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Research report

Vitamin D enhances antiepileptic and cognitive effects of lamotrigine in pentylenetetrazole-kindled rats

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ABSTRACT

Despite long use of antiepileptic drugs, it remains a challenge to achieve seizure control while reducing adverse effects and preventing cognitive impairment. Several lines of evidence suggest a role of vitamin D in epilepsy. So this study aimed to investigate the effect of vitamin D on epileptogenesis, cognitive dysfunction and antiepileptic activity of lamotrigine, in a rat model of chemical kindling. Rats were kindled by pentylenetetrazole injections every other day over four weeks, together with daily oral treatment by either vehicle, vitamin D, lamotrigine or combination of vitamin D and lamotrigine. The non-treated kindled rats developed generalized seizures and had poor cognitive performance in water maze, associated with prooxidative status; elevated malondialdehyde and nitric oxide with lowered glutathione levels; in brain tissues. Treatment with either vitamin D, lamotrigine or both leads to significant reduction of seizure activity score, improvement of cognitive performance, and amelioration of the disturbed oxidative stress biomarkers. These findings indicate that, vitamin D has anti-epileptic, cognitive improving and antioxidant effects, on its own and enhance the effects of lamotrigine, in a chronic model of epileptic seizures. Thus, vitamin D supplementation may be a useful addition to antiepileptic drugs improving seizure control and cognitive function in patients with epilepsy.

1. Introduction

Epilepsy is one of the most common neurological disorders, affecting 0.5–1% of the population (Haut et al., 2006). Antiepileptic drugs (AEDs) have been available for several decades, but their clinical use is frequently limited by occurrence of many adverse effects and drug-drug interactions. It has been estimated that, about 20–30% of patients with epilepsy have poor seizure control and become intractable, despite of optimal AED therapy (Weintraub et al., 2007). Moreover, clinical studies have shown that about half of the epileptic patients develop cognitive impairment (Motamedi and Meador, 2003), related to etiology of seizures and the use of current AEDs especially with the polytherapy (Xie et al., 2012). It therefore remains a challenge to treat epilepsy with a high degree of efficacy while reducing adverse effects and preventing cognitive impairment.

Brain tissues are highly vulnerable to the oxidative stress that has been shown to play an important role in the pathogenesis of seizures (Sudha et al., 2001). Oxidative stress is known to participate in pathways leading to neurodegeneration, which is the most important propagating factor in epileptogenesis and cognitive decline (Martinc et al., 2014). At the cellular level, seizure activity initiates significant calcium influx leading to biochemical cascades, which can induce reactive oxygen species (ROS) and trigger acute neuronal death (Fujikawa et al., 2000). Several substances with known antioxidative properties such as; Aloe vera (Rathor et al., 2013), alpha-tocopherol (Tomé et al., 2010), lipoic acid (Militão et al., 2010), curcumin (Ataie et al., 2010), melatonin (Solmaz et al., 2009), pentoxifylline (Tariq et al., 2008) and Centella asiatica (Gupta et al., 2003); have shown significant anticonvulsant activity. These agents can produce neuroprotective effects accompanied by reduction in oxidative stress parameters in the brain of various animal seizure models. However, it was demonstrated that some of the AEDs such as valproic acid (Martinez et al., 2004) and carbamazepine (Gilham et al., 2000), could be responsible for additionally increased oxidative stress and lipid peroxidation on

Abbreviations: AEDs, antiepileptic drugs; GABA, γ-aminobutyric acid; GSH, reduced glutathione; MDA, malondialdehyde; NMDA, *N*-methyl-D-aspartate; NO, nitric oxide; PTZ, pentylenetetrazole; ROS, reactive oxygen species; VDR, vitamin D receptors; Vit D, vitamin D.

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long-term use, possibly through metabolism to reactive epoxide intermediates. Therefore, it is reasonable to propose that neuroprotective antioxidants could be applied as an add-on therapy to epileptic patients, leading to reduced neurodegeneration, epileptogenesis and cognitive deterioration.

Both antiepileptic treatment and the comorbidities associated with epilepsy have a negative impact on vitamin D (vit D) levels. It has been observed that, vit D is deficient in about half of people with epilepsy (Teagarden et al., 2014). This vit D deficiency can affect many aspects of brain function and may affect the response to antiepileptic treatment (Cebeci, and Ekici, 2014). Epileptic seizures have been reported to show seasonal variation, peaked in January, reflecting fluctuations in vitamin D levels (Clemens et al., 2013). Poor vit D status has also been implicated in the pathogenesis of other neurological conditions such as Parkinson's disease and Alzheimer disease (Evatt et al., 2008), dementias (Breitling et al., 2012), and multiple sclerosis (Mowry, 2011). Several lines of epidemiological evidence together with some experimental data suggest a beneficial role of vit D in epilepsy (Holló et al., 2012). Acute anticonvulsant effect of vit D has been demonstrated in rats with electrical hippocampal seizures (Siegel et al., 1984), and in mice with pentylenetetrazole (PTZ)-induced convulsions (Kalueff et al., 2005). However, the effects of vit D in epileptogenesis, epilepsy treatment and associated cognitive impairment were not clarified.

So the present study used the PTZ-kindled rat model of epileptic seizures to investigate the effect of vit D on chemical epileptogenesis and antiepileptic activity of lamotrigine, The effects of vit D or lamotrigine or both drugs were assessed regarding kindling development, seizure activity, cognitive dysfunction and levels of oxidative stress biomarkers in the brain of kindled rats.

2. Results

The five rat groups were matching regarding their initial body weight at start of experiments. All rats showed significant weight gain during the study period. However, development of body weight were similar in the different groups with no significant differences between final body weights of the five rat groups ($p > 0.05$).

2.1. Effect on seizure activity and development of PTZ kindling

Chemical kindling was induced in all rats, developing abnormal movements and seizure activity initiated within 5 to 30 min after PTZ injection, in the form of head nodding, forelimb and hindlimb clonus, hyperextension of tail, loss of posture and myoclonic jerks progressing to a generalized seizure with an overall seizure score of 4.3 ± 0.28 and after a mean latency of 6.8 ± 0.5 min. The seizures lasted more than 1 h and 3 rats (25%) died within 24 h period following the last PTZ injection. Treatment with either vit D, lamotrigine or both along with PTZ injections lead to significant reduction in the seizure incidence (83%, 58%, 41%; respectively, $p < 0.05$) and seizure severity showing a mean score of 3.2 ± 0.22, 2.3 ± 0.20, 1.4 ± 0.11; respectively ($p < 0.05$). Also, animal mortality was significantly reduced to 8%, 0% and 8% in groups 3, 4 and 5, respectively. Addition of vit D with lamotrigine led to significantly improved anticonvulsant activity indicated by significantly lower seizure incidence and reduced seizure score in group 5 compared with group 4 treated with lamotrigine alone ($p < 0.05$). The normal control rats did not show any of the abnormal movements or seizure activity and no mortality observed in this group (Table 1 & Fig. 1).

2.2. Effect on cognitive performance in Morris water maze

Before cognitive testing, the motor abilities of various groups were similar and not impaired, as assessed during the day 1 free movement in the water maze. Animals treated by PTZ showed prolonged escape latency to reach the platform and spent less time in the target quadrant, during all sessions compared with the control rats ($p < 0.05$). This poor performance is clearly suggestive of impaired visual-spatial memory and cognitive deficit in non-treated kindled rats. The performance in water maze was markedly improved by concomitant treatment with either vit D or lamotrigine showing significantly shorter escape latencies compared with group 2 rats given PTZ only ($p < 0.05$). The combined treatment with both vit D and lamotrigine produced more marked improvement in cognitive performance, showing significantly shorter escape latency in group 5 rats compared with groups 3 and 4 treated with either vit D or lamotrigine alone ($p < 0.05$). However, all groups showed gradual improvement in performance over the 4 days of training period, with escape latencies in day 4 significantly shorter than latencies in day 1 for each group ($p < 0.05$) (Fig. 2a). The probe trial studies showed that rats treated with either vit D, lamotrigine or both, spent more time in the target quadrant compared with non-treated PTZ-kindled rats ($p < 0.05$) (Fig. 2b). The group treated by combined vit D and lamotrigine performed equivalently to the normal control group.

2.3. Effect on oxidative stress markers in brain tissues

2.3.1. Lipid peroxides levels

The lipid peroxide, MDA, levels were significantly increased in the brain tissues of PTZ-kindled rats compared with the control

Table 1
Anti-seizure effects of vitamin D, lamotrigine or both in the rat model of PTZ-induced epileptic seizures.

Group (n = 12)	1 (Normal)	2 (PTZ)	3 (PTZ-D)	4 (PTZ-L)	5 (PTZ-D + L)
Body weight:					
Initial body weight (g)	141 ± 8	145 ± 13	138 ± 11	136 ± 9	142 ± 10
Final body weight (g)	216 ± 17	208 ± 14	211 ± 16	203 ± 19	220 ± 12
Seizures:					
Seizure Latency (min)	0.0	6.8 ± 0.5‡	11.2 ± 0.7*	19.3 ± 1.2*,**	24.5 ± 1.6*,†
Seizure Incidence (%)	0.0	100%‡	83%*	58%*,**	41%*,†
Seizure Score (0 −5)	0.0	4.3 ± 0.28‡	3.2 ± 0.22*	2.3 ± 0.20*,**	1.4 ± 0.11*,†
Mortality (%)	0.0	3 (25%)‡	1 (8%)*	0 (0%)*	1 (8%)*

All values are expressed as Mean ± SEM, $p < 0.05$ is considered statistically significant.
PTZ: non-treated PTZ-kindled rats, PTZ-D: PTZ-kindled rats treated with vitamin D, PTZ-L: PTZ-kindled rats treated with lamotrigine, PTZ-D+L: PTZ-kindled rats treated with both vitamin D and lamotrigine.
‡ Significant change compared with group 1 (normal control).
* Significant change compared with group 2 (PTZ).
** Significant change compared with group 3 (PTZ-D).
† Significant change compared with group 4 (PTZ-L).