Journal of Clinical Neuroscience 20 (2013) 1564–1570

Contents lists available at SciVerse ScienceDirect

Journal of Clinical Neuroscience

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

Clinical Study

Expression analysis and clinical correlation of aquaporin 1 and 4 genes in human hippocampal sclerosis

N. Bebek [a,b,*], Ö. Özdemir [b], M. Sayitoglu [b], Ö. Hatırnaz [b], B. Baykan [a,b], C. Gürses [a], A. Sencer [c], A. Karasu [c,1], E. Tüzün [a,b], I. Üzün [d,e], S. Akat [d], N. Cine [f], G. Sargin Kurt [a], M. Imer [c], U. Ozbek [b], A. Canbolat [c], A. Gökyigit [a]

[a] Neurology Department, Istanbul Faculty of Medicine, Millet cad., 34390 Capa, Istanbul, Turkey
[b] Institute for Experimental Medicine, Istanbul University, Istanbul, Turkey
[c] Neurosurgery Department, Istanbul University, Istanbul, Turkey
[d] Council of Forensic Medicine, Ministry of Justice, Istanbul, Turkey
[e] Forensic Medicine Department, Faculty of Medicine, Mediterranean University, Antalya, Turkey
[f] Genetics Department, Kocaeli Medical Faculty, Kocaeli, Turkey

ARTICLE INFO

Article history:
Received 2 July 2012
Accepted 2 December 2012

Keywords:
Aquaporins
Epilepsy
Epileptogenesis
Gene expression
Human hippocampus
Mesial temporal sclerosis

ABSTRACT

Mesial temporal sclerosis (MTS) is the most frequent cause of drug resistant symptomatic partial epilepsy. The mechanism and genetic background of this unique pathology are not well understood. Aquaporins (AQP) are regulators of water homeostasis in the brain and are expressed in the human hippocampus. We explored the role of AQP genes in the pathogenetic mechanisms of MTS through an evaluation of gene expression in surgically removed human brain tissue. We analyzed *AQP1* and *4* mRNA levels by quantitative real-time polymerase chain reaction and normalized to *ABL* and *cyclophilin* genes, followed by immunohistochemistry for *AQP4*. Relative expressions were calculated according to the delta Ct method and the results were compared using the Mann-Whitney U test. Brain specimens of 23 patients with epilepsy who had undergone surgery for MTS and seven control autopsy specimens were investigated. Clinical findings were concordant with previous studies and 61% of the patients were seizure-free in the postoperative period. *AQP1* and *4* gene expression levels did not differ between MTS patients and control groups. Immunofluorescence analysis of *AQP4* supported the expression results, showing no difference. Previous studies have reported contradictory results about the expression levels of *AQP* in MTS. To our knowledge, only one study has suggested upregulation whereas the other indicated downregulation of perivascular *AQP4*. Our study did not support these findings and may rule out the involvement of AQP in human MTS.

1. Introduction

Mesial temporal sclerosis (MTS), also known as hippocampal sclerosis (HS), is the most frequent underlying pathology in drug resistant epilepsy. The epileptogenic focus mainly involves the hippocampal formation, which shows typical neuropathological features.[1] The anatomical and physiological structure of the hippocampus, particularly the large pyramidal cells in the CA1 region, is sensitive to external factors such as hypoxia during development. Therefore, early childhood risk factors causing neuronal damage are thought to initiate the process of epileptogenesis possibly in association with genetic factors.[2,3] A significant relationship between complicated febrile seizures (FS) and MTS has been reported.[4,5] A number of molecular studies on human brain tissue in MTS have been performed[6,7] with many gene expression studies carried out in animal models and using human biopsy specimens.[8,9] However, the mechanism and genetic background of this unique pathology is currently not elucidated.

Aquaporins (AQP), thought to be the regulators of water homeostasis in the brain, are expressed in the human hippocampus. They are transmembrane molecules and facilitate the movement of water across cellular compartments.[10] *AQP4* normally displays a higher distribution on the perivascular end feet membranes of astrocytes, and a significant increase in perivascular *AQP4* has been observed in the CA1 region of sclerotic hippocampi,[11] but the nature of this change – either genetic or acquired – is not known. It is well-known that *AQP4* is important in the complex pathways of water and ion homeostasis. It has been hypothesized that *AQP4* increases excitability by potassium (K^+) buffer modification, interleukin secretion enhancement, and calcium-induced release of

* Corresponding author. Tel.: +90 212 53 43 93; fax: +90 212 533 43 93.
E-mail address: nersesb@yahoo.com (N. Bebek).

[1] In memory of Dr. Aykut Karasu, a dear and invaluable colleague who was dedicated to epilepsy surgery. He oriented us to investigate the role of AQPs in hippocampal sclerosis. Unfortunately, he passed away before the termination of the project.

0967-5868/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jocn.2012.12.023

glutamate.[12,13] *AQP4* is expressed by astrocytes in the sclerotic CA1, and is furthermore positively correlated with glial fibrillary acidic protein. Moreover, an increased and highly differentiated distribution pattern of *AQP4* is associated with changes in the expression of dystrophin and dystrophin associated protein complex genes. Astrocytes in the sclerotic hippocampus may directly affect excitability by alteration of water homeostasis and K^+ buffering due to the redistribution of *AQP4* transporters.[14] In MTS, the T2-weighted MRI diffusion coefficient of the hippocampus is high which is thought to be due to the presence of increased water flow in the hippocampus.[11] Therefore we aimed to investigate the expression levels of aquaporins in the mesial temporal lobe tissue of patients with drug resistant epilepsy.

AQP4 is the predominant water channel in the brain and is concentrated in astrocytic foot processes at the blood–brain barrier.[12,15] Defects in other aquaporin genes are known to be related to some human diseases.[16] Aquaporin-1 (*AQP1*), also known as channel-forming integral membrane protein, was the first protein shown to function as a molecular water channel. *AQP1* is abundant in the choroid plexus. Scarcity or absence of *AQP1* has been reported in a few patients, resulting in restricted ability to concentrate the urine.[17] *AQP2* and *3* are most important in kidney function. Autoimmunity against *AQP4* is found in Devic's disease. A better understanding of these molecules and possible manipulation of their structures could help prevent brain edema due to brain tumor or infarction.[18]

In the field of epilepsy, information about the effects of *AQP* is limited. One study showed *AQP1* and *AQP4* expression in astrocytes located in the hippocampal tissues, but only *AQP4* expression was found to be increased in perivascular astrocytes.[11] Conversely, Eid et al. showed a loss of perivascular *AQP4* in epilepsy patients.[16] Jamali et al. reported elevated *AQP1* in microarray studies on hippocampal tissues, but these findings were not verified in an expression analysis.[6]

Our aim was to explore the role of the relevant *AQP* genes, namely *AQP1* and *4*, in the pathogenetic mechanisms of MTS through evaluation of the gene expression in surgically removed human brain tissue.

2. Material and methods

Brain tissue was surgically removed from 23 patients with epilepsy who had undergone surgery due to MTS, and from seven control autopsy specimens.

2.1. Brain tissue samples

2.1.1. Mesial temporal sclerosis samples

All patients were investigated presurgically using a detailed standard protocol including seizure semeiology, neurological examination, neuropsychological testing, neuroimaging, and long term non-invasive video-electroencephalogram (EEG) monitoring. All patients met the criteria for drug resistant mesial temporal lobe epilepsy.[19–21] The study was approved by the local Ethics Committee. All patients were informed about the procedure and written informed consent was obtained. Twenty-three patients with concordant findings in their presurgical evaluation underwent selective amigdalohippocampectomy (SAH) with or without anterior temporal lobectomy (ATL), carried out by an experienced neurosurgeon, and hippocampal tissue was removed (Table 1). A limited number of temporal polectomies were performed, just enough to identify the dura of the middle fossa and gain access to the temporal horn. During resection, biopsies were taken from the head and body of the hippocampus for examination as described previously.[22,23]

2.1.2. Control hippocampal samples

The control group was comprised of autopsy samples. Autopsies were performed within 24 hours of death. Only one of the autopsy controls was female and the average age was 50.1 ± standard deviation (SD) 19.3 (range, 21–73 years). The patients had no history of brain disease and suffered from sudden death without associated brain damage. The cause of death was cardiovascular disease in three patients, with penetrating injury, intoxication, hanging, and embolism due to fracture of the femur the causes in the remaining four patients. One additional patient who had died at age 1 due to gastroenteritis was excluded for better uniformity of age. Entire hippocampi were removed at autopsy from seven patients in association with The Council of Forensic Medicine. This part of the study was approved by The Education and Research Commission of The Council of Forensic Medicine.

All surgical and autopsy tissue samples were frozen immediately after removal in liquid nitrogen and stored at −80 °C.

2.1.3. RNA isolation and cDNA synthesis

The hippocampal tissues were homogenized in QIAzol Lysis Reagent (Qiagen, Venlo, Netherlands). Total RNA was isolated by RNeasy Lipid Tissue Kit (Qiagen). RNA samples were treated using DNAse (1 U/μg). RNA quality and quantity were checked with Nanodrop 1000 (Thermo Fisher Scientific, Waltham, MA, USA). 1 μg of total RNA was used for cDNA synthesis using random hexamers (Roche Diagnostics, Mannheim, Germany) and Moloney Murine Leukemia Virus reverse transcriptase (Thermo Fisher Scientific) according to the protocol of the manufacturer.

2.2. Analysis of gene expression by real time quantitative reverse transcription polymerase chain reaction

We analyzed *AQP1* and *4* besides two reference genes (*ABL* and *cyclophilin [CYBP]*) by real time quantitative reverse transcription polymerase chain reaction (PCR) which was carried out on the Light Cycler Instrument 480, with the LightCyler 480 Fast Start SYBR Green I Master Kit (Roche Diagnostics). The PCR conditions were prepared according to the instructions of the manufacturer; 5 pmol of primers and 200 ng of cDNA were used in each run and each sample was studied in duplicate. The specificity of the product amplification was confirmed by melting curve analyses and agarose gel electrophoresis. The PCR program was as follows: initial denaturation at 95 °C for 7 minutes, amplification for 5 s at 95 °C, 10 s at 56–60 °C and 10 s at 72 °C for 45 cycles, and melting curve for 15 s at 60 °C for one cycle. The suitable reference genes were selected by GeNorm version 3.4 (GeNorm, Amel Ardennes, Belgium), as described by Vandesompele et al.[24] The two most stable genes were selected (*ABL* and *CYBP*) for normalization. Relative expressions were calculated according to the delta Ct method, based on the mathematical model described by Livak et al.[25] REST 2005 (Beta V1.9.9, Corbett Life Science, Germany) and the Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) software were used.[26]

Relative expression results of control and MTS tissues were compared using the Mann-Whitney U (MWU) test. The association between expression values and clinical variables (such as sex, age, age at seizure onset, FS, seizure frequency, age at surgery, disease duration from onset to surgery, and outcome) were also evaluated using the MWU test.[6,25] A p value of <0.05 was regarded as statistically significant.

2.3. Immunofluorescence for aquaporin 4 expression

Five paraffin-embedded sections were obtained from both the MTS patients (n = 5) and the autopsy specimens (n = 5). The samples were deparaffinized and the antigens retrieved, as described