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Investigation of neuronal auto-antibodies in systemic lupus erythematosus patients with epilepsy

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ABSTRACT

Purpose: Epilepsy is an important feature for neuropsychiatric involvement in systemic lupus erythematosus (SLE) with unknown mechanism. Our aim was to investigate the presence of neuronal auto-antibodies (NAbs) in neuropsychiatric SLE (NPSLE).
Methods: Eighteen SLE patients (17 females, 1 male) experiencing recurrent seizures were enrolled to this study. Their clinical characteristics, EEG and MRI findings and follow-up information were evaluated from their files. Antibodies against voltage-gated potassium channel (VGKC)-complex antigens, contactin-associated protein-like 2 (CASPR-2), leucine-rich, glioma inactivated 1 (LGI1), glutamic acid decarboxylase (GAD), *N*-methyl-D-aspartate receptor (NMDA-R), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA-R) and type B gamma aminobutyric acid receptors ($GABA_B$-R) were screened in the sera of these patients. Moreover, indirect immunohistochemistry and immunocytochemistry tests were performed to reveal neuropil antibodies.
Results: Six out of 18 patients (33.3%) had various forms of NAbs. Among them, one patient had antibodies against GAD, one patient with hippocampal sclerosis on MRI was CASPR-2 antibody positive, whereas the remaining four patients showed hippocampal neuropil staining. We could not find a significant difference between seropositive and seronegative groups, regarding the clinical characteristics, EEG and MRI findings.
Conclusion: This study is the first to show hippocampal neuronal staining (4/18) reflecting antibodies against unknown neuronal cell surface antigens in SLE patients with epilepsy, besides the rare occurrence of GAD and CASPR2 antibodies. Further prospective studies are needed to search for new NAbs and uncover their pathogenic role in SLE associated with epilepsy.

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease affecting multiple organ systems. Central nervous system (CNS) involvement is a major cause of morbidity and mortality in SLE patients (Hanly et al., 2010). Epileptic seizures are among the most serious neuropsychiatric manifestations of SLE and constitute one of the diagnostic criteria for neuropsychiatric SLE (NPSLE) (Liang et al., 1999). They may occur at any time in the course of the disease, possibly by diverse mechanisms. In addition to being secondary to metabolic dysfunction, toxic effects of drugs lowering seizure threshold or stroke, seizures are also attributable to the primary disease activity of SLE (Andrade et al., 2008; Appenzeller et al., 2004). Autoimmunity related prothrombotic states leading to ischemic events or inflammatory mechanisms are considered to play the major role in the pathogenesis of seizures in NPSLE (Sciascia et al., 2014). A huge number of autoantibodies has been described in SLE and there is some preliminary evidence that antiphospholipid (aPL) antibodies, mainly lupus anticoagulant and high titres of anticardiolipin (aCL) IgG antibodies, are associated with epilepsy (Sherer et al., 2004). However, seizures develop only in a subset of patients having antiphospholipid syndrome (APS), whereas aPL-positivity occurs in all cases (Ganor et al., 2005). Therefore, it is not well established whether these antibodies are

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playing a role in the pathogenic mechanism of epilepsy or they are merely epiphenomena (Sciascia et al., 2014; Hanly et al., 2012; Verrot et al., 1997).

Since epilepsy is one of the major symptoms of NPSLE and temporal lobe epilepsy (TLE) is common in patients diagnosed with SLE experiencing seizures (with or without APS), our aim was to investigate the presence of auto-antibodies to hippocampal neurons and neuropils and to specific anti-neuronal antibodies (NAbs) recently found to be associated with epilepsy (Vanli-Yavuz et al., 2016; Ekizoglu et al., 2014). Furthermore, we aimed to disclose the clinical characteristics and prognosis of epilepsy of this subgroup of NPSLE patients according to the presence of NAbs.

2. Methods

Eighteen patients (17 females, 1 male) experiencing recurrent seizures, referred by the rheumatology clinics, with a diagnosis of SLE according to the Systemic Lupus International Collaborating Clinics (SLICC) classification criteria were enrolled in this retrospectively designed study. All had a follow-up duration of at least one year in our epilepsy center. The study protocol was approved by the Local Ethics Committee. The written informed consents were obtained from all patients.

The patients were examined and evaluated by a neurologist in terms of clinical features such as disease duration, age of seizure onset, seizure types and use of antiepileptic and immunomodulatory therapies. Their electroencephalography (EEG) and magnetic resonance imaging (MRI) findings were also reviewed.

Sera were obtained from the patients and age-gender matched 50 healthy individuals to investigate antibodies against voltage-gated potassium channel (VGKC)-complex antigens, contactin-associated protein-like 2 (CASPR-2), leucine-rich, glioma inactivated 1 (LGI1), glutamic acid decarboxylase (GAD), N-methyl-D-aspartate receptor (NMDA-R), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA-R) and type B gamma aminobutyric acid receptors (GABAB-R). A commercially available kit (Euroimmun, Luebeck, Germany) containing HEK293 cells transfected with plasmids containing the NR1/NR2 subunits of the NMDA-R, GluR1/GluR2 subunits of the AMPA-R, CASPR-2, LGI1 and GABAB-R were used for testing these ion channel antibodies. The binding was scored visually on a range from 0 (negative) to 4 (very strong), as described previously (Irani et al., 2010a). For the detection of antibodies to uncharacterized VGKC-complex antigens, a radioimmunoassay (RIA) kit was utilized (normal values <50 pM; RSR, Cardiff, UK). Antibodies in patients' sera were allowed to interact with detergent solubilized VGKC complexes extracted from rabbit brain tissue and complexed with ^{125}I-labeled α-dendrotoxin. After incubation at 2–8 °C overnight, the resulting antigen-antibody complexes were immunoprecipitated by the addition of anti-human IgG. After a second incubation of 1.5 h, assay buffer was added and the samples were centrifuged. Unbound ^{125}I-labelled α-dendrotoxin-VGKC complex was removed from the tubes by aspiration of the supernatant. The level of radioactivity remaining in the tube, which is proportional to the antibody level in the test sample, was measured with a gamma-counter and expressed as pM. GAD antibodies were measured quantitatively by an ELISA kit, as per manufacturer's recommendations (normal values <10 U/ml; Euroimmun, Luebeck, Germany). Sera were incubated with GAD coated onto a microplate and biotin-labeled GAD, consecutively. To detect the bound biotin, a third incubation was performed using enzyme-labeled avidin promoting a color reaction, the intensity of which is proportional to GAD antibody levels. The obtained optical density levels were converted to U/ml values using serial standard concentrations.

Furthermore an indirect immunohistochemistry test was performed to determine serum samples with neuropil antibodies, located in hippocampal molecular layer, the presence of which are suggestive of the positivity of a neuronal surface antibody. Whole rat brain was first treated with 4% paraformaldehyde overnight at 4 °C, then immersed in 40% sucrose overnight at 4 °C, and then snap frozen in liquid nitrogen. Seven micrometer-thick frozen sections were serially incubated with 0.3% H_2O_2 for 20 min, 10% goat serum for 1 h and serum samples (1:200) overnight at 4 °C. They were then incubated with biotinylated goat anti-human IgG (1:2000, Vector Laboratories, Burlingame, CA), and the immunoreactivity developed by serial incubation with avidin–biotin peroxidase (Vector Laboratories) for 1 h and diaminobenzidine (Dalmau et al., 2008).

Antibodies to neuronal surface antigens were detected by using cultured hippocampal neurons of P1 rat pups, as described (Irani et al., 2010b). The cultured neurons were incubated with patients' sera (1:250) for one hour at room temperature, followed by 3% formaldehyde fixation and by incubation with Alexa Fluor 488-conjugated anti-human immunoglobulin (IgG) (Invitrogen, Paisley, UK) for 45 min. Subsequently the cells were permeabilized with 0.3% Triton X-100 in phosphate buffered saline (PBS) for 15 min at room temperature and incubated with mouse monoclonal microtubule associated protein 2 (MAP-2) antibody (Sigma-Aldrich, Dorset, UK) (a marker of axonal and dendritic processes) (1:1000) for one hour at room temperature, followed by incubation with Alexa Fluor 568-conjugated anti-mouse IgG (Invitrogen) (1:1000) for 45 min. Images were photographed under a Zeiss fluorescence microscope with a digital camera using the Zeiss Axiovision software. The immunofluorescence results were assessed by two independent observers, who were blind to patients' identities. Moderate to strong Alexa Fluor 488-conjugated anti-human IgG-induced green color that co-localized with Alexa Fluor 568-conjugated anti-mouse IgG-induced red color was considered as positive.

Descriptive statistics were applied and the characteristics of the two groups of patients with and without serum NAbs were compared with chi-square, Fisher's exact and Mann Whitney *U* test, where appropriate. SPSS 20 software (SPSS Inc, Chicago, IL, USA) was used and the significance level was set at $p < 0.05$.

3. Results

The mean age of the included 18 patients was 46.8 ± 13 years and the average age of seizure onset was 31.6 ± 15.3 years. Eight patients (44.5%) presented with epilepsy and diagnosed thereafter with SLE (4.48 ± 0.97 years later), whereas 9 patients (50%) had the diagnosis of SLE before seizure onset and only one patient (5.5%) was diagnosed with concurrent SLE and seizure disorder. Three patients had only generalized convulsions (16.7%), one patient (5.5%) experienced only focal seizures evolving to bilateral convulsive seizures and the remaining 14 patients (77.8%) had focal seizures with or without loss of consciousness and evolution to bilateral convulsive seizures. Status epilepticus was not observed in any of our patients.

Thirteen patients (72.2%) were taking antiepileptic drugs (AEDs) such as levetiracetam, carbamazepine, oxcarbazepine, valproic acid and topiramate; 9 of them were under monotherapy during serum sampling. Overall 17 patients (94.5%) had history of using immune-modulating drugs such as methylprednisolone, azathioprine, cyclosporine, intravenous immunoglobulin. The prognosis of epilepsy was classified as benign (less than 1 seizure per year), moderate (more than 1 per year but less than 1 per month) or severe (more than 1 per month) regarding to the seizure frequency. Accordingly, 7 patients (39%) had benign, 9 (50%) patients had moderate and 2 (11%) patients had severe epilepsy prognosis.