MEMANTINE, AS A P-GLYCOPROTEIN EXPRESSION MODULATOR, ENHANCES LEVETIRACETAM THERAPEUTIC RESPONSE IN EPILEPTIC PATIENTS

SAHAR AHMED HARBY[1]*, RASHA A NASSRA[2], JAIDAA F MEKKY[3], SAMIA M ALI[1], CHERINE A ISMAIL[1]

[1]Department of Clinical Pharmacology, Faculty of Medicine, Alexandria University, Alexandria, Egypt. [2]Department of Medical Biochemistry, Faculty of Medicine, Alexandria University, Alexandria, Egypt. [3]Department of Neuropsychiatry, Faculty of Medicine, Alexandria University, Alexandria, Egypt. Email: sahar.ahmed15@alexmed.edu.eg

ABSTRACT

Objective: The principal aim of the present study was to assess the importance of multidrug transporter; P-glycoprotein (P-gp) as a potential therapeutic target in patients with epilepsy. Can P-gp transporter expression modulation by memantine add to the standard antiepileptic drugs (AEDs) response?

Methods: A cohort of 56 epilepsy patients was included in a 4 monthly visits prospective study. Patients were on levetiracetam (LEV) 1000 mg/day alone or combined with other AEDs. They were randomly assigned into two groups; LEV only group including LEV-treated patients and LEV + memantine group including patients on LEV with add-on oral memantine 10 mg/day until the end of the study. During monthly follow-up visits, therapeutic responses were evaluated for each patient by recording the monthly seizures frequency. Blood samples were drawn from every patient twice (on the first and last visits) for assessment of P-gp mRNA expression level.

Results: Fifty patients completed the study. At the end of 4th month, LEV only group showed a non-significant decrease in P-gp expression and seizure frequency compared to the 1st month, whereas, in LEV + memantine group, P-gp expression was significantly reduced and associated with significant seizure control.

Conclusion: Memantine by hindering P-gp overexpression was apt to enhance LEV efficacy and exhibit a better seizure control.

Keywords: P-glycoprotein, N-methyl d-aspartate receptor, Memantine, Levetiracetam, Epilepsy.

INTRODUCTION

Epilepsy is a chronic brain disease characterized by transient dysfunction of the central nervous system induced by abnormal discharge and threatens human health [1]. Approximately two-thirds of epilepsy patients can achieve satisfactory results through antiepileptic drug (AED) treatment, but the remaining third still cannot control their symptoms in spite of using multiple AED treatments and therefore may develop drug-resistant epilepsy (DRE) [2].

The resistance mechanism of DRE remains unclear. Until then, studies should be directed to deeply assimilate the pharmacological hypothesis that helps to elucidate the neurobiological basis for drug resistance, including the transporter hypothesis [3,4]. The transporter hypothesis of DRE refers to the excessive expression of certain blood-brain barrier (BBB) efflux transporters that can pump the drugs out of the brain cells, causing a reduction in the local concentration of AEDs at the epileptic lesion [1]. One of the major drug efflux transporters of concern in DRE is P-glycoprotein (P-gp) or ATP-binding cassette subfamily B member 1 protein [5]. Most AEDs have been documented as P-gp substrates with variable degrees, including levetiracetam (LEV) [6,7].

Preventing seizure-associated transporter upregulation, i.e., transporter modulation, might offer a unique alternate approach to overcome transporter-associated pharmacoresistance. Multiple signal transduction pathways may be involved in brain efflux transporter upregulation; the most evident is glutamate/N-methyl d-aspartate receptor (NMDA-R)/cyclooxygenase-2 (COX-2)/prostanoid E receptor 1 (EP1) signaling pathway [8]. In this pathway, seizures induce the neuronal and glial release of glutamate, which signals through the NMDA-R, COX-2, and EP1, resulting in increased expression of P-gp in brain capillaries [9]. In this view, memantine as a non-competitive NMDA-R antagonist is suggested to downregulate glutamate/NMDA-R /COX-2 /EP1 signaling pathway in resistant epilepsy [10]. The principal goal of the present study was to assess the importance of P-gp as a potential therapeutic target in patients with epilepsy. In this context, we aimed to evaluate the impact of memantine, as add-on therapy, on the therapeutic response to LEV in epileptic patients.

METHODS

The study protocol was performed in compliance with the ethical rules of the Helsinki declaration and was approved by the Local Ethics Committee of School of Medicine, Alexandria University. A written informed consent was provided by all participants involving the inclusion of the study data for research purposes.

Patients' inclusion criteria

The present work displayed a randomized prospective open-label study. Fifty-six patients with epilepsy with the age range of 18–60 years old were recruited from the epilepsy clinic of El-Hadara University Hospital in Alexandria. They had a distinct diagnosis of various seizure types. Only the patients that were on LEV treatment as a single agent or combined with other AEDs were included in the study.

Patients' exclusion criteria

Patients presenting with secondary epilepsy, significant co-morbidities, neuropsychiatric disorders, impaired hepatic and renal functions, or taking any neuropsychiatric medications other than AEDs were excluded from the study. Furthermore, pregnant and lactating patients and patients with uncontrolled epilepsy with the variation of their AEDs regimen [11,12].

Study design

Starting from January 2018, eligible epilepsy patients were randomly enrolled into one of two groups of 28 patients each; LEV only treated group, in which patients that were taking oral LEV (TIRATAM – Al Andalus Medical Company – Egypt) in a dose of 1000 mg/day with or without other AEDs continued taking their same regimen throughout the study. The second group was the LEV + memantine-treated patients that received oral memantine (ALZIXA – Mash Premiere – Egypt) 10 mg/day at a fixed time every night add-on their already prescribed LEV in a dose of 1000 mg/day until the end of the study [13,14].

Clinical follow-up and sampling

During monthly follow-up visits, the therapeutic responses were evaluated for each patient by recording the monthly seizures frequency (seizure calendar) [15]. The P-gp mRNA expression level was assessed twice for each patient on the first and last visits. The first visit sample was considered a baseline sample as it was taken before starting memantine. In this context, 3 mL of venous blood was withdrawn on EDTA for P-gp mRNA extraction from peripheral blood mononuclear cells (PBMCs) [16]. All samples were frozen at −80°C until analysis. The PBMCs were separated from blood samples by centrifugation for 30 min at 2500 rpm using 1.5 mL of Ficoll gradient and then cells were washed 3 times with ×1 cold PBS (pH 7.4) [17].

Assessment of P-gp expression in PBMCs by quantitative real-time polymerase chain reaction (PCR)

Total PBMCs RNA was extracted following the manufacturer's protocol (QIAGEN - Germany) and was used for reverse transcription using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific Inc.). Quantitative PCR was performed starting by amplification of the synthesized cDNAs using PCR related kits [17]. The specific primers sequence for P-gp were GTC TGG ACA AGC ACT GAA A (forward) and AAC AAC GGT TCG GAA GTT T (reverse). (GenBank accession number NM_000927.4) [16]. Verification of the PCR amplification product was performed on a 2% agarose gel stained with ethidium bromide. To confirm PCR product identity, a melting curve analysis was applied and a negative internal control was run with every PCR. Analysis of data was performed, where the level of expression of P-gp mRNA was determined by the comparative CT method for gene expression relative to the housekeeping gene GAPDH [18].

Statistical analysis

Data were analyzed using IBM SPSS software package version 20.0. The Kolmogorov–Smirnov test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), median, and interquartile range. p < 0.05 were considered significant. Wilcoxon signed ranks test was used for abnormally distributed quantitative variables, to compare between two periods. Mann–Whitney test was used for abnormally distributed quantitative variables to compare between two studied groups. Friedman test was used for abnormally distributed quantitative variables to compare between more than two periods and *post-hoc* test (Dunn's) for pairwise comparisons.

RESULTS

Patients' demographics and clinical features

Only 50 patients completed the study. The individual type of seizures and their LEV regimen together with their characteristic demographics was described in Table 1. Patients' detailed disposition flow in the study was demonstrated in Fig. 1.

P-gp mRNA expression level

There was a decrease in the 4th-month median P-gp expression level compared to the 1st month in the two patients' groups. This decrease was only significant in LEV + memantine group (Table 2). By comparing the percent change in P-gp expression level of the 4th month versus the 1st month between the two patients' groups, LEV + memantine-treated group showed a non-significant percent change compared to LEV only group (Fig. 2).

Seizure frequency/month

In LEV only group, no significant change was observed in the median number of seizures during the study. While in LEV + memantine-treated group, the median seizure frequency of the 3rd and 4th months showed a significant decrease compared to the 1st month reaching to a seizure-free status in the 4th month (Table 3). By comparing the percent change in seizure frequency of the 4th month versus the 1st month in the two patients' groups, LEV + memantine-treated group showed a significant percent reduction compared to LEV only group (Fig. 3).
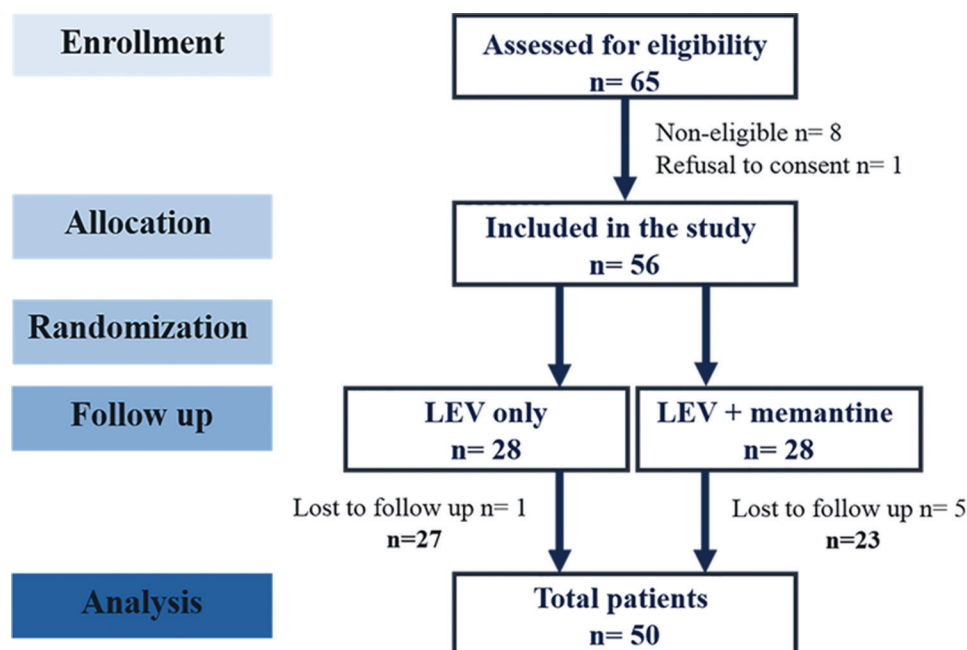

Fig. 1: Patients' disposition flow. Nine patients of 65 were excluded at the beginning of the study because they had one of the exclusion criteria or refused to consent. Only 50 patients of 56 completed the study and were included in the statistical analysis. n: Patients' number; LEV: Levetiracetam

DISCUSSION

A concern has been raised highlighting the intimate link of the multidrug transporter P-gp to the transporter hypothesis of pharmacoresistance in epilepsy compared to other BBB efflux transporters [19]. Consequently, the modulation of P-gp could be a plausible therapeutic target in epilepsy. Moreover, unraveling the role of glutamate/NMDA-R/COX-2/EP1 signaling pathway in the upregulation of the P-gp transporter and its potential link to the transporter hypothesis of AEDs resistance could aid in designing novel and more specific therapeutic strategies targeting this pathway. One of these strategies could be to block NMDA-R by the NMDA-R antagonist memantine.

Results of the present study revealed that the addition of memantine to LEV treatment in epileptic patients had significantly reduced the P-gp expression at the end of the 4th month compared to the baseline, which was lacking in the LEV only treated patients. Furthermore, the effect of memantine on P-gp expression was associated with a significant reduction in the number of seizures at the end of the study compared to the 1st month. The contrast of the seizure percent changes from the 1st to the 4th month between both groups revealed a significant percent reduction in the memantine add-on group versus that of LEV only treated patients.
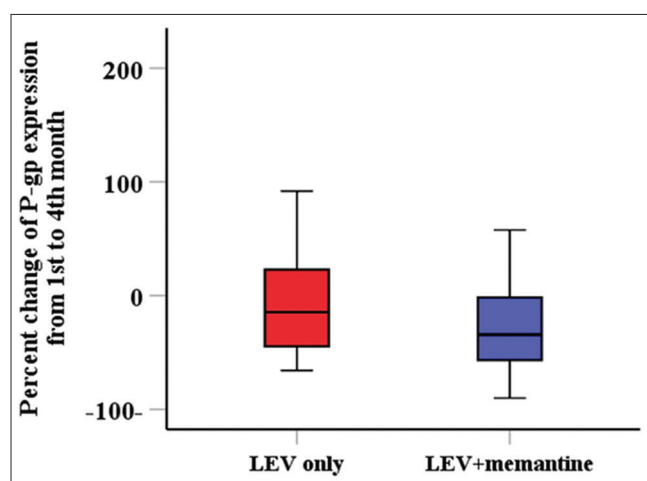

Fig. 2: Percent change of P-glycoprotein expression level from the 1st to the 4th month. LEV: Levetiracetam
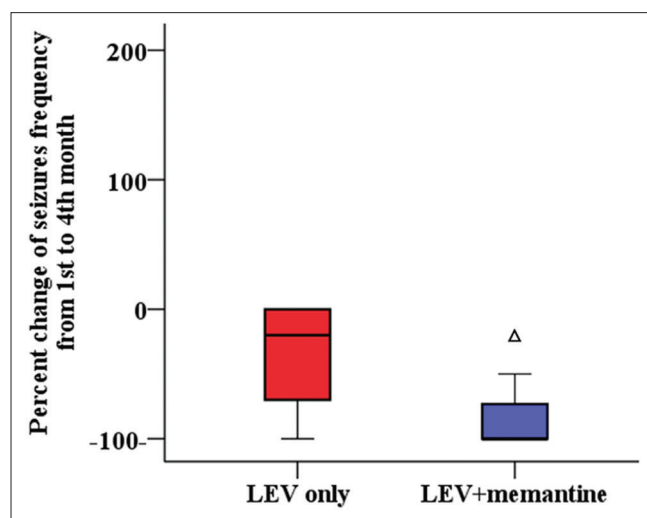

Fig. 3: Percent change of seizure frequency from the 1st to the 4th month. LEV: Levetiracetam, Δ: Significant difference as compared to LEV only group

These results emphasized on the importance of the previously reported role of glutamate/NMDA-R/COX-2/EP1 signaling pathway in pathogenesis of DRE as the blocking of NMDA-R by memantine was apt to halt the downstream signaling pathway and reduced the level of P-gp expression. This effect would increase LEV influx into the target tissues, as evidenced by the improvement in the therapeutic efficacy of LEV to the extent that a significant number of patients turned to be seizure free.

Alternatively, other mechanisms could also be suggested to reason the observed benefit of memantine rather than modulating P-gp expression. One of these mechanisms is the proposed neuroprotective effect of memantine that is speculated to aid in seizure control. Being a non-competitive antagonist at serotonin 5-HT$_3$ receptors, memantine can inhibit the promoting effect induced by 5-HT$_3$ receptors stimulation on seizure genesis. It has been reported that 5-HT$_3$ receptors facilitate neuronal excitation by transmembrane Na$^+$ and Ca^{+2} influx; thus, their inhibition may inhibit local excitation and spread of seizures. Moreover, 5-HT$_3$ receptors blockage has also been reported to diminish the elevated glutamate intracellular levels, oxidative stress, and caspase-3 activity; thus, it may significantly contribute to neuroprotection and seizures reduction [20,21].

Although the use of memantine as add-on AEDs is scarce in literature, the present findings are in accordance with a clinical study on epileptic encephalopathy, where off-label memantine was tried as adjuvant therapy in two children, who were refractory to conventional AEDs. Memantine led to a mild to moderate reduction in seizure burden as well as developmental improvements [22]. It seems likely that the currently observed benefit of memantine can be reproduced by its addition to other AEDs that are P-gp substrates, though this needs further confirmation. Another clinical study was using also memantine in adjuvant to AEDs, yet they were assessing its impact on cognitive

Table 1: Patients' demographics and clinical features

Demographic data/clinical features	Total (n=50)
Sex, n (%)	
Male	18 (36)
Female	32 (64)
Age (years)	
Range (median)	18–60 (25.0)
Types of seizures n (%)	
Generalized	26 (52)
Focal	9 (18)
Focal to bilateral tonic clonic	6 (12)
Myoclonic	5 (10)
Atonic	4 (8)
LEV regimen, n (%)	
LEV monotherapy	14 (28)
LEV add-on other AEDs	36 (72)

n: Patients' number, LEV: Levetiracetam, AEDs: Antiepileptic drugs, other AEDs include carbamazepine, valproate, phenytoin, and topiramate

Table 2: P-glycoprotein expression level of the 4th versus the 1st month

P-gp expression level	1st month	4th month	p
LEV only (n=27)			
Min.–Max.	0.09–6.29	0.11–5.89	0.079
Median (IQR)	1.19 (0.3–3.5)	1.11 (0.4–2.4)	
LEV+memantine (n=23)			
Min.–Max.	0.95–81.80	0.27–80.04	0.004**
Median (IQR)	8.17 (4.8–12.7)	4.02 (2.5–8.2)	

p: Value of significance for Wilcoxon signed ranks test for comparison of P-gp expression level between the 1st and 4th months. LEV: Levetiracetam, n: Number of patients. Min: Minimum, Max: Maximum, IQR: Interquartile range **: Statistically significant at p≤0.01. P-gp: P-glycoprotein

Table 3: Monthly seizure frequency

Seizure frequency/month	1st month	2nd month	3rd month	4th month	p
LEV only (n=27)					
Min.–Max.	0.0–15.0	0.0–30.0	0.0–35.0	0.0–35.0	0.235
Median (IQR)	3.0 (1.0–4.0)	1.0 (0.0–3.0)	1.0 (0.0–3.0)	2.0 (0.0–3.0)	
LEV+memantine (n=23)					
Min.–Max.	1.030.0	0.0–15.0	0.0–5.0	0.0–2.0	<0.001**
Median (IQR)	1.0 (1.0–3.50)	1.0 (0.0–3.50)	0.50 (0.0–1.0)	0.0 (0.0–1.0)	
p_{First}		0.114	0.001**	<0.001**	

p: Value of significance for Friedman test for comparing seizure frequency between the different 4 monthly visits. p_{First}: Value of significance for Dunn's multiple comparisons test for comparison between the 1st month versus each other months. LEV: Levetiracetam, n: Number of patients. Min: Minimum, Max: Maximum, IQR: Interquartile range **: Statistically significant at p≤0.01

function, memory, and quality of life. They declared that it has a favorable safety profile, which will encourage its safe use in epileptic patients [14].

On experimental level, in line, Schauwecker [23] demonstrated that NMDA-Rs antagonists reduced seizure-induced cell death in a mice model of status epilepticus. It is worth mentioning that the speculated beneficial effect of memantine in seizure control can indirectly downregulate P-gp expression per se. This is based on the evidence declaring that the neuronal injury induced by the seizures can upregulate P-gp expression, which depends on the intensity and the duration of the seizures [24].

It seems that the demonstrated valuable effect of memantine as add-on therapy to AEDs in epilepsy is not a group effect of all NMDA-R antagonists. In fact, Sveinbjornsdottir *et al*. [25] tested the effect of a competitive NMDA-Rs antagonist D-CPP-ene (SDZ EAA 494, (R)-4-(3-phosphono-2-propenyl)-2-piperazinecarboxylic acid) on patients with epilepsy. They declared that despite that this compound was well tolerated in healthy volunteers; it induced confusion and sedation in patients with epilepsy with no change or even deterioration of their seizure frequency. This contradiction could not only be due to the difference in structure of the NMDA-R antagonists and in their interaction with the receptors but also due to the difference in clinical setting, where Sveinbjornsdottir *et al*. [25] tried D-CPP-ene in only eight patients, who were all suffering from refractory epilepsy.

CONCLUSION

Memantine by hindering the overexpression of P-gp enhanced the anti-seizure effect of LEV. The present study provides preliminary clinical proof of the potential impact of P-gp transporter downregulation on seizure control in epilepsy patients. Targeting the glutamate /NMDA-R/ COX-2 /EP1 pathway can pave the way for the future development of safe and effective adjuvant AEDs therapy that can help to overcome the DRE challenge.

AUTHORS' CONTRIBUTIONS

The author declares that all the named authors have contributed equally to this article.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to disclose.

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