{-1}, alternating between the left and right cephalic vein.

Sample collection

Blood was sampled at selected time points between 0 and 120 h by transcutaneous puncture of a cephalic vein using a 21G or comparable Butterfly. Samples were drawn immediately prior to each infusion, and 5, 15 and 30 min, 1, 2, 3, 4, 6, 8, 12, 24 and 32 h after each infusion. Samples were also drawn on day 3 (48, 56 h), day 4 (72, 80 h) and day 5 (96, 120 h) after the second infusion. Blood was stabilized in 0.12 M sodium citrate (10:1), centrifuged and the plasma analysed for FVIII:C in a chromogenic assay [16]. Whole blood was analysed in WBCT and TEG® assays (see below). Samples were also taken immediately before each infusion and 72 h after the second infusion for analysis of the development of FVIII antibodies (Bethesda assay) [17].

Ex vivo whole blood clot formation (pharmacodynamic analysis)

The haemostatic process was recorded by a TEG® coagulation analyser (Haemoscope; Haemoscope Corporation, Chicago, IL, USA) and was initiated with kaolin (lot 1106-0605) as indicated by the manufacturer. Blood was analysed within 2 min of collection. TEG® recordings proceeded for 90 min and the TEG® reaction time (R-time), maximum clot firmness (MA) and angle were determined. WBCT was determined as described [18,19].

Pharmacokinetic analysis

The PK profiles of N8 and Advate® were evaluated by non-compartment analysis (NCA) using PK software WinNonLin on the basis of FVIII:C data. PK parameters analysed were maximum plasma concentration (C_{max}), area under the activity vs. time curve (AUC) extrapolated to infinity, half-life on the terminal part of the curve ($t_{½}$), clearance (Cl), mean residence time (MRT), volume of distribution at steady state (V_{ss}) and incremental recovery [C_{max}/dose, in units (U dL^{-1})/(U kg^{-1})] according to ISTH guidelines 2001. Pharmacokinetic parameters were compared using a paired two-sample Student's *t*-test (Microsoft Excel 2003).

Haematology

Blood samples were analysed for platelet counts, white cell counts and haematocrit at 0 h (before the first infusion), at 30 min, 1, 4 and 24 h after each infusion, and 72 h and 96 h after the second infusion.

Results

Haemophilia A dogs at baseline

Baseline characteristics of the three haemophilia A dogs are shown in Table 1. The haemophilia A dogs displayed prolonged WBCT and R-time by TEG®. If a clot was initiated at all, the clot development (angle) was very low. Consequently, the MA did not reach the normal range within the time of the analysis. Haematology parameters at baseline were within or close to the normal range (Table 1).

Pharmacokinetic analysis

N8 and Advate® showed similar FVIII:C profiles (Fig. 1). Plasma FVIII:C increased at the first sampling time point after the i.v. injection, then declined, exhibiting a biphasic disappearance. The estimated PK parameters for the individual dogs as well as the mean values [with 95% confidence intervals (CI)] are shown in Table 2. N8 and Advate® exhibited similar values for all PK parameters assessed, with no statistically significant differences between the three dogs ($P > 0.05$).

Surrogate pharmacodynamic assays

The prolonged WBCT at baseline normalized at the first time point (5 min) after injection of N8 or Advate®, then slowly increased (Fig. 2). N8 and Advate® both corrected WBCT to the range of normal dogs, i.e. N8 to 7.5–10.5 min and Advate® to 7.5–11.5 min (Table 3).

Examples of TEG® traces for dog M51 before and after Advate® and N8 administration are shown in Fig. 3B, C. Both compounds normalized the TEG® traces at the first sampling time (5 min). The clot time (R-time), clot development (angle) and maximal clot firmness (MA) 5 min after N8 or Advate® infusion (Table 3) were close to those reported in normal dogs (Table 1). In the three dogs, mean R-time stayed markedly shortened for up to 12 h; near-baseline R-times were reached by 72 h (Fig. 4a). These changes in R-time were similar to the changes in WBCT (Fig. 2). Also the angle (Fig. 4b) increased markedly by the first time point after infusion and then remained virtually unchanged for up to 12 h, after which a decrease was observed until the values returned to baseline by 72 h. The sustained normalization of the parameters obtained in the native whole blood clotting assays (WBCT, TEG®) up to 12 h is in contrast to the more rapid decrease of FVIII:C measurements in citrated plasma samples suggesting that the native whole blood clotting assays are more sensitive to the presence of low concentrations of FVIII than FVIII:C analyses.

Safety

There were no safety concerns during the study and both compounds were well tolerated. No significant changes occurred in platelet counts or white cell counts following administration of N8 or Advate® (data not shown). There was a slight decrease in the platelet count (to 149 $\times 10^3$ mm^{-3}) and haematocrit (to 34.2%) following the second infusion in dog K18, probably because of the multiple sampling as the values returned to normal within 48 h. Inhibitors to FVIII were not detected at any time points in the three dogs.

Discussion and conclusion

This cross-over study in haemophilia A dogs demonstrated that N8 and Advate® had similar safety, seen as an absence of adverse reactions. Furthermore, the data suggested similar efficacy for N8 and Advate®, as shown by PK based on FVIII:C measurements and surrogate PD parameters WBCT and TEG® profiles. In this study, N8 and Advate® exhibited a $t_{½}$ of 7.7–11 h and MRT of 11–14 h. The data are consistent with previously published FVIII data in dogs. In a previous cross-over study of haemophilia A dogs,

infusion of either ReFacto® (Genetics Institute, Andover, MA, USA) or Octonativ-M7® (Pharmacia, Stockholm, Sweden) at 125 IU kg^{-1} quickly corrected FVIII:C, WBCT and activated partial thromboplastin time (aPTT) [12]. The values for $t_{½}$ (11.7 and 10.8 h respectively) and MRT (15.9 and 14.3 h respectively) were in the same range as in this study.

Safety assessment in this study showed no adverse reactions with N8 or Advate®. There were no persistent changes in platelet counts, haematocrit or white blood cell count. Furthermore, inhibitors to FVIII were not detected during the study.

It is well established that haemophilia A dogs are predictive of the human condition [13]. Therefore, the PK and surrogate PD data in this study suggest that a comparable haemostatic effect of N8 and Advate® can be expected in patients with haemophilia A. This was confirmed in a very recently reported multinational, open-label, sequential trial of patients with severe haemophilia A, in which the PK profiles of N8 and Advate® were comparable, bioequivalence was demonstrated and there were no safety concerns [10]. The $t_{½}$ of N8 in humans was reported to 10.83 ± 4.95 h, which is similar to the $t_{½}$ in dogs reported here. A limitation of the present study is the small number of animals used. Furthermore, the dog model cannot predict immunogenicity in humans. Despite these limitations, the similar PK findings between haemophilia A dogs and patients support the suitability of the dog model.

In this study, both N8 and Advate® normalized impaired clot formation. The two FVIII compounds had a similar immediate effect and a similar duration of effect. Interestingly, the WBCT and TEG® parameters were normalized for ~12 h whereas a gradual decrease in FVIII:C was observed. The FVIII:C assay was sensitive to ~1% of normal FVIII levels (0.01 IU mL^{-1}), which was reached after 48 h, while the WBCT and R-time from the TEG® analysis did not return to baseline until 96–108 h and 60–72 h respectively. The data suggest that the WBCT and TEG® R-times are sensitive to very low levels of FVIII not measurable by FVIII:C.

In conclusion, based on the absence of adverse reactions and the PK and surrogate PD profiles, N8 has comparable safety and *ex vivo* efficacy to Advate® in haemophilia A dogs. These data predict that N8 will be as effective as Advate® in managing human haemophilia A.

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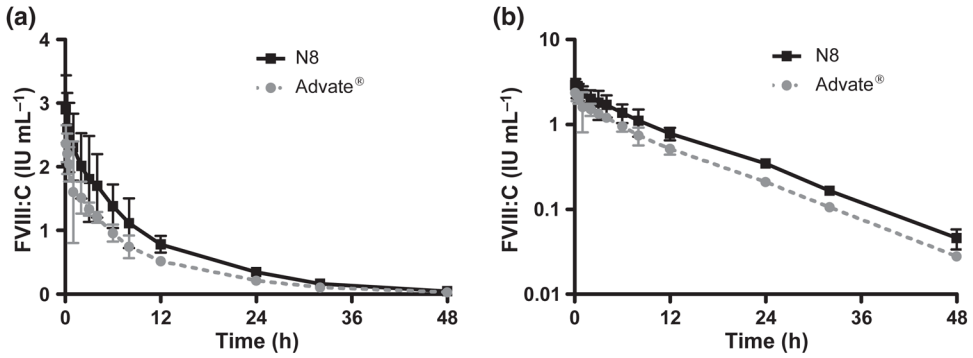

Fig. 1.

Mean (±SD) profile of FVIII:C vs. time on (a) a linear scale and (b) a log scale for three haemophilia A dogs following i.v. administration of N8 and Advate® (100 IU kg^{-1}).

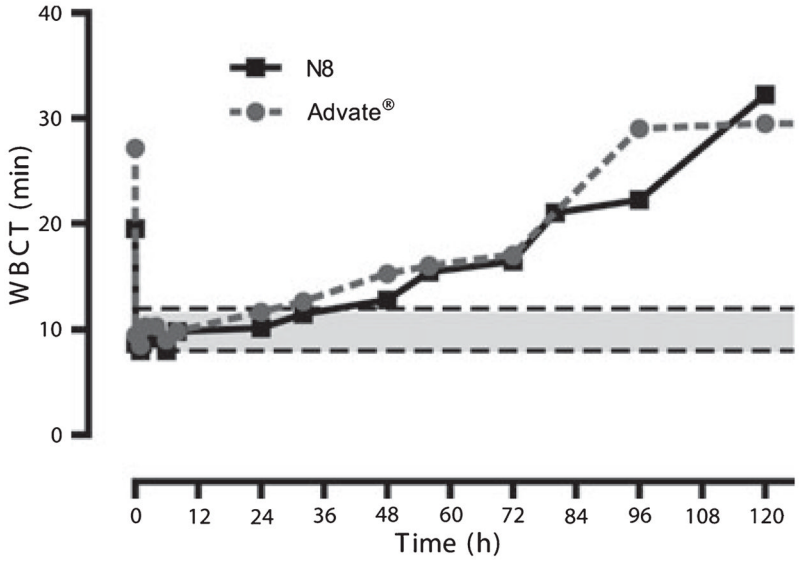

Fig. 2.

Mean profile of WBCT vs. time for three haemophilia A dogs following i.v. administration of N8 and Advate® (100 IU kg^{-1}). The shaded area indicates the normal range.

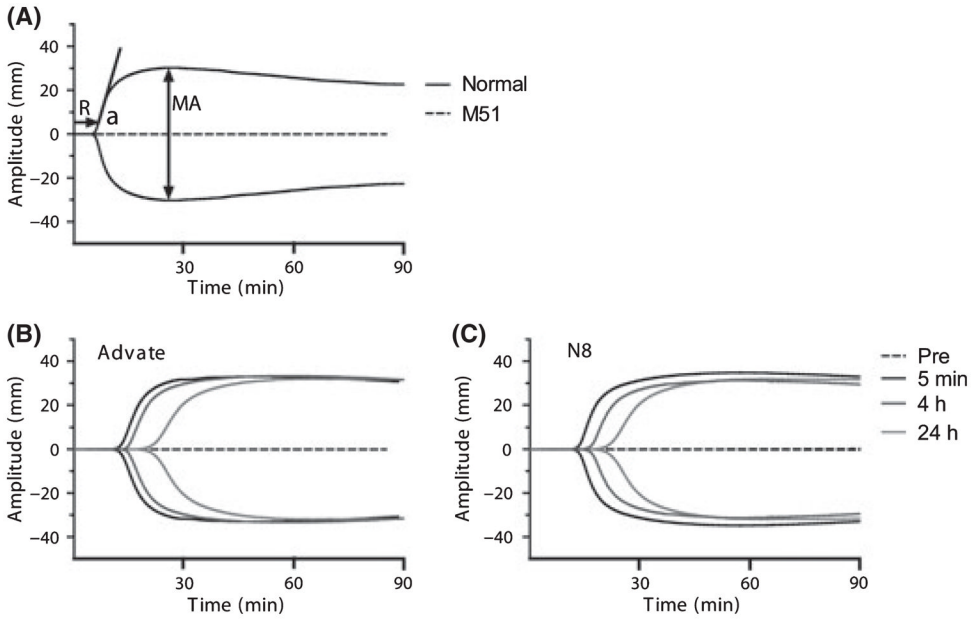

Fig. 3.

Thromboelastography (TEG®) traces of kaolin-initiated coagulation in native whole blood from a haemophilia A dog receiving Advate® and N8. (A) shows a TEG® trace from a normal dog (solid line) and a haemophilia A dog (dotted line) with indications of the TEG® parameters. R-time (R) is the latency time from clot initiation until an amplitude of 2 mm is reached. MA is the maximum amplitude, i.e. the maximum strength of the clot. Angle (a) is measured between R and the point where the amplitude reaches 20 mm and reflects the clot development. (B) and (C) are the TEG® traces for dog M51 before and following i.v. administration of Advate® and N8 (100 IU kg^{-1}).

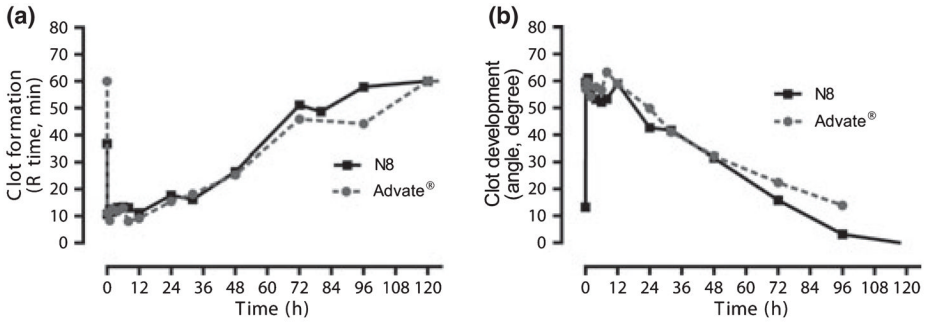

Fig. 4.

Mean profile of TEG® (a) clot formation (R-time) and (b) clot development (angle) vs. time for three haemophilia A dogs following i.v. administration of N8 and Advate® (100 IU kg^{-1}).

Table 1

Baseline characteristics of haemophilia A dogs.

	K18	M37	M51	Normal range
Sex	Male	Female	Female	–
Weight (kg)	19.8	14.9	16.4	–
Treatment (dose 100 IU kg^{-1})	Advate® then N8	N8 then Advate®	Advate®then N8	–
FVIII:C (IU mL^{-1})	<0.02	<0.02	<0.02	0.66–1.2 [20]
WBCT (min)	38.5	28.0	49.0	8–12
TEG®				
R-time (min)	>60	23	>60	6–12
Angle (degree)	0.4	2.3	ND	~68
MA (mm)	2.1	3.6	ND	~65
Haematology				
Platelet count (10^3 mm^{-3})	278	223	192	200–500
WBC (10^3 mm^{-3})	15.2	8.2	8.3	6.0–17.0
Haematocrit (%)	41.4	51.7	49.2	37–55
Haemoglobin (g dL^{-1})	13.8	17.0	16.5	12.0–18.0

ND, not detectable; WBCT, whole blood clotting time; TEG®, thromboelastography; R-time, reaction time; MA, maximum amplitude.

Table 2

Estimates of pharmacokinetic parameters after i.v. administration of 100 IU kg^{-1}N8 or Advate®.

	K18		M37		M51		Mean	
	N8	**Advate®**	**N8**	**Advate®**	**N8**	**Advate®**	**N8**	**Advate®**
C_{max} (IU mL^{-1})	2.3	2.7	3.4	2.2	3.0	2.2	2.9	2.4
AUC (h IU mL^{-1})	23.0	22.0	32.5	19.4	30.8	16.9	28.8	19.4
$t_{½}$ (h)	10.5	8.2	7.7	8.0	8.0	8.3	8.9	8.2
Clearance (mL h^{-1} kg^{-1})	4.3	4.5	3.1	5.2	3.3	5.9	3.6	5.2
MRT (h)	14	11	11	11	11	11	12	11
V_{ss} (mL kg^{-1})	62	47	32	56	36	67	43	56.7
Incremental recovery (U dL^{-1}/U kg^{-1})	2.3	2.7	3.4	2.2	3.0	2.2	2.9	2.4

C_{max}, maximum plasma concentration; AUC, area under the activity vs. time curve; $t_{½}$, half-life on the terminal part of the curve; MRT, mean residence time; V_{SS}, volume of distribution at steady state; C_{max}/dose, incremental recovery.

Table 3

Haemostatic response of haemophilia A dogs to infusions of N8 and Advate®. Values measured at first sampling point, $t = 5$ min.

Assay	N8	Advate®
WBCT (min)	7.5–10.5	7.5–11.5
TEG®		
R-time (min)	9.2–12.9	9.7–12.2
Angle (degree)	57.8–62.6	55.5–58.4
MA (mm)	56.9–67.8	62.0–63.4

WBCT, whole blood clotting time; TEG®, thromboelastography; R-time, reaction time; MA, maximum amplitude.