Clinical trial

Effect of isotretinoin treatment on plasma holotranscobalamin, vitamin B12, folic acid, and homocysteine levels: non-controlled study

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Abstract

Isotretinoin (Iso) has been used for the treatment of acne. Some previous studies reported elevated homocysteine (Hcy) levels after treatment with Iso. Some side effects have clinical presentations similar to vitamin B12, folic acid deficiencies, and hyperhomocysteinemia. In the present study we evaluated the plasma Hcy levels, the vitamins involved in its metabolism (vitamin B12 and folic acid), and holotranscobalamin (HoloTC), a transport system for vitamin B12 absorption in patients receiving Iso treatment for acne vulgaris. A total of 66 patients with acne vulgaris between the ages of 18 and 40 years were included. Screening for hemoglobin, creatinine, SGOT, SGPT, total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein cholesterol (VLDL-C), folic acid, vitamin B12, Hcy, and HoloTC were done just before initiation (pretreatment) and after four months of Iso treatment (posttreatment). Posttreatment vitamin B12, folic acid, and HoloTC levels were significantly lower while Hcy levels were significantly higher compared with initial values. Posttreatment total cholesterol, LDL-C, triglycerides, VLDL-C, SGPT, and SGOT levels were also higher, and HDL-C levels were lower compared with initial values while there was no change in hemoglobin levels during Iso treatment. We found that Iso usage might cause decreased vitamin B12, folic acid, and HoloTC. These Iso side effects might contribute to the missing link between Iso usage, hyperhomocysteinemia, and neuropsychiatric disorders. Trials may be made with the aim of demonstrating (clearly) if starting vitamin B12 and folic acid replacement therapies with Iso treatment initialization could be useful for preventing hyperhomocysteinemia and possibly related disorders.

Introduction

Oral isotretinoin (Iso, 13-cis-retinoic-acid) has been used for the treatment of severe acne for more than 35 years and indicated as first-line therapy for severe nodular acne and moderate to severe acne unresponsive to conventional therapy.¹ Iso is an effective and generally well-tolerated medication; however, it also has a broad side effect profile affecting mucocutaneous, musculoskeletal, ophthalmic, and central nervous systems, and some metabolic side effects such as dyslipidemia.² Most of the adverse effects are temporary and vanish after the drug is discontinued.³ Iso may also cause liver damage and as a result, elevated SGOT and SGPT levels.⁴,⁵ Treatment also has some neuropsychiatric side effects such as major depression, psychotic symptoms, suicidal ideation, and attempted suicides.⁶ Some previous studies reported elevated homocysteine (Hcy) levels after treatment with Iso.⁷,⁸ Hcy is metabolized to methionine via remethylation or to cysteine via vitamin B6-dependent transulfuration in the liver (Fig. 1). During transulfuration, Hcy is irreversibly catabolized into cysteine by cystathionine-β-synthetase enzyme. Most of the Hcy is remethylated, regenerating methionine, mainly due to the action of methionine synthetase, an enzyme that depends on the action of methylcobalamin (vitamin B12) as a co-factor and folic acid, in the form of 5-methyl-tetrahydrofolic acid, as a methyl group donor. 5,10-methylene-tetrahydrofolic acid is irreversibly reduced to 5-methyl-tetrahydrofolic acid in the presence of NADPH by the enzyme, 5,10-methylene-tetrahydrofolate reductase (MTHFR), an enzyme that also regulates the entry of folic acid into the Hcy remethylation...
Several drugs and liver dysfunction may adversely affect cystathionine-β-synthetase, thereby causing an increase in blood Hcy levels. Both vitamin B12 and folic acid are also required as cofactors for the enzyme Hcy methyltransferase, and any deficiency in either of these two vitamins results in hyperhomocysteinemia.

In observational studies, hyperhomocysteinemia was found to be a risk factor for coronary heart disease and might be associated with a decrease in cognitive function and dementia. It is clear from the literature that poor vitamin B12 status is accompanied by an increased prevalence of depressive and other neuropsychiatric disorders. Deficiency of the other essential vitamin for Hcy metabolism, folic acid, is associated with megaloblastic anemia, congestive heart failure, pigmentation, infertility, cervical dysplasia, neuropathy, psychiatric disorders, cognitive dysfunction, dementia, and some behavioral abnormalities. Folic acid and vitamin B12 replacement therapies are first-line therapeutic choices for elderly dementia.

Some Iso side effects have clinical presentations similar to vitamin B12 and folic acid deficiencies, and hyperhomocysteinemia. There is a case report in literature that reports vitamin B12 and folic acid deficiency in a patient receiving Iso treatment. Also, in a previous study authors reported folic acid deficiency developing under Iso treatment. In the present study, we evaluated the plasma levels of Hcy, the vitamins responsible for its metabolism, vitamin B12 and folic acid, and the transport system for vitamin B12 absorption, holotranscobalamin (HoloTC), in patients receiving Iso treatment for cystic acne.

Materials and methods
A total of 66 patients with acne vulgaris, who were admitted to the dermatology outpatient clinic, were included. The study group was selected from male or non-pregnant female patients between the ages of 18 and 40 years with moderate to severe nodulocystic acne. Females of childbearing age were included if they were using at least two separate and effective methods of birth control and had a negative serum pregnancy test one week before initiation of Iso therapy. Treatment was initiated on the second or third day of the menstrual cycle in these patients. Patients using any vitamin A supplements or having any of the following problems were excluded from this study: sensitivity or allergy to parabens; recent history of drug
or alcohol abuse (>80 g/d for the last 5 years); recent history of psychiatric disorders, mood or depressive disorders, significant depression, or a history of persistent symptoms of depression; previous therapy with oral retinoids; history of diseases known to affect vitamin B12 and/or folic acid metabolism (pernicious or megaloblastic anemia, celiac sprue, malabsorption syndromes, gastric or ileal resection); using multivitamin preparations containing folic acid, vitamin B12, or drugs affecting their metabolism for the last three months (phenytoin, cyclosporine, methotrexate, trimethoprim, barbiturates); hormone therapy for any reason in the last three months; pregnancy; cigarette smoking; thyroid dysfunction; and previously known diabetes mellitus. The local ethics committee approved the study. Each patient gave his or her written informed consent, and the study was conducted according to the rules of good clinical practice. Before enrollment, written informed consent was obtained from each patient.

Iso therapy was initiated at a dose of 0.5–0.75 mg/kg body weight. Treatment was continued for at least five months. The drug was administered twice daily with meals. Screening for biochemical parameters was done just before initiation (pretreatment) and after four months of Iso treatment (posttreatment). These parameters included folic acid, vitamin B12, Hcy and HoloTC, hemoglobin, creatinine, SGOT, SGPT, total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and very-low-density lipoprotein cholesterol (VLDL-C). Fasting blood samples were obtained by venipuncture of the large antecubital veins of patients without stasis and after a 12-hour fasting. The samples were then centrifuged immediately; the plasma was separated and stored at 80°C. In order to avoid variation, all samples were studied on the same day and using the same kit.

Statistical analyses were performed with SPSS software (Statistical Package for the Social Sciences, version 11.0; SSPS Inc, Chicago, IL, USA). All numerical variables are expressed as the mean ± standard deviation (SD). Normality of data was analyzed by using a Kolmogorov–Smirnov test. A paired sample t-test was performed for analyzing initial and final values of hormonal and biochemical data with homogenic variability. A Wilcoxon signed ranks test was used for analysis of data with non-homogenic variability. Power analysis was done by GPower software, version 3.1.2 (Universitat Kiel, Germany).

Results

A total of 66 patients were included (48 female, 18 male; age 22.3 ± 4.7 years; range 18–40 years). Pre- and post-treatment levels of biochemical parameters are summarized in Table 1. According to statistical analysis, posttreatment vitamin B12, folic acid, and HoloTC levels were significantly lower while Hcy levels were significantly higher compared with the initial values (Table 1). Posttreatment total cholesterol, LDL-C, triglycerides, VLDL-C, SGPT, and SGOT levels were also higher, and HDL-C levels were lower compared with initial values.

### Table 1 Comparison of pre- and posttreatment parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pretreatment values</th>
<th>Posttreatment values</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD (range)</td>
<td>Median (interquartile range)</td>
<td>Mean ± SD (range)</td>
</tr>
<tr>
<td>Homocysteine (mM)</td>
<td>12.9 (6.05)</td>
<td>15.5 (6.5)</td>
<td>&lt;0.05</td>
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<tr>
<td>Folic acid (ng/ml)</td>
<td>7.3 ± 2.3 (3.1–9.8)</td>
<td>5.7 ± 2.4 (2.4–11.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Vitamin B12 (pg/ml)</td>
<td>216.4 (111)</td>
<td>170.2 (133.7)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Holotranscobalamin (pw)</td>
<td>75.7 (25)</td>
<td>48.6 (23.4)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>SGOT* (IU/l)</td>
<td>15.8 ± 6.9 (6.0–26.0)</td>
<td>16.5 ± 9.4 (6.0–58.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SGPTb (IU/l)</td>
<td>19.9 ± 6.1 (10.0–45.0)</td>
<td>21.8 ± 6.3 (14.0–47.0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>13.9 ± 1.4 (11.4–17.0)</td>
<td>14.1 ± 1.8 (11.6–16.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>160.0 (31)</td>
<td>179.5 (50.5)</td>
<td>&lt;0.001</td>
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<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>84.0 ± 23.5 (65.0–179.0)</td>
<td>99.7 ± 37.2 (55.0–248.7)</td>
<td>&lt;0.0001</td>
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<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>49.8 ± 9.5 (36.0–69.0)</td>
<td>45.5 ± 12.8 (31.0–72.0)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>VLDL cholesterol (mg/dl)</td>
<td>16.6 ± 7.5 (10.2–23.8)</td>
<td>23.5 ± 12.8 (11.0–42.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>84.0 ± 44.4 (51.0–119.0)</td>
<td>110.3 ± 65.5 (55.0–211.0)</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very-low-density lipoprotein cholesterol. aGlutamicoxaloacetic transaminase. bGlutamic-pyruvic transaminase.
while there was no change in hemoglobin levels during Iso treatment (Table 1). The power analysis results were: 81.45% for folic acid; 78.1% for vitamin B12; 72.5% for Hcy; and 71.3% for HoloTC.

**Discussion**

Hyperhomocysteinemia can be a result of genetic disorders, dietary deficiencies, certain disease states, and drugs. Genetic deficiencies resulting in hyperhomocysteinemia are due to defects in the genes encoding for enzymes of Hcy metabolism, including cystathionine synthase, MTHFR, and methionine synthase.11,22 Folic acid and vitamin B12 and B6 deficiencies may also cause hyperhomocysteinemia.11,23 Hcy is metabolized in the liver and recycled into methionine by a transmethylation reaction requiring folic acid and vitamin B12 as methyl group donor and co-factor respectively; therefore, hepatic dysfunction and/or deficiency of B vitamins could cause decreased metabolism of Hcy and hyperhomocysteinemia.24

In the literature, a first study evaluating changes in plasma Hcy levels in 28 patients during Iso therapy for cystic acne was reported by Schulpis et al.8 They found that patients had an elevation of plasma Hcy, serum triglycerides, total cholesterol, LDL-C, and VLDL-C during Iso treatment. Based on these findings, the investigators concluded that elevated levels of Hcy despite normal values of the responsible vitamins for the amino acid metabolism might be a result of an inhibitor action of Iso on cystathionine-β-synthase. In another study, including 74 patients on Iso therapy for cystic acne, plasma Hcy, liver enzymes, triglycerides, total cholesterol, LDL-C, and VLDL-C levels increased significantly after treatment; however, there were no changes in the vitamin levels.7 Conversely, a 28-day treatment with Iso was reported to decrease plasma folic acid levels without any change in plasma Hcy levels.21 In two of these previous studies, authors concluded that there was no need for vitamin B12 or folic acid replacement during Iso treatment.7,8 Follow-up durations were shorter in the previous studies than ours (45 days vs. four months). In the present study, we found that folic acid, vitamin B12, and HoloTC levels decreased, whereas Hcy levels increased at the end of 4 months of Iso treatment. We also found that patients had increased total cholesterol, LDL-C, VLDL-C, and decreased HDL-C levels at the end of the treatment period. SGOT and SGPT levels also increased significantly compared with initial values. We think that longer treatment with higher cumulative doses of Iso might have caused subclinical vitamin B12 and folic acid deficiencies to have more effect on Hcy levels.

Iso is a commonly used drug that is usually well tolerated except for uncommon mucocutaneous side effects and atherogenic changes in the lipid profile. A previous study, including 13,772 patients, reported that elevations in triglyceride levels were the most common laboratory abnormalities, with 44% of patients demonstrating a value above the normal range during the treatment period. The authors also reported an 11% incidence of new transaminase elevations during the treatment period.25 Therefore, our findings of hyperlipidemia and elevated liver enzymes are clearly consistent with previous reports. In observational studies, hyperhomocysteinemia has been reported as a risk factor for coronary heart disease and found to be associated with endothelial damage and oxidative stress.26,27 It is clear that hyperhomocysteinemia can promote atherosclerosis, but the underlying pathophysiological mechanisms are not well understood.28 Moreover, the elevated lipids in addition to hyperhomocysteinemia could be associated with an increased risk of premature occlusive vascular disease in patients receiving Iso therapy.7

A less widely researched issue is the degree to which Iso can create psychiatric problems, including depression, psychotic symptoms, suicidal ideation, and attempted suicide. Previous animal studies have reported that retinoids can cross into the central nervous system and may alter mood regulation efforts by affecting dopamine signaling systems.2 Despite these possible links, there are no reported, exact and clear biological mechanisms that link Iso and depressive symptoms in the current literature.29 However, it is obvious that these neuropsychiatric problems are very similar to disorders secondary to hyperhomocysteinemia, vitamin B12, and folic acid deficiencies. There are some studies that reported the association between Hcy and neurotransmitters and demonstrated the antidepressant effects of folic acid and S-adenosylmethionine, a cofactor and an intermediate metabolite of the methionine-Hcy pathway.18 High Hcy levels were also found to be associated with a decrease in cognitive function and dementia.15–17 Vitamin B12 deficiency was also reported to be associated with hyperhomocysteinemia and some psychiatric disorders. Depressive and neuropsychological complaints can be caused by various mechanisms in these patients, including increased Hcy levels.18 Folic acid deficiency was also reported to cause neuropathy, psychiatric disorders, cognitive dysfunction, and dementia.16,18

To our knowledge, this is the first study investigating changes in HoloTC levels in patients receiving Iso treatment. After entering enterocytes, vitamin B12 is transferred to transcobalamin II (TC), a complex known as HoloTC. Then HoloTC enters the blood circulation and is finally taken up. About 50% of circulating vitamin B12 is in the form of HoloTC that represents metabolically active B12. Total vitamin B12 measurement is used cost-
effectively as the parameter of choice; however, it has limited sensitivity and specificity, especially in persons with vitamin B₁₂ concentrations < 400 pM. Clinical signs of vitamin B₁₂ deficiency can be seen in persons with vitamin B₁₂ concentrations within the normal reference range. Persons with normal concentrations of vitamin B₁₂ may have raised concentrations of methylmalonic acid (>300 nm) and lowered concentrations of HoloTC (<35 pM), indicating functional deficiency. A decreased serum HoloTC concentration is the earliest marker of vitamin B₁₂ deficiency and shows that the body does not have sufficient available vitamin B₁₂. At this early stage, clinical or hematological symptoms might not be present.

Neuropsychiatric side effects of Iso are very similar to the findings, which are seen in vitamin B₁₂ and folic acid deficiencies. These vitamin deficiencies lead to hyperhomocysteinemia, which is related to neuropsychiatric disorders and is considered an important risk factor for atherosclerotic vascular disease. Vitamin B₁₂ and folic acid deficiencies might be the missing link between Iso usage, hyperhomocysteinemia, and neuropsychiatric disorders. We think that studies with higher cumulative doses of Iso may reveal a stronger decline in vitamin B₁₂ and folic acid levels.

In conclusion, vitamin B₁₂ and folic acid deficiencies and hyperhomocysteinemia may be caused by Iso treatment in a short period of time. Longer treatment with Iso may reveal a different effect on these vitamins. Studies employing specific questionnaires are needed to understand the relation of these vitamin deficiencies with depression and cognitive function in patients treated with Iso. Further trials may be needed to demonstrate if starting vitamin B₁₂ and folic acid replacement therapies with Iso treatment initialization could be useful for preventing hyperhomocysteinemia and possibly related disorders.

References


