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Case Report / Olgu Sunumu

TRP Channels in Tension-Type Headache: A Pilot Study

Gerilim Tipi Baş Ağrısında TRP Kanalları: Pilot Çalışma

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Abstract

Tension-type headache (TTH) affects many individuals worldwide. Although the exact pathogenesis of TTH remains unclear, central, and peripheral mechanisms are considered to play a role in TTH 1. This pilot study aimed to investigate the role of transient receptor potential (TRP) channels in the development or chronic inflammation in TTH and to discuss the findings in the light of literature. This pilot study included a patient group comprising three patients with episodic TTH and three patients with chronic TTH (CTTH) aged 18-40 years with no comorbidities and a control group of three patients with no headache. Peripheral blood samples were obtained from all the participants, and both RNA and cDNA were isolated on the same day. The mRNA levels of pain-related TRP channels [TRPA1, TRP vanilloid-1 (TRPV1), TRPV3, TRPV4, TRPM3, and TRPM8] were measured by reverse transcriptase (RT)-quantititave polymerase chain reaction method and were normalized with the levels of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) transcript. Results were analyzed using statistical methods. All three groups were comparable with regard to demographic characteristics. No significant difference was found among the groups with regard to the mRNA levels of the TRP channels normalized by GAPDH, whereas the TRPM8 expression levels were not significantly lower in the CTTH group than in other groups (p=0.066). This study revealed that TRPM8 is likely to have a role in the pathogenesis of TTH, and this role of TRPM8 may be investigated by further studies.
Keywords: TRP channels, stension-type, headache

Öz

Gerilim tipi baş ağrısı (GTBA) dünya genelinde birçok kişiyi etkileyen bir hastalıktır. GTBA'nın patogenezi hala belirsizliğini korusa da santral ve periferal ağrı mekanizmalarının rolü olduğu düşünülmektedir. Bu pilot çalışmada biz transient reseptör potansiyel (TRP) kanallarının GTBA'nın gelişiminde ya da kronikleşmesinde rolünün varlığını araştırmayı ve literatür bulguları doğrultusunda tartışmayı amaçladık. Bu çalışma pilot olarak düzenlenmiştir ve hasta grupları 18-40 yaş arası, komorbiditesi olmayan üç epizodik GTBA'lı hasta, üç kronik GTBA'lı hasta ve üç herhangi bir baş ağrısı olmayan kontrol olarak dizayn edilmiştir. Periferal kan örnekleri alınarak aynı gün RNA ve cDNA izole edilmiştir. Ağrı ilişkili TRP kanallarının (TRPA1, TRPV1, TRPV3, TRPV4, TRPM3 ve TRPM8) mRNA seviyeleri ters transkriptaz-kantitatif polimeraz zincir reaksiyonu metoduyla ölçüldü ve gliseraldehid 3-fosfat dehidrogenaz (GAPDH) transkript düzeyine göre normalize edildi. İstatistiksel metodlarla sonuçlar analiz edildi. Üç grup da demografik karakterler açısından benzerdi. Gruplar arasında GAPDH ile normalize edilmiş TRP kanalarının mRNA seviyeleri arasında istatistiksel olarak anlamlı fark saptanmasa da TRPM8 mRNA ekspresyon düzeyleri kronik GTBA grubunda diğer gruplarla karşılaştırıldığında, istatistiksel olarak anlamlı olmasa da düşük elde edildi (p=0,066). Çalışmamız TRPM8'in GTBA patogenezinde rol oynayabileceğini düşündürmekte ve ileriki çalışmalarda TRPM8'in bu rolünün araştırılabileceğini önermektedir.
Anahtar Kelimeler: TRP kanalları, gerilim tipi, baş ağrısı

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Introduction

Tension-type headache (TTH) is a non-pulsating, pressing, or tightening headache. The pain in TTH may be unilateral or bilateral and is classified into four types: Infrequent episodic, frequent episodic, chronic, and probable (1). TTH is the most common primary headache and has a global prevalence of 38% and a lifetime prevalence of 46% in adults (2,3,4,5). Chronic TTH (CTTH) is reported to affect 0.5-4.8% of the population worldwide (5). Although the exact pathogenesis of TTH remains unclear, central, and peripheral mechanisms are considered to play a role in all TTH types. However, while peripheral pain mechanisms have a potential key role in episodic TTH (ETTH), central mechanisms such as central sensitization may be predominant in CTTH (1).

The transient receptor potential (TRP) ion channel family has been shown to play a role in central and peripheral sensitization. However, in spite of the wide heterogeneity of this family of ion channels, TRPs share a general role in sensory transduction, as they play a contributory role in hearing, vision, olfaction, touch, taste, and thermo- and osmosensation, thereby allowing cells to sense and respond to environmental changes (6,7). Members of the TRP family expressed in sensory neurons primarily contribute to the detection of harmful physical (thermal and mechanical) and chemical stimuli. Moreover, recent pathophysiological and pharmacological studies have suggested that TRP vanilloid-1 (TRPV1) and TRPA1, among the 6-7 TRPs expressed by nociceptors, are primary contributors of inflammatory and neuropathic pain (6).

Recent studies have also indicated that TRP channels, particularly TRPA1, TRP, TRPV1, TRPV3, TRPV4, TRPM3, and TRPM8, have a potential role in the formation of central sensitization in migraine and other pain types such as neuropathic pain. Based on the recent reports of TRP channel functions related to nociception and sensitization, we hypothesized that TRP channels might be actively involved in the pathogenesis of TTH. To the best of our knowledge, no study has reported the activities of these channels in TTH. Thus, this pilot study aimed to investigate the role of TRP channels in the development or chronic inflammation in TTH, which has an unclear pathogenesis, and to discuss the findings in the light of literature.

Case Report

The study included a patient group comprising three patients with ETTH and three patients with CTTH aged 18-40 years with no comorbidities and a control group of three patients with no headache. Patients were diagnosed based on the ICHD-3 beta criteria; CTTH was diagnosed in patients with headaches occurring on ≥15 days/month, for >3 months, and ETTH was diagnosed in patients with headaches occurring on <14 days/month.

Informed consent was obtained from each participant. Participants were selected from among patients who applied to Malatya Training and Research Hospital, Neurology Clinic and had a similar age distribution and demographic characteristics. All participants were non-smokers and had no history of cardiac diseases or additional pain syndromes such as migraine and neuropathic pain. Total RNA was isolated from blood samples using the QIAamp RNA Blood Mini Kit (Qiagen, Germany). The RNA concentration was determined by a Nanodrop spectrophotometer (DS-11 Fx+, Denovix). Genomic DNA traces were removed by DNAse treatment (Fermentas, MA, USA). Complementary DNA (cDNA) from total mRNA was synthesized by using the First Strand cDNA Synthesis Kit (Fermentas). cDNA samples were used as the template for the amplification reactions, and the *GAPDH* gene was used as the internal control. Triplicate RT-polymerase chain reaction (PCR) analyses were performed with the StepOne Plus real-time PCR system using Fermentas MaximaTM SYBR Green qPCR Master Mix (2x). The primers used for the amplification of target genes are shown in Table 1. Each reaction contained 20 ng of cDNA as the template, 1 µM of each primer, 1x Maxima SYBR Green/ROX qPCR Master Mix, and water to complete the volume to 15 µl. The $2^{-\Delta\Delta Ct}$ method was used to determine the relative expression of target genes. Mean values and percentages of data were evaluated. Fold changes of mRNA levels of TRP channels between groups were analyzed with the non-parametric Kruskal-Wallis test since the number of samples was <10 in each group, and there were more than two groups in the study. The level of significance was taken as $p<0.005$. Table 2 presents the demographic characteristics of the patients, and Table 3 shows the clinical characteristics of the patients with TTH.

Changes in the expression levels of TRP channels were calculated using the formulas under RT-PCR guidance. TRP

Table 1. Primers of TRP channels

TRP channel	Forward/backward	Primer sequence
TRPA1	Forward Backward	ATGAAGACAACGATGGGTGTACTCC CAGGTATTGATACGCCCATAACTGG
TRPV1	Forward Backward	GACCATCACAGTCAGCCCTGTTATC GCTCTCCAGATCCTGGCAGTTATTC
TRPV3	Forward Backward	GAGGACTTCAAGACGCAGAATGAC GAGCCGCTTCTCCTTGATCTCAC
TRPV4	Forward Backward	ACCAAATCTGCGCATGAAGTTC TCACTGGAGTGGTGACGATAGGTG
TRPM3	Forward Backward	TGGGACCATTGAGTTCCAAGGAG GGCTGGAGTTCAAAGTTCTGCAGG
TRPM8	Forward Backward	GCCCTGACATCTTCTGCCGTCAAG AGAGCGTAGGAGATGGCATTGCTC

TRP: Transient receptor potential

Table 2. Age and gender distribution of the control group and TTH patients			
	Episodic TTH patient (n=3)	**Chronic TTH patient (n=3)**	**Control group**
Sex, M/F	1/2	1/2	2/1
Average of age Minimum-maximum	25.33 22-32	26.66 18-37	24 23-25
TTH: Tension-type headache, M: Male, F: Female			

Table 3. Clinical characteristics of patients with TTH			
n		**Patients (n=6)**	
		%	
Localization	Right, unilateral	1	16.6
	Left, unilateral	1	16.6
	Bilateral	4	66.6
Character	Throbbing		-
	Compressor/oppressive	6	100
Nausea		0	0
Vomiting		0	0
Photophobia		0	0
Phonophobia		4	66.6
Increase with physical activity		0	0
Aura		0	0
Family history		2	33.3
TTH: Tension-type headache			

expression levels were normalized by the expression levels of glyceraldehyde 3-phosphate dehydrogenase *(GAPDH)*, used as the housekeeping gene. Accordingly, no significant difference was found among the three groups with regard to the expression levels of TRPA1, TRPV1, TRPV3, TRPV4, and TRPM3, whereas the expression of TRP cation channel subfamily M member 8 (TRPM8) did not significantly decrease in the CTTH group compared with other groups (Figure 1).

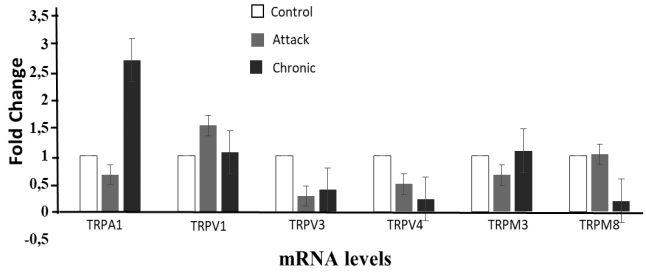

Figure 1. mRNA levels of TRPA1, TRPV1, TRPV3, TRPV4, TRPM3, and TRPM8 in patients with ETTH, CTTH, and control groups

TRP: Transient receptor potential, ETTH: Episodic tension-type headache, CTTH: Chronic tension-type headache

Discussion

In this study, the most important finding was the lack of a significant difference in the expression levels of the TRP channels involved in sensitization and nociception in the development or chronic inflammation in TTH.

ETTH, according to the ICHD-3 beta criteria, is defined as episodes of headache typically bilateral, pressing or tightening in quality, occurring on ≤14 days/month, and lasting for 30 min to 7 days. Infrequent ETTH are episodes of headache occurring on <12 days/year, while frequent ETTH are episodes occurring on 12 to 180 days a year. CTTH are unremitting episodes of headache typically bilateral, pressing or tightening in quality, and occurring on ≥15 days/month for >3 months (2). Although the exact pathophysiology of TTH remains unknown, peripheral mechanisms including pericranial tension, pain hypersensitivity, and muscle tension are considered to play a role. However, central mechanisms such as central sensitization have been blamed in the pathophysiology of CTTH (1). Previous migraine studies have reported that the sensitization of trigeminal neurons is considered the first step, and the development of cephalic allodynia and muscle tenderness is the second step during each attack (6). Nevertheless, evidence on the pathogenesis of other headache types is inadequate. By contrast, the literature suggests a possible relationship between the pathophysiology of TTH and myofascial structures. Accordingly, genetic studies have advocated familial aggregation of TTH and an inherited susceptibility (5). Moreover, previous studies have implicated genetic predisposition in migraine and TTH, whereby genetic predisposition was markedly

stronger in monozygotic and dizygotic twins with migraine and was only slightly stronger in monozygotic twins with TTH (8). By contrast, neurovascular inflammation has been recently implicated in the pathogenesis of CTTH (1). Therefore, in this study, we investigated TRP channels that have been implicated in the development of sensitization and neurogenic inflammation, based on literature data suggesting genetic susceptibility in TTH and pointing to the potential role of peripheral and central sensitization and neurovascular inflammation in the development of TTH despite its unclear pathogenesis.

The TRP channels are often described as cation-permeable channels and have three distinct cellular roles: (1) Molecular sensors (detectors or primary transducers) of chemical and physical stimuli, (2) downstream or secondary transducers of cell activation mediated by G protein-coupled receptors or ion channel receptors, and (3) ion transport channels responsible, e.g., for Ca2+ and Mg2+ homeostasis (9,10). To date, several diseases have been associated with the mutations of TRP channels, including TRP channelopathies, neurological disorders, kidney diseases, and complex skeletal dysplasias (11).

Mammalian TRPA1 is the only member of the *TRPA* gene subfamily, acting as a detector of mechanical stimuli and noxious cold (<17 °C) (9). TRPA1 is expressed abundantly in peptidergic nociceptors and has been activated by compounds in various spicy foods and by environmental irritants, such as industrial pollutants, tear gas, cigarette smoke, smoke from burning vegetation, and vehicle exhaust (6). This may explain the activation of headache by peripheral stimuli. Additionally, evidence shows that TRPA1 channels are involved in the development of inflammatory and neuropathic pain. The exposure of tissues containing peripheral or central sensory nerve terminals to TRPA1 agonists is known to trigger the release of calcium-dependent substance P, neurokinin A, and calcitonin gene-related peptide. Inhalation of several substances, which are identified as TRPA1 channel agonists, by patients with or without migraine, has been shown to cause migraine or non-migraine headaches, respectively. Sukenaga et al. (12) evaluated patients with neuropathic pain and reported that the methylation levels of the *TRPA1* gene increased in peripheral blood samples. Moreover, increased methylation levels were associated with worsened depression and anxiety symptoms. In this study, we assessed the expression of *TRPA1* channel in peripheral blood samples considering that *TRPA1* may be actively involved in TTH pathogenesis, which is known to be aggravated by several factors including emotional stress, fatigue, sleep deprivation, and anxiety. However, no significant difference was found between ETTH and other types with regard to *TRPA1* expression levels, and no significant change was noted in the TRPA1 expression levels in the CTTH group that could shed light on the pathogenesis of CTTH.

TRPM8 is another TRP channel expressed by nociceptors in addition to *TRPA1*. TRPM8 is a receptor of environmental cold temperatures and menthol and is activated by low temperatures and cooling agents such as menthol, eucalyptus, and icilin. Recent evidence suggests that TRPM8 is also expressed by peripheral sensory neurons in visceral organs. The TRPM8 channel is expressed by somatosensory neurons (6). TRPM8 is involved in innocuous cold transduction and plays a role in nociception and analgesia (9). By contrast, evidence shows that TRPM3 is incomparably activated by pregnenolone sulfate and is expressed in primary sensory neurons. In this study, no significant difference was found among the groups concerning the TRPM3 expression levels, while the TRPM8 expression levels were not significantly lower in the CTTH group than in other groups (p=0.066). This finding implies that although the TRPM8 channel may have no significant effect on TTH development, the inflammation in TTH may be associated with the reduction in TRPM8 activity.

Primary sensory neurons express four of six members of the TRPV subfamily, namely, TRPV1, TRPV2, TRPV3, and TRPV4, which are known to sense warm-hot temperatures and to function as chemosensory for various natural and synthetic ligands (6,13). TRP channels are sensitized after exposure to proinflammatory mediators and intracellular pathways (6,13,14). Recent pathophysiological and pharmacological studies have implicated that TRPV1 and TRPA1 channels, among the 6-7 TRPs expressed by nociceptors, are primary contributors of inflammatory and neuropathic pain (6). TRPV1 is a multifunctional channel involved in thermo- and chemo-sensation and acts as a receptor for various harmful stimuli (6,9). Moreover, TRPV1 is activated by ethanol and participates in the pathophysiology of migraine by causing CGRP release and meningeal vasodilatation (11). TRPV1 and TRPA1 actively participate in the transformation of acute inflammation into chronic inflammation and hyperalgesia in pancreatitis. By contrast, CGRP levels are increased in saliva during an acute migraine attack or in cranial blood during nitroglycerine-evoked cluster headache attacks (6). CGRP is expressed by the trigeminovascular pathway and is the main contributor of migraine attacks (11). Ashina et al. (15) evaluated the CGRP changes in TTH in antecubital vein blood by comparing a CTTH group (n=16) and a control group (n=16). No significant elevation in CGRP levels was found in the CTTH group when compared with the control group. Taken together, these findings indicate the lack of association between CGRP and TTH pathogenesis, and these findings were further supported by our findings about the lack of significant difference in CGRP-related TRPV1 expression levels between the patient groups and control group.

TRPV3 is expressed by primary sensory neurons, dorsal root ganglion, spinal motor neurons, and peripheral nerves. Incensole acetate is an activator of TRPV3 and increases c-fos expression in several brain areas, including areas that are associated with depression and anxiety, such as the central nucleus of the amygdala (13). Given that stress factors play a key role in TTH pathogenesis, we anticipated that the TRPV3 expression levels would be elevated in our patients; however, no significant difference in TRPV3 expression levels was found between the patient groups and control group.

The fourth member of the vanilloid subfamily of the TRP family, TRPV4, is a Ca2+-permeable cation channel expressed by both sensory and non-sensory neurons (9). TRPV4 is activated by hypo-osmotic stimuli that cause plasma membrane distortion (6,9). The arachidonic acid (AA) signaling pathway has been shown to modulate the activity of numerous TRP channels, particularly TRPV1 and TRPV4. Moreover, among the products of the AA signaling pathway, 12(S)-hydroxyeicosatetraenoic acid has been reported to activate TRPV1 and to increase the excitability of sensory nerve endings (13). Given that the TRPV channels are closely associated with the AA signaling pathway and that non-steroidal anti-inflammatory drugs are the primary treatment of headache, we anticipated finding increased TRPV1 and TRPV4 levels in our patient groups; however, no significant difference

was found in the expression levels of TRPV channels between the patient groups and control group.

This study was limited by the small sample size, which could be attributed to the scarcity of studies investigating the expression of TRP channels in TTH, non-availability of power analyses, and pilot design.

In conclusion, no significant difference was found among the three groups with regard to the expression levels of TRP channels, which have a potential role in the development or chronic inflammation in TTH. However, the TRPM8 expression levels decreased in the CTTH group relative to those in other groups. Based on the available literature data, it is wise to assert that TRP channels are actively involved in the pathophysiology of pain in TTH, although it is tempting to consider that they have no contribution to TTH pathogenesis and that the analysis of the expression levels of these channels in the peripheral blood may not provide conclusive findings. Therefore, future studies investigating the role of TRP channels in TTH pathogenesis are recommended to focus on the TRPM8 channel and to analyze the expression levels of these channels in the fluids/tissues other than the peripheral blood, such as the tissues in the peripheral/central nervous system and cerebrospinal fluid.

Ethics

Informed Consent: Informed consent was obtained from each participant.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Y.İ.G., Concept: Y.İ.G., İ.T., Design: Y.İ.G., İ.T., A.K., Data Collection or Processing: Y.İ.G., K.D., A.K., Analysis or Interpretation: Y.İ.G., İ.T., K.D., A.K., Literature Search: Y.İ.G., İ.T., A.K., Writing: Y.İ.G., İ.T., K.D., A.K.

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