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Effects of folic acid and lamotrigine therapy in some rodent models of epilepsy and behaviour

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Abstract

It has been suggested that a folic acid (FA) deficiency induced by antiepileptic drugs might be the basis for the neuropsychiatric toxicity associated with these drugs. In the present study, lamotrigine (LTG), one of the newer antiepileptic drugs, was evaluated for its effect on epilepsy, mood and memory in mice. Further, the effect of the addition of FA to LTG therapy was also investigated. The increasing current electroshock seizure test was used to evaluate the anticonvulsant effect of drugs, while the forced swimming test (FST) and spontaneous alternation behaviour (SAB) models were employed for assessing the effects on mood and memory, respectively. LTG exhibited a dose-dependent increase in seizure threshold, whereas FA did not have any effect. LTG did not affect, whereas FA decreased, behavioural depression in the FST in mice. Neither LTG nor FA affected memory scores in the SAB test. The combination of LTG and FA significantly reduced depression while enhancing the effects on memory and seizure threshold. The present observations have confirmed the antiepileptic action of LTG in yet another rodent model of epilepsy. Further, the results clearly demonstrate the additional benefits on epilepsy, mood and memory brought about by the inclusion of FA in the LTG regimen.

Introduction

Epilepsy refers to a disorder of brain function characterized by the periodic and unpredictable occurrence of seizures. Epilepsies are common and frequently devastating disorders, affecting 0.5–1% of the population. In underdeveloped countries, the years lived with disability from epilepsy were more than in developed countries (Murray & Lopez 1994). It is clear that, if untreated, epilepsy can be at the least disabling to the individual patient in particular and to the society at large.

The current treatment for epilepsy includes psychotherapy, surgery and pharmacotherapy, out of which pharmacotherapy remains the mainstay (Gupta & Malhotra 2000). A quick survey of the available antiepileptic drug (AED) preparations on the market reveals a stunning array of AEDs, with each new drug claiming to have better efficacy and safety. Although an empirical approach to AED development might have been responsible for so many drugs being made available in the past, a continuing search for the perfect AED (a drug with maximum efficacy and minimum toxicity) appears to be influencing the current turnover rate. Quite often, an epileptic patient suffers from neurobehavioural problems (e.g. impaired memory, depression), which may have a pathological and/or iatrogenic basis. Such patients would therefore need additional treatment, besides AED therapy, to correct the accompanying neurological deficits. For instance, impaired memory in epileptic patients can be treated with memory-enhancing drugs. Similarly, antidepressants may be used to correct behavioural depression. A better solution would be to use an AED that not only provides protection against seizure, but also has a positive effect on mood and memory. In this context, it is interesting to note that many of the AEDs themselves have beneficial effects on certain neurological aspects. For example carbamazepine is reported to have a stabilizing effect in mania (Schmitz 1999) and, similarly, of the newer drugs, lamotrigine is reported to be useful in bipolar depression (Sachs et al 2000). These observations suggest that a putative AED should be routinely screened for its neurological effects other than antiepileptic action as part of the drug development process.

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Pharmacotoxicology of AEDs is a major problem that often limits the usefulness of these agents. Among these adverse effects, neurotoxicology is generally the main limiting factor (Timmings 1997). Conventional AEDs, for example phenytoin and sodium valproate, may interfere with folate transport into the nervous system (Miller & O'Donnell 1983). It has been suggested that prolonged drug-induced folate deficiency may, as with folate deficiency in other clinical situations, sometimes lead to neuropsychiatric complications (Reynolds 1968, 1976). In the light of these observations, the influence of the addition of folic acid (FA) to an AED therapy was investigated in the present study.

Materials and Methods

Animals

Male albino mice of Swiss strain, 18–30 g, were used. Animals were housed in groups of 5–10 per cage, maintained at 20–30 °C, 50–55% humidity in a natural light and dark cycle, with free access to food and water. The experiments were performed during the light cycle in awake, freely moving animals that were adjusted to laboratory conditions before proceeding with the experiments. Animals were procured from the central animal house, Jamia Hamdard, New Delhi.

The project was undertaken with prior approval from the University Animals Ethics Committee and utmost care was taken to ensure that animals were treated in the most humane and ethically acceptable manner.

Drugs

The studies utilized the following drugs and chemicals: LTG (Lamitor tablets, Torrent); FA (Folvite tablets, Lederle). Drugs were diluted to the desired concentrations with distilled water and administered orally.

Experimental procedures

Increasing current electroshock seizures (ICES) test

The ICES test as proposed by Kitano et al (1996) and modified by Marwah et al (1998) was used to evaluate the anticonvulsant effect of the drugs. Starting with a current of 2 mA, electroshock was delivered to each mouse via ear electrodes as a single train of pulses (for 0.2 s) with linearly increasing intensity of 2 mA/2 s. The current at which tonic hind limb extension occurred was recorded as the seizure threshold current. If no tonic hind limb extension was observed by a current of 30 mA, electroshock was terminated and this cut-off current was used in the analysis. LTG alone and LTG + FA combinations were studied for their acute and chronic effects.

Forced swimming test (FST)

This test was based on the method of Porsolt et al (1977). Briefly, mice were trained to swim for 15 min in glass beakers (height 15 cm, diameter 11 cm) containing fresh water (22 ± 2 °C) up to a height of 6 cm. This constituted the pre-test session. Twenty-four hours later, each animal was re-exposed to the swimming condition in a similar environment in a 6-min test session. The animal's vigorous attempts to leave the swimming environment were interspersed with bouts of immobility signifying 'behavioural despair'. LTG alone and LTG + FA combinations were studied for their acute and chronic effects.

Spontaneous alternation behaviour (SAB) on plus maze

This was assessed using the method of Ragozzino et al (1998). The maze (height 50 cm) was constructed of wood, painted grey and contained a central platform (8 × 8 cm) from which radiated four symmetrical arms (23.5 cm long × 8 cm wide) with 10-cm walls. After being placed in the central platform, mice were allowed to traverse the maze freely for 6 min. The number and sequence of entries were recorded; an alternation was defined as entry into four different arms on overlapping quintuple sets. Five consecutive arm choices within the total set of arm choices made up a quintuple set. A quintuple set consisting of arm choices A, B, A, C, B was not considered an alternation. Using this procedure, possible alternation sequences are equal to the number of arm entries minus 4. The percentage alternation score is equal to the ratio of (actual alternation/possible alternation) × 100. LTG alone and LTG + FA combinations were studied for their acute and chronic effects.

Study design and drug treatment

ICES, FST and SAB

The acute effects of LTG (1.3, 2.6 and 5.2 mg kg^{-1}), FA (0.65 mg kg^{-1}) and LTG + FA (1.3 + 0.65 mg kg^{-1}), and the chronic effects of LTG (1.3 mg kg^{-1}), FA (0.65 mg kg^{-1}) and LTG + FA (1.3 + 0.65 mg kg^{-1}) were evaluated.

The LTG dose was based on the study by Miller et al (1986) to obtain a dose-dependent response in the acute and chronic experiments. The FA dose was calculated from the equivalent absolute human dose using surface area ratio of mouse to man (Ghosh 1984). The smallest significant antiepileptic dose of LTG at which there was significant efficacy (1.3 mg kg^{-1}) was combined with 0.65 mg kg^{-1} FA to study the effects of the combination on seizure threshold, SAB and behavioural despair.

All drugs were given orally in a volume of 10 mL kg^{-1}. Control animals received an equivalent volume of saline (0.9%).

In the chronic studies, drugs were administered for 21 days. All observations were made 1 h after LTG or FA treatment (on the same day for acute studies and on the Day 21 for chronic studies).

Observations from control groups were pooled together for a combined control group for each test (FST, ICES and SAB). There were at least five mice per group. Each mouse underwent only one type of treatment and test, and was not reused.

Statistical analysis

The results are presented as mean ± s.e.m. Data were analysed using one-way analysis of variance with Dunnet's *t*-test at the 95% confidence level.

Results

Effect of LTG and FA on seizure threshold in the ICES test

Acute effects

LTG (1.3, 2.6 and 5.2 mg kg^{-1}) significantly enhanced the seizure threshold as determined by analysis of variance and Dunnett's test ($F(5,48) = 29.33$, $P < 0.01$) with the higher dose providing greater enhancement. FA (0.65 mg kg^{-1}) did not affect seizure threshold (Table 1).

The combination of LTG (1.3 mg kg^{-1}) and FA (0.65 mg kg^{-1}) significantly enhanced the seizure threshold (vs 1.3 mg kg^{-1} LTG and vs 0.9% saline).

Chronic effects

In the chronic study, both LTG (1.3 mg kg^{-1}) and LTG + FA (1.3 + 0.65 mg kg^{-1}) significantly increased the seizure threshold in mice ($F(3,32) = 47.71$, $P < 0.01$, Table 1). FA (0.65 mg kg^{-1}) did not affect seizure threshold (Table 1).

Effect of LTG and FA on FST immobility

Acute effects

LTG (1.3, 2.6 and 5.2 mg kg^{-1}) did not affect the immobility time ($F(5,44) = 29.56$, Table 2). FA (0.65 mg kg^{-1}) moderately reduced the immobility time. The combination of LTG (1.3 mg kg^{-1}) and FA (0.65 mg kg^{-1}) significantly reduced the FST-induced immobility when compared with LTG (1.3 mg kg^{-1}) alone.

Chronic effects

As with the acute effects, chronic doses of LTG (1.3, 2.6, 5.2 mg kg^{-1}) did not affect the immobility time ($F(5,74) = 34.9$, Table 2). FA (0.65 mg kg^{-1}) significantly reduced the immobility time when compared with the saline-treated group. The combination of LTG (1.3 mg kg^{-1}) and FA (0.65 mg kg^{-1}) significantly reduced the immobility time compared with LTG (1.3 mg kg^{-1}) alone and the saline-treated group (Table 2).

Effect of LTG and FA on SAB

Acute effects

A low dose of LTG (1.3 mg kg^{-1}) moderately increased the alternation scores. FA (0.65 mg kg^{-1}) as well as higher doses of LTG (2.6 and 5.2 mg kg^{-1}) did not affect SAB. Similarly, the combination of LTG (1.3 mg kg^{-1}) and FA (0.65 mg kg^{-1}) did not affect SAB (Table 3).

Chronic effects

LTG (1.3 mg kg^{-1}) moderately reduced the alternation scores ($F(3,32) = 4.17$, $P < 0.01$, Table 3). FA (0.65 mg kg^{-1}) significantly increased the alternation scores when compared with the saline-treated group.

LTG (1.3 mg kg^{-1}) in combination with FA (0.65 mg kg^{-1}) did not significantly increase alternation when compared with the saline-treated group. However, the combination was significant in improving the alternation score when compared with animals receiving only LTG (1.3 mg kg^{-1}) treatment (Table 3).

Discussion

The present study confirms the antiepileptic efficacy of LTG in mice. In addition, it reveals some interesting neurobehavioural possibilities with LTG, given alone and in combination with FA.

LTG exhibited a dose-dependent increase in the seizure threshold in mice. These observations are consistent with the findings of Miller et al (1986) in the maximal electroshock seizure test. In the present study, FA on its own did not affect seizure threshold. However, when combined with LTG (1.3 mg kg^{-1}), it provided further enhancement of the seizure threshold. There has been little published research on the effect of administering folate supplements to patients with epilepsy. This is partly because of

Table 1 Effect of lamotrigine and folic acid on the increasing current electroshock seizure threshold of mice.

Drug treatment	Daily dose (p.o.)	Seizure threshold (mA) (mean ± s.e.m.)	
		Acute effect	Chronic effect[a]
0.9% saline	10 mL kg^{-1}	15.33 ± 0.42 (n = 24)	14.66 ± 0.38 (n = 18)
Lamotrigine	1.3 mg kg^{-1}	20.67 ± 0.66* (n = 6)	25.0 ± 1.69* (n = 8)
Lamotrigine	2.6 mg kg^{-1}	24.33 ± 1.96* (n = 6)	–
Lamotrigine	5.2 mg kg^{-1}	26.0 ± 1.69* (n = 6)	–
Folic acid	0.65 mg kg^{-1}	16 ± 0.632 (n = 6)	16.0 ± 0.46 (n = 8)
Lamotrigine + folic acid	1.3 + 0.65 mg kg^{-1}	26.4 ± 2.62*† (n = 6)	26.5 ± 1.37* (n = 8)
F values		F (5,48) = 29.33	F (3,32) = 47.71

*$P < 0.01$ vs respective saline-treated group. †$P < 0.01$ vs respective lamotrigine (1.3 mg kg^{-1})-treated group (analysis of variance followed by Dunnett's test). n = number of mice in each group. [a]Chronic treatment for 21 days.

Table 2 Effect of lamotrigine and folic acid on forced swimming test immobility in mice.

Drug treatment	Daily dose (p.o.)	Forced swimming test immobility (s) (mean ± s.e.m.)	
		Acute effect	Chronic effect[a]
0.9% saline	10 mL kg^{-1}	222.0 ± 2.0 (n = 25)	253.0 ± 6.0 (n = 40)
Lamotrigine	1.3 mg kg^{-1}	212.0 ± 5.0 (n = 5)	237.0 ± 11.8 (n = 8)
Lamotrigine	2.6 mg kg^{-1}	220.04 ± 9.0 (n = 5)	251.0 ± 8.5 (n = 8)
Lamotrigine	5.2 mg kg^{-1}	227.0 ± 8.0 (n = 5)	259.0 ± 7.5 (n = 8)
Folic acid	0.65 mg kg^{-1}	209.0 ± 11.5 (n = 5)	171.0 ± 9.5* (n = 8)
Lamotrigine + folic acid	1.3 + 0.65 mg kg^{-1}	175.0 ± 6.0*† (n = 5)	178.0 ± 6.5*† (n = 8)
F values		F (5, 44) = 29.56	F (5, 74) = 34.9

*$P < 0.01$ vs respective saline-treated group. †$P < 0.01$ vs respective lamotrigine (1.3 mg kg^{-1})-treated group (analysis of variance followed by Dunnett's test). n = number of mice in each group. [a]Chronic treatment for 21 days.

Table 3 Effect of lamotrigine and folic acid on spontaneous alternation behaviour in mice.

Drug treatment	Daily dose (p.o.)	Seizure threshold (mA) (mean ± s.e.m.)	
		Acute effect	Chronic effect[a]
0.9% saline	10 mL kg^{-1}	58.46 ± 5.15 (n = 25)	60.2 ± 3.94 (n = 18)
Lamotrigine	1.3 mg kg^{-1}	71.7 ± 3.56 (n = 5)	51.65 ± 5.81 (n = 6)
Lamotrigine	2.6 mg kg^{-1}	55.68 ± 7.82 (n = 5)	–
Lamotrigine	5.2 mg kg^{-1}	62.68 ± 5.21 (n = 5)	–
Folic acid	0.65 mg kg^{-1}	58.53 ± 9.31 (n = 5)	74.0 ± 3.23* (n = 6)
Lamotrigine + folic acid	1.3 + 0.65 mg kg^{-1}	57 ± 6.82 (n = 5)	63.36 ± 4.72† (n = 6)
F values			F (3, 32) = 4.17

*$P < 0.05$ vs respective saline treated group. †$P < 0.05$ vs respective lamotrigine (1.3 mg kg^{-1})-treated group (analysis of variance followed by Dunnett's test). n = number of mice in each group. [a]Chronic treatment for 21 days.

concerns about the proconvulsant properties of FA (Chanarin et al 1960; Reynolds 1968). The present study clearly demonstrates that, at the given dose (0.65 mg kg^{-1}), FA has no proconvulsant effect. On the contrary, it appears to improve the antiepileptic profile of LTG. Thus, it is likely that the proconvulsant effects of FA might be related to the dose employed. Further studies evaluating a graded, dose-dependent response with FA are warranted for a definite conclusion.

Depression is the most common psychiatric manifestation of epilepsy (Trimble & Reynolds 1976; Robertson & Trimble 1985), associated with the syndrome itself and its therapy (Lambert & Robertson 1999). LTG exhibited no significant effect on swimming induced immobility, whereas FA resulted in a significant reduction in immobility time. Interestingly, LTG (1.3 mg kg^{-1}) when given in combination with FA (0.65 mg kg^{-1}) resulted in a significant reduction in immobility. These findings suggest that the addition of folate to LTG therapy may provide an additional benefit in the treatment of depression associated with epilepsy (Edeh & Toone 1985; Froscher et al 1995).

Cognitive impairment is a problem frequently associated with epilepsy (Lesser et al 1986). There are various factors that contribute to the impairment of cognitive functions, the adverse effect of AEDs being one of them (Saber et al 1995). Cognitive functions are impaired at higher levels of AEDs and during polytherapy (Thompson & Trimble 1983). Compared with other AEDs, LTG has lesser potential for causing cognitive side-effects (Baxter et al 1990; Leach et al 1991). Consistent with this, in the present study, LTG (1.3, 2.6 and 5.2 mg kg^{-1}) did not affect the working memory in mice in the plus maze. These observations indicate that LTG has no deleterious effect on memory. Similarly, FA on its own did not affect working memory. However, in combination with LTG, it produced positive cognitive effects. The present effects of FA on memory agree well with earlier reports of an improvement in drive, speed of cerebration, alertness and concentration in epileptic patients receiving folate supplements (Reynolds 1968).

Conclusion

To the extent that several epileptic patients require treatment for accompanying neuropsychiatric disorders, it would be worthwhile to carry out routine screening of AEDs for additional psychopharmacological benefits. Further, there is evidence to show that the addition of

FA to an AED regimen can greatly reduce the AED-associated neurotoxicity. The results of the present study using LTG and FA (at the doses employed) can be summarized as follows: (i) LTG increased seizure threshold, whereas FA had no effect in the ICES test in mice; (ii) LTG exhibited no change, whereas FA decreased swimming-induced immobility in mice; (iii) neither LTG nor FA affected SAB in mice; and (iv) the combination of LTG and FA significantly reduced immobility while enhancing alternation scores and the seizure threshold.

The present observations have confirmed the antiepileptic action of LTG in a rodent model of epilepsy. The results clearly demonstrate the additional benefits on epilepsy, mood and memory brought about by the inclusion of FA in the LTG regimen. Thus, the use of FA as an adjunct in antiepileptic therapy warrants further study.

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