Intranasal Heparin Tolerability Study
IND 152357

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Test Article: **Tolerability of topically applied intranasal heparin**

Drug supplier: **National Center for Natural Products Research**

Clinical Phase: **Phase 0 - exploratory**

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1.0 General Information

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2.0 Background Information

2.1 Introduction and Background

The recent emergence of Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) in Wuhan,China in late 2019 and its subsequent spread to the rest of the world has created a pandemic situation unprecedented in modern history [1-4]. SARS-CoV-2 is a betacoronavirus closely related to SARS-CoV; however, significant differences in the Spike glycoprotein (SGP) are present in SARS-CoV-2 that may drive differences in the attachment and entry process. In SARS-CoV, the SGP binds to its cognate receptor, human angiotensin converting enzyme 2 (hACE2). The bound virus is then endocytosed into the cell, where SGP is acted upon by the endosomal protease TMPRSS2 to allow envelope fusion and viral entry.

While ACE2 has been confidently identified as the viral receptor, many viruses (including some betacoronaviruses) will use cellular polysaccharides as cellular attachment coreceptors, allowing the virus to adhere to the surface of the cell and increasing the local concentration of viral particles to increase effective infection rates. Sequence analysis of SGP of SARS-CoV-2 suggest that this virus has evolved to have additional potential glycosaminoglycan (GAG) binding domains compared to SARS-CoV. GAGs are a family of linear sulfated polysaccharides found on the surface of virtually all mammalian cells, and commonly includes chondroitin sulfate (CS) and heparan sulfate (HS). Previous studies using isolated SARS-CoV-2 SGP monomer or trimer and surface plasmon resonance (SPR) have shown that SARS-CoV-2 SGP has high affinity to heparin [5-6], a specialized member of the HS family that is highly sulfated and commonly used clinically as an anti-coagulant drug. It was also reported that heparin was capable of inhibiting infection of SARS-CoV-2 in Vero cell culture in a dose-dependent fashion [6]. These results support a model of SARS-CoV-2 attachment and entry illustrated in **Figure 1**, where SARS-CoV-2 initially binds to HS in the nasal epithelium glycocalyx, where it then binds hACE2 and is endocytosed.

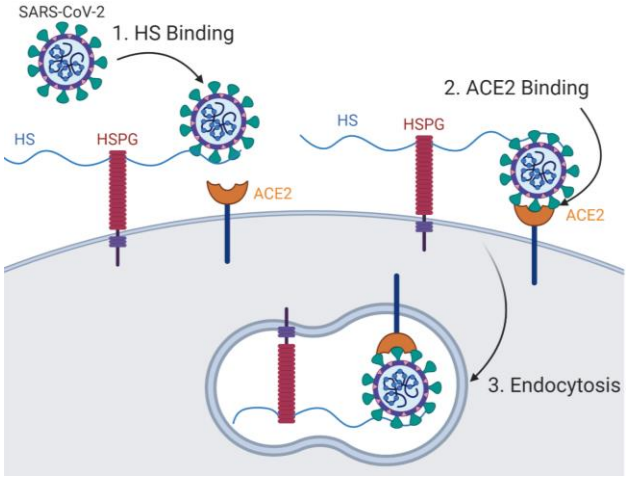


Figure 1: Model of SARS-CoV-2 attachment and entry. Binding of virus to HS in the glycocalyx increases the local concentration of virus, improving binding to hACE2.

Based on this model, disruption of the initial interaction between SARS-CoV-2 SGP and HS could be an effective method for preventing COVID-19 or treating the disease, especially in its earliest stages. Heparin is an FDA-approved drug commonly used via intravenous infusion, intravenous injection or deep subcutaneous injection as an anti-coagulant, where it acts in the blood to disrupt the coagulation cascade. Heparin has numerous side effects, including bleeding and heparin-induced thrombocytopenia, all of which also occur due to heparin activity in the blood. Moreover, heparin is used in COVID-19 treatment where evidence of microclotting (such as high D-dimer levels) is present. Because of this, use of heparin as an anti-viral through a route of administration that allows for significant distribution to the blood carries risks, especially for projected use outside of a closely-observed clinical setting.

Previous studies have examined large doses of heparin via an inhalation route in both humans [7-8] and rodents [9] indicate that even very large doses of heparin administered via inhalation has very poor serum bioavailability, and no reported toxicity. These results suggest that COVID-19 treatment by heparin via an intranasal or inhalation route could avoid dangerous side effects or complications with anti-coagulation treatments while potentially still providing a prophylactic or therapeutic benefit. Heparin's inability to affect blood coagulation when administered intranasally is due to the molecule's large molecular weight which precludes its active and passive transport across the epithelial barrier of the nasal passages. Recent published reports indicate that the nasal epithelium is a probable major portal for initial infection and transmission based on viral loads in both symptomatic and asymptomatic patients [10-11], as well as expression patterns of both the hACE2 receptor and the TMPRSS2 protease [12]. This suggests that a self-administered nasal spray of heparin may be a simple, safe and effective prophylactic to lower the rates of SARS-CoV-2 transmission. A previously disclosed patent indicated that heparin formulated in 15% ethanol had only 0.02% systemic bioavailability [13]; this small amount of bioavailability is likely due to the presence of the 15% ethanol. Single intranasal administration of saline heparin in a rat model resulted in no noted toxicity and very poor serum bioavailability [9]. We are aware of no published studies of repeated dosing of intranasal heparin.

2.2 Preliminary Studies

In vitro potency and efficacy. In order to test the potency and efficacy of heparin, we developed an *in vitro* model. We created a pseudotyped third-generation lentiviral system that expresses the SARS-CoV-2 SGP, which drives cell attachment and entry in SARS-CoV-2. Upon attachment and entry into target cells (in this case, HEK293T cells), the pseudotyped system will drive expression of green fluorescent protein (GFP). Therefore, the number of cells that have been infected by the pseudotyped virus can be determined by counting the number of cells that fluoresce. Using this assay, we tested the ability of various sulfated polysaccharides, including heparin, to block infection. The results of this assay for heparin (160 USP/mg) are shown in **Figure 2**.

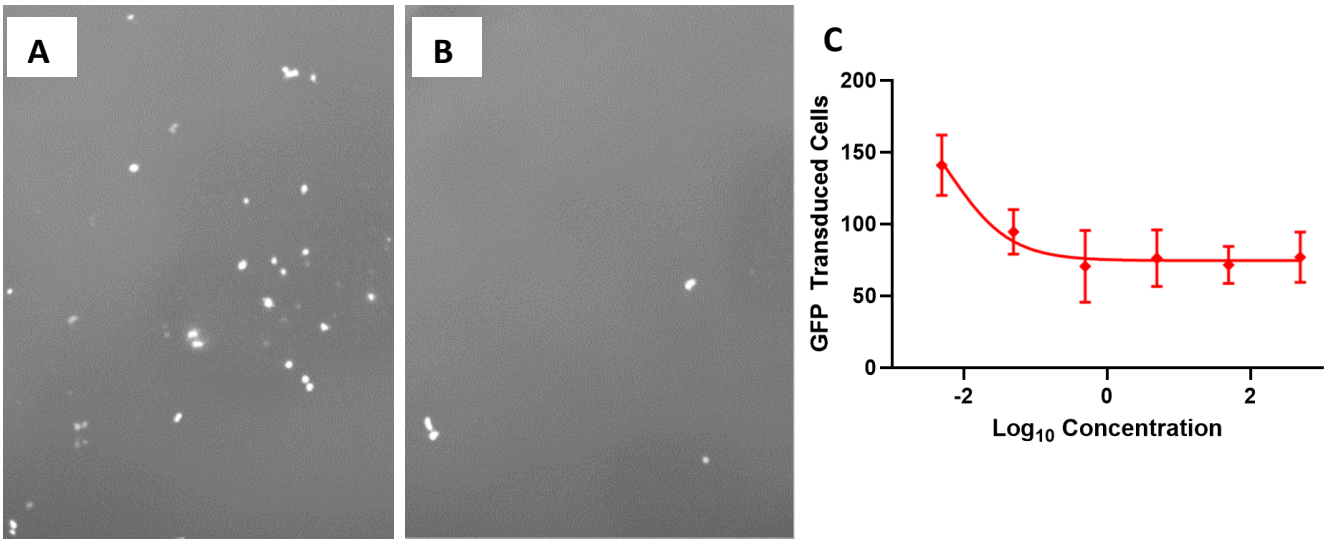


Figure 2. SARS-CoV-2 SGP pseudotyped lentiviral assay for heparin inhibition of viral attachment and entry. A. Representative fluorescent microscopy of inhibition assay results from chemically N-desulfated heparin, which removes the anti-viral activity. Each spot is an infected cell. **B.** Representative fluorescent microscopy of inhibition assay results from heparin. Almost all infection has been stopped. **C.** Dose response curve of heparin for doses from 500 mg/L to 5 µg/L.

Heparin showed high potency, with an IC_{50} of 5.99 µg/L (95% CI: 2.90 – 11.96 µg/L), or 0.96 U/L. Based on the average molecular weight of heparin of 15,000 g/mol, this equals an IC_{50} of ~400 pM. This is 10x higher than the K_D of heparin for SARS-CoV-2 SGP measured by surface plasmon resonance [5].

Mouse toxicology of intranasally administered heparin

In order to test the toxicity of repeated intranasally administered doses of heparin, we carried out a two-week blinded toxicology study in C57BL/6 mice. Mice were administered 12 µL (6 µL/nostril) of a heparin sodium saline formulation identical to the FDA-approved formulation used for deep subcutaneous injection (5 mg/mL sodium chloride, 1% benzyl alcohol, pH 5.0 – 7.5, adjusted using sodium hydroxide and/or hydrochloric acid as needed). Four groups of six mice each, plus one control group of six mice, were used to test heparin. Each group was given 12 µL of heparin daily for two weeks at concentrations of 5000 mg/L, 500 mg/L, 50 mg/L or 5 mg/L, with the control group given the vehicle. Given the average mouse nasal cavity volume of 32 mm^3, this results in a peak nasal cavity concentration of 1875 mg/L, well above the highest dose tested in the *in vitro* pseudotype study. Mice were monitored daily for bleeding, nasal discharge, nose irritation or discoloration, and weight loss. At the end of the two-week study, the samples were unblinded and the mice exposed to the highest dose were tested for anosmia. All mice were euthanized, and a necropsy was performed to identify any gross tissue abnormalities. Blood was used to perform an activated partial thromboplastin time (aPTT) test to look for anticoagulant activity, as well as look for decreases in platelet count to indicate heparin-induced thrombocytopenia (HIT). In no cases were any evidence of toxicity found at any dose tested.

3.0 Study Objectives

This exploratory clinical trial is designed to assess tolerability of increasing doses of intranasally administered heparin sodium in saline solution. Baseline values for aPTT and complete blood count will be obtained from six subjects. Heparin will be administered in increasing concentrations using one 0.1 mL spray per nostril (0.2 ml total) on a daily basis. Major signs of toxicity will be clinically relevant changes in aPTT time, clinically relevant decrease in platelet count, signs of anosmia 30 minutes after administration, or epistaxis. Dosing will start at 1000 units of heparin administered (500 U in each nostril) and then escalated to 2000 units. Signs of toxicity include a clinically relevant aPTT time (> 90s) or decrease in platelet count (below clinical lab normal limit). Tolerance of each dose during the acute phase will be used to determine the dose tested for a 14-day self-administered chronic phase to determine any adverse effects of repeated dosing.

4.0 Study Overview

4.1 Description of Design of Study

This study is a single center, prospective, Phase 0 exploratory tolerability trial. A total of 6 healthy subjects (3 M and 3 F) will be enrolled into the study. This study will evaluate the acute and multi-day (14 days) tolerability of intranasally administered heparin.

5.0 Test Article

5.1 Investigational Product

The study will assess the single and multi-dose tolerability of intranasal administration of an aliquot of an FDA-approved heparin sodium injection (5,000 USP units/mL or 10,000 USP units/mL) to deliver 1000U or 2000U of heparin sodium. Doses will be prepared and administered as follows:

1000 U = (2 x 0.1 mL of 5000U/mL or one spray of 500 U in each nostril)
2000 U = (2 x 0.1 mL of 10000U/mL or one spray of 1000 U in each nostril)

In order to minimize the need for formulation stability testing, we are limiting our studies to formulations of heparin currently FDA-approved for administration by injection. In order to maximize patient compliance for self-administration, we are testing formulations that require only a single 100 µL spray per nostril. Heparin sodium injection is currently available in concentrations up to 10,000 units/mL, or 1,000 units/spray. As the formulation is liquid, we decided that nasal cavity volume was the appropriate measure to determine tolerable concentration. Our initial mouse studies found that

doses up to 337.5 U of heparin per cubic centimeter of nasal cavity volume each day were tolerated for the entire 14 days of the study, based on an average mouse nasal cavity volume of 32 mm^3 [14]. In humans, this corresponds to a dose of up to 7,155 U/day based on an average human nasal cavity volume of 21.2 cm^3 [15], well above the maximum dose for a single spray per nostril at the highest available heparin concentration (2,000 U/day).

Our pharmacokinetic goal was to maintain a concentration of heparin ≥ 0.267 U/cm^3 nasal cavity volume for at least eight hours (preferably twelve hours). *In vivo* imaging studies in a mouse model indicate that between 1% and 4% of the applied 2.67 U dose remained in the nasal cavity after twelve hours. If this elimination model holds in humans, a 1000 U dose (the lower dose to be tested) would results in a concentration between 0.47 and 1.89 U/cm^3 nasal cavity volume after twelve hours, meeting our pharmacokinetic goal. The next lower concentration of heparin currently commercially available (1000 U/mL, or a 200 U dose), would result in a lower range of retained concentration below our target concentration after twelve hours (0.094 to 0.378 U/cm^3). Both the 1000 U dose (47.2 U heparin per cm^3 nasal cavity volume) and the 2000 U dose (94.3 U heparin per cm^3 nasal cavity volume) are well below our highest tested and tolerated dose in mice of 337.5 U heparin per cm^3 nasal cavity volume, and represent the highest two doses that can be achieved from a single 100 µL spray per nostril of currently available FDA-approved heparin sodium formulations.

5.3 Labeling of Investigational Product

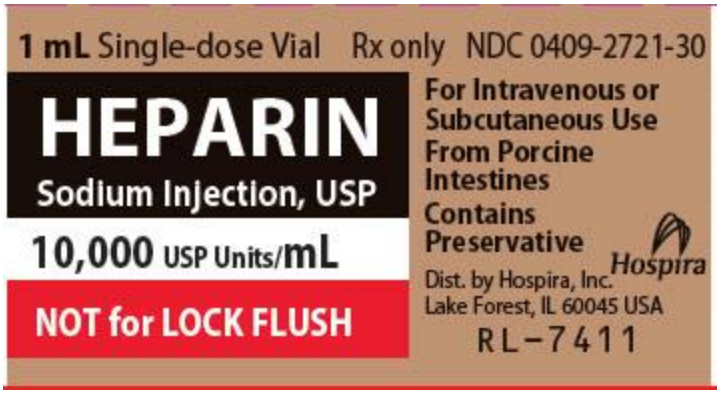
Heparin Sodium (Injection, USP) is a sterile preparation of heparin sodium derived from porcine intestinal mucosa in water for injection as an anticoagulant. The drug preparation is available in various potencies. Potency is determined by a biological assay using a USP reference standard based on units of heparin activity per milligram.

5,000-Units Solution
National Drug Code: 71288-402-10 (71288-402-11 unit of sale)

Each mL contains: 5,000 USP Heparin Units (porcine); 7 mg sodium chloride (to render isotonic); 0.01 mL benzyl alcohol. Hydrochloric acid and/or sodium hydroxide may have been added for pH adjustment (5.0-7.5). Note that 50,000 USP units per 10 mL equals 5,000 per mL.

5,000-Units Heparin Sodium, Injection Solution Label

10,000-Units Solution
National Drug Code: 00409-2721-30
Each mL contains: 10,000 USP Heparin Units (porcine); 5 mg sodium chloride (to render isotonic); 0.01 mL benzyl alcohol. Hydrochloric acid and/or sodium hydroxide may have been added for pH adjustment (5.0-7.5).



10,000-Units Heparin Sodium, Injection Solution Label

Heparin single & daily dose Nasal Sprayers

LOT: xxx-xxx

HEPARIN SODIUM INTRANASAL SOLUTION

Heparin Concentration: **5,000 USP units/mL**

CAUTION: *New Drug—Limited by United States law to investigational use.*

Instill one (1) spray into each nostril every day at _____

For Questions or Concerns: Contact Kerri A. Harrison RN, CCRC 662-915-2103

Please return empty container to the Research Clinic

Store at room temperature.

National Center for Natural Products Research
University, MS 38677

Date_____ Test Subject ID #_____

5,000-units Label

LOT: xxx-xxx

HEPARIN SODIUM INTRANASAL SOLUTION

Heparin Concentration: **10,000 USP units/mL**

CAUTION: *New Drug—Limited by United States law to investigational use.*

Instill one (1) spray into each nostril every day at _____

For Questions or Concerns: Contact Kerri A. Harrison RN, CCRC 662-915-2103

Please return empty container to the Research Clinic

Store at room temperature.

National Center for Natural Products Research
University, MS 38677

Date_____ Test Subject ID #_____

10,000-units Label

5.4 Supplier

The test article (Heparin Sodium USP) will be obtained from UM Pharmacy Health Services.

5.4.1 Preparation of Investigational Product

NCNPR personnel will prepare the investigational product within a Class II Type A2 biosafety cabinet in the clinical laboratory where the study will take place.

The test article will be prepared by transferring (4 mL) of sterile heparin sodium injection (5,000 USP units/mL or 10,000 USP units/mL) at ambient room temperature into intranasal spray bottles, one bottle for each individual subject. The sprayer has been verified to dispense 100 ± 20 µL heparin per spray before delivery of the test article to subjects. The ability of the sprayer to dispense the appropriate 100 µL of heparin per spray has been tested by weighing the bottle before and after dispensing and using the change in mass to determine the amount of heparin dispensed (92± 8 uL/spray).

The test article will be administered within 4 weeks of preparation, and the remainder discarded after study completion.

6.0 Study Population

This study will be conducted in healthy volunteers, that are not currently taking prescription anti-coagulants or blood thinners, not currently taking any nasal medication, and ***that have no symptoms of COVID-19.***

An equal number of males and females will be included in this study. Racial distribution within this protocol is expected to reflect the racial distribution of the campus of the University of Mississippi, residents of the city of Oxford, MS, Lafayette County, MS or the surrounding counties in northeast Mississippi. The student body of the University of Mississippi, the population of Oxford, Lafayette County, and surrounding counties is predominately Caucasian (72%) with a large minority of African-Americans (24%), Asians (2%) and Hispanics (2%). Attempts will be made to recruit subjects of all ethnicities; however, actual racial distribution will be dependent upon personal choice of each subject.

The University currently has more than 23,000 students (undergraduate and graduate) enrolled with faculty and staff totaling almost 1,600. Lafayette County has a population of approximately 50,000. Advertisements, both print and media (email, campus intranet, and social media), will be used to inform and recruit subjects about the study as well as criteria for qualification and enrollment.

6.1 Inclusion criteria:

6.1.1 Normal, healthy adults aged 18 to 65 years

6.2 Exclusion criteria

6.2.1 Allergy to Heparin

6.2.2 Currently taking any prescription blood thinners or anti-coagulants, aspirin, Ibuprophen, or other Non steroidal Anti-Inflammatory medications (NSAIDS), or currently taking any intranasal medication

6.2.3 Subject is a regular/heavy consumer of alcohol (3 or more drinks in a day or >14 drinks in a week for males; 2 or more drinks in a day and >7 drinks/week for females)

6.2.4 Known history of anemia, thrombocytopenia, heparin-induced thrombocytopenia (HIT) or other blood disorder (epistaxis)

6.2.5 Autoimmune disorders

6.2.6 Known history of neurologic/psychiatric disorders

6.2.7 Report of an active infection

6.2.8 Subject is pregnant or breast-feeding, or is expecting to conceive during the study.

6.2.7.1 Subjects of child bearing potential will use (or is currently using) during the study, one of the following acceptable methods of contraception:

- Male Sterilization (vasectomy)
- Female Sterilization (tubal ligation, hysterectomy)
- Intrauterine Device IUD or other Implant
- Oral Contraceptive, Injectable Contraceptive
- Contraceptive Patch/Ring
- Diaphragm

- Male Condom
- Sponge/Spermicide

7.0 Administration of Investigational Product

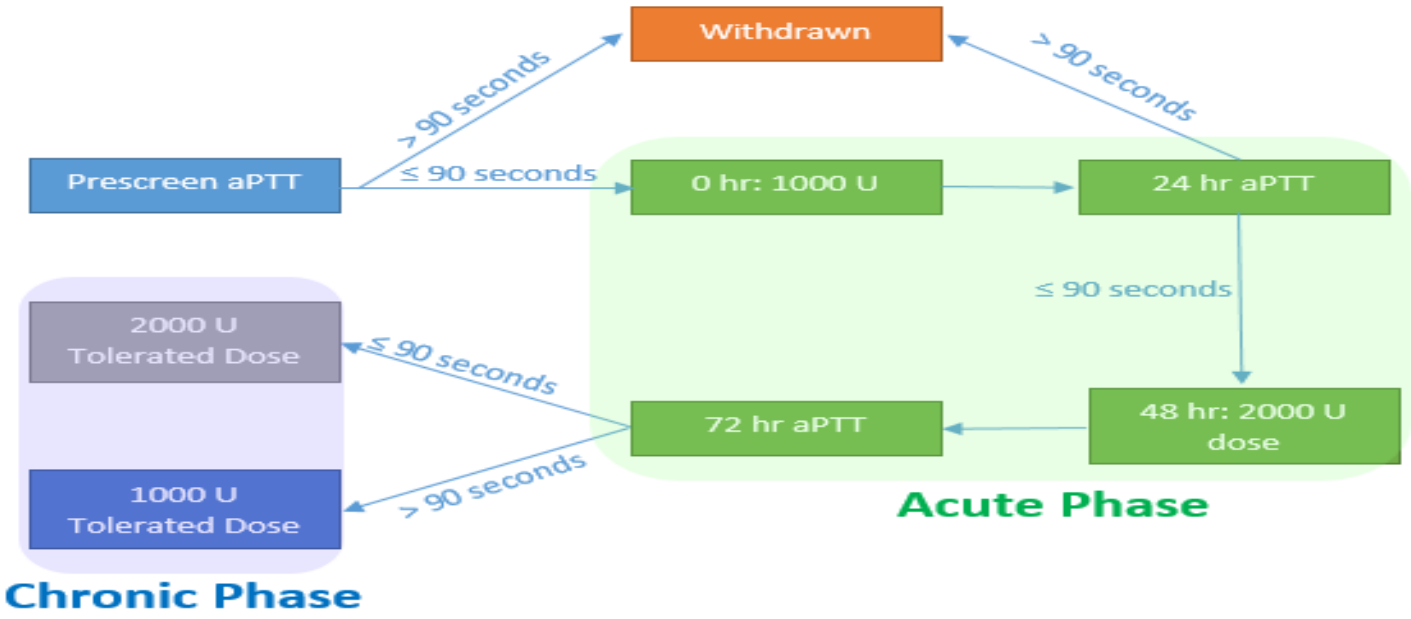
7.1 Administration Regimens

This study is a single center, prospective Phase 0 tolerability proof-of-concept trial of topical intranasal heparin solution. Six (3 females, 3 males), individuals will be enrolled into the study Each test subject (all 6 subjects) will adhere to the following procedures for both acute and chronic dosing

Table 1: Test subject dosing schedule over 5 weeks

WEEK 1		PRESCREENING WORK UP						
TIME COMMITMENT		1.5HR	15MIN	1.5HR	15MIN			
		MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY	SUNDAY
ACUTE PHASE	ACTIVITY	DAY 0	DAY 1 24 HOURS	DAY 2 48 HOURS	DAY 3 72 HOURS	DAY 4 96 HOURS	DAY 5 120 HOURS	DAY 6 144 HOURS
Week 2	PRE-DOSE DRAW	VS	aPTT/VS	VS	aPTT/VS **********	**********	WASHOUT**********	
	DOSE	0.1mL/1000u(500u/nostril)		0.1mL/2000u(1000u/nostril)				
	POST DOSE	1HR OBS.		1HR OBS.		FOLLOW UP CALL		
			1.5HRS					
		MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY	SUNDAY
ACUTE PHASE		Day 7 168 HOURS	DAY 1 CHRONIC PHASE	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6
Week 3	PRE-DOSE DRAW		VS					
	DOSE	*****WASHOUT*******	0.1mL/1000u(500u/nostril) OR 0.1mL/2000u(1000u/nostril)	*0.1mL/1000u(500u/nostril) OR 0.1mL/2000u(1000u/nostril)	*0.1mL/1000u(500u/nostril) OR 0.1mL/2000u(1000u/nostril)	*0.1mL/1000u(500u/nostril) OR 0.1mL/2000u(1000u/nostril)	*0.1mL/1000u(500u/nostril) OR 0.1mL/2000u(1000u/nostril)	*0.1mL/1000u(500u/nostril) OR 0.1mL/2000u(1000u/nostril)
	POST DOSE		1HR OBS.			FOLLOW UP CALL		
		MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY	SUNDAY
CHRONIC PHASE		DAY 7	DAY 8	DAY 9	DAY 10	DAY 11	DAY 12	DAY 13
Week 4	PRE-DOSE DRAW							
	DOSE	*0.1mL/1000u(500u/nostril) OR 0.1mL/2000u(1000u/nostril)	*0.1mL/1000u(500u/nostril) OR 0.1mL/2000u(1000u/nostril)	*0.1mL/1000u(500u/nostril) OR 0.1mL/2000u(1000u/nostril)	*0.1mL/1000u(500u/nostril) OR 0.1mL/2000u(1000u/nostril)	*0.1mL/1000u(500u/nostril) OR 0.1mL/2000u(1000u/nostril)	*0.1mL/1000u(500u/nostril) OR 0.1mL/2000u(1000u/nostril)	*0.1mL/1000u(500u/nostril) OR 0.1mL/2000u(1000u/nostril)
	POST DOSE	FOLLOW UP CALL		FOLLOW UP CALL		FOLLOW UP CALL		
		1.5HRS	1HR					
		MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY	SUNDAY
CHRONIC PHASE		DAY 14	DAY 15	DAY 30				
Week 5	PRE-DOSE DRAW	aPTT/VS	aPTT,PT/INR,CBC/VS/MD EVAL	aPTT,PT/INR,CBC/VS/MD EVAL				
	DOSE	0.1mL/1000u(500u/nostril) OR 0.1mL/2000u(1000u/nostril)						
	POST DOSE	1HR OBS.						
* DENOTES DOSING AT HOME								

Table 2 Dose Calculations base on aPTT results



7.2 Data Collection at Enrollment and Follow-up

For this study, subject data will be collected by paper source documents and transferred to a secure electronic data system (e.g. Excel).

The investigator will ensure the data are recorded on the eCRFs as specified in the study protocol. He will ensure the accuracy, completeness, and timeliness of the data recorded.

8.0 Study Conduct

All recruitment and enrollment procedures will follow approved UM IRB Appendix G COVID-19 Procedures. Initial recruiting interviews will be conducted by phone, email or video. Potential subjects will be asked about COVID-19 exposure before an in-person interview is scheduled. **Those that report COVID-19 like or respiratory symptoms WILL NOT BE SCHEDULED or ENROLLED**

8.1 Procedures

8.1.1 Subject Enrollment and Screening

A. ***Prior to enrollment, the research team will:***

1. Complete COVID screening (subjects will report to clinic with proper face mask, have their temperature take by clinic RN, complete the COVID-19 Related exposure questionnaire, and sign the COVID-19 Compliance, Liability Waiver, and Assumption of the Risk form, see Appendix G)

2. Subjects that complete the above and are asymptomatic and afebrile, will be eligilble for enrollment, as long as they meet other inclusion/exclusion criteria

3. Determine initial eligibility prior to performing any study-specific procedures.

4. Obtain signed informed consent from the subject before any study-specific procedures are performed.

5. Review subject eligibility.

6. Assign the potential subject a unique enrollment number. If a subject withdraws from participation in the study, then his/her enrollment code cannot be reused.

B. Once the consent form is signed and the subject is enrolled into the study, the research team will*:*

1. Collect demographics, medical history and concomitant medications/supplements.

2. Obtain vital signs & complete a physical examination (per study Physician).(see section 9.1)

3. Obtain blood samples (18 mL hematology, aPTT and PT/INR and hCG (females only) for baseline and inclusion/exclusion assessments at least one week prior to dosing. (see section 9.3) aPTT results must be 60 seconds or less to be enrolled.

4. Schedule the dosing day. Test subjects will report to the Research Clinic each day at specified time to receive dose.

5. Once eligibility is conformed, subjects will be instructed to avoid alcohol consumption during the study, as it can compromise study findings.

6. The subject is also instructed to report to the research team having more than 2 drinks a day at any time during the study progress.

8.1.2 Initiation of Investigational Product: Initial Dose/Day 0 (Acute Phase)

Once the eligibility of the subject is confirmed, the research team will:

1. COVID Assessments as outlined in 8.1.1 prior to entering clinic

2. Prior to dosing, obtain a set of vital signs. (see section 9.1) Test subjects will remain in clinic for about an hour post dose for observation.

3. Collect 7 mL of blood for aPTT assessments and platelet count, **(at least one week *prior to dosing).*** For those test subjects with an aPTT result of 60 seconds or above (normal range is 30-40 seconds), will not receive the initial single dose.

4. Obtain a urine sample for hCG on female Test subjects

5. Administer one 0.1 mL spray of heparin nasal solution in each nostril to deliver a total dose of 1000U (500U per nostril/0.1mL per nostril to equal total dose of 0.2mL)

6. Observe test subject for 1-hour post dose for signs of adverse events (e.g. nose bleed)

7. Dismiss test subject with instructions to call with concerns/questions (emergency number will be provided), and to return the following day at designated time for blood draw, assessments

8.1.3 24-Hour Follow Up

1. COVID Assessments as outlined in 8.1.1 prior to entering clinic

2. Obtain a set of vital signs (see section 9.1)

3. Evaluate the test subjects for any adverse event or new concomitant medications

4. Collect 7 mL of blood for aPTT assessment and platelet count

5. Dismiss test subject with instructions to call with concerns/questions (emergency number will be provided), and to return the following day at designated time for dosing

8.1.4 48-Hour Follow Up/Dose Escalation

1. COVID Assessments as outlined in 8.1.1 prior to entering clinic

2. Obtain a set of vital signs (see section 9.1)

3. Evaluate the test subjects for any adverse event or new concomitant medications

4. Obtain a urine sample for hCG on female Test subjects

5. If the test subject's aPTT result is 90 seconds or less (result from 24 hour follow up), administer one 0.1 mL spray of heparin nasal solution in each nostril to deliver a total dose of 2000U (1000U/0.1mL per nostril to equal total dose of 0.2mL)

6. For those test subjects with an aPTT value >90 seconds (normal range is 30-40 seconds), they will not receive the second single dose. (see section 9.0).Those subject will be withdrawn from the study, and have follow up aPTT labs until result returns to baseline

7. Observe test subject for 1-hour post dose

8. Dismiss test subject with instructions to call with concerns/questions (emergency number will be provided), and to return the following day at designated time for blood draw and assessments.

8.1.5 72-Hour Follow Up

1. COVID Assessments as outlined in 8.1.1 prior to entering clinic

2. Obtain a set of vital signs (see section 9.1)

3. Evaluate the test subjects for any adverse event or new concomitant medications

4. Collect 7 mL of blood for aPTT assessment and platelet count

5. Dismiss test subject with instructions to call with concerns/questions (emergency number will be provided). The test subject will return at the designated date and time for initiation of Multi-day dosing , blood draw and assessments

8.1.6 Five Day Wash-Out Period

All subjects will undergo at least a 5 day wash-out period. Washout starts at Day 3/72 hour post dose through Day 7/168 hours post dose in acute phase. *(see scheme 1, in section 7.0)*. Test subjects will return to the research center after that time-period (appointment schedule will be given at 72 hour follow up) .

8.2 Initiation of Multi-day Dose (Chronic Phase)

8.2.1 Dosing Schedule

1. The highest acute dose that has no clinically relevant impact on aPTT (i.e., aPTT ≤90 seconds) will be used for the multi-day phase of this study

2. The test subject will receive one spray of heparin nasal solution into each nostril to deliver a total dose of either 1000U, or 2000U daily for 14 days. ***The administered daily dose (and volume) will depend upon the results of the acute phase. The highest dose in which aPTT ≤ 90 seconds is observed will be used. (Some subjects may only tolerate 1000U, while others may easily tolerate 2000U. Still others may not tolerate 1000U and will be removed from the study. See Table 2) Subjects will receive the dose at which no clinically relevant increase in aPTT (i.e., ≤90 seconds) is observed (see Section 9.0)***

3. The initial dose (Day 0) and last dose (Day 14) will be administered at the NCNPR Clinical Studies Facility. All other doses will be self-administered by subjects at home.

8.2.2 Initial Daily Dose/Day 0

1. COVID Assessments (as outlined in 8.1.1) prior to entering clinic

2. Prior to dosing, obtain a set of vital signs. (see section 9.1) Test subjects will remain in clinic for about an hour post dose for observation.

3. Evaluate the test subjects for any adverse event or new concomitant medications since last dose

4. Obtain a urine sample for hCG on female Test subjects

5. If the test subject's aPTT result is 90 seconds or less for at least one of the two acute doses (result from 72 hour follow up, section 8.1.5), administer heparin nasal solution into each nostril to deliver a total dose of either 1000U or 2000U, per study RN. ***The administered daily dose (and volume) will depend upon the results of the acute phase (i.e. dose yielding aPTT ≤90 seconds) as outlined in 8.2.1.***

6. Observe test subject for 1-hour post dose

7. Schedule subject for 14 day clinic visit. Test subjects will report to the Research Clinic at specified date/time for blood draw and assessments.

8. Test subjects will be given a 14 day dose nasal sprayer, along with "dosing diary" to record time of administration and number of intranasal sprays, during self-administer at home. (instructions will be given to each Test Subject)

9. Instruct subject on proper self-administration of intranasal dose. Subject will administer the dose once daily at the same time each day

10. Dismiss test subject with instructions to call with concerns/questions (emergency number will be provided), and to return in fourteen days (visit 2) at designated time for blood draw & assessments.

11. Test subject will be called every three days for updates and subjective reports (adverse events)

8.2.3 <u>Day 14 Follow Up/Dose 14</u>

1. COVID Assessments (as outlined in 8.1.1) prior to entering clinic

2. Obtain a set of vital signs (see section 9.1)

3. Evaluate the test subjects for any adverse event or new concomitant medications

4. Collect 7mL of blood for aPTT assessment and platelet count

5. Administer one 0.1 mL spray of heparin nasal solution into each nostril to deliver a total dose of either 1000U or 2000U, per study RN. ***The administered daily dose will depend upon the results of the acute phase (i.e., aPTT ≤ 90 seconds) as outlined in 8.2.1.***

6. Observe test subject for 1-hour post dose

7. Dismiss test subject with instructions to call with concerns/questions (emergency number will be provided), and to return in 24 hours (visit 3) at designated time for final blood draw & assessments.

8.2.4 <u>Day 15 Post study Follow up</u>

1. COVID Assessments (as outlined in 8.1.1) prior to entering clinic

2. Obtain a set of vital signs (see section 9.1)

3. Evaluate the test subjects for any adverse events

4. Collect 7mL of blood for aPTT and PT/INR assessment and platelet count.

5. Dismiss subject. Schedule for a 30 day safety assessment.

8.2.5 30 Day Post study Follow up

1. COVID Assessments (as outlined in 8.1.1) prior to entering clinic
2. Obtain a set of vital signs (see section 9.1)
3. Evaluate the test subjects for any adverse events
4. Collect 7mL of blood for aPTT assessment, platelet count, and PT/INR (pre and post study only for baseline evaluations)
5. Dismiss subject. Concludes study

9.0 Safety Assessments

Safety assessments will be completed as part of this single and multi-dose study. Safety will be assessed prior to test article administration and at 24, 48 and 72 hours post dose, in the acute phase. For the chronic phase, safety asssessments will be assessed prior to test article administration and on days 14, 15, and 30. Each subject will be called every three (3) days for updates and subjective reports (adverse events). Subject safety will be assessed by monitoring adverse events, clinical laboratory tests, vital signs, and physical examinations.

Throughout the study laboratory values will be monitored closely by the Investigator and study Physician to detect changes induced by the test article. *Subjects with an aPTT value of 60 seconds or greater (normal range is 30-40 seconds) will not recieve the initial daily dose (one 0.1 mL spray of heparin nasal solution in each nostril to deliver a total dose of 1000U [500U per nostril/0.1mL per nostril to equal total dose of 0.2mL]), OR proceed to dose 2 (one 0.1 mL spray of heparin nasal solution in each nostril to deliver a total dose of 2000U [1000U/0.1mL per nostril to equal total dose of 0.2mL])*, in the acute phase.

In the multi-day, or chronic phase, each individual test subject's dose will be based on the highest acute dose received that produced ***no clinically relevant impact on aPTT*** (i.e., aPTT ≤ 90 seconds). This dose (1000U or 2000U) will be used for the multi-day phase of this study.

9.1 Vital Signs

Vital signs (temperature, respiratory rate, blood pressure, heart rate, SpO2) will be collected prior to the initiation of the test article and with the Safety assessment visits at 24, 48 and 72 hours post dose, in the acute phase. During the chronic phase, vital signs will be assessed prior to test article administration, and on days 14, 15, 30-post per study RN.

9.2 Physical Examination

A physical examination will be performed during the screening period, prior to test article initiation, and post study visit, per Study RN & MD.

9.3 Laboratory Parameters

Laboratory parameters will be evaluated during screening and daily, to include the following:

- Complete Blood Count (WBC, RBC, Hematocrit, Hemoglobin, MCV, Platelet Count)
- aPTT

- PT/INR (baseline and post study assessments)
- Serum hCG baseline assessment only
- Urine hCG prior to each in clinic dose

10.0 Blood Sample Processing

10.1 Activated Partial Thromboplastin Time (aPTT)

Blood samples for aPTT will be collected at baseline, prior to single dose, 24, 48, and 72-hours post dose, and again pre multi-day dose, and days 14, 15 and 30

10.2 Collection and Processing of Blood Samples

For aPTT assessment, 3mL of venous blood will be collected and will be determined per Lab Corp before and at 24, 48, and 72 hours after each heparin administration as well as pre multi-day dosing, and on days 14, 15, and 30.

For Safety Assessments (CBC & Other studies-see section 9.3),4 mls of venous blood will be collected in appropriate vacutainer and stored as per Lab processing sight specifications. These specimens will be sent off site for analysis.

11.0 Adverse/Serious Adverse Events

11.1 Clinical Adverse Events

Clinical Adverse Events will be monitored throughout the study. However, such events are not anticipated during this trial due to the low systemic exposure of the test article being administered. However, local side effects of intranasal heparin, such as epistaxis or nasal congestion may result.

For any abnormal lab values, test subjects will be evaluated for any sign and symptoms, and labs will be redrawn, until stabilized or returned to baseline. Subjects will be referred to the primary physician for any continued care required.

In the event of an Emergency, there will be an onsite RN for the duration of the study, the study Physician will present on the day of dosing. The use of emergency equipment (defibrillator) and 911 would be used as necessary, deemed prudent by study physician and investigators.

Unanticipated problems related to the research involving risks to subjects or others that occur during the course of this study (including SAEs) will be reported to the IRB. AEs that are not serious but that are notable and could involve risks to subjects will be summarized in narrative or other format and submitted to the IRB at the time of continuing review.

11.2 Adverse Event Reporting

AE's will be monitored and recorded in the source document to include the description of the event, onset date, stop date, and outcome. The subjects will be instructed to inform the study personnel or clinical staff of any AEs experienced during the trial. In addition, subject safety will be assessed by monitoring clinical laboratory tests, vital signs and physical examinations. Any subject who has an AE will be evaluated by the investigator and will be treated accordingly. The designated study physician will review each event and assess its relationship to the drug treatment.

11.2.1 Time-Frame for Reporting

Under 21 CFR 312.32(c), the Principal Investigator will notify the FDA within 7 calendar days an event that meets all three definitions of a suspected adverse reaction, serious adverse reaction and unexpected adverse reaction via a secure e-mail account with the FDA. The Principal Investigator will evaluate the available information and decide whether there is a reasonable possibility that the test article causes the adverse event, and therefore, that the event meets the definition of suspected adverse reaction. In addition, the investigator will identify in each IND safety report all IND safety reports previously submitted to the FDA concerning a similar suspected adverse reaction in light of previous, similar reports of any other relevant information. As per 21 CFR part 312, the investigator will conduct ongoing safety evaluations, including a periodic review and analyses of the entire safety database, not only for IND safety reporting purposes, but also to update investigator brochures, protocols, and consent forms with new safety information.

11.2.2 Regulatory Reporting

Reporting of SAEs by the Investigator to the IRB will be done in accordance with the standard operating procedures and policies of the IRB. Adequate documentation will be maintained showing that the IRB was properly notified

11.3 Follow-Up of Adverse Events

Any SAE or AE assessed after the last dose of the test article will be followed until either resolution of the event or determination by the Investigator that the event has become stable or irreversible. Follow-up data concerning reported SAE must also be reported to the FDA.

12.0 Discontinuation and Replacement of Subjects

12.1 Discontinuation

Subject(s) may be discontinued from study at any time if the subject or the investigator is of the opinion that continuing participation in the study is not in the interest of the subject. Subjects on their own may withdraw from study with reason or without providing any reason. If reasons for subject withdrawal from study is known, such reasons shall be documented.

Other reasons why subjects may be withdrawn from study include:

1. Subject withdrawal of consent
2. Subject not adhering to study plan
3. University request for study termination
4. Changes in subject's condition that meets exclusion criteria, e.g. pregnancy
5. If withdrawn from study due to adverse events, subject(s) will be treated by the investigators and monitored till adverse effects are resolved.

12.2 Replacement

Subjects who withdraw from the study may be replaced if so considered by the investigators, by another qualified subject.

13.0 Data Handling and Record Keeping

In compliance with ICH guidelines, the investigator will maintain copies of all source documents that support data collected from each patient and all study documents as specified in ICH Section 8, Essential Documents for the Conduct of a Clinical Trial. The investigator will take measures to prevent accidental or premature destruction of these documents.

All data containing identifiable health information will be collected and stored in a locked cabinet located in the UM SOP Research Center. In addition, all electronic data in the form of Microsoft excel spreadsheet, word documents etc. will be stored in a password protected encrypted computer.

14.0 Institutional Review Board (IRB)/Ethics

The protocol complies with the principles of the Declaration of Helsinki, 1964. In addition, the protocol complies with the laws and regulations of the state of Mississippi.
The collection and processing of personal data from subjects enrolled in this study will be limited to that data which is necessary to investigate the efficacy, safety, quality, and utility of the investigational test article used in this study.

The data will be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations.

Only approval by the Institutional Review Board (IRB), explicit consent for the enrollment in this research study, and processing of personal data will be obtained from the participating subject before collection of data.

The subject has the right to request through the investigator access to his/her personal data and the right to request rectification of any data that is not correct or complete. Reasonable steps will be taken to respond to such a request, taking into consideration the nature of the request, the conditions of the study, and the applicable laws and regulations.

Data will be kept confidential and if published in a scientific journal, will be done without identification of the individual patients.

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