Experimental pain models for the evaluation of next-generation analgesics in clinical pharmacology studies

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CHAPTER 9

General discussion and conclusion

Scientific progress, and in particular development of drugs, has been accelerating with unprecedented speed. Where discovery of medicines initially was based on herbal knowledge (e.g., aspirin has been formulated from the willow bark), drugs are now discovered by high-throughput screening of libraries containing candidate molecules for their biological activity (i.e., combinatorial chemistry) or screening of molecules for their interaction with a biomolecule proposed to yield therapeutic benefit (i.e., rational drug design). Pharmaceutical and biotechnological companies utilize these approaches and leverage improved knowledge of biological targets to discover and develop novel, often highly selective (analgesic) drugs that are expected to yield improved clinical utility over classical medicines with fewer dose-limiting adverse effects.

By redefining drug discovery, drug development strategies should be revised as well. Biological processes are known to vary widely between species, which is also true for pain signalling. An example described in this thesis is the clear difference in availability of voltage-gated sodium channel (Na_V)1.8 and Na_V1.9-positive sensory neurons between humans and mice. [1] A wide range of preclinical models have been developed to mimic human pain disease phenotypes, but their predictive value is questionable. [2] Although animal models remain a vital tool in drug testing, they commonly are not equipped to accurately predict the full nature of a drug's therapeutic effects. [3] As a further complication, costs associated with human trials are ever increasing, [4] warranting careful decision making on a drug's potential early in the clinical development process.

By including biomarkers that allow for measuring pain signalling in early-phase drug studies, important data on (dose-dependent) effects can be generated, which can save costs in later-phase trials. It must be noted that, while of importance, biomarkers mostly are models for clinically relevant endpoints at best, e.g., in healthy volunteers they can only mimic a specific part of a certain (pain) pathology. Another challenge is that many novel drugs are increasingly target-selective, and may have effects on (pain) pathways that often have yet to be clinically proven relevant. Previously validated methods should therefore be scrutinized for their validity to establish Proof-of-Mechanism or Proof-of-Concept (POM, POC; **Chapter 1**) of new drug classes. In parallel, improved selectivity necessitates further refinement of human experimental models to more accurately represent aspects of clinical disease or symptoms targeted. Based on these advances, the studies described in this thesis were conducted: a

quest for finding suitable biomarkers, by developing and testing models for usability to evaluate Na_V inhibitors, the third-to-most studied analgesic drug class in early-phase drug development (**Chapter 1**).

In **Chapter 1**, we defined that a proper (analgesic) biomarker should be able to demonstrate a clear, consistent drug response across different studies, and should demonstrate it consistently for drugs of the same class. [5] By using PainCart – the fixed-sequence nociceptive test battery employed in the studies described in this thesis –, in combination with either the topical 1% capsaicin cream model or ultraviolet (UV)B model that were developed previously, [6] we assessed in **Chapters 2-4** which currently available methods are suitable to consistently demonstrate effects of Na_V inhibitors on nociceptive thresholds. Altered cold pressor pain thresholds proved to be the most reproducible biomarker, by responding to three Na_V inhibitors (VX-128, VX-150 and mexiletine) and aligning with our hypothesis described in **Chapter 1**. In that Chapter, we also suggested to include the capsaicin model in studies evaluating Na_V inhibitors, but observed that neither of the selective Na_V1.8 inhibitors tested with that model (i.e., VX-150 nor VX-128; **Chapter 2** and **3**, respectively) affected capsaicin-induced heat pain thresholds. This may be explained as Na_V1.7 rather than Na_V1.8 is linked to inherited erythromelalgia ('man on fire' syndrome), supported by the finding that selective Na_V1.7 inhibitors PF-05089771 reduced burning-like symptoms in a phase II trial. [7,8] It may, however, also be concluded that the topical 1% capsaicin cream model is suboptimal for studying analgesics, as the same model failed to show effects of tramadol or duloxetine. [6] This led us to performing the study described in **Chapter 8**.

A pharmacological biomarker should also clearly (and when applicable, dose-dependently) respond to therapeutic dose levels of drugs. [5] The tests included in the PainCart battery have previously been profiled using a variety of registered analgesics, including the Na_V-blocking antiepiLEPtic phenytoin that significantly affected nociceptive thresholds in the electrical stair pain paradigm. [9] It was, however, concluded that insufficient plasma concentrations of phenytoin were reached, preventing use of that data for evaluation of this biomarker criterion. In **Chapter 4** we noted that the cold pressor test significantly responded to therapeutic doses of Na_V inhibitor mexiletine, but not lacosamide. Based on the differential characteristics of mexiletine and lacosamide – preferential modulation of Na_V1.8 versus Na_V1.7, respectively – we discussed that

biomarker selection should depend on which Na_V-subtype is primarily targeted. Corroborated by evidence from the VX-128 and VX-150 studies (**Chapter 2 and 3**), this thesis supports the use of the cold pressor test as biomarker for $Na_V1.8$-induced analgesia. The effects (or lack thereof) of lacosamide on nociceptive thresholds using PainCart provide further evidence that evoked pain models may not be suitable for evaluating $Na_V1.7$-preferential analgesics: others could also not demonstrate analgesics effects of lacosamide on experimental pain models, and no effects could be demonstrated of selective $Na_V1.7$ inhibitor PF-05089771. [6,10] While at time of discovery, in 2006, the role of $Na_V1.7$ in pain signalling was considered a major breakthrough, $Na_V1.7$ inhibitors have withhold their pain potential as none has been registered as of yet. [11] Without evidently efficacious $Na_V1.7$ inhibitors available to test, it remains difficult to draw unambiguous conclusions on the validity of currently available methods for that subtype. It may be that other methods such as the nerve excitability threshold tracking test are more suitable, as it demonstrated POM of lacosamide on, e.g., motor nerve excitability and sodium channel conductance. [12]

Having established that there is still ample room for improvement in the development of suitable biomarkers for profiling of (selective) Na_V inhibitors in healthy volunteers, hyperalgesia-inducing methods were considered. As stated in **Chapter 1**, a suitable biomarker should have a plausible relationship with the pharmacology of the tested drug class, and with the disease pathophysiology. A key aspect in many types of chronic pain such as fibromyalgia and neuropathic pain syndromes, is central sensitization – defined by the International Association for the Study of Pain (IASP) as *'an increased responsiveness of nociceptors in the central nervous system to either normal or sub-threshold afferent input'*. [13,14] Central sensitization may manifest as symptoms such as hyperalgesia and allodynia. [13] These relate to conditions caused by nociceptor hyperexcitability, a mechanism targeted by Na_V-inhibiting drugs – and by selective $Na_V1.8$ inhibitors in particular. [15] Inducing hyperalgesia in healthy volunteers therefore was determined as potentially leading to suitable pharmacodynamic biomarkers for Na_V inhibitors – and/or other analgesic drug classes.

Wishing to further expand our knowledge on hyperalgesia testing and expand our range of models in healthy volunteers, we examined whether two distinct models – total sleep deprivation (TSD) and topical application of 1% capsaicin ethanolic solution – could be used in experimental

clinical trial-context to induce hyperalgesia and/or allodynia. We noted that TSD induced sex-dependent hyperalgesia on cold-, heat- and pressure pain, and altered the conditioned pain modulation response (**Chapter 6**), as well as nociceptive processing (**Chapter 7**). The 1% capsaicin ethanolic solution model was found to increase sensitization to heat and induce secondary allodynia (**Chapter 8**). Those results confirmed suitability of these methods in healthy volunteer drug studies, but follow-up studies with pharmacological interventions are warranted to adequately test if they are sensitive to drug effects as well.

The role of Na_V-1.7 and Na_V-1.8 in inflammatory pain is through modulation by kinases such as PKA (protein kinase) and P38 MAPK (mitogen-activated protein kinase). [16] Following injury or inflammation, various inflammatory cells and mediators (e.g., macrophages, neutrophils, mast cells) are recruited to the affected tissue that subsequently increase the level of a set of kinases, including P38 MAPK. Na_V-1.7 and Na_V-1.8 – channels that are upregulated in nociceptors that innervate the affected tissue – become phosphorylated and modulated, resulting in increased ectopic action potential generation and ultimately to hyperalgesia and allodynia. [16] The human endotoxemia model (i.e., systemically administering lipopolysaccharide (LPS)) can be used to induce systemic inflammation and P38 MAPK signalling. [17] We tested in **Chapter 5** whether this could translate into a systemic inflammatory hyperalgesia model. However, LPS was not able to evoke clear, consistent and dose-dependent, inflammatory hyperalgesia, failing as challenge model to be part of a suitable biomarker for profiling effects of analgesics.

In the present thesis, we have attempted to address certain issues that analgesic drug developers are facing in the early-phases of clinical drug development, by reviewing applicable tools for the top-10 most-developed analgesics in early-phase clinical development (**Chapter 1**), by using those methods to profile investigational and registered analgesic compounds (**Chapters 2-4**), and finally by exploring other tools that may further improve predictability of a drugs' anti-hyperalgesic effects in healthy volunteers (**Chapters 5-8**). While two methods are suitable for further testing, we need to note that – except for the sleep deprivation model – most studies were only performed in male volunteers, and only mimicked one or few aspect(s) of the complex and multifactorial symptom that is pain. As such, psychological and psychosocial factors that play a role in pain chronification were left out-of-scope. Mostly as they are (yet) unfeasible

and/or unethical to test in study context (e.g., exposing healthy subjects to irreversible or prolonged pain), but also because of the exploratory nature of this research. Novel technologies including augmented/virtual reality (VR) may serve a purpose here, as they may aid in further refining methods and assessing aspects of pain that have been infeasible to test without putting the safety of volunteers at risk. While VR in pain research till date primarily has been used to temporarily inhibit the pain perception by introducing immersive images (e.g., an interactive snowy canyon environment during the treatment of burn wounds), [18] VR simulation possibly may also be used as biomarker to enhance the pain experience and assess the affective component of pain perception. Preliminary results from a study using such a method at the Centre for Human Drug Research (CHDR) seem promising and suggest for a follow-up study to evaluate drug effects targeting affective pain mechanisms. [19]

In an industry with exhaustive lead times such as the pharmaceutical sector, improving methods is key in reducing the time needed to bring medical products onto the market. Recently, the European Medicines Agency released a guidance to help developers navigate through the most important regulatory requirements in the clinical development trajectory of advanced therapy medical products (ATMPs), stipulating to answer important questions about the drug's therapeutic potential in a timely manner. [20] Although most analgesics are not identified as ATMPs, the same approach should apply. Drug developers and clinical researchers that aid in this process, are advised to design early-phase studies in such a way that allow to demonstrate POM and/or POC early-on in healthy volunteer studies, or in well-chosen patient (sub-)populations – but not to test neither and leave questions unanswered till late. The results described here offer an opportunity to aid in this process and refine pain research, in an effort to bring therapies with improved clinical efficacy to the pain patients in need.

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