Seizure 48 (2017) 79–88

Contents lists available at ScienceDirect

Seizure

journal homepage: www.elsevier.com/locate/yseiz

Up-regulated BAFF and BAFF receptor expression in patients with intractable temporal lobe epilepsy and a pilocarpine-induced epilepsy rat model

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ARTICLE INFO

Article history:
Received 11 November 2016
Received in revised form 23 March 2017
Accepted 25 March 2017
Available online xxx

Keywords:
BAFF
BAFFR
Temporal lobe epilepsy
Immune and inflammatory reaction
NF-κB pathway

ABSTRACT

Purpose: Some studies have suggested that BAFF and BAFFR are highly expressed in the central nervous system (CNS) and participate in inflammatory and immune associated diseases. However, whether BAFF and BAFFR are involved in the pathogenesis of epilepsy remains unknown. This study aimed to investigate the expression of BAFF and BAFFR proteins in the brains of patients with temporal lobe epilepsy (TLE) and in a pilocarpine-induced rat model of TLE to identify possible roles of the BAFF–BAFFR signaling pathway in epileptogenesis.
Methods: Real-time quantitative polymerase chain reaction (RT-qPCR), western blot, immunohistochemistry, and double-immunofluorescence were performed in this study.
Results: The results showed that BAFF and BAFFR expression levels were markedly up-regulated in intractable TLE patients and TLE rats. Moreover, BAFF and BAFFR proteins mainly highly expressed in the membranes and cytoplasms of the dendritic marker MAP2 in the cortex and hippocampus.
Conclusion: Therefore, the significant increased in BAFF and BAFFR protein expression in both TLE patients and rats suggest that BAFF and BAFFR may play important roles in regulating the pathogenesis of epilepsy.

1. Introduction

Epilepsy is the most common serious neurological disease. Although the majority of patients suffering from epilepsy are well-managed with anti-epileptic drugs (AEDs), approximately 30% of people affected by epilepsy still have recurrent seizures and are drug resistant, which can result in a progression to intractable epilepsy (IE) [1–4]. Patients with intractable temporal lobe epilepsy are usually excellent candidates for epilepsy surgery, which is efficacious in up to 70% of cases [5]. IE can give rise to serious clinical problems. Therefore, there is an urgent need for more effective therapeutic strategies to address intractable epilepsy, which is mainly represented by temporal lobe epilepsy (TLE) [4]. The roles of immune processes in many central nervous system (CNS) diseases, such as CNS vasculitis, multiple sclerosis, and acute disseminated encephalomyelitis, have been widely investigated [1–3]. In recent years, an increasing body of clinical and experimental evidence has strongly supported the hypothesis that immune processes within the brain might participate in the pathophysiology of seizures and epilepsy, and some studies have reported the existence of immunological alterations in patients with epilepsy [3,6–8]. Furthermore, immune-modulating treatments, including corticosteroids and adrenocorticotropic hormone, have been proven successful in treating specific epilepsy syndromes and epileptic encephalopathies, such as Lennox Gastaut syndrome (LGS), Landau-Kleffner syndrome (LKS) and acute symptomatic seizures complicating bacterial and viral meningitis/meningoencephalitis, that are resistant to conventional anticonvulsants and anti-epileptic drugs (AEDs), supporting the involvement of the immune system in epilepsy [9–11]. Adrenocorticotropic hormone (ACTH) and corticosteroids are also the first-line treatment options for epilepsies during early childhood in the majority of cases [12,13]. Furthermore, the discovery that pro-inflammatory cytokines are produced in response to seizures in patients with TLE implies that there may be a connection between inflammation and the pathogenesis of epilepsy [14–16]. In particular, pro-inflammatory cytokines (interleukin IL-1, IL-6, and tumor necrosis factor), growth factors, and neurotrophins

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http://dx.doi.org/10.1016/j.seizure.2017.03.016
1059-1311/

were increased in the hippocampi and cortices of epileptic rats. Moreover, recent studies have indicated that growth factors and cytokines can regulate the downstream events that follow seizures [17,18].

B cell-activating factor (BAFF), also known as B lymphocyte stimulator (BLyS), is a highly conserved member of the tumor necrosis factor superfamily of cytokines and is mainly expressed by innate immune cells, including monocytes, macrophages, dendritic cells, and other non-hematopoietic cells [19,20]. BAFF selectively stimulates B lymphocyte proliferation and immunoglobulin production, which could consequently modulate B cell function [21]. The physiological activity of BAFF is reportedly mediated through the three following receptors: B cell-activating factor receptor (BAFFR), transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI), and B cell maturation antigen (BCMA) [20,22–24]. Among these receptors, BAFFR is the predominant BAFF receptor expressed on peripheral B cells in both humans and mice. In humans, the BAFF–BAFFR interaction is critical to support neural cell survival and is effective in T cell co-stimulation [20,21,25]. Furthermore, BAFFR can modulate B cell survival and differentiation, including activation of the nuclear factor-kappa B (NF-κB) pathway [26,27]. Therefore, BAFF and BAFFR regulate CNS activity mainly by promoting immune and inflammatory reactions. In humans, BAFF serum levels are significantly increased in patients with systemic lupus erythematosus, Sjögren's syndrome and rheumatoid arthritis compared to those in healthy controls [3,28,29]. Although BAFF and BAFFR are involved in a variety of CNS inflammatory diseases, BAFF and BAFFR have not been assessed in studies of epilepsy to date.

In our present study, we investigated BAFF and BAFFR expression in the temporal neocortex of patients with TLE. In addition, BAFF and BAFFR expression levels in the hippocampi and adjacent cortices of rats in a model of TLE were investigated using real-time quantitative polymerase chain reaction (RT-qPCR), double immunofluorescence, immunohistochemistry and Western blot analyses.

2. Materials and methods

2.1. Human brain tissue and clinical data

Twenty-four patients undergoing surgery for medically intractable TLE and eight patients undergoing non-epilepsy-related surgery were randomly selected from our epilepsy brain bank, which includes 300 patients with intractable TLE. The study protocol complied with the guidelines for research administration involving human subjects as proposed by the Code of Ethics of the World Medical Association and the Committee on Human Research of Chongqing Medical University. Prior to surgery, brain magnetic resonance imaging (MRI), video-electroencephalogram (EEG), sphenoidal electrode monitoring, and intraoperative electrocorticography were used to locate the epileptogenic zones. All patients were refractory to combination therapy with at least three or more AEDs, including valproic acid, phenytoin, phenobarbital, carbamazepine, lamotrigine, or topiramate. The diagnostic criteria for intractable TLE were based on the 1981 International Classification of Epilepsy Seizures from the International League Against Epilepsy (ILAE), and the patients had typical clinical presentations and characteristic EEGs. Table 1 summarizes the clinical features of the patients. After temporal neocortex lesion resection, electrodes for intraoperative electrocorticography were placed on the remaining edge of the tissue to ensure that the lesion had been

Table 1
Clinical features of the patients with TLE.

Subjects	Sex (M/F)	Age (years)	Course (years)	Seizure type	AEDS	Resected tissue	Pathology
P1	M	20	13	SPS,CPS	LEV,OXC,CBZ	TNL	NL,ND
P2	F	32	21	SGS	VPA,PB,CBZ	TNL	G,ND
P3	M	28	20	GTCS	VPA,CBZ,CZP	TNR	G,NL
P4	M	34	13	CPS	VPA, CZP,LEV	TNR	G,ND
P5	F	14	10	CPS,SGS	TPM,OXC,VPA	TNR	NL
P6	M	17	12	SPS,SGS	OXC,CPZ,CBZ	TNL	G,NL
P7	F	23	19	CPS	VPA,CBZ,TPM	TNR	G,ND
P8	M	12	9	GTCS	VPA,PB,CBZ,PHT	TNL	G,NL
P9	F	31	20	SGS	VPA,PHT,LTG	TNR	G,NL
P10	F	27	7	SPS	LTG,CBZ,OXC	TNL	G,ND
P11	M	17	10	CPS	VPA,TPM,CBZ	TNR	G,NL
P12	M	14	10	SGS	CBZ,VPA,TPM	TNR	G,NL
P13	M	19	14	GTCS	VBZ,TPM,CZP	TNR	G,NL
P14	F	9	4	CGS,SGS	LEV, LTG,PB	TNL	NL,ND
P15	F	21	17	SPS	CBZ,VPA,TPM	TNR	G,NL
P16	M	36	25	SPS	VPA,CBZ,CZP	TNL	G,NG
P17	F	33	27	SPS,SGS	CBZ,VPA,TPM	TNR	G,NL
P18	M	24	18	CPS	CBZ,VPA,TPM,LTG	TNL	G,NL
P19	F	15	11	SGS	PB,CBZ,PHT,LTG	TNL	G,NL,ND
P20	M	8	3	CPS	VPA,PB,CBZ,LTG	TNR	G
P21	M	15	6	GTCS	VPA,PB,TPM	TNR	G,NL
P22	F	13	8	SPS,SGS	VPA,TPM,CBZ	TNL	G,NL
P23	F	10	5	SGS	LEV,CBZ,CPZ	TNR	G,NL
P24	F	27	20	CPS	PHT,PB,CBZ,VPA	TNL	G,NL,ND
C1	M	23	0	None	None	TNL	N
C2	F	17	0	None	None	TNR	N
C3	M	29	0	None	None	TNL	N
C4	M	32	0	None	None	TNR	N
C5	F	34	0	None	None	TNL	N
C6	M	19	0	None	None	TNR	N
C7	F	22	0	None	None	TNL	N
C8	F	37	0	None	None	TNR	N

Sex: F, female; M, male; CPS, complex partial seizure; GTCS, generalized tonic-clonic seizure; SGS, secondarily generalized seizure; SPS, simplex partial seizure. AEDs, antiepileptic drugs; CBZ, carbamazepine; CLB, clonazepam; GBP, gabapentin; LTG, lamotrigine; OXC, oxcarbazepine; PB, phenobarbital; PHT, phenytoin; TPM, topiramate; VPA, valproic acid. Resected tissue: TNL, left temporal neocortex; TNR, right temporal neocortex. Pathology: NL, neuronal loss; ND, neuronal degeneration; G, gliosis.