

The Mechanism of Eptinezumab-Jjmr Targeting CGRP In the Treatment of Migraine

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Abstract: Migraine is a neurologic illness characterized by episodic headaches, nausea, and heightened sensitivity to sensory stimulations that affects about 12% of the population. Recently a relatively new and fully humanized monoclonal antibody named Vyepiti (Eptinezumab-jjmr, ALD304) was developed to inhibit the activities of Calcitonin gene-related peptide (CGRP), which can induce headaches, as a preventative treatment for migraine. The effectiveness of Eptinezumab-jjmr is reflected in its special molecular chemical structure. By adding specific FcRn on the basis of IgG1, Eptinezumab-jjmr can bind to both α - and β -forms of the CGRP ligand, resulting in steric hindrance and removing the excessive CGRP that is released at the trigeminal sensory nerve fibers, to prevent nociceptive transmission. Here we provide the background of migraine and the specific molecular structure of CGRP and Eptinezumab-jjmr to illustrate the mechanism of Eptinezumab-jjmr. This approach may be applicable to the development of other monoclonal antibodies, providing a new approach for analytical structural modification for a wider range of biological aspects.

1. Introduction

Migraine, a common type of neurologic chronic disease related to cerebral neurovascular dysfunction that people get repeatedly, occurs with nausea, vomiting, or sensitivity to light. There are 35 million sufferers worldwide and 13% of women between ages 15 and 45. Migraine as the second leading cause of disability worldwide with a strong chronically paroxysmal recurrences have been classified by The World Health Organization as equaling disease with dementia, quadriplegia and several mental illnesses. Nowadays, scientists have made a breakthrough in the understanding and treatment of this pathogenesis of this disease. One of the famed treatment methods is preventive medication. CGRP is a neuropeptide present in human cell bodies and its receptor was identified as a trigger for the discovery of migraine. Eptinezumab-jjmr, humanized monoclonal antibodies, is a prescription medicine used for the preventive treatment of migraine in adults and is the first intravenous (IV) therapy approved by the FDA, blocking the activity of the CGRP. In this study, we will short discuss the background information, but focus more effort on pathology and methodology of migraine will be investigated in detail, including the CGPR target and Eptinezumab-jjmr structure and route and method of administration.

2. Background of Migraine

Migraine is the most common type of primary headache. Clinically, it is characterized by moderate to severe episodic, pulsatile-like headache, which is usually lateral and lasts 4-72 hours. It may be exacerbated by nausea and vomiting, light and sound stimulation, or daily activities. About 60% of

migraine patients have a family history, and their migraine risk is 3-6 times higher in their relatives than in the general population [1]. The pathogenesis of migraines is still not very clear. Previous theories were established surrounding blood vessel dilations and trigeminal neurovascular inflammation.

Vascular action causes headaches by dilating blood vessels. Intracranial pain-sensitive tissue such as cerebrovascular, meningeal vessels, venous sinus, its perivascular nerve fibres and trigeminal nerve may be the physiological basis and pain conduction pathway of migraine. Electrical stimulation of the trigeminal ganglia can lead to aseptic inflammation of the epidural vessels. Trigeminal nerve vascular reflex theory of migraine, that migraine is the trigeminal nerve afferent fibre terminal release P substance (SP) and other neurotransmitters, efferent nerve action on intracranial and external blood vessels, causing headache and vascular dilation.

Certain foods and pharmaceuticals, such as tyramine-carrying cheese, meat and preserved foods containing nitrite preservatives, phenethylamine-containing chocolate, food additives such as monosodium glutamate (MSG), red wine, and wine, can cause migraines. Oral contraceptives and vasodilators like nitro-glycerine are among the medications available. Stress, overwork, emotional agitation, excessive or insufficient sleep, menstruation, and strong light are all examples of environmental and mental conditions that can cause agitation [2].

There are two types of treatment: drug therapy and non-drug therapy. Physical therapy can be combined with magnetic treatment, oxygen therapy, psychological counseling, and other non-drug therapies to relieve pressure, maintain a healthy lifestyle, and prevent all types of migraine triggers. There are two types of drug treatment: paroxysmal treatment and preventive treatment. Nonspecific pain medications including nonsteroidal anti-inflammatory medicines (NSAIDs) and opioids, as well as specific pharmaceuticals like ergot and triptans, are used in treatment. Drugs should normally be used as soon as symptoms appear for optimal results. For individual treatment, drug selection should be based on the severity of the headache, any accompanying symptoms, past medication, and other factors. Eptinezumab-jjmr has an interesting role in curing patients. According to recent studies, thus we need to learn more about its effects and mechanisms.

3. The relationship of Calcitonin gene-related peptide (CGRP) and Migraine

3.1 CGRP receptor

CGRP is a neuropeptide that is found in 36–40% of cell bodies in the human trigeminal ganglion and is involved in pain transmission [3]. Its levels rise during a migraine attack. It could possibly play a function in the initiation of migraine attacks. The CGRP receptor has recently been identified as a hot target for migraine research. CGRP inhibitors work by blocking the action of CGRP, a tiny protein found in abundance in the sensory neurons that feed the head and neck. Migraine treatment includes the use of CGRP inhibitors. CGRP inhibitors are divided into two categories: CGRP receptor antagonists (gepants) and monoclonal antibodies, which are the most recent migraine treatments. The gepants are small molecule CGRP antagonists and work by blocking the CGRP receptor.

Gepants are small chemical migraine relievers and preventers that inhibit the CGRP receptor. Gepants, unlike monoclonal antibodies, penetrate the brain swiftly and work quickly; nevertheless, because they are metabolized in the liver, they have a higher risk of interactions and possibly liver damage. mUbrovelvy (ubrogepant), Nurtec ODT (rimegepant sulfate), and Qulipta (ubrogepant sulfate) are the two that have been approved (atogepant). A monoclonal antibody is a group of identical proteins designed to target a single chemical in the body. Aimovig (erenumab-aooe), Ajovy (fremanezumab), Emgality (galcanezumab-gnlm), and Vyapti are the four monoclonal antibodies approved so far for CGRP inhibitors (eptinezumab-jjmr).

3.2 CGRP antagonist

Olcegepant (BIBN4096BS), the first CGRP antagonist created for human usage, was tested in a proof-of-concept multinational, multicenter, double-blind, randomized clinical research. The rate of sustained response over 24 hours, the frequency of recurrence of headache, improvement in nausea,

photophobia, phonophobia, and functional ability, as well as the time to meaningful alleviation, all indicated substantial superiority over placebo [4].

Merck developed telcagepant (MK-0974), an oral CGRP receptor antagonist that was tested in many large multicenter trials. Following that, based on Merck's experiments. It found that telcagepant had significantly lower 2-hour pain free rates than placebo, as well as no phonophobia, photophobia, or nau-sea. Despite the fact that telcagepant and zolmitriptan had similar efficacy, tecagepant had a considerably superior side effect profile. Telcagepant was employed in a subsequent class II inquiry. Zolmitriptan was utilized in the same way. Telcagepant was given twice daily in a subsequent class II study. Patients had elevated blood alanine aminotransferase levels as a result of the drug [5]. Despite the conclusion of phase 3 acute therapy studies, the medication was pulled from the registration procedure.

Then, in early Phase 2 trials, MK-3207, an anti-migraine CGRP receptor antagonist, was revealed to be superior to placebo at the highest dose tested. However, one patient receiving MK-3207 experienced an adverse event of increased aspartate aminotransferase, forcing the decision to discontinue MK-3207 development [6]. However, compared to CGRP antagonists, a fresh approach on the rise may be beneficial for patients managing migraines: CGRP antibodies.

3.3 CGRP antibodies

PET images were recently used to explore the site of action of CGRP receptor antagonists. [7]. The MK4232 CGRP receptor PET tracer was developed to detect CGRP receptor occupancy in the brain at clinically effective anti-migraine plasma drug levels of the CGRP receptor antagonist telcagepant. MK-4232 has a strong affinity for recombinant human CGRP-R. MK-4232 prefers the CGRP-R (CLR/RAMP1) receptor over the adrenomedullin 2 receptor (CLR/RAMP3) receptor, which has a high affinity for the related human amylin 1 (AMY1; CTR/RAMP1) receptor, and is antagonistic to the human amylin 3 (AMY3; CTR/RAMP3) receptor. PET imaging investigations revealed that CGRP antagonists may have a peripheral mechanism of action, which has driven the development of CGRP monoclonal antibodies (mAb) methods. As a result, novel peripheral target techniques may be effective in migraine treatment. Because of the inhibition of neurogenetic vasodilation, CGRP antibodies may be ideal as a treatment until 2008. Migraine prophylactics are medications that are used to prevent migraines. [8].

mAbs are very target specific and do not cause toxicity because they are metabolized to amino acids and eliminated through the liver and kidney. Monoclonal antibodies have a lengthy half-life, which eliminates prophylactic drug compliance issues. Arteaus Therapeutics' LY2951742, Alder Biopharmaceuticals' ALD403, Labrys Biologics' TEV-48125 (formerlyLBR-101), and Amgen's TEV-48125 (formerlyLBR-101) are among the fore monoclonal antibodies aimed against CGRP in various phases of clinical development (AMG 334). These four monoclonal antibodies (mAbs) are all designed to treat episodic migraine. Chronic migraine is likewise targeted by LBR-101 and AMG334[9]. CGRP-antibodies' specific method of action is uncertain. Antibodies seldom pass the blood–brain barrier (BBB), showing that extracranial nociceptors are the site of action. Some think that during migraine attacks, the BBB is more permeable and that core areas of action are more accessible. Why some people respond entirely is a mystery. Future investigations are needed to clarify the site of action for CGRP antibodies in the CDRP antibody trials [10].

4. Eptinezumab-jjmr Mechanism of Action

Eptinezumab-jjmr is a type of monoclonal antibody. It can bind to CGRP and prevent CGRP function to the trigeminal nerves. Eptinezumab-jjmr was selected from several nonclinical monoclonal antibody candidates from rabbits that were immunized with CGRP. In transfected model systems and cell lines, rat FcRn has a higher apical-to-basal transcytosis than human FcRn. Although rabbits are not rodents, in consideration of the process of humanizing the antibody onto human genes, it is more appropriate to choose rabbits as the select objects. It is essential to say that although a small number of rabbit amino acids are ultimately included in the monoclonal antibody, the rabbit structure and

sequence usage are highly “human-like”, and have little effect on the human body. Affinity, immunogenic recognition (Fc activity), clearance mechanisms (FcRn activity and glycosylation pattern), formulation solubility, and bioavailability are all factors considered during the design process. Each of these qualities was designed to support appropriate dose levels, administration route/schedule, and duration of action in order to meet efficacy, safety, and ease-of-use goals [11].

In terms of the structure of compounds, Eptinezumab-jjmr was engineered on an IgG1 scaffold, and IgG1 interacted well with FcR. IgG are classical Fc receptors. There is evolutionary diversity of antibody isotypes. The establishment of highly variable antigen binding areas is accompanied by the development of limited sets of consistent heavy chains during the evolution of antibody repertoires. The antibody isotype (IgA, IgD, IgE, IgG, and IgM in humans) is determined by these continuous heavy chains, which can be further defined for various isotypes (e.g., in humans as IgG1 to IgG4, IgA1 and IgA2) (Figure 1). The human IgG1 isotype dominates the spectrum of already licensed therapeutic antibodies, and it appears to be the most promising antibody isotype for tumor immunotherapy. In mouse models, human IgG1 antibodies bind efficiently to activating murine Fc receptors on effector cells and appear to be efficacious. IgG1 antibodies have been shown to interact well with both humans and the murine neonatal Fc receptor, in addition to their potential effector effects (FcRn). IgG1 molecules are protected from destruction by binding to FcRn, which extends their serum half-life. Human IgG1 antibodies also showed promising biotechnological properties, such as high production rates in Oma cells that were transfected. These features made it possible to establish Good Manufacturing Practice (GMP) in order to acquire the best medicinal medicines. Importantly, human IgG1 antibodies are frequently employed as the foundation for Fc engineering efforts aimed at improving therapeutic antibody effector functions, stability, or pharmacokinetic features.

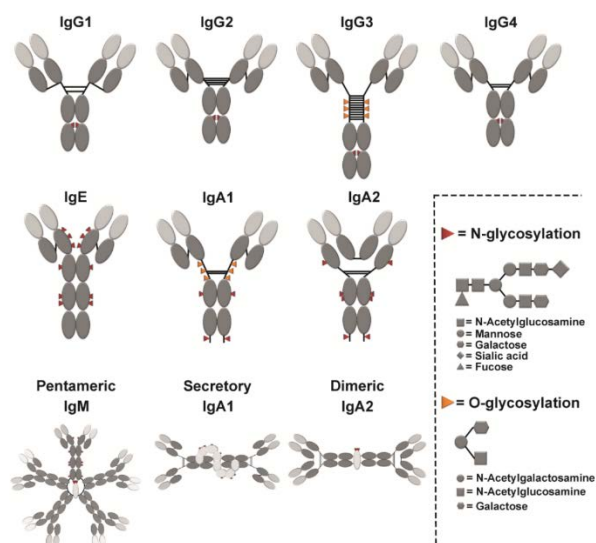


Figure 1: The schematic representation of antibody isotypes and subclasses [12].

FcR (FcRn) is a member of the large and functionally diverse MHC molecule family. FcRn, unlike other MHC family members, has low variety and is unable to present Ags. Instead, it has a high affinity for binding IgG and albumin. FcRn also plays a function in immunity at mucosal and systemic levels by influencing the lifespan of IgG and participating in innate and adaptive immune responses. The ability of FcRn to rescue albumin and IgG from early degradation provides a promising strategy for modifying medicine plasma half-lives, even though the details of its biology are still unknown. The cornerstone for generating novel or modified drugs with higher FcRn-binding capability is the FcRn-dependent redirection of IgG and albumin away from lysosomal degradation, resulting in longer serum half-lives and potentially better pharmacokinetics. [13]. Throughout life, FcRn contributes to effective human immunity by recycling IgG and extending its half-life in circulation (Romanian and Akilesh 2007). In the structure of Eptinezumab-jjmr, the interaction between Fc and its receptor is a glycan-glycan interaction. mAbs, which lack glycosylated Fc regions, tend to lose their effector functions. Therefore, glycoengineering of mAbs can enhance therapeutic efficacy and safety [12].

With a rising number of antibodies available against a wider range of target antigens, choosing the right antibody isotype for a particular therapeutic application may become critical to an antibody's therapeutic success. Investing in this still unknown field of antibody immunotherapy could thus represent a potential source of revenue for the increasingly crowded 'antibody space.'

After the design of Eptinezumab-jjmr's structure, the key of Eptinezumab-jjmr blocked the contact between CGRP and nerve was based on the fact that the All 6 CDRs of Eptinezumab-jjmr (H1, H2, H3, L1, L2, L3) make extensive contact with CGRP (Figure 2). The binding pocket interaction between Eptinezumab-jjmr and CGRP consists of 5 hydrogen bonds and 25 hydrophobic interactions, most of which are between CGRP and the CDRs. The Eptinezumab-jjmr Fab region is in touch with eleven of the 12 CGRP amino acids present in the crystal structure.

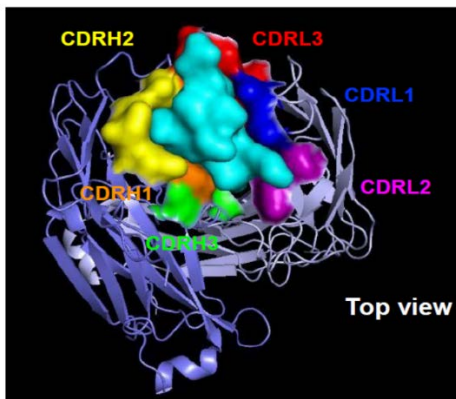


Figure 2: All 6 CDR loops in the Eptinezumab-jjmr Fab region bind to CGRP.
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The complementarity determining region (CDR) is a loop area of an antibody's V-DOMAIN (Variable (V) Domain) that is delimited by the IMGT unique numbering system for V domains [14]. Heavy and light chain heterodimers, which are frequently coupled with disulfide links laid out differently, make up all human isotypes. The ranges of light and heavy chains are different (vL and vH, respectively). A constant domain (cL) exists in light chains, while heavy chains have four constant domains for IgE and IgM and three for all other isotypes. The functional and pharmacokinetic properties of each antibody are determined by the different heavy chains [12]. Both VL and VH have 3 HVR, which together constitute the antigenic binding site of Ig, to determine antibody specificity. They constitute the antigenous binding site of the antibody, and correspond solely to the corresponding antigenic determinant, and have specific binding, which is the structural basis of antigen-specific binding antigen. In that case, the hypervariable region is also called the complementary determinant region of the antibody molecule [15].

FcRn binds to IgG with a 1:1 or 2:1 stoichiometry under unbalanced or equilibrium conditions, according to structural analyses. On the other hand, an FcRn receiver attaches itself to an albumin molecule. FcR connects to each of its two ligands via contacts on opposing surfaces, allowing FcRn to link IgG and albumin at the same time without competition or cooperation. The biochemical and crystallographic findings show that neither the FcRn nor the IgG undergo large structural changes during fixation at pH 6. Bonding is enabled by the protonation of histidine residues (H310, H435, H436) at the IgG1 hinge region CH2-CH3. FcRn's acidic C-terminal $\alpha 2$ domain residues (E117, E132, and D137), as well as the first residue of $\beta 2m$, can form salt bridges at the FcRn-Fc contact.

It's interesting that the FcRn-binding sites in IgG clash with IgG Fc binding to staphylococcal protein A. FcRn-albumin binding, on the other hand, is mostly hydrophobic and is thought to be maintained via a pH-dependent hydrogen-bonding network within each protein. The albumin third domain (DIII) comprises three histidine (H464, H510, and H535) residues, which are necessary for pH-dependent FcRn binding. FcRn has two tryptophan (W53 and W59) residues. Unlike FcRn-IgG interactions, hydrophobic FcRn-albumin binding has no pH-dependent intermolecular salt bridges. FcRn has a stronger affinity for IgG than albumin, according to the findings. FcRn and its ligands

interact in unique ways, which support a diverse spectrum of physiologically relevant FcRn-driven functions. Eptinezumab-jjmr binds to the C-terminal end of the -CGRP peptide, with heavy and light chain CDRs contributing significantly. Eptinezumab-jjmr helps to sequester CGRP and block downstream biologic action in this situation (Figure 3).

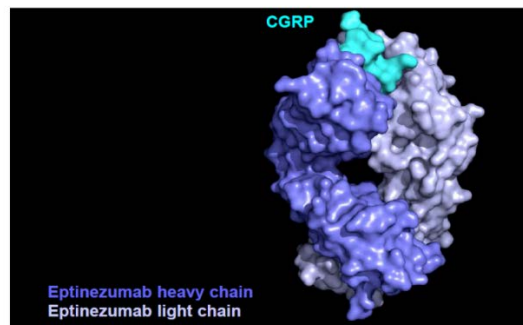


Figure 3: CGRP molecular structure shown in a space filling.

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In population pharmacokinetics and exposure response analysis, Eptinezumab-jjmr exhibits linear pharmacokinetics, achieving a C_{max} of approximately 30 minutes, coinciding with the end of IV administration. Pharmacokinetic analyses of Eptinezumab-jjmr support a dosing schedule of every 12 weeks, with no adjustment for patient characteristics. Duration of effect was demonstrated for both 100 mg and 300 mg doses. When administered intravenously, this anti-CGRP mAb results in 100% bioavailability at the end of the ~30-minute infusion [16]. Although the link between pharmacodynamic activity and the mechanism(s) by which Eptinezumab-jjmr exerts its clinical effects is unknown, it has shown a significant reduction in monthly migraine days in two phase 3 clinical trials and aims to raise expectations with positive 75 percent and 100 percent responder rate results [11]. Therefore, VYEPT has good market expectations.

5. Conclusions

Migraine as a neurologic disorder reduces life quality of a large population. Fortunately, CGRP was identified by scientists as a target for treatment. Two therapeutic options are that CGRP small molecule antagonists and monoclonal antibodies could interact with CGRP. Monoclonal antibodies were advantaged by their high binding specificity and low hepatic toxicity. There are currently just a few monoclonal antibodies against CGRP, and the location of action of CGRP antibodies is unknown. Further study was required to improve antibody therapies because of the failure of bypassing blood-brain barrier to demonstrate the efficacy of antibodies in CNS. As Eptinezumab-jjmr considered as administered intravenously, its demonstrated extensive binding capability with CGRP, as all CDR loops of Eptinezumab-jjmr Fab region binds to CGRP. The pharmacokinetics of Eptinezumab-jjmr showed rapid onset of effect. Through pharmacokinetics analysis, Eptinezumab-jjmr coincides with the end of IV administration and the analysis supports a relative stable dosing schedule for different patient characteristics. It was determined that the effective dose was 100 mg and 300 mg. In short, Eptinezumab-jjmr improves life qualities for many patients and becomes a successful drug on the market expectation.

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