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Original Research Article

Evaluation of antidepressant activity of l-methylfolate per se and its interaction with escitalopram in mice

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ABSTRACT

Background: Depression is a worldwide illness in the current population. Low levels of L-methylfolate are linked to depression. Present study evaluates the anti-depressive activity of acute and chronic administration of L-methylfolate per se in forced swimming test (FST) and tail suspension test (TST) and its interaction with escitalopram in albino mice.

Methods: For this 30 swiss albino mice were divided randomly into five groups (n=6) as group I (control,10ml/Kg, p.o) - 2% suspension of gum acacia, group II - escitalopram suspension (10mg/kg, p.o), group III- L-methylfolate suspension (3mg/kg, p.o), group IV- L-methylfolate (3mg/kg, p.o) plus escitalopram (5mg/kg, p.o), group V- L-methylfolate(3mg/kg, p.o) plus escitalopram(10mg/kg, p.o), for forced swimming test. In tail suspension test again, mice were divided in five groups as above except that the dose of L-methylfolate was reduced to 1.25mg/kg. The pharmacologically validated models forced swimming test and tail suspension test were performed in mice to evaluate acute and chronic antidepressant activity of L-methylfolate and its combination with escitalopram respectively, after performing an acute toxicity study.

Results: L-methylfolate and L-methylfolate plus escitalopram (10mg/Kg and 5mg/Kg, p.o) showed acute and chronic antidepressant activity in albino mice in FST and TST respectively. In human L-methylfolate is only active form of folic acid that readily crosses the blood brain barrier and utilized by the CNS. It regulates the bioavailability of critical cofactor BH4, required by enzymes synthesizing monoamines whose deficiency leads to depression.

Conclusions: Hence, this study suggests antidepresant activity of L-methylfolate per se and as adjuvant with escitalopram when initiated from initiation of antidepressant therapy. Also, L-methylfolate opens the possibility of reducing the dose of antidepressant when used as adjuvant.

Keywords: Escitalopram, Forced swimming test, L-methylfolate, Major depressive disorder, Tail suspension test

INTRODUCTION

Major depressive disorder (MDD) is an illness that can have lasting (nearly every day for at least 2 weeks) emotional and physical manifestations, such as feeling of worthlessness, helplessness, hopelessness, guilt of indecision, change in sleep habits, loss of concentration, loss of energy, loss of interest, loss of pleasure, agitation, mental and motor slowing, drug or alcohol abuse and social withdrawal and solitariness.[1]

As per Age-standardised disability-adjusted life year (DALY) rates per 100,000 inhabitants India is ranked 5th world wide for major depression, US being on number one. Depression is estimated to affect nearly 340 million people worldwide and is projected to be the second leading cause of disability in the world by the year 2020.[2] Population

studies have consistently shown major depression to be about twice as common in women as in men, affecting 7-12% of men and 20-25% of women.[3,4] Studies suggest that 3-5% of children and adolescents suffer from clinical depression, and 10-15% have some depressive symptoms.[5,6] About 1-5% of elderly people suffer from depression.[7] People are most likely to suffer their first depressive episode between the ages of 30 and 40 years, and there is a second, smaller peak of incidence between ages 50 and 60 years.[8]

The causes of depression are not fully known. Depression is most likely due to a combination of genetic, biologic, and environmental factors. Studies have found that close relatives of patients with depression are 2 to 6 times more likely to develop the condition than individuals without a family history. Biologic Factor, the basic biologic causes of depression is strongly linked to abnormalities in the delivery of certain key neurotransmitters in the brain. The most important neurotransmitter in depression is serotonin. Imbalances in the brain's serotonin levels can trigger depression and other mood disorders. Other neurotransmitters involved in depression include dopamine, norepinephrine, epinephrine and acetylcholine. In women, the female hormones estrogen and progesterone may play a role in depression. Environmental Factors, including drugs can affect brain chemicals and trigger depression. These medications include certain types of drugs used for acne, high blood pressure, contraception, Parkinson's disease, inflammation, gastrointestinal relief and other conditions.

While MDD is sometimes viewed as one of the most "treatable" conditions, it tends to be recurrent.[9] Conventional pharmacological treatment begins with monotherapy of antidepressant, but this approach is often ineffective in achieving an adequate clinical response. Regardless of the standard antidepressant medication used to start treatment, initial monotherapy compounds have comparable limitations in their overall efficacy.[9] The Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study concluded that full remission and also maintenance of remission was less than 30% with initial monotherapy. There was an augmentation of results when lithium, nortryptyline, MAO inhibitors were added to initial monotherapy by 16, 20 and 14% respectively.[10-12]

Depression has been considered one of the most "treatable" of all illnesses, with authors commonly citing study response rates of 50% to 70%. However, "response" has traditionally meant a reduction in score (usually 50%) on a Hamilton Rating Scale for Depression (HDRS) or a Montgomery-Åsberg Depression Rating Scale (MADRS). A reduction in score may reflect improvement in symptoms but not full remission. In a long-term study following MDD patients, 76% of subjects who did not attain full remission (HDRS >7) had relapsed by month 15, while a HDRS of ≤7 was associated with a far lower likelihood of relapse (25%), by month 15.[13] Thus, if recovery is only partial, patients remain at high risk for a relapse, possibly as severe as their initial episode.Patients who experience full remission of MDD early in the course of treatment are more likely to remain well compared to those who showed only symptomatic improvement.[14] So, the goal of therapy should be achieving and maintaining remission, rather than a reduction in severity of symptom. When no response is reported after an adequate time (generally accepted to be at least 4 weeks), and side effects are minimal, increasing the dose is a common strategy. However, if no response is reported but side effects are significant, switching agents would be preferred over dose escalation because most side effects are dose-dependent. For partial and non-responders, augmentation of antidepressants may have several advantages over dose escalation or switching. It eliminates the need to taper some medications that pose a risk of withdrawal, and augmentation may allow the patient to build on the partial response already achieved rather than risk losing that response, which can occur when switching the primary agent to a newer choice. Further, some augmentation agents can often ameliorate the side effects of primary agents (such as sexual side effects) or have other benefits (e.g., lower anxiety or help with insomnia).

Several factors, including poor long-term adherence to pharmacological therapies, have contributed to low remission rates of MDD. Discontinuation of antidepressant medication due to adverse events, the most common being sexual dysfunction and weight gain is associated with poor outcomes. Some patients prematurely terminate initial monotherapy due to perceived lack of efficacy before having had a chance to experience the potential benefits of further therapeutic steps, including the use of combination or adjunctive therapies with better safety and tolerability.[9] There is an urgent need for innovative pharmacological approaches to treat MDD that increase the chance of early and complete remission. Few controlled clinical trials have been conducted to evaluate combination or adjunctive therapies implemented at the start of treatment. Administering combination or adjunctive agents at the initiation of treatment in lieu of sequenced treatment trials represents a major paradigm shift in the treatment of MDD.[13] Combination therapies may work synergistically to regulate the availability of monoamines and result in a broader spectrum of action, enhancing antidepressant efficacy and long-term results.[14] Six clinical trials suggest that combinations from the initiation of drug therapy may lead to more rapid clinical outcomes, higher remission rates, and lower relapse rates when compared with sequentially administered single antidepressants.[15,16] Evidences from open and blinded studies has demonstrated the efficacy of L-methylfolate per se and in combination with antidepressants at initiation of therapy in reducing depressive symptoms, improving cognitive function, and reducing somatic symptoms in depressed patients with normal and low folate levels.[17-19] L-methylfolate, the bioavailable form of folate, is required in the central nervous system (CNS) to aid in the synthesis of monoamines, such as serotonin, norepinephrine, and dopamine.[20] Suboptimal serum and red blood cell (RBC)

folate levels have been associated with more severe symptoms of depression, poorer response to antidepressant drugs, longer duration of illness, later onset of clinical improvement, and greater treatment resistance.[21,22] Individuals with low RBC folate are six times more likely to be nonresponders to antidepressant therapy and less likely to achieve and maintain remission compared to those with normal concentrations.[23] Patients known to be at risk for suboptimal CNS folate status and monoamine deficiency include older individuals, individuals with a history of poor nutrition (chronic dieting, anorexia, bulimia), individuals with a history of tobacco use or excess alcohol intake, women of childbearing age, and individuals who take medications that interfere with folate metabolism (lamotrigine, valproate, oral contraceptives, metformin, methotrexate, warfarin, fenofibrates, certain retinoids).[24,25] Depressive episodes are also linked with the common inborn error of metabolism "methyltetrahydrofolate reductase polymorphism (MTHFR C677T)" associated with reduced L-methylfolate.[26,27] There is growing recognition that the combination of L-methylfolate plus an antidepressant from initiation of treatment may result in greater efficacy and more rapid improvement in depressive symptoms compared to standard antidepressant monotherapy.[9,28]

METHODS

The study was done at Mammalian and Experimental Pharmacology Lab, Department of Pharmacology, M.Y Hospitals and M.G.M Medical College, Indore. Forced swimming test and tail suspension test were performed in mice to evaluate acute and chronic antidepressant activity of L-methylfolate and its combination with escitalopram respectively, after performing an acute toxicity study. The procedures in this study were performed in accordance with the CPCSEA Guidelines for the Care and Use of Laboratory Animals. The protocol of the study was approved by the Animal Ethics Committee of the Institution and all efforts were made to minimize animal suffering and to reduce the number of animals used in the experiments.

Acute toxicity study

As per OECD, 2006 guidelines acute toxicity study was done in female mice.[29] The animals were observed for any gross behavioral changes, sedation, morbidity and mortality. Based on preliminary studies doses of 1.25 and 2.5mg/kg (ten times of human dose) were selected for further experiments.

Animals

Adult Swiss albino mice weighing 18-25gms, 30 for each experimental model of depression were taken. Swiss albino mice were weighted and marked at different sites for identification. They were separated into five groups of six mice each with equal number of male and non pregnant female mice. Mice were maintained at constant room temperature (22-25ºC) with free access to water and food, under a 12:12 h light/dark cycle (lights on at 05:00 pm) in cages, separated by sex. Animals (male and female mice were homogeneously distributed among groups) were acclimatized to the laboratory for at least 1 h before testing and were used only once throughout the experiments. All manipulations were conducted in the light phase, between 09:00 am and 04:00 pm.

Forced Swimming Test[30]

A group of 30 mice was divided at random into five groups (n=6) and treated as group I (control) - 2% solution of gum acacia suspension in a dose of 10ml/Kg, group II - escitalopram suspension in dose of 10mg/kg, group III- L-methylfolate suspension in dose of 3mg/kg, group IV- L-methylfolate in dose of 3mg/kg plus escitalopram in dose of 5mg /kg, group V- L-methylfolate in a dose of 3mg/kg plus escitalopram in a dose of 10mg /kg. All the drugs were administered orally 1 hour prior to the FST. Mice were placed in the acrylic cylinder for 15 min (pre- test session) 24 hours before the test session. After the pre-test session mice were again exposed to the same conditions for 15 min (test session). Basal readings (pre-test readings) were taken then drugs were administered and again the test session repeated to record post treatment effects. The mouse was judged immobile if it remained floating in the water, except for small movements to keep its head above the water.

Tail suspension test (TST)[31]

A group of 30 mice were divided at random into five groups (n=6) and with the help of feeding syringe were treated as group I - gum acacia suspension 2% in a dose of 10 ml/Kg, group II - escitalopram suspension in dose of 10mg/kg, group III - L-methylfolate suspension in dose of 1.25mg/kg, group IV - L-methylfolate in dose of 1.25mg/kg plus escitalopram in dose of 5mg /kg, group V - L-methylfolate in a dose of 1.25 mg/kg plus escitalopram in a dose of 10mg/kg. All drugs were administered orally. The solutions were administered once daily between 1-3 pm over a period of 21 days. Mice were suspended (pre test session) for 5 min on a wire tied between two stands by an adhesive tape placed one cm from the tip of the tail individually 24 hours before the test session. Twenty four hours after the pre test session the mice were again exposed to the same conditions for 5 min (test session). Between the pre-test session and main session drug solutions were administered orally three times as follows: just after the pre test session, 5 h before the main test and 1 h before the main test. The mice are suspended individually on a metal wire tied between two stands. This wire lies 50 cm above the table top. The duration of immobility is recorded for a period of 5 minute. Mice are considered immobile when they hang passively and completely motionless for 60 sec at a stretch. The test was performed between 1-3 p.m. Test was performed on the 7th, 14th and 21st day during the course of study after recording basal values on day 0.

RESULTS

Escitalopram (10mg/Kg) did not reduce immobility period during evaluation of acute antidepressant activity in FST. Whereas L-methylfolate (3mg/Kg) per se, L-methylfolate plus escitalopram (3mg/Kg+10mg/Kg) and L-methylfolate plus escitalopram (3mg/Kg+5mg/Kg) demonstrated reduced immobility period in mice in FST model when analysed by paired t-test (dependent) (Table 1). From this it was concluded that escitalopram did not show any acute antidepressant activity. Whereas L-methylfolate, L-methylfolate plus escitalopram (10mg/Kg) and L-methylfolate plus escitalopram (5mg/Kg) showed acute antidepressant activity in mice and can be seen in Table 1.

Table 1: The influence of L-Methylfolate and its combination with escitalopram on immobility time in forced swimming test.

Group	Dose (mg/Kg) orally	Immobility period for 15 min (sec)	
		Pre treatment	Post treatment
Control	10ml/Kg	729.00± 2.47	727.83±2.27
Escitalopram	10	729.83±2.79	720.00±3.45
L-methylfolate	3	731.17±2.104	648.00±3.89[a]
L-methylfolate + Escitalopram	3 + 5	731.83±2.04	652.33±4.65[a]
L-methylfolate + Escitalopram	3 + 10	729.33±2.90	643.33±5.51[a]

Paired t-test (Dependent)
Values are Mean± SEM, n=6, df = 5
ap<0.001 compared to pretreatment immobility period
The column represents the mean duration of immobility recorded in a 15 min observation period

When results of forced swimming test were analysed by one way analysis of variance [ANOVA] followed by bonferronis multiple comparison test it was found that L-methylfolate showed antidepressant activity per se. Its antidepressant activity is greater in its combination with 10 and 5mg/Kg of escitlopram as compared to escitalopram alone (Table 2).

L-methylfolate (1.25mg/kg) also reduced the immobility period though less than escitalopram and in its combination with 10 and 5mg/Kg of escitalopram in mice in TST model used for evaluation of chronic antidepressant activity. Results were analysed by one way analysis of variance (ANOVA) followed by bonferronis multiple comparison test,that is L-methylfolate showed chronic antidepressant activity per se, but less than escitalopram. Its antidepressant activity is greater in its combination with 10 and 5 mg/Kg of escitlopram as compared to escitalopram alone (Table 3).

Table 2: The influence of L-Methylfolate and its combination with escitalopram on immobility time in FST.

Group	Dose (mg/kg) orally	Immobility period for 15 min (sec)
Control	10ml/kg	727.83± 2.27
Escitalopram	10	720.00 ±3.45
LMF	2.5	648.00± 3.89 [a,b]
LMF+Escitalopram	2.5 + 5	652.33±4.65 [a,b]
LMF+Escitalopram	2.5 + 10	643.33± 5.51 [a,b]

One way F 1344; ANOVA P <0.001
One way ANOVA followed by Post hoc bonferonni comparison test
Values are Mean±SEM, n=6, df= 4, 25
a p<0.001 as compared to control; b p<0.001 as compared to Escitalopram
The column represents the mean duration of immobility recorded in a 5 min observation period

Table 3: The influence of L-Methylfolate and its combination with escitalopram, on immobility time in TST.

Groups	Dose mg/kg	Pre Treat.	Immobility period for 5 min (sec)		
			7 days	14 days	21 days
Control	10	207.50±2.34	209.17±0.91	210.67±1.40	210.50±1.80
Escitalopram	10	209.00 ±1.52[a]	132.83±2.05[a]	129.67±3.07[a]	128.33±2.81 [a]
L-methylfolate	1.25	209.83±2.16 [a]	184.17±1.49[a]	181.00±0.57[a]	182.00±1.39 [a]
L-methylfolate + Escitalopram	1.25+ 5	210.83±1.77,[ab]	100.50±5.03[a,b]	100.33±3.60[a,b]	104.17±4.68[a,b]
L-methylfolate + Escitalopram	1.25+10	210.50±8.21[a,b,c]	76.00±4.76[a,b,c]	74.67±4.30[a,b,c]	76.83±4.28[a,b,c]

One way F 0.331 281 364 284; ANOVA p >0.005 <0.001 <0.001 <0.001; One Way ANOVA followed by Post hoc Bonferonni multiple comparison test
Values are Mean±SEM, n=6, df 4, 25; a p<0.001 as compared to control; b p<0.001 as compared to Escitalopram; c p<0.001 as compared to L-methylfolate + Escitalopram (1.25+5); The column represents the mean duration of immobility recorded in a 5 min observation period

DISCUSSION

Considering the lack of preclinical studies dealing with the antidepressant-like activity of L-methylfolate, this study therefore sought to investigate the effect of this vitamin in the forced swimming test (FST) and the tail suspension test (TST) in mice.

Folic acid (folate) is a water-soluble B-vitamin whose biologically active form is L-methylfolate, which participates in the transfer of 1-carbon units (such as methyl, methylene, and formyl groups) to the essential substrates involved in the synthesis of DNA, RNA, and proteins.

Ingested folic acid can be converted to its physiological forms. This process is initiated by dihydrofolate reductase in a two-step reaction; the first step, conversion to dihydrofolate (DHF) is a slow and rate-limiting step. In the second, more rapid, step dihydrofolate is further reduced to THF. THF is then converted into L-methylfolate, the form that is normally found in the circulation and in tissues. L-methylfolate is also replenished by the conversion of folinic acid (5 formyltetrahydrofolate), an active metabolite of folic acid. Because de novo folate synthesis is not present in the CNS, it depends on adequate folate transport across the blood-brain barrier. L-methylfolate is the only bioactive form of folate that can cross BBB.[32] Within neurons; part of the folate pool will be catabolized by oxidation to dihydrofolates and folic acid, which can be reconverted to THF by dihydrofolate reductase.

Preclinical studies have shown that systemic and central administration of folic acid produces antidepressant-like effect in two predictive models of antidepressant activity, the forced swimming test (FST) and tail suspension test (TST).[33]

In this study we performed the forced swimming test (FST) and tail suspension test using the active form of folic acid that is L-methylfolate. Acute effects of L-methylfolate were evaluated by the forced swimming test (FST) model and its chronic effects were demonstrated by tail suspension test model of depression in which mice were treated for 21 days with the test and standard drugs and evaluation was done on days 7, 14 and 21.

For statistical analysis, Comparisons between experimental and control groups were performed by Paired t-test and One-Way ANOVA followed by bonferroni comparison test when appropriate $P < 0.05$ was considered significant.

Acute study using forced swim test when analysed through paired t-test, df = 5, $p<0.001$) demonstrated that L-methylfolate, and the combination of L-methylfolate plus escitalopram in both the doses 10mg/Kg and 5mg/Kg showed significant antidepressant activity. Escitalopram did not show any acute antidepressant activity.

In acute study using forced swim test when analysed through one-way analysis of variance (ANOVA, $F_{4,25}$= 1344, $P < 0.001$), L-Methylfolate demonstrated significant reduction in immobility period compared to control and escitalopram monotherapy ($p<0.001$). Its combination with 5 and 10mg/kg of escitalopram also reduced immobility period significantly compared to control and escitalopram alone. L-Methylfolate combination with escitalopram did not show significant difference in immobility period when compared to L-Methylfolate alone.

In chronic study using tail suspension test, ANOVA ($F_{4,25}$= 281, 364, 284, $P < 0.001$), it was found that escitalopram (10mg/Kg) shortens the total duration of immobility; L-methylfolate also shortens the total duration of immobility which showed that LMF itself got antidepressant like properties, but less than escitalopram. L-methylfolate plus escitalopram (10mg/Kg) shortens the total duration of immobility more than escitalopram alone.

L-methylfolate with half dose of escitalopram (5mg/Kg) also shortens the total duration of immobility more than escitalopram alone at days 7, 14 and 21. These observations support the antidepressant effects of L-methylfolate alone and in combination with antidepressant escitalopram when administered at the start of treatment, as total duration of immobility resembles the pattern of depression seen in human beings. L-methylfolate also showed the possibility of reducing the dose of antidepressant when used as adjuvant, which will help to reduce the adverse effects related to the use of antidepressants.

Depression is well known to involve dysregulation of one or more monoamines serotonin (5-HT), norepinephrine (NE), and dopamine (DA). L-methylfolate acts as an important regulator of a critical cofactor BH_4 needed for neurotransmitter synthesis.[34] L-methyloflate combines with BH_2 to synthesize BH_4 utilizing methyltetrahydrofolate reductase enzyme. BH_4 is a necessary cofactor for the rate-limiting enzyme hydroxylase for serotonin, dopamine and norepinephrine synthesis.

Another mechanism of antidepressant activity of L-methylfolate is its role in the homocysteine cycle.[35] High CNS homocysteine levels are associated with depression, dementia, and stroke, as well as negative symptoms of schizophrenia. Homocysteine is transformed to methionine utilizing B_{12} and L-methylfolate, both necessary cofactors for this transformation. Methionine is then converted to s-adenyl-methionine, which serves as the methyl donor for all three monoamines-serotonin, norepinephrine, and dopamine. Thus, patients with low CNS L-methylfolate are less able to convert homocysteine to methionine, the first necessary step of the homocysteine cycle.

CONCLUSION

L-methylfolate demonstrated antidepressant properties per se. Combination of L-methylfolate with escitalopram (10mg/Kg) demonstrated a significantly improved antidepressant activity compared with escitalospram alone. The combination also showed an improved antidepressant activity when the dose of escitalopram was decreased to half (5mg/Kg). L-methylfolate thus opens the possibility of reducing the dose of antidepressant escitalopram when used as adjuvant, which will help to reduce the adverse effects related to the use of antidepressants. It can be concluded that, initiating antidepressant therapy along with L-methylfolate is more effective for treating depression.

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