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ORIGINAL COMMUNICATION

Synaptic vesicle protein 2A tumoral expression predicts levetiracetam adverse events

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Abstract

Objective The efficacy of levetiracetam (LEV) in controlling seizures in patients with brain tumor-related epilepsy (BTRE) depends on tumoral expression of synaptic vesicle protein 2A (SV2A). Although LEV is generally well tolerated, neuropsychiatric adverse events (NPAEs) might occur, limiting compliance and seizure control. We aimed to assess the influence of tumoral SV2A expression on the occurrence of LEV-related NPAEs in patients with glioma.

Methods Specimens from patients enrolled in the multicenter COMPO study, with glioma and BTRE treated with LEV, undergoing neurosurgery were retrieved. Immunohistochemistry-based expression of SV2A in tumoral and peritumoral tissue was scored in a four-point scale from absent (score = 0) to strong (score = 3). Low immunoreactivity (IR) corresponded to scores < 2. Staining ratios (tumoral SV2A IR/peritumoral SV2A IR) were grouped into low (≤ 0.5) and high (> 0.5). NPAEs were assessed longitudinally with the Neuropsychiatry Inventory 12 test (NPI-12).

Results Overall, 18 patients were eligible for analysis. All received LEV monotherapy, with 67% developing NPAEs. Patients with NPAEs had significantly lower median SV2A intensity score compared to patients without NPAEs (score 1 vs 0, $p = 0.025$). Low staining ratio (≤ 0.5) associated with higher NPAE occurrence compared to SR > 0.5 (85.7% vs 0%, $p < 0.01$). A SR ≤ 0.5 predicted a consistent increase in risk of NPAEs (OR 45.0; 95% CI 1.8–1128; $p = 0.02$).

Conclusions Our results suggest that SV2A expression in tumoral and peritumoral tissue correlates with the occurrence of LEV-related NPAEs. Thus, considering that SV2A expression also influences LEV effectiveness, SV2A staining might help in tailoring treatment to patients.

Keywords Brain tumor · Glioma · Epilepsy · Antiepileptic drugs · Adverse events

Introduction

Patients with brain tumor can develop brain tumor-related epilepsy (BTRE) in up to 50% of cases, needing antiepileptic drug (AED) treatment [1]. Levetiracetam (LEV), counting on easy titration and few interaction with chemotherapy, is the first-line AED [1]. LEV binds with high affinity the synaptic vesicle protein 2A (SV2A), blocking pre-synaptic neurotransmitter release [1]. Hence, a possible relationship between SV2A expression and the effectiveness of LEV was postulated. Indeed, among patients with glioma-related epilepsy, the response to LEV has been shown to directly depend on the tumoral expression of SV2A [2]. In particular, a low expression of SV2A in the tumor leads to a reduced binding of LEV at the epileptic focus, translating into ineffectiveness in terms

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of seizure control [2]. However, it is unknown whether LEV-induced neuropsychiatric adverse events (NPAEs) also depend on SV2A expression.

Longitudinal studies support a higher occurrence of neuropsychiatric adverse events (NPAEs) among patients receiving LEV for BTRE [3, 4]. In a multicenter study, we reported a sevenfold increase in NPAEs risk in BTRE patients receiving LEV compared to those receiving alternative AED, independently from tumor site [3]. Thus, our aim was to test if low SV2A expression could predict NPAE occurrence during LEV treatment.

Materials and methods

The cohort was derived from the reported multicenter prospective COMPO study, addressing AED tolerability in BTRE [3]. Briefly, patients aged > 18 receiving monotherapy for BTRE with operable brain tumor were screened for NPAEs using Neuropsychiatric Inventory 12 test (NPI-12) at baseline and follow-up [3]. Other adverse events were defined with iLAEP [5]. For this preliminary study, we included specimens from (1) patients enrolled at the University Hospital of Perugia, (2) with tumor classified as diffuse astrocytic or oligodendroglial tumors (WHO 2016 classification) [6], (3) receiving LEV for BTRE.

Tissue preparation and immunohistochemistry

Tumor/peritumoral tissues were routinely collected during neurosurgical procedures, and assessed for SV2A expression following described methods [2]. Tissues were formalin fixed and paraffin embedded. Four-micrometer sections were placed on slides with permanent positively charged surface. BOND-III fully automated immunohistochemistry stainer (Leica Biosystems) carried out immunostains, using incubation with proteinase K at 37 °C for 10 min as antigen retrieval, followed by primary antibody (anti-SV2A antibody, Rabbit polyclonal; dilution 1:300; Abcam) incubation for 30 min. Finally, the ready-to-use Bond™ Polymer Refine Detection System detected antigen–antibody reaction. Semiquantitative evaluation of immunoreactivity (IR) was performed on each section with high-power non-overlapping fields (diameter 0.55 mm). Staining intensity was evaluated as 0 = absent; 1 = weak; 2 = moderate; 3 = strong. The resulting IR score was grouped for tumoral and peritumoral tissue in low intensity (IR score < 2: absent/weak) and high intensity (IR score ≥ 2: moderate/strong) [2] (Fig. 1). Staining ratio (SR = tumoral IR score/peritumoral IR score) was calculated and dichotomized in low SR (≤ 0.5) and high SR (> 0.5).

Outcomes

Main outcome was NPAEs occurrence, assessed through NPI-12 as previously reported [3]. Further outcomes were NPAE severity and LEV efficacy [3]. SV2A expression, including SV2A IR and SV2A SR, was tested for correlation with specified outcomes.

Statistical analysis

Statistical analysis was performed with SPSS 25.0. Categorical data are presented as number and percentage, and continuous variables as mean and standard deviation (SD). Descriptive statistics are presented as means ± standard deviations for continuous variables, while categorical ones are presented as counts and percentages. Student's *t* test was used to compare continuous variables and the Chi-square test was used to compare categorical data. Non-parametric methods included Mann–Whitney test for continuous/ordinal variables and Fisher's exact test for categorical ones. Odds ratios (OR) were calculated with 2 × 2 contingency tables with Haldane–Anscombe correction if needed; $p < 0.05$ was defined as statistically significant.

Results

Overall, 18 specimens were eligible for analysis. Demographic data of the study cohort are displayed in Table 1. Eight patients (44%) were diagnosed with glioblastoma multiforme, six patients with oligodendroglioma (33%), three (16.7%) with astrocytoma and one (5.5%) with oligoastrocytoma, NOS [6]. SV2A tumoral IR score ranged from absent (38.9%) to strong (5.6%). Staining ratio (SR) ranged from 0 to 1 (mean 0.29 ± 0.28) (Table 1). Overall, 67% of the cohort referred NPAEs, with only 33% of the cohort being NPAEs free, with no relation to LEV dosage or tumor localization to the frontal lobe. The most common NPAE was agitation/aggressiveness ($n = 9$, 50%), followed by irritability ($n = 8$, 44%) and anxiety ($n = 7$, 39%).

Patients with NPAEs had significantly lower median tumoral SV2A IR compared to patients without NPAEs (1 vs 0, $p = 0.025$). In addition, patients with low-intensity tumoral SV2A (IR score < 2) had more NPAEs compared to patients with high-intensity score (80% vs 0%, $p < 0.05$) (Fig. 2a). Compared to SR > 0.5, low staining ratio (≤ 0.5) associated with NPAE occurrence (85.7% vs 0%, $p < 0.01$) (Fig. 2b). A SR ≤ 0.5 predicted a consistent increase in risk of experiencing NPAEs (odds ratio 45.0; 95% CI 1.8–1128; $p = 0.02$).

All patients were seizure free or achieved a > 50% seizure reduction during the study period. Afterwards, two patients

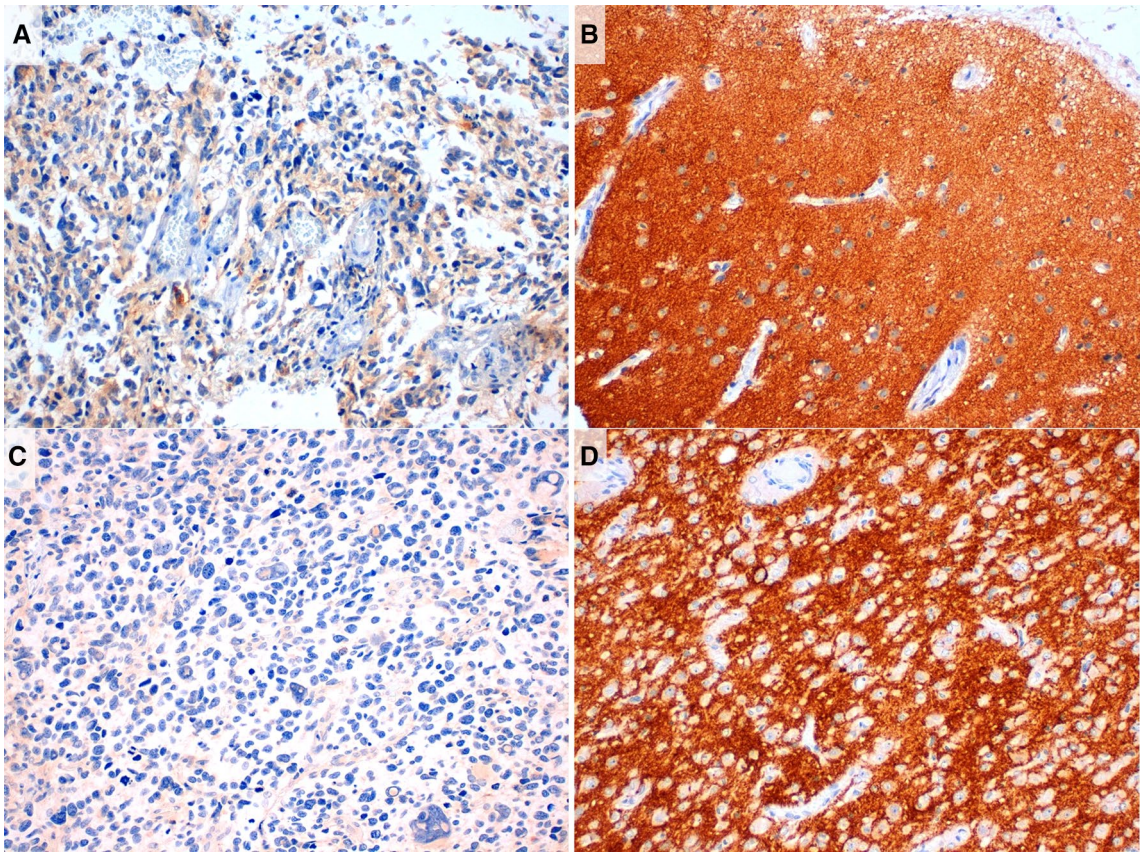

Fig. 1 SV2A immunoreactivity. Glioblastoma with high tumor (IR = 2) (**a**) and peritumor SV2A (IR = 3) expression (**b**), with high staining ratio (SR > 0.5), in a patient without NPAEs; glioblastoma with low tumor (IR = 0) (**c**) and high peritumor SV2A (IR = 3) (**d**) expression, resulting in low staining ratio (SR < 0.5), in a patient with severe NPAEs. Original magnification 200× (**a**–**d**)

Table 1 Characteristics of the study cohort ($n = 18$)

Age	55.3 ± 14.6
Male gender	11 (61.1%)
Histotype, *n* (%)	
Glioblastoma multiforme	8 (44.4%)
Astrocytoma	3 (16.7%)
Oligodendroglioma	6 (33.3%)
Oligoastrocytoma, NOS	1 (5.5%)
Tumor location, *n* (%)	
Frontal	9 (50%)
Non-frontal	9 (50%)
Tumoral SV2A staining intensity	
Absent (0)	7 (38.9%)
Weak (1)	8 (44.4%)
Moderate (2)	2 (11.1%)
Strong (3)	1 (5.6%)
Neuropsychiatric adverse events (NPAEs), *n* (%)	
Absent	6 (33.3%)
Present	12 (66.7%)

required add-on treatment: both had low tumoral SV2A-IR, SR ≤ 0.5, and NPAEs. The small sample size did not allow to detect significant differences regarding LEV effectiveness depending on SV2A tumor expression.

Discussion

LEV efficacy in BTRE depends on tumoral expression of SV2A [2]. Our study shows that also NPAEs depend on tumoral expression of SV2A. Indeed, low tumoral expression of SV2A, as well as low tumoral/peritumoral staining ratio (SR), were associated with occurrence of NPAEs. A SR ≤ 0.5 associates with a 45-fold increase in NPAE occurrence. The fact that such relationship seems so consistent despite a very small sample is worth consideration. In BTRE, LEV frequently causes NPAEs [4]. The mechanisms underlying NPAEs are largely unexplored, and susceptibility to adverse events varies across conditions [7]. In the specific case of LEV for BTRE, given low ligand (SV2A) expression in the tumor, a redistribution of LEV to non-tumoral site might be postulated, with over-binding to healthy neurons.

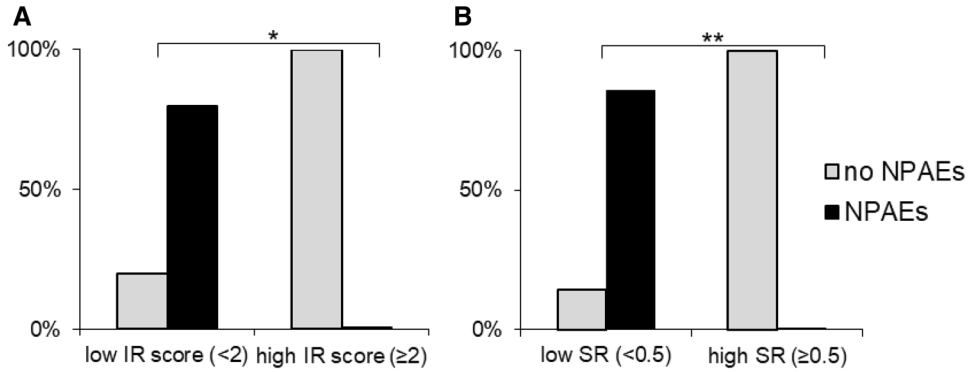

Fig. 2 Neuropsychiatric adverse event (NPAE) occurrence depending on tumor SV2A immunoreactivity (IR) score (**a**) and staining ratio (SV2A tumor IR score/SV2A peritumor IR score) (**b**). *IR* immunoreactivity, *NPAEs* neuropsychiatric adverse events, *SR* staining ratio = tumoral SV2A IR score/peritumoral SV2A IR score. $^{*}p < 0.05$, $^{**}p < 0.01$ (Fisher's exact test)

Such atypical LEV redistribution might participate in NPAE increase. In addition, since low SV2A expression leads to lower LEV efficacy, it is likely that patients with low SV2A expression are prescribed with dosages higher than patients with high SV2A expression. The present study was underpowered to detect differences in LEV dosage, but such hypothesis should be further investigated in longitudinal studies.

Previous reports highlighted that SV2A expression predicted LEV efficacy with 91% accuracy, suggesting that SV2A expression assessment could be implemented to guide treatment choice [2]. Our results demonstrate that, beyond treatment efficacy, SV2A expression also influences NPAEs. Hence, the assessment of SV2A expression might improve the risk/benefit evaluation of LEV prescription for BTRE.

The small sample size is a main limitation to this study. However, deriving the sample from an already reported multicenter study through strict inclusion criteria allowed the selection of a specific population, reinforcing reproducibility. Moreover, given that our findings integrate previous reports on LEV efficacy [2], the hypothesis emerging from this study is worth consideration.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Ethical standards The study was approved by the Internal Advisory Board [3] and complied with the 1964 Declaration of Helsinki and its later amendments.

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