PERSPECTIVE

Repositioning imatinib for spinal cord injury

Efforts to pharmacologically restore central nervous system (CNS) function after injury has historically focused on promoting nerve growth with nerve growth factors such as nerve growth factor (NGF), neurotrophin 3 (NT3) and brain-derived neurotrophic factor (BDNF). However, spinal cord injury researchers have become increasingly aware of the roles of secondary degenerative events, occuring after the primary insult, and aggravating outcome. This knowledge has led to protective treatments at an acute stage of the injury, leading to better functional recovery. While such therapeutic treatments do not "cure" the disability caused by the injury, they can be seen as a first line of treatment to spare as much as possible of the original circuitry. Inhibition of certain growth factors emerged as one potential therapeutic intervention to avoid excessive endogenous reactivity to the injury. This was the focus of our research when we became aware of the therapeutic potential of the drug imatinib. Below follows a short account and a discussion of our findings, supporting the repositioning of imatinib for clinical trials in acute spinal cord injury. For a more specific and in depth interpretation of many of the experimental findings, we refer to our original papers (Abrams et al., 2012; Kjell et al., 2015).

One feature of acute secondary pathology of any trauma or injury to the CNS is a breach of the blood-brain barrier (BBB) that allows free passage of molecules otherwise restricted to the blood circulation, into damaged CNS parenchyma. The excitotoxicity that follows is considered one primary reason why additional neurons and glial cells enter necrosis or apoptosis after the primary insult. Re-establishing the BBB or blood-spinal cord barrier (BSCB) is hence considered a protective treatment alternative with the potential to be beneficial for almost any CNS injury. A seminal study from Eriksson, Lawrence, and coworkers determined that platelet-derived growth factor receptor (PDGFR)-alpha inhibition potently restricts BBB leakage and hence could reduce its negative consequences in a model of stroke (Su et al., 2008). In addition, they found that imatinib, a small molecule receptor tyrosine kinase (RTK) inhibitor known to inhibit PDGFRs, similarly was able to reduce BBB permeability (**Figure 1**). Imatinib was initially developed to inhibit constitutive activation of Abl/Bcr in chronic myelogenous leukemia and is currently an FDA approved drug for this indication, as well as for mastocytosis, hypereosinophilia, dermatofibrosarcoma protuberans and gastrointestinal stromal tumors. Imatinib was thus a candidate drug that seemed like it could deal with one of the major predicaments following injury to the spinal cord, and one with which many of the hurdles associated with untested drugs could be avoided. This prompted our group to carry out a series of experiments that today, 7 years later, has strengthened imatinib as a candidate for clinical trials in spinal cord injury.

For our first series of experiments, we decided to administer imatinib during the first 5 days after injury with the hope of reducing what had been defined to be the period of largest amount of vascular permeability (Popovich et al., 1996; Abrams et al., 2012; Figley et al., 2014). The treatment was initiated 30 minutes after injury to have the highest probability of detecting any potential protective capability. Imatinib was given by gavage, and we used a rat spinal cord contusion model in order for the studies to be translationally relevant. We found that the treatment improved bladder recovery and hindlimb locomotor function. Furthermore, at the end of the treatment period (5 days after injury), we found the BSCB to be normalized and the pathology to be generally reduced. Also, volumes of cavities and injured areas were reduced (grey and white matter included). At the endpoint of the experiments (8 weeks), we found that the treatment had lead to an increased amount of spared axons and myelin at the center of the injury site.

We focused the next series of experiments on imatinib's potential translational value (Kjell et al., 2015) and extended the treatment period to 14 days. In rats, BSCB permeability is normalized by day 14, in terms of preventing passage of molecules above 1 kDa to enter the parenchyma (Popovich et al., 1996; Figley et al., 2014). The pathological processes are expected to be extended in time in humans and thus the length of treatment may also need to be longer in humans than in rats in order to include the period of extensive BSCB permeability. In consultation with our clinical colleagues, we decided it was of primary importance to investigate a 4-hour delay for the initial administration of the treatment, to be considered clinically relevant. We also tested longer delays until treatment, such as 8 and 24 hours, to possibly increase patient inclusion numbers. We found improvements of both bladder recovery and locomotor function with a 4-hour delay until start of treatment, and improved bladder recovery also when start of treatment was delayed by 8 or 24 hours. Importantly, neither the extended time period nor the treatment itself was found to cause any obvious detrimental side effects, no sensory disturbances (but rather moderate improvements of sensory functions), and the weights of the treated animals were never negatively affected. It was interesting that the groups of animals that due to treatment improved hind limb locomotion, also improved weight gain, perhaps due to increased amount of muscle mass.

The pathological progression is extended in time in humans in comparison to rodents as evidenced by metabolic rate data and biochemical markers in the cerebrospinal fluid (CSF). This suggests that experimental results can be extrapolated so that longer delays until start of treatment may still have positive effects in humans (*e.g.*, four times the delay in the experimental setting). With a 4-hour delay in rat we could confirm axon sparing by imatinib at early time points (24 hours and 7 days after injury) coupled to a permanent reduction of pathology. Since tissue rescue seems time dependent, we argue that for clinical application the aim should still be to give the first imatinib dose within 4 hours after injury. However, since we found functional benefits with extended delays and humans do seem to offer extended time windows, we find reason to also include patients that may receive the first drug treatment with longer delays. Interestingly, it seems imatinib treatment will thus be used

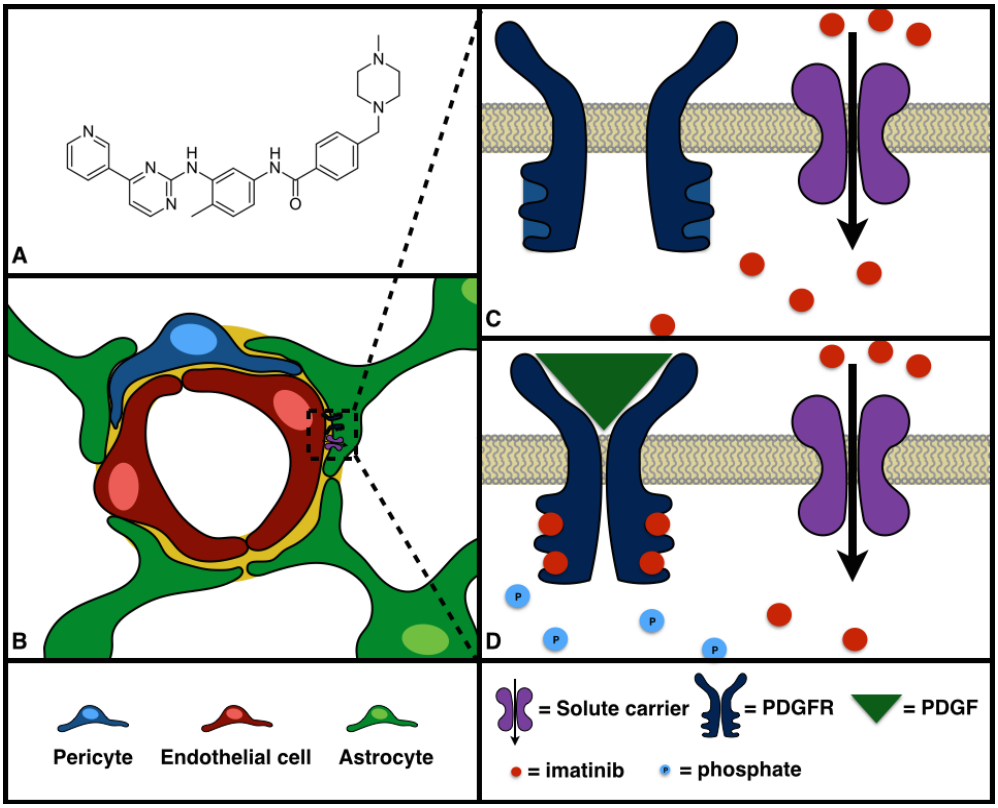

Figure 1 Imatinib and platelet-derived growth factor receptor (PDGFR) inhibition.
(A) Chemical structure of imatinib. (B) Schematic representation of a cross-section of spinal cord vasculature with endothelial cells surrounding the lumen. Onto the basement membrane, surrounding the endothelial cells, are pericytes and astrocyte end-feet maintaining blood-spinal-cord-barrier integrity. (C) Solute carriers (*e.g.*, OCT1) transport imatinib into the cell. (D) Imatinib binds to phosphorylation sites on the RTK of the tyrosine kinase receptor PDGFR and inhibits receptor signaling following dimerization due to ligand (PDGF) binding.

with a similar recommended time window and treatment period as is used for Riluzole, a repositioned sodium channel blocker that inhibits excitotoxicity, currently in the beginning of a phase IIb/III trial (Fehlings et al., 2015). It would be of interest to experimentally test if imatinib and Riluzole, arguably acting on different biological mechanisms, might have additive effects.

There have been several sizable clinical trials in spinal cord injury in the past, and while others are ongoing, no drug treatment has yet been proven sufficiently effective to become standard of care for acute spinal cord injury. A major issue in smaller and even bigger trials of protective treatments has been to determine effects of treatment, because recovery trajectories can vary markedly from the initial diagnosis. Here, biochemical markers have received much attention since they may inform about different aspects of the pathological progression. Analytes can be readily obtained (CSF and blood), and such procedures are relatively inexpensive. Biochemical markers can be divided into (1) diagnostic/disease markers, (2) efficacy markers, and (3) prognostic markers. A biochemical biomarker indicative of neuronal damage is thus a biomarker that can belong to both category 1 and 3. There are diagnostic markers, such as neurofilament and S100β, available for clinical use; however, their sensitivity as prognostic markers for spinal cord injury patients remains debated. In our second study (Kjell et al., 2015), we focused on finding a category 2 biomarker of drug bioactivity, which could potentially be used as a tool to define cohorts responsive to the drug. Furthermore, such an efficacy biomarker may also have prognostic value in a patient population. Our search for one or several potential efficacy biomarkers for imatinib in serum encompassed 12 inflammatory markers of which we found 3 candidates (MCP-1, MIP-3α and IL-8) to be increased in blood by the end of the first week of treatment. These three candidate biomarkers could therefore possibly serve as efficacy biomarkers in patients and be informative by comparisons between early and later time points in individual patients. We also found that the analytical strength was increased by combining all three markers. Importantly, we found a study describing results similar to ours using biochemical analysis of serum from leukemia patients on continuous imatinib treatment, strengthening the prospect of our results to be reflected in a spinal cord injury patient population (Hayashi et al., 2012).

Our initial hope of finding a biomarker among inflammatory cytokines was spurred by several studies reporting effects of imatinib on cytokine profiles, but also on the different immune cell populations (Hayashi et al., 2012; Adzemovic et al., 2013). In our second study, the increased concentration of cytokines was reflected in a markedly increased activation of macrophages in lymphatic organs. This drug-specific effect of imatinib has, to the best of our knowledge, not previously been reported for imatinib. Thus emerged an interesting discrepancy between an increased peripheral inflammatory response and the reduced inflammation we observed at the site of injury. In addition to finding a reduced macrophage load at the injury site, we also found that the treatment altered macrophage activation. Whether these delayed occurrences were crucial for the improved outcome in terms of functional recovery remains unknown.

RTK inhibitors such as imatinib will have different effects on different cell types. Even a single mechanism of action may cause different sets of effects in different cells. Recently, a study impressively determined opposing effects of the drug

epothilone B, a microtubule stabilizing drug repositioned for spinal cord injury, on neurons (axons) and fibroblasts due to differences in an intrinsic cellular mechanism (Ruschel et al., 2015). In this study, it seems that the divergent cellular effects of treatment may converge in terms of promoting axon regeneration. For imatinib, there are also additional direct effects on pericyte activation and migration, probably due to antagonizing PDGFR-beta signaling, and thus imatinib possibly reduces the contribution of pericytes to scar formation after spinal cord injury (Su et al., 2008; Göritz et al., 2011; Abrams et al., 2012).

Together, the above results warrant consideration for clinical trials with imatinib. A first trial, for the specific situation of future treatment of spinal cord injury, should focus on safety, drug bioavailability and tolerability of treatment. By selecting patients with cervical spinal cord injury, it would be possible in later trials to detect positive effects of tissue rescue of patient value immediately above injury. Thus in cervical spinal cord injury, a potential protective effect following imatinib treatment could result in clinical benefits even if the spinal cord is completely interrupted. Furthermore, it has been found that "complete" injuries typically still have some spared axons crossing the injury site and that voluntary motor function may be restored with therapeutic intervention (Angeli et al., 2014). Imatinib treatment may thus better the chances for this category of patients.

Since gastrointestinal motility may be considerably affected after cervical injury, the drug concentrations in plasma should be continuously measured. Imatinib is absorbed throughout the gastrointestinal system although mainly in the intestines, but to what extent plasma levels will be delayed or reduced with gastrointestinal dysmotility is unknown. Indeed, intravenous administration may be a second option; however, it would require additional preparation since no such drug composition is commercially available.

A first trial of imatinib in spinal cord injury should also aim at determining serum concentrations of the three efficacy biomarkers identified in our second study (Kjell et al., 2015), and thus possibly determine what time points could be used in potential future trials. Combined with data on blood levels of imatinib, efficacy markers may detect any possible differences between individuals in terms of drug bioactivity.

There are currently a number of drugs in clinical trial or close to it, several of them repositioned. In imatinib we believe we have found an additional promising therapeutic candidate, together with candidate efficacy biomarkers. Previous trials have proved the challenge is daunting. Nevertheless, taking all current approaches into account, there is now room for cautious optimism that there will be drugs available in the not too distant future, able to dampen the lasting effects of acute spinal cord damage.

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Jacob Kjell[*, †], **Lars Olson**

Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden

[†]*Current address: Department of Physiological Genomics, Ludwig-Maximilians-Universität München (LMU), Munich, Germany*

***Correspondence to:** Jacob Kjell, Ph.D., jacob.kjell@ki.se, jacob.kjell@med.uni-muenchen.de.*
***Accepted:** 2015-09-17*
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