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Folic acid supplementation: The new dawn for postmenopausal women with hot flushes

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Abstract

Hot flushes, experienced by 75% of menopausal women, are associated with estrogen deprivation. Estrogen was shown to ameliorate hot flushes by interacting with monoamine neurotransmitters in the brain; reducing noradrenaline and increasing serotonin. Hormone replacement therapy (HRT), the first treatment option, causes concerns over possible increased risks particularly breast cancer. Folic acid is involved in the biosynthesis of serotonin and nordrenaline, which is responsible for its effects on mood and cognition, and degrees of folate inadequacy, not severe enough to produce megaloblastic anaemia, were found to be associated with depression and cognitive malfunctioning. Also, increased age was observed to relate to reduced serum and cerebrospinal fluid folic acid levels. There is emerging evidence that folic acid supplementation ameliorates hot flushes by the same mechanism as estrogen. To explore this hypothesis, a multi-centre, double-blind, placebo-controlled randomized is being set up to compare the effect of 5 mg folic acid *vs* placebo in reducing the frequency and severity of hot flushes in postmenopausal women, and on the blood level of serotonin and noradrenaline. If folic acid supplementation is demonstrated to be effective, this will be a turning point in the clinical practice since it represents a cheap, safe and well-tolerated alternative to HRT.

Key words: Folic acid; Hot flushes; Menopause; Noradrenaline; Serotonin

Core tip: Hormone replacement therapy usage by postmenopausal women with hot flushes causes concerns over possible increased risks particularly breast cancer. The improved longevity of women in general and breast cancer survivors in particular, and the limited success shown by the non-hormonal alternatives made it imperative to find a therapy that is effective and safe. It is hypothesized that folic acid supplementation may ameliorate hot flushes by the same mechanism as estrogen supplementation, *i.e.*, by reducing noradrenaline and increasing serotonin neurotransmitters. This article discusses the rationale, potential role, mechanisms of action and safety issues related to its use in these women.

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CURRENT DILEMMA

Hot flushes, the most characteristic menopausal symptom, are experienced by up to 75% of menopausal women, and in half of them symptoms are severe enough to seek medical advice[1]. Hot flushes are associated with estrogen deprivation and they are the most commonly reported side effect of the selective estrogen receptor modulators[2]. Therefore, hormone replacement therapy (HRT)

is the first treatment option[3]. However, the perception of the risks and benefits of HRT had changed since the publication of Women's Health Initiative Trial in 2002[4], and an increasing number of women are seeking alternatives for conventional HRT because of the concerns over possible increased risks particularly breast cancer[3]. In addition, there is evidence that non-hormonal mechanisms play an important role in the pathophysiology of hot flushes[3,5].

Treatment of breast and endometrial cancer frequently results in the loss of ovarian function and menopausal symptoms. Symptoms of iatrogenic menopause are usually more intense than those of natural menopause due to sudden onset of symptoms, younger age, and the physical and psychological impacts such as body image concerns and sexual dysfunction[6]. Furthermore, the improved longevity of breast cancer patients and the increased use, in recent years, of aromatase inhibitors over tamoxifen, leading to profound estrogen deprivation[7,8], made it imperative to find a therapy that is effective and safe. In addition, the use HRT in breast and endometrial cancer survivors is not welcomed by most women and doctors because of the potential stimulation of residual cancer and the induction of new hormone-sensitive disease[9]. The non-hormonal alternatives which are commonly proposed to these women showed a limited success[3,10-12].

HOT FLUSHES - THE MECHANISM AND ROLE OF ESTROGEN

The entire episode of hot flushes usually lasts no more than 1-3 min. The frequency can range from 5 per year to 50 per day, with great variations among individuals or even within an individual, although 5-10 times per day is more common. They generally persist for 1 to 5 years, but in some women they can continue for as long as 44 years. There is no accepted metric for measuring severity of hot flushes[13].

Hot flushes exact aetiology is not yet understood. Although many theories were postulated to explain the pathophysiology, none of them could explain all aspects of hot flushes. Estrogen replacement was shown to ameliorate hot flushes by interacting with monoamine neurotransmitters in the brain; noradrenaline and serotonin [5-hydroxytryptamine (5-HT)][1,2].

It was hypothesized that hot flushes are triggered within the hypothalamus by α_2-adrenergic receptors on noradrenergic neurons. There is evidence to suggest increased central noradrenergic activity in women suffering from flushes, leading to disturbances in the thermoregulatory centre which is probably responsible for the occurrence of flushes[14]. It was found that yohimbine, an α_2-adrenergic antagonist, increased central noradrenaline release, provoking hot flushes, while clonidine, an α_2-adrenergic agonist, reduced central noradrenaline release, raised the sweating threshold and lowered the shivering threshold, leading to amelioration of flushes[14-16]. This theory was further supported by finding significantly elevated plasma levels of 3-methoxy 4-hydroxy phenyl glycol (MHPG), the end metabolite of brain noradrenaline in women with hot flushes[17,18].

Furthermore, it was found that 5-HT$_{1A}$, 5-HT$_{2A}$ and 5-HT$_7$ receptors are implicated in hypothalamic control of temperature[2,19]. It was hypothesized that hot flushes are the net result of activation of estrogen withdrawal induced up regulated 5-HT$_{2A}$ receptors in the hypothalamus by mild internal or external stimuli such as high ambient temperatures, anxiety, coffee, or alcohol, resulting in a hyperthermic response[5]. Estrogen affects the function of serotonin neural system, and the blood levels of serotonin fluctuate with the circulating levels of estrogen. In spontaneous and surgically menopausal women, it was found that blood levels of serotonin were reduced by about 50% when compared to premenopausal controls and estrogen replacement restored levels to normal[20]. Further, estrogen replacement in postmenopausal women augmented serotonergic activity, increased the excretion of 5-hydroxyindoleacetic acid (5-HIAA; the main metabolite of serotonin)[21] and increased the expression of tryptophan hydroxylase, the key enzyme in serotonin biosynthesis[19]. In addition, a number of serotonergic compounds such as serotonin re-uptake inhibitors fluoxetine, venlafaxine, sertraline and paroxetine, and the serotonin disinhibition mianserin and mirtazapine were shown to reduce both the number and intensity of hot flushes[5].

FOLIC ACID: WHAT CLINICIANS NEED TO KNOW?

Background

Folic acid, a water-soluble B-Vitamin, serves as the parent for a large family of compounds having similar nutritional value to which the generic term "folates" is applied[22]. As per the definition of a vitamin, it cannot be synthesized *de novo,* and must be derived from diet or supplementation. Dietary folates is found in leafy green vegetables, legumes, beans, liver, citrus fruits and yeast[23]. The name "folate" derives from the Latin for leaf *(folia)* since leafy green vegetables do contain folate[24,25]. Multiple biochemical conversions are required for dietary folates to become tetrahydrofolates; the metabolically active and tissue-usable forms. Folates are involved - *via* donation of a methyl group - in numerous biochemical pathways including monoamine neurotransmitters synthesis, which is responsible for its effects on mood and cognition[23-25].

Pharmacokinetics

Folic acid, the synthetic molecule, is highly absorbed (85%-95%) when compared to the dietary form (50%). Folate absorption takes place in the lumen of the proximal small intestine. After assimilation by the intestinal epithelial cells, a substantial fraction of the absorbed folate is methylated and reduced, partly through the action of "methylene tetrahydrofolate reductase enzyme", to 5-methyl tetrahydrofolate, which is the main circulating form of folic acid[23]. Vitamin B$_{12}$ is involved in the methyla-

tion of homocysteine to methionine, which is needed to convert 5-methyl tetrahydrofolate to tetrahydrofolate[22]. The peak folate serum level after oral administration is reached within 30 to 60 min. The average value of folic acid in serum is 7-36 nmol/L. Tetrahydrofolate and its derivatives are distributed in all body tissues. The liver, the principal storage site, contains half of the total body stores followed by erythrocytes. The normal erythrocyte level is about 320-1300 nmol/L[25,26].

Safety

Folic acid is usually well-tolerated with no adverse effects associated with the consumption of excess folates from food in human[24,27]. Daily oral supplements of 5-10 mg synthetic folic acid appear to be well tolerated and rarely cause side effects in healthy individuals[28-30]. A few cases of allergic reactions have been reported including skin rash, swelling of the face, lips, tongue or throat, or bronchospasm[26,31,32]. Caution is necessary in administering folic acid supplements alone in megaloblastic anaemia. If the cause is vitamin B12 deficiency, the megaloblastic anaemia may be corrected , but any nuerological manifestations (*e.g.*, subacute combined degeneration of the cord) are likely to get worse[24]. Folic acid supplements should be used with cautions also in patients with epilepsy because seizures activity may be induced since it reduces the serum level of some anti-convulsants[28]. A recent meta-analysis found no increase in overall and site-specific cancer incidence in the randomized controlled trials of folic acid supplementation at doses higher than those from fortification. It included 13 trials (with 49621 participants) that compared folic acid *vs* placebo, had treatment duration of at least 1 year, and included at least 500 participants. It was found that, during an average treatment duration of 5.2 years, folic acid supplementation increased folate serum concentrations by 4-fold (573 nmol/L for the folic acid groups *vs* 135 nmol/L for the placebo groups), but had no significant effect on overall cancer incidence (1904 cancers in the folic acid groups *vs* 1809 cancers in the placebo groups, RR = 1.06, 95%CI: 0.99-1.13, $P = 0.10$). There was also no trend towards greater effect with longer treatment durations[33].

HOW FOLIC ACID COULD AFFECT BRAIN FUNCTION

Folic acid is essential for the functioning of the nervous system. It is necessary for the biosynthesis of the monoamine neurotransmitters serotonin, noradrenaline and dopamine. 5-methyltetrahydrofolate, participates in re-methylation of the amino acid metabolite homocysteine, creating methionine. The downstream metabolite of methionine; S-adenosylmethionine, is involved in numerous one-carbon methylation reactions in the body, including those that create neurotransmitters, *i.e.*, S-adenosylmethionine must be present as a methyl donor for both the serotonin and catecholamine pathways to function properly. After donation of its methyl group, S-adenosylmethionine becomes homocysteine[23,34,35]. At this point, homocysteine must either be further metabolized to become cysteine, taurine, and glutathione or re-methylated to become methionine again. Re-methylation is done *via* "methionine synthetase", which facilitates the donation of a methyl group from vitamin B12 (which gets its methyl group from 5-methyltetrahydrofolate). Therefore, some researchers believe homocysteine is simply a marker of folate and/or B12 deficiency. Without the participation of 5-methyltetrahydrofolate in this process, S-adenosylmethionine and neurotransmitter levels decrease in the cerebrospinal fluid[23,34,35].

5-methyltetrahydrofolate also appears to stabilize, enhance production of, or possibly act as a substitute for tetrahydrobiopterin (BH4), which is an essential nutrient cofactor in the biosynthesis monoamine neurotransmitters serotonin, dopamine, noradrenaline, and adrenaline[23,34,35]. It appears to be important in regenerating BH4, which is highly susceptible to oxidation. In the absence of an adequate amount of BH4, 5-methyl tetrahydrofolate may substitutes for BH4 in the "hydroxylase enzymes" involved in monoamine neurotransmitters synthesis[36,37].

Folic acid deficiency in depression and old age

Over the past four decades, degrees of folate deficiency not severe enough to produce megaloblastic anaemia, were found to be associated with psychological symptoms, particularly depressive symptoms (*e.g.*, apathy, fatigue, insomnia, irritability and concentration difficulties) and impaired cognitive functioning[38-40]. Up to 71% of individuals with severe folic acid deficiency were found to have depression[41], and a French study found a significant association between high folate intake and a lower risk of depression in middle-aged men and women[42]. Bottiglieri *et al*[34], in a study of 46 inpatients with severe depression, found that 52% of them were having high homocysteine. Further, depressed patients with increased serum homocysteine had significantly lower (1) serum, red blood cell and cerebrospinal fluid folate; (2) cerebrospinal fluid S-adenosylmethionine; and (3) the metabolites of serotonin, noradrenaline and dopamine.

Several studies reported that low blood levels of folate and vitamin B12, and high levels of homocysteine were correlated with depression especially in the elderly[34,40,41]. A recent meta-analysis of 11 studies ($n = 15315$) found a significant relationship between the risk of depression and low folate status[43,44], and it was reported that 15%-38% of adults with severe depression had borderline or low serum and red blood cell folic acid[23,45-47]. It is estimated that 20%-30% of individuals with depression have also high homocystein levels[35,43,48-50]. Investigations revealed a connection between high homocysteine levels and brain dysfunction, including cognitive function, dementia, Alzheimer's disease, and depression[23] because it has a neurotoxic effect through several mechanisms, including impaired methylation, excitotoxicity, oxidative stress and hypoxia in the central nervous system[49]. Folate deficiency, by elevating homocysteine levels, may have a

role in depression[51], and folic acid supplementation was shown to reduce elevated homocysteine levels[52].

Increased age has been observed to relate to reduced serum and cerebrospinal fluid folic acid concentrations, and with increased, homocysteine levels at the same time[43]. The results of the studies are conflicting as regards the relation between depression and serum folic acid level in elderly population with some failing to identify a relationship[51,53] and others showing an inverse relationship[54,55]. It may be argued that folic acid deficiency in individuals with depression, particularly the elderly, might be attributed to poor nutrition, medication, chronic disease, increased needs, or malabsorption; however, low folic acid levels were observed in overweight individuals with depression, and in individuals who had gained weight inadvertently[40,47].

FOLIC ACID MAY CURE HOT FLUSHES: THE CLINICAL EVIDENCE SO FAR

The scientific literatures were searched using NHS evidence website: www.evidence.nhs.uk on March 4, 2013. Then under Journals and Databases, "Healthcare Databases Advanced Search" was accessed and the following databases were searched; AMED, BNI, CINAHL, EMBASE, HEALTH BUSINESS ELITE; HMIC, MEDLINE, PsycINFO. The key words for search were: "folic acid and menopausal women" and "folic acid and hot flushes". The search result was combined using "OR" and retrieved 44 articles. Only one original article[56], an abstract[57] and a hypothesis[58] were found (n = 3). The rest of the articles have been excluded; duplicates (n = 22) or non-relevant articles (n = 19).

Gaweesh *et al*[56], in a small prospective cohort study, examined the effect of folic acid 5 mg supplementation *vs* placebo for 4 wk on the occurrence of hot flushes in 46 healthy postmenopausal Egyptian women. In the treatment group, there was significant improvement of symptoms and significant lowering in plasma levels of MHPG. There was significant negative correlation between clinical improvement in hot flushes and the plasma level of MHPG. The improvement was described as "good" on complete disappearance of hot flushes, and "moderate" when the frequency and intensity of the flushes were satisfactorily reduced. The level of improvement was subjectively decided by women based on their overall feeling as regards the number and intensity of hot flushes. In the treatment group, 9 (39.1%), 6 (26.1%) and 8 (34.8%) women had good, moderate and no improvement, respectively. The equivalent figures for the control group were 1 (5.3%), 2 (10.5%) and 16 (84.2%), respectively. The number of women who had good improvement was significantly higher in the treatment group (P = 0.01), but the difference between the two groups as regards moderate improvement did not reach statistical significance (P = 0.26). The number of women who had no improvement was significantly higher (P = 0.002) in the control group. On comparing the mean plasma levels of MHPG before and after treatment in both groups, a significant lowering in mean level was found in the treatment group (t = 6.12, mean % change = -24.1 ± 17.9, P < 0.001) when compared with the control group (t = 1.72, mean % change = -5.59 ± 16.4, P = 0.10). In the treatment group, the test of correlation [Spearman's rank correlation coefficient (r)] showed a significant negative correlation between clinical improvement in hot flushes and the plasma level of MHPG (r = -0.453, P = 0.03).

Although these results are encouraging, the study had many limitations. First, the study was underpowered with small number of participants which is not sufficient to generalize the results. Second, folic acid supplementation was given for a short duration disallowing evaluation of its benefit on the medium and long terms. Last, the bias in allocation and assessment cannot be excluded since it is not a randomized double blind controlled study.

The second study, which was published as an abstract and included two groups (n = 20 each), investigated the effect of 5 mg folic acid supplementation for 4 wk *vs* no treatment. The treatment group demonstrated an average of 57% reduction in the frequency in hot flushes by the 4th week of treatment, while no change was observed in the control group[57].

HOW MIGHT FOLIC ACID AMELIORATE HOT FLUSHES?

Hot flushes possibly occur because of the increased central noradrenergic activity leading to disturbances in the thermoregulatory centre[14,17,18], and/or activation of estrogen withdrawal induced up-regulated 5-HT$_{2A}$ receptors in the hypothalamus by mild internal or external stimuli resulting in a hyperthermic response[5]. Animal studies reported that folic acid, like estrogen, reduced noradrenaline secretion[59,60], and increased serotonin activity[59]. It was found that folic acid administered to mice produced an antidepressant-like effect mediated by an interaction with the noradrenergic receptors (α_1 and α_2) and serotonergic receptors (5-HT$_{1A}$ and 5-HT$_{2A/2C}$)[59].

It was suggested that the link between folate and noradrenaline and serotonin metabolism is probably through BH4 since there is a significant positive correlation between its CSF levels with that of 5-HIAA and red cell folate in patients with severe depression[34,61]. As previously mentioned, 5-methyltetrahydrofolate appears to stabilize, enhance production of, or possibly act as a substitute for BH4, which an essential nutrient cofactor in the biosynthesis of serotonin and noradrenaline[23,34,35]. 5-methyltetrahydrofolate causes a significant reduction in the noradrenaline secretion to only 12.9% of control release, probably by duplicating the rate limiting behaviour of a synthetic pteridine cofactor "DL,2-amino-4-hydroxy-6,7,dimethyltetrahydropteridine"[60]. Further, folate deficiency was associated with decreased serotonin activity[38], and supplementation with folic acid increased CSF levels of 5-HIAA in folate deficient patients with depression[62]. Interestingly, it was found that the regional

distribution of 5-methyltetrahydrofolate in the brain was similar to that of serotonin[63].

Slopien *et al*[64] suggested that there might be a role for folate and possible methionine metabolism involvement in the development of depression in postmenopausal women, and it was also reported that there is an association between hot flushes and high rate of depression both in postmenopausal[65-67] and perimenopausal women[68].

THE FUTURE

It is plausible to assume that folic acid supplementation objectively ameliorates hot flushes by the same mechanism as estrogen replacement, *i.e.*, by interacting with monoamine neurotransmitters in the brain; namely noradrenaline and serotonin. It lowers noradrenaline and increases serotonin activities. Nevertheless, there is a need for well designed studies: (1) To investigate the effect of folic acid supplementation on the frequency and severity of hot flushes; (2) To explore whether symptomatic postmenopausal women are deficient in folate, and which patients are most suitable for folic acid therapy. It should be borne in mind that folate levels in the normal range might still be inadequate for the purpose of methyl donation and neurotransmitter synthesis in some individuals[23]; (3) To find out the optimum dose and the proper duration of therapy. Although 5 mg folic acid supplementation is considered as the standard dose, some investigators alleged that small doses up to 2 mg administered over a long time span may be preferable because the entry of folate in the nervous system is limited by the blood brain barrier, thus rendering large quantities inefficient[41]; and (4) To study the correlation between folate levels and monoamine neurotransmitters serotonin and noradrenaline.

To resolve some of these issues, a multi-centre, double-blind, placebo-controlled randomized, phase III trial is being set up and sponsored by "University of Birmingham" and "Sandwell and West Birmingham Hospitals NHS Trust", United Kingdom to directly compare the effect of 5mg folic acid *vs* placebo in reducing the frequency and severity of hot flushes in postmenopausal women, and on the blood level of monoamine neurotransmitters serotonin and noradrenaline. If folic acid supplementation is demonstrated to be effective, this will be a turning point in the clinical practice worldwide since it represents a cheap, safe, well-tolerated alternative to the conventional HRT, particularly in breast and endometrial cancer survivors who have no options at the moment but to live with their disabling symptoms.

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