Oral and Intravenous Pharmacokinetics of NVN1000, a Nitric Oxide Donating Macromolecule, in Miniature Swine

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ABSTRACT

NVN1000 is a nitric oxide (NO) releasing polysiloxane macromolecule that has demonstrated both antimicrobial and anti-inflammatory effects in vitro and in vivo and is currently in clinical development for the treatment of acne vulgaris. NVN1000 contains the NO donating moiety *N*-Methylaminopropyl-trimethoxysilane-diazeniumdiolate (MAP3-NONOate) and release of NO from this macromolecule is proton initiated. In the blood, endogenous and exogenous NO rapidly reacts with hemoglobin to produce methemoglobin (metHb) and is also metabolized to form nitrate. Hydrolyzed MAP3 (hMAP3) is a unique and quantifiable analyte of NVN1000 exposure. Additional surrogate analytes include increases in elemental silicon, plasma nitrate and methemoglobin. The objective of this study was to determine a quantitative correlation between these analytes after oral (PO) and intravenous (IV) administration in Hanford minipigs. Animals received a single administration of NVN1000 at 20 mg/kg (IV), 30 or 100 mg/kg (PO). Serial blood samples were collected via jugular vein catheters predose and up to 48 hours postdose.

For all four analytes, the IV $t_{1/2}$ was ≤ 4.5 hrs. The linear association (r^2) between hMAP3 and silicon concentrations was 0.99 where a 0.42 ng/mL increase in silicon correlated to a 1 ng/mL increase in hMAP3; r^2 for hMAP3 and nitrate association was 0.91 where a 0.31 ng/mL increase in nitrate correlated to a 1 ng/mL increase in hMAP3. There was a linear association (r^2 > 0.8) between nitrate and metHb concentrations at 20 mg/kg IV, 100 mg/kg PO, and when all three dose levels were combined. In this model, a systemic nitrate increase >1400 ng/mL would be required for an absolute 1% increase in metHb. These correlations show tight associations between the active and inactive components of the NVN1000 molecule as it is metabolized *in vivo*. As hMAP3 is not endogenous, small changes in hMAP3 concentrations can be quantitated, making it the more appropriate analyte to demonstrate NVN1000 systemic exposure.

NITRICIL™ & ASSOCIATED ANALYTES

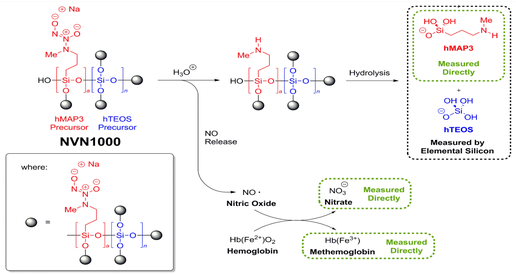


Figure 1. NVN1000 Macromolecular Structure and Products of *in situ* Hydrolysis. NVN1000 is a nitric oxide-releasing macromolecule comprised of a polysiloxane backbone that contains covalently bound N-diazeniumdiolate nitric oxide donors throughout the polymeric structure. At the time of topical administration nitric oxide is released yielding hMAP3 (hydrolyzed methylaminopropyltrimethoxysilane). In vivo nitric oxide can be rapidly bound by hemoglobin yielding methemoglobin or metabolized to nitrate. Systemic absorption and NVN1000 metabolism are measured by quantifying plasma hMAP3, elemental silicon, plasma nitrate, and blood methemoglobin levels.

MATERIALS & METHODS

Study Overview: Three naïve female Hanford miniature swine were employed for this crossover study. Each pig received each of the three different doses of NVN1000 dose levels, in cross over fashion, separated by at least a seven day washout period. The study design and PK blood sampling times are presented in Tables 1 and 2.

Dose Procedures: Two routes of administration were used for this study. For PO dosing, animals were transferred from their pens to slings. The appropriate volume of NVN1000 formulation was administered via an oral gavage tube and followed immediately with a water flush to rinse the tubing. For IV dosing, the appropriate volume of NVN1000 formulation was administered to a jugular vein vascular access port (VAP).

Methemoglobin Collection & Analysis: Whole blood samples were obtained via accessed VAP and were stored refrigerated in collection tubes containing K_2EDTA as an anticoagulant until analysis. Analysis of metHb concentration was performed within 48 hrs of collection.

Nitrate, Silicon, and hMAP3 Sample Collection & Analysis: At least 5 mL of whole blood was obtained from each animal via VAP and transferred to Royal Blue Top Vacutainer tubes, containing K_2EDTA, designated for trace elemental analysis. Samples were inverted by hand twice following collection and subsequently stored refrigerated until further processing. Each blood sample was centrifuged at ~3,000 rpm for 15 minutes at 4°C. Plasma was divided into six aliquots (three sets of two tubes). Plasma for nitrate analysis was stored in glass HPLC vials, for silicon analysis in nitric acid-washed cryovials, and for hMAP3 in polypropylene cryovials.

Pharmacokinetic Analysis: Non-compartmental analysis was applied to plasma concentration-time data. Pharmacokinetic analysis was performed using Phoenix WinNonlin 6.4. PK parameters were calculated separately for each dose level and animal using the raw data and nominal timepoints. Prior to PK analysis, to account for physiologic nitrate, silicon, and methemoglobin baseline values, the predose concentration of each animal's analyte concentration-time series, was subtracted from all post dose concentration values. If the resulting concentration value fell below zero, the original reported concentration remained with the PK data set. All predose hMAP3 values, reported as BQL, were interpreted as 0 ng/mL. All BQL values, reported for nitrate and silicon, were regarded as one half of the compound's assay LLOQ value. This was done to account for endogenous nitrate and silicon concentrations that are present as a result of normal dietary intake.

STUDY DESIGN

Table 1. NVN1000 Dose Regimens

Week	# of Animals*	Formulation			Target Dose Amount (mg/kg)
		Name	Route	Target Dose Concentration	
1	3	NVN1000	PO	60 mg/mL	30
2	3	NVN1000	PO	200 mg/mL	100
3	3	NVN1000	IV	80 mg/mL	20

Table 2. Pharmacokinetic (PK) Profile Blood Sampling Times

Route	Dose	Timepoints
Oral	30 mg/kg or 100 mg/kg	Predose, 0.5, 1, 2, 4, 6, 8, 12, 24, and 48 hours after oral dose administration
IV	20 mg/kg	Predose, 2, 10, and 20 minutes and 0.5, 1, 3, 6, 12, 24, and 48 hours after IV dose administration

RESULTS

Figure 2. Mean Plasma Concentration-Time Curves in Hanford Miniature Swine Receiving NVN1000 via IV or PO administration. For each analyte, the mean plasma concentration-time curve was determined following either IV or PO administration of NVN1000 **A)** hMAP3, **B)** silicon, and **C)** nitrate,

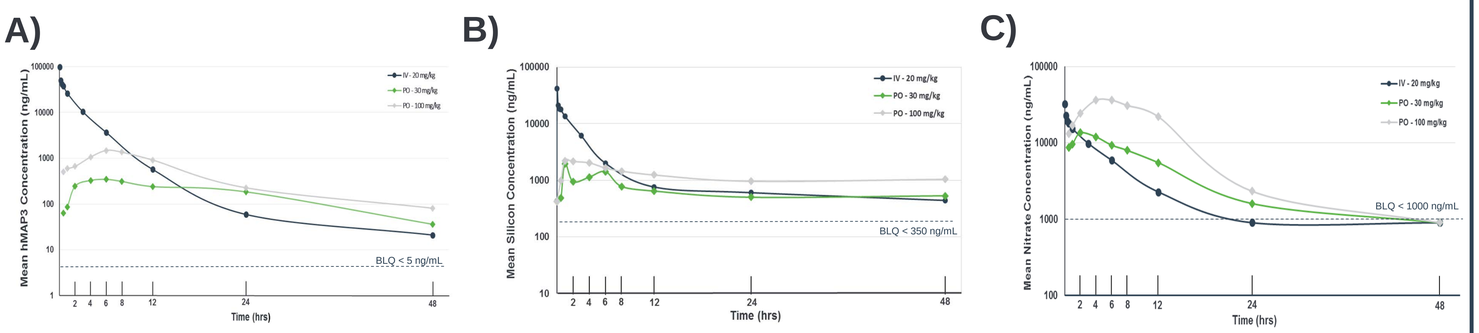


Figure 3. IV Dose Stratified Concentration-Concentration Linear Regression Plots. At the 20 mg/kg IV dose there were significant trends and linear associations between **A)** mean plasma silicon concentration and mean plasma hMAP3 concentration, as well as between **B)** mean plasma nitrate concentration and mean hMAP3 concentration. p<0.001

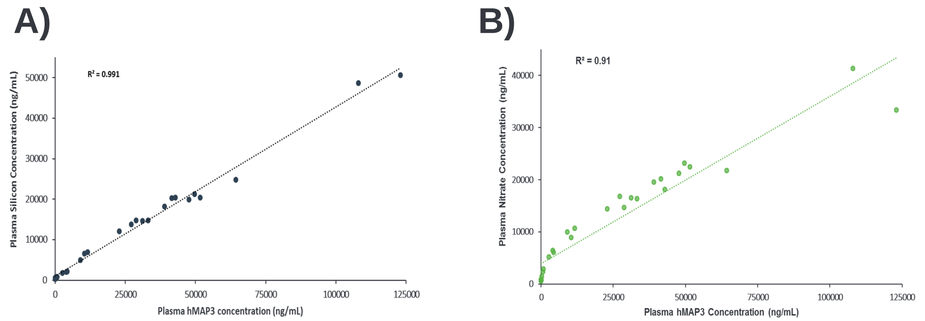
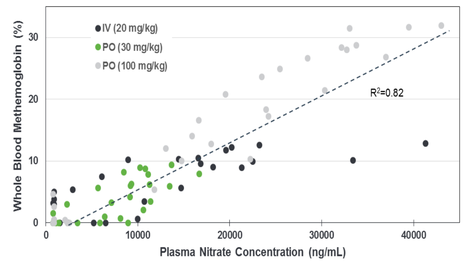


Figure 4. Mean Methemoglobin Concentration- Mean Nitrate Concentration Linear Regression Plot. When the mean plasma nitrate and mean whole blood methemoglobin percentages were combined, without regard to dose or dose route, there was a significant trend between plasma nitrate concentration and whole blood methemoglobin percentage. p<0.001



CONCLUSIONS

- Both PO and IV administration of NVN1000 dose levels of 20 mg/kg IV, 30 mg/kg PO, and 100 mg/kg PO demonstrated measurable levels of hMAP3 and increases of nitrate, elemental silicon, and methemoglobin over endogenous levels.
- For all 4 analytes, the *in vivo* half-life after IV administration of NVN1000 averaged ≤ 4.5 hrs demonstrating rapid normalization of plasma or blood levels to predose baseline levels.
- There were significant trends and linear associations between plasma nitrate concentration and whole blood methemoglobin, and when all 3 dose levels were combined, for every 1 ng/mL increase in plasma nitrate concentration whole blood methemoglobin increased by 0.0007%.
  - These trends indicated that a systemic increase in plasma nitrite level of >1400 ng/mL would be required for an absolute 1% increase in whole blood methemoglobin levels.
- Unlike elemental silicon and nitrate, hMAP3 is not endogenous and small changes in plasma concentrations (BLQ < 5 ng/mL) can be quantitated making hMAP3 the more appropriate analyte to measure for demonstration of NVN1000 systemic exposure.
  - The tight associations between the active and inactive components of NVN1000, as it is metabolized *in vivo*, support the measure of hMAP3 to quantitate NVN1000 systemic exposure.