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Replacement therapy for vitamin B12 deficiency: comparison between the sublingual and oral route

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Aims To compare the efficacy of sublingual and oral administration of 500 µg of cobalamin in subjects with cobalamin deficiency.

Materials and results Thirty subjects with low serum concentrations of cobalamin participated in the study. Subjects were randomly allocated to receive one tablet daily of 500 µg cobalamin sublingually or orally, or two tablets daily of a vitamin B complex. Serum cobalamin concentrations before treatment were 94 ± 30 pmol l^{-1}, 108 ± 17 pmol l^{-1} and 98 ± 14 pmol l^{-1} in the sublingual B12, oral B12 and oral B-complex groups, respectively. After 4 weeks, concentrations rose to 288 ± 74 pmol l^{-1}, 286 ± 87 pmol l^{-1} and 293 ± 78 pmol l^{-1}, respectively. The increase in each group across time was statistically significant ($P = 0.0001$, differences [95% confidence intervals] 194.2 (114.5, 273.9), 178.3 (104.2, 252.4), and 195.1 (135.0, 255.2) pmol l^{-1}, respectively). There was no significant difference in concentrations between the treatment groups.

Conclusion A dose of 500 µg of cobalamin given either sublingually or orally is effective in correcting cobalamin deficiency.

Keywords: cobalamin deficiency, oral, sublingual

Introduction

Cobalamin (vitamin B12) deficiency is caused by pernicious anaemia, food–cobalamin malabsorption, vegetarianism, and other deficiency states. It has a reported prevalence of 3–29% [1]. The usual treatment for cobalamin deficiency consists of intramuscular injections of the drug. However, these can be painful, are difficult to give to disabled or elderly patients, and are costly if administered by health professionals [2]. In the 1960s, Swedish investigators treated 64 patients with pernicious anaemia with 1000 µg of oral cyanocobalamin, and all showed clinical and haematological remission [3]. About 1% of cobalamin is absorbed orally in subjects without intrinsic factor. The daily requirement of cobalamin is 1.0–2.5 µg, and thus, large oral doses may meet these needs [4]. This hypothesis was confirmed by several more recent trials with sublingual or oral doses of cobalamin between 1000 and 5000 µg [5–9].

The purpose of the present study was to compare the efficacy of sublingual and oral administration of a smaller dose of cobalamin (500 µg) in achieving adequate cobalamin concentrations in subjects with mild cobalamin deficiency without anaemia. We also compared two oral preparations, namely pure cobalamin and a vitamin B complex containing cobalamin, thiamine, and pyridoxine, in order to determine if the combination of cobalamin and pyridoxine, which both serve as cofactors in the metabolism of homocysteine, is superior to cobalamin alone.

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Methods

Subjects

Thirty subjects with a serum cobalamin concentration <138 pmol l^{-1} (normal range 138–781 pmol l^{-1}) were recruited for the study from a screening centre at Rabin Medical Center in Israel. Subjects were randomized to receive sublingual therapy or one of two oral preparation regimens. Twenty-three subjects agreed to a Schilling test. Briefly, after a 12-h fast, each subject swallowed a capsule containing 0.25 µg of cyanocobalamin radio-labelled with ^{58}Co, followed immediately by an intramuscular injection of 1000 µg of cyanocobalamin. Urine excreted during the 24-h period after the capsule swallow was collected for counting. ^{58}Co urine excretion of less than 10% (normal 10–40%) was considered pathological [10].

Prior to the study, all subjects gave written informed consent. The study was performed with the approval of the Helsinki ethics committee of the Rabin Medical Center.

Protocol

The study was conducted over an 8-week period. Subjects received 500 μg of cyanocobalamin in one of three forms: (i) a sublingual preparation [one 500 μg sublingual tablet (Dot); Twinlab, Ronkonkoma, New York, USA]; (ii) an oral preparation (one 500 μg tablet; GNC, Greenville, South Carolina, USA); and (iii) an oral vitamin B complex preparation (two tablets, each containing 250 μg cobalamin, 100 mg thiamine and 250 mg pyridoxine; Tribemin,® Bat-Yam, Israel). All formulations were administered daily, with breakfast. At the end of each week, a pill count was performed to assess compliance.

Complete blood count and serum cobalamin measurements were obtained before the study and at the end of weeks 1, 2, 3, 4 and 8 of treatment. Serum folate and plasma homocysteine concentrations as well as urine methylmalonic acid (MMA) concentrations were measured before and 4 weeks after treatment. Serum cobalamin and folate concentrations were determined by enzyme immunoassay (Axsym System; Abbott, USA) [11, 12], plasma homocysteine concentrations by fluorescent polarization immunoassay (IMX System; Abbott) [13], and urine concentrations of MMA by gas chromatography–mass spectrometry [14, 15].

Coefficients of variation for low and high control standards of serum cobalamin, serum folate, plasma homocysteine and urine MMA were ±9.7%; ±6.70%, ±10.1%; ±4.3%, 5.4%; 4.9%, ±11.29%; and ±6.24%, respectively. The lower limits of detection of the assays were 44.2 pmol l^{-1}, 2.04 nmol l^{-1}, 0.5 μmol l^{-1} and 0.1 mg g^{-1} creatinine^{-1} for serum cobalamin, serum folate, plasma homocysteine and urine MMA, respectively.

Statistical analysis

Continuous variables are shown as means ± standard deviations. To analyse the distribution of categorical variables, the chi-Squared test or Fisher's exact test was used, as appropriate. A Student's *t*-test was used to compare differences between continuous parameters. Treatment groups were compared by analysis of variance with the Duncan multiple comparison option. To compare data across time, analysis of variance with the Dunnett's multiple comparison option was used, and 95% confidence intervals for the differences between weeks were calculated. $P \leq 0.05$ was considered statistically significant.

Results

Eighty percent, 70%, and 70% of subjects were male in the sublingual B12, oral B12 and oral vitamin B complex groups, respectively. The mean age of each group was 44.5 ± 14.7, 50.2 ± 15.1 and 49.7 ± 11.6 years, respectively. Based on pill count, the compliance with treatment was 99.3 ± 1.5%, 97.8 ± 3.8% and 97.1 ± 6.7%, respectively. The number of strict vegetarians or low meat consumers was 6, 5, and 5, respectively. There were no significant differences between the groups in gender distribution, mean age or compliance.

Haematocrit and mean corpuscular volume (MCV) were within normal range in all subjects at baseline and showed no change after 4 and 8 weeks of treatment. In all three treatment groups, cobalamin concentrations returned to the normal range within 4 weeks of treatment ($P = 0.0001$) and were maintained after 8 weeks (Table 1). Most of the increase was achieved by the end of the first week of treatment (Figure 1). There was no statistically significant difference in serum cobalamin concentrations between the groups either before treatment or after weeks 4 and 8. Baseline serum folate concentrations were normal in all subjects and did not change after cobalamin treatment.

Plasma homocysteine concentrations and urine MMA concentrations were within the normal range in all groups (Table 1). After 4 weeks of cobalamin treatment, the concentrations of both metabolites decreased, but not significantly, except for borderline *P* values in the sublingual group ($P = 0.056$ and $P = 0.052$ for homocysteine and MMA concentrations, respectively).

Five of the 23 subjects who agreed to a Schilling test had abnormal findings. Both this subgroup and the 18 subjects with a normal Schilling test had low serum cobalamin concentrations at baseline, which increased significantly to normal range within 4 weeks of treatment [276 ± 69 *vs* 96 ± 15 pmol l^{-1} (CI 95% 130.5, 231.3); $P = 0.0001$ and 262 ± 70 *vs* 99 ± 15 pmol l^{-1} (CI 95% 48.1, 277.9); $P = 0.0028$ for subjects with a normal and abnormal Schilling test, respectively]. There was no significant difference in cobalamin concentrations between the subjects with a normal and abnormal Schilling test.

Discussion

Cobalamin is traditionally administered by intramuscular injections. However, it has recently been shown [9] that the sublingual route is equally effective. In this prospective study of 30 subjects with vitamin B12 deficiency, we found that sublingual and oral administration of 500 μg of cobalamin was equally effective in correcting cobalamin concentrations. Most of the increase in cobalamin

Table 1 Cobalamin, folate and metabolite concentrations before and after treatment.

	Sublingual B12	*Oral B12*	*Oral B complex*	*P-value*[1]
Serum cobalamin (pmol l^{-1})				
Baseline	94 ± 30	108 ± 17	98 ± 14	NS
Week 4	288 ± 74	286 ± 87	293 ± 78	NS
Week 8	279 ± 75	241 ± 73	266 ± 56	NS
P-value[2]	0.0001	0.0001	0.0001	
95% CI[4]	(114.5, 273.9)	(104.2, 252.4)	(135.0, 255.2)	
95% CI[5]	(105.5, 264.9)	(59.1, 207.3)	(107.6, 227.8)	
Serum folate (nmol l^{-1})				
Baseline	15.8 ± 5.6	19.0 ± 7.3	15.6 ± 10.2	NS
Week 4	15.3 ± 4.7	19.7 ± 7.1	15.4 ± 8.9	NS
P-value[3]	NS	NS	NS	
95% CI[4]	(−5.3, 4.3)	(−6.1, 7.4)	(−9.5, 8.6)	
Plasma homocysteine (µmol l^{-1})				
Baseline	16 ± 5	15 ± 5	22 ± 16	NS
Week 4	12 ± 3	13 ± 4	15 ± 7	NS
P-value[3]	0.056	NS	NS	
95% CI[4]	(−7.8, 0.1)	(−5.7, 2.9)	(−18.4, 4.7)	
Urine MMA (mg g^{-1} creatinine)				
Baseline	1.8 ± 1.1	1.2 ± 0.7	2.2 ± 2.1	NS
Week 4	1.0 ± 0.6	1.0 ± 0.5	1.0 ± 0.7	NS
P-value[3]	0.052	NS	NS	
95% CI[4]	(−1.6, 0.01)	(−0.7, 0.4)	(−2.5, 0.4)	

Data are mean ± SD. [1]Comparison between treatment groups; [2]comparison between data in weeks 4 and 8 *vs* baseline; [3]comparison between data in week 4 *vs* baseline; [4]95% confidence interval between data in week 4 *vs* data at baseline; [5]95% confidence interval between data in week 8 *vs* data at baseline. MMA, methylmalonic acid. Normal serum cobalamin concentration: 138–781 pmol l^{-1}; normal serum folate concentration: 6.8–38.5 nmol l^{-1}; normal plasma homocysteine concentration: 5–20 µmol l^{-1}; normal urine MMA concentrations 0–3.5 mg g^{-1} creatinine.

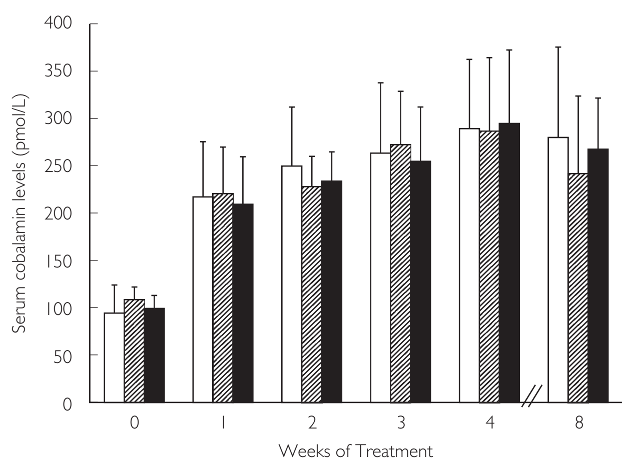

Figure 1 Serum cobalamin concentrations (mean ± SD) produced by the three therapeutic regimens during 8 weeks of treatment. SL vitamin B12 (□), ORAL vitamin B12 (▨) and ORAL vitamin B complex (■).

concentrations was achieved by the end of the first week of treatment (Figure 1). Previous studies used sublingual or oral doses of 1000–5000 µg [5–9]. Our study shows that as little as 500 µg is enough to correct cobalamin deficiency. All our subjects had a very low serum cobalamin concentration at entry to the study (mean 100 pmol l^{-1}) and all were in a preclinical state of cobalamin deficiency. None had specific symptoms of cobalamin deficiency or anaemia. Plasma homocysteine concentrations as well as urine MMA concentrations were within the normal range. After 4 weeks of cobalamin treatment, there appeared to be a trend towards lower concentrations for both these metabolites. The difference did not achieve statistical significance, except in the sublingual group, where the decrease was of borderline significance. Since the low concentrations of cobalamin in our subjects were found as part of a screening program, in the absence of specific clinical symptoms, we assume that the subjects were at an early stage of negative cobalamin balance [16]. We are aware that some of the subjects may have had genetically low concentrations of transcobalamin I (TCI), which is now known to be a benign condition that most probably does not need medical treatment [17]. However, because of its rarity, it is highly unlikely that all 30 subjects had inherited TCI deficiency.

About 50% of our subjects were vegetarians, and this probably explains their cobalamin deficiency. Five subjects had an abnormal Schilling test, two of whom had high concentrations of antiparietal cell antibodies. All five subjects had a similar treatment response to those with a normal Schilling test.

Pyridoxine (vitamin B6) acts as a cofactor in the *trans*-sulphuration pathway of homocysteine to cysteine. The role of pyridoxine treatment in the prevention or treatment of hyperhomocysteinaemia is controversial [18–20]. Ten of the subjects in our study received 500 mg of pyridoxine with cobalamin. Their homocysteine concentrations did not change significantly, and were not different from the other groups.

In summary, a dose of 500 µg of cobalamin given either sublingually or orally, is apparently effective in correcting cobalamin deficiency in subjects with early-stage disease.

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