Nutrition xxx (2010) 1–4

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Rapid communication

Vitamin C provision improves mood in acutely hospitalized patients

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ARTICLE INFO

Article history:
Received 4 December 2009
Accepted 26 May 2010

Keywords:
Ascorbic acid
Malnutrition
Nutritional supplements
Scurvy
Vitamin D

ABSTRACT

Objective: Hypovitaminosis C and D are highly prevalent in acutely hospitalized patients, but the clinical significance of these biochemical abnormalities is not known. Because deficiencies of vitamin C and D have been linked to psychologic abnormalities, vitamin C or D provision could improve the mood state of acutely hospitalized patients.
Methods: Double-blind clinical trial of the effect of vitamin C (500 mg twice daily) or vitamin D (1000 IU twice daily) on mood, as assessed with a validated instrument, the Profile of Mood States.
Results: Vitamin C therapy increased plasma ($P < 0.0001$) and mononuclear leukocyte ($P = 0.014$) vitamin C concentrations and was associated with a 34% reduction in mood disturbance ($P = 0.013$). Vitamin D therapy increased plasma 25-hydroxyvitamin D concentrations ($P = 0.0004$), but had no significant effect on mood.
Conclusions: Treatment of hypovitaminosis C improves the mood state of acutely hospitalized patients.

Introduction

Hypovitaminosis C is highly prevalent in acutely hospitalized patients [1–4]. Thus, a recent population-based survey disclosed hypovitaminosis C in 60% of the patients on the acute medical wards of a Montreal teaching hospital, but in only 16% of people attending the hospital's outpatient test center [4]. Hypovitaminosis C usually implies vitamin C deficiency, but the response to systemic inflammation could redistribute the vitamin into leukocytes or other tissues without necessarily indicating nutritional deficiency. It is also possible, however, that systemic inflammation increases vitamin C catabolism and induces true biochemical deficiency when vitamin C provision is inadequate [5].

We recently investigated the metabolic origin of hypovitaminosis C in acutely hospitalized patients by characterizing their responses to vitamin C provision. Because psychologic abnormalities are a feature of vitamin C deficiency [6–8], an assessment of mood was included. Vitamin C therapy was associated with a 35% reduction in average mood disturbance [5]. However, because all study participants received vitamin C, this striking improvement in mood could have been a placebo response.

This article describes the results of a double-blind clinical trial of vitamin C therapy to examine whether our earlier observation would be reproduced in a new patient sample and, if so, whether it was a placebo response. The trial was both explanatory, in that its aim was to understand a biological phenomenon by testing whether a specific biological response could be explained by exposure to a particular therapy, and pragmatic, in that it was a feasible intervention in typical patients in a common clinical setting, rendering the results clinically applicable [9,10]. Vitamin D was selected as a plausible alternative treatment because, as with vitamin C, biochemical vitamin D deficiency is highly prevalent in acutely hospitalized patients [11] and has been linked to abnormal mood [12–14].

Methods

Study design

Over a 6-wk period in July and August, all the patients on eight active medical and surgical teaching units of a university teaching hospital were considered for enrollment if they were mentally competent and fluent in French or English. Patients in the intensive care unit or being considered for transfer there were not eligible for enrollment. Prospective participants were informed they could be at risk of vitamin C and D deficiency and offered enrollment in the study, which involved double-blind paired randomization to treatment with 500 mg vitamin C twice daily or 1000 IU vitamin D twice daily for up to 10 d. By rule, patients

This work was supported by the Lotte and John Hecht Memorial Foundation, a grant from McGill University, and a medical student research bursary from the Faculty of Medicine, McGill University.

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0899-9007/$ - see front matter © 2010 Published by Elsevier Inc.
doi:10.1016/j.nut.2010.05.016

Table 1
Baseline characteristics of the participants*

Variable	Initial study group	Study completed group		
		All patients	Vitamin C	Vitamin D
Number of participants	55	32	15	17
Male sex (%)	54.5	59.4	53.3	61.1
Smoker (%)	16.4	18.8	6.7	29.4
Days in hospital at enrolment		19 ± 15†	21 ± 17†	17 ± 14
Blood hemoglobin (g/L)	109 ± 25.8	106 ± 27.7	109 ± 17.9	105 ± 34.5
Serum albumin (g/L)	32.7 ± 6.2	31.4 ± 5.6	31.9 ± 6.3	30.7 ± 4.9
Plasma ascorbic acid (μmol/L)	26.6 ± 20.3	24.3 ± 18.0	26.3 ± 20.1	22.5 ± 16.5
Plasma total vitamin C (μmol/L)	30.9 ± 21.8	28.5 ± 20.8	31.8 ± 23.7	25.6 ± 18.1
Subnormal vitamin C (% of patients)	56	63	60	65
MML ascorbic acid (mmol/L)	4.00 ± 3.12	4.54 ± 3.60	3.73 ± 2.27	5.18 ± 4.35
MML total vitamin C (mmol/L)	4.97 ± 3.27	5.53 ± 3.90	4.80 ± 2.53	6.06 ± 4.73
Plasma 25-OH vitamin D (nmol/L)	41.2 ± 47.0	51.2 ± 20.0	51.4 ± 20.2	51.0 ± 20.6
Subnormal vitamin D (% of patients)	84	81	80	82
TMD score	21.9 ± 21.7	25.7 ± 24.6	28.6 ± 21.8	23.1 ± 27.2

MML, mixed mononuclear leukocytes; TMD, total mood disturbance (a higher score indicates more severe mood disturbance)

* Values are means ± SD. There were no significant differences between the initial study group and study completed group nor between the vitamin C and vitamin D groups.

† Excludes one patient under acute care for 120 d.

already prescribed supplemental vitamin D in hospital were assigned to the vitamin C group (no patients had been prescribed supplemental vitamin C). Treatment was considered complete if five or more days of vitamin therapy were completed. Before and after 5 to 10 d of vitamin administration, participants completed a mood assessment questionnaire and had a blood sample drawn for the analyses described below. This study was approved by the Research Ethics Committee of Montreal's Jewish General Hospital.

Blood samples, laboratory methods, and procedures

Morning fasting blood samples were drawn prior to any vitamin administration into two 4 mL EDTA Vacutainer tubes and immediately pushed into crushed ice in a black box where they remained for no more than 2 h before delivery to the research laboratory by one of the investigators. After the blood cells were separated in a refrigerated centrifuge, and working under dim light, 0.5 mL fresh plasma was mixed with 0.5 mL 10% (w/v) metaphosphoric acid in 2 mmol/L disodium EDTA, kept on ice for 5 min, then centrifuged at 16 000 × *g* for 10 min at 4°C. The resulting acidified protein-free supernatant was transferred into duplicate screw-cap vials, flash-frozen in dry ice/ethanol, and kept at −80°C until analysis (normally within 1 mo).

Mixed mononuclear leukocytes (MML) were isolated by centrifuging 4 mL fresh whole blood through a BD Vacutainer CPT Cell Preparation Tube (BD-Canada, Mississauga, ON) for 30 min at 1750 × *g* at 22°C. Approximately 0.1 mL of the resulting MML fraction was counted by hemocytometer and another 0.4 mL was washed in 5 mL of ice-cold 137 mmol/L sodium chloride 10 mmol/L sodium phosphate (pH 7) and centrifuged at 380 × *g* for 10 min at 4°C. The cell pellet was resuspended in 0.4 mL 1 mmol/L EDTA, mixed with an equal volume of 10% metaphosphoric acid 2 mmol/L EDTA, and processed and stored like a plasma sample.

At the time of analysis, samples of acidified deproteinized plasma or MML were allowed to thaw under observation on ice under dim light and then treated as described by Lykkesfeldt [15]. To analyze ascorbic acid, 0.05 mL of sample was mixed with 0.20 mL 1 mmol/L EDTA. To analyze total vitamin C (ascorbic acid plus dehydroascorbic acid), 0.05 mL of sample was treated with 0.025 mL 2.5 mmol/L tris-(2-carboxyethyl)-phosphine hydrochloride in 800 mmol/L TRIS buffer (pH 9), mixed, and allowed to stand in the dark for 5 min at room temperature, followed by the addition of 0.175 mL McIlvaine buffer (0.28 mol/L citric acid in 0.56 mol/L dibasic sodium phosphate, pH 4.5). The sample was then centrifuged at 4°C and kept on ice prior to injection on the HPLC column within 15 min. The HPLC conditions and analysis by electrochemical detection were as previously described [4].

A plasma total vitamin C concentration <28.4 μmol/L was regarded as consistent with vitamin C depletion and a concentration <11.4 μmol/L was regarded as deficient [4,5]. The vitamin C content of MML was calculated in units of mmol/L of cell volume; to convert to nmol/10^8 cells, multiply by 25 [5]. Plasma 25-hydroxyvitamin D (25OHD) was analyzed by enzyme immunoassay (Immunodiagnostic Systems, Tyne and Wear, UK). In keeping with recent concepts, a concentration less than 75 nmol/L was considered deficient [16].

Profile of Mood States

The Profile of Mood States (POMS) is a widely used 65-item questionnaire that measures mood in healthy, physically ill, and psychiatric populations; the instrument generates a total mood disturbance score [17]. Brief versions of the POMS have been developed to accommodate the limited reserve of physically ill patients and found to be practical and valid [17–19]. The POMS-B was chosen as the most appropriate instrument to assess the mood of acutely hospitalized patients because it is a validated, extensively used, broad-spectrum tool that can be conveniently and quickly administered even to sick, hospitalized patients [20]. English Canadian and Canadian French versions of the 30-item POMS-B were purchased and used (MultiHealth Systems Inc, Toronto, ON). One person (M.Z.) carried out all the mood assessments; neither she nor any patient knew their treatment assignment nor biochemical vitamin status. Patients completed the questionnaire by hand or had it read to them; their assessment was based on how they felt on the day of measurement. Both mood assessments were carried out in the same manner and at the same time of day.

Statistical analyses

Significant differences in unpaired samples were tested for using the Mann-Whitney or Fisher's exact test as appropriate ($P < 0.05$). The Wilcoxon matched pairs test was used to detect significant differences in paired comparisons. Results are expressed as means ± SD.

Results

Of 88 patients considered for inclusion, 55 were mentally competent, fluent in French or English, understood the nature of the research, signed the informed consent document, and began the study; they are referred to as the *initial study group*. (Reasons for refusal included reluctance to take more pills, fear of interaction with ongoing treatment, feeling overwhelmed with their general condition, and mistrust of research.) No patient had petechiae, purpura, hemorrhagic gingivitis, or hemarthrosis. Fifty-six percent of plasma total vitamin C concentrations were <28.4 μmol/L and 9% were <11.4 μmol/L; 84% of 25OHD concentrations were <75 nmol/L. Twenty enrolled participants were discharged from hospital before completing 5 d of treatment. One withdrew; one was non-compliant, and one never began the study because of a nursing error. Thus, 32 patients completed the study.

The 32 participants in the *study-completed group* were similar to the initial study group in age, sex, and other parameters (Table 1). In particular, 62.5% of their plasma vitamin C concentrations were <28.4 μmol/L and 12.5% were <11.4 μmol/L; 81% of their plasma 25OHD concentrations were <75 nmol/L. The clinical diagnoses were as follows: solid tumor or hematologic malignancy (34% of patients), cardiovascular disease (31%), diabetes mellitus (16%), infectious disease (25%), gastrointestinal

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Table 2
Metabolic and mood effects of vitamin C and D therapy

Variable	Vitamin C therapy			Vitamin D therapy		
	Initial	Final	*P*	Initial	Final	*P*
Plasma ascorbic acid (μmol/L)	26.3 ± 20.1	94.6 ± 35.5	<0.0001	22.5 ± 16.5	22.4 ± 17.5	0.6025
Plasma total vitamin C (μmol/L)	31.8 ± 23.7	101.1 ± 34.5	<0.0001	25.6 ± 18.1	25.6 ± 18.0	0.7764
MML ascorbic acid (mmol/L)	3.73 ± 2.27	8.56 ± 5.55	0.0029	5.18 ± 4.35	4.28 ± 2.31	0.4263
MML total vitamin C (mmol/L)	4.80 ± 2.53	9.29 ± 5.39	0.0137	6.06 ± 4.73	4.89 ± 2.47	0.3575
Plasma 25OH vitamin D (nmol/L)	51.4 ± 20.2	50.0 ± 15.7	0.7615	51.0 ± 20.6	61.8 ± 17.2	0.0004
TMD score	28.6 ± 21.8	18.8 ± 19.4	0.0134	23.1 ± 27.2	22.4 ± 22.4	1.000
Treatment duration (d)		8.7 ± 1.6			7.5 ± 1.9	

disease (12%), renal failure (12%), other (19%). At time of enrollment, four patients were receiving 400 to 800 IU/d vitamin D supplementation (mean 700 IU/d) and one was receiving 10,000 IU/wk; their mean 25OHD concentration (58.0 nmol/L) was not significantly different from that of the 27 patients not prescribed a vitamin D supplement (49.9 nmol/L, $P = 0.253$). The rule-directed allocation of these patients to the vitamin C group did not result in significant differences in the characteristics of the vitamin C and vitamin D treatment groups (Table 1).

As shown in Table 2, vitamin C provision for an average of 8.7 d more than tripled plasma vitamin C concentrations ($P < 0.0001$) and nearly doubled MML concentrations ($P = 0.014$). This treatment was associated with a 34% reduction in the total mood disturbance score ($P = 0.013$). Vitamin D administration for an average of 7.5 d increased the average plasma 25OHD concentration by 20% ($P = 0.0004$), but had no significant effect on mood.

Discussion

Hypovitaminosis C is highly prevalent in acutely hospitalized patients [1–4]. We previously observed a large and statistically significant improvement in the mood of such patients after vitamin C treatment, but the study was not controlled [5]. In this trial we aimed to determine whether this large improvement in mood would be reproduced in a new sample of patients and, if so, whether it was a placebo response. We therefore carried out a double-blind comparison trial in which the comparator treatment was vitamin D. Vitamin D was chosen as a plausible alternative treatment because, as with vitamin C, biochemical vitamin D deficiency is very common in acutely hospitalized patients [11] and has been linked to abnormal mood [12–14]. Vitamin C treatment was associated with an improvement in mood of comparable magnitude to our earlier study. No improvement in mood occurred with vitamin D treatment, thus making the placebo response an unlikely explanation for the effect of vitamin C.

Acutely hospitalized patients experience emotional distress for many reasons. The observation that vitamin C provision improved mood in these patients may therefore be considered surprising, but it is not implausible. There is a well-known relationship between vitamin C deficiency and psychologic state [6–8]. Vitamin C is involved in neuronal transmission and neurotransmitter metabolism, and its cerebrospinal fluid concentration is approximately threefold higher than, and tightly linked to, its plasma concentration [5]. If subnormal vitamin C concentrations in the cerebrospinal fluid adversely affect brain function, their replenishment could improve mood. Indeed, whether hypovitaminosis C is caused by redistribution of the vitamin into tissues, increased catabolism, or both, a predictable consequence is reduced vitamin C availability to the brain.

Vitamin D provision at the currently recommended upper intake level [21] increased plasma 25OHD concentrations, but not enough to reach the target of 75 nmol/L, and no significant change in the total mood disturbance score occurred. Vitamin D could play an important role in brain function [22], and there is some evidence that biochemical vitamin D deficiency impairs mood [12–14,22]. One clinical trial suggested that mood can improve after as few as 5 d of vitamin D therapy [23], but we consider the duration of the present trial too short, and the dose of vitamin D too low [21], to justify any conclusion about the relationship between biochemical vitamin D deficiency and mood in acutely hospitalized patients.

This study has limitations, one being its small size. Small clinical trials can play a vital role in the early assessment of novel ideas when they are well designed and interpreted [24]. The number of patients enrolled in the present study was known to be sufficient to test our hypothesis, since the magnitude of the treatment effect and its variability were known from our earlier study [5]. In this connection, it is noteworthy that the 34% improvement in mood associated with vitamin C therapy in this study is closely similar to the 35% improvement that occurred in our earlier, unblinded study, which involved the same treatment in similar patients in the same hospital [5]. Given the homogeneity of these studies— as well as the lack of a change in mood in response to vitamin D, which argues against a placebo effect—it is reasonable to combine the total mood disturbance scores of the vitamin C-treated patients in a single analysis of 49 patients. This analysis indicates that the effect of vitamin C provision on mood is extremely unlikely to have occurred by chance ($P = 0.0003$).

A second limitation is that 5 of the 32 study participants had already been prescribed a vitamin D supplement by their physician at the time of enrollment, and these patients were automatically assigned to the vitamin C treatment group. This non-random assignment could decrease the likelihood that the two groups were similar, notwithstanding their similarity with regard to the parameters indicated in Table 1. We judged this prespecified treatment assignment to be reasonable, because the alternative would have been to exclude such patients from study, potentially introducing another kind of bias. A post-hoc analysis restricted to the 10 vitamin C-treated patients who were assigned strictly by randomization indicates that, except for inadequate statistical power in this small sample, the average total mood disturbance score and its decrease with vitamin C therapy were similar to those in the original group (baseline score 28.8 ± 21.9, posttreatment score, 21.0 ± 21.22; $P = 0.0827$). This post-hoc analysis does not support the supposition that prior treatment with vitamin D in a subset of patients accounted for the overall favorable effect of vitamin C.

A final concern is patient heterogeneity. Heterogeneity is unavoidable in research carried out in the complex milieu of a modern acute-care hospital, and especially in research that

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deals with malnutrition, a condition that crosses medical and surgical diagnostic boundaries. Heterogeneity is often a feature of pragmatic clinical trials [10]. Our trial was both explanatory, in that its aim was to understand a biological process by testing the hypothesis that a specific biological phenomenon could be explained by exposure to a particular therapy, and pragmatic, in that it was a clinically feasible intervention in typical patients in a common clinical setting, rendering the results clinically applicable [9,10]. Clinical trials with a pragmatic orientation are increasingly advocated in the evidence-based medicine literature [9,10]. It remains possible that specific patients with different clinical diagnoses and types and severities of metabolic stress would respond differently to the correction of hypovitaminosis C or D than was observed in this study. In particular, the participants in this study were mentally competent. People with hypovitaminosis and concurrent psychiatric disorders might respond differently to vitamin C supplementation.

In conclusion, this and other recent studies document a high prevalence of hypovitaminosis C in acutely hospitalized patients. Whether the explanation is tissue redistribution, insufficient vitamin C provision in a setting of increased catabolism, or both, a consequence of in-hospital hypovitaminosis C could be cerebral hypovitaminosis C with a resulting mood disorder that could be easily ameliorated or prevented by adequate vitamin C provision.

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