Folic acid safety and toxicity: a brief review

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ABSTRACT

Oral folic acid (pteroylglutamic acid) is generally regarded as not toxic for normal humans but it may cause neurological injury when given to patients with undiagnosed pernicious anemia. The vitamin should be given with caution to drug-treated epileptic patients because seizure control may be affected. Some studies suggest that folic acid supplements interfere with intestinal zinc absorption in humans and animals but others do not confirm such an effect. The weight of current evidence favors the view that daily supplements of 5–15 mg folic acid do not have significant adverse effects on Zn nutriture in healthy nonpregnant subjects. Because antifolate medications are now being used to treat a wide range of malignant and nonmalignant disorders, further investigation is needed concerning folate metabolism and the safety of supplements in patients with these disorders.

KEY WORDS Folic acid, safety, toxicity, pernicious anemia, epilepsy, zinc and folate interaction, antifolate

Introduction

When synthetic folic acid (oxidized pteroylglutamic acid) was first introduced into clinical medicine 43 y ago, there was only meager information regarding human requirements. Lacking firm knowledge about its concentration in foods, bioavailability, tissue content, and turnover, early investigators empirically chose dose levels now known to be far in excess of nutritional requirements. Fortunately, good effects outweighed the bad (if any). In the treatment of tropical sprue, Suarez et al (1) employed oral doses of 100 mg daily for 2 wk or single oral doses of 500 mg which are, respectively, ≥ 200–1000 times present-day estimates of the nutritional requirement, yet no toxicity was observed. In a well-respected textbook of pharmacology (2) the following statement appears: “Oral folic acid is not toxic for man. Even with doses as high as 15 mg per day, there have been no substantiated reports of side effects.”

Hunter et al (3) in 1970 described mental changes, sleep disturbances, and gastrointestinal symptoms over a 1-mo period in 14 normal volunteers who ingested 15 mg folic acid/d. Several others reported no toxic side effects with folic acid supplementation in normal subjects (4–6). Sheehy (4) described a man who took 60 mg folic acid/d for 3 y with no apparent toxic effect. Taylor et al (7) described renal hypertrophy and epithelial cell hyperplasia in rats after large doses of folic acid but there have been no reports of renal toxic effects in humans. In a brief review of the toxicity of folic acid and other vitamins, DiPalma and Ritchie (8) concluded that most water-soluble vitamins are nontoxic in ordinary doses but they noted that “even folic acid may be toxic in exceptional circumstances.”

Neurologic disease in pernicious anemia

Undoubtedly the most dreaded toxic effect of folic acid is its ability to mask the anemic manifestations of pernicious anemia while allowing the neurological disease (posterolateral spinal cord degeneration [PCD]) to progress. Fortunately this devastating complication is unlikely to occur with the amounts of folate in ordinary diets and in over-the-counter vitamin supplements. Moreover, the diagnosis of pernicious anemia can be ex-

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cluded with a reasonable degree of certainty if the serum vitamin B-12 is normal. Because vitamin B-12 assays are now widely available in diagnostic laboratories, the test should probably be performed as a precautionary measure before pharmacologic doses of folic acid are prescribed.

Because of a virtual absence of data on the prevalence of folate-induced PCD, an informal survey was conducted in 1971 (CE Butterworth Jr, unpublished observations, 1988). A first-class letter was sent to the entire membership of the American Society of Hematology (~1200 members) with a stamped, self-addressed post card asking, “Have you personally cared for any patient during the last ten years whose illness was, in your opinion, made worse by the daily ingestion of 400 micrograms (or less) of folic acid, in addition to amounts present in the diet? Yes ____ No ____.” There were 860 responses, representing 72% of the mailing. Of these, 98% had no knowledge of anyone made worse by a supplement of 400 μg folic acid/d during the previous 10 y. A follow-up inquiry requesting further information was then sent to each of the 20 respondents who gave a “yes” answer to the question. There were seven responses of which only three qualified as cases of PCD during the 10-y period: one with a daily intake of 0.1 mg, one with 5.0 mg, and one unknown. The 5.0-mg dose was prescribed by a physician. There were no peer-reviewed publications of case reports. Quite obviously there are many weaknesses in a study of this type but it suggests that aggravation of pernicious anemia and PCD associated with the use of over-the-counter folic acid supplements is extremely rare (ie, one or two cases nationwide in a 10-y period). Sixty-five percent of respondents reported that they had encountered subjects whose illness might have been improved or prevented by a 400-μg supplement of folic acid.

In one of the early references to neurologic injury in pernicious anemia, Heine and Welch (9) made the following statement, “... we have treated and maintained 47 patients with synthetic folic acid for periods up to one year. Of this number only 2 others (in addition to the patient described) have exhibited definite neurologic relapse.” The one case described in detail by Heine and Welch (9) displayed dramatic improvement in the anemia and improved sense of well-being over a period of 79 d. At that time his four remaining teeth were extracted and he was placed on a soft diet, after which his neurologic condition rapidly deteriorated. Questions may be asked as to whether poor diet and/or anesthesia (perhaps with nitrous oxide) played significant contributory roles in the neurological deterioration. One is also led to wonder if some of the other 47 patients would have fulfilled present-day criteria for the diagnosis of pernicious anemia. Because of ethical considerations it is likely that the precise incidence of folate-induced, subacute combined degeneration of the spinal cord in pernicious anemia will never be known.

**Folate interaction with anticonvulsant drugs**

Chanarin et al (10) in 1960 first described the possibility that folic acid supplements in high doses may reverse the effectiveness of anticonvulsant medication. Since that time published reports as to whether folic acid therapy affects fit frequency have been controversial (8). In a study on the effect of intravenous folic acid on drug-treated epileptics, it was observed that some subjects appear to be relatively sensitive and others more tolerant to the infusion (11). Six subjects received 75 mg folic acid intravenously in a period of 30 min without electroencephalogram (EEG) changes, seizure, or other ill effects. One subject demonstrated a transiently abnormal EEG pattern with characteristic spikes but no seizure activity or other ill effects after receiving 150 mg folic acid in 30 min. The eighth patient displayed a burst of spikes and slow waves on the EEG but no seizure after receiving 7.2 mg folic acid intravenously over a period of 3 min. Typical seizures and EEG tracings followed subsequent infusions of 14.4 and 19.2 mg folic acid. It may be concluded that some individuals with drug-controlled epilepsy are sensitive to rapid intravenous infusions of as little as 7.0 mg folic acid. In contrast, others tolerated up to 20 times this amount without seizure activity or other ill effects.

**Folic acid–zinc interactions**

During the last several years interest has developed in relationships between folic acid and zinc (12–19). There is good evidence that bovine hepatic folic acid conjugase is a Zn metalloenzyme (20) and that folic acid polyglutamates are not well absorbed by the intestine in Zn-deficient humans (21). However, there are conflicting reports as to whether folic acid supplements exert deleterious effects on Zn nutrition in man. Milne et al (12) in 1984 reported that a folic acid supplement of 400 μg every other day, in addition to a dietary folate intake of 150–180 μg/d, was associated with increased fecal losses of Zn. However, urinary losses appeared to compensate for fecal losses so that net Zn balance was not affected. Nevertheless the authors cautioned about “the possibility of adverse effects from supplemental pteroylglutamic acid.”

Also in 1984, in a detailed and complex study of 450 pregnant women, Mukherjee et al (13) observed a high degree of correlation between the occurrence of pregnancy complications or fetal distress and the combination of low plasma Zn and high folate concentration in the maternal blood sample. They postulated that folic acid supplementation and iron supplements may each interfere with the intestinal absorption of Zn. They suggested further that prenatal vitamin supplements, which not uncommonly contain 800–1000 μg folic acid, inhibit intestinal absorption of Zn, which in turn was responsible for the observed fetal distress.

Simmer et al (14) recently gave a cautiously affirmative answer to the question, are Fe-folate supplements
harmful? Zn absorption was assessed by measuring plasma Zn concentration after an oral dose of Zn sulfate (25 mg Zn) rather than by metabolic balance. Comparisons were made of the area under the curve and the peak concentration at 1, 2, 3 or 4 h, a procedure that is subject to error because of variations in gastric emptying, peripheral uptake, and renal excretion. It does not give information concerning long-term absorption or net metabolic balance. The test was performed in 10 healthy pregnant women and repeated after 2 wk of oral supplementation with a commercial product containing 350 μg folic acid and 100 mg ferrous Fe. The average dietary Zn intake of the subjects was 12.1 mg/d (60% of Recommended Dietary Allowances [22] based on 7-d diet histories). A similar test was performed in a group of eight men and two nonpregnant women with twice as much Zn sulfate (50 mg Zn) after a 2-wk period of oral supplementation with 350 μg folic acid, rather than the folic acid and Fe combination. Treatment depressed the bioavailability of Zn in both groups, according to the stated criteria (14). The effect was more pronounced with the folic acid and Fe combination than with folic acid alone. It should be noted that the Zn-absorption test was performed after a period of supplementation; the Zn and folic acid were not administered simultaneously. More data are needed concerning possible effects of folic acid supplementation on Zn balance in pregnant women.

Ghishan et al (15) described two animal experiments and a series of in vitro studies designed to examine relationships between Zn and folate. Perfusion of 30-cm segments of rat small bowel in situ revealed diminished mucosal uptake of radioactive Zn when folate was also present in the lumen. Previous intraperitoneal injection of folate did not block mucosal Zn uptake. For reasons that are not stated, the reciprocal relationship, ie, the effect of Zn on radioactive folate absorption was not studied in the same system but rather in an in vitro preparation of everted rat jejunal segments. It was observed that a 1000-fold molar excess of Zn did not significantly alter mucosal-to-serosal transport of radioactive folate; however, inhibition was observed when the molar ratio of Zn to folate was 2500:1. Ghishan et al (15) also described in vitro experiments concerning the effect of charcoal-binding and pH. The findings suggest that Zn and folate form an insoluble complex at low pH but the complexes dissolve when the pH is raised above 6.0.

Keating et al (16) also studied the Zn-folate interaction in Sprague-Dawley rats and humans but under somewhat different circumstances. After intragastric administration of 65Zn to rats, the researchers observed no significant differences in hepatic and renal Zn retention at molar ratios of either 1:20 or 2:1 for folic acid:Zn. A similar lack of effect on growth pattern and Zn uptake into bone was observed with diets representing these same molar ratios of folic acid:Zn. When six healthy young men took 25-mg oral doses of Zn with or without 10 mg folic acid, similar patterns of increase in plasma Zn were observed, with peak levels at 2 h after ingestion of the dose.

Fuller et al (17) determined the effect of folic acid in the diet on tissue folate and Zn levels in rats during pregnancy and lactation. High-folic-acid diets (100 mg folic acid added to 1 kg basal diet) fed for over 40 d significantly increased blood folate levels but did not change Zn concentrations in various tissues. The authors suggest that if the results are applicable to humans, then some reassurance is provided that prenatal folate supplementation does not necessarily cause Zn depletion in pregnant women.

A study currently in progress has afforded the opportunity to compare plasma and erythrocyte concentration of Zn in a group of young women receiving a 10-mg daily supplement of oral folic acid with an identical group receiving a placebo (18). Fifty subjects with cervical dysplasia, of whom 27 received folic acid and 23 received placebo, were evaluated at the end of 2 mo. At the end of 4 mo, 12 of the same subjects in the folic acid group and 9 in the placebo group were available for another comparison. In spite of a dramatic difference in plasma and erythrocyte folate concentration between the supplemented and placebo groups (p < 0.001 at both 2 and 4 mo), there was no significant difference between the two groups with regard to Zn. Subsequently, 49 subjects in the folic acid–supplemented group and 58 subjects in the placebo groups completed the 6-mo randomized, double-blind clinical intervention trial. The median value for Zn concentration in plasma and erythrocytes was virtually identical in the two groups of subjects and remained virtually unchanged throughout 6 mo of folic acid supplementation.

A recently published abstract by Krebs et al (19) reports no impairment of absorption of a stable isotope of Zn (95Zn) in three normal adults when administered with a simultaneous dose of 30 mg folic acid. Another group of eight subjects aged 24 ± 12 y with the fragile-X syndrome who had received 16 ± 5 mg folic acid/d for 1–4 y was also studied; mean fasting Zn concentrations in plasma were not significantly different from values from adult control subjects. Neutrophil Zn levels and the activities of two Zn-dependent enzymes (serum alkaline phosphatase and erythrocyte δ-aminolevulinic acid dehydratase) were also within normal limits in folic acid-supplemented subjects.

Divergent experimental techniques

It is difficult to reconcile the conflicting results reported in the literature regarding a deleterious effect of folic acid supplements on Zn nutriture. A major source of confusion is the noncomparability of results due to widely different experimental techniques. For example, some human studies have employed plasma Zn response curves after an oral dose of Zn with or without folate. This approach is subject to error because of variations
in such factors as gastric emptying, intestinal absorption rate, and excretion by the kidney. Some have compared increases in blood levels of Zn but not folic acid, and vice versa, when both had been administered orally. Other studies compared increases in plasma Zn after a 1-2-wk period of supplementation with folic acid (ie, both nutrients were not administered simultaneously). These results must be regarded with skepticism until confirmed by more reliable procedures. Investigations which in one way or another reflect long-term metabolic balance in man (12, 18, 19) or animals (16, 17) have not indicated deleterious effects of folic acid supplements on Zn. A summary of some published studies of Zn-folate interactions is presented in Table 1.

Antifolate medications

Virtually nothing is known about the safety or toxicity of oral folic acid supplements in humans who are being treated with drugs known to interfere with folate metabolism. A wide variety of folic acid antagonists is being used to treat such diverse illnesses as cancer (23), leukemia (24, 25), psoriasis (26), rheumatoid arthritis (27, 28), polymyositis (29, 30), dermatomyositis (30, 31), Reiter disease (32), Wegener granulomatosis (33), sarcoidosis (34), bronchial asthma (35), sclerosing cholangitis (36), primary biliary cirrhosis (37), bacterial infection (38), malaria (39, 40), hypertension (41), Crohn disease (42), ulcerative colitis (25), gout (43), and epilepsy (10). A partial list of these diseases along with the antifolate medication commonly used in their treatment is presented in Table 2. Methotrexate, pyrimethamine, trimethoprim, and trimetrexate are all known to be inhibitors of dihydrofolate reductase. Recently a new dihydrofolate reductase inhibitor, trimetrexate, has been used successfully in the treatment of Pneumocystis carinii pneumonia in patients with AIDS (44). Species differences exist regarding enzyme susceptibility to inhibition but little is known regarding differences between human individuals or in different human tissues. The use of so-called folic acid antagonists in these disorders might seem to imply that an overabundance of folate in the diet would be harmful. There is little or no evidence to support such a view, nor is it known if folic acid supplements negate the therapeutic effectiveness of these medications. Indeed, because folic acid is itself an inhibitor of dihydrofolate reductase (45, 46) it could quite possibly be not only safe but beneficial in the treatment of these disorders. Alternatively it may be possible to titrate dosages of folic acid and antifolate medication to spare certain pathways while inhibiting others. For example, sulfasalazine is not only an inhibitor of dihydrofolate reductase but also of methyltetrahydrofolate reductase, serine transhydroxymethylase (47), and human jejunal brush border conjugase (48). Colchicine suppresses blood folate levels in mice, dogs, and humans (43) although the mechanism is

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### Table 1

<table>
<thead>
<tr>
<th>System</th>
<th>Molar ratio of Zn to folate*</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human: metabolic balance</td>
<td>135:1</td>
<td>Increased fecal Zn; net balance unaffected</td>
<td>12</td>
</tr>
<tr>
<td>Human: plasma Zn response curve</td>
<td>750:1</td>
<td>Flat response curve after oral Zn dose</td>
<td>14</td>
</tr>
<tr>
<td>Rat: gut perfusion</td>
<td>1:4.5</td>
<td>Zn uptake inhibited</td>
<td>15</td>
</tr>
<tr>
<td>Rat: everted gut sac</td>
<td>1000:1, 2500:1</td>
<td>Folate uptake not inhibited, Folate uptake inhibited</td>
<td>15, 15</td>
</tr>
<tr>
<td>Rat: growth, tissue uptake</td>
<td>20:1</td>
<td>Growth not inhibited; bone Zn uptake normal</td>
<td>16</td>
</tr>
<tr>
<td>Human: plasma Zn response curve</td>
<td>17:1</td>
<td>Normal curve of plasma Zn response after oral dose</td>
<td>16</td>
</tr>
<tr>
<td>Rats: tissue Zn levels</td>
<td>4.4:1, 13.4:1, 1.2:5, 1.2:1</td>
<td>No depression of tissue Zn</td>
<td>17</td>
</tr>
<tr>
<td>Human: red blood cell and plasma Zn</td>
<td>7:1</td>
<td>No depression of blood Zn after 2 and 4 mo</td>
<td>18</td>
</tr>
<tr>
<td>Human: plasma Zn, Zn metalloenzyme, absorption of Zn</td>
<td>7:1</td>
<td>No depression of blood Zn and metalloenzyme activity after 1-4 y, normal Zn absorption</td>
<td>19</td>
</tr>
</tbody>
</table>

* Normal value is 250:1 based on Recommended Dietary Allowances (22) (15 mg [230 μmol] Zn and 0.4 mg [0.91 μmol] folic acid).

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### Table 2

<table>
<thead>
<tr>
<th>Disease</th>
<th>Drug</th>
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<tbody>
<tr>
<td>Cancer, leukemia</td>
<td>Methotrexate</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>Methotrexate</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>Methotrexate</td>
</tr>
<tr>
<td>Bronchial asthma</td>
<td>Methotrexate</td>
</tr>
<tr>
<td>Bacterial infection</td>
<td>Trimethoprim</td>
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<tr>
<td>Malaria</td>
<td>Pyrimethamine</td>
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<tr>
<td>Hypertension</td>
<td>Triamterene</td>
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<tr>
<td>Crohn disease</td>
<td>Sulfasalazine</td>
</tr>
<tr>
<td>Gout</td>
<td>Colchicine</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>Phenytoin</td>
</tr>
<tr>
<td>AIDS</td>
<td>Trimetrexate</td>
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</table>
not established. It is not known whether the beneficial effects of colchicine therapy in gout are due to suppression of folate-mediated pathways of purine biosynthesis or to some more general antiinflammatory action. The safety of folate supplementation remains an open question for patients with the diseases listed in Table 2, whether or not they are receiving antifolate medication.

Summary

Daily oral supplements of 5–10 mg folic acid appear to be well-tolerated and without toxicity in normal non-pregnant subjects; some studies suggest the desirability of ensuring an adequate intake of Zn if folate supplements are to be used during pregnancy. Neurologic injury may occur when folic acid supplements are given to patients with undiagnosed pernicious anemia. This is probably quite rare and avoidable with more widespread use of screening tests for serum vitamin B-12. Folic acid supplements should be used with caution in patients with epilepsy because seizure activity may be induced in some (but not all) drug-controlled subjects. Although high concentrations of folate may interfere with the intestinal absorption of Zn in experimental animals, the weight of current evidence indicates that daily oral supplements of 5–15 mg folate do not adversely affect Zn balance in normal humans over periods of 6 mo to 4 y. Further investigation is needed concerning folate metabolism in a wide range of clinical disorders now being treated with antifolate medications.

References

46. Morales DR, Greenberg DM. Purification and properties of dihydrofolate reductase of sheep liver. Biochim Biophys Acta 1964;85:360-76.