Effect of Administration Route on the Pharmacokinetics of Cobalamin in Elderly Patients: A Randomized Controlled Trial

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ABSTRACT

Background: The gold standard for cobalamin deficiency treatment is administration of cobalamin by intramuscular injection. The injection is painful and inconvenient, particularly for elderly persons. Cobalamin might also be administered intranasally. Previous studies do not provide insight into the pharmacokinetics of intranasal cobalamin administration in comparison with cobalamin injection.

Aim: To quantify the pharmacokinetics of intranasal and intramuscularly administered cobalamin to determine if intranasal administration might be an alternative for intramuscular administration.

Methods: Ten inpatients and outpatients of a geriatrics unit were recruited and randomly assigned to receive a single dose of 1000 μg cobalamin administered either by intranasal spray or intramuscular injection (5 per group). Inclusion criteria were written informed consent, age > 65 years, and a cobalamin serum concentration < 200 pmol/L. Total cobalamin serum concentrations were determined 10 times within 48 hours after administration. The differences in Cmax, Tmax, and AUC0–48 h per administration route were statistically compared using ANOVA.

Results: The average Cmax was 1 nmol/L after intranasal and 38.5 nmol/L after intramuscular administration. The average Tmax for intranasal and intramuscular administration was 42 minutes versus 342 minutes, respectively, and the AUC0–48 h was 1.3 μmol/L/min versus 45.4 μmol/L/min, respectively. These values also differed significantly (P < 0.05). The estimated bioavailability of the intranasal administration was 2%.

Conclusions: The pharmacokinetics of intranasal and intramuscular cobalamin administration in elderly, cobalamin-deficient patients differ significantly. However, the estimated 2% bioavailability of cobalamin after intranasal administration makes intranasal cobalamin administration a potentially interesting administration route for elderly patients. Netherlands Trial Registry identifier: NTR 3005.

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Introduction

Cobalamin deficiency in human beings may take 3 to 4 years to develop due to the large cobalamin stores in the liver. Nevertheless, approximately 10% to 15% of the population aged 65 years and older has cobalamin (vitamin B12) deficiency. An even higher prevalence of 30% to 40% is reported for malnourished and sick elderly people. Cobalamin serves as a cofactor for methionine synthase and L-methylmalonyl-coA mutase. People with cobalamin deficiency may experience megaloblastic anemia, subacute combined degeneration of the spinal cord, peripheral polynuropathy, cognitive impairment, and mental changes. Cobalamin deficiency is due to malabsorption of food-bound cobalamin and/or insufficient dietary intake. Food-bound cobalamin is released by pepsin in the acidic environment in the stomach. Subsequently, cobalamin is bound to intrinsic factor in the duodenum. The cobalamin-intrinsic factor complex then binds to the ileal endocytic cubam receptor. The cubam receptor consists of 2 proteins: cubulin and amnionless. Together these proteins take part in the endocytosis of the intrinsic factor-cobalamin complex, which is followed by degradation of intrinsic factor in lysosomes and the release of cobalamin into plasma in complex with transcobalamin II. Cobalamin malabsorption can therefore be caused by lack of intrinsic factor, decreased pepsin or acid secretion, or other defects in the cobalamin uptake system.

Repeated administration of cobalamin by way of intramuscular injection has been the gold standard for cobalamin deficiency...
treatment for many years. These intramuscular injections have several disadvantages. Injections are painful; injection-related adverse reactions (such as intramuscular hematoma) may occur, especially in elderly persons who are frequently treated with anticoagulant medication; and health care professionals are usually needed to administer the injections. The latter increases the burden of the treatment considerably. A more convenient and safer treatment would be advantageous to both patients and the health care system in general.

Food-bound cobalamin is actively absorbed. In contrast, crystalline cyanocobalamin administered via capsules is absorbed by way of passive diffusion. Although approximately only 1% of an oral dose of crystalline cyanocobalamin is absorbed, oral administration of cyanocobalamin is effective in normalizing serum cobalamin levels.6 Oral treatment might not be an option in patients who have an absorption disorder or are unable to take oral medication.8–10 Intranasal administration of cobalamin could be a suitable alternative to both cobalamin injections and oral administration in elderly patients.

Absorption of intranasally administered cobalamin has been demonstrated in studies.11–14 However the results of those studies do not provide insight into the pharmacokinetics of intranasally administered cobalamin in elderly, cobalamin-deficient patients or in comparison with cobalamin injection. Insight into the pharmacokinetics is necessary to determine if intranasal administration could be an alternative to intramuscular administration in elderly patients. Also, insight into the pharmacokinetics could be used to develop a dosing regimen. The objective of our pilot study was to quantify the pharmacokinetics of intranasally and intramuscularly administered cobalamin in elderly, cobalamin-deficient patients to determine if intranasal administration might be an alternative for intramuscular administration.

Materials and Methods

Design, setting, and study population

Ours was a randomized, open, comparative pilot study conducted between September 2011 and January 2012 in Haarlem, the Netherlands. Patients were recruited from the in- and outpatient geriatrics department of the Kennemer Gasthuis, a teaching hospital with a total of 450 beds of which 15 are geriatrics beds (3250 geriatric outpatient visits a year). Cobalamin serum concentration measurements are part of standard care at this clinic. Eligible patients were aged 65 years or older who had a cobalamin serum concentration <200 pmol/L. Patients also had to be able to give written informed consent. Patients with chronic rhinitis, a running nose, or concomitant use of intranasally administered medication were excluded from study participation to minimize possible influences on intranasal absorption. In addition, patients who were hemodynamically unstable, had clinically significant infections, or were severely malnourished were excluded from participation for ethical reasons. Simplified Nutritional Appetite Questionnaire was used to determine a patient’s nutritional status.15 Patients with a score of 3 were excluded. Patients with a glomerular filtration rate ≤20 mL/min/1.73 m² were also excluded from study participation, because cobalamin is exclusively excreted through the kidneys. The study protocol (NL33450.029.11) was approved by the Medical Ethics Committee of the Vrije Universiteit Amsterdam and the Kennemer Gasthuis Haarlem.

Randomization and intervention

Patients were randomly assigned to 1 of the 2 study groups, to receive a single dose of 1000 μg cobalamin administered by intranasal spray or by intramuscular injection. A research nurse administered either 2 mL of a 500 μg/mL hydroxocobalamin solution for injection (Hydrocobamin, Nycomed BV, Hoofddorp, the Netherlands) in the muscle of the upper left or right arm, or 1 puff (0.1 mL) 500 μg cyanocobalamine (Nascobal, Par Pharmaceutical Companies Inc, Woodcliff Lake, New Jersey) in each nostril. The intranasal spray was primed before each administration. Blood samples were obtained at 0, 15, 30, 60, 120, 240, 480, 1440 (24 hours), and 2880 minutes (48 hours) following administration. These blood samples were drawn using an intravenous cannula inserted into a forearm vein. The cannula was inserted in the arm not used for injection if the patient received the cobalamin by way of intramuscular injection.

Analysis

Total cobalamin serum concentrations were determined using a competitive immunoassay vitamin B₁₂ kit with a measuring range between 22 and 1476 pmol/L (Roche Diagnostics GmbH, Mannheim, Germany) on a Roche Modular Analytics E170 immunoassay analyzer (Roche Diagnostics GmbH, Mannheim, Germany). The mean coefficient of variation in human serum of this assay is 1.9% for repeatability and 4.7% for intermediate precision. Samples were diluted with Elecsys Diluent Universal (Roche Diagnostics GmbH, Mannheim, Germany) in case the cobalamin serum concentration exceeded the upper range of the assay.

A concentration-time graph was constructed for each patient using PK Solutions (version 2.0, Summit Research Services, Montrose, California). For each patient Cₘₐₓ, Tₘₐₓ, and AUC₀–₄₈ h were determined using PK Solutions. The average of Cₘₐₓ, Tₘₐₓ, and AUC₀–₄₈ h for the group receiving cobalamin intranasally and the group receiving cobalamin intramuscularly were also calculated using PK Solutions. Bioavailability of the intranasal administration was estimated by dividing the mean AUC₀–₄₈ h after intranasal administration by the mean AUC₀–₄₈ h after intramuscular administration.

In our pilot study a sample size of 10 was presumed sufficient to establish if intranasal administration could be a potential alternative for intramuscular administration in elderly patients. Data were tested for deviations from a normal distribution. Subsequently differences between both groups of patients in Cₘₐₓ, Tₘₐₓ, and AUC₀–₄₈ h were tested for significance using ANOVA. The ANOVA was done using PASW Statistics (version 18, IBM-SPSS Inc, Armonk, NY). A P value ≤0.05 was considered statistically significant.

Results

Thirteen patients gave written informed consent. Three patients were excluded from the final analysis. One patient did not receive the study medication because the intravenous cannula could not be inserted. Analyses of blood samples of 2 patients failed due to incorrect handling. Ten patients were therefore included in the final analysis. The mean age of patients was 81 years (range = 70–91 years), the mean weight was 76 kg (range = 60–93 kg), and the mean baseline cobalamin serum concentration was 165 pmol/L (range = 100–250 pmol/L). Five patients received cobalamin by way of intranasal spray and 5 patients by way of intramuscular injection. Between the intranasal and intramuscular groups there were no significant differences in mean baseline cobalamin serum concentration, body mass index, and age (Table I).

The concentration-time graphs of the different patients following intranasal and intramuscular administration are presented in Figure 1. Similar overall changes in serum cobalamin concentration over time were determined among patients in the intranasal administration group (Figure 1A). The Cₘₐₓ varied between 0.5 and
2 nmol/L and the time to maximum concentration was comparable among the patients with minor differences in \( T_{\text{max}} \). The AUC_{0-48 h} among patients varied between 0.67 and 2 \( \mu \text{mol/L/min} \).

Patients in the intramuscular administration group did not show similar overall changes in serum cobalamin concentration over time (Figure 1B). The maximum concentration in this group also varied between 22 and 72 nmol/L. Contrary to the intranasal administration group, the \( T_{\text{max}} \) varied considerably among these patients, ranging between 60 and 480 minutes. Patients in this group had AUC_{0-48 h} that varied noticeably between 8.7 and 76 \( \mu \text{mol/L/min} \).

The average concentration-time graphs after intranasal and intramuscular administration are presented in Figure 2. After intranasal administration a significantly \((P < 0.05) \) lower C_{max} (ie, 1 vs 38.5 nmol/L) was determined compared with intramuscular administration. Cobalamin absorption was significantly \((P < 0.05) \) faster following intranasal administration (ie, \( T_{\text{max}} \) 42 vs 324 minutes, respectively). The bioavailability of cobalamin after intranasal administration was estimated to be 2% compared with intramuscular administration. AUC_{0-48 h} was significantly \((P < 0.05) \) smaller after intranasal compared with intramuscular administration. Both the intranasal and intramuscular administrations were well tolerated. No adverse events were reported in either administration group.

### Table I

Baseline characteristics of patients receiving intranasal and intramuscular cobalamin administration.

|                          | Intranasal administration (range) | Intramuscular administration (range) |
|--------------------------|----------------------------------|-------------------------------------|
| Cobalamin concentration (pmol/L) | 160 (100-200)                  | 170 (110-250)                      |
| Body mass index          | 26 (23.5-32.9)                  | 24 (23.5-24.9)                     |
| Age, y                   | 83 (78-91)                      | 78 (70-84)                         |
| Gender, male/female      | 4/1                             | 2/3                                |

Figure 1. Concentration-time graphs of patients receiving 1000 \( \mu \text{g} \) cobalamin by way of (A) intranasal or (B) intramuscular administration.
Discussion

In our pilot study, significant pharmaco-kineti-c differences between intranasal and intramuscular cobalamin administration in elderly, cobalamin-deficient patients were established. After intranasal administration the Cmax and AUC0-48h were lower, and Tmax was reached faster, compared with intramuscular adminis-tration. The estimated cobalamin bioavailability after intranasal administration was 2%.

Our study has several limitations. Only 10 frail elderly patients were included because of the invasive nature of the study. Also, a crossover design was considered unethical. Because cobalamin levels can be raised for a long period of time—especially after intramuscular injection—a wash-out period of 2 to 3 months would have been necessary in a crossover design to rule out influences from 1 administration upon the other. During this period patients would not have been treated properly for their cobalamin deficiency.

Furthermore, 2 different cobalamin forms (ie, hydroxocobalamin [injection] and cyanocobalamin [nasal spray]) were used in our study. Cyanocobalamin intranasal spray was used because there was no registered hydroxocobalamin intranasal spray available. Although the molecular structures of hydroxocobalamin and cyanocobalamin are almost identical, hydroxocobalamin is believed to be better retained by the body due to better receptor binding. A better availability to cells has not been established and both forms are registered for cobalamin deficiency treatment.16,17

The immunoassay used to measure cobalamin serum concentrations measures total serum cobalamin—both protein-bound and free cobalamin—and does not differentiate between hydroxocobalamin and cyanocobalamin. The bioavailability of the cobalamin injection is assumed to be 100%. The bioavailability of intranasally administered cobalamin in our study was estimated to be 2%, which is comparable to previous studies, in which a bioavailability of 2% and 5% were found.11

In our study, vast interpatient differences were identified within both the intranasal and the intramuscular administration group. Interver-satility variability was expected for intranasal adminis-tration due to differences in nasal mucosa and mucociliary clearance. The large interpatient differences found in the intramuscular administration group were unexpected. As can be seen in Figure 1B, cobalamin absorption following intramuscular administration differed among the patients. This might have been caused by changes in muscle mass due to aging.18

Here, a serum cobalamin cut-off value of 200 pmol/L was used to identify a cobalamin deficiency. Generally a serum cobalamin concentration <148 pmol/L is used to identify cobalamin deficiency. Because elderly persons may already be cobalamin deficient while having a cobalamin serum concentration >148 pmol/L, other serum cobalamin cut-off values for elderly persons have been proposed.19-21

An average Cmax of 38,500 pmol/L was established after intramuscular cobalamin administration. The therapeutic cobalamin serum concentration range is generally considered to be between 148 and 664 pmol/L. The high cobalamin serum concentrations following intramuscular cobalamin administration seem to be of little clinical significance. In several studies daily oral administration of even low cobalamin doses have been shown to be effective in normalizing cobalamin serum concentrations.22-26 Some studies have also demonstrated oral cobalamin administration to be at least as effective as intramuscular administration for treatment of cobalamin deficiency.27,28 Like intranasal administration, the maximum concentration after oral administration is lower compared with intramuscular administration. In a recent study a Cmax of 100 pmol/L was reached after a single oral cobalamin dose of 1000 μg; this is comparable to the average Cmax of 1040 pmol/L after intranasal administration established in this study.29 The bioavailability of cobalamin after oral administration is approxi-mately 1 %, which is also comparable to the bioavailability after intranasal administration found in this study.

We compared intranasal cyanocobalamin and intramuscular hydroxycobalamin administrations to gain insight into the pharmaco-kineti-cs, to determine if intranasal cyanocobalamin administration could be an alternative for intranasal administration. Absorption of intranasally administered cobalamin has been examined in healthy elderly volunteers and in patients with ileal resections or Crohn’s disease. Reproducible nasal hydroxycoba-lamin absorption has been shown in these studies.11,12 Similarly, our study shows reproducible nasal absorption of a single dose of 1000 μg cyanocobalamin in elderly, cobalamin-deficient patients. Also, the Tmax of 42 minutes determined in our study is comparable to the Tmax of 35 minutes found in healthy elderly volunteers.

Figure 2. Average concentration time curves following intranasal and intramuscular cobalamine administration, respectively. Following intranasal administration, mean (SD) Tmax was 42 (16) minutes, Cmax was 1.0 (0.6) nmol/L, and AUC0-48h was 1.3 (0.6) μmol/L/min. Following intramuscular administration, mean (SD) Tmax was 324 (215) minutes, Cmax was 38.5 (20.5) nmol/L, and the AUC0-48h was 61 (19) μmol/L/min.

![Figure 2](image-url)
Conclusions

Despite its limitations our study shows that there are significant pharmacokinetic differences between intranasal and intramuscular cobalamin administration. The bioavailability of 2% after intranasal administration is comparable to the bioavailability after oral administration. Intranasal cobalamin administration was well tolerated and no adverse events were reported. Hence, intranasal administration seems a potentially interesting alternative to intramuscular cobalamin administration. Additional research is needed to assess the pharmacokinetics of repeated intranasal cobalamin administration to determine an intranasal dosing regimen. A clinical study will also have to demonstrate efficacy and safety of repeated intranasal cobalamin administration in cobalamin-deficient elderly persons.

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Conflicts of Interest

The authors have indicated that they have no conflicts of interest regarding the content of this article.

References

1. Wolters M, Ströhle A, Hahn A. Cobalamin: a critical vitamin in the elderly. Prev Med. 2004;39:1256–1266.
2. André E, Loukil NH, Noel E, et al. Vitamin B₁₂ (cobalamin) deficiency in elderly patients. Can Med Assoc J. 2004;171:251–259.
3. Dali-Youcef N, Andrès E. An update on cobalamin deficiency. Crit Rev Clin Lab Sci. 2005;42:515–528.
4. Carmel R. Malabsorption of food cobalamin. Baillieres Clin Gastroenterol. 1995;9:833–854.
5. Walraven van C, Austin P, Naylor CD. Vitamin B₁₂ injections versus oral supplements. How much money could be saved by switching from injections to pills? Can Fam Physician. 2001;47:79–86.
6. Vidal-Alaball J, Butler C, Cannings-John R, et al. Oral vitamin B₁₂ versus intramuscular vitamin B₁₂ for vitamin B₁₂ deficiency (Review). Cochrane Database Syst Rev. 2005;20:CD004655.
7. André E, Forberg-Hill H, Meceli M. Efficacy of oral cobalamin (vitamin B₁₂) therapy. Expert Opin Pharmacother. 2010;11:249–256.
8. Singh S, Handy S. Dysphagia in stroke patients. Postgrad Med J. 2006;82:383–391.
9. Kalf JG, De Swart B, Bloem BR, Munneke M. Prevalence or oropharyngeal dysphagia in Parkinson’s disease: a meta-analysis. Parkinsonism Relat Disord. 2012;18:311–315.
10. Vavricka SR, Rogler G. Intestinal absorption and vitamin levels: is a new focus needed? Dig Dis. 2012;30(Suppl 3):73–80.
11. Van Asselt D, Merkus F, Russel F, Hoefnagels W. Nasal absorption of hydroxocobalamin in healthy elderly adults. Br J Clin Pharmacol. 1998;45:83–86.
12. Bruins Slot W, Merkus F, Van Deventer S, Tygat G. Normalization of plasma vitamin B₁₂ concentration by intranasal hydroxocobalamin in vitamin B₁₂-deficient patients. Gastroenterology. 1997;113:430–433.
13. Monto RW, Rebeck J. Observations on the mechanism of intranasal absorption of vitamin B₁₂ in pernicious anemia. Blood. 1955;10:1151–1155.
14. Kuy Van der P-HM, Merkus FWJM, Lohman JJHM, et al. Hydroxocobalamin, a nitric oxide scavenger, in the prophylaxis of migraine: an open, pilot study. Cephalalgia. 2002;22:513–519.
15. Wilson MM, Thomas DR, Rubenstein LZ, et al. Appetite assessment: simple appetite questionnaire predicts weight loss in community-dwelling adults and nursing home residents. Am J Clin Nutr. 2005;82:1074–1081.
16. Hall CA, Begley JA, Green-Collogan PD. The availability of therapeutic hydroxocobalamin to cells. Blood. 1984;63:335–341.
17. Herbert V, Zalusky R, Skegg HS. Retention of injected hydroxocobalamin versus cyanocobalamin versus liver extract-bound cobalamin. Am J Clin Nutr. 1963;12:445–450.
18. Doherty TJ. The influence of aging and sex on skeletal muscle mass and strength.Curr Clin Nutr Metab Care. 2001;4:503–508.
19. Wiersinga W, De Rooij S, Huijmans J, et al. Diagnosis of Vitamin B₁₂ deficiency revised [in Dutch]. Ned Tijdschr Geneeskd. 2005;149:2789–2794.
20. Lindenbaum J, Rosenberg IH, Wilson PW, et al. Prevalence of cobalamin deficiency in the Framingham elderly population. Am J Clin Nutr. 1994;60:2–11.
21. Snow CF. Laboratory diagnosis of Vitamin B₁₂ and folate deficiency. A guide for the primary care physician. Arch Intern Med. 1999;159:1289–1298.
22. Seal RC, Metz J, Flicker L, Meloy J. A randomized, double blind, placebo-controlled study of oral vitamin B₁₂ supplementation in older patients with subnormal or borderline serum vitamin B₁₂ concentrations. J Am Geriatr Soc. 2002;50:146–151.
23. Andres E, Kaltenbach G, Noel E, et al. Efficacy of short-term oral cobalamin therapy for the treatment of cobalamin deficiencies related to food-cobalamin malabsorption: a study of 30 patients. Clin Lab Haematol. 2003;25:161–166.
24. Blacher J, Czernichow S, Raphael M, et al. Very low oral doses of vitamin B₁₂ increase serum concentrations in elderly subjects with food-bound vitamin B₁₂ malabsorption. J Nutr. 2007;137:373–378.
25. Eusen SJPM, Groot de LCPGM, Clarke R, et al. Oral cyanocobalamin supplementation in older people with vitamin B₁₂ deficiency. A dose finding trial. Arch Intern Med. 2005;165:1167–1172.
26. Dhonukshe-Rutten RAM, Zutphen van M, Groot de LCPGM, et al. Effect of supplementation with cobalamin carried either by milk product or a capsule in mildly cobalamin-deficient elderly Dutch persons. Am J Clin Nutr. 2005;82:568–574.
27. Bolaman Z, Kadikoylu G, Yukelsen V, et al. Oral versus intramuscular cobalamin treatment in megaloblastic anemia: a single-center, prospective, randomized, open-label study. Clin Ther. 2003;25:3124–3134.
28. Kuzminski AM, Del Giacco EJ, Allen RH, et al. Effective treatment of cobalamin deficiency with oral cobalamin. Blood. 1998;92:1191–1198.
29. Castelli C, Wong D, Friedman K, Riley G. Pharmacokinetics of oral cyanocobalamin formulated with sodium N-[8-(2 hydroybenzoyl)amino]caprylate (SNAC): an open-label, randomized, single-dose, parallel-group study in healthy male subjects. Clin Ther. 2011;33:934–945.