CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

215875Orig1s000

NON-CLINICAL REVIEW(S)

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
Food and Drug Administration
Center for Drug Evaluation
and
Research Office of
Drug Evaluation I
Division of Cardiovascular and Renal Products

Memorandum

Date: January 12, 2023

From: Rama Dwivedi
Pharmacologist

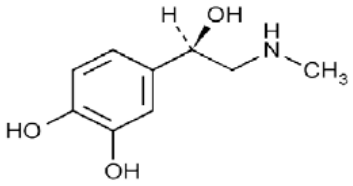
Xuan Chi
Supervisory
Pharmacologist

To:

Application No: NDA-215875 (SN 0001)

Drug Product: Epinephrine in 0.9% Sodium Chloride Injection, 8 mcg/mL, 16 mcg/mL, 20 mcg/mL, 32 mcg/mL, and 40 mcg/mL (also known as NVK019 in nonclinical studies)

Chemical Name(s): 1,2- Benzenediol, 4-[(1R)-1-hydroxy-2-(methylamino)ethyl]-, or (-)-3,4-Dihydroxy-α-[2-(methylamino)ethyl]benzyl alcohol



Molecular Formula: $C_9H_{13}NO_3$
Molecular Weight: 183.2
CAS No: 51-43-4
Indication: Indicated to increase mean arterial blood pressure in adult patients with hypotension associated with septic shock
Subject: *In vitro* Evaluation of the Influence of NVK019 on Platelet Aggregation, Red Blood Cell Hemolysis, Plasma Flocculation in Humans and Local Tolerance
Applicant: Nevakar Injectables, Inc. (Nevakar)

Reference Listed Drug: ADRENALIN® (NDA 204200-Par Pharmaceuticals, Inc)

Background Information
In accordance with 21 CFR§312.54 Federal Food, Drug, and Cosmetic Act, Nevakar Injectables, Inc. (Nevakar), submitted the NDA 215875 (505(b)(2)) for the approval of Epinephrine in 0.9% Sodium Chloride Injection, 8 mcg/mL, 16 mcg/mL, 20mcg/mL, 32 mcg/mL, and 40 mcg/mL to increase mean arterial blood pressure in adult patients with hypotension associated with septic shock. Nevakar's Epinephrine in 0.9%Sodium Chloride Injection is a drug-device combination product presented as a ready-to-use sterile solution in 250 mL IV infusion bags, presented at concentrations of 8 mcg/mL, 16 mcg/mL, 20 mcg/mL, 32 mcg/mL, and 40 mcg/mL.

Nevakar has not conducted any nonclinical or clinical studies with Epinephrine in 0.9% Sodium Chloride Injection and relies on findings of safety and effectiveness for the reference listed drug (RLD), ADRENALIN® (epinephrine injection) 1 mg/mL, approved under NDA 204200 (Par Pharmaceutical, Inc.) as 1 mg/mL single dose vials (NDA 204200) and 30 mg/30 mL (1 mg/mL) multiple dose vials (NDA 204640).

To evaluate the effect of NVK019 on red blood ***cell hemolysis*** and platelet aggregation, Navakar conducted an ***in vitro*** red blood cell hemolysis and plasma flocculation study in human.

In addition, Nevakar also conducted the ***Local Tolerance Study*** via Intravenous (IV) and Paravenous Administration (PV) of NVK019 in New Zealand White Rabbits to determine the local tolerance of NVK019 injection.

Results of the red blood cell hemolysis, platelet aggregation, plasma flocculation in human and local tolerance study in rabbits are reviewed as below.

In Vitro Evaluation of the Influence of NVK019 on Platelet Aggregation, Red Blood Cell Hemolysis and Plasma Flocculation in Humans

Sponsor:	Nevakar Injectables, Inc.
Test Facility Study No.:	6000804
Conducting laboratory and location:	(b) (4)
Date of Initiation:	March 16, 2021
Date of Completion:	March 26, 2021
GLP compliance:	Yes
QA statement:	Yes

Key Study Findings
Whole blood samples were collected, and processed for hemolysis, flocculation, and osmolality study as: Group 1 = no treatment control, Group 2 = hemolysis positive control (20% saponin), Group 3 = flocculation positive control (20% Intralipid®),Group 4 = negative control (0.9% NaCl), Group 5 = reference item/vehicle control (9 mg/mL Sodium Chloride, 0.01 mg/mL Disodium Edetate in Sterile Water for Injection (USP) (pH=4.0) and Groups 6 - 8 = NVK019 at final concentrations in whole blood of 0.014, 0.140, and 1.40 µg/mL.

Based on the results of the present study, there were no hemolysis, no flocculation, no change in plasma osmolality observed with NVK019 (at concentration of 0.014, 0.140, and 1.40 µg/mL) in male and female human blood samples. NVK019 induced increase in platelet

aggregation induced by collagen agonist and dose-related spontaneous platelet aggregation at the concentration of 0.014, 0.14, and 1.4 µg/mL in human Platelet-Rich-Plasma (PRP) samples, which were expected and have been reported previously with epinephrine (Spalding et al. 1998; Solinger et al.1973; Jakobs et al., 1978).

Objective

The objective of this study was to evaluate the effects of NVK019 on hemolysis, flocculation, and platelet aggregation in human blood samples.

Methods

Male and female subjects (non-smokers who did not take non-steroidal anti-inflammatory drugs, prescription drugs, aspirin, or acetaminophen in the last 7 days) were fasted for 4 hours prior to blood draw. Platelet-rich plasma (PRP) was prepared from the whole blood and kept at room temperature until spiked with controls, reference item, or test item, on the day of collection. Blood samples were collected as per protocol below:

Sample Collection

Subjects	
All	X
Time Point:	On days of testing
Fasting:	At least 8 hours
Anticoagulant:	Sodium heparin
Special Requirements:	BD Vacutainer® blood collection tubes
Processing:	Heparinized blood samples for each donor were prepared in glass tubes

Experimental Design and sample Preparation for Hemolytic Assay

Experimental Design

Group No.	**Treatment**	**Final Whole Blood Test Item Concentration (µg/mL)**	**Number of Human Subjects**	
			Male	**Female**
1	Whole Blood Control	0	3	3
2	20 % Saponin (Hemolysis Control)	0	3	3
3	20% Intralipid® (Flocculation/Turbidity Control)	0	3	3
4	Negative Control (0.9% NaCl)	0	3	3
5	Reference Item (Vehicle Control)	0	3	3
6	NVK019 (0.4 µg/mL)	0.014	3	3
7	NVK019 (4 µg/mL)	0.140	3	3
8	NVK019 (40 µg/mL)	1.4	3	3

Control and Samples Preparation

Group No.	**Treatment**	**Test Item Solution (µL)**	**Reference Item (vehicle control) (µL)**	**0.9% NaCl (µL)**	**20% Saponin (µL)**	**20% Intralipid® (µL)**	**Whole Blood (mL)**	**Total Volume (mL)**	**Test Item Final Conc. (µg/mL)**
1	Whole Blood Control	-	-	-	-	-	2.0	2.0	0
2	20% Saponin (Hemolysis Control)	-	-		200	-	1.8	2.0	0
3	20% Intralipid® (Flocculation/Turbidity Control)	-	-	150	-	50	1.8	2.0	0
4	Negative Control (0.9% NaCl)	-	-	70	-	-	1.930	2.0	0
5	Reference Item (Vehicle Control)	-	70	-	-	-	1.930	2.0	0
6	NVK019 (0.40 µg/mL)	70	-	-	-	-	1.930	2.0	0.014
7	NVK019 (4 µg/mL)	70	-	-	-	-	1.930	2.0	0.140
8	NVK019 (40 µg/mL)	70	-	-	-	-	1.930	2.0	1.4

Experimental Design for Optimization Phase

Experimental Design for Optical Method Platelet Aggregation – With Agonist

Group No.	Treatment	Final Std PRP Collagen Concentration (µg/mL)	Number of Human Subjects		Experimental Conditions
			Male	Female	Low Dose Agonist Collagen [a]
1	Negative Control (0.9% NaCl)	0	3	3	Spiked
2	Collagen (250 µg/mL)	25	3	3	Spiked
3	Collagen (300 µg/mL)	30	3	3	Spiked
4	Collagen (350 µg/mL)	35	3	3	Spiked
5	Collagen (200 µg/mL)	20	3	3	Spiked

Std PRP = Standardized Platelet Rich Plasma

[a] Each of the above groups was tested with the individual samples from 3 male and 3 female subjects spiked in duplicate for each experimental condition.

Experimental Design for Platelet Aggregation Assay

Experimental Design for Optical Method Platelet Aggregation – With Agonist

Group No.	Treatment	Final Std PRP Test Item Concentration (µg/mL)	Number of Human Subjects		Experimental Conditions
			Male	Female	Low Dose Agonist Collagen [a, b]
1	Negative Control (0.9% NaCl)	0	3	3	Spiked
2	Reference Item (Vehicle Control)	0	3	3	Spiked
3	NVK019 (0.35 µg/mL)	0.014	3	3	Spiked
4	NVK019 (3.5 µg/mL)	0.140	3	3	Spiked
5	NVK019 (35 µg/mL)	1.4	3	3	Spiked

Std PRP = Standardized Platelet Rich Plasma

[a] Collagen (250 µg/mL) for a final PRP agonist concentration of 25 µg/mL was used as low dose agonist.

[b] Each of the above groups was tested with the individual samples from 3 male and 3 female subjects spiked in duplicate for each experimental condition.

Experimental Design for Optical Method Platelet Aggregation – Without Agonist

Group No.	Treatment	Final Std PRP Test Item Concentration (µg/mL)	Number of Human Subjects		Experimental Conditions
			Male	Female	$CaCl_2$
6	Positive control (Collagen 1.9 mg/mL)[&]	0	3	3	Spiked
7	Negative Control (0.9% NaCl)	0	3	3	Spiked
8	Reference Item (Vehicle Control)	0	3	3	Spiked
9	NVK019 (0.35 µg/mL)	0.014	3	3	Spiked
10	NVK019 (3.5 µg/mL)	0.140	3	3	Spiked
11	NVK019 (35 µg/mL)	1.4	3	3	Spiked

Std PRP = Standardized Platelet Rich Plasma; $CaCl_2$ = Calcium Chloride

The Std PRP samples were recalcified as an event target 5 minutes post start of platelet aggregation monitoring using a stock solution of $CaCl_2$ at 0.125M.

Each of the above groups was tested with the individual samples from 3 male and 3 female subjects spiked in duplicate for each experimental condition.

[&] Collagen (1900 µg/mL) for a final PRP agonist concentration of 190 µg/mL was used as positive control.

Samples were prepared as follows:

Control and Sample Preparation for Optical Platelet Aggregation – PAP-8E (Optimization Phase)

Group No.	Test Material	Std PRP (µL)	NaCl (µL)	Low Dose Agonist (µL)[a]	Final Collagen Conc. (µg/mL)	Total Volume (µL)
1	Negative Control (0.9% NaCl)	215	35	-	0	250
2	Collagen (250 µg/mL)	215	10	25	25	250
3	Collagen (300 µg/mL)	215	10	25	30	250
4	Collagen (350 µg/mL)	215	10	25	35	250
5	Collagen (200 µg/mL)*	215	10	25	20	250

Conc. = concentration; Std PRP = Standardized Platelet Rich Plasma

[a]Each of the above groups was tested with the individual samples from 3 male and 3 female subjects spiked in duplicate for each experimental condition.

*See Appendix 1

Control and Sample Preparation for Optical Platelet Aggregation – PAP-8E – With Agonist

Group No.	Test Material	Std PRP (µL)	Reference Item (µL)	NaCl (µL)	Test Item (µL)	Low Dose Agonist (µL)[a]	Final Conc. (µg/mL)	Total Volume (µL)
1	Negative Control (0.9% NaCl)	215	-	10	-	25	0	250
2	Reference Item (Vehicle Control)	215	10	-	-	25	0	250
3	NVK019 (0.35 µg/mL)	215	-	-	10	25	0.014	250
4	NVK019 (3.5 µg/mL)	215	-	-	10	25	0.140	250
5	NVK019 (35 µg/mL)	215	-	-	10	25	1.4	250

Conc. = concentration; Std PRP = Standardized Platelet Rich Plasma
[a] Collagen (250 µg/mL) for a final PRP agonist concentration of 25 µg/mL was used as low dose agonist.
Each of the above groups was tested with the individual samples from 3 male and 3 female subjects spiked in duplicate for each experimental condition.

Control and Sample Preparation for Optical Platelet Aggregation[a] – PAP-8E – Without Agonist

Group No.	Test Material	Std PRP (µL)	Reference Item (µL)	NaCl (µL)	Test Item (µL)	Collagen 1.9 mg/mL (µL)[&]	$CaCl_2$ (µL)	Final Conc. (µg/mL)	Total Volume (µL)[b]
6	Positive control (Collagen 1.9 mg/mL)	225	-	-	-	25	10	0	250
7	Negative Control (0.9% NaCl)	225	-	25	-	-	10	0	250
8	Reference Item (Vehicle Control)	225	10	15	-	-	10	0	250
9	NVK019 (0.35 µg/mL)	225	-	15	10	-	10	0.014	250
10	NVK019 (3.5µg/mL)	225	-	15	10	-	10	0.140	250
11	NVK019 (35 µg/mL)	225	-	15	10	-	10	1.4	250

Conc. = concentration; Std PRP = Standardized Platelet Rich Plasma; $CaCl_2$ = Calcium Chloride
The PRP samples were recalcified as an event target 5 minutes post start of platelet aggregation monitoring using a stock solution of $CaCl_2$ at 0.125M

[a] Prepared for duplicate assay
[b] Total volume prior to the addition of $CaCl_2$
[&] Collagen (1900 µg/mL) for a final PRP agonist concentration of 190 µg/mL was used as positive control

Results

Effect of NVK019 on Human Red Blood Cell Hemolysis and Plasma Flocculation

The hemolysis and flocculation/turbidity results from three male and three female human whole blood samples after addition of either reference item (vehicle control) or NVK019 are presented below:

The percent hemolysis of each spiked sample (except for Groups 1 and 3) was calculated as follows:

$$\% \text{ Hemolysis} = \frac{(100\text{-hematocrit }\%) \times \text{Plasma Hemoglobin}^{@}\ (g/dL)}{\text{Negative Control Whole Blood Hemoglobin}^{\#}\ (g/dL)}$$

@ Plasma hemoglobin from spiked sample.
# Negative control of the same donor.

The result from the hematology analyzer was used if the hemoglobin level was ≥ 0.2 g/dL; if the result was < 0.2 g/dL, the hemolytic index result, converted to g/dL was used to calculate the % hemolysis level.

Expected Results for Positive Controls (20% Saponin; 20% Intralipid)

Hemolysis Control (20% Saponin)

H^{+++}

Hemoglobin concentration: ≥ 5 g/dL

Hemolytic index: ≥ 5000

% hemolysis: ≥ 80%

Flocculation Control (20% Intralipid®)

L^{+++}

Ratio of net turbidity index of the positive control over the plasma control: > 5

The Test Item was considered to have a significant *in vitro* effect on whole blood if all the following observations were obtained:

Hemolytic effect:

$\geq H^{++}$;

hemoglobin concentration: ≥ 0.5 g/dL;

hemolytic index: ≥ 500;

% hemolysis: ≥ 5%.

Flocculating effect:

The test item was considered to have a flocculation effect if a dose-dependent trend was observed in the difference in net turbidity index for the three tested concentrations.

***In Vitro* Evaluation of the Influence of NVK019 on Human Red Blood Cell Hemolysis**

Males

Group	Parameter	Identification	H1	H2	H3	Mean	SD
4	Whole Blood Hemoglobin (g/dL)	Negative Control - 0.9% NaCl	13.9	14.7	14.7	14.4	0.5
1	Whole Blood Hematocrit (%)	Non-Spiked Whole Blood	42.2	45.0	45.4	44.2	1.7
2		Positive Control - 20% Saponin	0.1	0.0	0.0	0.0	0.1
4		Negative Control - 0.9% NaCl	40.2	42.9	43.4	42.2	1.7
5		Reference Item (Vehicle Control)	41.2	43.1	42.8	42.4	1.0
6		NVK019 - 0.014 µg/mL	41.4	42.4	43.3	42.4	1.0
7		NVK019 - 0.140 µg/mL	40.5	42.1	43.3	42.0	1.4
8		NVK019 - 1.4 µg/mL	40.8	42.2	44.2	42.4	1.7
1	Plasma Hemoglobin (g/dL)	Non-Spiked Whole Blood	0.0	0.0	0.0	0.0	0.0
2		Positive Control - 20% Saponin	13.4	14.1	13.9	13.8	0.4
4		Negative Control - 0.9% NaCl	0.0	0.0	0.0	0.0	0.0
5		Reference Item (Vehicle Control)	0.0	0.0	0.0	0.0	0.0
6		NVK019 - 0.014 µg/mL	0.0	0.0	0.0	0.0	0.0
7		NVK019 - 0.140 µg/mL	0.0	0.0	0.0	0.0	0.0
8		NVK019 - 1.4 µg/mL	0.0	0.0	0.0	0.0	0.0
1	Plasma Hemolytic Index	Non-Spiked Whole Blood	16	10	14	13	3
2		Positive Control - 20% Saponin	11880	12420	11820	12040	330
4		Negative Control - 0.9% NaCl	42	36	32	37	5
5		Reference Item (Vehicle Control)	36	23	22	27	8
6		NVK019 - 0.014 µg/mL	51	41	38	43	7
7		NVK019 - 0.140 µg/mL	46	34	37	39	6
8		NVK019 - 1.4 µg/mL	51	44	40	45	6

In Vitro Evaluation of the Influence of NVK019 on Human Red Blood Cell Hemolysis

Males

Group	Parameter	Identification	H1	H2	H3	Mean	SD
1	Plasma Hemolytic	Non-Spiked Whole Blood	0.016	0.010	0.014	0.013	0.003
2	Index	Positive Control - 20% Saponin	11.880	12.420	11.820	12.040	0.330
4	(Hemoglobin	Negative Control - 0.9% NaCl	0.042	0.036	0.032	0.037	0.005
5	equivalent in g/dL)	Reference Item (Vehicle Control)	0.036	0.023	0.022	0.027	0.008
6		NVK019 - 0.014 µg/mL	0.051	0.041	0.038	0.043	0.007
7		NVK019 - 0.140 µg/mL	0.046	0.034	0.037	0.039	0.006
8		NVK019 - 1.4 µg/mL	0.051	0.044	0.040	0.045	0.006
1	Plasma Hemolysis	Non-Spiked Whole Blood	N	N	N	N/A	N/A
2		Positive Control - 20% Saponin	H^{+++}	H^{+++}	H^{+++}	N/A	N/A
4		Negative Control - 0.9% NaCl	N	N	N	N/A	N/A
5		Reference Item (Vehicle Control)	N	N	N	N/A	N/A
6		NVK019 - 0.014 µg/mL	N	N	N	N/A	N/A
7		NVK019 - 0.140 µg/mL	N	N	N	N/A	N/A
8		NVK019 - 1.4 µg/mL	N	N	N	N/A	N/A
2	% Hemolysis	Positive Control - 20% Saponin	96.3	95.9	94.6	95.6	0.9
4		Negative Control - 0.9% NaCl	0.2	0.1	0.1	0.1	0.1
5		Reference Item (Vehicle Control)	0.2	0.1	0.1	0.1	0.1
6		NVK019 - 0.014 µg/mL	0.2	0.2	0.1	0.2	0.1
7		NVK019 - 0.140 µg/mL	0.2	0.1	0.1	0.1	0.1
8		NVK019 - 1.4 µg/mL	0.2	0.2	0.2	0.2	0.0

N/A = Not applicable

In Vitro Evaluation of the Influence of NVK019 on Human Red Blood Cell Hemolysis

Females

Group	Parameter	Identification	H4	H5	H6	Mean	SD
4	Whole Blood Hemoglobin (g/dL)	Negative Control - 0.9% NaCl	11.9	9.7	13.4	11.7	1.9
1	Whole Blood	Non-Spiked Whole Blood	38.7	33.6	40.2	37.5	3.5
2	Hematocrit	Positive Control - 20% Saponin	0.0	0.0	0.0	0.0	0.0
4	(%)	Negative Control - 0.9% NaCl	36.9	31.2	39.1	35.7	4.1
5		Reference Item (Vehicle Control)	36.2	32.0	38.5	35.6	3.3
6		NVK019 - 0.014 µg/mL	36.3	31.2	39.4	35.6	4.1
7		NVK019 - 0.140 µg/mL	35.9	31.7	39.3	35.6	3.8
8		NVK019 - 1.4 µg/mL	36.5	30.9	38.7	35.4	4.0
1	Plasma Hemoglobin	Non-Spiked Whole Blood	0.0	0.0	0.0	0.0	0.0
2	(g/dL)	Positive Control - 20% Saponin	11.0	9.4	12.5	11.0	1.6
4		Negative Control - 0.9% NaCl	0.0	0.0	0.0	0.0	0.0
5		Reference Item (Vehicle Control)	0.0	0.0	0.0	0.0	0.0
6		NVK019 - 0.014 µg/mL	0.0	0.0	0.0	0.0	0.0
7		NVK019 - 0.140 µg/mL	0.0	0.0	0.0	0.0	0.0
8		NVK019 - 1.4 µg/mL	0.0	0.0	0.0	0.0	0.0
1	Plasma Hemolytic	Non-Spiked Whole Blood	3	4	9	5	3
2	Index	Positive Control - 20% Saponin	9560	7480	10880	9307	1714
4		Negative Control - 0.9% NaCl	22	14	28	21	7
5		Reference Item (Vehicle Control)	13	10	27	17	9
6		NVK019 - 0.014 µg/mL	19	19	31	23	7
7		NVK019 - 0.140 µg/mL	19	17	41	26	13
8		NVK019 - 1.4 µg/mL	24	25	39	29	8

***In Vitro* Evaluation of the Influence of NVK019 on Human Red Blood Cell Hemolysis**

Females

Group	Parameter	Identification	H4	H5	H6	Mean	SD
1	Plasma Hemolytic	Non-Spiked Whole Blood	0.003	0.004	0.009	0.005	0.003
2	Index	Positive Control - 20% Saponin	9.560	7.480	10.880	9.307	1.714
4	(Hemoglobin	Negative Control - 0.9% NaCl	0.022	0.014	0.028	0.021	0.007
5	equivalent in g/dL)	Reference Item (Vehicle Control)	0.013	0.010	0.027	0.017	0.009
6		NVK019 - 0.014 µg/mL	0.019	0.019	0.031	0.023	0.007
7		NVK019 - 0.140 µg/mL	0.019	0.017	0.041	0.026	0.013
8		NVK019 - 1.4 µg/mL	0.024	0.025	0.039	0.029	0.008
1	Plasma Hemolysis	Non-Spiked Whole Blood	N	N	N	N/A	N/A
2		Positive Control - 20% Saponin	H^{+++}	H^{+++}	H^{+++}	N/A	N/A
4		Negative Control - 0.9% NaCl	N	N	N	N/A	N/A
5		Reference Item (Vehicle Control)	N	N	N	N/A	N/A
6		NVK019 - 0.014 µg/mL	N	N	N	N/A	N/A
7		NVK019 - 0.140 µg/mL	N	N	N	N/A	N/A
8		NVK019 - 1.4 µg/mL	N	N	N	N/A	N/A
2	% Hemolysis	Positive Control - 20% Saponin	92.4	96.9	93.3	94.2	2.4
4		Negative Control - 0.9% NaCl	0.1	0.1	0.1	0.1	0.0
5		Reference Item (Vehicle Control)	0.1	0.1	0.1	0.1	0.0
6		NVK019 - 0.014 µg/mL	0.1	0.1	0.1	0.1	0.0
7		NVK019 - 0.140 µg/mL	0.1	0.1	0.2	0.1	0.1
8		NVK019 - 1.4 µg/mL	0.1	0.2	0.2	0.2	0.1

N/A = Not applicable

Based on the measurements of hemoglobin released from red blood cells to plasma, there were no hemolytic effect observed with NVK019 at final concentrations in the blood of 0.014, 0.140, and 1.4 µg/mL when compared with the reference vehicle control. No hemolysis was observed by visual macroscopic evaluation for both males and females.

***In Vitro* Evaluation of the Influence of NVK019 on Human Plasma Flocculation**

Males

Group	Parameter	Identification	H1	H2	H3	Mean	SD
1	Visual Flocculation	Non-Spiked Whole Blood	N	N	L^{+}	N/A	N/A
3	(Plasma)	Positive Control - 20% Intralipid®	L^{+++}	L^{+++}	L^{+++}	N/A	N/A
4		Negative Control - 0.9% NaCl	N	N	L^{+}	N/A	N/A
5		Reference Item (Vehicle Control)	N	N	L^{+}	N/A	N/A
6		NVK019 - 0.014 µg/mL	N	N	L^{+}	N/A	N/A
7		NVK019 - 0.140 µg/mL	N	N	L^{+}	N/A	N/A
8		NVK019 - 1.4 µg/mL	N	N	L^{+}	N/A	N/A
1	Plasma Turbidity	Non-Spiked Whole Blood	14	7	19	13	6
3	(660 nm / 700 nm)	Positive Control - 20% Intralipid® [a]	46	92	34	57	31
4		Negative Control - 0.9% NaCl	11	9	19	13	5
5		Reference Item (Vehicle Control) [b]	0	1	0	0	1
6		NVK019 - 0.014 mg/mL [b]	0	0	0	0	0
7		NVK019 - 0.140 mg/mL [b]	4	0	0	1	2
8		NVK019 - 1.4 mg/mL [b]	4	1	0	2	2

a = Ratio of positive control over non-spiked plasma control
b = Result corrected for negative control
N/A = Not applicable

***In Vitro* Evaluation of the Influence of NVK019 on Human Plasma Flocculation**

Females

Group	Parameter	Identification	H4	H5	H6	Mean	SD
1	Visual Flocculation	Non-Spiked Whole Blood	N	N	N	N/A	N/A
3	(Plasma)	Positive Control - 20% Intralipid®	L^{+++}	L^{+++}	L^{+++}	N/A	N/A
4		Negative Control - 0.9% NaCl	N	N	N	N/A	N/A
5		Reference Item (Vehicle Control)	N	N	N	N/A	N/A
6		NVK019 - 0.014 µg/mL	N	N	N	N/A	N/A
7		NVK019 - 0.140 µg/mL	N	N	N	N/A	N/A
8		NVK019 - 1.4 µg/mL	N	N	N	N/A	N/A
1	Plasma Turbidity	Non-Spiked Whole Blood	8	10	9	9	1
3	(660 nm / 700 nm)	Positive Control - 20% Intralipid® [a]	81	56	74	70	13
4		Negative Control - 0.9% NaCl	6	10	9	8	2
5		Reference Item (Vehicle Control) [b]	2	0	0	1	1
6		NVK019 - 0.014 mg/mL [b]	1	0	0	0	1
7		NVK019 - 0.140 mg/mL [b]	0	1	0	0	1
8		NVK019 - 1.4 mg/mL [b]	3	3	0	2	2

a = Ratio of positive control over non-spiked plasma control
b = Result corrected for negative control
N/A = Not applicable

Based on the visual flocculation evaluation and the measurement of sample turbidity index, there was no flocculation and no dose-related increases in net turbidity index with NVK019 at final concentrations of 0.014, 0.140, and 1.4 µg/mL in human blood samples.

***In Vitro* Evaluation of the Influence of NVK019 on Human Plasma Osmolality**

Males

Group	Parameter	Identification	H1	H2	H3	Mean	SD
4	Osmolality (mOsm/kg H_20)	Negative Control - 0.9% NaCl	288	293	293	291.3	2.9
5	(Plasma)	Reference Item (Vehicle Control)	295	292	290	292.3	2.5
6		NVK019 - 0.014 µg/mL	292	292	292	292.0	0.0
7		NVK019 - 0.140 µg/mL	291	292	290	291.0	1.0
8		NVK019 - 1.4 µg/mL	292	292	290	291.3	1.2

***In Vitro* Evaluation of the Influence of NVK019 on Human Plasma Osmolality**

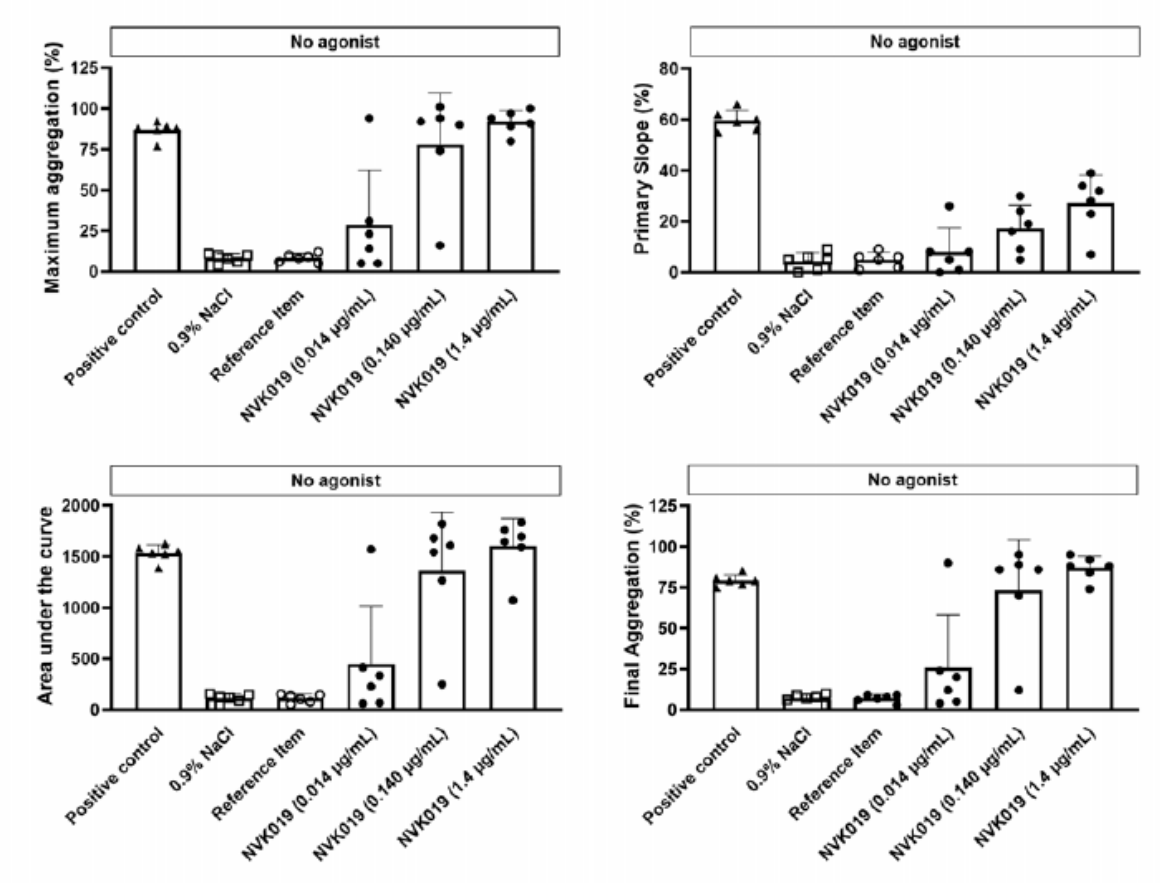
Females

Group	Parameter	Identification	H4	H5	H6	Mean	SD
4	Osmolality (mOsm/kg H_20)	Negative Control - 0.9% NaCl	289	283	286	286.0	3.0
5	(Plasma)	Reference Item (Vehicle Control)	286	285	284	285.0	1.0
6		NVK019 - 0.014 µg/mL	287	280	286	284.3	3.8
7		NVK019 - 0.140 µg/mL	284	281	280	281.7	2.1
8		NVK019 - 1.4 µg/mL	285	285	287	285.7	1.2

There were no change in plasma osmolality observed in human samples spiked with NVK019 at final concentrations in the blood of 0.014, 0.140, and 1.4 µg/mL compared to reference vehicle control.

Spontaneous Platelet Aggregation
Spontaneous platelet aggregation was observed in samples spiked with NVK019 at 0.014 µg/mL (+244% Maximum Aggregation; +294.4% Area Under The Curve), compared to samples spiked with reference item.

Maximal spontaneous platelet aggregation was observed in samples spiked with NVK019 at 0.140 µg/mL (+834% Maximum Aggregation; +1103.2% Area Under The Curve) and NVK019 at 1.40 µg/mL (+1002% Maximum Aggregation; +1314.4% Area Under The Curve)



Increased platelet aggregation were observed as expected and has previously been demonstrated that adrenaline (epinephrine) induces spontaneous platelet aggregation (Spalding et al. 1998; Solinger et al.1973; Jakobs et al., 1978).

Local Tolerance Study

NVK019: Single Dose Local Tolerance Study via Intravenous and Paravenous Administration in New Zealand White Rabbits

Sponsor:	Nevakar Injectables, Inc.
Test Facility Study No.:	1602-21033
Conducting laboratory and location:	(b) (4)
Date of Initiation:	February 17, 2021
Date of Completion:	August 31, 2021
GLP compliance:	Yes
QA statement:	Yes

Key Study Findings

NVK019 (1.2 mcg/kg) was administered into the right and left marginal ear veins of male rabbits via a bolus intravenous (IV) or perivascular (PV) Injection. A full gross necropsy mortality, physical examinations, body weights, body weight changes, food consumption, gross pathology findings, and histopathology findings. Results of Local Tolerance Study in rabbits demonstrate that NVK019 administration at doses up to 1.2 mcg/kg had no effect on mortality, physical examinations, body weights, body weight changes, food consumption, gross pathology, or histopathology.

Conclusion

There were no effects observed because of the addition of NVK019 (0.014 – 1.4 µg/mL) to whole human blood on hemolysis, plasma osmolality and flocculation parameters.

Increased platelet aggregation induced by Collagen agonist and dose-related spontaneous platelet aggregation were observed in human PRP samples spiked with NVK019 (at 0.014, 0.14, and 1.4 µg/mL). The effects on platelet aggregation in vitro have been previously reported with epinephrine and no additional concern has been identified in this study.

Results of Local Tolerance Study in rabbits demonstrated that NVK019 administration at doses up to 1.2 mcg/kg by intravenous (IV) or perivascular (PV) injection had no effect on mortality, physical examinations, body weights, body weight changes, food consumption, or macroscopic or microscopic observations.

The NVK019 (Epinephrine in 0.9% Sodium Chloride Injection 8 mcg/mL, 16 mcg/mL, 20 mcg/mL, 32 mcg/mL, and 40 mcg/mL) is therefore, safe for human use by IV administration from the Pharm/Tox perspective.

REFERENCES

Spalding et al.: Mechanism of Epinephrine-Induced Platelet Aggregation. Hypertension. Feb;31(2):603-7 (1998).

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