

Pharmacokinetics of Oral Cyanocobalamin Formulated With Sodium N-[8-(2-hydroxybenzoyl)amino]caprylate (SNAC): An Open-Label, Randomized, Single-Dose, Parallel-Group Study in Healthy Male Subjects

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ABSTRACT

Background: Vitamin B12 (cobalamin) deficiency may be caused by inadequate dietary intake of B12 or by conditions that result in malabsorption of the vitamin. Crystalline vitamin B12, usually in the form of cyanocobalamin, is administered parenterally (ie, intramuscularly) or orally for treating deficiency states. Intramuscular administration is widely accepted as a treatment method. Oral B12 supplementation is also used, but it is considered to be less reliable.

Objective: This study was conducted to compare the pharmacokinetics and tolerability of 2 oral formulations of cyanocobalamin—a marketed cyanocobalamin tablet (immediate-release B12 5 mg) and cyanocobalamin formulated with a proprietary carrier, sodium N-[8-(2-hydroxybenzoyl)amino]caprylate (SNAC)—to establish the feasibility of using an absorption enhancer with B12 to improve uptake of the vitamin. This was the first clinical study conducted with the cyanocobalamin/SNAC coformulation.

Methods: An open-label, randomized, single-dose, parallel-group study was conducted in healthy male subjects. Subjects were randomly assigned to 1 of 4 treatment groups: Treatment A subjects (n = 4) received 2 tablets of 5-mg cyanocobalamin formulated with 100-mg SNAC as part of a dose range-finding arm included to determine a dose to provide a measurable concentration of vitamin B12 at all time points when tested with the available vitamin B12 assay; treatment B subjects (n = 6) received 1 tablet of 5-mg cyanocobalamin formulated with 100-mg SNAC; treatment C subjects (n = 6) received 1 commercially available 5-mg cyanocobalamin tablet; and treatment D subjects (n = 4) received commercially available 1-mg cyanocobalamin IV. Treatment A was completed 3 weeks before treatments B, C, and D were studied. Human serum B12 was analyzed by chemilumines-

cence assay method. Validation procedures established that samples could be diluted up to 100 times without any effects on accuracy and precision. The pharmacokinetic properties of vitamin B12 were characterized by noncompartmental analysis. Vitamin B12 absolute bioavailability estimates were calculated between the oral (A, B, and C) and IV (D) treatments using non-baseline-adjusted vitamin B12 concentrations as well as baseline-adjusted vitamin B12 concentrations, with or without body weight adjustments. Tolerability was evaluated through review or monitoring of medical history, physical examination findings, concomitant medications, vital signs, laboratory tests (hematology, serum chemistry, and urinalysis values), electrocardiography, adverse events, and serious adverse events.

Results: Twenty healthy male subjects, aged 20 to 45 years, participated in this study. Based on data from treatment A, a 5-mg cyanocobalamin dose was selected for use with treatments B and C. The oral cyanocobalamin formulation containing SNAC had greater mean absolute bioavailability than the commercial oral formulation (5.09% vs 2.16%, respectively), calculated on AUC_{0-last} values uncorrected for baseline, weight, or body mass index. It also had a reduced T_{max} compared with the commercial formulation (0.5 hours vs 6.83 hours, respectively). The K_e was similar between treatments (0.028 1/h vs 0.025 1/h). Comparable results were achieved using corrected values. The cyanocobalamin/SNAC formulation was well tolerated, and there were no reported adverse events.

Conclusions: An oral formulation of 5-mg cyanocobalamin containing 100-mg SNAC, an absorption en-

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hancer, provided significantly improved bioavailability and a significant decrease in T_{max} for B12 in a small study of normal healthy subjects compared with a commercially available 5-mg cyanocobalamin oral formulation. Both oral formulations and commercial 1-mg cyanocobalamin IV were well tolerated. ClinicalTrials.gov identifier: NCT01311739. (*Clin Ther.* 2011;33:934–945) © 2011 Elsevier HS Journals, Inc. All rights reserved.

Key words: B12, cobalamin, cyanocobalamin, oral, pharmacokinetics, SNAC.

INTRODUCTION

Vitamin B12 deficiency is a clinically important condition that occurs when vitamin stores are depleted through inadequate dietary intake or impaired vitamin absorption. Vitamin B12 is most abundant in foods of animal origin; therefore, vegetarian diets predispose to B12 deficiency.¹ Defective absorption is generally attributable to a specific failure of extraction and transport of B12 from dietary sources, as, for example, with gastric achlorhydria and inadequate intrinsic factor production, or to more generalized disturbances of gastrointestinal structure and function, such as gastric resection, ileal resection, Crohn's disease, and bacterial overgrowth of the intestine.

Supplemental vitamin B12, in the form of crystalline cobalamin (common forms include cyanocobalamin, methylcobalamin, and hydroxycobalamin), is administered either parenterally (ie, intramuscularly) or orally to treat vitamin B12 deficiency. Unlike dietary B12, which is protein bound and requires pepsin and acid conditions in the stomach for release and subsequent binding to intrinsic factor,² crystalline cobalamin exists in the free unbound state. Crystalline cobalamins are known to be absorbed by passive diffusion to the extent of ~1% over a range of ~100 μg to 5 mg.³ However, dietary B12 is absorbed through the action of intrinsic factor and its receptors, a mechanism that normally limits B12 uptake to between 5 and 10 $\mu\text{g}/\text{d}$.⁴ Intramuscular administration is widely accepted as a method of treating B12 deficiency. Oral B12 supplementation, which originated in the 1960s, continues to be studied.³ An area of interest in the use of oral supplements has been in the elderly, in whom food-cobalamin malabsorption becomes increasingly prevalent after 50 years of age.⁵ In recent years, doses of 1000 to 2000 $\mu\text{g}/\text{d}$ have been used in

clinical studies to restore B12-deficient patients to the normal range. Successful treatment of patients with B12 deficiency has been observed also with lower doses, but the proportion of patients responding decreases.^{6,7}

An earlier preclinical study in rats⁸ reported that the extent of vitamin B12 absorption was significantly ($P < 0.05$) enhanced when cyanocobalamin was administered in combination with the absorption enhancer sodium N-[8-(2-hydroxybenzoyl)amino]caprylate (SNAC). The present 4-arm, open-label, randomized, single-dose, parallel-group study investigated the pharmacokinetic profile of a new formulation* of cyanocobalamin containing SNAC in 20 healthy males. SNAC has been tested in nonclinical^{9,10} and clinical studies as a drug delivery agent at dose multiples exceeding those used for cyanocobalamin delivery.¹¹ The 2 ingredients in this new formulation, cyanocobalamin and SNAC, are both designated generally recognized as safe (GRAS) according to US Food and Drug Administration regulatory definitions pertaining to food and food additives. In both cases this status was granted independently of the present clinical study.

SUBJECTS AND METHODS

Study Design

This was an open-label, randomized, single-dose, parallel-group pharmacokinetic (PK) study conducted at a single center (MDS Pharma Services, Neptune, New Jersey) between May 22, 2008 and June 16, 2008. Our objective was to assess the PK profile and tolerability of oral cyanocobalamin coformulated with SNAC and a commercial 5-mg, immediate-release formulation[†] of cyanocobalamin. The formulations are identified as cyanocobalamin/SNAC and commercial cyanocobalamin. The study was approved by the independently functioning institutional review board (IRB) of MDS Pharma Services and was conducted in compliance with Section 56 of Title 21 of the Code of Federal Regulations (CFR) and in accordance with the clinical research guidelines established by the Medical Research Council of Canada, the basic principles of the Declaration of Helsinki defined in US 21 CFR Part 312.20, the requirements of Directive 2001/20/EC

*Trademark: Eligen B12[®] (Emisphere Technologies, Inc, Cedar Knolls, New Jersey).

†Trademark: Vitalabs[®] Immediate Release B-12 (Vitalabs, Inc, Jonesboro, Georgia).

(Europe), the principles enunciated in the Declaration of Helsinki (Edinburgh, 2000), and the ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice.

The selected doses of 10 mg (2×5 -mg cyanocobalamin/100-mg SNAC oral dose) and 1 mg (IV dose) were expected to yield B12 concentrations exceeding the endogenous baseline and consequently to allow full PK assessments. The design of the study allowed for a decrease in the oral dose of cyanocobalamin if, following the pilot part of the study (treatment A, conducted 3 weeks before the remaining treatments), it was determined that relevant concentrations for PK analysis could be achieved following a 5-mg dose.

Subjects

Recruitment was conducted using the MDS Pharma Services database. Subjects were called and asked whether they wanted to participate in the study. During enrollment subjects were asked to read, understand, and sign the IRB-approved informed consent form (ICF). In addition to elucidating the purpose and conduct of the study, the ICF clearly stated the subjects' responsibilities, which included following general clinic rules, following study instructions given by the staff, following study restrictions, reporting any changes in physical or mental condition during the study, reporting any side effects experienced during the study, and giving true and complete answers to questions during the study. Subjects were compensated, and payment was prorated at the completion of each period (completion of confinement for dosing/sampling and completion of study). Subjects assigned to IV dosing were paid slightly more than subjects assigned to oral dosing.

Healthy male subjects aged 18 to 45 who weighed between 60 and 100 kg and had a body mass index (BMI) between 18 and 30 kg/m^2 were eligible to receive treatments A, B, C, or D, as per inclusion criteria, if they had normal organ function, including renal and hepatic function; normal vital signs and electrocardiography (ECG) results; normal results on routine hematology and clinical chemistry tests; and normal levels of serum B12 (193–982 pg/mL), methylmalonic acid (0.0 – $0.4 \text{ } \mu\text{mol/L}$), and homocysteine (5 – $12 \text{ } \mu\text{mol/L}$). Exclusion criteria included current use of acetaminophen or nonsteroidal antiinflammatory drugs, antibiotics, antacids, multivitamins, or nutritional supple-

ments and absolute platelet count below $100 \times 10^9/\text{L}$.

Eligible subjects entered the clinic the evening before study product administration (check-in) and remained in the clinic for at least 24 hours postdose (2-night confinement) until the conclusion of the treatment and assessments. Subjects were discharged after the last blood sample was taken and the appropriate safety parameters (eg, vital signs, ECG) remained within acceptable ranges. Clinical laboratory tests, urine drug screen, and urine alcohol test were performed prior to dosing. While in the clinic, all subjects began a 10-hour overnight fast prior to dosing. Vital signs and baseline PK blood samples were taken within 30 minutes before study product administration. Vital signs were taken, adverse event (AE) monitoring was performed, and blood samples were drawn for up to 24 hours after study product administration to measure the PK properties of cyanocobalamin and obtain tolerability data. Standard meals were given for the duration of confinement beginning at 4 hours postdose.

Test Formulations

The study product used for treatments A and B was a cyanocobalamin/SNAC tablet containing 5-mg cyanocobalamin and 100-mg SNAC (Emisphere Technologies, Inc, Cedar Knolls, New Jersey; lot no. 112-08-01, expiration date April 23, 2009). The comparator product was a commercial 5-mg, immediate-release cyanocobalamin tablet (Vitalabs, Inc, Jonesboro, Georgia; lot no. 22083, expiration date March 2010). The IV product was a commercial 1-mg cyanocobalamin solution (1 mg/mL) (American Regent, Inc, Shirley, New York; lot no. 8071, expiration date January 2010).

Treatments

Treatment A was a single oral dose of cyanocobalamin/SNAC administered to 4 human subjects in the fasted state as 2 tablets taken with 50 mL of water for a total dose of 10-mg cyanocobalamin and 200-mg SNAC. Treatment A was administered as a screening step to enable selection of a dose resulting in an increase in serum B12 concentrations that would be measurable above the baseline value for a period of 24 hours and be suitable for PK evaluation. This dose range-finding arm was completed 3 weeks before treatments B, C, and D were studied.

Treatment B, the test treatment, was a single oral dose of 5-mg cyanocobalamin/100-mg SNAC administered in the fasted state to 6 subjects as 1 tablet taken with 50 mL of water.

Treatment C, the comparator treatment, was a single oral dose of commercial 5-mg cyanocobalamin alone administered in the fasted state to 6 subjects as 1 tablet taken with 50 mL of water.

Treatment D, the reference treatment, was a single 1-mg IV dose of commercial cyanocobalamin administered in the fasted state to 4 subjects. Each subject received a 1-mL IV injection of a 1-mg/mL solution, resulting in a total dose of 1-mg cyanocobalamin.

Blood Sample Processing

Following each treatment, A, B, C, or D, 25 blood samples were drawn by venipuncture for B12 analysis within 30 minutes predose and at minutes 2, 5, 10, 20, 30, 40, and 50 and hours 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 16, 20, and 24 postdose. Five mL of whole blood were collected into a pre-labeled 5-mL red top or serum separator tube (SST tube) at each sampling time point under fluorescent lighting, repeatedly inverted as per manufacturer's instructions and allowed to clot for 30 minutes prior to centrifugation at 3000 rpm for 7 minutes at 5°C. The samples were removed from the centrifuge under yellow light and transferred to 2 1-mL transfer tubes prior to placement in a freezer at -20°C. Approximately 2 mL of serum was collected per sample. Each serum sample was split in half (~1 mL per aliquot). One duplicate sample was shipped to MDS Bioanalytical Laboratories (Lincoln, Nebraska) for B12 analysis and the other was retained as a backup sample. Sampling in subjects who received the IV dose was performed in the arm not used for dosing.

Sample Analysis

The B12 assay used in this study to analyze samples following treatment A, B, C, or D is a competitive multistep assay that involves an alkaline denaturation of endogenous binding proteins using a dithiothreitol (DTT) and a sodium hydroxide/potassium cyanide (NaOH/KCN) solution and immunoreaction steps, during which the released vitamin B12 from test samples competes with immobilized vitamin B12 for binding to hog intrinsic factor. The Immulite 2000 analyzer and vitamin B12 reagent kit used were purchased from DPC (now Siemens Medical Solutions, Flanders, New Jersey).

The following steps were followed to operate the instrument as described in the Immulite 2000 operators manual (document no. 600149-03-D), Section 6 (Routine Operations)¹²: (1) Calibrate the vitamin B12 channel of the Immulite 2000 using the low and high adjustors (included in the DPC Immulite 2000 Vitamin B12 Reagent Kit; used as described in the package insert PIL2KVB-22, 2006-04-18¹³); (2) Transfer at least 350 µL of each standard, quality control (QC), and unknown patient samples into appropriate sampling tubes to be loaded onto the instrument; (3) Place each sample tube on the instrument in the same order as the runsheet; (4) Program the instrument to analyze each sample for Vitamin B12. The assay was performed and validated by MDS Pharma Services (report on file).¹⁴

During standard kit validation, a set of calibration verifiers (160–1120 pg/mL) were run at the beginning of each curve, along with at least 6 replicates of QC samples at 3 concentrations (178, 423, and 799 pg/mL or 178, 423, and 1012 pg/mL). The 5 calibration standard sets (160–1120 pg/mL) assayed over a period of 4 days determined the interday and intraday reproducibility, along with benchtop stability, freeze/thaw stability, and refrigeration stability. Long-term storage stability at -20°C was determined at 178, 423, and 799 pg/mL. Due to the higher than expected vitamin B12 in clinical samples, a new calibration standard range was necessary. Therefore, for V2 (update) validation, VB12_V09 was run on July 17, 2008 to validate new standard 5 (1120 pg/mL) and new high QC (1012 pg/mL). During the validation, QC samples were stored in a freezer set at -20°C.

Before validation, the vitamin B12 channel of the Immulite analyzer was calibrated. Two non-zero adjustors were used to correlate the counts per second (cps) of the instrument's electronically stored master standard. The analyte concentration is determined from that adjusted 2-point curve, as it relates to the master standard curve. From these 2 known values, the instrument established a response factor that it used to calculate the concentration of vitamin B12 in any given samples using the immunometric (competitive) assay equation:

$$\text{CPS} = P_2 + \frac{P_1 - P_2}{P_4} \left(1 + (\text{Dose}/P_3) \right)$$

where P_1 is maximum cps; P_2 is minimum cps (NSB); P_3 is dose at half the maximum cps, and P_4 is slope of the logit-log plot.

Table I. Vitamin B12 method validation: dilution linearity results.

Target Values (pg/mL)	2806	4881	9032	9032	42,234	83,737
DF	5	10	20	40	100	100
Result 1	2787	5034	9142	8553	43,097	107,774
Result 2	3174	5059	9027	8599	43,940	113,955
Result 3	2740	5121	9043	8204	41,199	106,446
Result 4	2794	4738	9377	9210	40,343	100,434
Result 5	3066	4651	8988	8294	41,678	100,120
Result 6	2767	4947	8405	8481	40,857	98,083
Mean	2888	4925	8997	8557	41,852	104,469
SD	184	189	322	354	1388	6013
%RSD	6.4	3.8	3.6	4.1	3.3	5.8
Mean % Recovery	102.9	100.9	99.6	94.7	99.1	124.8
N	6	6	6	6	6	6

DF = degrees of freedom; RSD = relative standard deviation.

The vitamin B12 sample concentrations were then printed out on the instrument's printer. The protein-based verifiers prepared for this validation were used only to verify the linear range of the assay, with no standard curve generated. The following performance parameters were validated and documented in the validation report: specificity, quantification limit, calibration range, intraday and interday accuracy and precision, dilutional linearity, and stability (refrigerations, on-system, benchtop, freeze/thaw, and long-term).

Percent recoveries at all calibration verifier levels were between 97.1% and 113.5%, and the relative standard deviation (%RSD) was between 2.4% and 9.6%. All verifiers were within acceptance criteria. Precision and accuracy was evaluated by analyzing 4 replicates of 3 concentrations of the QC verifiers on the same day (intraday accuracy and precision) or on different days (interday accuracy and precision). The 3 concentrations were 178, 423, and 799 pg/mL. Intraday and interday accuracy and precision were verified within the serum vitamin B12 concentration range of 160 to 1120 pg/mL. The percent recoveries and %RSD for replicates at each level were within acceptance criteria. Samples could therefore be diluted up to 100 times without any effects on accuracy and precision. The sample with a target value of 83,727 pg/mL ($\times 100$ dilution) showed a lack of accuracy (124.8% recovery). This higher than expected recovery appeared to be sample related, rather than dilution factor related,

as the sample with a target value of 42,234 pg/mL and the same dilution factor (100) did show acceptable accuracy and precision. Table I summarizes the results for dilutional linearity.

Pharmacokinetic Analysis

The PK properties of B12 were characterized by noncompartmental analysis using WinNonlin Professional 5.2 (Pharsight, St. Louis, Missouri). The nominal PK sampling times were used for PK analysis unless the actual sampling times deviated significantly from the nominal time points according to the statistical analysis plan. Serum PK parameters determined for B12 included C_{max} , T_{max} , AUC_{0-last} , $AUC_{0-\infty}$, $t_{1/2}$, and K_e .¹⁵ Serum B12 PK parameters were also calculated using baseline-adjusted B12 values. Baseline-adjusted individual serum B12 concentrations were obtained by subtracting the predose concentration from postdose concentration at each time point. Baseline-adjusted PK analysis was performed based on baseline-adjusted serum B12 concentration-time data. Slightly negative values obtained following baseline adjustment were treated as zero for the PK analysis. K_e and other relevant PK parameters ($t_{1/2}$ and AUC) were not estimated in cases in which the terminal phase of the log-concentration-time profile exhibited a linear decline phase with regression coefficients < 0.85 . At least 3 or more data points (excluding C_{max}) in the terminal phase were used for K_e calculations. Baseline cobalamin mea-

surements were limited to a single sample per subject because high (5-mg) doses of cyanocobalamin were administered.¹⁶ Circadian changes in B12 do not occur except as a possible consequence of plasma volume changes during bedrest.¹⁷ In these circumstances, repeated measures of baseline B12 were not considered necessary. This was confirmed by the study data, which demonstrated that baseline corrected C_{max} values for B12 greatly exceeded baseline values (mean baseline screening value for all subjects, 512 [216] pg/mL; mean C_{max} value for the test formulation, 12,847 [6613] pg/mL; mean C_{max} value for the commercial formulation, 1239 [450] pg/mL).

B12 absolute bioavailability (%F) estimates were performed on AUC_{0-last} values (% of F where $F = AUC_{oral} * Dose_{IV} / AUC_{IV} * Dose_{oral}$) between the oral (A, B, and C) and IV (D) treatment formulations. The AUC_{0-last} parameter was calculated using non-baseline-adjusted cyanocobalamin concentrations as well as baseline-adjusted B12 concentrations, with or without body weight adjustments.

Tolerability

All subjects were included in the tolerability analysis. Clinical tests for inclusion/exclusion criteria and tolerability were conducted by a CLIA- and CAP-certified laboratory (MDS Pharma Services, Neptune, New Jersey). A responsible physician was present on site for the duration. A product safety physician with expertise in the new formulation was on call. Tolerability was evaluated through the monitoring or review of medical history findings, physical examination findings, concomitant medications, vital signs (including blood pressure, respiratory rate, heart rate, and temperature), laboratory tests (hematology, serum chemistry, and urinalysis values), ECG, AEs, and serious adverse events. Vital sign measurements (systolic blood pressure, diastolic blood pressure, heart rate, respiration rate, and temperature) were performed in the sitting position at screening, check-in (day 1, predose), and at approximately 1, 2, 3, 5, 8, 12, 16, and 24 (end of study) hours postdose. Clinical laboratory tests were performed at screening, check-in (day 1, predose), and end of study and were evaluated by the investigator. ECG was performed at screening and end of study. Physical examinations were conducted at screening, check-in, and end of study. Tolerability analyses were performed and a report was prepared at MDS Pharma Services, Department of Clinical

Pharmacology. AEs were coded using the *Medical Dictionary for Regulatory Activities* (MedDRA) 11.0.¹⁸ AEs were collected by the nursing staff trained to address the subject with open-ended questions, such as "How are you feeling this morning?". If the subject offered a complaint, the nurse inquired further regarding onset, associated symptoms, precipitating or aggravating or relieving factors, and resolution time. Data for AEs were analyzed using the treatment-emergent AE (TEAE) philosophy. TEAEs were defined as AEs that emerged during treatment, having been absent at pretreatment, or that worsened in severity or frequency relative to the pretreatment state. Predose values were defined as the last observation obtained prior to day 1 dosing. Shift tables describing out-of-range shifts from predose to postdose were created by treatment group. The shift tables were generated by the project programmer. Another peer programmer and project statistician reviewed these tables. The medical writer then used these tables to write the safety text. The principal investigator reviewed and evaluated the original values. Out of normal range and clinically significant laboratory values were listed by subject. For vital signs, descriptive statistics (N, mean, SD, median, minimum, and maximum) were reported for vital sign measurements and change-from-predose (before dosing of each period) values by time point and treatment group. For ECG, descriptive statistics (N, mean, SD, median, minimum, and maximum) were reported for ECG measurements (ventricular rate, PR, QRS, QT, and QTc [using Bazett's formula]) and change from screening values by treatment group. ECG results were also classified as normal or abnormal (clinically significant and not clinically significant) and a shift table was created to describe shifts from screening to end of study by treatment group. Individual clinically significant results were to be discussed. For physical examinations, a shift table was created to describe shifts from check-in to end of study by treatment group for each body system examined. All medications, as documented by the investigator, were coded using the WHO Drug Dictionary, October 2007.¹⁹ Data listing of prior and concomitant medications were provided. Descriptive statistics were calculated for continuous demographic variables (age, height, weight, and BMI) and frequency counts were tabulated for categorical demographic variables (race and gender).

Statistical Methods

Pharmacokinetics

Serum B12 concentrations and PK parameters were summarized using descriptive statistics (mean, SD, %CV, geometric mean, N, median, minimum, and maximum). Mean serum B12 concentration-time curves were presented graphically on a linear scale (with and without SD) and semi-log scale (with and without SD). Individual serum B12 concentration-time curves were also graphically presented. A statistical analysis using a 2-sample *t* test was performed on individual values to determine the effects of SNAC on oral B12 absorption (ie, C_{max} , T_{max} , and AUC).²⁰ The significance criterion was set at 0.05. To this purpose, PK parameters from the commercial cyanocobalamin formulation were compared with the cyanocobalamin/SNAC formulation.

Tolerability

To review the safety profile, summary tables and change from baseline tables, including descriptive statistics for serum chemistry, hematology, and urinalysis, were created. Shift tables describing out-of-range shifts from baseline to postdose were also created. All ECG results were summarized using descriptive statistics and were classified as normal or abnormal, and a shift table was created to describe shifts from predose to end of study.

Descriptive statistics for vital sign measurements at each time point and change from baseline to each time point were also created. Physical examination results were reviewed by summarizing the data by a shift table, showing shifts in normal/abnormal status from check-in to end of study.

RESULTS

Demographic and Other Baseline Characteristics

Twenty healthy male subjects, aged 20 to 45 years, participated in this study. Demographic characteristics and group mean B12 baseline concentrations are shown in Table II. The mean age was 30 years, the mean weight was 76.7 kg (range, 61.3–94.4 kg), and the mean height was 174 cm (range, 163–180 cm). Body weight was between 60 and 100 kg, and the mean BMI was 25.4 kg/m² (range, 21.8–30.0 kg/m²). Regarding race, 15 subjects were black, 3 subjects were Caucasian, 1 subject was Hispanic, and 1 was European/Middle Eastern.

All subjects satisfied all inclusion and none of the exclusion criteria, with brief verification at check-in (day 1, predose).

Pilot Arm

A 10-mg cyanocobalamin/SNAC oral dose (treatment A: 2 × 5-mg cyanocobalamin/100-mg SNAC) administered prior to the other treatments was used as a pilot arm to identify an oral cyanocobalamin dose that would achieve serum concentrations of B12 greater than the endogenous baseline for a full 24-hour PK evaluation. Assuming linear PK properties, the results suggested that a 5-mg dose would be sufficient for treatments B and C in the treatment period (Table III).

Vitamin B12 Pharmacokinetics

Serum cobalamin PK parameters following treatments A, B, C, and D are shown in Table III. All values are reported as mean (SD). Treatment B had significantly greater C_{max} and AUC_{0–last} and a shorter mean T_{max} compared with the treatment C commercial formulation ($P < 0.01$). The same result was observed after baseline adjustment.

The exposure to 5-mg cyanocobalamin/SNAC (treatment B: 1 tablet of 5-mg cyanocobalamin/100-mg SNAC) and 10-mg cyanocobalamin/SNAC (treatment A: 2 × 5-mg cyanocobalamin/100-mg SNAC) appears to be dose proportional. However, because only 2 dose levels were used in this study and a different number of tablets was administered for each dose, dose proportionality should be confirmed in subsequent studies.

The mean serum B12 concentration versus time semi-log profiles for the 2 5-mg oral formulations and 1-mg IV formulation are shown in the Figure. The 5-mg cyanocobalamin/SNAC formulation achieved mean T_{max} earlier (0.50 [0.21] hours vs 6.83 [3.19] hours) and had a mean C_{max} approximately 10-fold higher than the commercial 5-mg cyanocobalamin formulation (12,847 [6613] pg/mL vs 1239 [450] pg/mL). Elimination of B12 occurred at approximately the same rate for both formulations. Mean K_e was 0.025 [0.009] and 0.028 [0.006], whereas $t_{1/2}$ was 30.06 [8.24] hours vs 25.95 [6.07] hours for treatments B and C, respectively. As noted in Table III, it was possible to estimate the elimination phase in 5 out of 6 subjects for treatment B and in 3 out of 6 subjects in treatment C.

Estimates of %F were performed for all oral treatments (A, B, and C) and are shown in Table IV. All

Table II. Participant demographic characteristics and baseline cyanocobalamin concentrations.

Parameter	Treatment				Overall
	A	B	C	D	
Gender, no.					
Male	4	6	6	4	20
Race, no.					
Black	4	3	5	3	15
Caucasian	0	1	1	1	3
European/Middle Eastern	0	1	0	0	1
Hispanic	0	1	0	0	1
Age, y					
Mean (SD)	33 (7)	29 (8)	33 (9)	24 (4)	30 (8)
Range	28-43	22-43	23-45	20-30	20-45
Weight, kg					
Mean (SD)	69.5 (5.3)	83 (11.0)	74.1 (8.5)	78.5 (13.5)	76.7 (10.5)
Range	65.0-77.0	67.1-94.4	61.3-85.0	66.0-93.3	61.3-94.4
Height, cm					
Mean (SD)	170 (6)	176 (4)	172 (5)	176 (3)	174 (5)
Range	163-175	170-180	165-178	173-180	163-180
BMI, kg/m ²					
Mean (SD)	24.0 (1.8)	26.7 (3.0)	25.1 (3.2)	25.2 (3.3)	25.4 (2.9)
Range	21.8-25.8	23.0-30.0	22.3-29.8	22.1-28.7	21.8-30.0
Cyanocobalamin baseline, pg/mL					
Mean (SD)	534 (247)	518 (193)	387 (118)	666 (294)	512 (216)
Range	341-878	267-837	218-474	408-1084	218-1084

BMI = body mass index.

values are reported as mean (SD). Estimates of %F were also performed using baseline-adjusted B12 concentrations and with or without body weight and BMI adjustments (Table IV).

Statistical comparison of B12 %F with and without baseline adjustment and either with or without body weight adjustment in Table IV indicates that 5-mg cyanocobalamin/SNAC (treatment B) had significantly greater %F compared with treatment C ($P < 0.005$). B12 %F was slightly less following baseline adjustment compared with the non-baseline-adjusted data (4.24 [1.28]% vs 1.40 [0.66]%, for baseline-adjusted treatments B and C, respectively; 5.09 [1.53]% vs 2.16 [0.78]%, for baseline-unadjusted treatments B and C, respectively). In treatment C, however, B12 non-baseline-adjusted %F was significantly greater than baseline-adjusted %F after values were normalized for BMI

(2.15% vs 1.27%; $P < 0.05$). Generally, %F was not affected by body weight or BMI adjustment.

Tolerability

There were no AEs reported in this study with any treatment. Mean clinical laboratory values, vital signs, and ECG parameters remained within normal limits at the end of study, with minimal changes from baseline and no important treatment-related differences observed. No changes in physical examination results were reported. All treatments were well tolerated by the healthy male participants, with no apparent difference in safety profile by treatment. Administration of all study treatments, including the single oral doses of cyanocobalamin/SNAC up to 10 mg/200 mg (treatment A), appeared to be well tolerated by the participants.

Table III. Pharmacokinetic parameters of non-baseline-corrected serum cyanocobalamin following treatments A, B, C, and D.

Pharmacokinetic Parameters	Treatment A*		Treatment B†		Treatment C‡		Treatment D§	
	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	n
C_{max} , pg/mL	28,175 (13,681)	4	12,847 (6613)	6	1239 (450)	6	221,287 (80,248)	4
T_{max} , h	0.54 (0.32)	4	0.50 (0.21)	6	6.83 (3.19)	6	0.05 (0.03)	4
AUC_{0-last} , pg/mL/h	127,494 (65,790)	4	54,609 (16,405)	6	23,165 (8382)	6	214,738 (44,614)	4
$AUC_{0-\infty}$, pg/mL/h							235,165 (43,854)	3
K_e , 1/h	0.03 (0.010)	3	0.025 (0.009)	5	0.028 (0.006)	3	0.048 (0.018)	3
$t_{1/2}$, h	25.31 (8.8)	3	30.06 (8.24)	5	25.95 (6.07)	3	15.53 (4.70)	3

SNAC = sodium N-[8-(2-hydroxybenzoyl)amino]caprylate.

*Treatment A: 2 × 5-mg cyanocobalamin/100-mg SNAC tablets.

†Treatment B: 1 × 5-mg cyanocobalamin/100-mg SNAC tablet.

‡Treatment C: 1 × 5-mg cyanocobalamin tablet.

§Treatment D: 1-mg cyanocobalamin IV (1 mg/mL solution).

||Values missing or not reportable.

DISCUSSION

A tablet formulation of cyanocobalamin containing SNAC, an absorption enhancer, (cyanocobalamin/

SNAC), was compared with a marketed immediate-release tablet containing the same quantity of cyanocobalamin (5 mg) in a small single-dose study in

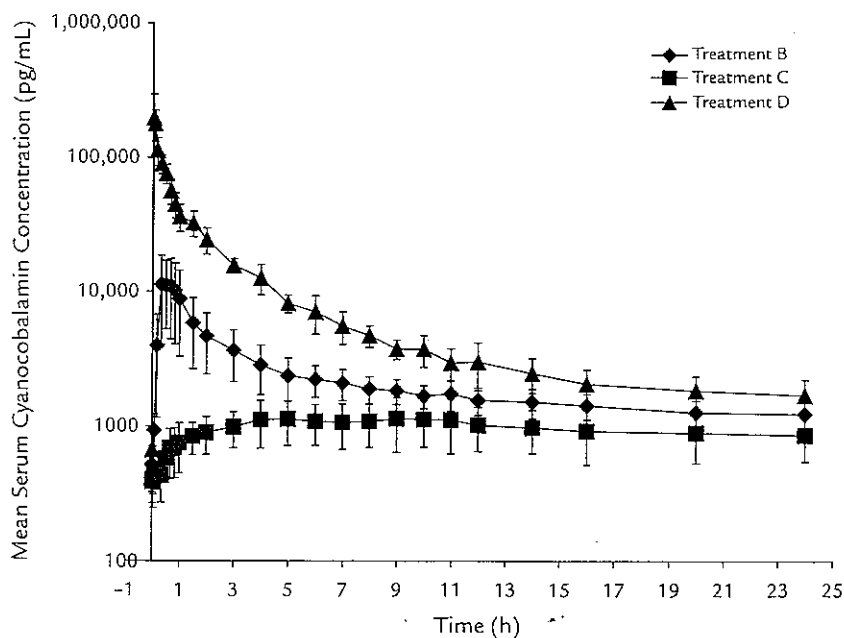


Figure. Time course of mean (SD) serum concentrations of treatments B, C, and D administered to healthy male subjects (logarithmic scale).

Table IV. Treatment B versus treatment C percent bioavailability (%F) non baseline-adjustment.

Parameter	Treatment B (n = 6)	Treatment C (n = 6)
%F (AUC _{last})	5.09	2.16
T Test value	4.18	
P-value	0.0019	
%F (AUC _{last_D})	5.5	2.02
T Test value	4.36	
P-value	0.0014	
%F (AUC _{last_BMI})	4.65	2.15
T Test value	4.02	
P-value	0.0044	

%F (AUC_{last})- Percent bioavailability without body weight adjustment.

%F (AUC_{last_D})- Percent bioavailability with body weight adjustment.

%F (AUC_{last_BMI})- Percent bioavailability with BMI adjustment.

normal healthy male subjects. This study found that SNAC enhanced the absorption of cyanocobalamin. The cyanocobalamin/SNAC formulation also resulted in a shorter T_{max} compared with the commercial cyanocobalamin formulation. The group mean T_{max} was 0.5 hours for cyanocobalamin/SNAC and 6.8 hours for the commercial cyanocobalamin formulation. In addition, cyanocobalamin/SNAC mean peak B12 concentrations were >10-fold higher (12,847 pg/mL vs 1239 pg/mL). The calculated $t_{1/2}$ of B12 was similar with both formulations. Absolute bioavailability of B12 with the SNAC formulation was calculated from non-baseline-adjusted data to be 5.09% and was significantly greater ($P < 0.05$) than the commercial formulation (2.16%). There were no tolerability-related findings in the study.

The study population consisted of 75% black males and no women. Studies of ethnic differences in use of cobalamin and its metabolism conducted in the United States^{21,22} have indicated that blacks have significantly higher endogenous cobalamin levels than Caucasians, and a trend toward higher endogenous cobalamin levels in female subjects has also been reported. The design of the present study did not evaluate the impact of gender and ethnicity of the subjects. How-

ever, the PK response to both treatments in the present study exceeded baseline values in all cases for the duration of the study and clearly demonstrated differences in bioavailability. Individual B12 predose values ranged from 218 to 1084 pg/mL in this small and selected healthy subject study population. The choice to test only 1 gender was made to limit variability, given the small sample size.

The oral cyanocobalamin dose of 5 mg used in the control group (treatment C) resulted in B12 %F of slightly more than 2% in the present healthy male and predominantly black population. To our knowledge, this is the first study reporting %F of oral formulations. A study conducted in 1968 reported 1.2% bioavailability in a wide range of cyanocobalamin doses (100 μ g-100 mg) in a Swedish population.³ In this paper the method used to calculate bioavailability was based on the relationship between dose and urinary excretion of radioactive B12.

SNAC has reportedly low toxicity for animals. In a 13-week general toxicity study in rats, a no-adverse-effect level of 1000 mg/kg has been reported.⁹

The specific mechanism of action of SNAC enhancement of cyanocobalamin absorption has not been studied. However, studies with several different polar drug molecules have shown a single pattern of SNAC action, which may also be relevant to cyanocobalamin. Laboratory studies of insulin,²³ heparin,²⁴ cromolyn,²⁵ and human growth hormone²⁶ when coformulated with SNAC have shown that the carrier aids gastrointestinal absorption by fluidization of gastrointestinal epithelial cell membranes and transcellular uptake of both drug and carrier. Membrane fluidization by SNAC is concentration dependent and reversible.

Clinical studies of SNAC administered with heparin resulted in enhanced heparin absorption and rapid onset of action as measured by pharmacodynamic response (activated factor Xa).¹¹ The T_{max} of both heparin and SNAC was measured in healthy male subjects following a single dose of SNAC/heparin.²⁷ The T_{max} values for SNAC and heparin were similar at 0.50 hours and 0.58 hours, respectively.

Both oral and intramuscular routes of administration of B12 supplementation are in clinical use. In most countries, including the United States, intramuscular treatment is the classical treatment for B12 deficiency.² Oral treatment is more convenient and less expensive to administer, but questions remain

about its reliability.²⁸ Based on a search of MEDLINE, there appear to be no other reports of effective enhancement of oral B12 delivery with permeation enhancers.

Further controlled clinical studies in B12-deficient patients of cyanocobalamin/SNAC and current B12 replacement regimens are needed to establish the effectiveness of this new approach to normalizing serum B12 and the major biomarkers of B12 deficiency, methylmalonic acid and homocysteine.² The present study was designed with a small group size of 4 to 6 mainly black males and, therefore, confirmatory studies should be conducted with a larger and more diverse population.

A single measurement of baseline serum vitamin B12 was taken from the members of the study population. In similar studies involving the PK analysis of endogenous substances, several measurements of baseline values are recommended to improve accuracy.¹⁷ However, a single measurement is adequate when doses are high and the concentrations produced clearly exceed baseline, as in the present study.

Crystalline B12 has an oral bioavailability of ~1% over a wide dose range.³ Dose proportionality of the test formulation has not been established. However, in the present study, doses of 5 and 10 mg resulted in an approximate doubling of AUC and C_{max} (Table III).

Changes in the PK profile of crystalline B12 when dosed in the test formulation consisted of an earlier T_{max} and greater bioavailability compared with the conventional oral formulation. Similar PK profiles have been observed with other drug formulations using the same technology.²³⁻²⁷ However, studies to determine the changes, if any, to B12 site of absorption and uptake mechanism, both potentially important considerations for a new B12 oral therapy, have not been performed.

CONCLUSIONS

An oral formulation of cyanocobalamin (5 mg) containing SNAC (100 mg), an absorption enhancer, provided significantly improved bioavailability and a significant decrease in time to T_{max} in a small study of normal, healthy male subjects compared with a commercially available oral formulation (5 mg cyanocobalamin). Both oral formulations and an IV cyanocobalamin formulation (administered at a 1-mg dose), were well tolerated.

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Carol Thomas-Sharp monitored the study; Karen Brazzillo and Kiran Chaudhary were responsible for project management. Moses Oyewumi, Matt Gurler (deceased), Jun Liao, and Prateek Bhargava contributed with clinical trial dose preparation. James Sherry, MD, PhD, was product safety physician. Joe Fotso reviewed the validation report for B12 analysis from MDS Pharma Services. MDS Pharma Services conducted this clinical study, performed the bioanalytical determinations, and wrote the study report.

Dr. Castelli and Ms. Wong designed the study, reviewed the pharmacokinetics and safety analysis, contributed to the interpretation of data, and drafted the manuscript. Ms. Friedman monitored the study. Dr. Riley made substantial conceptual contributions and revisions.

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