CGRP Receptor Antagonism and Migraine

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Summary: Calcitonin gene-related peptide (CGRP) is expressed throughout the central and peripheral nervous systems, consistent with control of vasodilatation, nociception, motor function, secretion, and olfaction. αCGRP is prominently localized in primary spinal afferent C and AΔ fibers of sensory ganglia, and βCGRP is the main isoform in the enteric nervous system. In the CNS there is a wide distribution of CGRP-containing neurons, with the highest levels occurring in striatum, amygdala, colliculi, and cerebellum. The peripheral projections are involved in neurogenic vasodilatation and inflammation, and central release induces hyperalgesia. CGRP is released from trigeminal nerves in migraine. Trigeminal nerve activation results in antidromic release of CGRP to cause non-endothelium-mediated vasodilatation. At the central synapses in the trigeminal nucleus caudalis, CGRP acts postjunctionally on second-order neurons to transmit pain signals centrally via the brainstem and midbrain to the thalamus and higher cortical pain regions. Recently developed CGRP receptor antagonists are effective at aborting acute migraine attacks. They may act both centrally and peripherally to attenuate signaling within the trigeminovascular pathway. **Key Words:** Migraine, CGRP, trigeminovascular, CGRP receptor antagonists.

INTRODUCTION

Primary head-pain syndromes such as migraine and cluster headache are common. Early studies suggested that the trigeminovascular system plays a key role in their pathogenesis. The classic observations of Ray and Wolff[1] showed that mechanical, thermal, or electrical stimulation of large cerebral arteries, venous sinuses, and dural (meningeal) arteries induced extracranial referred painful sensations. They suggested that the main head pain–producing structures are associated with the intracranial vessels. The theory hypothesized that activation of nociceptors, located in the walls of intracranial vessels that respond with local antidromic release of neuronal messengers and connect via the trigeminal nerve with second-order neurons in the trigeminocervical complex in the brainstem, is the main source of head pain.

The pain-sensitive supratentorial structures are innervated by sensory nerve fibers arising from pseudounipolar neurons with their cell bodies in the first division (ophthalmic branch) of the trigeminal ganglion, which connect to the CNS at second-order sensory neurons within the brainstem trigeminal nucleus caudalis and at $C_{1\text{-}2}$. Antidromic or local mechanical stimulation of sensory nerve endings causes dilatation of peripheral vessels via the release of substance P and calcitonin gene-related peptide (CGRP) from the trigeminovascular system in humans.[2] The vasomotor response of the sensory nerves in the peripheral circulation has a counterpart in the cerebral circulation with the trigeminal system. The nerve fibers and the cell bodies contain a number of messenger molecules, but CGRP is the one most frequently expressed in humans.[3] Moreover, release of CGRP from perivascular nerves in the meninges (dura mater) and in the cerebral circulation[2,4–6] has been conclusively demonstrated experimentally and shown to evoke vasodilatation and migraine.

Nevertheless, a number of questions remain. First, is vascular activation the only route of triggering migraine? Second, what is the mechanism for the associated symptoms, such as photophobia, phonophobia, and nausea? Third, why are migraines often triggered by menstrual periods, hunger, and changes in sleep, triggers that do not appear to be trigeminovascular-related? Recent advances in understanding of CGRP biology, central pain processes, and cerebellum biology now suggest that the pathophysiology of migraine is far more complex than was originally thought, and that vascular activation may be just one of many factors involved in migraine pathogenesis.

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CGRP is a 37-amino-acid neuropeptide, identified in the early 1980s.[7] The calcitonin gene was unexpectedly found to encode two different mRNAs, and these encode either calcitonin or αCGRP mRNA, depending on anatomical localization. Whereas the calcitonin gene transcript dominates in the thyroid, αCGRP is the predominant expression product in the nervous system. A second CGRP gene has been discovered that forms βCGRP, which is expressed primarily in the enteric sensory system, in the gut and inner organs. In humans, the two forms αCGRP and βCGRP differ by three amino acids, but have indistinguishable biological effects in the vasculature. Primary neurons express more αCGRP than βCGRP, whereas enteric neurons almost exclusively express βCGRP.[8]

Peptides in the family that includes adrenomedullin, amylin, and calcitonin possess diverse biological functions within the central and peripheral nervous systems.[9,10] CGRP is widely expressed in both central and peripheral nervous systems.[11] In the periphery, the expression is in both unmyelinated C-fibers and thinly myelinated Aδ fibers innervating almost all organs including epidermis,[12] sweat glands,[13] skeletal muscles,[14] airway, and enteric system, in addition to the vasculatures. Furthermore, CGRP-positive vagal nerve fibers have also been identified.[15] In the CNS, CGRP-containing cell bodies have been found in a number of areas that may play an important role in migraine pathophysiology, such as the hypothalamus (trigger), superior colliculi (visual symptoms), inferior colliculi (phonophobia), brainstem and trigeminal complex (head pain, nausea), and cerebellum.[11]

Specifically, these areas include the medial preoptic area, the anterior hypothalamic nuclei, the periventricular, the perifornical area, and in the lateral hypothalamus–medial forebrain bundle area, the premammillary nucleus, the medial amygdaloid nucleus, the ventromedial nucleus of the thalamus, the hippocampus and the dentate gyrus, the periventricular gray, and the area around the fasciculus retroflexus (parafascicular area) extending laterally over the lemniscus medialis. In the mesencephalon, CGRP-positive cells are found in the peripeduncular area ventral to the medial geniculate body, extending dorsally along its medial aspects. CGRP-containing cell bodies are seen in the paratrigeminal nucleus, as well as in the superior colliculus.[16] Peptidergic fibers containing CGRP have been found to innervate the anterior pituitary of monkey, dog, rat, and human.[17–20] Notably, there is high expression of CGRP in the cerebellum and inferior olivary complex, suggesting that it may play an important modulatory role.[21]

The best known function of CGRP is its effect on the peripheral vasculature. It acts on smooth muscle cells and causes vasodilatation via a nonendothelial mechanism through activation of adenylate cyclase.[22] The release of perivascular peptides relaxes cerebral arteries concomitant with stimulation of cAMP accumulation or release of an endothelium-derived relaxing factor in the cat.[23] In the periphery, CGRP also has been known to modulate neuromuscular junctions by locally inhibiting the expression of acetyl cholinesterase,[24–27] which is involved in inflammation within airways,[28,29] gastric secretion,[30] and intestinal mobility.[31] Centrally, evidence is emerging that CGRP may play an important autocrine and paracrine function in many areas. Activation of CGRP receptors on cultured trigeminal ganglion neurons increased endogenous CGRP mRNA levels and promoter activity.[32] CGRP has also been shown to differentially regulate cytokine secretion via activation of neuron–glia signaling in cultured trigeminal ganglion glia[33] and to regulate glial inducible nitric oxide synthase (iNOS) and NO release.[34]

CGRP may also be involved in a variety of stress responses, and may play a pivotal role in stress-induced suppression of the gonadotrophin releasing hormone (GnRH) pulse generator in the rat by suppressing luteinizing hormone pulses and increasing Fos expression within the medial preoptic area and paraventricular nucleus.[35]

The potential role of CGRP in migraine pathophysiology was first suggested in 1984.[23] There is a dense supply of thin CGRP-containing nerve fibers in intracranial vessels, and these originate in the trigeminal ganglion.[36,37] The CGRP-positive perivascular nerve fibers in intracranial vessels (dural as well as cerebral) originate in the first division of the trigeminal ganglion; the other branches of the fifth cranial nerve supply other parts of the head with sensory innervation. CGRP and substance P are colocalized, but there is significantly more CGRP immunoreactivity. CGRP is the strongest vasodilator found to date, acting via a nonendothelial mechanism and using adenylate cyclase as second messenger.[23,38] Electrical field stimulation or capsaicin treatment[39,40] causes local vasodilatation and release of CGRP from the perivascular nerve fiber endings. These effects are attenuated by administration of a CGRP blocker acting postsynaptically[4–6,41,42] or a triptan acting at presynaptic sites.[4,43] A growing body of evidence suggests a pivotal role for CGRP in the pathogenesis of primary headaches.[38] The trigeminal nerve fibers mediate dilatation of brain vessels and increases in cerebral blood flow,[38] and have an important role in the trigeminovascular reflex.[37,40] Although CGRP has a number of effects, its most pronounced actions are intracranial vasodilatation and transmission of nociception.[19]

The CGRP-containing nerve cell bodies are pseudounipolar and constitute more than 40% of the neurons in the trigeminal ganglion; they have functional connections with neurons in the trigeminal nucleus caudalis and in related extensions down to the $C_{1\text{-}2}$ level, and also in

the perivascular nerve fiber network of intracranial vessels.[38] Early horseradish peroxidase tracing studies showed anatomical connections between meningeal (dural) vessels and the trigeminal ganglion;[44] subsequent retrograde tracing with True Blue in combination with neuropeptide immunocytochemistry revealed that the sensory fibers and the trigeminal neurons colocalize CGRP and substance P,[45,46] and that denervation (trigeminal nerve lesion) abolished these neuropeptides from the perivascular nerves.[36]

Recent retrograde tracing from intra- and extracranial arteries and the superior sagittal sinus have shown that the perivascular sensory C-fibers terminate in lamina I/II and the mechanoceptor Aδ-fibers in lamina III/IV of the brainstem.[17,18,47] The tracing studies furthermore suggest a somatotopic organization of the perivascular nerve fiber projections in the brainstem. The peripheral part of the trigeminal system innervates intracranial vessels via its ophthalmic division, and contains the neuronal messengers CGRP, substance P and neurokinin A.[48] In addition, there is a population of cell bodies in the trigeminal ganglion that store nitric oxide synthase, pituitary adenylate cyclase activating peptide, dynorphin, galanin and nociceptin.[20] The trigeminovascular system[37,40] has a primary involvement in cranial sensory functions, but also acts as a vasodilator pathway with antidromic release of CGRP, putatively as a response to localized cerebrovascular constriction. In the brainstem it is primarily the C-fibers that contain CGRP and these connect in layers I/II with secondary neurons that have pre- and/or postjunctional CGRP receptors.

NEUROTRANSMITTER RELEASE IN MIGRAINE

A number of neurotransmitters have been examined for a role in the initiation of primary headache attacks, but without convincing success. The role of the sensory nerves located around the intracranial vessels has been analyzed in humans in conjunction with stimulation of the trigeminal ganglion; such stimulation resulted in unilateral blood flow increases, release of CGRP and substance P, and ipsilateral facial flushing.[38] In addition, glycerol injection into the trigeminal ganglion induced a slight increase in human cerebral blood flow,[49] and cutaneous stimulation in patients with trigeminal neuralgia resulted in facial flushing and was associated with CGRP release.[50] These studies strongly suggest that there is release of CGRP from the trigeminovascular system during activation of the trigeminal ganglion.

In spontaneous migraine attacks, there is significant release of CGRP but not of any other neuropeptide.[38,51,52] Current theories propose that migraine is a primary brain disease due to mutations in ion channel genes rendering CNS neurons unstable and capable of initiating a migraine attack. To date, however, this is proven only for hemiplegic migraine, despite a decade of research since the original finding.[53,54] The current view of migraine pathophysiology furthermore suggests that the trigeminal system is an essential part of the disease expression. Hypothetically, the mechanisms involve activation of the trigeminovascular reflex as a defense mechanism against cerebrovascular constriction elicited either due to spreading depression[55–58] or other localized cerebrovascular vasomotion.[59] The cerebral circulation requires high and constant flow and metabolism; hypothetically, cerebral vasoconstriction is sensed by the trigeminal sensory nerve fibers with a subsequent antidromic release of CGRP to maintain local brain blood flow within normal limits. The trigeminal activation results in dromic activation of neurons in the trigeminal nucleus caudalis with subsequent second-order neuron involvement and mediation of the central aspects of pain within the two regions of termination: sensory C-fibers in lamina I/II and Aδ-mechanoreceptor fibers in lamina III–IV.[48]

The results from trigeminal ganglion stimulation in trigeminal neuralgia patients led us to investigate neuropeptides associated with the autonomic and sensory nerves during migraine attacks.[38] The concentration of neuropeptide Y (marker for sympathetic nerves), vasoactive intestinal peptide (parasympathetic activity), and CGRP and substance P (markers for sensory nerves) were analyzed in the cranial venous outflow. There were no changes in the levels of neuropeptide Y, vasoactive intestinal peptide, or substance P during migraine attacks; however, marked increases in CGRP levels were observed in patients during attacks of migraine with aura or without aura.[52] The release of CGRP rather than substance P may be explained by the fact that the intracranial circulation is preferentially innervated by CGRP-containing sensory fibers from the trigeminal ganglion.[3,45] In line with these observations, substance P receptor antagonists have no antimigraine effects.

In the clinical setting, nitroglycerine is used to elicit migraine-like attacks.[60] Further experiments have provided supportive data demonstrating a linear correlation between the increased levels of CGRP and the intensity of the headache.[61,62] It is worth noting that low pain results in no significant increase in venous CGRP.[62,63] In addition, nitroglycerine did not elicit cluster headache attacks if the patient was not in a prone status (i.e., a state in which the disease was active and a small stimulation could then elicit the full cluster headache attack). Studies of the perfused middle cerebral artery[4,61,64] showed that CGRP does not readily pass the blood–brain barrier, which agrees well with this supposition; the nerve fibers are situated in the adventitia and act on the receptors located in the smooth muscle cells. Thus, a low degree of perivascular CGRP release likely occurs in mild to mod-

erate attacks, but it is necessary to have a large release to measure the peptide in the cranial venous effluent. Any negative data would fall into this category.[65]

This view is supported by human studies of subarachnoid hemorrhage, in which the CGRP increase in the cranial venous outflow and in the CSF correlated with the degree of vasoconstriction measured with transcranial Doppler.[66,67] In addition, after sumatriptan or rizatriptan administration, the plasma levels of CGRP returned to control levels, with successful amelioration of the headache.[4,61,64] These results have been confirmed in experimental studies using zolmitriptan, rizatriptan, sumatriptan, and dihydroergotamine. The 5-$HT_{1B/1D}$ receptors are expressed on human trigeminal ganglion cells and on trigeminal sensory fibers,[68] thus providing sites for presynaptic inhibition of the CGRP release and of contractile 5-HT_{1B} receptors in intracranial arteries.[69] The question remains, however, as to whether the vasomotor effect of the triptans is of pivotal importance for their antimigraine effect.

What triggers the activation of the vascular nociceptors? One hypothesis suggests that vasoconstriction initiated when, for example, cortical spreading depression is registered by the nociceptors, which respond with an antidromic release of CGRP and a dromic neuronal signal to the trigeminal nucleus caudalis. A CGRP receptor antagonist at either site (central or peripheral) would block the postjunctional receptors, and the triptans would inhibit the release of neuronal CGRP; however, both principles may work in the same direction to alleviate the signaling within the trigeminovascular system.

CGRP RECEPTORS

Early pharmacological studies focused on the use of CGRP agonists and the CGRP fragment $CGRP_{8-37}$ to discriminate between CGRP receptor subtypes.[19] The use of peptide antagonists to classify CGRP receptors in tissue preparations in particular has been criticized, based on the observation that experimental conditions may affect the actions of these peptides and thereby limit their ability to reliably discriminate CGRP receptor subtypes.[70]

Both human and rat calcitonin-like receptor (CLR) have been cloned; CLR is a seven-transmembrane domain G-protein-coupled receptor, which shares 55% sequence identity with the calcitonin receptor.[71] Functional CGRP and adrenomedullin receptors are derived from CLR, and the phenotype is determined by coexpression with a receptor activity modifying protein (RAMP). Coexpression of CLR with RAMP1 results in CGRP receptor pharmacology, whereas coexpression with RAMP2 or RAMP3 yields an adrenomedullin receptor or possibly a combined receptor (CGRP and adrenomedullin). Apart from contributing to the receptor specificity, the RAMPs enable expression of CLR on the cell surface.[72] The CGRP receptor requires another accessory protein for proper biological function, the receptor component protein (RCP); this RCP protein does not function as a molecular chaperone, but is involved in coupling of the receptor to downstream signaling pathways (e.g., adenylate cyclase activity).

CGRP receptors have classically been subdivided into two classes, $CGRP_1$ and $CGRP_2$, but a recent classification proposes only a single CGRP receptor.[73] To address the question of where CGRP receptors are localized in the trigeminovascular system, immunocytochemistry studies have been performed. In intracranial blood vessels, CGRP receptor components were found in the smooth muscle cells.[74] In addition, we have observed both CLR and RAMP1 in human trigeminal ganglion cells. Although CGRP can be seen in thin sensory fibers in the layers I/II of the trigeminal nucleus caudalis region, it has been difficult to obtain positive staining of the CGRP receptor components in this region (L. Edvinsson, unpublished data). A recent report suggests presynaptic and postsynaptic CGRP receptors in this region.[75]

The basic mechanisms of vascular headaches involve the presence of CGRP receptor components in cerebral and middle meningeal arteries.[74] Thus, human middle cerebral and middle meningeal arteries and brain microvessels express mRNA for CLR, RAMP1–3, and RCP. Cultured smooth muscle cells and brain microvascular endothelial cells express mRNA for all components except RAMP3.[74] The mRNA for the entire CGRP receptor is expressed on human intracranial arteries. Functional CGRP receptors are localized on the vascular smooth muscle cells of the cranial arteries in particular, because the responses of cerebral veins to CGRP are very weak.[37,76] Pharmacological studies of cerebral and meningeal arteries revealed that CGRP receptors dominate by inducing stronger dilatation of cerebral than of the middle meningeal artery, whereas the responses to amylin and adrenomedullin are minor and mediated by the CGRP receptor. The CGRP-induced relaxation in humans is endothelium independent and occurs in parallel with activation of adenylyl cyclase. In addition, CGRP receptor mRNA and mRNAs encoding CLR and all three RAMPs have been shown in the smooth muscle cells of human cranial arteries[74,77,78]; however, it is RAMP1 that determines the functional phenotype.

CGRP RECEPTOR ANTAGONISTS

Based on the role of CGRP in migraine, and the desire for a nonvasoconstrictor treatment, CGRP receptor antagonism was proposed as a potential therapeutic target.[38] $CGRP_{8-37}$ was initially investigated in vitro and in vivo, but this antagonist has a short half-life and cannot be absorbed orally. A breakthrough in the CGRP field

	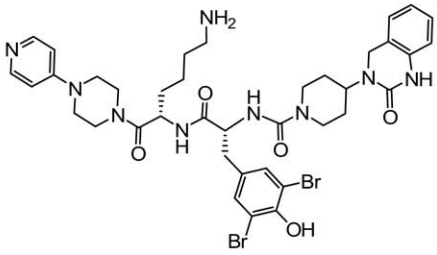	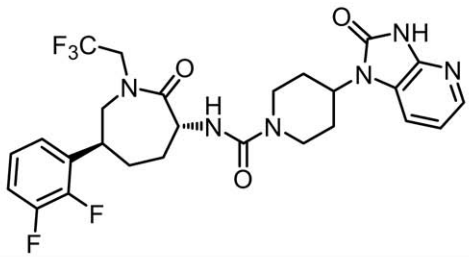
	Olcegepant	Mk-0974 telcagepant
Ki, nM	0.010 nM	0.77
MW	870	566
Clinical Dose	2.5 mg IV	140 to 280 mg

FIG. 1. Molecular structure, inhibition constant (K_i), molecular mass, and clinical dose for olcegepant and telcagepant (MK-0974), two calcitonin gene-related peptide (CGRP) receptor antagonists.

came with the development of a series of potent small molecule CGRP receptor antagonists (FIG. 1), namely, olcegepant[79] and some molecular modifications of this compound.[80] Among the more potent of these, olcegepant demonstrates extremely high affinity for the human CGRP receptors, with a pA_2 value in the range of picomoles per liter.[81,82] The antagonist is 3 log units more potent in human tissues, compared with values seen in experimental animals. The reason for this was revealed by Mallee et al.[83]: the high affinity of olcegepant was dictated strictly by hRAMP1. The region between amino acids 66 and 112 is critical for determining the pharmacology of these small molecule antagonists. The exact molecular mechanism by which RAMP1 modulates antagonist binding sites depends on a mutation of one nucleotide in RAMP1.[83]

A major potential advantage of a CGRP receptor blocker is the lack of direct vasoconstrictor action, but blocking the receptor of a strong vasodilator involves a theoretical risk. In experimental studies, denervation or CGRP receptor antagonism did not change resting cerebral blood flow or metabolism, cerebral autoregulation, or responses to changes in blood gases.[4,84] Olcegepant did not change the diameter of the superficial temporal or the middle cerebral artery, or alter regional and global cerebral blood flow.[85,86] It was concluded that olcegepant does not affect the resting tone in the majority of investigated vessels, which gives olcegepant an advantage compared with other antimigraine compounds such as triptans and ergot derivatives.

The site of action of the antagonist is still not clear. With a closed cranial window model[42] olcegepant was found to inhibit dilatation of dural (meningeal) arteries after intravenous systemic CGRP administration and neuronal CGRP from perivascular nerves after transcranial electrical stimulation. These findings are in agreement with previous studies, because olcegepant inhibits CGRP-induced hypotension and trigeminal ganglion–stimulated increase in facial blood flow in experimental studies.[79] In contrast, the antagonist did not significantly inhibit changes in the tone of cerebral arterioles or in local cortical cerebral blood flow.[42] This indicates that the effect of the compound is mainly extracerebral and that the antagonist does not readily pass the blood–brain barrier, which correlates with results from clinical study.[86]

There is recent support for this view. Perfusion of the isolated middle cerebral artery showed that neither CGRP nor olcegepant passed the blood–brain barrier.[87] In healthy volunteers, the CGRP-antagonist prevented CGRP-induced headache and associated CGRP symptoms (flushing and sensation of heat). The increase in middle cerebral artery diameter and in cerebral blood flow was not significantly inhibited by olcegepant, but the drug blocked CGRP-induced dilatations of the superficial temporal and radial arteries.[86]

CLINICAL STUDIES OF CGRP RECEPTOR ANTAGONISTS

The ability of CGRP receptor antagonists to treat acute migraine attack was initially established with olcegepant (BIBN 4096 BS). In a proof-of-concept study, intravenously administered olcegepant was effective in relieving acute migraine pain and associated symptoms and was well tolerated, with no cardiovascular or cerebrovascular effects.[88] The results with olcegepant were encouraging, but migraine treatments are administered primarily on an outpatient basis, and it was therefore important to develop CGRP receptor antagonists that

could be taken orally or via some other route that avoids the need for patients to inject themselves.

Telcagepant (MK-0974), the first orally available CGRP receptor antagonist, is currently in phase III trials for treatment of acute migraine. It is a potent selective antagonist of the human (K_i = 0.77 nmol/L) and rhesus monkey (K_i = 1.2 nmol/L) CGRP receptors, but displays >1500-fold lower affinity for the canine and rat receptors as determined via [^{125}I]-human CGRP competition binding assays.[89] Telcagepant potently blocked α-CGRP-stimulated cAMP responses in human CGRP receptor-expressing HEK293 cells with an IC_{50} of 2.2 ± 0.29 nmol/L. The unbound fraction in plasma was 4.1% in human plasma. In human cerebral and middle meningeal arteries in vitro assay, telcagepant showed a pA_2 value of 8.83 and 8.03 (approximately corresponding to 1 and 10 nmol/L), respectively, in blocking α-CGRP-induced vasodilatation.[90]

In clinical pharmacology studies, telcagepant was rapidly absorbed, with a T_{max} of ~1.5 hours. The terminal half-life was ~6 hours. The C_{max} and $AUC_{0-\infty}$ reached by the 300 mg was ~4 μmol/L and 14 μmol/L · hr, respectively. A greater than dose-proportional increase in $AUC_{0-\infty}$ was observed. After twice-daily dosing, with each dose separated by 2 hours, steady state was achieved in approximately 3 to 4 days, with an accumulation ratio of ~2. There were no clinically meaningful pharmacokinetic differences across age and gender. The pharmacokinetics were not affected by migraine-associated gastrostasis.[91] No consistent clinically relevant effects on electrocardiography parameters, blood pressure, or heart rate were observed in single- and multiple-dose clinical pharmacology studies.[92] Furthermore, an interaction study showed that telcagepant by itself does not elevate mean arterial blood pressure and that coadministration of telcagepant with sumatriptan resulted in elevations in mean arterial blood pressure similar to that after administration of sumatriptan alone in migraineurs during the interictal period.[93]

The effectiveness of telcagepant in treating acute migraine was tested in a phase IIb dose-finding study in which doses from 25 to 600 mg were explored.[94] Telcagepant doses of 300 mg to 600 mg were shown to be effective in treating both acute migraine pain and migraine associated symptoms. Telcagepant was well tolerated, with an adverse event rate similar to placebo. The efficacy and safety profiles of telcagepant were confirmed subsequently in three additional large pivotal phase III acute efficacy migraine trials involving a total of 3293 telcagepant treated patients.[94–96] All three trials demonstrated that both telcagepant 300 mg capsule or 280 mg tablet and 150 mg capsule or 140 mg tablet were effective in treating migraine pain (2-hour pain freedom and pain relief, 2- to 24-hour and 2- to 48-hour sustained pain freedom, Table 1) and migraine associated symptoms (photophobia, phonophobia and nausea, Table 2). (Note that 300 mg and 150 mg capsules are bioequivalent to 280 mg and 140 mg tablets, respectively). In addition, the multiple attack study[96] showed that telcagepant 140 mg and 280 mg were more consistently effective than a control group (comprising mostly placebo) in treating migraine pain across four attacks (measured by the proportions of patients who had 2-hour pain freedom or relief in at least three out of four attacks).

These trials also confirmed that telcagepant was generally well tolerated, with an adverse event rate similar to placebo. Compared with triptans, telcagepant appeared to have fewer of the adverse events commonly associated with triptans, such as asthenia, chest discomfort, fatigue, myalgia, dizziness, paraesthesia, and throat tightness. Similarly, in a long-term safety study, telcagepant was used by 640 patients for their acute migraine attacks for up to 18 months. Telcagepant was efficacious and well tolerated in long-term intermittent treatment.[97] However, in a phase II migraine prophylaxis study in which patients were treated with twice daily doses of telcagepant, some patients showed elevated transaminases after more than 2 weeks of treatment. None of these patients fulfilled Hy's law criteria (i.e., elevated bilirubin ≥2× upper limit of normal accompanied by elevated transaminases of ≥3× upper limit of normal). The exposure achieved in this study was much higher than the acute migraine dose, because of accumulation of drug with daily treatment. Similar hepatic signals were not seen with acute intermittent therapy, suggesting that the potential for hepatic toxicity may be time- and dose-dependent.

Although the overall proportions of responders in the phase III trials were similar between telcagepant and triptans, a subgroup analysis based on a prospectively defined question showed that the population responding to telcagepant may be different from that responding to triptans. Many patients who did not report a good response to triptans did appear to be responsive to telcagepant for their acute migraine attacks (Ho, submitted), suggesting that the there may be heterogeneity in acute migraine patients and that not all patients will respond equally to different treatments.

HOW DO CGRP ANTAGONISTS ACT IN MIGRAINE?

Although it has been demonstrated that CGRP antagonism is an effective way to abort acute migraine attacks and treat migraine associated symptoms, questions remain as to how CGRP receptor antagonists work. Given the high potency of telcagepant, it was expected at first that relatively low doses would likely be efficacious if blocking of peripheral CGRP receptors were sufficient for antimigraine actions. Indeed, in a capsaicin-induced vasodilatation study, it was shown that the EC_{90} for blocking the peripheral CGRP receptor–mediated vaso-

Table 1. *Summary of Pain-Related Efficacy Endpoints in Three Pivotal Telcagepant (MK-0974) Efficacy Studies*

Protocol	Dose*	*N*	*n/m*	Observed % (95% CI)	*P*-value†
			2-hour pain freedom		
PN011	150	333	57/331	17.2 (13.3–21.7)	<0.01
	300	354	95/353	26.9 (22.4–31.9)	<0.001
	placebo	348	33/343	9.6 (6.7–13.2)	
PN016	150	381	88/380	23.2 (19.0–27.7)	<0.001
	300	371	88/369	23.8 (19.6–28.5)	<0.001
	placebo	365	39/365	10.7 (7.7–14.3)	
PN031	140	559	122/556	21.9 (18.6–25.6)	<0.001
	280	537	134/534	25.1 (21.5–29.0)	<0.001
	placebo	542	55/539	10.2 (7.8–13.1)	
			2-hour pain relief		
PN011	150	333	165/331	49.8 (44.3–55.4)	<0.001
	300	354	194/353	55.0 (49.6–60.2)	<0.001
	placebo	348	95/343	27.7 (23.0–32.8)	
PN016	150	381	205/380	53.9 (48.8–59.0)	<0.001
	300	371	205/369	55.6 (50.3–60.7)	<0.001
	placebo	365	120/365	32.9 (28.1–38.0)	
PN031	140	559	326/556	58.6 (54.4–62.8)	<0.001
	280	537	303/534	56.7 (52.4–61.0)	<0.001
	placebo	542	180/539	33.4 (29.4–37.6)	
			Sustained pain freedom from 2 to 24 hours		
PN011	150	333	35/328	10.7 (7.5–14.5)	<0.01
	300	354	71/351	20.2 (16.1–24.8)	<0.001
	placebo	348	17/343	5.0 (2.9–7.8)	
PN016	150	381	62/378	16.4 (12.8–20.5)	<0.001
	300	371	63/365	17.3 (13.5–21.5)	<0.001
	placebo	365	26/363	7.2 (4.7–10.3)	
PN031	140	559	86/553	15.6 (12.6–18.8)	<0.001
	280	537	101/529	19.1 (15.8–22.7)	<0.001
	placebo	542	35/537	6.5 (4.6–8.9)	
			Sustained pain freedom from 2 to 48 hours		
PN011	150	333	25/324	7.7 (5.1–11.2)	<0.05
	300	354	64/347	18.4 (14.5–22.9)	<0.001
	placebo	348	14/342	4.1 (2.3–6.8)	<0.001
PN016	150	381	50/370	13.5 (10.2–17.4)	<0.001
	300	371	57/362	15.7 (12.1–19.9)	<0.001
	placebo	365	21/360	5.8 (3.6–8.8)	<0.001
PN031	140	559	74/552	13.4 (10.7–16.5)	<0.001
	280	537	94/526	17.9 (14.7–21.4)	<0.001
	placebo	542	33/536	6.2 (4.3–8.5)	<0.001

Data are based on nominal time points of 2, 2.5, 3, 4, 6, 8, and 24 hours for PN011 and 2, 2.5, and 24 hours for PN016 and PN031 in conjunction with the appropriate recurrence question at 24 or 48 hours post dose.
CI = confidence interval; *N* = number of patients treated; *n/m* = number of patients achieving the indicated endpoint divided by the number of patients in the full analysis set population.
*The 150-mg and 300-mg doses of MK-0974 were formulated as capsules. The 140-mg and 280-mg doses of MK-0974 were formulated as tablets.
†Significant values for MK-0974 vs. placebo based on a logistic regression model adjusting for treatment, geographic region (U.S., ex-U.S.), baseline headache severity (moderate, severe) and age.

dilatation was at or below 900 nmol/L. The relatively flat concentration–response curve above 900 nmol/L indicated that at or above this plasma concentration, telcagepant was maximally blocking the peripheral CGRP receptor in humans. Thus, it was surprising that relatively high doses of telcagepant (150 mg and 300 mg) were necessary to achieve antimigraine efficacy.[95,98] At these doses, the mean plasma concentrations are approximately twofold to fourfold higher than 900 nmol/L, suggesting that larger doses than those producing maximal peripheral CGRP receptor inhibition are necessary for antimigraine efficacy. Similarly, this dose is also orders of magnitude higher than the pA_2 of telcagepant in inhibiting αCGRP-induced vasodilatation in human cerebral and coronary arteries in vitro (8.0 to 8.3, approximately corresponding to 1 and 10 nmol/L).

So, can telcagepant act centrally given that it is a P-glycoprotein substrate? The in vivo CSF level of tel-

Table 2. *Response Rate for Migraine-Associated Symptoms in Three Pivotal Telcagepant (MK-0974) Efficacy Studies*

Protocol	Dose*	*N*	*n/m*	Observed % (95% CI)	*P*-value†
			Absence of photophobia		
PN011	150	333	149/331	45.0 (39.6–50.6)	<0.001
	300	354	180/353	51.0 (45.6–56.3)	<0.001
	placebo	348	99/342	28.9 (24.2–34.1)	
PN016	150	381	176/380	46.3 (41.2–51.5)	<0.001
	300	371	179/369	48.5 (43.3–53.7)	<0.001
	placebo	365	119/365	32.6 (27.8–37.7)	
PN031	140	559	290/554	52.3 (48.1–56.6)	<0.001
	280	537	280/534	52.4 (48.1–56.7)	<0.001
	placebo	542	219/539	40.6 (36.5–44.9)	
			Absence of phonophobia		
PN011	150	333	178/331	53.8 (48.2–59.2)	<0.001
	300	354	204/353	57.8 (52.4–63.0)	<0.001
	placebo	348	126/342	36.8 (31.7–42.2)	
PN016	150	381	192/380	50.5 (45.4–55.7)	<0.05
	300	371	206/369	55.8 (50.6–61.0)	<0.001
	placebo	365	152/365	41.6 (36.5–46.9)	
PN031	140	559	341/555	61.4 (57.2–65.5)	<0.001
	280	537	317/534	59.4 (55.1–63.6)	<0.001
	placebo	542	261/537	48.6 (44.3–52.9)	
			Absence of nausea		
PN011	150	333	221/330	67.0 (61.6–72.0)	<0.01
	300	354	229/352	65.1 (59.8–70.0)	<0.01
	placebo	348	189/342	55.3 (49.8–60.6)	
PN016	150	381	260/379	68.6 (63.7–73.2)	<0.001
	300	371	258/369	69.9 (65.0–74.6)	<0.001
	placebo	365	196/365	53.7 (48.4–58.9)	
PN031	140	559	403/553	72.9 (69.0–76.5)	<0.001
	280	537	383/534	71.7 (67.7–75.5)	<0.001
	placebo	542	338/538	62.8 (58.6–66.9)	

CI = confidence interval; *N* = number of patients treated; *n/m* = number of patients with absence of photophobia, phonophobia, or nausea at 2 hours post dose divided by the number of patients in the full analysis set population.
*The 150-mg and 300-mg doses of MK-0974 were formulated as capsules and are bioequivalent to the 140-mg and 280-mg doses, respectively, formulated as tablets.
†Significant values for MK-0974 vs. placebo based on a logistic regression model adjusting for treatment, geographic region (U.S., ex-U.S.), baseline headache severity (moderate, severe) and age.

cagepant was evaluated in cisterna magna-ported rhesus monkeys as a surrogate to the clinical experience, and pharmacokinetic parameters were determined in cerebral CSF and plasma after oral dosing in rhesus monkey. A CSF/plasma ratio (%) was computed as an index of CNS penetrability. The CSF/plasma ratio was ~1.4% (C_{max} = 8.7 μmol/L plasma and 127 nmol/L CSF), suggesting that telcagepant has brain penetration potential. At this penetration, the telcagepant CSF level could be as high as 60 nmol/L which is above the K_i (0.77 nmol/L) and IC_{50} (2.2 nmol/L) of telcagepant. Thus at clinical doses, based on data from rhesus CSF study, telcagepant could potentially achieve a relevant CSF concentration.[99] Nevertheless, one must interpret this data with caution, because CSF levels should not be equated with receptor occupancy.

Similarly, the CGRP blocker olcegepant has poor penetration across the blood–brain barrier, and cannot be used as an oral drug because of its dipeptide structure. Although systemic olcegepant effectively blocks the CGRP-induced temporal artery dilatation, it does not modify the tone of cerebral vessels.[86] In agreement with this observation, recent studies on the isolated middle cerebral artery revealed that luminal olcegepant did not block abluminal CGRP-induced vasodilatation.[87] If, however, the olcegepant is given by local microiontophoresis (to circumvent the blood–brain barrier), it is a potent inhibitor of activated trigeminocervical neurons in vivo,[100] or if given systemically in very high doses.[101] These studies demonstrate the presence of functional CGRP receptors on the second-order trigeminal neurons.

An in vivo C_{max} of >200 nmol/L is necessary to show clinical efficacy.[88] The K_i of olcegepant is in the 0.01 nmol/L range (revealing a difference in inhibition concentration of >20,000), which points to the need to use a high dose of the antagonist to act on receptors located inside of the blood–brain barrier and thus effectively

rules out the neurogenic inflammation theory in the meningeal circulation as the prime target.[87]

Why are such high doses of CGRP receptor antagonists required for acute migraine efficacy? It could be that, in a migraine population, higher doses are needed to maximally inhibit the peripheral CGRP receptor in a majority of the patients. Another possibility is that peripheral CGRP receptor inhibition may not be sufficient for antimigraine efficacy, and that central engagement may also be required. Given the widespread distribution of CGRP receptors in several migraine-relevant brain areas (e.g., the hypothalamus, cerebellum, periaqueductal gray, superior and inferior colliculi, in addition to the trigeminal complex), there are many potential ways that CGRP antagonism could abort acute migraine attacks. Evidence is now emerging that the role of CGRP may play more of a modulator role in the CNS. This may explain the favorable tolerability of CGRP receptor antagonists.

Like other neuromodulatory neuropeptides, CGRP is often coexpressed with other classic neurotransmitters. CGRP has been shown to modulate the cholinergic system by antagonizing neuronal nicotinic acetylcholine receptors in the autonomic nervous system. Through this action, CGRP may inhibit background synaptic noise at cholinergic synapses, thus contributing to the fine-tuning of nicotinic synaptic transmission.[102] CGRP has been shown to modulate pain transmission by increasing discharge frequency of wide dynamic neurons in the dorsal horn, thereby facilitating the transmission of nociceptive signals.[103]

In cultured trigeminal neurons, CGRP activates the transcription of the *P2RX3* gene and enhances P2X3 trafficking to the neuronal membrane. Both effects are achieved through protein kinase A–dependent mechanisms. P2X3 is the receptor for ATP, which is a mediator involved in mediating chronic pain. Activation of trigeminal neurons by CGRP during migraine could enhance the sensitivity of the trigeminal system to pain-producing ATP, resulting in stronger and more sustained pain signaling in the brain.[104] This mechanism provides a potential molecular substrate whereby CGRP could contribute to trigeminal sensitization and, hence, to allodynia and hyperalgesia in migraine. Supporting a role for CGRP in this form of trigeminal plasticity is the observation that the CGRP receptor antagonist $CGRP_{8\text{-}37}$ reduces mechanical allodynia and hyperalgesia in rat.[105]

Finally, CGRP may modulate neuronal function through interaction with glial CGRP receptors.[106] CGRP is thought to be secreted by neurons and then to activate the surrounding glia cells to express molecules such as nitric oxides, which in turn can activate many pronociceptive mediators This modulating effect of CGRP on glial function is not only limited to trigeminal ganglia, but has also been demonstrated in other brain areas, such as the cerebellum[107–110] and neuromuscular junctions.[25]

The cerebellum is known to be important in modulating many cortical motor and sensory inputs. Subtle clinical cerebellar alterations have been found in migraine.[110] Moreover, it is known that the cerebellum exerts an inhibitory control on the cerebral cortex. Abnormalities in visual and motor cortex excitability consistent with a lack of inhibitory effect have been described in migraine. Cerebellar conditioning transcranial magnetic stimulation showed a significant deficit of cerebellar inhibition in migraine patients, compared with controls,[111] suggesting that migraine patients may have a deficit in filtering sensory inputs. The deficit in filtering somatosensory, visual, and auditory inputs may lead to head pain, photophobia, and phonophobia respectively. CGRP receptors are located in cerebellar Purkinje cell cytoplasm and dendrites and Bergmann glia cells. CGRP receptors are also located in inhibitory interneurons.[107] CGRP, suppressed both the spontaneous firing rate of olivary neurons and the enhanced activity induced by application of excitatory amino acids.[21] These findings suggest that CGRP may be involved in neuron–glia interactions influencing neuronal activity and that CGRP may play an important role in the cerebellum's ability to modulate sensory inputs.

Although CGRP has been shown to facilitate nociceptive transmission through neuromodulation, in some parts of the brain it may be antinociceptive. For example, intracerebroventricular injection of CGRP produced an antinociceptive effect in rat[112] and mouse. CGRP produced significant antinociceptive effects in the nucleus raphe magnus of rat, an action that appeared to involve opioid receptors,[113] and in the nucleus accumbens. Although these observations could reflect a physiological action of CGRP, the doses that were used in these studies were quite high, raising the possibility that desensitization and internalization of CGRP receptors led to reduced responsiveness to CGRP.

CONCLUSIONS

The rationale for the development of specific CGRP receptor antagonists is firmly based on translational research. CGRP receptor antagonism is now regarded as a prime target for the development of novel antimigraine therapies. There is an excellent correlation between CGRP release and pain in migraine headache, which points toward the potential usefulness of a specific CGRP antagonist in the treatment of primary headaches.[38] The demonstration that triptans have the ability to inhibit the release of CGRP supports this view, but triptans suffer from significant potential cardiovascular risks.[114] The progress in the demonstration of the unique molecular biology and functional organization of the

CGRP family of receptors provides the basis for an understanding of the elements of the CGRP receptor function. Clinical trials with olcegepant and telcagepant have demonstrated that CGRP antagonism is an effective acute antimigraine mechanism and has no significant acute side effects. At present, there is no evidence that the novel CGRP receptor antagonists have direct contractile effects, and this may provide a significant advantage over the triptans.

Much remains to be learned about the contributions of peripheral and central CGRP biology to migraine pathophysiology. Future research and development of PET ligands may shed some light on this issue and help further our understanding of migraine pathophysiology.

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