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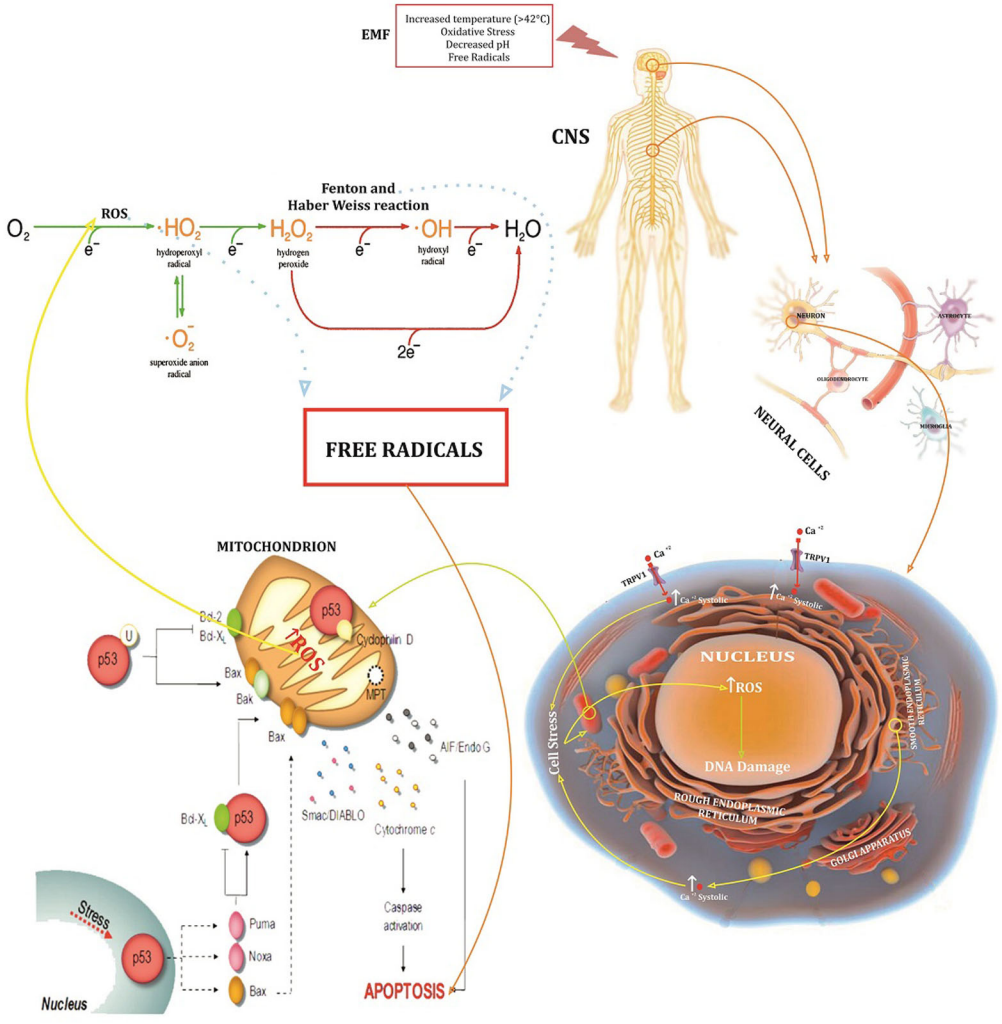

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AIM AND SCOPES
Journal of Cellular Neuroscience and Oxidative Stress is an online journal that publishes original research articles, reviews and short reviews on the molecular basis of biophysical, physiological and pharmacological processes that regulate cellular function, and the control or alteration of these processes by the action of receptors, neurotransmitters, second messengers, cation, anions, drugs or disease.

Areas of particular interest are four topics. They are;

A- Ion Channels (Na^+- K^+ Channels, Cl^- channels, Ca^{2+} channels, ADP-Ribose and metabolism of NAD^+, Patch-Clamp applications)

B- Oxidative Stress (Antioxidant vitamins, antioxidant enzymes, metabolism of nitric oxide, oxidative stress, biophysics, biochemistry and physiology of free oxygen radicals)

C- Interaction Between Oxidative Stress and Ion Channels in Neuroscience
(Effects of the oxidative stress on the activation of the voltage sensitive cation channels, effect of ADP-Ribose and NAD^+ on activation of the cation channels which are sensitive to voltage, effect of the oxidative stress on activation of the TRP channels in neurodegenerative diseases such Parkinson's and Alzheimer's diseases)

D- Gene and Oxidative Stress
(Gene abnormalities. Interaction between gene and free radicals. Gene anomalies and iron. Role of radiation and cancer on gene polymorphism)

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Biophysics	Biochemistry
Biology	Biomedical Engineering
Pharmacology	PhysiologyGenetics
Cardiology	Neurology
Oncology	Psychiatry
Neuroscience	Neuropharmacology

Keywords
Ion channels, cell biochemistry, biophysics, calcium signaling, cellular function, cellular physiology, metabolism, apoptosis, lipid peroxidation, nitric oxide, ageing, antioxidants, neuropathy, traumatic brain injury, pain, spinal cord injury, Alzheimer's Disease, Parkinson's Disease.

RESEARCH ARTICLE

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Expression of HIF-1α, TFRC-1, and TIM-2 relative mRNA levels in PTZ-kindling model of epilepsy

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List of Abbreviations ;

CNS, *Central nervous system;* ***HIF,*** *Hypoxia inducible factor;* ***I.P.****, Intraperitoneally****;* ***mRNA****, Messenger ribonucleic acid;* ***PTZ****, Pentylenetetrazol;* ***qPCR****, Quantitative polymerase chain reaction;* ***RNA****, Ribonucleic acid;* ***RS****, Racine scoring;* ***RT-PCR****, Real time polymerase chain reaction;* ***Tfr-1****, Transferrin-1 receptor;* ***TFRC****, Transferrin receptor gene;* ***TIM****, T cell immunoglobulin and mucin region.*

Abstract

Epilepsy is a neurological disease characterized by abnormal electrical activity and recurrent seizures in the central nervous system (CNS). Changes in hypoxia and iron metabolism can stimulate seizures through the CNS and cardiac system. Cardiovascular system disorders such as arrhythmias also accompany this process. It was aimed to examine the genes of TFRC-1 and TIM-2, which contribute to ion homeostasis by providing hypoxia inducible factor (HIF) -1 and intracellular iron flow, through the kindled model of pentylenetetrazol (PTZ). HIF-1α, TFRC-1 and TIM-2 gene expressions were investigated in both brain and heart tissue by RT-PCR method. As a result of the data, TIM-2 expression significantly decreased in the brain ($p<0.01$) and cardiac ventricle tissue ($p<0.05$) in female rats. TFRC-1 gene expression decreased in female brain tissue ($p<0.05$). Our findings suggest that TFRC-1 and TIM-2 gene

modulation may have therapeutic potential in epilepsy patients. In addition, TFRC-1 and TIM-2 genes may contribute to ferroptosis and oxidative stress mechanisms known to be associated with the seizure process by regulating iron transfer into the cell. It is crucial to conduct new studies on behalf of the future to elucidate iron metabolism in epilepsy through the TIM-2 and TFRC-1 genes.

Keywords: Epilepsy; PTZ-kindling model; HIF-1α; TFRC-1; TIM-2.

Introduction

Epilepsy is characterized by seizures that occur as a result of the imbalance between cerebral inhibition and arousal in the CNS (Steinlein 2004). It is one of the most common neurological diseases worldwide and affects more than 70 million people (Thijs et al. 2019). Epileptic seizures are recurrent paroxysmal events (occurring in sudden and temporary crises) that reflect the neural mechanisms of pathogenesis (Neligan et al. 2012). These seizures are associated with functional defects in the expression of genes responsible for controlling the neural signaling, synaptic structure, cell death, and inflammation (Ma 2018). However, the etiology of a significant portion of epilepsy cases is not fully known yet (Brodie et al. 2018).

The brain carries out changes in specific gene expressions to protect from non-physiological epileptic conditions. These changes result from the excitation of the transcriptional response coordinated to minimize brain damage (Simon 2016). This coordinated transcriptional response in epilepsy is not limited to brain cells, but also creates a transcriptional response in heart myocytes (Biet et al. 2015; Brewster et al. 2016; Lai et al. 2018).

Epidemiological studies have shown that epilepsy patients have a higher prevalence of heart disease compared to the normal population (Kadima et al. 2013; Keezer et al. 2016). Genetic defects can lead to abnormal cardiovascular dysfunction (Marino and Digilio 2000). These disorders can affect the development of both the heart and the brain (Miller and Vogel 1999; McQuillen and Miller 2010). In addition, hypoxia inducible factor (HIF), a gene that has been observed to affect the CNS and cardiovascular system, is associated with epileptic seizures (Jiang et al. 2016; Auzmendi et al. 2018).

The HIF-1 gene is an essential gene responsible for inducing tissue repair in response to the presence of oxygen (Darby and Hewitson 2016). HIF-1α is the part that is sensitive to oxygen. The gene shows activation in case of hypoxia, and this may lead to overexpression in the HIF-1α gene (Yu et al. 1998; Shimoda and Semenza 2011). HIF-1α gene expression can be observed in cardiovascular diseases such as heart failure and pulmonary hypertension (Schultz et al. 2006). On the other hand, high HIF-1α expression levels have been reported in brain tissues in epilepsy (Li et al. 2014; Jiang et al. 2016). The HIF-1α gene in the brain can cause iron overload with different interactions on neurons (Merelli et al. 2018).

Iron is essential for life but may have a toxic effect (Gorter et al. 2005). Iron causes seizures by catalyzing oxidative stress and ferroptosis (Mao et al. 2019; Nakamura et al. 2019). In addition, ferritin, which is an iron storage protein, increases in the hippocampus in epilepsy (Lakaye et al. 2000). In this context, receptors responsible for intracellular iron homeostasis constitute mechanisms for controlling iron levels in cells (Gorter et al. 2005).

T cell immunoglobulin and mucin region (TIM) -2 receptors bind ferritin and form the main mechanism in iron uptake. TIM-2 is a specific ferritin receptor expressed in many systems such as immune system cells, liver and kidney tissues, including CNS cells (Todorich et al. 2008). Transferrin-1 receptor (Tfr-1) is a receptor encoded by the TFRC-1 gene and might be stimulated by HIF-1α (Merelli et al. 2018). Decreased iron concentration in the cell caused by Tfr-1 inactivation is associated with heart problems that can cause death (Gorter et al. 2005). Since the transport of iron will be modulated by these receptors, studying the TFRC-1 and TIM-2 genes in epilepsy will help clarify the mechanism.

In this study, the HIF-1α gene in the brain and heart and the Tfr-1 and TIM-2 receptors genes involved in iron metabolism were investigated in epileptic rats.

Material and Method

The methodology of this entire study was summarized in a diagram in **Figure 1.**

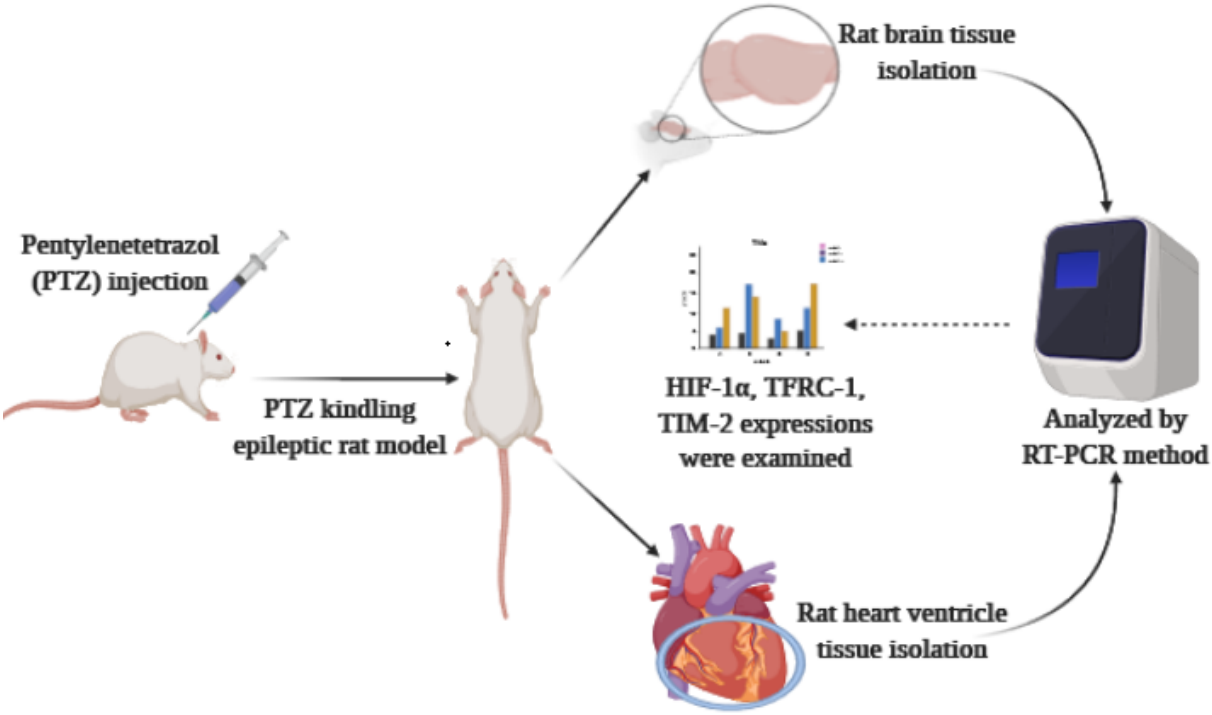

Figure 1. *Establishment of PTZ kindling pattern, followed by analysis of HIF-1α, TFRC-1 and TIM-2 gene expressions. In the epilepsy model created as a result of PTZ injection, the isolation of rat brain and heart ventricle tissues and RT-PCR method was applied and data were analyzed.*

Animals

The animals were ordered to create an experimental epilepsy model at the Kayseri Erciyes University Research Center. The experimental protocol of this study was approved by the Animal Ethics Committee of the Kayseri Erciyes University (ethics committee decision number: 2019/027, approved 13 February 2019). A total of 44 adult male and female Wistar-albino rats weighing between 280-400 g were randomly divided into 4 groups. Rats were placed in cages at controlled temperatures (24 °C ± 2 °C). They were given free access to water and food and kept on a 12-hour light-dark cycle.

Animal Model

PTZ Kindling Model

The PTZ (P6500, Sigma-Aldrich, St. Louis, MO, USA) agent was dissolved in isotonic saline (0.9% NaCl) and a 1% solution concentration was established. 35 mg/kg PTZ injection contains 1% PTZ agent. It was prepared in a 10 ml volume of 229 mg PTZ. This preparation helped to prepare 35 mg/kg PTZ. A dose of 35 mg/kg of 1% solution of PTZ was administered I.P. every two days to create chronic epilepsy seizures in the rat. The solution was injected into rats three days a week (Monday, Wednesday and Friday) for one month (a total of 13 injections, the last injection being 50 mg/kg). Rat behaviors were observed 30 minutes after injection according to the protocol, and epileptic seizure scoring was performed (**Table 1)**.

Number of injections	Racine scoring of epileptic female rats	Racine scoring of epileptic male rats
1.	0,7	1,16
2.	1,17	1,77
3.	2,17	2,12
4.	1,88	2,06
5.	2,47	2
6.	2,52	2,75
7.	2,94	2,81
8.	2,97	3
9.	3	3,26
10.	3,11	3,31
11.	3,23	3,12
12.	4,4	4,38
13.	5,2	5,31

Table 1: *Racine scoring of epileptic rats.*

Racine Scoring System

Racine scoring (RS) is one of the most commonly used tools to assess seizure intensity in rodent experimental epilepsy models (Racine 1972). RS, which categorizes the stages of seizures,

Stage 0: No answer,

Stage 1: Twitching in the ear and face,

Stage 2: Myoclonic jerks in the body,

Stage 3: Standing up on hind legs,

Stage 4: Tonic-clonic seizures with the animal falling to the ground,

Stage 5: Recurrent (generalized) severe tonic-clonic seizures.

Real time polymerase chain reaction (RT-PCR)

This study was conducted to detect and compare gene expression levels in rat brain and cardiac ventricle tissue samples. Tissue samples were firstly homogenized, RNA isolation (#11828665001, Roche) was performed with manual isolation, the obtained RNAs were converted into cDNA, their purity levels and amounts were measured.

For amplification, qPCR study was performed with 2X Sybr Green Master Mix qPCR kit (BIONEER) and HIF-1α, TIM-2 and TFRC-1 primers, and the gene expression levels were compared to each other and the reference gene among tissues as control and patient groups.

qPCR Kit and PCR Conditions

The WizPureq PCR Master Mix (SYBR) we have used recommends adding 1-100 ng of cDNA to prepare a reaction volume of 50-100 µl. The cDNAs we obtained from our extraction device were diluted to appropriate concentrations. BIONEER Brand WizPure qPCR Master Mix (SYBR) was used in our Real Time PCR studies.

In the expression test experiments performed after Absolute Quantification study (in Quantitative Analysis), primers and sample cDNA (or PCR Grade water for Negative Control) were added to ready-made reaction tubes, the final sample volume was completed to 20 µl with PCR Grade water. Before the tubes were loaded into the Q-PCR device, BIONEER Brand ExiSpin Model Vortex-Mixer was used, which automatically performs both vortex and spin processes in order to ensure homogeneity.

Protocol of Real Time PCR

PCR process was performed with BIONEER BRAND EXICYCLER96 REAL TIME PCR device. The prepared mixes were placed in BIONEER Brand ExiCycler96 Model Q-PCR device (after homogeneity was achieved with the BIONEER Brand ExiSpin Model Vortex-Mixer device) in 0.2 ml PCR tubes covered with optic transparent film. Since the amplification conditions of the experiments (temperature, time and number of cycles required for Syber Green Master Mix) were already optimized by BIONEER (the manufacturer of Devices, Master Mixes and Primer-Probes), significant results were obtained from the studies.

Statistical Analysis

SPSS program (SPSS for Windows, SPSS Inc, Chicago, IL, version 24.0) was used for statistical analysis. Results are shown as the average standard error margin. Inter-group comparison was made with the one-way ANOVA test and Independent sample T-test was used for binary comparisons. Statistical value of $p<0.05$ was considered as significant.

Results

Scoring of PTZ Kindling Epilepsy Model

After PTZ treatment, kindling epileptic seizures were gradually induced. At the 13th injection, generalized tonic-clonic seizures, corresponding to stage 5 of the Racine scaling system score, were observed in both male and female rats (**Figure 2**) (5.31±0.58 and 5.2±0.47). The latency of the first seizure was calculated for both groups. Female rats with PTZ had their first seizure in 266±66 s, while PTZ-affected male rats had their first seizure in 308±95 s. There was no significant change in the body weight of the rats during the PTZ injection procedure.

Findings of RT-PCR

HIF-1α, TFRC-1 and TIM-2 gene expression on brain tissue isolated from the PTZ-induced epilepsy model was shown in **Figure 3**. TFRC-1 gene expression was significantly decreased in female rats compared to the control group ($p <0.05$), but no significant result was obtained in male rats. TIM-2 expression was significantly decreased in female rats compared to the control group ($p <0.01$), and no significant data was observed in male rats. In addition, HIF-1α had no a significant difference in both female and male rats.

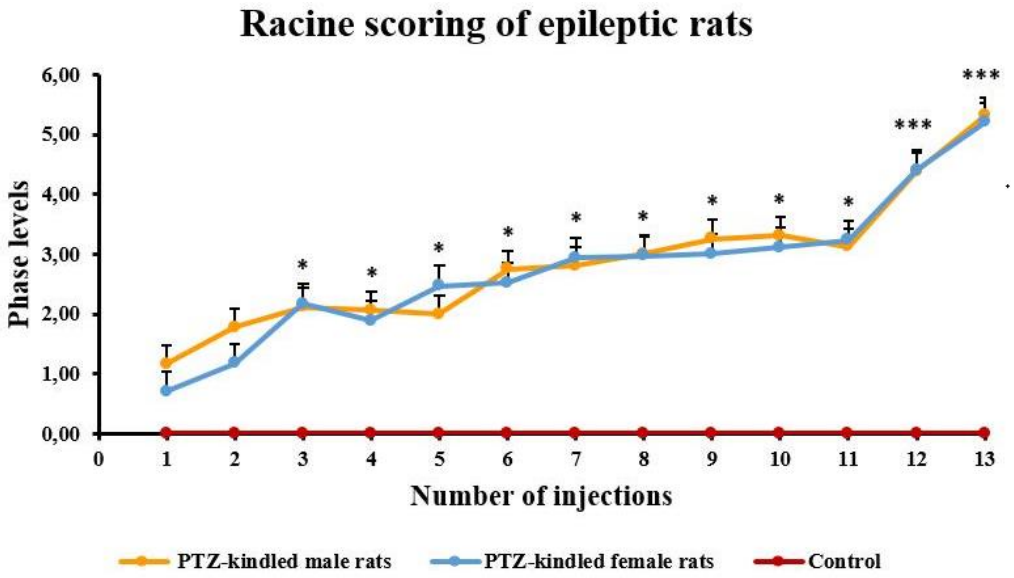

Figure 2. Racine score exhibited by male (orange) and female (blue) rats after each injection of PTZ (*p<0.05; ***p<0.001).

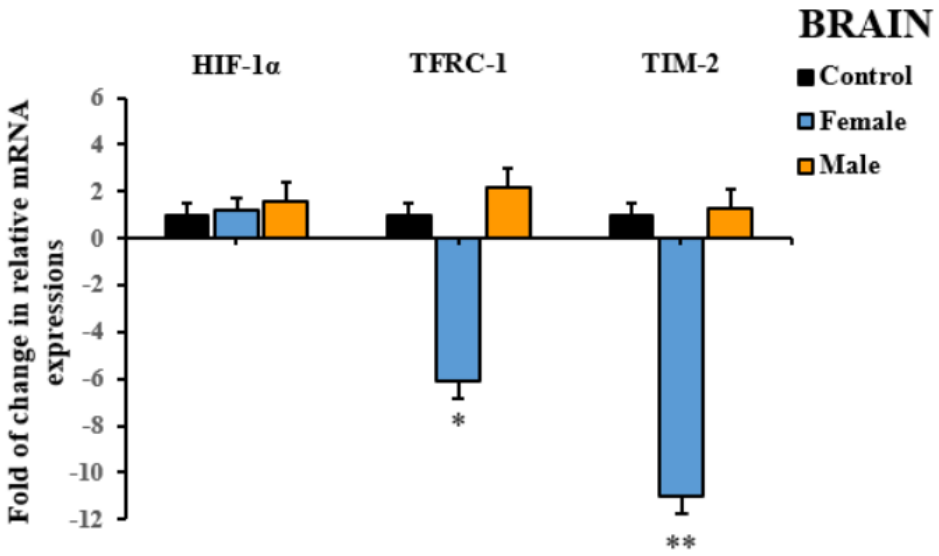

Figure 3. HIF-1α, TFRC-1 and TIM-2 gene expression levels graph obtained from brain tissue by RT-PCR analysis. The black column refers to the control group, the blue column to the female rat, the orange column to the male rat (*p<0.05; **p<0.01).

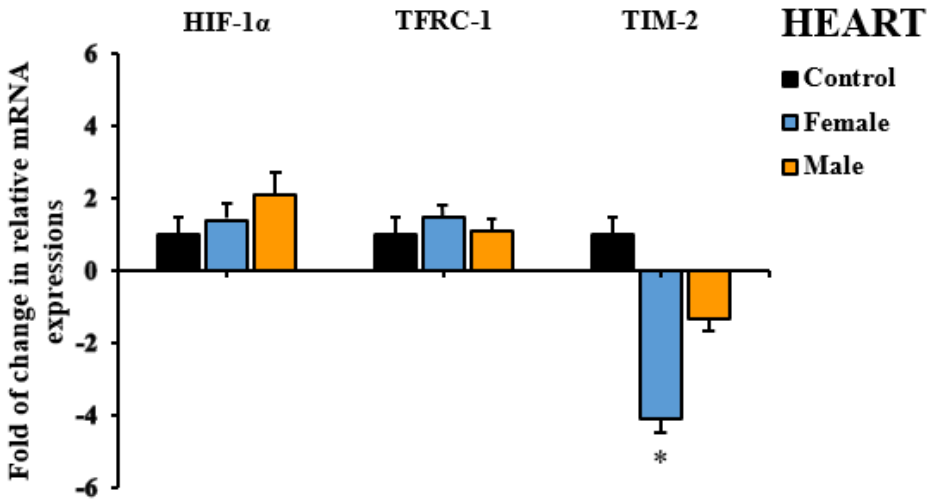

Figure 4. HIF-1α, TFRC-1 and TIM-2 gene expression levels graph obtained from cardiac ventricle tissue by RT-PCR analysis. The black column refers to the control group, the blue column to the female rat, the orange column to the male rat (*p<0.05).

In our study, as a second target, HIF-1α, TFRC-1 and TIM-2 gene expression analysis was performed on the cardiac ventricle tissue and was shown in **Figure 4**. TIM-2 expression in cardiac tissue significantly decreased in female rats ($p<0.05$). HIF-1α expression was not provided significant data in both male and female rats compared to the control group. When TFRC-1 gene expression was examined, no significant results were obtained in both genders.

Discussion

Ferritin receptor encoded by the TIM-2 gene and Tfr-1 transferrin receptor encoded by the TFRC-1 gene play a role in iron homeostasis in cells (**Figure 5B**) (Xu et al. 2015; Merelli et al. 2018). Our results show that TFRC-1 and TIM-2 gene expressions are significantly reduced in female rat brain tissue in epilepsy (**Figure 3**). The decrease in TIM-2 gene expression we have observed may be a counter-pathogenesis response of neurons to reduce epileptic activity by reducing iron flow into the cell.

As a control mechanism to compensate for seizures that may occur with increased iron concentration in neurons, cells may exhibit a decrease in TFRC-1 and TIM-2 gene expression. Pathogenesis may be minimized by a natural process by trying to reduce the intracellular iron concentration. In addition, the receptors encoded by these genes in the iron-induced ferroptosis mechanism known to be associated with epilepsy (Feng et al. 2020; Mao et al. 2019; Nakamura et al. 2019) may contribute to seizure formation by regulating intracellular iron homeostasis (**Figure 5B**).

The formation of reactive oxygen products and neuropathy findings (Cozzi et al. 2010) as a result of the decrease in Tfr-1 expression of Cozzi et al. supports the role of this gene in epilepsy, in line with the decrease in expression in the TFRC-1 gene we obtained in our study. In the pathogenesis of seizures, a mechanism that triggers oxidative damage by disrupting Tfr-1-related iron homeostasis can be considered (**Figure 5B**). Another striking data is that a significant decrease in genes encoding transferrin and ferritin receptors was observed only in female rats. This process suggests that TIM-2 and TFRC-1 genes may have a regulation mechanism by steroid hormones such as estrogen and progesterone. Studies to analyze the regulatory effect of hormone differences between sexes on TIM-2 and TFRC-1 genes can provide meaningful data.

It has been reported that sex differences can cause changes in gene expression in tissues (Kassam et al. 2019). Sex differences involved in gene expression have been observed in a number of tissues, including the liver, heart, and brain (Ellegren and Parsch 2007; Parsch and Ellegren 2013; Rinn and Snyder 2005). Male and female gender can also differ in terms of many pathologies such as cancer (Naugler et al. 2007), cardiovascular (Lerner and Kannel 1986) and neurological diseases (Gillies et al. 2014). Accordingly, in our study, using both male and female rats, the discrimination in terms of gender in the change in targeted gene expression was reported.

We present a new approach to epilepsy with the relationship between HIF-1 and iron metabolism in the brain (**Figure 5A**) (Merelli et al. 2018) mentioned in the literature, the unchanged amount of HIF-1α we obtained and the results of the decrease in TIM-2 and TFRC-1 gene expression. Based on our results, in future studies, in addition to the brainstem, TIM-2 and TFRC-1 genes from the peripheral nervous system, especially in the vagus nerve, can be examined and neurological approaches can be developed.

As a result of studies, cardiovascular problems contribute to mortality in epilepsy (Neligan et al. 2011). Another parameter we examined in our study is TIM-2 and TFRC-1 genes found in cardiac ventricles. We reported that the expression level of the TIM-2 ferritin receptor gene was significantly decreased in female rats (**Figure 4**). Therefore, pathogenesis may be triggered by changing the iron homeostasis in cardiomyocytes.

Availability of iron ions is of great importance for cardiomyocytes. Cardiac iron deficiency leads to situations such as oxidative phosphorylation, metabolic changes, and disruption of mitochondria (Xu et al. 2015). Therefore, in epilepsy, cardiac iron deficiency may occur with a decrease in TIM-2 ferritin receptors, and this may contribute to seizures by creating cardiac function abnormalities (**Figure 5B**).

According to the data we have obtained, targeting iron metabolism via TIM-2 and TFRC-1 encoded transferrin and ferritin receptors may have therapeutic potential for epilepsy. Due to the significant TIM-2 and TFRC-1 expression changes we have observed in both the brain and the heart, the seizure prognosis can evolve into a more significant state by using specific receptor agents. The reduction of TIM-2 receptor in both tissues may

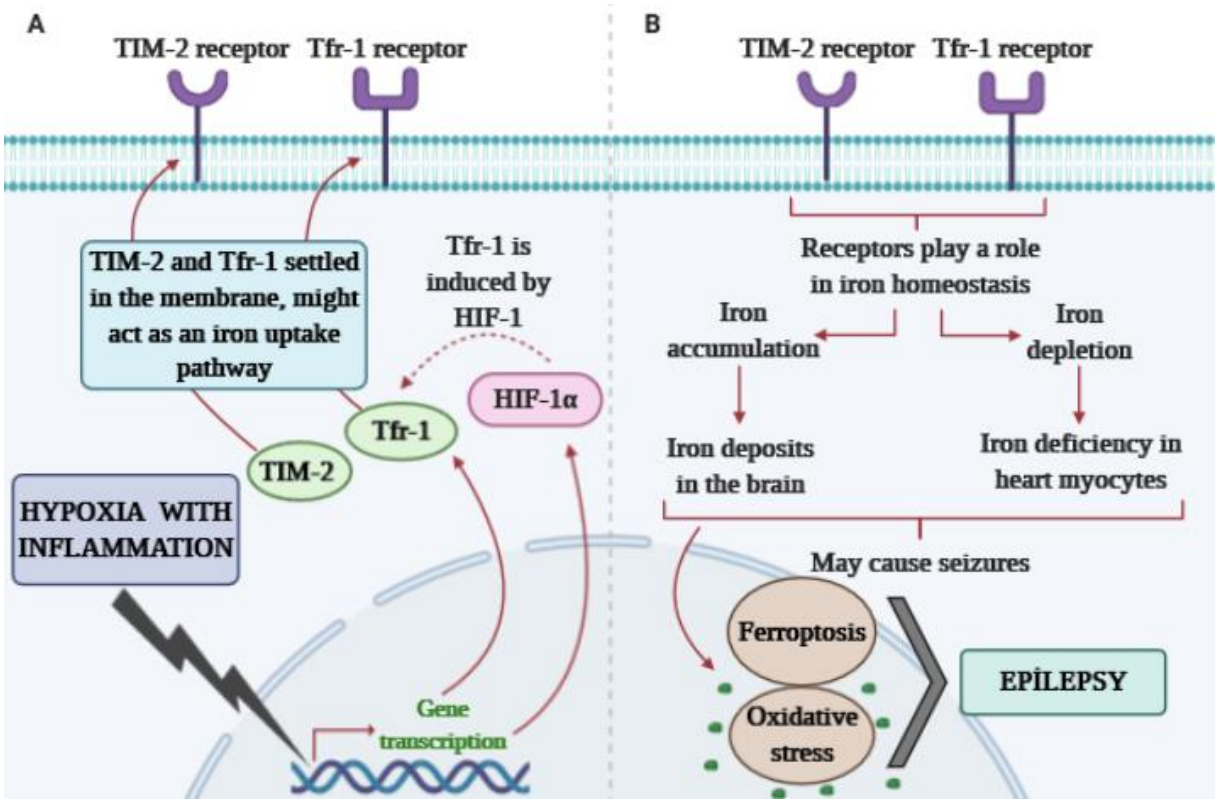

Figure 5. General epilepsy mechanism of TIM-2 and Tfr-1 receptors and related genetic process. (A) The mechanisms of induction of TIM-2 and Tfr-1 receptors suggested in previous studies were shown. The association of hypoxia and inflammation creates an increase in the expression of these receptors. It is also stated that HIF-1α induces the Tfr-1 receptor. (B) Iron imbalance that may be caused by modulation of TIM-2 and Tfr-1 receptors can cause seizures.

indicate that this receptor has a wider role in iron transport in epilepsy. TFRC-1 expression, which encodes the Tfr-1 receptor, decreases only in the brain. This may indicate that the TFRC-1 gene may have local effects on the brain tissue in epilepsy, while the TIM-2 receptor may cause pathology in the cardiac system as well as the neuronal system. On behalf of the TIM-2 receptor, the change in expression in both the brain and the heart can be targeted primarily in therapeutic approaches and a wider systemic effect can be created. In addition, due to the inability to obtain significant data for the HIF-1α gene, efficient results may not be observed by targeting this gene therapeutically in epilepsy.

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Authors' contributions

HED, AO, SRS and FK wrote the main manuscript text. HED prepared the figures and tables. EA designed the experiment and revised the manuscript. SY were responsible for the isolation of targeted tissues from rats.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

References

Auzmendi J, Buchholz B, Salguero J, Cañellas C, Kelly J, Men P, Zubillaga M, Rossi A, Merelli A, Gelpi RJ, Ramos AJ, Lazarowski A. (2018). Pilocarpine-Induced Status Epilepticus Is Associated with P-Glycoprotein Induction in Cardiomyocytes, Electrocardiographic Changes, and Sudden Death. Pharmaceuticals (Basel) 11.

Biet M, Morin N, Lessard-Beaudoin M, Graham RK, Duss S, Gagné J, Sanon NT, Carmant L, Dumaine R. (2015). Prolongation of action potential duration and QT interval during epilepsy linked to increased contribution of neuronal sodium channels to cardiac late Na+ current: potential mechanism for sudden death in epilepsy. Circ Arrhythm Electrophysiol. 8:912-920.

Brewster AL, Marzec K, Hairston A, Ho M, Anderson AE, Lai YC. (2016). Early cardiac electrographic and molecular remodeling in

a model of status epilepticus and acquired epilepsy. Epilepsia. 57:1907-1915.

Brodie MJ, Zuberi SM, Scheffer IE, Fisher RS. (2018). The 2017 ILAE classification of seizure types and the epilepsies: what do people with epilepsy and their caregivers need to know? Epileptic Disord. 20:77-87.

Cozzi A, Rovelli E, Frizzale G, Campanella A, Amendola M, Arosio P, Levi S. (2010). Oxidative stress and cell death in cells expressing L-ferritin variants causing neuroferritinopathy. Neurobiol Dis. 37:77-85.

Darby IA, Hewitson TD. (2016). Hypoxia in tissue repair and fibrosis. Cell Tissue Res. 365:553-562.

Ellegren H, Parsch J. (2007). The evolution of sex-biased genes and sex-biased gene expression. Nat. Rev. Genet. 8,689–698.

Feng H, Schorpp K, Jin J, Yozwiak CE, Hoffstrom BG, Decker AM, Rajbhandari P, Stokes ME, Bender HG, Csuka JM, Upadhyayula PS, Canoll P, Uchida K, Soni RK, Hadian K, Stockwell BR. (2020). Transferrin Receptor Is a Specific Ferroptosis Marker. Cell Rep. 30(10):3411-23. e7.

Gillies GE, Pienaar IS, Vohra S, Qamhawi Z. (2014). Sex differences in Parkinson's disease. Front. Neuroendocrinol. 35,370–384.

Gorter JA, Mesquita AR, van Vliet EA, da Silva FH, Aronica E. (2005). Increased expression of ferritin, an iron-storage protein, in specific regions of the parahippocampal cortex of epileptic rats. Epilepsia. 46:1371-1379.

Jiang G, Zhou R, He X, Shi Z, Huang M, Yu J, Wang X. (2016). Expression levels of microRNA-199 and hypoxia-inducible factor-1 alpha in brain tissue of patients with intractable epilepsy. Int J Neurosci. 126:326-334.

Kadima NT, Kobau R, Zack MM, Helmers S. (2013). Comorbidity in adults with epilepsy--United States, 2010. MMWR Morb Mortal Wkly Rep. 62:849-853.

Kassam I, Wu Y, Yang J, Visscher PM, McRae AF. (2019). Tissue-specific sex differences in human gene expression. Hum. Mol. Genet. 28(17), 2976-2986.

Keezer MR, Sisodiya SM, Sander JW. (2016). Comorbidities of epilepsy: current concepts and future perspectives. Lancet Neurol. 15:106-115.

Lai YC, Li N, Lawrence W, Wang S, Levine A, Burchhardt DM, Pautler RG, Valderrábano M, Wehrens XH, Anderson AE. (2018). Myocardial remodeling and susceptibility to ventricular tachycardia in a model of chronic epilepsy. Epilepsia open 3:213-223.

Lakaye B, de Borman B, Minet A, Arckens L, Vergnes M, Marescaux C, Grisar T. (2000). Increased expression of mRNA encoding ferritin heavy chain in brain structures of a rat model of absence epilepsy. Exp Neurol. 162:112-120.

Lerner DJ, Kannel WB. (1986). Patterns of coronary heart disease morbidity and mortality in the sexes: a 26-yearfollow-up of the Framingham population. Am. Heart J. 111,383–390.

Li J, Jiang G, Chen Y, Chen L, Li Z, Wang Z, Wang X. (2014). Altered expression of hypoxia-Inducible factor-1α participates in the epileptogenesis in animal models. Synapse. 68:402-409.

Ma Y. (2018). The Challenge of microRNA as a Biomarker of Epilepsy. Curr Neuropharmacol. 16:37-42.

Mao XY, Zhou HH, Jin WL. (2019). Ferroptosis Induction in Pentylenetetrazole Kindling and Pilocarpine-Induced Epileptic Seizures in Mice. Front Neurosci. 13:721.

Marino B, Digilio MC. (2000). Congenital heart disease and genetic syndromes: specific correlation between cardiac phenotype and genotype. Cardiovasc Pathol. 9:303-315.

McQuillen PS, Miller SP. (2010). Congenital heart disease and brain development. Ann N Y Acad Sci. 1184:68-86.

Merelli A, Rodríguez JCG, Folch J, Regueiro MR, Camins A, Lazarowski A. (2018). Understanding the Role of Hypoxia Inducible Factor During Neurodegeneration for New Therapeutics Opportunities. Curr Neuropharmacol. 16:1484-1498.

Miller G, Vogel H. (1999). Structural evidence of injury or malformation in the brains of children with congenital heart disease. Semin Pediatr Neurol. p 20-26.

Nakamura T, Naguro I, Ichijo H. (2019). Iron homeostasis and iron-regulated ROS in cell death, senescence and human diseases. Biochim Biophys Acta Gen Subj. 1863:1398-1409.

Naugler WE, Sakurai T, Kim S, Maeda S, Kim K, Elsharkawy AM, Karin M. (2007). Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. Science. 317, 121–124.

Neligan A, Bell GS, Johnson AL, Goodridge DM, Shorvon SD, Sander JW. (2011). The long-term risk of premature mortality in people with epilepsy. Brain. 134:388-395.

Neligan A, Hauser WA, Sander JW. (2012). The epidemiology of the epilepsies. Handb Clin Neurol. 107:113-33.

Parsch J, Ellegren H. (2013). The evolutionary causes and consequences of sex-biased gene expression. Nat. Rev.Genet. 14, 83–87.

Racine, RJ. (1972). Modification of seizure activity by electrical stimulation: II. Motor seizure. Electroencephalogr Clin Neurophysiol. 32, 281–294.

Rinn JL, Snyder M. (2005). Sexual dimorphism in mammalian gene expression. Trends Genet., 21, 298–305.

Schultz K, Fanburg BL, Beasley D. (2006). Hypoxia and hypoxia-inducible factor-1alpha promote growth factor-induced proliferation of human vascular smooth muscle cells. Am J Physiol Heart Circ Physiol. 290:H2528-2534.

Shimoda LA, Semenza GL. (2011). HIF and the lung: role of hypoxia-inducible factors in pulmonary development and disease. Am J Respir Crit Care Med. 183:152-156.

Simon RP. (2016). Epigenetic modulation of gene expression governs the brain's response to injury. Neurosci Lett. 625:16-19.

Steinlein OK. (2004). Genetic mechanisms that underlie epilepsy. Nat Rev Neurosci. 5:400-408.

Thijs RD, Surges R, O'Brien TJ, Sander JW. (2019). Epilepsy in adults. Lancet. 393:689-701.

Todorich B, Zhang X, Slagle-Webb B, Seaman WE, Connor JR. (2008). Tim-2 is the receptor for H-ferritin on oligodendrocytes. J Neurochem. 107:1495-1505.

Xu W, Barrientos T, Mao L, Rockman HA, Sauve AA, Andrews NC. (2015). Lethal Cardiomyopathy in Mice Lacking Transferrin Receptor in the Heart. Cell Rep. 13:533-545.

Yu AY, Frid MG, Shimoda LA, Wiener CM, Stenmark K, Semenza GL. (1998). Temporal, spatial, and oxygen-regulated expression of hypoxia-inducible factor-1 in the lung. Am J Physiol. 275:L818-826.