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Laboratory studies

Higher expression of monocyte chemoattractant protein 1 and its receptor in brain tissue of intractable epilepsy patients

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ABSTRACT

We aimed to explore the pathogenesis of monocyte chemoattractant protein-1 (MCP1) and CC chemokine receptor 2 (CCR2) in brain tissue of patients with intractable epilepsy (IE). Hippocampi or temporal lobe tissues were obtained from 40 patients with IE and five patients without IE who had undergone surgical decompression and debridement. The levels of MCP1 and CCR2 were evaluated using immunohistochemistry. Pearson correlation analysis was employed to evaluate the correlation between levels of MCP1 and CCR2 in IE with or without hippocampal sclerosis (HS) and the disease duration, along with age. Higher levels of MCP1 (11.68 ± 4.68% *versus* 1.72 ± 1.54%) and CCR2 (11.54 ± 4.65% *versus* 1.52 ± 1.29%; $P < 0.05$) were observed in IE patients compared to controls. Expression levels of MCP1 (R = 0.867) and CCR2 (R = 0.835) in IE patients with HS were correlated with the disease duration. However, no correlation was found in IE patients without HS. There was also no correlation between levels of MCP1 and CCR2 in IE patients with age, either with HS or without HS. These results suggest that MCP1 and its receptor may play a role in the pathogenesis and progression of IE.

1. Introduction

Epilepsy is a common disease with serious health ramifications. It is characterized by spontaneous recurrent seizures owing to hyperexcitability and hypersynchrony of brain neurons [1]. Epilepsy often requires long-term drug treatment and affects both an individual's family and society. Despite the availability of a large number of antiepileptic drugs, approximately 30% of epilepsy patients have drug resistance or serious adverse reactions [2]. Mortality is higher in patients with epilepsy than in the general population, especially the risk of sudden unexpected death in epilepsy in intractable epilepsy (IE) [3–6]. The molecular mechanisms responsible for this disease remain elusive [7,8].

Currently, accumulating evidence suggests that both the central nervous system and the immune system may be involved in epilepsy [9,10]. The pathological mechanisms of cytokines in epilepsy have gained increasing attention [11–16]. Studies have indicated that epileptic seizures could produce cytokines, and these cytokines can lead to autoimmune-mediated damage of brain cells and intracellular calcium overload in neurons and glial cells, which may in turn cause epilepsy [11,17,18]. Somera-Molina et al. [19] confirmed that proinflammatory cytokine production and microglial activation was increased after kainic acid-induced seizures. Xu et al. [20] demonstrated that monocyte chemotactic protein-1 (MCP-1, also known as CC motif chemokine 2 [CCL2]), CC motif chemokine receptor 2 (CCR2) and CCR2A were involved in pilocarpine-induced status epilepticus in mouse hippocampi. Kan et al. [21] suggested that the CCL4 expression was increased concomitant with a persistent up-regulation of CCR5 in rat hippocampus after status epilepticus (SE). SE is characterized by continual seizure activity that can vary widely in the intensity of convulsions [22]. All these findings highlight the fact that a variety of chemokines and receptors have a role in the epileptogenic process.

Notably, the cytokine MCP1 and its receptor CCR2 have been shown to be important components in the pathophysiology of epilepsy [23–25]. MCP1 and CCR2 expression are observed in multiple brain regions including the hippocampus [26,27]. Hung et al. [28] found that the MCP1 gene was significantly up-regulated in the dentate gyrus after SE in rats. The interaction of MCP1 and CCR2 mediates monocyte infiltration in neuroinflammation and was implicated in epileptogenesis in a rat experimental model [29].

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Despite such progress, only limited clinical data on the role of MCP1 and CCR2 expression in the human epileptic brain is available.

Wu et al. [30] demonstrated that MCP1 expression in the brain tissue of patients with IE was altered and played an important role in the pathogenesis of IE. MCP1 and CCR2 expression in human brain tissue was investigated in the present study. In addition, the correlation between levels of MCP1 and CCR2 with the disease duration was investigated depending on the presence or absence of hippocampal sclerosis (HS), along with correlations between levels of MCP1 and CCR2 with patient age. HS is a neuropathological condition characterized by severe neuronal cell loss and gliosis in the hippocampus [31]. HS has several pathological subtypes in patients with temporal lobe epilepsy, and changes of neuronal loss and gliosis in the amygdala virtually never occur in the absence of HS [32]. The results of our study may contribute to the identification and characterization of the mechanism(s) responsible for this challenging disease, as well as the prevention and treatment of epilepsy.

2. Material and methods

2.1. Patient selection and tissue collection

Between December 2006 and June 2010, 31 hippocampal tissues and nine temporal lobe tissues were obtained from 40 patients with IE. The disease was diagnosed at the First Affiliated Hospital of Harbin Medical University, China, according to the International League Against Epilepsy criteria for presurgical evaluation [33] (Table 1). Three hippocampal tissue samples and two temporal lobe tissue samples obtained from five patients (four males, one female, aged 16–35 years) were used as the control group. These patients underwent decompression and debridement due to cerebral hemorrhage or severe brain trauma, but had no history of epilepsy, familial genetic disease, or other neurological disorders. This study was approved by the ethics committee of the First Affiliated Hospital of Harbin Medical University, and written informed consent was obtained from each patient for the use of their brain tissue for research.

All patients were assessed using comprehensive epilepsy testing, including clinical characterization, video-electroencephalogram telemetry, and psychiatric and neuropsychological assessments. HS was present if a visual inspection of MRI revealed both hippocampal atrophy and T2 signal abnormalities. To further confirm HS, an experienced neuropathologist microscopically examined specimens for characteristic patterns of neuronal loss. Patients with bilateral HS or dual pathology (for example, HS accompanied by other epileptogenic lesions) were excluded. Twelve of the patients in this study were diagnosed with HS.

Table 1
Clinical data for the patients with intractable epilepsy included in this study

Patient	Age (years)	Sex	Disease duration (years)	Epileptic seizure type	Excision region
1	41	M	22	SPS, GTCS	HIP
2	16	M	15	SGS	HIP
3	32	M	17	SGS	HIP
4	12	M	7	CPS	HIP
5	15	F	12	SGS	HIP
6	20	F	20	SGS	HIP
7	18	M	17	CPS	HIP
8	20	M	6	SPS, GTCS, TS	HIP
9	23	F	22	SGS	HIP
10	28	F	13	SGS	HIP
11	14	M	3	CPS	HIP
12	9	M	9	CPS, TS	HIP
13	22	M	6	SGS	HIP
14	12	M	12	SGS, GTCS	HIP
15	25	M	9	SGS	HIP
16	24	F	11	SGS	HIP
17	20	M	13	CPS	HIP
18	16	F	15	SGS	HIP
19	46	F	19	SGS	HIP
20	16	M	5	SGS	HIP
21	18	F	8	SGS	HIP
22	20	F	6	SGS	HIP
23	22	F	10	CPS	HIP
24	16	M	8	SGS, GTCS	HIP
25	17	F	16	CPS	HIP
26	28	M	25	CPS	HIP
27	19	M	1	CPS	HIP
28	40	M	21	SGS	HIP
29	21	F	4	SGS	HIP
30	14	F	12	CPS	HIP
31	20	F	16	SGS, GTCS	HIP
32	26	M	7	TS	TL
33	18	F	5	SGS	TL
34	8	M	8	SGS	TL
35	37	M	4	CPS	TL
36	16	F	10	CPS	TL
37	33	M	32	SGS	TL
38	24	F	9	SGS	TL
39	57	F	5	CPS	TL
40	37	M	7	CPS	TL

CPS = complex partial seizure, F = female, GTCS = generalized tonic-clonic seizure, HIP = hippocampi, IE = intractable epilepsy, M = male, SGS = second generalized seizure, SPS = simple partial seizure, TL = temporal lobe, TS = tonic seizure.

2.2. Histology

The resected tissues were fixed in 10% paraformaldehyde for 24 hours, and embedded in paraffin. The fixed tissues were prepared for serial cross-sections (4 μm thick). Hematoxylin and eosin staining was performed to observe the morphologic changes of these tissues with a light microscope (Nikon, Tokyo, Japan).

2.3. Immunohistochemistry

Tissue slices (3 μm) were washed three times in 0.1 M phosphate buffered saline (PBS; 10 minutes for each wash), and endogenous peroxidase activity was blocked with 3% H_2O_2. Tissue sections were stained simultaneously to minimize variation in the immunohistochemical reactions. Sections were incubated with polyclonal rabbit antibodies against human MCP-1 (ab9669; 1:100; Abcam, Cambridge, UK) and CCR2 (ab21667, 1:200; Abcam) overnight at 4°C. After washing three times in 0.1 M PBS for 5 minutes, sections were incubated with secondary antibodies (goat anti-rabbit immunoglobulin G, 1:2000; Vector Laboratories, Burlingame, C(A) USA). Then the immunoperoxidase complexes were visualized using 0.5% 3,3′-diaminobenzidine hydrochloride (DAB; Sigma, St. Louis, MO, USA) diluted in PBS. For the control group, the primary antibody was omitted and 0.1 M PBS was substituted.

Positive staining was indicated by the presence of brown particles seen using a microscope (Leica DM-1000; Leica Microsystems, Germany) and stained cells were counted in five randomly selected fields of vision (original magnification × 400). The mean number of cells expressing MCP1 and CCR2 was recorded. Immunohistochemical staining results were subsequently interpreted independently by two pathologists who were blinded to the clinical parameters of the individual cases. The semi-quantitative scoring method described by McCarthy et al. [34] was used to determine positive MCP1 and CCR2 staining. Briefly, scoring for MCP1 and CCR2 staining was determined by dividing the number of positively-stained cells by the total number of cells and then multiplying by 100, giving a percentage estimate of positive cells.