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Original Research Article

Safety, tolerability and pharmacokinetic profile of recombinant human tissue kallikrein, DM199, after intravenous and subcutaneous administration in healthy volunteers

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ABSTRACT

Background: DM199 is a recombinant form of human tissue kallikrein (KLK1) that is being developed for ischemia-related diseases such as acute ischemic stroke. KLK1 is an important serine protease that promotes vasodilation and microcirculation in multiple tissues. Preclinical stroke studies demonstrate that KLK1 treatment promotes vasodilation, angiogenesis, prevents inflammation, and cerebral cell death.
Methods: The safety and tolerability, as well as the pharmacokinetic profile of DM199 was investigated in a Phase 1B clinical trial following an intravenously (IV) or subcutaneous (SC) administration. Part A was a single ascending IV infusion of DM199 and Part B involved a single IV infusion and single SC dose in two different groups.
Results: For both routes of administration, DM199 was found to be safe and well tolerated at all doses given, albeit the PK profile differed between routes with respect to the time to maximum concentration and plasma half-life. Importantly, DM199 did not influence blood coagulation parameters, suggesting it could be safely used with other stroke treatments like tPA.
Conclusions: Together, these results support the design of future studies to test the efficacy of DM199 in ischemic related diseases including in acute ischemic stroke (AIS).

Keywords: Tissue kallikrein, Safety, Pharmacokinetic, Stroke

INTRODUCTION

Kallikreins are a structurally related family of serine proteases, which have a wide variety of functions in the mammalian system. The kallikrein proteins arise from 15 genes, all found on human chromosome 19, and one prominent variant is referred to as human tissue kallikrein (KLK1). The most well characterized activity of KLK1 is the enzymatic cleavage of low molecular weight kininogen (LMWK) to produce kinins, including bradykinin (BK). Kinins exert their biological effects through the kallikrein-kinin system (KKS) via G-coupled protein BK receptors (BK1R and BK2R). These receptors are located on neurons, endothelial lining, intestines, uterus, heart/aorta and kidney.[1,2] Additionally, both *in vitro* and *in vivo* studies show that KLK1 itself directly activates the BK receptors even in the absence of kinins, suggesting that KLK1 may have additional activity beyond that mediated by BK.[3-5]

The KKS is important for the regulation of blood pressure, inflammation vascular permeability and vasodilation.[6] In fact, kinins are among the most potent vascular endothelium activators and promote blood flow by inducing rapid vasodilation and longer-term angiogenesis.[6,7] Furthermore, KLK1 protects against cerebral cell death injury and down regulates inflammatory mediators in an animal model of stroke.[8,9] For these reasons, KLK1 is a promising therapeutic candidate following cerebral injury, such as acute ischemic stroke (AIS).

Currently, human urinary kallikrein (HUK) is used in The People's Republic of China as a treatment for AIS, however, as a urine-sourced protein it has multiple purity and manufacturing drawbacks. DM199 is a recombinant form of endogenous human KLK1 and is being developed as a drug to treat diseases associated with ischemia, including AIS and diabetic nephropathy (DN). Here we report the outcome of a Phase 1 clinical trial that investigated DM199 using the intravenous (IV) and subcutaneous (SC) routes of administration. The primary objective of this study was to evaluate the safety of a single dose of DM199 following a 30-minute IV infusion or a single SC dose in normal healthy subjects. Additionally, this study characterized the pharmacokinetic (PK) profile generated by each dosing route. The results show that DM199 is safe and well tolerated after both IV and SC administration, and the PK profile after SC dosing is prolonged compared to that observed after IV administration.

METHODS

This was an open label, Phase 1B single center, two-part study in normal healthy subjects. The study period occurred between September 2016 to March 2017. A total of 36 subjects were randomized at a single study center (Linear Clinical Research, Nedlands, Australia). The protocol and details of the inclusion and exclusion criteria can be found at www.clinicaltrials.gov, unique identifier NCT02868996. Novotech (Pyrmount, Australia) was responsible for all patient monitoring, data collection and data analysis. The clinical study protocol was approved by a private IRB (Bellberry Limited) and reported to the Therapeutic Goods Administration (TGA) prior to eligibility screening. Table 1 summarizes the demographics of the study volunteers.

Study design

This study was conducted as two separate parts. Part A was a single ascending dose of IV DM199 given over a 30-minute infusion in a planned four-sequential cohort with a minimum of three and a maximum of six subjects per dose in the event that a possible dose limiting toxicity (DLT) was observed. Data for each dose was reviewed by the safety review committee (SRC), and if no more than one subject experienced a DLT at a given dose levels, enrollment for the next higher dose group commenced. Plans were in place for testing intermediate lower doses in case 2 DLTs were observed at one of the doses. Since no DLTs were observed, subjects received single doses of DM199 at 0.25 µg/kg, 0.50 µg/kg, 0.75 µg/kg and 1.0 µg/kg as originally planned. There were three subjects in each dosing cohort. In Part B, an additional 24 subjects were enrolled and randomized to receive either a single 30-minute IV infusion of DM199 at 0.75 µg/kg (n=12) or a single 3 µg/kg SC dose (n=12) of DM199. Following review of the data in Part A from the SRC, the 0.75 µg/kg dose was selected as the optimal IV dose for Part B. Previous clinical experience with 3 µg/kg, SC provided the safety and PK rationale for using this dose in the SC arm of the study (MTD; manuscript in prep).

Vital signs

Vital signs were taken for all subjects at each dose level throughout the study: at screening, pre-treatment, and Days 1-4, Day 15 and Day 30. Vital sign measurements included a physical examination (at pre-treatment, Day 4, 15 and 30) as well as a 12-lead electrocardiogram (ECG; at pre-treatment, Day 1, 4, and 15). Vital sign measurements also included supine and standing systolic and diastolic blood pressure, pulse, body temperature and respiratory rate.

Adverse event reporting

All subjects who received DM199 were included in the safety and adverse events (AE) analysis. Safety was monitored throughout the study and based on self-reported AEs, clinical laboratory evaluations, vital signs and 12-lead ECGs.

Another component of adverse event analysis was hematology parameters. This included a blood coagulation profile, which measured prothrombin time (PT) and activated partial thromboplastin time (aPTT), both measurements of the time it takes for blood to clot. Additionally, a standardized measure of PT, the International Normalized Ratio (INR) was measured. Hematology labs were taken at screening, on Day -1, at pre-dose and four hours post dose on Day 1, on Day 4 and at follow-up.

Pharmacokinetic assessment

All subjects receiving active DM199 and having measurable plasma concentrations were included in the PK population. Plasma samples for PK analysis were collected a multiple time points before and after DM199 dosing for 72 hours post-dose in Parts A and B. Tetra Q Research (Infrastructure Centre, Brisbane, Queensland, Australia) analyzed all plasma samples and performed data analysis for this study. The plasma concentrations of DM199 were measured using an ELISA-based assay with DM199 specific monoclonal antibody. The fully

validated ELISA had a lower limit of quantification equaling 0.391 ng/ml.

Statistics

Adverse events

Data was summarized by system organ class (SOC) and preferred term (PT). AEs were defined as pre-treatment existing conditions that worsened after study drug administration, or events that occurred during the study or after the administration of the drug. An overall summary of AEs included the number and percent of subjects reporting at least one AE: serious AE, grade 3 AE or higher, AE related to study treatment (possible or probable), and serious AE related to study treatment. In this study, only AEs that the study's medical professional deemed possibly or probably related to study drug are reported.

Pharmacokinetic analysis

Plasma concentrations of DM199 were summarized descriptively by dose level and nominal sampling time. For Part A and B, PK data was only reported if all three subjects in the dosing groups showed measurable DM199 levels at a given time point. Plasma PK parameters were estimated using validated PK software (Phoenix WinNonlin version 6.3) by a standard non-compartmental model. For IV administration, the length of the infusion (30 minutes) was included for the PK parameter estimation. For each dosing route, descriptive statistics were conducted for the PK parameters, including AUC_{0-t} (area under the curve from time 0 (time of dosing) to the last time point measurable), AUC_{0-inf} (AUC from time point 0 to extrapolated to infinity), C_{max} (maximum observed concentration) AUC_{0-24} (AUC from time point 0 to 24 hour post-dose), T_{max} (the first time when C_{max} was observed), $T_{1/2,app}$ (apparent elimination half-life), CL/F (apparent clearance) and V_z/F (apparent total volume of distribution at the terminal phase).

The absolute bioavailability (F) was estimated by Analysis of Variance (ANOVA) model by comparing the logarithm transformed, dose normalized AUC parameters between SC and IV dosing route. The absolute bioavailability was expressed as the ratio of the geometric least-squares means of the formulations (SC/IV) along with its corresponding 90% confidence interval (CI). The ratio and confidence intervals were expressed as a percentage relative to the IV route, and absolute bioavailability analysis was normalized to AUC parameters. For exploratory purposes, the normalized C_{max} parameter was also used to estimate bioavailability.

RESULTS

Adverse events

In Part A, a total of 33 AEs were reported by nine subjects across all four dose cohorts. There were no AEs reported that were considered serious or greater than Grade 2. Six of the subjects reported a mild AE and three reported a moderate AE. Of the reported AEs, four were reported as possibly related to study drug and three were reported as probably related to study drug. The most common organ class was Nervous System Disorder, with seven of the nine subjects reporting an AE from this class. The most common AE was headache followed by erythema at the injection site.

In Part B, a total of 55 AEs were reported by 23 subjects. There were no serious or higher than Grade 2 AEs reported. Out of the 23 subjects, ten reported moderately severe AEs. Both dose cohorts had at least one subject with an AE that was related to study drug. The most common organ class was again Nervous System Disorder with headache and dizziness being the most common.

Table 1: Volunteer demographics.

Treatment	Statistics	Part A	Part B
Sex			
Male	n (%)	4 (33.33%)	12 (50.0%)
Female	n (%)	8 (66.7%)	12 (50.0%)
Age (yr)	mean±SD	26.4±6.87	27.2±6.69
	min-max	19-43	18-45
Height (cm)	mean±SD	172.1±9.22	173.9±8.92
	min-max	161-189	162-192
Weight (kg)	mean±SD	70.84±12.50	72.43±12.69
	min-max	52.7-91.6	52.0-98.1
BMI (kg/m^2)	mean±SD	23.79±2.75	23.80±2.65
	min-max	19.2-27.96	19.37-27.46

Table 1 shows patient demographics in Part A (a) and Part B (b) volunteers. Subject sex, age, height, weight and BMI were all recorded. Each mean, standard deviation (SD), and minimum (min) – maximum (max) range includes both male and female measurements.

Table 2: Study drug related adverse events.

System/Organ	DM199 IV 0.25 µg/kg (n=3)	DM199 IV 0.50 µg/kg (n=3)	DM199 IV 0.75 µg/kg (n=15)	DM199 IV 1.0 µg/kg (n=3)	DM199 SC 3.0 µg/kg (n=12)
All body systems	1	2	7	2	5
Gastrointestinal disorders (Dyspepsia, Vomiting, Diarrhea)	1	0	1	0	0
Nervous system disorders (Dizziness, Dysgeusia, Headache, Presyncope, Paraesthesia, Somnolence)	0	2	6	2	5
Vascular disorder (Orthostatic hypotension)	0	1	0	0	0
General disorders/administration site conditions (Chest discomfort)	0	0	1	0	0

There were no deaths or serious adverse events reported in the study. Table 2 lists all the AEs in each dose cohort related to study drug. Each AEs was grouped together in categories based on physiological system. Numbers represent the absolute number of subjects that reported a specific AE in each category. Part A and B are combined.

Hematology/laboratory assessment

Overall, there were no dose-dependent trends noted in the laboratory assessment including blood coagulation. Furthermore, all coagulation results were within normal reference ranges except for one instance of a prothrombin time reported at 14 seconds for a single subject in the Part A dose cohorts 0.50 µg/kg four hours post dose.

Pharmacokinetics

Part A

After initiation of IV dosing, plasma concentrations peaked rapidly and reached C_{max} at the end of the 30 minutes infusion for all dose cohorts (Figure 1). DM199 plasma concentrations declined exponentially after T_{max} for all dose groups. The duration of plasma concentrations above the BLOQ were greater with higher doses. At the lowest IV dose of 0.25 µg/kg, only one of the 3 participants had plasma concentrations above the BLOQ beyond the 1-hour post dose time point. This patient had plasma concentrations >1.2 ng/ml both pre-dose (time 0) and 72 hours (Day 4) after the IV infusion dose. It is feasible that the DM199 assay also detects native KLK1 present in the sample, and this subject's baseline KLK1 levels were higher than typical. In comparison, the other two participants in this dosing group did not show any quantifiable levels beyond one hour post the infusion. Thus, no IV AUC and other parameters could be calculated from these two participants, and the group mean could not be calculated for this lowest IV dose, except for the C_{max} values as summarized in Table 3. With the baseline adjustment for the C_{max} of the 0.25 µg/kg, the dose-normalized geomean C_{max} ranged from 3.87 to 4.65 for the IV dose from 0.25-1 µg/kg, close to dose-proportional. Similarly, the dose-normalized AUC_{inf} is also roughly dose-proportional for the IV doses from 0.5 to 1 µg/kg (Table 3), with volume of distribution (V_z) and total plasma clearance (CL) in the comparable range. Although the mean AUC_{inf} in the 0.75 µg/kg dose group seems to be higher than dose-proportional, this was not statistically significant due to inter-subject variability.

Table 3: Pharmacokinetic data from part A.

Parameter	DM199 IV 0.25 µg/kg (n=3)	DM199 IV 0.50 µg/kg (n=3)	DM199 IV 0.75 µg/kg (n=3)	DM199 IV 1.0 µg/kg (n=3)
$AUC_{0-\infty}$ (hr*ng/mL)	NR	8.28 (± 6.4)	26.80 (±18.93)	16.56 (±4.02)
C_{max} (ng/ml)	1.67 (±1.44)	1.96 (±0.41)	3.54 (±0.77)	4.19 (±0.72)
T_{max} (hr)	NR	0.50 (±0.00)	0.51 (±0.009)	0.50 (±0.00)
$T_{1/2, app}$ (hr)	NR	3.56 (±2.22)	8.31 (±5.3)	4.07 (±0.73)
V_z/F (mL/kg)	NR	23131.02 (±2896.49)	24729.82 (±6216.31)	25915.14 (±4904.37)
CL/F (mL/h/kg)	NR	6069.20 (±3945.50)	2586.28 (±1602.00)	4434.92 (±0.693.52)

AUC=the area under the plasma concentration curve until the terminal phase, Cmax=maximal plasma concentration, Tmax=time to Cmax, T1/2, app=apparent half life time, Vz/F=apparent total volume distribution, CL/F=apparent total plasma clearance. NR=not reported (n<3)

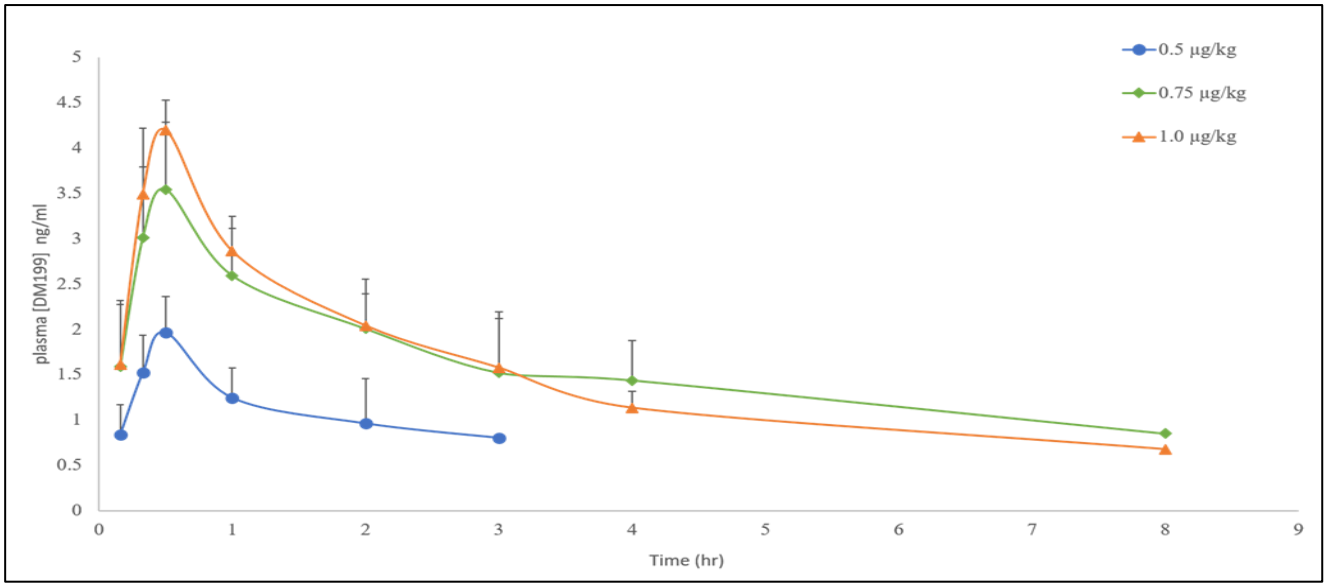

Figure 1: Plasma concentrations in following a 30 minutes IV infusion of DM199.

Observed plasma concentrations of DM199 after a 30-minute IV infusion for four dose levels. The Y-axis represents baseline mean concentrations of DM199 measured in serum (ng/ml) and the X-axis represents time after initial dose measured in hours. Each point represented the mean concentration of DM199 plus the standard deviation. The first three hours of data from dose group of 0.5 µg/kg are graphed here.

Part B

Following IV infusion of 0.75 µg/kg, mean plasma concentrations reached peak around 30 minutes (at the end of infusion). Similar to the IV dose cohorts in Part A, mean plasma concentrations declined rapidly after C_{max} and reached the BLOQ at 24 hours after dosing. Conversely, the 3 µg/kg SC group, DM199 mean concentrations gradually peaked between 8 and 36 hours after dosing. Either elimination or absorption for the SC dose was prolonged compared to the IV dose, with higher mean concentrations of plasma DM199 still observed at 72 hours after dosing (Figure 2). In fact, none of the 12 subjects in the SC study arm reached BLOQ plasma DM199 concentrations by the final time point at 72 hours. It should be noted that a previous human clinical trial has demonstrated the apparent/observed half-life of single SC doses of DM199 is greater than 72 hours (manuscript in prep). All pharmacokinetic parameters are listed in Table 4. In addition to the observed prolonged T_{max} and reduced CL/F than the CL following the IV dose, the mean SC Vz/F was ~3-fold higher than the mean V_z following the IV infusion dose of 0.75 µg/kg.

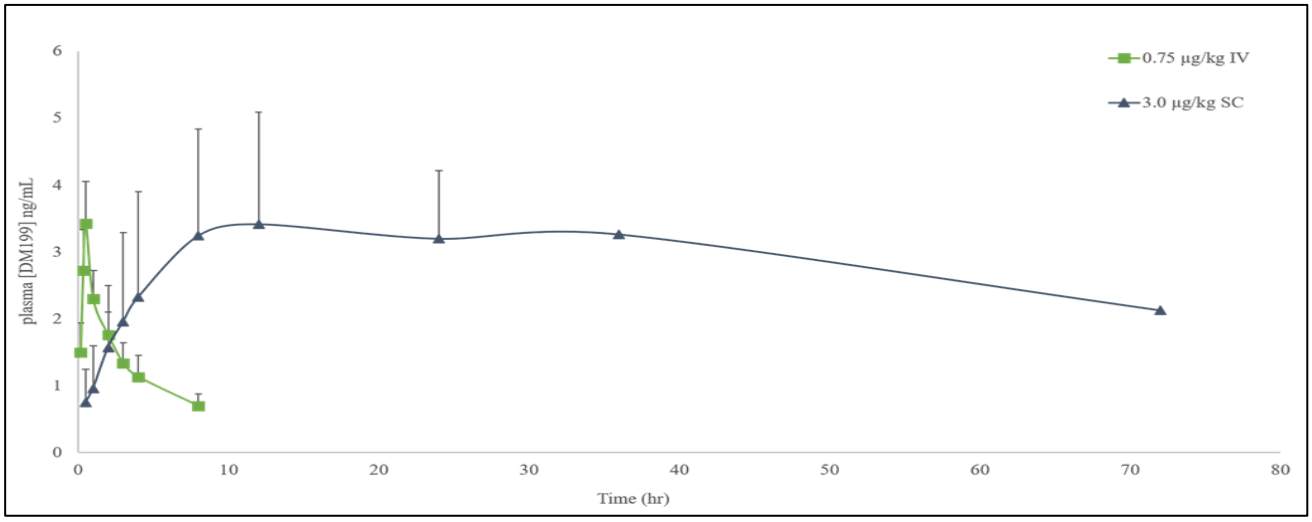

Figure 2: Mean baseline concentrations following IV or SC administration.

Observed plasma concentrations of DM199 after a 30-minute IV infusion or single SC injection. The Y-axis represents baseline adjusted concentrations of DM199 measured in serum (ng/mL) and the X-axis represents time after initial dose measured in hours. Each point represented the mean concentration of DM199 plus the standard deviation.

Table 4: Pharmacokinetic data from part B.

Parameters	0.75 µg/kg IV (n=12)	3.0 µg/kg SC (n=12)
$AUC_{0\text{-}inf}$ (hr*ng/ml)	20.63 (±15.5)	372.87 (±245.17)
C_{max} (ng/ml)	3.44 (±0.62)	3.72 (±1.53)
T_{max} (hr)	0.50 (±0.06)	21.02 (±12.28)
V_z/F (ml/kg)	27329 (±6769.53)	84148.60 (±63184.81)
CL/F (ml/h/kg)	3552.20 (±1637.53)	699.64 (±260.81)

AUC=the area under the plasma concentration extrapolated to infinity, C_{max}=maximal plasma concentration, T_{max}=time to C_{max}, Vz/F=apparent total volume distribution, CL/F=apparent total plasma clearance.

Table 5: Summary of absolute bioavailability (F) for DM199.

Parameter (Dose normalized)	Geometric least square means 3 µg/kg SC (n=12)	Geometric least square means 0.75 µg/kg IV (n=12)	Geometric means ratio (SC vs IV) (90% CI)	P-value
$AUC_{0\text{-}t}$ (hr*ng/ml)	0.863 (0.664, 1.124)	0.227 (0.174, 0.295)	3.806 (2.622, 5.522)	<0.0001
$AUC_{0\text{-}inf}$ (hr*ng/ml)	1.558 (1.030, 2.356)	0.323 (0.241, 0.432)	4.828 (2.908, 8.016)	<0.0001
$AUC_{0\text{-}24h}$ (hr*ng/ml)	0.300 (0.241, 0.373)	0.214 (0.172, 0.267)	1.398 (1.026, 1.907)	0.0767
C_{max} (ng/ml/µg)	0.016 (0.014, 0.019)	0.064 (0.054, 0.075)	0.253 (0.202, 0.317)	<0.0001

ANOVA model: Log (dose Normalized PK parameter) per Dosing-Route. AUC0-inf= the area under the plasma concentration extrapolated to infinity, AUC0-t = Area under the curve to terminal phase, AUC0-24h= area under the curve to 24 hours post dose. Cmax=maximal plasma concentration.

Absolute bioavailability of subcutaneous DM199

The estimated ratio of the least-squares means of the formulations (SC/IV) for dose normalized is listed in Table 5. Interestingly, ratios were above the value of one, particularly for $AUC_{0\text{-}inf}$ and $AUC_{0\text{-}t}$. This is different than what is most often seen, since IV administration typically has 100% bioavailability and SC should be less than 100%, creating a ratio less than one. However, it is interesting to note that the SC bioavailability based on $AUC_{0\text{-}24}$ and C_{max} was ~1 and 0.253, respectively.

DISCUSSION

This is the first published clinical study investigating the safety, tolerability and the PK profile of DM199 administered both IV and SC in healthy subjects. The primary objective of this study was to establish the safety and tolerability of DM199 following a single IV and SC dose. Both routes of administration were found to be safe with no serious AEs and few drug related AEs reported. The other objective of the current study was to establish the PK profile following each route of administration. The two routes differed, specifically in the time peak plasma concentration was reached. IV dosing showed shorter time to reach peak DM199 plasma concentrations compared to SC dosing. Finally, there was no effect on blood coagulation following either dose, suggesting DM199 can be used with other drugs that may affect blood coagulation and are often prescribed following AIS.

Administration of a single IV infusion of DM199 at four different dose levels was found to be safe and well tolerated, with only a few of the AEs deemed to be related to the drug. A single SC dose of DM199 (3.0 µg/kg) was also found to be safe and well tolerated. The most common AE was headache, and no severe AEs were observed. The AEs following the IV route of administration were similar to the AEs reported following SC dosing. This shows that DM199 is safe and well tolerated at proposed therapeutic doses using different routes of administration. In fact, previous studies investigating the PK profile of human urinary kallikrein (HUK), show that 0.15 PNAU/body dose (approximately 0.75 µg/kg) of HUK increases plasma kallikrein concentrations to approximately 1 ng equivalent of HUK/ml.[10] This is a common dose used in The People's Republic of China for AIS and equivalent to the enzymatic activity of DM199 at 0.75 µg/kg. The C_{max} plasma concentrations reached about 3 ng/mL similar to the C_{max} concentrations seen here following 3 µg/kg SC.[10] Since 0.15 PNAU is the common dose used in China for treatment for AIS, this data suggests the 3 µg/kg SC dose of DM199 would be roughly equivalent to the standard HUK dose.

The peak DM199 plasma levels following IV and SC dosing differed. Mean plasma levels were maintained for a longer period (up to 72 hours) with a gradual increase in mean plasma concentrations (8-36 hours) following SC dosing. In comparison, IV administration reached peak concentration at the end of 30-minutes infusion and decreased exponentially following the end of the infusion time, where the BLOQ level was reached by 24 hours post-dose for all IV dosing cohorts. Interestingly, the absolute bioavailability for the SC dose based on dose normalized AUC parameters was atypical. Commonly,

plasma exposures following SC dosing are less than those of IV dosing, generating bioavailability ratios of less than unity. In the current study, plasma DM199 was eliminated rapidly for the IV infusion route and slower for the SC route, with the dose normalized $AUC_{0\text{-}inf}$ >4-fold higher in the SC dose group. The underlying cause of this phenomenon is not clear at present time. Considering the SC bioavailability ratio based on $AUC_{0\text{-}24}$ is close to 1 and based on C_{max} is only 0.253, the apparent higher bioavailability ratio of DM199 at the SC dose of 3 µg/kg may be related to prolonged SC absorption, and/or slower elimination then that following the lower IV dose at 0.75 µg/kg. However, the dose-dependent increase in the IV infusion dose of 0.5-1 µg/kg is close to be dose-proportional. Alternatively, the V_z/F following the SC dose seems to be 3-fold higher than that of IV V_z, it is possible that the drug distribution is dramatically different following the SC route compared to that following IV thus the elimination rate was reduced. Further evaluation is needed to clarify this hypothesis.

Finally, clinical hematology was specifically evaluated to investigate the effect of treatment on blood coagulation parameters. Interestingly, there were no differences between any dosing groups or administration routes. DM199 did not show any abnormal blood clotting profiles compared to normal ranges. This suggests that DM199 could be administered along with other medications that do influence blood clotting, such as tissue plasminogen activator (tPA).

DM199 is a recombinant form of KLK1 that is being developed to treat ischemic related diseases including AIS. KLK1 generates kinins throughout the body and their receptors (BK1R and BK2R) are localized to neurons, endothelial lining, intestines, uterus, heart/aorta and kidney.[1,2] Kinins and the KKS play an important role in local vasodilation and angiogenesis.[6,7] Other studies confirm kinins play an important role in reducing inflammation and protection from cell death as well.[8-9] In this way, DM199 is a promising candidate for the treatment of ischemic diseases including AIS. Currently, the primary drug treatment for AIS is a single injection of tPA, which enzymatically breaks up the clot to establish normal blood flow. However, tPA has a limited time window of effectiveness, and treatment with DM199 could have a longer time frame for safely and effectively starting treatment. In China, urinary derived KLK1 (Kallikang ®) is used in the treatment of stroke. Clinical studies in China not only show a therapeutic effect of urinary KLK1 following stroke but also demonstrate that treatment can be administered up to 48 hours post incident without compromising efficacy.[11,12] Again, due to the vasodilatory, anti-inflammatory, and cellular protection actions of KLK1, DM199 is a promising therapeutic in AIS.[6,8-9] Mechanistically, KLK1 treatment has been shown to upregulate endothelial nitric oxide synthase (eNOS) mRNA and protein to improve blood flow, and activation of BK2R leads to transactivation of vascular endothelial growth factor (VEGF) receptor and release of VEGF promoting angiogenesis and neurogenesis.[13-15] The therapeutic mechanism of action following KLK1 treatment in AIS, the increased therapeutic time window, along with no apparent effect on the coagulation time in this study all support the use of DM199 following AIS in most patients, including those who have already received tPA.[12]

CONCLUSION

The current study shows DM199 1) is safe and well tolerated following a single IV or SC administration, 2) has a PK profile following SC and IV dosing that differs significantly and 3) does not influence blood coagulation measures suggesting it may be safe to use in conjunction with other stroke therapies such as tPA. Further studies will be conducted to confirm the PK profile, test larger cohorts of AIS patients, and firmly establish the safety and efficacy in AIS patients that have been treated with tPA. The safety profile established here (within the proposed therapeutic doses), the PK evaluation, the null effect in hematology parameters, along with the mechanism of action of KLK1, suggest DM199 would be a promising candidate for the treatment in AIS.

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Conflict of interest: Dr. Alexander-Curtis, Dr. Verdoorn, and A. Seeklander are employees of DiaMedica Therapeutics, Inc. Dr. Duan is a consultant hired by DiaMedica Therapeutics, Inc. Dr. Salman, S. Scott, N. Norton, and Dr. Wong are employees of the third-party company hired by DiaMedica Therapeutics to conduct the execution of the study
Ethical approval: All procedures were approved by a private IRB (Bellberry Limited) and reported to the Therapeutic Goods Administration (TGA) prior to eligibility screening

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