Review

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Posted Date: 4 January 2023

doi: 10.20944/preprints202301.0034.v1

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Exploring Novel Therapeutic Targets in the Common Pathogenic Factors in Migraine and Neuropathic Pain

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Abstract: Migraine and neuropathic pain (NP) are evocative of painful, disabling, chronic conditions which exhibit resembling symptoms and thus considered to share a common etiology. Calcitonin gene-related peptide (CGRP) has gained credit as a target for migraine management; nevertheless, the efficacy and the applicability of CGRP modifiers warrant search for more effective therapeutic targets for pain management. This scoping review overviews human studies of common pathogenic factors in migraine and NP to explore potential novel therapeutic targets. CGRP causes inflammation in the meninges; monoclonal antibodies and inhibitors target CGRP. Glutamate-induced hyperexcitability and subsequent sensitization are closely linked to an alteration of the tryptophan (Trp)-kynurenine (KYN) metabolic system; the Trp-KYN system may serve as a potential target. Microglial overaction is observed in migraine and NP; modifying the microglial activity may be a possible approach. Cytokine-induced inflammation is a leading hypothesis of the pathogenesis of the conditions; alleviating neuroinflammation may complement a pain-relieving armamentarium. Transient receptor potential (TRP) ion channels evoke the release of several substances; TRP ion channels may potentially emerge as new targets. The endocannabinoid system plays a major role in the pain trafficking pathway; modification of the system may open a new path toward discovery of new analgesics. Here we highlight the mechanism of those common pathogenic factors to explore therapeutic targets for innovative pain management in migraine and NP.

Keywords: migraine; neuropathic pain; calcitonin gene-related peptide (CGRP); kynurenine; glia; cytokines; neuroinflammation; transient receptor potential (TRP) ion channels; endocannabinoids

1. Introduction

Migraine and neuropathic pain (NP) are chronic pain syndromes with extensively studied pathogenesis. While their clinical manifestations strongly differ, their pathophysiology has common roots. Cause-based treatment of these two devastating, painful neurological diseases is still unsatisfactory. Migraine is one of the frequent primary headache disorders with typical clinical features like unilateral throbbing or pulsating, moderate to severe headache with concomitant symptoms such as nausea, vomiting, photophobia, phonophobia, osmophobia and allodynia. Its main subtypes are migraine without aura (M0) and with aura (MA). Episodic (EM) or chronic (CM) forms can be differentiated based on the number of migraine days per month [1].

NP is a chronic secondary pain condition caused by a lesion or disease in the central or peripheral somatosensory system [2–4]. It is characterized by burning and lancinating pain with abnormal sensation, such as paresthesia, dysesthesia, or allodynia. We underline the difference of

characteristics of these two painful conditions as follows quality of the pain (throbbing in migraine and burning in NP), the associated symptoms of migraine (nausea/vomiting, photophobia and phonophobia) which are absent in NP, the action of triptans (very effective in migraine, ineffective in neuropathic pain), the effect of nonsteroidal anti-inflammatory drugs (effective in migraine, ineffective in pure neuropathic pain). These two different clinical entities meet via their common pathomechanisms, like hyperexcitability and sensitization, which involve neuropeptides (mainly calcitonin gene-related peptide, CGRP), the glutamatergic system and microglia activation, pro- and anti-inflammatory cytokines and transient receptor potential (TRP) ion channel alterations and involvement of the endocannabinoid system [5–16].

CGRP is a vasodilator neuropeptide that plays a crucial role in the pathomechanism of migraine. CGRP is well-documented in pain transmission in the somatosensory nervous system. The latest therapeutic innovation is based on human and fully humanized monoclonal antibodies (mAbs) targeting CGRP and CGRP receptors. Clinical trials in EM and CM patients revealed high efficacy and safety of these pharmacons [17]. Clinical data showed that anti-CGRP mAbs provide strongly efficacious preventive treatment for both EM and CM. In NP co-existing CM only one study showed decrease in Neuropathic Pain Scale scores [18]. The glutamate system is involved in the pain process of hyperexcitability and sensitization. Kynurenines (KYNs) play pivotal roles in this process, since kynurenic acid (KYNA) is one of the rare endogenous antagonists of excitatory glutamatergic N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. The function of the KYN pathway in migraine and NP, based on the available human data and clinical trials, are summarized in this review [7,19].

Microglia are part of neuron-microglia interactions. In migraineurs, altered levels of S100B, a sensitive marker of glial cell injury, have been demonstrated, which point to the role of microglia in hyperactivation of the trigeminovascular system. Microglia play a fundamental role in NP transmission and in the sensitization process in the nervous system [9,10,20]. There is evidence that cytokines have pathophysiological roles in pain genesis and transmission. The key pro-inflammatory cytokines are interleukin-1 (IL-1), IL-2, IL-6, IL-17, IL-18 (previously interferon gamma) and tumor necrosis factor alpha (TNF-alpha), while the anti-inflammatory ones are IL-4, IL-10, and IL-37. There are controversial results with cytokines in the field of migraine, while in NP both pro- and anti-inflammatory cytokines are of significant importance [15,21].

TRP channels are involved in pain mechanisms. Several clinical observations have indicated that different agents (e.g., herbs, food, environment) have the ability to influence migraine headaches via modulation of subclasses of TRP superfamilies (TRP-ankyrin 1 - TRPA-1; TRP-vanilloid 1 - TRPV-1; TRP-melastatin 8 - TRPM-8) [13,22]. Research targeting TRP led to the innovation of the high concentration (8%) capsaicin dermal patch for different types of NP, such as painful diabetic neuropathy (PDN), postherpetic neuralgia (PHN) and human immunodeficiency virus (HIV)-associated neuropathy [23]. Elements of the endocannabinoid system exert antinociceptive effects through the activation of cannabinoid receptors. Favorable preclinical results, which showed decreased trafficking of pain transmission, were clinically confirmed in migraine patients. In the field of NP, only a few clinical studies are available [24].

In this review we overview the human data relevant to pathomechanisms, clinical trials, and therapeutic considerations in migraine and NP.

2. Calcitonin gene-related peptide function in migraine and neuropathic pain

2.1. Migraine

The first clinical data on the importance of the trigeminovascular system in the pathomechanism of migraine revealed that CGRP plasma concentration was elevated in the external jugular vein during a migraine attack (Table 1) [25]. Later, increased CGRP plasma levels were also observed in the cubital vein during the ictal period, as compared with the interictal period [26]. Experimental work on human trigeminal ganglia demonstrated the distribution of CGRP and CGRP receptors [27,28].

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Table 1. Selected human clinical data related to CGRP in migraine and neuropathic pain.

Migraine						ref.
EM				CM		
M0		MA				
Ictally	Interictally	Ictally	Interictally	Ictally	Interictally	
↑ (plasma from external jugular vein)	nd	nd	nd	nd	nd	[25]
↑ (plasma from cubital vein)	nd	nd	nd	nd	nd	[26]

Neuropathic pain		ref.
Peripheral NP	Central NP	
↑ (nerve fibers) painful neuroma	nd	[47]
↑ (serum) CRPS	nd	[46]
↑ (keratinocyta) PHN	nd	[44]

↑: increased concentration; ↓: decreased concentration; Abbreviations: CGRP: calcitonin gene-related peptide, CM: chronic migraine, CRPS: complex regional pain syndrome, EM: episodic migraine, M0: migraine without aura, MA: migraine with aura, NP: neuropathic pain, nd: no data available, PHN: postherpetic neuralgia.

The trigeminovascular system forms a bridge between cerebral dura mater and the vasculature of the meninges, cortex and second-order nociceptive neurons of the trigemino-cevical complex (TCC) [29–31]. One putative mechanism for the activation of the trigeminovascular system suggests that the peripheral branch of the pseudounipolar neurons of the trigeminal ganglion is triggered by cortical spreading depression, affecting different brain areas, like frontal regions. Due to this process, CGRP released in the peripheral and central branches of trigeminal neurons, lead to vasodilation and neurogenic inflammation in the meninges and the activation of second-order sensory neurons in the TCC. Second-order neurons then activate the third-order neurons in the thalamus [17,32].

The clinical sign of this activation and hyperexcitability is allodynia, which is a painful response to an innoxious stimulus. Allodynia can have cephalic or extracephalic localization [33]. A double-blind cross-over clinical trial revealed that intravenous infusion of alpha-CGRP versus placebo caused delayed migraine-like headache in migraineurs [34]. This was the first clinical observation which clearly demonstrated that CGRP can induce migraine attacks, leading to the development of small molecule CGRP receptor antagonists, the gepants. The second generation gepants (orally administered ubrogepant, rimegepant, atogepant) and the third generation version (intranasal application vazogepant) are available for acute and/or prophylactic treatment of migraine [35–37].

A novel pharmacological innovation produced human and fully humanized mAbs against CGRP and CGRP receptors to prevent EM and CM. Eptinezumab, fremanezumab and galcanezumab selectively bind to CGRP itself as a ligand, while erenumab competitively and reversibly targets CGRP receptor components. All of the above mentioned mAbs are highly effective and safe in the prophylaxis of both EM and CM [5,17,38–41].

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2.2. Neuropathic pain

Human studies have found that nerve fibers in the spinal cord lamina I, III and V had CGRP-like immunoreactivity, and their receptors are widely distributed in the pain pathways of the nervous system [42,43]. Experimental data showed that CGRP can sensitize nociceptors and also can induce central sensitization [43]. Allodynia is a clinical feature of central sensitization and is one of the most common sensory abnormalities indicating NP. In spite of these preclinical findings, available clinical data in this area are very sparse.

In skin biopsy samples of PHN patients, increased CGRP levels were found in keratinocyte [44] (Table 1). Complex regional pain syndrome (CRPS) has two types, both occuring after trauma, and peripheral nerve injury exists only in CRPS type 2. The latest classification by the International Association for the Study of Pain (IASP) suggests that CRPS type 2 may be associated with neuropathic mechanisms [45]. Clinical studies have demonstrated increased CGRP serum levels in patients suffering from CRPS [46]. In painful neuroma patients, higher densities of CGRP-immunoreactive nerve fibers were observed in comparison to controls [47]. A retrospective clinical study validated the effectiveness of CGRP-targeting mAbs in CM patients who also suffered from NP. Interestingly, in these patients the anti-CGRP treatment significantly improved the Neuropathic Pain Scale scores. The limitation of this study is that it was open-label, not placebo-controlled trial and the numbers of the patients were very low [18]. Allodynia is the common clinical feature of both migraine and NP. Peripheral and central sensitization, allodynia and responsiveness to anti-CGRP mAbs point to the potential common role of CGRP both in migraine and NP.

3. Kynurenine function in migraine and neuropathic pain

3.1. Migraine

The KYN pathway is the metabolic pathway of tryptophan catabolism. The determinative tryptophan degradation product is L-KYN, which serves as a precursor for KYNA. KYNA is one of the rare endogenous antagonists of excitatory amino acid receptors. By affecting glutamate receptors, it has a role in pain processing and neurogenic inflammation [6-8, 48, 49], as well as in cognitive dysfunctions. The sites of central sensitization are the second-order neurons of the TCC. This sensitization is induced by the release of glutamate from C-fibers of the central branch of pseudounipolar trigeminal neurons. Calcium ion influx and opened calcium storage in the cells result in increased intracellular calcium ion levels, which activate protein kinase C and lead to the phosphorylation of NMDA receptors. This process results in increased glutamate sensitivity, which is the basis for the hyperexcitability of the neurons [6,50].

Related to the above-mentioned process, clinical studies were performed using different body fluids including plasma, serum, cerebrospinal fluid (CSF), and saliva. Higher plasma glutamic acid levels were observed both during attacks and pain-free periods in M0 and MA patients (Table 2) [51]. High levels of glutamic

acid in platelets were detected in patients with MA compared to M0 patients and healthy controls. Furthermore, glutamic acid platelet concentrations were higher ictally in MA patients [52].

Table 2. Selected human clinical data related to the glutamate and the kynurenine system in migraine and neuropathic pain.

Migraine					ref.
EM				CM	
M0		MA			
Ictally	Interictally	Ictally	Interictally		

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↑ glutamic acid (plasma)	↑ glutamic acid (plasma)	↑ glutamic acid (plasma)	↑ glutamic acid (plasma)	nd	[51]
nd	nd	↑ glutamic acid (platelet)	↑ glutamic acid (platelet)	nd	[52]
↓ glutamic acid (plasma)	nd	↓ glutamic (plasma)	nd	nd	[53]
↑ glutamic acid (CSF)	nd	↑ glutamic acid (CSF)	nd	nd	[53]
nd	↑ glutamic acid (saliva)	nd	nd	nd	[54]
nd	nd	nd	nd	↑ glutamic acid (CSF)	[55]
nd	nd	nd	nd	↓ KYNA (serum)	[66]
nd	↓ L-KYN, KYNA, anthranilic acid, picolinic acid, 5-hydroxy-indoleaceticacid (plasma)	nd	nd	nd	[67]
Neuropathic pain					**ref.**
Peripheral NP			**Central NP**		
↑ L-glutamate (plasma) in CRPS ↓ L-TRP (plasma) in CRPS ↑ the KYN/TRP ratio			nd		[73]
negative correlation (TRP serum level and pain intensity) in temporomandibular disorders myalgia positive correlation the KYN/TRP ratio and pain intensity) in temporomandibular disorders myalgia			nd		[74]

↑: increased concentration; ↓: decreased concentration; Abbreviations: CM: chronic migraine, CRPS: complex regional pain syndrome, CSF: cerebrospinal fluid, EM: episodic migraine, KYNA: kynurenic acid, L-KYN: L-kynurenine, M0: migraine without aura, MA: migraine with aura, NP: neuropathic pain, nd: no data available, Trp: tryptophan.

In EM M0 and MA patients, plasma levels of glutamic acid were lower during attacks, while CSF concentrations of glutamic acid were higher in migraineurs than in controls [53]. Interictally, in

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the saliva of M0 patients, elevated glutamic acid concentrations were reported [54]. High glutamate concentration in the CSF of CM patients compared to controls was also demonstrated [55].

Imaging studies in migraine patients attempted to find a link between the glutamatergic system and specific brain regions. Altered excitatory neurotransmitter distribution in the anterior cingulate cortex and insula of migraineurs was observed by magnetic resonance imaging spectroscopy (MRI) [56]. Meta-analysis of MRI spectroscopy data during pain-free periods in patients suffering from M0, MA or CM revealed increased glutamate concentrations in particular brain regions [57,58]. In M0 patients, during a resting state functional MRI, altered periaqueductal gray matter functional connectivity (as a brainstem migraine generator and a pain modulator) was detected and correlated with plasma tryptophan concentration, both of which were higher in migraineurs than controls [59–61].

A rare subtype of MA, familial hemiplegic migraine (FHM) can be divided into three subclasses: FHM1, FHM2 and FHM3. The following genetic mutations led to the alteration of the glutamate system: In the patients suffering from FHM1, *CACNA1A* (encoding the α1 subunit of neuronal Ca,2.1. Ca^{2+} channels) gene disruption results in glutamate release from presynaptic nerve terminals. In FHM2 patients, the *ATP1A2* gene (encoding the α2 subunit of Na^{+}/K^{+} ATPase pumps) is damaged and indirectly reduces uptake of glutamate from the synaptic cleft in astrocytes. In FHM3, the *SCNA1* (encoding the pore-forming α1 subunit of neuronal Nav1.1 Na^{+} channels) gene lesion can reduce firing of inhibitory interneurons and can increase glutamate levels in the synaptic cleft [62–65]. By studying these rare subtypes of MA, the role of glutamate has become better known.

CM is a distinct subclass of migraine, which develops if the patient suffers from more than 15 headache days per month, of which at least 8 days are with M0 or MA, for three consecutive months [1]. In CM, which is a devastating form of migraine headache that greatly affects quality of life, altered KYN pathway metabolites and reduction in serum levels of KYNA have been observed [66].

In a well-designed clinical trial examining female M0 patients during headache-free periods, plasma concentrations of tryptophan metabolites (L-KYN, KYNA, anthranilic acid, picolinic acid, 5-hydroxy-indoleacetic acid) were significantly decreased. The diminished peripheral tryptophan catabolism during the interictal period might act as a trigger for the migraine attack [67]. The first in-human, phase 1, open-label, single ascending dose study of L-KYN administered via intravenous infusion in healthy volunteers revealed that L-KYN was safe and well-tolerated [68].

3.2. Neuropathic pain

A clinical sign of central sensitization is the phenomenon of allodynia, which mirrors the activation of the glutamatergic system in NP [6,19,69]. Overactive glutamatergic transmission via NMDA receptors is the basis of central sensitization in NP. Blocking the allosteric glycine B co-agonist site on NMDA receptors leads to antagonism of the glutamate system. L-4-chlorokynurenine, a novel oral prodrug, is a potent and selective glycine B site inhibitor.

A cross-over randomized controlled trial (RCT) revealed that NGX426, an oral AMPA-kainate receptor antagonist, reduced capsaicin-induced pain and hyperalgesia in healthy volunteers [70]. A Phase 2 outpatient RCT examining LY545694 tosylate, a potent and selective ionotropic glutamate receptor antagonist, in PDN patients did not demonstrate a difference compared to the placebo [71]. A dose-escalation RCT demonstrated a consistent reduction of allodynia and mechanical and heat hyperalgesia in an intradermally capsaicin-induced pain model in healthy volunteers [72].

In CRPS patients, plasma levels of L-glutamate significantly increased, whereas L-trp significantly decreased when compared to controls. The L-KYN to L-trp (KYN/Trp) ratio exhibited a significant increase in patients. A significant correlation between overall pain and plasma levels of L-glutamate and the KYN/Trp ratio was detected. A correlation between the decrease in plasma L-tryptophan concentration and disease duration was also observed in CRPS patients (Table 2) [73].

An exploratory pilot study involving female patients with NP-like syndromes, such as temporomandibular disorders myalgia and fibromyalgia, showed associations between the KYN/Trp ratio and pain intensity. In temporomandibular disorders myalgia, a significant negative correlation between plasma tryptophan concentration and worst pain intensity, and a positive correlation

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between the KYN/Trp ratio and both worst and average pain intensity were observed. Women suffering from fibromyalgia exhibited significantly lower plasma tryptophan levels than controls [74].

In addition to neuropeptides, the pathomechanism of hyperexcitability and sensitization is an overactivated glutamate system, both in migraine and NP. Alteration of the KYN system has been reported in these two painful clinical conditions. Metabolites of the KYN pathway might have future therapeutic potential for migraine and NP.

4. Glial function in migraine and neuropathic pain

4.1. Migraine

The trigeminovascular system is the backbone of the most accepted hypothesis for migraine pathogenesis. The center of this system is the trigeminal ganglion, which involves pseudounipolar neurons and satellite glial cells. There are strong data that glial cells have a role in peripheral sensitization and neuroinflammation, which lead to migraine chronification and the development of autonomic symptoms during migraine attacks [75,76].

S100B is a calcium-binding protein in the cytoplasm of glial cells in the nervous system, and it is a sensitive marker for glial cell injury. Clinical studies used it as a biomarker for the detection of glial involvement in the pathomechanism in EM and CM patients, ictally and interictally. Unfortunately, the results are controversial. In a clinical study which was performed during and after migraine attacks (2-4 days), the serum concentration of S100B was elevated (Table 3) [77]. A trial of M0 patients revealed increased serum S100B levels in both ictal and interictal phases [20]. A cross-sectional prospective study of M0 and MA patients revealed that serum S100B levels were significantly lower than in controls, and the two study groups did not differ [78]. A pilot RCT in CM patients revealed that ibudilast (a potential glia inhibitor) treatment did not reduce the frequency of headache, but it was well-tolerated [79]. In a case-control trial, serum levels of S100B were analyzed in EM and CM patients, and the results showed no interictal S100B elevation [80]. In EM and CM patients, the serum level of S100B was significantly higher compared to controls, and there was no difference between the two patient groups [81].

Table 3. Selected human clinical data related to glia function in migraine and neuropathic pain.

Migraine					**ref.**
EM				**CM**	
M0		**MA**			
Ictally	**Interictally**	**Ictally**	**Interictally**		
↑ S100B (serum)	↑ S100B (serum)	nd	nd	nd	[77]
↑ S100B (serum)	↑ S100B (serum)	nd	nd	nd	[20]
nd	↓ S100B (serum)	nd	↓ S100B (serum)	nd	[78]
nd	↑ S100B (serum)	nd	nd	↑ S100B (serum)	[80]
nd	↑ S100B (serum)	nd	nd	↑ S100B (serum)	[81]
Neuropathic pain					**ref.**
Peripheral NP			**Central NP**		

 doi:10.20944/preprints202301.0034.v1

activated glial cells (PET): in thalamus, anterior and posterior central gyrus, paracentral lobule	nd	[90] [9]

↑: increased concentration; ↓: decreased concentration; Abbreviations: CM: chronic migraine, CRPS: complex regional pain syndrome, CSF: cerebrospinal fluid, EM: episodic migraine, KYNA: kynurenic acid, L-KYN: L-kynurenine, M0: migraine without aura, MA: migraine with aura, NP: neuropathic pain, nd: no data available, PET: positron emission tomography.

4.2. Neuropathic pain

In a case of peripheral nerve lesion (peripheral arm of neurons of the dorsal root ganglia), one of the main consequences was ATP release from the central terminals in the dorsal horn of the spinal cord. ATP acts on microglia via purinergic P2X4 receptors and results in the release of brain-derived neurotrophic factor from the activated glial cells. This trophic factor stimulates second-order neurons via the activation of tyrosine kinase B receptors. The result of this process is central sensitization of the second-order neurons leading to the development of allodynia as a main clinical sensory sign of NP. It also leads to overactivation of the third-order neurons in the thalamus [6,82–87]. Based on these findings, NP can be considered a gliopathy [88, 89].

An early PET study, using a sensitive *in vivo* marker of glial cell activation, demonstrated activated glial cells in the contralateral thalamus after limb amputation, which pointed to a long-term transsynaptic glial response in the central nervous system (CNS) following peripheral nerve injury (Table 3) [90]. A functional imaging technology using novelly synthesized glia-PET tracers emphasizes the importance of neuron-microglia interactions in the mechanism of NP [9].

Preclinical studies confirmed that opioids could activate microglia *via* the toll-like receptor 4 and the myeloid differentiation factor 2 receptor complex. The consequence was an activated mitogen-activated protein kinase (MAPK) system, which resulted in interleukin gene activation, leading to neuroinflammation [91,92].

Motor cortex stimulation is a potential therapeutic method for relief of NP. In a clinical study, epidural strips were implanted over the motor cortex in central post-stroke NP patients and one trigeminal nerve injury NP patient. A comparison of postoperative PET with preoperative scans demonstrated significant decreases in a tracer, [(11)C]diprenorphine, binding to opioid receptors in different brain areas. Binding changes in the anterior middle cingulate cortex and periaqueductal gray matter were significantly correlated with pain relief [93]. A brain imaging study (integrated PET/MRI) with the new generation ligand 11C-PBR28 of translocator protein (TSPO), as a marker of glial activation, demonstrated increased binding to the pain matrix in chronic low back pain patients [94]. Peripheral benzodiazepine receptor, 18 kDa TSPO, is upregulated in activated microglia. PET imaging studies using a specific tracer related to TSPO showed higher activation of the thalamus, anterior and posterior central gyri and paracentral lobule in pain patients *versus* controls[9].

The inhibition of the activated microglia in NP might be a novel therapeutic target. A CNS glial modulator, propentofylline, administered orally, failed to decrease pain in PHN patients in a proof-of-concept clinical trial (Protocol SLC022/201, EudraCT number 2008-002108-24). Activated p38 MAPK in spinal microglia was detected in peripheral nerve injury-associated NP [95]. A RCT in patients suffering from peripheral NP following nerve injury treated with p38 MAPK inhibitor (dilmapimod) revealed significantly decreased daily average pain scores [95]. An *in vitro* study revealed that human and rodent microglia responded differently to propentofylline (SLC022) [96]. Another p38 MAPK inhibitor, losmapimod, which was investigated in a RCT in patients with NP from lumbosacral radiculopathy, failed to decrease pain intensity [97].

In a prospective, open-label, pilot RCT in PDN patients, minocycline, a tetracycline antibiotic as a microglial inhibitor, significantly improved Visual Analog Scale scores [98]. In another clinical trial involving NP patients, minocycline failed to decrease pain intensity [99]. The neuron-microglia interaction is a rate-limiting step in sensitization, both in migraine and NP. The overactivated glial

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cells have potential effects in the trigeminal ganglia during the pain process in migraine, while they show activation in the dorsal horn of the spinal cord and in the thalamus in NP. However, the results regarding glial biomarkers, like S100B protein, are controversial in migraine. Modern functional imaging techniques are available to detect the presence of hyperactivated glia in the CNS in NP patients. Unfortunately, thus far, microglia inhibitors have failed to reduce pain intensity in these conditions.

5. Cytokine function in migraine and neuropathic pain

5.1. Migraine

In the late eighties and early nineties, clinical studies demonstrated that intravenously administered TNF produced headache in tumorous patients [100–103]. There is growing evidence that cytokines play a role in a genesis of migraine pain. They are released by neurons, microglia, astrocytes, macrophages, mast cells and T-cells. Human studies reflect that the pro-inflammatory cytokines are TNF-alpha, IL-1beta, IL-6 and IL-18 [15,76]. Given that, a balance of pro- and anti-inflammatory cytokines is important for neural functions. The pro-inflammatory cytokines may have a role in inducing nausea and headache during a migraine attack by increasing arachnoid acid production [15]. Several trials investigated the pro- and anti-inflammatory cytokines in plasma, saliva and CSF of migraineurs. In an early clinical study, there were no differences in plasma IL-1 and TNF during migraine attacks compared to headache-free periods in M0 and MA patients (Table 4) [105].

Table 4. Selected human clinical data related to cytokines in migraine and neuropathic pain.

Migraine					**ref.**
EM				**CM**	
M0		**MA**			
Ictally	**Interictally**	**Ictally**	**Interictally**		
=IL-1, TNF (plasma)	=IL-1, TNF (plasma)	=IL-1, TNF (plasma)	=IL-1, TNF (plasma)	nd	[104]
↑ IL-6 (urine) =IL-1beta (urine) ↓ TNFalpha (urine)	↑ IL-6 (urine) =IL-1beta (urine) ↓ TNFalpha (urine)	nd	nd	nd	[105]
nd	=TNFalpha, IL-6 (serum)	nd	=TNFalpha, IL-6 (serum)	nd	[106]
nd	↑ TNFalpha, IL-1beta, IL-10 (plasma)	nd	nd	nd	[106]
↑ TNFalpha (serum)	nd	nd	nd	nd	[108]
↑ IL-10, IL-6 (serum)	nd	nd	nd	nd	[109]
↑ IL-1 receptor	nd	↑ IL-1 receptor	nd	nd	[108]

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antagonist (CSF)		antagonist (CSF)			
=TNFalpha (serum)	nd	nd	nd	nd	[110]
↑ IL-1beta, IL-6 (serum) ↑ Il-10 (serum)	nd	nd	nd	nd	[111]
↑ IL-6 (serum)	nd	↑ IL-6 (serum)	nd	nd	[112]
↑ TNFalpha , IL-1beta, IL-6 (serum)	nd	nd	nd	nd	[113]
nd	↓ IL-6 (mRNA and serum)	nd	nd	nd	[114]
nd	nd	nd	nd	↑ TNFalpha, L-6 (serum)	[115]
nd	↑ IL-1beta (saliva)	nd	nd	nd	[116]
↑ IL-18 (serum)	↑ IL-18 (serum)	↑ IL-18 (serum)	↑ IL-18 (serum)	nd	[117]
nd	nd	nd	↑ IL-6 (mRNA)	nd	[124]
nd	↑ IL-4, IL-18, TGFbeta, TNFalpha (mRNA) =IL-1beta, IL-17, IL-2 (mRNA)	nd	↑ IL-4, IL-18, TGFbeta, TNFalpha (mRNA) =IL-1beta, IL-17, IL-2 (mRNA)	nd	[125]
Neuropathic pain					**ref.**
Peripheral NP			**Central NP**		
↑ TNFalpha expression (Schwann cells)			nd		[132]
↑ IL-2, TNFalpha (mRNA, plasma) ↓ IL-4, IL-10 (mRNA, plasma)			nd		[133]

 doi:10.20944/preprints202301.0034.v1

↑ TNFalpha (serum) in PDN	nd	[134]
↑ TNFalpha (plasma) in PDN	nd	[135]
↑ IL-6 (serum) in painful DSPN	nd	[140]
↑ TNF-alpha, IL-1beta (mRNA) ↓ IL-10 (mRNA) =IL-4 (mRNA) (in NP after peripheral nerve lesion)	nd	[16]

↑: increased concentration; ↓: decreased concentration; =: no change; Abbreviations: CM: chronic migraine, CSF: cerebrospinal fluid, DSPN: distal sensori-motor polyneuropathy, EM: episodic migraine, IL: interleukin, M0: migraine without aura, MA: migraine with aura, mRNA: messenger ribonucleic acid, NP: neuropathic pain, nd: no data available, PDN: painful diabetic neuropathy, TGF: tumor growth factor, TNF: tumor necrosis factor

A clinical trial analyzed IL-1beta, IL-6 and TNF-alpha in 24-hour urine samples in female migraineurs, during menstrual and non-menstrual migraine attacks and headache-free periods and compared them with non-headache controls. Mean IL-6 levels in urine were higher in all three samples of migraineurs *versus* controls, while IL-1beta levels showed no difference. TNF-alpha values were very low in menstrual migraineurs compared to controls [105].

Another clinical trial failed to demonstrate differences in serum concentrations of TNF-alpha and IL-6 between M0 and MA patients compared to controls, however the soluble receptor TNF-RI tended to be lower [106]. A clinical study of migraine patients revealed that the plasma levels of TNF-alpha, IL-1beta and IL-10, as an anti-inflammatory cytokine, were significantly higher ictally *versus* interictally [107]. TNF-alpha levels in internal jugular blood of M0 patients was elevated during ictal periods [108]. A clinical trial demonstrated that IL-10 serum levels were higher during migraine attacks *versus* the intervallum period and healthy controls. Furthermore, IL-6 serum concentrations were increased in migraineurs compared to controls [109]. A pilot study of EM patients demonstrated no significant difference in the serum levels of TNF-alpha during the attacks or headache-free periods [110]. A case-control study investigating newly diagnosed migraine patients revealed significantly higher serum IL-1beta and IL-6 concentrations, while IL-10 serum levels were lower compared to healthy controls [111]. A clinical study investigating M0 and MA patients in both attack and pain-free periods revealed that serum levels of IL-6 were significantly higher in migraine patients during attacks compared to controls [112].

A prospective, case-control RCT in migraineurs concluded that the serum concentrations of TNFalpha, IL-1beta and IL-6 were significantly higher during the migraine attack compared to controls [113]. In the MOXY study, which studied female migraineurs responsive to adjunctive cervical non-invasive vagus nerve stimulation, interictal saliva ELISA assays of IL-1beta showed significantly elevated values both pre- and post-VNS procedure compared to healthy controls [114]. The evaluation of the inflammatory state in migraineurs *versus* healthy controls in a case-control study demonstrated that the serum levels of TNF-alpha and IL-6 were significantly increased in CM as opposed to EM patients and controls [115]. The SalHead longitudinal prospective cohort study, analyzing salivatory IL-6 and IL-1beta levels, observed non-significant differences at different time-points (headache-free period *versus* during headache *versus* one day after headache) between migraine patients and tension-type headache patients. Salivatory levels of IL-1beta showed the highest discriminative value between headache patients and healthy controls [116]. Investigation of serum IL-18 (previously interferon-gamma) levels in M0 and MA patients, ictally and interictally,

 doi:10.20944/preprints202301.0034.v1

revealed that they were higher in migraineurs than in the control group. IL-18 serum concentrations did not show differences ictally *versus* interictally [117].

An interesting study, analyzing the pro- and anti-inflammatory cytokine levels in the CSF, revealed that IL-1 receptor antagonist levels were elevated in M0 and MA patients during attacks compared to controls. There were significant differences in the CSF levels of certain cytokines (IL-1 receptor antagonist, monocyte chemoattractant protein-1 and transforming growth factor-beta1) in migraine and episodic tension-type headache compared to pain-free controls. The intrathecal pro-inflammatory monocyte chemoattractant protein-1 level was correlated with IL-10 anti-inflammatory cytokine in MA patients [118].

The analysis of genetic variations of cytokines provided useful data regarding the neurinflammation process of migraine. A genetic study showed significant differences in the TNF-alpha -308G/A and IL-1beta +3953C/T gene polymorphisms in migraineurs *versus* control subjects [119]. A meta-analysis from 2011, focusing on TNF-alpha 308G>A and TNFbeta 252A>G gene polymorphisms among migraine patients, concluded no overall association between the above-mentioned gene variants and migraine [120]. Another meta-analyis, published in 2014, revealed that TNF-beta 252A>G gene polymorphism was not associated with overall migraine risk [121]. A clinical study analyzing omega-3 fatty acids and nano-curcumin supplementation targeting TNF-alpha gene expression and serum concentrations in migraine patients demonstrated that the TNF-alpha messenger ribonucleic acid (mRNA) was significantly downregulated, and the serum level of TNF-alpha was decreased [122].

A RCT in EM patients revealed downregulated IL-6 mRNA and decreased IL-6 serum concentrations [123]. Investigation of the IL-6 coding gene in peripheral blood of M0 and MA patients demonstrated no significant differences in expression of IL-6 between total migraine patients and healthy controls. However, the expression of IL-6 was significantly higher in MA patients *versus* controls [124]. A clinical study investigating cytokine-coding gene expression in blood among M0 and MA patients revealed that the expression of IL-4, tumor growth factor-beta (TGF-beta) and TNF-alpha was increased in patients compared to controls, but there was no difference in expression levels of IL-1beta, IL-17 and IL-2. Expression of IL-18 was also higher in migraineurs (lower in women than in men) compared to healthy controls [125]. A genetic study focusing on the TNF-alpha gene polymorphisms (rs1800629 and rs1799724) among Jordanian migraineurs showed significant associations with migraine occurrence [126]. For future therapeutic innovations in migraine, IL-37, as an anti-inflammatory cytokine, may be a crucial player. IL-37, as a natural inhibitor of immune response and inflammation, can diminish pro-inflammatory IL-1 activation and upregulate the anti-inflammatory IL-10 [15]. A recent meta-analysis of peripheral inflammatory cytokines in migraine concluded that IL-1beta, IL-6 and TNF-alpha serum levels were higher when compared to healthy controls, while IL-2 and IL-10 (an anti-inflammatory cytokine) did not show significant differences [127].

5.2. Neuropathic pain

There is increasing evidence that cytokine expression is a contributor to NP [128]. In the development of NP, TNF-alpha, IL-1, and IL-6 may have fundamental roles in inflammation [129]. Cytokine action sites involve peripheral nerve endings, dorsal root ganglia, the synaptic junction in the dorsal horn of the spinal cord and distinct regions of the brain (like the hippocampus, locus coeruleus, red nucleus) [129–131].

A clinical investigation of nerve biopsies in painful and non-painful neuropathic patients revealed upregulated TNF-alpha expression in human Schwann cells in the painful group (Table 4) [132]. A clinical study focusing on mRNA expression and plasma protein levels of cytokines in patients who have painful *versus* painless neuropathies demonstrated that both measured parameters of pro-inflammatory cytokines (IL-2 and TNF-alpha) were increased in the painful patient group, while IL-4 and IL-10, as anti-inflammatory cytokines, were lower in this group compared to painless patients [133]. A clinical investigation of PDN and painless diabetic neuropathic patients indicated

increased TNF-alpha serum levels in the neuropathic group compared to non-neuropathic and healthy groups [134].

A prospective genetic study analyzing local (skin) and systemic (plasma) cytokine gene expression in patients suffering small fiber sensory neuropathy revealed that local gene expression of IL-6 and IL-8 (chemokine) were significantly increased (5-fold), while only mildly elevated gene expression of IL-2 and IL-10 was detected in the plasma (2-fold) [128].

A cross-sectional study revealed that plasma TNF-alpha levels and immunoreactivity for TNF-alpha were higher in patients with severe pain, based on VAS in PDN patients, compared with controls [135]. A prospective RCT in patients suffering low back and leg pain, caused by lumbar disc herniation and lumbar spinal canal stenosis, who were treated with epidurally administered etanercept (an anti-TNF mAb) *versus* dexamethasone, demonstrated that etanercept significantly decreased both leg and low back pain [136]. The same clinician group published the results of a clinical trial using epidurally applied tocilizumab onto the spinal nerve as an anti-IL-6 receptor antibody for patients with low back and radicular leg pain caused by lumbar spinal stenosis. They concluded that infiltration of tocilizumab was more effective than dexamethasone in these patient groups [137]. A double-blind, placebo-controlled trial evaluating the analgesic effect of losmapimod (a p38 alpha/beta inhibitor) in patients with NP after peripheral nerve injury revealed that losmapimod statistically did not differ from analgesic response to the placebo [138].

A prospective study of patients with painful or painless peripheral neuropathy demonstrated that painful neuropathies are associated with increased pro-inflammatory IL-6 and anti-inflammatory IL-10 gene expression in the sural nerve [139].

A clinical trial including patients with painful distal sensorimotor polyneuropathy (DSPN) from the German KORA F4 survey found positive associations between serum concentrations of IL-6 and painful DSPN, whereas no associations were observed for IL-18, TNF-alpha and IL-1 receptor antagonists [140]. A parallel-group RCT, in patients suffering central NP associated with spinal cord injury, revealed that in the anti-inflammatory diet treatment group the serum levels of pro-inflammatory cytokines, such as interferon-gamma (later named IL-18), IL-1beta and IL-6 were decreased [141]. A cross-sectional study assessing different serum biomarkers, including cytokines (oncostatin, TNFSF10, TNFSG12, TNFSF14), in patients with diabetic polyneuropathy did not find differences in biomarker levels between painful and painless DSPN patients [142].

A genetic trial focused on patients with and without NP after peripheral nerve lesion revealed that in white blood cells the gene expression of TNF-alpha was higher in patients with pain compared to those without. IL-1beta gene expression was higher in painful patients compared to controls. IL-10 showed lower gene expression than in the control group, and IL-4 gene expression was not different between the control and painless patients [16].

A pilot RCT in patients with peripheral NP due to PHN examined mRNA expression of IL-6 in two study groups. In group 1, PHN-related NP patients received pregabalin monotherapy alone, while Group 2 patients were treated with a combination of pregabalin together with cognitive behavioral therapy. The results showed that patients in Group 2 had significant downregulation of IL-6 mRNA expression [143].

A recent meta-analysis focused on the association of pro-inflammatory (TNF-alpha, IL-2, IL-6, IL-18) and anti-inflammatory (IL-10) cytokines, as systemic inflammatory biomarkers in painful and painless diabetic neuropathy. It concluded that serum levels of pro-inflammatory markers were increased, while anti-inflammatory ones were lower in painful compared to painless diabetic polyneuropathy [21].

In migraine, whether in ictal or interictal phases, the data regarding cytokines are controversial, but pro-inflammatory cytokines tend to be elevated in human clinical trials. In the NP patients, the levels of pro-inflammatory cytokines also showed elevation compared to controls in the majority of the clinical studies.

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6. Transient ion channel function in migraine and neuropathic pain

6.1. Migraine

TRP ion channels are non-selective cation channels and can be divided into six subfamilies: TRPV (vanilloid), TRPA (ankyrin), TRPM (melastatin), TRPC (canonical), TRPP (polycystin) and TRPML (mucolipin) [144]. Preclinical studies have concluded the putative role of TRPs in migraine. The activation of TRPs (TRPA-1, TRPV-1) in the TCC results in CGRP and substance P release and depletion from the central branch of trigeminal nerve endings. The consequences are overexcited, second-order, pain-processing neurons in the TCC, which lead to central sensitization. The peripheral parts of the trigeminal nerve terminals, projecting into cerebral dura mater and the vasculature of the meninges, contain TRPs. The activation of TRPs leads to CGRP release, which can act on its receptors on the smooth muscle cells of blood vessels, resulting in strong vasodilation [144,145].

Capsaicin, as a potent and highly selective TRPV-1 receptor agonist, is a chemical compound isolated from chili pepper. A RCT using intranasal civamide (a synthetic stereoisomer of capsaicin) for M0 and MA patients during headache attack revealed decreased pain severity at 2 hours post-dose in 55.6% of patients, and 22.2% of patients were pain-free [146]. A double-blind study in CM patients demonstrated that repeated intranasal capsaicin application had favorable effects [147]. A single-blind, placebo-controlled, cross-over study in small numbers of M0 patients, who were treated with topical capsaicin (0.1%) jelly, led to relief of arterial pain by at least 50% [148].

TRPM-8, a non-selective cation channel, can be activated by cold temperature and menthol. A triple-blind RCT revealed that a 10% menthol solution applied to the forehead and temporal skin areas was significantly superior to the placebo at 2-hour pain freedom [149].

Extensive preclinical studies focusing on TRP ion channels concluded that TRPA-1 and TRPV-1 could play crucial roles in the activation of several substances (as migraine triggers) that evoke migraine pain. Odors (cigarette smoke, formalin, *Umbellularia californica* – *'headache tree'*) are triggering and worsening factors for migraine attack via TRPA-1 receptor agonism. Other agents, such as *Tanacetum parthenium* (feverfew) and *Angelica sinensis* (dong quai, female ginseng), as desensitizing TRPA-1 receptor agonists, are migraine preemptive factors. A well-known migraine trigger factor, glyceryl trinitrate (nitroglycerine-NTG) as a nitric oxide donor, is also a TRPA-1 receptor agonist [22]. Long-term recognized migraine trigger factors are alcohol-containing drinks (ethanol), which are TRPV-1 receptor agonists. Capsaicin, as a pungent ingredient of paprika (*Capsicum*), is a desensitizing agonist of the TRPV-1 receptor [22].

An unusual clinical study, examining scalp arteries (superficial temporal and occipital arteries) of CM patients, demonstrated significantly increased TRPV-1-like immunoreactive nerve fiber density in the wall of arteries of CM patients *versus* control (Table 5) [150].

Table 5. Selected human clinical data related to TRP ion channels in migraine and neuropathic pain.

Migraine					**ref.**
EM				**CM**	
M0		**MA**			
Ictally	**Interictally**	**Ictally**	**Interictally**		
nd	nd	nd	nd	↑ TRPV-1-like immunoreactive nerve fibers density in the wall of scalp arteries	[150]
Neuropathic pain					**ref.**

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Peripheral NP	Central NP	
↓ pain intensity after 8% capsaicin patch treatment (in post-herpetic neuralgia, chronic postsurgical NP, post-traumatic NP, PDN, HIV-associated NP, painful radiculopathy, trigeminal neuralgia, chemotherapy-induced NP)	nd	[23] [159] [160] [161]

↑: increased concentration; ↓: decreased concentration; =: no change; Abbreviations: CM: chronic migraine, EM: episodic migraine, HIV: human immunodeficiency virus, IL: interleukin, M0: migraine without aura, MA: migraine with aura, NP: neuropathic pain, nd: no data available, PDN: painful diabetic neuropathy, TRP: transient receptor potential

A recent pilot study, searching for predictors of migraine chronification, investigated the 1911A>G single nucleotide polymorphism (SNP) in the TRPV-1 gene in patients with EM and CM compared to healthy subjects. The results showed that genotype frequency distribution in EM was comparable with controls, while it differed significantly in CM patients [151]. Another genetic study, among Spanish migraine patients, demonstrated association with migraine and SNPs of the TRPV-1 and TRPV-3 receptor genes [13,152]. The above discussed clinical data led to the design of early phase clinical trials targeting thermoTRP channels for migraine treatment, such as an oral TRPV-1 receptor antagonist (NCT00269022), a TRPM-8 receptor agonist (topical menthol 6%) (NCT01687101) and a TRPM-8 receptor antagonist (oral AMG 333) (NCT01953341) [153]. Final results are not yet available.

6.2. Neuropathic pain

Nociceptors are special afferent sensory neurons which convey thermal, mechanical and chemical stimuli. The members of the TRP family are densely expressed on nociceptors. Therefore, they have fundamental roles in nociception and NP transmission [154–157]. A high concentration (8%) capsaicin patch reversibly de-functionalizes nociceptive nerve terminals [14,158]. Based on the latest Cochrane Database, the 8% capsaicin patch is effective, well-tolerated and safe for treatment of PHN, HIV-neuropathy and PDN (Table 5) [23]. Clinical trials revealed that 8% capsaicin patch significantly reduced the average pain intensity in chronic postsurgical NP [159]. A retrospective observational study, collecting different types of peripheral NP patients (PHN, chronic postsurgical NP, post-traumatic NP, PDN, HIV-associated NP, painful radiculopathy, trigeminal neuralgia), demonstrated a reduction in pain intensity and pain area after 8% capsaicin patch application [160]. The 8% capsaicin patch can provide pain relief for up to 3 months or longer after a single 30-60 min application in chemotherapy-induced NP [161].

Early phase clinical studies targeting thermoTRP channels for NP treatment, including an intranasal TRPV-1 receptor agonist in PHN (NCT01886313), a subcutaneous TRPV-1 receptor inhibitor (parentide) in non-specified NPs (EP002846-21), an oral TRPV-3 receptor antagonist for non-specified NPs (NCT01463397) and a TRPM-8 receptor agonist (topical menthol 7%) for chemotherapy-induced peripheral neuropathy (NCT0185567) are under way [153]. Published data are not yet available.

Capsaicin, as a potent TRPV-1 receptor agonist, can decrease the intensity of pain either in migraine or NP *via* modulation of the release of pain-related neuropeptides from nociceptors. In migraine, several agents targeting TRPA-1 and TRPV-1 receptors can trigger or preempt the headache attacks. The high concentration (8%) capsaicin patch is strongly recommended for treatment of peripheral NPs such as PDN, PHN and HIV-neuropathy. Early phase clinical trials are ongoing both in migraine and NPs.

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7. Endocannabinoid Function in Migraine and Neuropathic Pain in Humans

7.1. Migraine

Endocannabinoids are endogenous cannabis-like substances. Chemically, they are characterized as small molecules and they are derived from arachidonic acid. As neurotransmitters, the endocannabinoids are part of the biological endocannabinoid system and they act on cannabinoid receptors (cannabinoid receptor (CBR) type 1 and CBR type 2-CBR-2). Their main endogenous ligands are anandamide (N-Arachidonoylethanolamine) and 2-arachidonoylglycerol (2-AG). Moreover, the system involves enzymes that regulate the synthesis and degradation of the ligands. CBR-1 is located in the nervous system – mainly in the brain, while CBR-2 is found in immune system [24].

In the endocannabinoid system, one of the main catabolic enzymes is fatty acid amide hydrolase (FAAH), which catabolizes fatty acid ethanolamides, such as anandamide. Other enzymes of this system include monoacylglycerol lipase (MAGL), diacylglycerol lipase alpha, diacylglycerol lipase beta and alpha/beta hydrolase domain 6. Fatty acid ethanolamides, together with 2-AG, are the main endocannabinoid signaling lipids interacting with CBR-1 and CBR-2 [24,162]. The endocannabinoid system seems to be dysfunctional and dysregulated in migraine. It interacts with migraine-related pathways, like the serotonin system (5-HT1A, 5-HT2A receptors), the migraine generator (periaqueductal grey matter), meningeal vessel dilatation and activation of the TCC [163,164].

In a clinical trial, the activity of FAAH and the specific anandamide membrane transporter (AMT) was measured in platelets taken from the peripheral blood of M0 patients and healthy controls. The results showed significant sex differences in the activity of FAAH and AMT in both study groups, namely an increase in the activity of FAAH and AMT was found only in female, but not male, M0 patients (Table 6) [165]. A study focused on the examination of anandamide, palmitoylethanolamide (PEA) and 2-AG concentrations in the CSF of patients with CM compared to control subjects. The results showed that CSF concentrations of anandamide were significantly lower, while those of PEA were significantly higher in CM patients *versus* the non-migraineur control group. 2-AG concentrations were below detection level in both patient and control groups [166].

Table 6. Selected human clinical data related to endocannabinoids in migraine and neuropathic pain.

Migraine					**ref.**
EM				**CM**	
M0		**MA**			
Ictally	**Interictally**	**Ictally**	**Interictally**		
nd	↑ FAAH and AMT (platelet) (only in female patients)	nd	nd	nd	[165]
nd	nd	nd	nd	↓ anandamide (CSF) ↑ PEA (CSF)	[166]
nd	nd	nd	nd	↓ AMT and FAAH (platelet)	[167]

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nd	nd	nd	nd	↓ anandamide and 2-AG	[168]
nd	=anandamide (plasma)	nd	=anandamide (plasma)	nd	[171]
Neuropathic pain					**ref.**
Peripheral NP		**Central NP**			
nd		=mean pain intensity after ultramicronized sublingually PEA treatment (NP associated with spinal cord injury)			[180]

↑: increased concentration; ↓: decreased concentration; =: no change; Abbreviations: 2-AG: 2-arachidonoylglycerol, AMT: anandamide membrane transporter, CM: chronic migraine, CSF: cerebrospinal fluid, EM: episodic migraine, FAAH: fatty acid amide hydrolase, M0: migraine without aura, MA: migraine with aura, NP: neuropathic pain, nd: no data available, PDN: painful diabetic neuropathy, PEA: palmitoylethanolamide

A comparative clinical trial demonstrated that the levels of AMT and FAAH were significantly reduced in platelets of CM patients compared to EM patients and the control group, and this was observed for both sexes [167]. A clinical study investigating the levels of anandamide, 2-AG and serotonin in platelets of CM patients and healthy controls found that anandamide and 2-AG platelet levels were significantly lower in CM patients *versus* controls. Furthermore, serotonin levels in platelets were also strongly reduced in the CM group and were correlated with 2-AG levels [168]. An observational, cross-sectional study comparing the binding of CBR-1, as detected by positron emission tomography (PET), among female migraine patients demonstrated a global increase, which was most pronounced in the anterior cingulate, mesial temporal, prefrontal, and superior frontal cortices of the brain in migraineurs compared to healthy controls [169].

A clinical trial was designed for migraineurs with medication-overuse headache, before and after withdrawal treatment. The results demonstrated a marked facilitation in spinal cord pain processing (increased temporal summation threshold of the nociceptive withdrawal reflex and reduction in related pain sensation) before withdrawal treatment when compared with controls. The acute significant reduction of FAAH activity in platelets was coupled with a reduction in the facilitation of pain processing after *versus* before withdrawal treatment [170]. A clinical study concluded that the plasma levels of anandamide and related N-acylethanolamines and linoleic acid-derived oxylipins did not show any differences between M0 *versus* MA patients and migraine *versus* healthy controls [171].

Results from a genetic study demonstrated a significant haplotypic effect of *CNR1* (the gene of CBR-1) on headache with migraine symptoms (e.g., nausea, photophobia, disability) only when using extreme trait combinations (0 symptom *versus* 3 symptoms) [172]. Later, the same research group reported that variants in the *CNR1* gene predisposed to headache with nausea in the presence of life stress. None of the SNPs showed primary genetic effects on possible migraine [173]. A RCT testing the effects of a 12-week aerobic exercise plan on plasma anandamide concentration and its relationship with clinical and cardiorespiratory outcomes in EM patients revealed plasma anandamide level reduction both in migraine and control exercise groups. Significant correlations were found between the reduction in abortive medication used and cardiorespiratory fitness and reduced anandamide plasma levels [174].

A recent pilot study in EM and CM with medication overuse headache patients demonstrated higher CBR-1 and CBR-2 gene and protein expression in peripheral blood mononuclear cells compared to controls. FAAH gene expression was lower in migraine groups compared to controls. Gene expression of MAGL was significantly higher in migraineurs [175]. In a clinical trial of EM

 doi:10.20944/preprints202301.0034.v1

patients and healthy controls, plasma anandamide and PEA, an anandamide activity enhancer, levels, and spinal sensitization were evaluated in a validated human model of migraine based on systemic NTG administration. After NTG administration, anandamide plasma levels were increased in both groups, while increased PEA plasma levels were detected only in the EM group [176].

7.2. Neuropathic pain

Endocannabinoids exert effects on a wide range of biological cell functions, like exocytosis, proliferation, differentiation, and control of pain transmission *via* inhibiting the ascending stimulatory and activating the descending inhibitory pain pathways [177,178]. An early clinical study of patients suffering painful carpal tunnel syndrome demonstrated that treatment with PEA (daily administered 600 mg or 1200 mg for 30 days) significantly reduced median nerve latency time during nerve conduction tests [179]. In a later RCT, ultramicronized PEA treatment (administered sublingually) was investigated in patients with spinal cord injury-associated NP. The results showed no difference in mean pain intensity between ultramicronized PEA and the placebo treatment (Table 6) [180]. Based on promising preclinical data with FAAH and MAGL, clinical trials with MAGL inhibitors are ongoing. A randomized, placebo-controlled, optimized titration study with a MAGL inhibitor (ABX-1431) in PDN patients (NCT03447756) and a double-blind, placebo-controlled, cross-over trial in central (multiple sclerosis-associated) NP patients (NCT03138421) are being conducted. In both clinical trials, favorable safety profiles were observed. Detailed results related to efficacy are not yet available [24]. The importance of the endocannabinoid system in pain modulation has been known since the early 1990s. Based on clinical data of the ligands and enzymes in this system, a correlation has been shown with migraine. Only limited clinical data on how compounds of this system affect NP are available. In the future, CBR antagonists and FAAH and MAGL enzyme inhibitors might be promising therapeutic targets in treatment of both migraine and NP.

8. Conclusion

In this review we have summarized the available human clinical data and novel therapeutic approaches which overlap between migraine and NP, focusing on CGRP, KYNs, microglia, cytokines, TRP ion channels and endocannabinoids. Potential common factors in the pathomechanisms of migraine and NP are hyperexcitability and peripheral and central sensitization, which result in allodynia as a common clinical symptom of both painful disorders. One of the fundamental players of this process is CGRP. There are data from clinical trials indicating that CGRP-targeting mAbs treatment reduces pain intensity in both migraine and NP. Another participant in this process is an overactivated glutamate system. Altered metabolites in the KYN pathway have been observed both in migraine and NP, thus KYNs might offer valuable therapeutic targets for both entities. Common anatomical sites of neuron-microglia interactions are the trigeminal and dorsal root ganglia, the dorsal horn of the spinal cord and the thalamus. There are available human data which show overactivated glial function in the above-mentioned pain-related regions, so the innovation of microglial inhibitors as therapeutic targets, both in migraine and NP, is needed in the near future.

The results of preclinical studies indicate significant roles of pro- and anti-inflammatory cytokines in the hypothesized inflammatory processes of migraine and NP. Despite controversial human data regarding migraines, in NP elevated pro-inflammatory cytokines levels have been detected. Distinct TRP ion channels have marked roles in the pathomechanisms of both migraine and NP. Results of the clinical research have led to therapeutic options for migraines, while evidence in several types of peripheral NP highly recommends them. The endocannabinoids show shared mechanisms for migraine and NP *via* trigeminovascular and pain transmission systems. Future therapeutic possibilities might include CBR antagonists and FAAH and MAGL modulators.

In this review, human data on potential common factors in migraine and NP were analyzed, which support the hypothesis that these painful disorders have shared pathomechanical backgrounds. In the near future all of the substances discussed above have the potential to serve as biomarkers and novel therapeutic targets in both migraine and NP.

 doi:10.20944/preprints202301.0034.v1

Author Contributions: Conceptualization, J.T., D.S., A.C., and L.V.; writing—original draft preparation, J.T., D.S., and A.C.; writing—review and editing, M.T. and L.V.; visualization, Á.S.; supervision, L.V.; project administration, L.V.; funding acquisition, L.V.All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by OTKA-138125-K, TUDFO/47138-1/2019-ITM, and ELKH-SZTE Eötvös Loránd Research Network and the University of Szeged.

Acknowledgments: We are grateful to Jennifer Tusz, a native English language speaker for the corrections (e-mail: jentusz@gmail.com).

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

2-AG	2-arachidonoylglycerol
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMT	anandamide membrane transporter
CBR	cannabinoid receptor
CGRP	calcitonin gene-related peptide
CM	chronic migraine
CNS	central nervous system
CRPS	complex regional pain syndrome
CSF	cerebrospinal fluid
EM	episodic migraine
FAAH	fatty acid amide hydrolase
FHM	familial hemiplegic migraine
HIV	human immunodeficiency virus
IL	interleukin
KYN	Kynurenines
KYNA	kynurenic acid
L-KYN	L-kynurenine
M0	migraine without aura
MA	migraine with aura
mAbs	monoclonal antibodies
MAGL	monoacylglycerol lipase
MAPK	mitogen-activated protein kinase
mRNA	messenger ribonucleic acid
nd	no data available
NMDA	glutamatergic N-methyl-D-aspartate
NP	neuropathic pain
NTG	nitroglycerine
PDN	painful diabetic neuropathy
PEA	palmitoylethanolamide
PET	positron emission tomography
PHN	postherpetic neuralgia
RCT	randomized controlled trial

TCC	trigemino-cevical complex
TNF-alpha	tumor necrosis factor alpha
Trp	tryptophan
TRP	transient receptor potential
TRPA-1	transient receptor potential ankyrin 1
TRPM-8	transient receptor potential melastatin 8
TRPV-1	transient receptor potential vanilloid 1
TSPO	translocator protein

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