Patient-derived neuronal models for pharmacogenetic pain treatment of sodium channelopathies

CHAPTER 7

SIGNIFICANCE STATEMENT

CLINICAL AND SCIENTFIC IMPACT

Chronic pain affects nearly 1 in 4 individuals worldwide [1-3], and despite its global spread, treatments for this condition are still largely ineffective and carry serious side effects [4, 5]. Recent drug developments have focused on inhibitors of voltage-gated sodium channels (VGSC), which, when dysfunctional, are associated with several human pain syndromes [6-8]. Different classes of VGSC blockers have emerged, including anticonvulsants, local anesthetics, and isoform-selective blockers. Lacosamide, an anticonvulsant and emerging candidate for treating pain [9], has shown variable efficacy in patients with $Na_V1.7$-related small fiber neuropathy (SFN) [10]. To date, the peripheral mechanisms underlying lacosamide treatment response modulation are still poorly understood. With limited number of individuals responsive to available medications, unraveling the contributing factors interfering with pain targets and drug modulation is highly warranted. In **chapter 2**, we recapitulated inter-individual variations in lacosamide response in patients with $Na_V1.7$ mutations. Our results support the utilization of a pre-clinical biophysical assessment of specific mutations to select compounds to predict individualized patient response. Furthermore, our -and similar- findings can be extrapolated back to the clinic for patients genotyped with already-assessed $Na_V1.7$ mutations, allowing clinicians to anticipate their response to, in this case, lacosamide. We also linked specific genetic mutations to distinct pharmacological phenotypes in different SFN patients, which can be incorporated in future studies to develop better-suited treatment strategies. Epilepsy research has taught us important lessons on the extrapolation of genomic data for pharmacotherapy, by titrating dosages based on $Na_V1.7$ splicing variants [11]. A comparable, easy-to-use pharmacogenomic-based model is proposed here for pain, which can as well be implemented to study a wide range of mutations and drugs, whereby the biophysical signature correlating to a positive drug response can guide treatment.

Understanding the mechanisms of action of analgesics, such as lacosamide and its isoform-selectivity, is critical to make informed decisions when treating specific patients and could aid future drug developments. Lacosamide has been shown to exert its effects in an unconventional manner compared to traditional pore-blocker sodium channel inhibitors [12-16]. Solving its mechanistic basis can further explain inter-individual variability in efficacy and tolerability in treated patients [10]. **Chapter 3** provides a description of a working model of lacosamide and reports a novel binding region in $Na_V1.7$, in the voltage-sensing domain 4 (VSD4), which, alongside an intact local anesthetic site within the channel's pore, is required for lacosamide to exert its therapeutic functions. The role of the VSD4 seems to be determinant to lacosamide-isoform specificity at clinically-achievable concentrations. By unraveling the interaction between lacosamide and the VSD4, **chapter 3** sheds a light on possible implications in other sodium channelopathies, where other VGSC isoforms are affected, for example in patients with VGSC-related epilepsy or cardiac disease. By establishing the binding properties of lacosamide, we can make better predictions on drug efficacy and tolerability in patients. Our findings also highlight the pivotal role the

VSD play in initiating and maintaining lacosamide functions at therapeutic concentrations, which may be relevant in patients with mutations located in the VSD. Specifically, our results show that in cells expressing the W1538R variant, the mutation-related lacosamide block can be overcome by increasing dosage. Therefore, genomic evaluation of neuropathic pain patients, whereby specific genetic variants in $Na_V1.7$ are detected, can be used as a dosing guideline.

Modeling genetic diseases is a tedious task, as there is a constant need for further developing cellular systems mimicking those in physiological conditions. The use of patient-specific cells can extend our understanding of pain pathophysiological mechanisms [17-20] and drug response [17, 21, 22]. In **chapter 4**, we initiated the development of a dorsal root ganglion (DRG)-targeted *in vivo* and *ex vivo* differentiation method, as a model of "humanized" cells, to offer a more translational approach, which could potentially help increase the success rates in clinical trials. While we did not succeed in forcing iPSC differentiation *in vivo* yet, successful optimization of our iPSC methods could inform us on iPSC patterns of migration, speed of differentiation and body absorption. Furthermore, this may allow drug screening in iPSCs, which will in turn extend our knowledge on iPSC-derived cells metabolism in response to drug exposure, and further delineate the responsiveness of alternative variants to different VGSC-blocking treatments. Furthermore, our results were educational on inter-species physiological interactions and on iPSC behaviors and parameters that affect DRG teratoma development. Our study provided substantial data on the differentiation and migration properties of undifferentiated cells in the rat DRG and its potential for tumorigenicity, as well as its influence on non-transplanted adjacent organs and overall physiological functions. Although beyond the scope of this thesis, these findings can serve as a basis for stem cell therapy in the DRG on how to avoid cell graft rejection and tumor formation, which can have major implications for advances in regenerative medicine. Furthermore, our model provides important insights to the study of iPSCs that are transplanted into a specific organ and can be used to propel research efforts in other pain-related or unrelated diseases.

Besides concerns associated with iPSC immaturity, the lack of functional expression of the $Na_V1.8$ and $Na_V1.9$ sodium channels in iPSC-derived sensory neurons (iPSC-SN) still represent a prominent caveat to the study of painful sodium channelopathies in iPSC models [18, 23]. These two channels are particularly important to pain signaling as they contribute to a large portion of the sodium current transmission and cellular excitability [24, 25]; therefore, without their physiological input, the recorded current is corrupted and not representative of the patient's cells true electrophysiological properties. In **chapter 5**, we described for the first time the relationship and contribution of human $Na_V1.8$ and $Na_V1.9$ channels to human neuronal excitability as pain correlates. Furthermore, not only were we able to produce $Na_V1.8$ and $Na_V1.9$ currents, but we also developed a brand-new model allowing the study of $Na_V1.8$ and $Na_V1.9$ mutations in iPSC-SNs via dynamic clamp, which was never possible before. Moreover, this technique can be used to introduce several mutations simultaneously,

in the same patient-specific cells, covering a wider range of genetic variants and possible interactions. In turn, patients who carry genetic variants in the two channels can be evaluated in the future. Additionally, the overall cellular excitability of patient iPSC-SNs with $Na_V1.7$ mutations might be restored to similar levels that would have been recorded in the donor's neurons, and thus, aid find better fitted treatments. Our ability to modulate $Na_V1.8$ and $Na_V1.9$ via dynamic clamp may further guide future therapeutic considerations in drug development and clinical trial dosing. Specifically, dynamic clamp of iPSC-SNs might bridge the gap in knowledge regarding the precise degree of channel inhibition needed to induce therapeutic relief without total loss of pain sensation [26, 27].

Taken together, the findings from this thesis have set the stage for using pharmacogenomic-guided tools in patient-specific iPSC-SNs to solve pain mismanagement while providing novel strategies to implement personalized therapy in pain research.

REFERENCES

1. Reid, K.J., et al., *Epidemiology of chronic non-cancer pain in Europe: narrative review of prevalence, pain treatments and pain impact.* Curr Med Res Opin, 2011. **27**(2): p. 449-62.
2. Kennedy, J., et al., *Prevalence of persistent pain in the U.S. adult population: new data from the 2010 national health interview survey.* J Pain, 2014. **15**(10): p. 979-84.
3. McCarberg, B.H. and R. Billington, *Consequences of neuropathic pain: quality-of-life issues and associated costs.* Am J Manag Care, 2006. **12**(9 Suppl): p. S263-8.
4. Nishikawa, N. and M. Nomoto, *Management of neuropathic pain.* J Gen Fam Med, 2017. **18**(2): p. 56-60.
5. Finnerup, N.B., et al., *Pharmacotherapy for neuropathic pain in adults: a systematic review and meta-analysis.* Lancet Neurol, 2015. **14**(2): p. 162-73.
6. Bennett, D.L., et al., *The Role of Voltage-Gated Sodium Channels in Pain Signaling.* Physiol Rev, 2019. **99**(2): p. 1079-1151.
7. Dib-Hajj, S.D. and S.G. Waxman, *Sodium Channels in Human Pain Disorders: Genetics and Pharmacogenomics.* Annu Rev Neurosci, 2019.
8. Dib-Hajj, S.D., et al., *The Na(V)1.7 sodium channel: from molecule to man.* Nat Rev Neurosci, 2013. **14**(1): p. 49-62.
9. Carona, A., et al., *Pharmacology of lacosamide: From its molecular mechanisms and pharmacokinetics to future therapeutic applications.* Life Sci, 2021. **275**: p. 119342.
10. de Greef, B.T.A., et al., *Lacosamide in patients with Nav1.7 mutations-related small fibre neuropathy: a randomized controlled trial.* Brain, 2019. **142**(2): p. 263-275.
11. Raymond, C.K., et al., *Expression of alternatively spliced sodium channel alpha-subunit genes. Unique splicing patterns are observed in dorsal root ganglia.* J Biol Chem, 2004. **279**(44): p. 46234-41.
12. Errington, A.C., et al., *The investigational anticonvulsant lacosamide selectively enhances slow inactivation of voltage-gated sodium channels.* Mol Pharmacol, 2008. **73**(1): p. 157-69.
13. Niespodziany, I., et al., *Comparative study of lacosamide and classical sodium channel blocking antiepileptic drugs on sodium channel slow inactivation.* J Neurosci Res, 2013. **91**(3): p. 436-43.

14. Sheets, P.L., et al., *Differential block of sensory neuronal voltage-gated sodium channels by lacosamide [(2R)-2-(acetylamino)-N-benzyl-3-methoxypropanamide], lidocaine, and carbamazepine.* J Pharmacol Exp Ther, 2008. **326**(1): p. 89-99.
15. Wang, Y., et al., *Development and characterization of novel derivatives of the antiepileptic drug lacosamide that exhibit far greater enhancement in slow inactivation of voltage-gated sodium channels.* ACS Chem Neurosci, 2011. **2**(2): p. 90-106.
16. Rogawski, M.A., et al., *Current understanding of the mechanism of action of the antiepileptic drug lacosamide.* Epilepsy Res, 2015. **110**: p. 189-205.
17. Cao, L., et al., *Pharmacological reversal of a pain phenotype in iPSC-derived sensory neurons and patients with inherited erythromelalgia.* Sci Transl Med, 2016. **8**(335): p. 335ra56.
18. Meents, J.E., et al., *The role of Nav1.7 in human nociceptors: insights from human induced pluripotent stem cell-derived sensory neurons of erythromelalgia patients.* Pain, 2019. **160**(6): p. 1327-1341.
19. Mis, M.A., et al., *Resilience to Pain: A Peripheral Component Identified Using Induced Pluripotent Stem Cells and Dynamic Clamp.* J Neurosci, 2019. **39**(3): p. 382-392.
20. Yuan, J.H., et al., *KCNQ variants and pain modulation: a missense variant in Kv7.3 contributes to pain resilience.* Brain Commun, 2021. **3**(3): p. fcab212.
21. Capurro, A., et al., *Nav1. 7 gating in human iPSC derived sensory neurons: an experimental and computational study.* bioRxiv, 2020.
22. Namer, B., et al., *Pain relief in a neuropathy patient by lacosamide: Proof of principle of clinical translation from patient-specific iPS cell-derived nociceptors.* EBioMedicine, 2019. **39**: p. 401-408.
23. Eberhardt, E., et al., *Pattern of Functional TTX-Resistant Sodium Channels Reveals a Developmental Stage of Human iPSC- and ESC-Derived Nociceptors.* Stem Cell Reports, 2015. **5**(3): p. 305-13.
24. Herzog, R.I., T.R. Cummins, and S.G. Waxman, *Persistent TTX-resistant Na+ current affects resting potential and response to depolarization in simulated spinal sensory neurons.* J Neurophysiol, 2001. **86**(3): p. 1351-64.
25. Renganathan, M., T.R. Cummins, and S.G. Waxman, *Contribution of Na(v)1.8 sodium channels to action potential electrogenesis in DRG neurons.* J Neurophysiol, 2001. **86**(2): p. 629-40.
26. McDonnell, A., et al., *Efficacy of the Nav1.7 blocker PF-05089771 in a randomised, placebo-controlled, double-blind clinical study in subjects with painful diabetic peripheral neuropathy.* Pain, 2018. **159**(8): p. 1465-1476.
27. Siebenga, P., et al., *Lack of Detection of the Analgesic Properties of PF-05089771, a Selective Na(v) 1.7 Inhibitor, Using a Battery of Pain Models in Healthy Subjects.* Clin Transl Sci, 2020. **13**(2): p. 318-324.