ErbB4 Selective Small Molecule Inhibitors & Chronic Pain Analgesia

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Abstract

Pain is a vital biological process that serves as a warning to prevent further bodily harm and helps in the healing process by protecting the damaged or injured part of the body. When this pain persists and does not subside after the injury or disease heals for 3 months, that is referred to as chronic pain. Chronic pain affects 18.9% of Canadians and has a huge economic burden. Yet, to date, there has been no long-term effective treatments for chronic pain. Opioids are prescribed most, and while they have short-term efficacy, tolerance builds up, and efficacy drops. Recent studies have implicated the ErbB4 receptor with pain management. This paper discusses the summer project I carried out and its future direction. It also reviews the possibility of small molecule inhibitors of ErbB4 receptors serving as a pharmacological intervention for chronic pain relief.

Introduction

Pain is a vital biological process that serves as a warning to prevent further bodily harm and helps in the healing process by protecting the damaged or injured part of the body. Normally, pain subsides as the injury or disease heals, but through unknown pathological processes, pain may persist after the injury and/or disease has resolved. Any type of pain which lasts longer than 3 months is referred to as chronic pain (Treede et al., 2015). In Canada, it is estimated that around 18.9% of adults older than 18 years have chronic pain and it has a huge economic burden that is estimated to be around $37 billion per year – exceeding that of cancer, heart disease and HIV combined (Schopflocher et al., 2011; Moulin et al., 2002; Lynch, 2011). Despite its prevalence and its negative impact on society, chronic pain is still not properly managed (Hogan

et al., 2016; Green & Hart-Johnson, 2010; Groenewald et al., 2014). Currently, opioids are the most prescribed analgesics that are used to manage chronic pain (Rosenblum et al., 2008; Gaskell et al., 2016; Dhalla et al., 2009). While opioids are effective for short-term pain management, they quickly build up tolerance and lose efficacy. In return, due to the decreased efficacy, the doses given to patients need to increase to maintain the same pain relief, and that in turn increases addiction risk and overdose. Therefore, other pain management strategies and drugs need to be discovered to resolve this current opioid epidemic.

Growth factors are vital for development they affect our growth, homeostasis, and affects many cellular functions (Wieduwilt & Moasser, 2008). Growth factors carry this out through cell-cell communication which underlies many important processes such cell fate determination, cell apoptosis and its survival, specializing of tissues, and migration of cell (Wieduwilt & Moasser, 2008). Growth factors bind to their receptors extracellularly and trigger a cascade that leads to the activation of intracellular messengers that carry out changes in the cell or through the internalization of the receptor and translocation to the nucleus to cause transcriptional changes (Wieduwilt & Moasser, 2008). Among these many receptors for growth factors, our focus will be on the receptor tyrosine kinase family, specifically the Epidermal growth factor receptor family, also known as the ErbB family. This family consist of four receptors: ErbB1, ErbB2, ErbB3, and ErbB4 (Wieduwilt & Moasser, 2008).

Due the various contributions these growth receptors have on important cell functions and the development and maintenance of many tissues, they are extensively implicated in various diseases. Diseases include psoriasis, peripheral neuropathies, Alzheimer's disease, schizophrenia, and cancer (Wieduwilt & Moasser, 2008). More recently, ErbB receptors have been implicated with pain management (Yu et al., 2021; Kersten et al., 2015; Wan et al., 2021).

Inhibitors targeting the tyrosine kinase site of these receptors have been found to be effective analgesics in mice for inflammatory and neuropathic pain and their activation leads to enhanced pain (Yu et al., 2021). Specifically, the ErbB4 receptor has been implicated in schizophrenia, Parkinson's disease, and we have data showing that inhibition of ErbB4 reduces chronic pain hypersensitivity in mice. Therefore, the identification of a selective small molecule inhibitor for ErbB4 with high binding affinity would serve as a potential candidate to treat and manage chronic pain and other debilitating diseases.

Pain relief agents currently on the market

Currently, there are several families of agents that possess clinical utility as pain therapeutics. They have varying degrees of efficacy for different pain states and adverse event profiles that often limit their utility (Dunwoody et al., 2008; Joshi & Ogunnaike, 2005; Goldberg & McGee, 2011). Management of inflammatory pain typically involves nonsteroidal anti-inflammatory drugs (NSAIDs), for example, inhibitors of cyclooxygenases (COX-1 and/or COX-2) and opiate-based drugs (Ong et al., 2007; Brunton, 2011). For pain associated with nerve injury, treatment options include anti-depressants to block monoamine uptake (amitriptyline, duloxetine, venlafaxine) (Sindrup et al., 2005; Finnerup et al., 2010), anticonvulsants to block sodium channels (lidocaine, carbamazepine) (Mika et al., 2013; Wallace et al., 1996; Dworkin et al., 2007; Wiffen et al., 2014), calcium channel blockers (e.g. ziconotide, gabapentin) (Wiffen et al., 2013; Moore et al., 2014) or increasing extracellular levels of the inhibitory transmitter gamma aminobutyric acid (GABA) (tigabine) (Dalby, 2003; Todorov et al., 2005). To a lesser extent, opioids have been used and topical medications such as lidocaine and capsaicin (Derry & Moore, 2014; Smith & Brooks, 2014). Often, combinatorial therapies that employ multiple agents with distinct targets and non-overlapping side effect profiles achieve improved therapeutic benefit when treating

neuropathic conditions (Chaparro et al., 2012). Of interest is whether any of these agents might be able to prevent the transition from an acute reversible injury state to a chronic pain state (Clarke et al., 2012). While these pain relief agents have demonstrated efficacy, it is often found that, even with aggressive management, the patient's pain remains of a similar magnitude as the initial pain state (Häuser et al., 2010; Moore et al., 2009). It is often difficult to know whether efficacy is restricted by the limitation of the drug to interact with the target or whether side effects preclude administration of higher doses (Woolf, 2010). Therefore, the development of novel therapeutics to manage pain has been a focus of significant investment and effort (Woolf, 2010; Kissin, 2010).

ErbB Receptors

As mentioned earlier, the ErbB receptors belong to the receptor tyrosine kinase family of receptors. The receptors bind to 11 separate but structurally similar growth factors that affect different cell processes and mediate development, homeostasis, and pathologies (LINGGI & CARPENTER, 2006). The receptors are made of a single chain transmembrane glycoprotein (LINGGI & CARPENTER, 2006). These chains contain a domain that is exposed to the extracellular space (ectodomain), this is the ligand-binding domain (Wieduwilt & Moasser, 2008). They also contain a transmembrane domain, juxtamembrane section, a tyrosine kinase domain, and a tyrosine-containing C-terminal tail. Binding of ligand to the ectodomain causes the receptor to undergo a homodimer formation with another ErbB receptor of the same kind or a heterodimer with a different kind of ErbB receptor (Wieduwilt & Moasser, 2008). This formation of a dimer is vital for the activation of the tyrosine kinase domain on the receptor and that leads to the phosphorylation of the C-terminal tail (Wieduwilt & Moasser, 2008). The residues then lead to the direct or indirect activation of various components of many signaling pathways (Wieduwilt & Moasser, 2008). Specifically, ErbB4 is a fully functional receptors as it can bind to both ligands

and undergo autophosphorylation, it can also undergo homodimerization or heterodimerize with ErbB2 (Wieduwilt & Moasser, 2008).

ErbB4 receptor and Neuropathic pain

Recent studies have specifically implicated the ErbB4 receptor with chronic pain. In one study, researchers assessed the association of mechanical allodynia in neuropathic pain with the ErbB4 receptors in spinal parvalbumin (PV) interneurons (Yu et al., 2021). Mechanical allodynia is a sensation of pain that is caused by mechanical stimulation that is not painful, such as light touch. PV interneurons are part of the inhibitory circuits for nociceptive signals reaching the spinal cord, most are GABA-immunoreactive, and all are glycine-immunoreactive neurons (Laing et al., 1994). ErbB4 has been found to be selectively expressed in these interneurons (Yu et al., 2021).. After inducing chronic pain in mice, the results showed that the chronic pain model used, increased the activation of ErbB4 receptors in the spinal cord (Yu et al., 2021). Furthermore, when ErbB4 receptor gene expression is silenced, it either prevented the induction of mechanical allodynia or attenuated it (Yu et al., 2021). Activation of ErbB4 receptor with NRG1 lowered the pain threshold in mice. Therefore, this study implicates the ErbB4 receptor in PV interneurons in the regulation of mechanical allodynia (Yu et al., 2021). Another study carried out also demonstrated that regulation of NRG1-ErbB4 signaling in the spinal cord could reduce pain hypersensitivity and lead to analgesia in inflammatory pain (Wan et al., 2021). Therefore, targeting small molecule inhibitors towards the ErbB4 receptor shows promise as a possible pharmaceutical treatment. Furthermore, it offers a pain relief pathway that avoids the opioid receptors, avoiding the tolerance and drop of efficacy associated with drugs that target them.

Methodology

I have outlined below some experiments that can be used to identify small molecule inhibitors for the ErbB4 receptors. There is cell culture involved in growing HEK-293 cells and then transfecting them with the receptors. Upon successful transfections, inhibitors can then be tested for their efficacy in binding. Unfortunately, due to many COVID-19 restrictions, I was unable to fully carry out these experiments.

Cell Culture

To start off, *in vitro* cell culture is carried out using HEK-293 (human embryonic kidney cells) were cultured in 100mm x 15 mm petri dishes. These cells were maintained in the University of Toronto Mississauga Cell Culture Facility in an incubator at 37 degrees Celsius and 5% CO_2. The cells were grown in a solution of DMEM containing 10% fetal bovine serum (FBS) to promote rapid growth and 1% penicillin/streptomycin mix to prevent bacterial growth.

Transfection

When cells reach the optimal confluency, they can then undergo DNA transfections. A full-length ErbB4 cDNA templated should be obtained. This DNA fragment can then be cloned into an expression vector. The cells can then be transfected with the expression vector to express ErbB4 receptors. To assess the transfection efficiency, a human ErbB4 ELISA kit can be used to quantitatively determine how many ErbB4 receptors are found in the cell culture, coupled with immunofluorescence staining and imaging, and finally, western blots.

Selective Small Molecule Inhibitors

The inhibitors were developed by the company Atomwise which develops test compounds through an artificial intelligence virtual screen based on predicted binding sites.

cAMP Production Assay

After assessing that the transfections were successful, the binding affinity of the inhibitors to the receptor needs to be assessed. A cAMP assay kit is used. The well plates containing the cells are incubated with the reagents found in the kit for 2 hours at room temperature. The cells can then be treated with varying concentration of the inhibitors for 30 minutes and then undergoing a challenge with the agonist (NRG1). Some of the experiments are ran without the antagonist to assess the effect of the agonist alone. cAMP levels can then be assessed through its luminescence about 10 minutes are treating with the agonist through a cell imaging machine. A serial dose curve for each inhibitor can then be constructed which will determine the EC50 value. EC50 is the concentration at which of the maximal effect is induced. Furthermore, the agonist concentration can also be varied to perceive the change in the EC50 value with changing competitive agonist concentrations. A Schild plot can then be created to assess the affinity of the inhibitors for the receptor. Also, through these experiments various other affinity values can be calculated such as the dissociation constant, IC50, and the inhibition constant, to further assess each inhibitor's ability to bind to receptors.

Future Directions

This is a very recent area of research, so not much is known about the ErbB4 receptor and its implication with various signaling pathways and its possible relief of pain. However, it shows a lot of promise and also utilizes a pathway that does not involve opioid receptors, which could help reduce the opioid epidemic in the future. One area that is required to be researched more is the downstream effects that the inhibition of ErbB4 receptors might have on cells. It is known that ErbB4 plays a big role in development and homeostasis, so it would be essential to know if its inhibition has any detrimental effects. Second, if some inhibitors are found to be effective with

good binding affinity, their affinity towards other ErbB receptors needs to be assessed and specific inhibitors that are specific to ErbB4 only need to be selected. Finally, these inhibitors need to be tested *in vivo* on mice to test their effectiveness. While various drugs are very successful during the in vitro stage, the transfer of these drugs into animals is a very complex matter and needs to be tested accurately to consider moving to clinical trials.

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