PDE4 gene inhibition

Impact

Societal impact

One of the major hurdles regarding the treatment of neurodegenerative and demyelinating disorders, is the impairment of endogenous recovery and repair processes in these diseases. Chronic neuroinflammation, persistent demyelination and nutrient deprivation leaves neurons vulnerable, ultimately leaving to neurodegeneration. Unfortunately, current treatment strategies are insufficient in providing long term neuroprotection and -repair. Hence, there is an urgent medical need for efficient therapeutic strategies capable of supporting glial cell functioning, thereby allowing neuroreparative processes to occur.

Target group - MS patients

MS is the most common neurodegenerative disease in young adolescents and affects 1 in 1000 people in Belgium and over 2.5 million people worldwide (600 000 in Europe). Globally, over 1 million patients suffer in particular from secondary PMS. Currently, the treatment of MS patients comprises the administration of immunomodulatory and immunosuppressive drugs. The disease-modifying therapies either decrease relapse rate or reduced relapse severity by dampening the immune response. Unfortunately, these drugs are only partly effective in RRMS patients as they target the overreactive immune response and are unable to halt or reverse disease progression. Therefore, more efficient anti-inflammatory treatments or remyelinating therapies are highly needed. In Belgium, the first line treatment includes dimethyl fumarate, teriflunomide, glatiramer acetate and interferon-β (47-50). When the first line drugs are ineffective at reducing clinical symptoms, second line treatment like fingolimod, cladribine, ocrelizumab or natalizumab are initiated (51-53). Compared to the first line treatment, second line treatment strategies are often more effective but are accompanied with a higher risk of adverse events. Even though all disease modifying treatments are currently approved for treating RRMS patients, only ocrelizumab has been approved for treating progressive MS patients (53). The average yearly costs for therapeutic management of a MS patient is estimated at €37.948, which increases up to €63.047 with progressive severity of MS. This constitutes 1.8% of the total economic costs for brain disorders. These numbers would translate in a yearly health cost of €350 million for pMS patients in Belgium.

Target group - SCI patients

Yearly, 250.000 to 500.00 people worldwide suffer from **SCI**. A SCI can be either traumatic (e.g. falls, violent crimes or vehicle accidents) or non-traumatic (e.g. infection or tumor) in nature and mainly affect young males although with the increase in activity more and more elderly are also at risk (57). Current treatment strategies nowadays include surgical intervention to stabilize and decompress the spinal cord to limit additional damage, physical rehabilitation and corticosteroid drug administration (e.g. methylprednisolone) to temper secondary injury responses (57, 484-486). However, long term perspective and recovery is limited as no regenerative and therefore curative treatment exists for managing SCI, rendering the yearly cost of SCI care management to be around €35.000 yearly per patient in Belgium.

Target group - stroke patients

Globally, **stroke** is the second highest cause of disability and death. Stroke can be broadly classified into hemorrhagic and ischemic stroke. While hemorrhagic stroke is caused by a bleeding in the brain, ischemic stroke, which account for about 71% of all strokes, is caused by a blockage of blood flow to the brain (75). Approximately 1 out of 4 adults are estimated to experience a stroke throughout their lifespan rendering a total of over 80 million stroke survivors globally. For ischemic stroke specifically, 9.8 million people globally are affected per year leading to over 2.7 million deaths yearly (82, 83). Nowadays, the status of stroke treatment only includes recombinant tissue plasminogen activators (rtPAs) and mechanical thrombectomy surgery to treat the acute phase (78). However, the narrow therapeutic window (within 4.5 hours following stroke onset for rtPAs and 6-24 hours for endovascular thrombectomy) limits the application potential of the current treatment strategies, highlighting the importance of investigating novel therapeutic strategies for ischemic stroke (78, 85-87). The lifetime costs per person are averaged to be over €38.700 for ischemic stroke management in Belgium and account for 45% of acute-care costs, 20% of nursing home costs and aggregated lifetime costs and 35% for long-term ambulatory care (487, 488).

Target group - peripheral nerve injury and CMT patients

In the PNS, **peripheral nerve injuries** caused by trauma are estimated to have a worldwide prevalence of 13 to 23 in 1.000.000 people (489). The core strategy for treating peripheral nerve injuries include surgical coaptation of nerve ends with or without nerve grafts or nerve transfers. However, still 50% of patients do not benefit from surgical nerve reconstruction leaving them with a dysfunctional nerve function (93). Combining socio-economical costs with patient treatment costs renders the lifetime socio-economic burden of peripheral nerve injuries over €45.000 (490). A second peripheral neuropathy is **CMT**. CMT is the most common hereditary motor and sensory neuropathy of the PNS and has an estimated worldwide prevalence of 1 in 2500 (94). Currently, treatment strategy mainly include symptom management by physiotherapy and controlling neuropathic muscle and joint pain using non-steroidal anti-inflammatory drugs or tricyclic antidepressants (101). Rarely, surgery is required to correct CMT induced deformities. However, no effective treatments have been developed for CMT patients. The total annual costs of management CMT are estimated to be around €20.000 yearly per patient (based on results from Germany) (491).

Taken together, even though significant progress has been made in the field of many neurodegenerative disorders, as of to date, no effective treatments have been developed yet. Therefore, developing a **new drug-based therapy** capable of supporting glia functioning, thereby inducing neuroreparative processes would dramatically reduce the emotional and socio-economic burden for patients, caretakers and government.

PDE4 (gene) inhibition to treat neurodegenerative and demyelinating disorders

The full PDE4 inhibitor roflumilast, targeting all PDE4 subtypes and isoforms, is currently FDA-approved and marketed for treating chronic obstructive pulmonary disease (COPD) patients. However, 25% to 30% of the patients treated with roflumilast experience side effects and often need to stop their treatment (492). Roflumilast drug-repurposing for treating neuro-inflammatory and neurodegenerative disorders is therefore not possible due to the high drug concentration required for sufficient CNS penetration, which is accompanied with even more severe dose-limiting toxicities including emesis. Recently, a phase II double-blinded clinical trial with the small molecule ibudilast, which inhibits PDE4

as well as PDE10, toll-like-receptor-4 (TLR4) and the macrophage migration factor (MIF), was conducted to evaluate its activity and safety in PMS (SPRINT-MS) and ALS patients (COMBAT-ALS) (340, 493). The SPRINT-MS study preliminary reported that the rate of brain atrophy was significantly slowed down by 48% in PMS patients treated with ibudilast. Although generally well tolerated, patients treated with ibudilast did report a higher incidence of gastrointestinal disorders (304, 340). Unravelling more specific players within this therapeutic cascade can therefore hold the key for safely modulating CNS-related processes. In line, **within this dissertation**, we demonstrate that PDE4 gene specific inhibition possesses potent therapeutic potential for treating neurodegenerative and demyelinating disorders without having pre-clinical indications of emesis related side effects. By attenuating neuroinflammation, PDE4B specific inhibition diminished neuroinflammatory insults in an animal model for MS. In line, prophylactic PDE4B inhibition reduced neutrophil activation in an animal model for ischemic stroke. Furthermore, PDE4D specific inhibition stimulates myelin and neuronal regeneration in an animal model for MS, demyelinating CNS disorders , spinal cord injury and peripheral neuropathies. These findings paved the way towards multiple patents on both the use of selective PDE4D inhibitors against demyelinating diseases and the composition of matter for the development of such selective PDE4D inhibitors (EP18165843.6 PCT/EP2019/05495 WO 2019/193091 and EP21177320.5). Furthermore, our pending patents are underlying the setup of a drug platform for PDE4D inhibitors as **spin off finality** to translate the preclinical findings into a clinical application for a variety of neurodegenerative diseases. However, due to the preclinical nature of the studies conducted within this dissertation, it remains highly essential to validate and generalize the observed biological effects in other confirmatory studies.

Scientific impact

Over the past years, PDE4 research is often kept fundamental in nature due to the described side effects accompanied by full PDE4 inhibition. However, the new concept introduced here that inhibiting PDE4 subtypes (PDE4B or PDE4D specific inhibition) and even isoforms can outperform the therapeutic properties of pan PDE4 inhibitors while being tolerable, opens new perspectives for past and future research. Diminishing neuroinflammation by inhibiting PDE4B or stimulating neuronal and myelin regeneration are not only crucial for treating MS, SCI, stroke and peripheral neuropathies but can be therapeutically valuable for other neurodegenerative and demyelinating research fields (e.g. leukodystrophies and amyotrophic lateral sclerosis). Therefore, the data obtained within this dissertation can lead to long term novel research projects investigating the therapeutic potential of PDE4 gene specific inhibitors in other disease domains. Furthermore, we are the first to show the PDE4D isoform expression profile in human area postrema derived neurons. This information is highly valuable for designing and the clinical development of next generation PDE4D inhibitors with distinct isoform specificity to circumvent gastro-intestinal side effects. Finally, on a technical level, we successfully demonstrated the myelination potential of human iPSC-derived OPCs, by implementing the microfiber myelination assay, in which remyelinating drugs can be functionally evaluated, which is of added value to both the scientific and industrial community active in the field of remyelination

Taken together, the methodology implemented in this dissertation combined with the scientific findings will ultimately lead to an academic and societal breakthrough.