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THE METABOLIC INTERMEDIARY ROLE OF FOLACIN AND COBALAMIN AS CYTOSOLIC METHYL DONORS IN ACTIVATION OF ONE-CARBON TRANSFER PATHWAY IN DEPRESSIVE DISORDER

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Abstract-
Objective: The purpose of the present study was to assess the effect of folic acid (folacin) and vitamin B12 (cobalamin) deficiency or supplementation on regulation of the one-carbon metabolism (OCM) in depression in adult male rats.
Methods: Fifty adult male Albino rats (Sprague-Dawely) strain, mean weight varied between 160.2 to 163.7 g were fed on basal control diet, folic acid deficient diet (FD), folic acid supplemented diet (100mg folic acid/kg diet) (FS), vitamin B12 deficient diet (B12D), and Vit.B12 supplemented diet (0.5mg vit.B12/kg diet) (B12S) throughout the experimental period (21days). Serum was analyzed for content of folic acid, vitamin B12, homocysteine (Hcy), phospholipids (PL), malondialdehyde (MDA), and reduced glutathione (GSH). Brain was analyzed for serotonin (5-HT), dopamine (DA), and norepinephrine (NE) contents.
Results: Serum folic acid, vitamin B12, PL concentrations and brain levels of 5-HT, DA, and NE were reduced significantly ($p<0.05$) in rats fed diets deficient in folic acid (FD) or vitamin B12 (B12D). Dietary supplementation of folic acid or vitamin B12 resulted in significant increase in these values when compared to control group. Serum Hcy levels were significantly increased ($p<0.05$) in rats fed diets deficient in either folic acid or vitamin B12. Dietary supplementation of folic acid or vitamin B12 reduced Hcy concentration. Serum MDA levels were significantly increased by feeding FD diet. Folic acid supplementation reduced serum MDA level, while folic acid deficiency resulted in significant reduction in serum GSH content. There were no significant differences in the levels of serum MDA, and GSH in rats fed B12D or B12S diets as compared with control rats.
Conclusion: The present study concluded that, folate and vitamin B12 are involved in the one-carbon metabolism necessary for the production of monoamine transmitters and prevention of depression in adult male rats. Also, the deficiency of the vitamins may interfere with methylation reactions.
Keywords- Cobalamin, Depression, Folacin, folic acid, Homocysteine, One Carbon-Metabolism, S-adenosylmethionine, Vitamin B12

Introduction

One-carbon metabolism is a network of interrelated biochemical reactions that involve the transfer of one-carbon groups from one site to another [1]. The dietary components of interest that mediate, or in some manner facilitate, one-carbon metabolism are the B-vitamins, folate (folacin or vitamin B9) and vitamin B-12 (cobalamin) [2]. Folacin and cobalamin are integrally involved in one-carbon metabolism necessary for the production of monoamine transmitters, and deficiency of the vitamins may interfere with methylation reactions [3]. Several studies have shown high prevalence of folate and vitamin B12 deficiency in depression [3-5]. Depression is the most common serious psychiatric disorder. Some causes of depression (genetic) are not liable to change, whereas some others are modifiable [6]. Nutrition can play an important role in preventing depression. In fact, nutrient deficiencies can affect mental and cerebral mechanisms resulting in mood disorders such as depression [7]. There are similarities and differences in the clinical presentation observed in folate and vitamin B12 deficiency, which stem largely in part to their intimate metabolic relationship [8]. The one central biochemical reaction that unifies folate and vitamin B12 metabolism involves the methylation of homocysteine (Hcy) to methionine, which is catalyzed by methionine synthetase [9]. Elevated blood levels of Hcy have been associated with several psychiatric and neurodegenerative disorders including depression [10]. It has been suggested that, there is a link between folate deficiency and impaired metabolism of serotonin, dopamine, and noradrenaline (norepinephrine), which have been implicated in mood disorders [11].

In a vitamin B12-dependent reaction, folic acid plays a pivotal role in one-carbon metabolism (acts as a donor of single-carbon groups). Folate is reduced to dihydrofolate and then further to tetrahydrofolate (THF) by the enzyme dihydrofolate reductase using NADPH as the reducing agent [12]. More recently, the total plasma homocysteine level was shown to be a sensitive marker of folate and vitamin B12 deficiency, and higher concentrations of homocysteine were observed in depressed patients [12]. The purpose of the present study was to study the intermediary role of folacin and cobalamin as cytosolic methyl donors in activation of one-carbon metabolism in depressive disorder in adult male rats.

Materials and Methods

Materials: Folic acid (Folacin) and vitamin B12 (Cobalamin) were purchased from El-Gomhouria Company for chemicals, Cairo, Egypt.

Experimental animals and diets: Five groups each of ten adult male Albino rats (Sprague-Dawely) strain, mean weight varied between 160.2 to 163.7 g. The animals were 6 weeks old at the beginning of the experiment. They were obtained from Helwan breeding farm, Cairo, Egypt. Animals received human care, housed individually in stainless steel cages with wire mesh bottoms and maintained at temperature 25°C±3°C, humidity 50%± 5% and light dark cycle held constant 12/12h. Food and water were provided ad libitum during the experimental period (21 days). The rats were fed on basal control diets [13] and classified as follows:

G1: Basal Control diet (C) [2 mg folic acid/kg diet + 0.01 mg vit.B12/kg diet]

G2: Folic acid deficient diet (FD) [basal control folic acid deficient diet]

G3: Folic supplemented diet (FS) [basal control diet +100 mg folic acid/kg diet]

G4: Vitamin B12 deficient diet (B12D) [basal control vitamin B12 deficient diet]

G5: Vitamin B12 supplemented diet (B12S) [basal control +0.5 mg vit.B12/kg diet]

Blood Analysis: At the end of the experiment (21 days), rats were fasted for 12 hrs, then the animals were scarified under ether anesthesia and blood samples were taken from hepatic portal vein in centrifuge tubes. The tubes centrifuged at 10000 x g for 15 minutes to provide serum needed for the biochemical analysis. Serum was separated immediately and stored at -20°C until analysis. The concentration of serum homocysteine (Hcy) was determined by enzymatic procedure [14]. The serum levels of folic acid and vitamin B12 were measured by direct chemiluminescence immunoassay [15, 16]. The concentration of serum malondialdehyde (MDA) was determined by colorimetric procedure using kit of Biodiagnostic [17]. Reduced glutathione (GSH) level was measured by colorimetric method kit developed by Biodiagnostic [18]. Serum phospholipids (PL) were measured by colorimetric method kit of Biodiagnostic [19].

Brain Analysis: At the end of the experiment, rats were sacrificed by decapitation, brains removed as rapidly as possible, dissected and stored at -20°C until assay. Brain serotonin, dopamine, and norepinephrine contents were extracted and determined by spectrofluorometric method [20].

Statistical Analysis: Analysis of variance (ANOVA; F-test) followed by the least significant difference (LSD) test was used to determine statistical significance ($P<0.01$) between the treatment groups. Statistical analysis was done by using SPSS 11.5 statistical software.

Results

Feed intake and gain weight were significantly ($p<0.05$) reduced in rats fed either folic acid deficient or vitamin B12 deficient diets as compared to control rats (Table 1). There were no significant differences in values of brain weights among all the experimental groups. Feed intake and gain weight values showed no changes in rat groups fed on diets supplemented with high doses of folic acid (100 mg folic acid/kg diet) or vitamin B12 (0.5 mg vit.B12/kg diet) as compared to control group (Table 1).

Serum folic acid and vitamin B12 concentrations were decreased significantly ($p<0.05$) in rats groups fed on diets deficient in folic acid (FD) or vitamin B12 (B12D) respectively as compared to control group. On the other hand, these values were significantly increased by folic acid and vitamin B12 supplementation (FS and B12S) when compared to all tested groups (Table 2). Serum homocysteine (Hcy) levels were significantly increased ($p<0.05$) in rats fed on folic acid or vitamin B12 deficient diets (FD or B12D respectively) as compared to control group. Dietary supplementation of folic acid or vitamin B12 reduced significantly ($p<0.05$) serum homocysteine concentration as compared with deficient and control groups (Table 2).

Levels of brain serotonin (5-HT), dopamine(DA), and norepinephrine (NE) were reduced significantly ($p<0.05$) in rats fed on diets deficient in folic acid or vitamin B12 as compared to control group. Dietary supplementation of folic acid or vitamin B12 resulted in significant increase in these values when compared to deficient and control groups (Table 3).

Serum concentration of phospholipids (PL) was significantly decreased in rats fed diets deficient in folic acid or vitamin B12 as compared to control group. While supplementation of folic acid and vitamin B12 at high doses increased serum phospholipids significantly ($p<0.05$) when compared with control rats. Serum and malondialdehyde (MDA) levels were significantly increased by deficiency of folic acid. Dietary supplementation of folic acid reduced serum malondialdehyde levels as compared to control rats. Supplementation or deficiency of vitamin B12 had no effect on serum levels of MDA, and GSH as compared to control rats (Table 4). Serum a level of reduced glutathione (GSH) was significantly reduced in rats fed diets deficient in folic acid. Dietary supplementation of folic acid increased GSH content significantly ($p<0.05$) as

compared with control, B12D, and B12S groups (Table 4).

Discussion

Folate functions as a family of cofactors that carry one-carbon (C1) units required for the synthesis of thymidylate, purines, and methionine, and required for other methylation reactions [3]. Folate status is an important determinant of plasma homocysteine; low serum folate is linked with high plasma homocysteine. Vitamin B12 also influences homocysteine levels, although its relevance is less critical than that of folate [9]. When general nutritional status was studied, the results showed that supplementation with folic acid did not affect normal growth in rats. Food intake and gain weight values showed no changes in rats fed on diets supplemented with high doses of folic acid (100 mg folic acid/kg diet) or vitamin B12 (0.5 mg vit.B12/kg diet). Compared with folate, low serum B12 concentration (140.2 pg/ml) was found in rats fed diet deficient in vitamin B12 (B12D) appeared to distinguish depressed from nondepressed rats.

The results also showed that the level of serum folate reduced significantly in rats fed diet deficient in folic acid (FD) (2.1 ng/ml). Lower serum folate concentration has been found to be associated with increased severity in the symptoms of depression [21]. Response to antidepressant medication has also been found to be dependent upon folate status and enhanced by folic acid supplementation [22]. The results of the present study showed a marked decrease in serum folic acid and vitamin B12 and an increase in serum homocysteine (Hcy) levels in folic acid and vitamin B12 deficient rats. High serum homocysteine has been associated with reduced levels of CSF amine metabolites [23]. The neurotoxic effect of homocysteine may relate to its metabolism to S-adenosylhomocysteine (SAH), which has been shown to inhibit monoamine neurotransmitter metabolism and phospholipids methylation [24]. Hyperhomocysteinaemia may occur as a result of inherited disorders that alter enzyme activity in the transulfuration and remethylation pathways. Alternatively nutritional deficiencies of cobalamin or folic acid can result in the blockade of homocysteine pathways because those enzymes depend on these vitamins as co-factors [25]. Homocysteine, a thiol containing amino acid, is an intermediate metabolite of the indispensable amino acid methionine. Hcy is formed by demethylation of methionine and is catabolized to cystathionine and cysteine or remethylated to methionine by folate and vitamin B12-dependent reaction [26]. Hyperhomocysteinemia, folate and vitamin B12 deficiency were all significantly related to depressive disorders [27].

The rise in serum homocysteine in the present study indicates a failure of methylation of homocysteine to methionine due to a shortage of supply of methyl groups from methyl folate or, more rarely in depression, lack of the vitamin B12 cofactor for this methylation reaction [28]. Methionine is in turn the immediate precursor of SAM, the methyl donor in many methylation reactions in the brain involving monoamines, neurotransmitters, proteins, nucleoproteins, and membrane phospholipids. It is, therefore, of interest that the failure of one carbon metabolism in depressed rats with folate deficiency associated with a high plasma homocysteine, was also accompanied by a significant fall in CSF SAM. Similar to folic acid, SAM has been reported to have effects on mood [29]. The results of the present study also shows that rats with high serum homocysteine concentrations have significantly lower concentrations of the CSF monoamines suggesting impairment in the metabolism of serotonin, dopamine, and noradrenaline, all of which have been implicated in the biology of affective disorders [30]. Serotonergic, dopaminergic, and noradrenergic systems share many similar synthetic and degradative enzymes and a reduction in all three monoamines may indicate an effect at some common metabolic point [30]. There are several suggested mechanisms by which folic acid may affect the central nervous system pathways pertaining to depression. It may influence the synthesis of neurotransmitters through the promotion of the synthesis of tetrahydro biopterins (BH4), the rate limiting pteridine cofactor required in the synthesis of monoamine neurotransmitters [26]. BH4 is a co-factor in the conversion of phenylalanine to tyrosine and in the hydroxylation of tyrosine and tryptophan, which are rate-limiting steps in the synthesis of dopamine and noradrenaline [26]. Another suggested mechanism is the role of folic acid in methylation reactions in the central nervous system [31]. An adequate amount of folate and vitamin B12 are required in the regulation of neurotransmitter metabolism [29].

The results of the present study showed that, the levels of brain serotonin (5-HT), dopamine (DA), and norepinephrine (NE) were reduced significantly in rats fed diets deficient in folic acid or vitamin B12 (Table 3). Cobalamin deficiency has been shown to cause hyperhomocysteinemia and lipid peroxidation, suggesting that cobalamin deficiency can result in oxidative stress-induced cell death from high intracellular concentrations of homocysteine [32]. Vitamin B12 can enhance the effectiveness of folate to reduce high blood homocysteine levels and oxidative stress. Hcy is a highly reactive amino acid and is known to produce endothelial cell injury through oxidant-mediated mechanisms in cellular and animal studies [33]. The results of the present study showed that, dietary supplementation of folic acid has been shown to reduce serum Hcy levels and increase GSH content [34].

Folate is believed to be central methyl donor required for mitochondrial protein and nucleic acid synthesis through its active form, 5-methyltetrahydrofolate (CH3THF). Methyl group (CH3) is transferred to homocysteine from CH3THF via the formation of methylcobalamin intermediate. The reaction is catalyzed by methionine synthetase to form methionine. Methionine could then be used for cysteine synthesis required for GSH and phospholipids synthesis [35]. The results of the present study showed that, serum phospholipids were reduced

significantly in rats fed diets deficient in folic acid (FD) or vitamin B12 (B12D). Methionine is necessary to guarantee sufficient S-adenosylmethionine synthesis that participates in most cellular transmethylation reactions including phosphatidylethanolamine (PE) methylation [36]. The results of the present study showed that, supplementation of folic acid prevent lipid peroxidation and reduced MDA content. Folic acid can act as a direct antioxidant and scavenger molecule. Furthermore it can prevent microsomal lipid peroxidation and can scavenge and repair peroxyl, hydroxyl and thiol radicals [37]. The present study concluded that, folate and vitamin B12 play an important role regulation and activation of the one-carbon metabolism and methylation reaction necessary for the production of neurotransmitters and prevention of depression.

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Table 1: Effect of folic acid and vitamin B12 on gain weight, feed intake, and brain weight

	Basal	Folic Acid		Vitamin B_{12}	
	Control (C)	Deficiency (FD)	Supplemented (FS)	Deficiency (B_{12}D)	Supplemented (B_{12}S)
Gain weight (g)	40.9±2.2[a]	16.6±1.5[b]	37.8±2.3[a]	14.4±1.3[b]	35.3±3.1[a]
Feed intake (g/d)	17.8±1.2[a]	12.5±0.9[b]	18.4±1.3[a]	13.2±1.1[b]	16.9±1.2[a]
Brain weight [N.S] (g)	1.55±0.05	1.53±0.04	1.58±0.05	1.54±0.02	1.56±0.04

Values are expressed as mean± SD, n=10, N.S= Non significant
There was significant difference at p< 0.05 between means have the different alphabetic superscript in the same row

Table 2: Effect of folic acid and vitamin B12 on serum folic acid, vitamin B12, and homocysteine levels

	Basal	Folic Acid		Vitamin B_{12}	
	Control (C)	Deficiency (FD)	Supplemented (FS)	Deficiency (B_{12}D)	Supplemented (B_{12}S)
Serum Folic Acid (ng/ml)	5.5±0.6[a]	2.1±0.1[b]	18.9±1.9[c]	5.2±0.5[a]	5.1±0.5[a]
Serum Vitamin B_{12} (pg/ml)	213.5±6.4[a]	198.7±5.4[a]	205.1±8.9[a]	140.2±4.7[b]	362.8±12.1[c]
Serum Homocysteine (mmol/L)	13.2± 0.9[a]	17.8±1.6[b]	9.8±0.7[c]	16.3±1.5[b]	11.5±0.8[c]

Values are expressed as mean± SD, n=10
There was significant difference at p< 0.05 between means have the different alphabetic superscript in the same row

Table 3: Effect of folic acid and vitamin B12 on brain neurotransmitters

	Basal	Folic Acid		Vitamin B_{12}	
	Control (C)	Deficiency (FD)	Supplemented (FS)	Deficiency (B_{12}D)	Supplemented (B_{12}S)
Serotonin (µg/g tissue)	0.988±0.04[a]	0.275±0.02[b]	1.356±0.07[c]	0.546±0.03[d]	1.125±0.01[e]
Dopamine (µg/g tissue)	0.604±0.03[a]	0.225±0.01[b]	0.912±0.05[c]	0.375±0.03[d]	0.822±0.06[e]
Norepinephrine (µg/g tissue)	1.655±0.08[a]	0.800±0.03[b]	1.895±0.15[c]	0.725±0.05[d]	1.720±0.02[e]

Values are expressed as mean± SD, n=10
There was significant difference at p< 0.05 between means have the different alphabetic superscript in the same row

Table 4: effect of folic acid and vitamin B12 on serum phospholipids (PL), malondialdehyde (MDA), and reduced glutathione (GSH) levels

	Basal	Folic Acid		Vitamin B_{12}	
	Control (C)	Deficiency (FD)	Supplemented (FS)	Deficiency (B_{12}D)	Supplemented (B_{12}S)
Phospholipids (mg/dl)	101.5±4.6[a]	77.8±2.5[b]	112.6±6.2[c]	80.9±2.9[b]	106.8±4.1[c]
Malondialdehyde (nmol/ml)	1.39±0.06[a]	3.22±0.20[b]	1.10±0.05[c]	1.40±0.04[a]	1.34±0.03[a]
Reduced Glutathione (GSH) (nmol/ml)	1.55±0.09[a]	1.20±0.04[b]	1.85±0.20[c]	1.40±0.06[a]	1.58±0.45[a]

Values are expressed as mean± SD, n=10
There was significant difference at p< 0.05 between means have the different alphabetic superscript in the same row