Normalization of Plasma Vitamin B_{12} Concentration by Intranasal Hydroxocobalamin in Vitamin B_{12}–Deficient Patients

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Background & Aims: **Patients with previous stomach and terminal ileum resections are often treated with intramuscular vitamin B_{12} injections. Disadvantages are, on a worldwide scale, the frequent need for medical personnel to administer injections and the sometimes painful way of application. This study was designed to investigate the feasibility of intranasal hydroxocobalamin suppletion in cobalamin-deficient patients and to assess whether intranasal hydroxocobalamin application could be an alternative for intramuscular injection.** ***Methods:*** **Six patients with plasma cobalamin concentrations of <200 ng/L were recruited. A dose of 1500 μg hydroxocobalamin was applied intranasally at days 0, 14, and 21. Plasma cobalamin concentrations were determined 1 hour after hydroxocobalamin application and on days 0, 7, 21, 28, and 35.** ***Results:*** **All patients showed substantial increase of cobalamin concentrations 1 hour after intranasal application. In these 6 patients, there was an eightfold increase of mean baseline cobalamin concentrations. All patients showed a sustained increase of baseline cobalamin concentrations 1 week after prior intranasal application of hydroxocobalamin. No side effects were noted.** ***Conclusions:*** **Intranasal application of hydroxocobalamin in cobalamin-deficient patients results in fast nasal absorption and leads to sustained increase of baseline cobalamin concentrations.**

Vitamin B_{12} or cobalamin mainly exists as hydroxocobalamin, methylcobalamin, and adenosyl cobalamin. These forms of vitamin B_{12} are almost entirely found in animal tissues. Meat, milk, egg, and fish are good sources of vitamin B_{12}. Approximately 1–2 μg/day are required to provide our normal needs. Vitamin B_{12} is important for the function of bone marrow, central nervous system, and gastrointestinal tract. It is involved in fat, protein, and carbohydrate metabolism. Vitamin B_{12} deficiencies can originate from insufficient intake of cobalamins, from insufficient deliverance of food-related cobalamins because of lack of gastric acid, from absent or abnormal intrinsic factor production by the stomach, or from pancreatic insufficiency that leads to inadequate breakdown of R protein–cobalamin complex in the duodenum. Vitamin B_{12} deficiencies can also develop in patients who lack or have diseased parts of the terminal ileum,[1] during bacterial overgrowth or in patients with Zollinger–Ellison syndrome. The prevalence of cobalamin deficiency in the general population is not known. From the Framingham study, we do know that a well-defined ambulatory elderly population showed cobalamin deficiency in nearly 12% of the cohort.[2] It is thought that atrophic gastritis plays a role in the pathogenesis of this cobalamin deficiency. After resection of stomach or terminal ileum, cobalamin deficiency often ensues, necessitating suppletion by intramuscular injections of hydroxocobalamin. Major drawbacks of intramuscular injections are the general need for medical personnel on a wordwide scale and the fact that they are sometimes painful. A more convenient and less costly alternative treatment would be advantageous to health care and could improve the compliance of the patient. Recently, a formulation for nasal administration of hydroxocobalamin has been developed. It provides a fast nasal absorption of hydroxocobalamin with a maximum plasma cobalamin level reached within 30–60 minutes.[3] This study was designed to investigate the feasibility of vitamin B_{12} suppletion by using intranasal application of hydroxocobalamin in cobalamin-deficient patients and to assess whether intranasal hydroxocobalamin application could be an alternative for the monthly parenteral administration scheme.

Materials and Methods

Patients

Six patients were recruited from the outpatient clinic. Four patients with vitamin B_{12} deficiency had undergone ileal

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resections (length of ileal resection varied from 40 to 90 cm) in the past because of Crohn's disease, 1 patient had Crohn's disease and recurrent inflammatory activity of the terminal ileum, and 1 patient had small bowel abnormalities caused by chronic radiation enteritis. All patients had an initial plasma cobalamin level of <200 ng/L. During the study period, all patients with Crohn's disease were in remission. Patients with prior nasal surgery or with nasal diseases and patients who used transnasal medication were excluded.

Study Design

The nasal hydroxocobalamin contained 750 μg hydroxocobalamin per 70 μL preserved solution. The metered dose spray device used was obtained from Valois (VP 7/70, Merli le Roy, France). The investigator administered a dose of 1500 μg (one puff of 70 μL in each nostril) hydroxocobalamin intranasally at days 0, 14, and 21. Plasma cobalamin concentrations were determined by radioimmunoassay (Solid Phase No Boil Dualcount Kit; Diagnostic Products Corp., Los Angeles, CA) at days 0, 7, 21, 28, and 35 after the first intranasal application. Plasma cobalamin levels were also assessed 1 hour after each application of hydroxocobalamin in all patients. Normal plasma cobalamin levels were defined between 200 and 700 ng/L. Plasma cobalamin concentrations could be accurately determined up to 2400 ng/L. All values of >2400 ng/L are presented as 2400 ng/L.

Results

Plasma Cobalamin Concentrations After Intranasal Hydroxocobalamin Application

Figure 1 shows increases of baseline cobalamin concentration before and 1 hour after nasal application of hydroxocabalamin at days 0, 14, and 21. In all patients, there was a large increase in cobalamin concentrations 1 hour after intranasal hydroxocobalamin application. The mean baseline cobalamin concentration before hydroxocobalamin application on days 0, 14, and 21 was 199 ng/L, and the mean cobalamin concentration 1 hour after intranasal administration was 1713 ng/L. The application of hydroxocobalamin caused approximately an eightfold increase of mean baseline cobalamin concentrations.

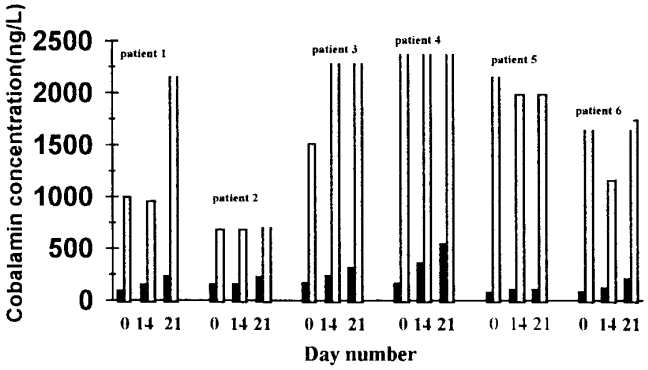

Figure 1. Increase of plasma cobalamin concentration 1 hour after 1500 μg of intranasal hydroxocobalamin on days 0, 14, and 21 in 6 patients. ■, Before 1500 μg of hydroxocobalamin; □, after 1500 μg of hydroxocobalamin.

Table 1. Plasma Cobalamin Concentrations Before Intranasal Administration of 1500 μg Hydroxocobalamin on Days 0, 7, 14, 21, 28, and 35

Patient	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35
1	96	172	153	232	306	211
2	158	186	157	230	250	231
3	173	259	243	321	433	337
4	168	367	366	546	635	475
5	81	130	108	134	172	154
6	83	150	120	211	229	195
Mean (n = 6)	127	211	191	279	338	267
SD	47	97	107	159	190	129

Long-term Effect of Three Consecutive Intranasal Doses of Hydroxocobalamin on Plasma Cobalamin Concentrations

Table 1 shows plasma cobalamin concentration at days 0, 7, 14, 21, 28, and 35 during application of 3 × 1500 μg intranasal hydroxocobalamin on days 0, 14, and 21. All patients showed a sustained increase of baseline plasma cobalamin concentration measured 1 week after intranasal application of 1500 μg hydroxocobalamin. The mean increase at day 7 was 84 ng/L, at day 21, 88 ng/L, at day 28, 59 ng/L. Figure 2 shows mean plasma cobalamin concentrations with SD in 6 patients at days 0, 7, 14, 21, and 28. The nasal administration of hydroxocobalamin was well tolerated, and no signs of irritation or nasal sensitivity were noted.

Discussion

To our knowledge, this is the first report to document nasal hydroxocobalamin absorption in vitamin B_{12}–deficient patients. Despite the increase in the use of nasal formulation for systemic drug delivery, much still has to be learned about the exact mechanism of nasal drug absorption. The absorption of water-soluble compounds is dependent on several factors, including contact time with the nasal mucosa and the molecular size of the compound. An inverse relationship has been established between the molecular weight of a drug and the proportion of the intranasal dose absorbed.[4] A log-plot of nasal absorption vs. molecular weight shows good bioavailability for all compounds up to a molecular weight of about 1000 daltons, but bioavailability decreases with increasing molecular weights.[5] This study provides evidence

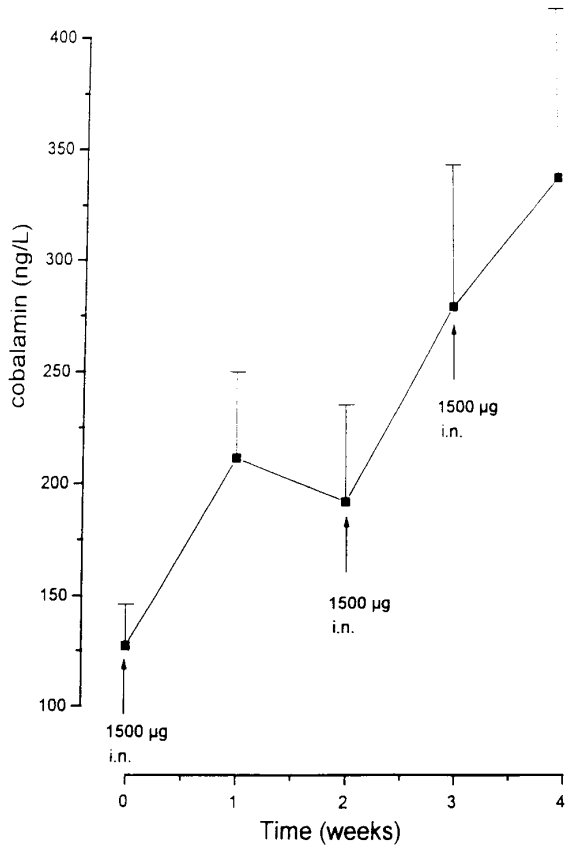

Figure 2. Mean increase (±SD) of cobalamin plasma concentration in 6 patients after intranasal (i.n.) administration of 1500 µg hydroxocobalamin on days 0, 14, and 21.

that hydroxocobalamin, with a molecular weight of 1346 daltons, is well absorbed nasally. Also other hydrophilic compounds of comparable size, such as oxytocin (mol wt, 1007 daltons), desmopressin (mol wt, 1069 daltons), buserelin (mol wt, 1238 daltons), and nafarelin (mol wt, 1321 daltons), are absorbed nasally. These drugs have already been on the market for many years in nasal dosage forms. The nasal absorption of such relatively large hydrophylic drugs is thought to be diffusion through aqueous channels (tight junctions) in the epithelial membrane.

In this preliminary study, intranasal application of one dose of hydroxocobalamin in cobalamin-deficient patients leads to an eightfold increase of mean baseline cobalamin concentration 1 hour after application, indicating rapid absorption of significant amounts of hydroxocobalamin in cobalamin-deficient patients. A single intranasal dose resulted in a sustained increase of cobalamin concentration 1 week after application in all 6 patients.

Cyanocobalamin was the first vitamin B_{12} isolated and introduced for parenteral use in 1948.[6] In the early 1950s, a few investigators proposed an alternative route for administering vitamin B_{12}, in particular inhalation, insufflation, or instillation of cyanocobalamin.[7–9] The formulations used consisted of low concentrations of cyanocobalamin in isotonic saline solution or lactose powder. Although these applications were reported to be effective and safe, none of these proposals found a follow-up in clinical practice. Apparently the results were not convincing, and the proposed formulations were not very practical. Recently, a nasal gel containing cyanocobalamin has become available commercially. In a letter to the editor, this gel in a dose of 400–500 µg cyanocobalamin was claimed to be effective.[10] We are not aware of any clinical studies that show the efficacy of this product in treating vitamin B_{12}–deficient patients. Hydroxocobalamin binds more extensively to plasma proteins and has a longer half time in the body than cyanocobalamin.[11] As a result, hydroxocobalamin is better retained in the body and, therefore, requires less frequent dosing. Moreover, cyanocobalamin is contraindicated in patients with tropical amblyopia and simultaneous tobacco usage and in patients with pernicious anemia with optic neuropathy; hence, hydroxocobalamin is the drug of choice in restoring vitamin B_{12} deficiencies.[12,13]

This study shows reproducible nasal hydroxocobalamin absorption and sustained increase of hydroxocobalamin levels in B_{12}-deficient patients. The intrasubject and intersubject differences in the amount of nasally absorbed hydroxocobalamin measured 1 hour after nasal application can be explained by differences in nasal mucocilary clearance and variations in spraying technique.

The sustained increase in baseline cobalamin concentration 1 week after nasal application of hydroxocobalamin indicates that hydroxocobalamin has not been eliminated from the body. Our preliminary data suggest that a weekly intranasal application of 1500 µg hydroxocobalamin would be sufficient to restore cobalamin deficiencies completely. Prolonged studies during several months are needed to confirm this conclusion.

In conclusion, the application of intranasal hydroxocobalamin in cobalamin-deficient patients results in a fast and adequate absorption through the nasal mucosa and leads to a sustained increase of baseline cobalamin concentration. Further studies are needed to investigate the possibility of long-term intranasal application of hydroxocobalamin as maintenance therapy in patients who need vitamin B_{12} parenterally.

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