Nasal absorption of hydroxocobalamin in healthy elderly adults

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Aims To investigate the nasal absorption of hydroxocobalamin in 10 healthy elderly adults.

Methods In a cross-over study, blood samples were collected before administration of the drug and after 10, 20, 30, 40, 60, 120, 180 and 240 min. The plasma cobalamin concentration was determined by competitive radioisotope binding technique.

Results The maximal plasma cobalamin concentration ($C_{\max}$) after nasal administration of 750 μg hydroxocobalamin was $1900 \pm 900 \text{ pmol l}^{-1}$ (mean $\pm$ s.d.). The maximal plasma cobalamin concentration was reached in $35 \pm 13 \text{ min}$ ($t_{\max}$). The $C_{\max}$ after nasal administration of 1500 μg hydroxocobalamin was $3500 \pm 2500 \text{ pmol l}^{-1}$ with a $t_{\max}$ of $28 \pm 16 \text{ min}$. Both the AUC(0,240 min) and AUC(0,∞) increased significantly with an increase of the dose from 750 μg to 1500 μg ($P=0.037$ and $P=0.028$, respectively). The nasal spray was well tolerated. No signs of irritation or local sensitivity were noted.

Conclusions The nasal absorption of hydroxocobalamin in healthy elderly adults is rapid, high and well tolerated.

Keywords: hydroxocobalamin (vitamin B12) nasal spray, nasal absorption, elderly volunteers, plasma cobalamin concentrations

Introduction

Cobalamin (vitamin B12) deficiency is especially common among elderly people. Studies indicate that as many as 15% are cobalamin deficient [1, 2]. The treatment of choice is the administration of cobalamin by intramuscular injections [3, 4]. Usually, the patient is put on a lifetime regimen of monthly maintenance injections. Intramuscular injections, however, have disadvantages: they are inconvenient, painful and the frequent need for the assistance of health professionals makes the patient dependent and increases the cost of treatment considerably. These drawbacks could encourage noncompliance. A more convenient and cost-effective alternative would be advantageous to health care in general and patients in particular.

The search for alternative routes of cobalamin administration began soon after cyanocobalamin was isolated and introduced for parenteral use in 1948 [5]. Nasal inhalation, insufflation or instillation of cyanocobalamin were proposed in the early 1950s [6–8]. The formulations for nasal inhalation or instillation consisted of low concentrations of cyanocobalamin in isotonic saline solution or lactose powder. Although these formulations were reported to be effective in the treatment of pernicious anaemia, none of these proposals found a follow-up in clinical practice, in industry or in the scientific literature. Recently a gel for nasal

cyanocobalamin instillation has become commercially available. The gel, in a dose of 400–500 μg cyanocobalamin, has been claimed to be safe and effective [9]. We are not aware of studies that show the safety and efficacy of the nasal gel in treating cobalamin deficiency. A practical problem with the use of a gel is that it sometimes dries up and sticks to the unit-dose tubes. In the sixties oral cyanocobalamin administration for the treatment of pernicious anaemia was proposed [10–12]. Despite the claim that doses of 150 μg to 1000 μg daily are effective, it is rarely used [13].

A disadvantage of all these forms of treatment is that they contain cyanocobalamin while hydroxocobalamin is the drug of choice. Since the fifth World Health Organization Model List of Essential Drugs, hydroxocobalamin, and not cyanocobalamin, has been the cobalamin included in the list [14]. Hydroxocobalamin binds more extensively to plasma proteins and has a longer half-life in the body than cyanocobalamin. As a result, hydroxocobalamin is better retained in the body and therefore requires less frequent dosage [15]. Moreover, cyanocobalamin is contraindicated in patients with tobacco and tropical amblyopia and optic neuropathy in pernicious anaemia. A plea for the withdrawal of cyanocobalamin has been made [16, 17].

Recently, a formulation for the nasal administration of hydroxocobalamin has been developed [18]. Here we present the results of our exploratory study on the absorption of nasally administered hydroxocobalamin in healthy elderly adults. To our knowledge this is the first report to document nasal hydroxocobalamin absorption in humans.

Methods

Subjects

Healthy elderly adults were recruited from the general population by means of advertisements in local newspapers. The following exclusion criteria were applied: use of vitamin supplements containing cobalamin, use of nasal medication, or acute or chronic rhinitis. Ten subjects were selected, six females and four males, age 71.6 ± 4.6 year (mean $\pm$ s.d.).

Protocol

The study protocol was approved by the Committee for Experimental Research with Humans of the University Hospital Nijmegen. All subjects gave written informed consent. On the first test day subjects were randomly allocated to a dose of 750 μ g or 1500 μ g hydroxocobalamin. On the second test day (after 163 ± 10 days, range 147–175 days) subjects received the alternate dose. Blood samples were obtained through an indwelling cannula inserted into a forearm vein of each subject. After a blood sample was collected the cannula was flushed with 1 ml of heparinized salt solution (150 IE heparin in 0.9% NaCl solution) in order to maintain patency. Before each blood sample was collected the first 1–2 ml blood were discarded. EDTA-plasma was collected before and after drug administration at the following time points: 0, 10, 20, 30, 40, 60, 120, 180 and 240 min.

The hydroxocobalamin formulation contained 750 μ g hydroxocobalamin per 70 μ l in a preserved solution and the nasal spray device was a metered pump (Valois, France, VP 7/70). The investigator administered 750 μ g (one puff) or 1500 μ g (one puff in each nostril) hydroxocobalamin nasally. The nasal dispenser was weighed before and after administration of the drug to ensure that the dose had actually been given.

The plasma cobalamin concentration was determined by competitive radioisotope binding technique using purified hog intrinsic factor as cobalamin binder (Solid Phase DualCount, Diagnostic Products Corporation, Los Angeles, California). The within-assay coefficient of variation was 3.1–3.5% and the between-assay coefficient of variation was 3.3–6.7%. The assay has a detection limit of 37 pmol l⁻¹. In case the plasma cobalamin concentration fell outside the range of the assay, i.e. >1700 pmol l⁻¹, the samples were diluted with zero-diluent and assayed again.

Pharmacokinetic analysis

Pharmacokinetic parameters of cobalamin were analyzed by model-dependent and model-independent methods. Because a semi-logarithmic plot of the concentration–time data suggested a one-compartment model with first-order absorption, data were analyzed according to this model by weighted (1/y) nonlinear regression with use of PCNONLIN (version 3.0, Scientific Consulting Inc., Apex, N.C.). The following first-order absorption equation, which takes the endogenous concentration of cobalamin into

account, was applied:

$$C(t) = A \cdot (e^{-k_{el} \cdot t} - e^{-k_a \cdot t}) + C_b$$

$$A = \frac{F \cdot D \cdot k_a}{V (k_a - k_{el})}$$

in which A is the intercept of the elimination phase on the y-axis, k_{el} and k_a are the rate constants of elimination and absorption, V is the volume of distribution, D is the dose, F is the bioavailability and C_b is the baseline plasma cobalamin concentration. The following pharmacokinetic parameters were calculated: total area under the curve, $AUC(0, \infty) = A(1/k_{el} - 1/k_a)$; total area under the first moment curve, $AUMC(0, \infty) = A(1/k_{el}^2 - 1/(k_a k_{el}))$; apparent total plasma clearance, $CL/F = D/AUC$; apparent volume of distribution, $V/F = D \cdot AUMC / (AUC)^2 - D / (AUC \cdot k_a)$; absorption half-life, $t_{1/2,a} = \ln 2 / k_a$; elimination half-life, $t_{1/2,z} = \ln 2 / k_{el}$; mean residence time, $MRT = AUMC / AUC$; mean absorption time, $MAT = MRT - 1/k_{el}$; time to reach peak plasma concentration, $t_{max} = \ln(k_a / k_{el}) / (k_a - k_{el})$; and the peak plasma concentration, C_{max} , is calculated after substitution of the value for t_{max} in the first order absorption equation.

The goodness of fit to the plasma concentration data was evaluated through the deviations between observations and model-predicted values, expressed as

$$r^2 = 1 - \Sigma(\text{Dev})^2 / \Sigma(\text{Obs})^2$$

AUC and AUMC were also calculated model-independently by application of the linear trapezium method (from 0 to 240 min) after correction for baseline plasma cobalamin concentrations, with extrapolation to infinity by using the log-linear terminal slope of the plot.

Statistical analysis

The results are presented as means $\pm$ s.d. The Wilcoxon Signed Rank test was used for comparing continuous variables of paired samples. A P value of 0.05 or less was considered statistically significant.

Results

Figure 1 shows the mean plasma concentrations of cobalamin after nasal administration of 750 μ g and 1500 μ g to 10 healthy elderly adults. The pharmacokinetic parameters are summarized in Table 1. Model-independent analysis revealed virtually the same values (data not shown). The goodness of fit expressed as the mean coefficient of determination was 0.98 ± 0.14 (range 0.939–0.998), indicating that the model described the data accurately. We assumed that the descending part of the plasma curves represents the elimination phase of hydroxocobalamin, because the rate constants k_{el} and k_a in the model are not uniquely identifiable. The only way to be sure of this assumption is to compare the calculated values with the elimination rate constant after intravenous administration. Literature data of intravenously administered hydroxocobalamin in dosages yielding plasma concentrations comparable to our data are not available. Although the model fitted the data very well it should be noticed that the extrapolated part of the AUC contributes

Figure 1 Mean and s.d. plasma cobalamin concentration after nasal administration of 750 µg (●) and 1500 µg (○) hydroxocobalamin in healthy elderly adults.

to a large extent to the total AUC (44–55%). Extrapolation of the terminal phase of the plot to infinity may be an underestimation of the actual AUC, because an additional, slower phase, might have been missed. However, since plasma concentrations of all individuals at both dosages had nearly returned to their baseline values after 48 h (data not shown), the influence of such an underestimation on the total AUC would be relatively minor.

The nasal absorption of hydroxocobalamin was fast. The $C_{\max}$ after nasal administration of 1500 µg hydroxocobalamin was approximately twice as high as the $C_{\max}$ after nasal administration of 750 µg hydroxocobalamin. However, due to intra- and intersubject variability the difference in $C_{\max}$ was not statistically significant ($P=0.09$). Nevertheless, both

the AUC(0,240 min) and AUC(0,∞) increased significantly with the increase in dose ($P=0.04$ and $P=0.03$, respectively).

The nasal spray was well tolerated. No signs of irritation or local sensitivity were noted.

Discussion

The results of our study show that nasal hydroxocobalamin absorption is rapid, high, and safe. The absorption of water-soluble compounds depends on several factors like the contact time with the nasal mucosa and the size of the compound. There is an inverse relationship between the molecular weight of a drug and the absorbed proportion of the intranasal dose [19]. Nasal absorption decreases with increasing molecular weight but the absorption of compounds up to about 1000 daltons is good [20]. Nevertheless, this study demonstrates that the absorption of hydroxocobalamin with a molecular weight of 1346 daltons is fair. Other hydrophilic compounds of similar weight like oxytocin (MW=1007), desmopressin (MW=1069), buserelin (MW=1238), and nafarelin (MW=1321) are also absorbed nasally. These products have been on the market for some years. The nasal absorption of relatively large hydrophilic compounds is thought to occur by diffusion through aqueous channels in the epithelial membrane [19]. The role of the cobalamin-binder haptocorrin, which has been demonstrated in nasal secretion, [21] in the nasal absorption of hydroxocobalamin is unclear.

Our study presents the first results on the nasal absorption of hydroxocobalamin in healthy elderly volunteers. We found that nasally administered hydroxocobalamin is rapidly absorbed, that the amount absorbed increases significantly with increasing dose and that it is well tolerated. The plasma concentrations achieved after nasally administered hydroxocobalamin are in the therapeutic range. We did not compare the intranasal administration with the currently used intramuscular route. However, based on values in literature it can be estimated that the bioavailability of nasal hydroxocobalamin is approximately 2–5%. Plasma concen-

Table 1 Pharmacokinetic parameters of nasal hydroxocobalamin.

Parameter	Nasal hydroxocobalamin		P-value
	750 µg	1500 µg	
$C_{\max}$ (pmol l ⁻¹)	1900 ± 900	3500 ± 2500	0.09
$t_{\max}$ (min)	35 ± 13	28 ± 16	0.17
Cb (pmol l ⁻¹)	280 ± 170	240 ± 70	1.00
AUC(0,240 min) (pmol ml min ⁻¹)	270 ± 160	580 ± 460	0.04
AUC(0,∞) (pmol ml min ⁻¹)	480 ± 350	1220 ± 1130	0.03
$t_{1/2,a}$ (min)	7.6 ± 3.3	5.3 ± 3.6	0.15
$t_{1/2,z}$ (min)	170 ± 60	200 ± 70	0.06
MRT (min)	260 ± 80	300 ± 110	0.08
MAT (min)	11 ± 5	8 ± 5	0.15
CL/F (ml min ⁻¹)	2.4 ± 2.3	2.1 ± 1.8	0.72
V/F (l)	0.52 ± 0.49	0.50 ± 0.33	0.88

Data are means ± s.d. AUC(0,240 min) was calculated by applying the linear trapezoidal rule. $C_{\max}$, peak plasma concentration; Cb, endogenous plasma cobalamin concentration; $t_{\max}$, time to reach the peak plasma concentration; AUC, area under the plasma concentration-time curve; $t_{1/2,a}$, absorption half-life; $t_{1/2,z}$, elimination half-life; MRT, mean residence time; MAT, mean absorption time; CL/F, apparent total plasma clearance; V/F, apparent volume of distribution.

trations achieved after intramuscular injections of comparable dosages are approximately 10 times higher than after nasal administration [22, 23]. However, with an intramuscular injection of 1000 µg about 50% of free hydroxocobalamin is lost into urine [24]. Another, although less frequently applied route is oral administration. Clinical and haematological remission has been reported in patients with pernicious anaemia treated with 150 µg day⁻¹, 300 µg day⁻¹ or 500 µg day⁻¹ of oral hydroxocobalamin [10–12]. The achieved plasma concentrations after oral administration of hydroxocobalamin are about 10 to 20 times lower than after nasal administration of 750 µg and 1500 µg, respectively [10–12].

The rather large intra- and intersubject differences in the amount of nasally absorbed hydroxocobalamin can be explained by differences in mucociliary clearance or variations in spraying technique and deposition pattern. These variables will have to be investigated in more detail in a next study. The nasal absorption of hydroxocobalamin is so high that weekly application could prove a good alternative for monthly injections. In a pilot study during 1 month of four patients with Crohn's disease the mean serum cobalamin concentration increased significantly after weekly administration of 1500 µg of the same formulation [25]. A large clinical study will have to demonstrate that weekly nasal administration of hydroxocobalamin to cobalamin deficient patients results in normalization of the plasma cobalamin level and reversal of clinical consequences of cobalamin deficiency.

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