

The Development of New Drug Treatments for Functional Recovery After Neurological Damage Based Upon a Novel Hypothesis Driven-Technological Platform

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Abstract: The global pharmaceutical industry is facing unprecedented challenges that are increasingly influenced by financial, political, demographical and ecological issues. As shown by the significant reduction of new drug applications or NDAs (U.S. Food and Drug Administration-approved new molecular entities) in recent years, the industry is profoundly affected by the increasing cost of development of new drugs, the large number of existing drugs that have begun to go off patent (reduced revenues), increasingly stringent regulations and corresponding increased development time. As a response, we have created a technological platform aimed at accelerating the development of new drug treatments for central nervous system indications. It allows a rapid identification of leads and drug candidates suitable for clinical development based upon a novel *in vivo* drug screening and hypothesis-driven approach that reduces the time of drug discovery and development. Furthermore, the platform is adapted to specifically identify synergistic (or new) effects induced by combination products with known molecular entities rather than with new molecular entities which reduces risks and costs of development. In less than five years, we have identified with minimal investment, two (2) combination product candidates for functional recovery after spinal cord injury that are suitable for preclinical and clinical development. These results provide evidence suggesting that it is feasible with alternative approaches and small research facilities to efficiently identify leads and drug candidates and, thus, to provide the industry with new drug treatments suitable for clinical development.

Keywords: Combination product, neurology, neuroscience, drug, technological platform.

INTRODUCTION

New Drug Applications (NDAs) in the United States (U.S.) mean new drugs (often new molecular entities) approved by the Food and Drug Administration (FDA) as a new pharmaceutical for sale and marketing. The efficient development and approval of new drugs is vital to the health of patients. However, the process underlying NDA approvals and new drug treatments in the U.S. has undergone significant changes and, since the mid-90s, the number of NDA approvals (specifically small molecule therapeutics) has drastically reduced from approximately 40 per year to less than 20 per year [1]. Multiple factors may have contributed to this large reduction in new approved drugs. For instance, it has been reported that the cost of research and development for a new drug has reached unsustainable levels in the last 10 years. On average, it now costs above \$1.7 billion U.S. dollars to develop a new drug treatment [2].

Since the *Vioxx* withdrawal in 2004, there has been clear evidence suggesting a toughening regulatory environment in the U.S. for drug approvals which, in turn, can only promote already long development time (on average between 12 and 14 years) and failure to develop new drug treatments. In addition to these factors, since 2008, most countries have been facing the worst global financing crisis since 1929. The window of opportunity for small-to-mid size drug companies

to initial public offerings (IPOs) and private equity transactions has consequently been largely reduced [3].

The picture is not rosy also for large pharmaceutical companies that have begun to see many of their block busters (best selling drugs) coming off patent. For instance, GlaxoSmithKline has already lost 4.5 billion dollars in revenues to generic competition over the last three years due to their drugs coming off patent [4]. All in all, the pharmaceutical industry is currently seeking for new approaches to rapidly identify new drugs. It has become urgent both for patients and these companies to find new solutions capable to increase and accelerate NDA approvals which are needed to refill pipelines, restore revenues levels, and support strong R&D activities.

SPINAL CORD INJURY (SCI)

For more than a century, the spinal cord has been considered as a simple relay between brain cells and effective organs (muscles, skin, etc.). However, recent findings have provided clear evidence supporting the idea that the spinal cord is also a 'command center' involved in the control and modulation of several functions. It is made approximately of one billion neurons [5, 6] and is now recognized as a key structure of the central nervous system (CNS) in the control of both simple and complex motor functions. It is known to mediate simple spinal reflexes (e.g., monosynaptic excitatory, reciprocal inhibitory, withdrawal and crossed-extension reflexes) as well as more complex functions such as locomotion, bladder/bowel control, and sexual function [7]. The spinal cord is constituted of 31

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segments (8 cervical, 12 thoracic, 5 lumbar, 5 sacral and 1 coccygeal) with peripheral input and output *via* the spinal nerves. Recent studies have revealed the existence of 1.3 million SCI patients in North America (approximately 1 250 000 Americans, 50 000 Canadians) (www.christopherreeve.org). Healthcare for these individuals creates a significant economic burden, not to mention the physiological, psychological and social suffering of these people day after day.

It is an irreversible condition for which no cure has yet been developed. Surgery (e.g., removal of bone fragments, decompression) and administration of methylprednisolone (corticosteroid) are essentially the only care (acute care intervention) provided to stabilize their condition [8]. Depending on the completeness or incompleteness of the lesion, SCI leads respectively to a total (47%) or partial loss (53%) of sensory and motor functions below injury level. Patients with cervical or thoracic injuries become either tetraplegics (also called quadriplegics) or paraplegics whereas injuries at lower levels are rare [9]. Despite injury level or extent, most SCI patients will experience significant functional losses (e.g., locomotion, respiration, bowel/bladder control, sexual reproduction), and often life-threatening health complications (e.g., immunological, hormonal, autonomic, cardiovascular, bone, and muscular problems) [10-13].

SPINAL NETWORK-MEDIATED FUNCTIONS

The lumbar spinal cord segments are known to contain several neuronal networks involved in the control of specific motor functions. These networks often referred to as central pattern generators (CPGs) are generally considered as specialized networks that can produce oscillatory motor output in the absence of any oscillatory input. To date, CPGs for locomotion (walking, running), scratching, micturition, and ejaculation have been clearly identified [7].

The locomotor CPG is by far the best studied spinal cord network. Early evidence of a locomotor CPG emerged a century ago from the pioneer work of Sherrington and Graham-Brown [17,18]. Although, several locomotor pattern modulating drugs had been found since then (e.g., clonidine, cyproheptadine), none was shown to display potent CPG activating effects and corresponding weight-bearing stepping movements in non-stimulated, untrained and completely paraplegic animals [14,19,20]. No clear ejaculation or micturition network-activating drugs have yet been reported [21]. Given that compelling evidence of these networks in most mammalian species including humans exists, we set ourselves the goal not to discover drugs that can repair the spinal cord *per se* but, instead, drugs that can temporarily restore functions by specifically, properly and potently re-activating each of these networks independently and despite the spinal cord lesion.

NEW TECHNOLOGICAL PLATFORM

To reach this goal, we have created a research platform aimed to ease and accelerate drug screening studies in SCI animal models. It is essentially designed to facilitate the identification of lead compounds and drug treatment candidates that can restore either partially or completely functions after SCI by acute activation temporarily of

specific spinal cord networks (e.g., for locomotor movement generation or ejaculatory motor pattern). Our specific objectives were: 1) to remain focused, 2) to identify potent drugs, 3) to develop safer drugs, 4) to reduce R&D costs, 5) to accelerate their identification. To achieve these objectives, we develop an approach based on hypothesis-driven experiments conducted directly in an *in vivo* model using only existing molecular entities with known safety profiles.

Specifically, we developed a new mouse model of complete paraplegia in which surgical procedures are fast and reliable. It is a no-laminectomy spinal cord-transected model where a complete spinal cord transection is performed intervertebrally between the 9th and 10th vertebrae in adult CD1 mice (Charles River Laboratories, St-Constant, Quebec)[14-16]. This model is reliable (compared with contusion models), affordable (compared with other species, e.g., rats, dogs, cats, etc.), fast (complete surgical procedures in ten min per animal) and convenient (low aggressiveness compared with C57BL/6). In contrast with larger species, mice can be housed up to five animals per cage which constitutes a fair advantage when conducting large-scale drug-screening studies. Although most experimental animal models of SCI are compression or contusion models (useful for studying inflammation, cell death, regeneration or sprouting at the lesion site), they are known for being relatively unreliable and timely (i.e., dependent upon the surgeon, the SCI device, the animal species or strains, etc.) which is not ideally suited for large scale studies [14]. Other advantages, pre-op or post-op procedures have been described elsewhere [14]. A complete transection model was ideally suited also to eliminate unrelated effects induced by systemic drug administration (e.g., brain-mediated effects onto spinal networks through spared descending fibers). Indeed, testing drugs administered intraperitoneally or subcutaneously instead of intrathecally was critically important to speed up testing procedures and to increase reliability of administration. Since a large variety of brain-permeable drugs (i.e., that can cross the blood brain barrier) have been synthesized and made commercially available over the years, it is now possible to test a plethora of relatively selective ligands belonging to different families of compounds (see below).

As mentioned earlier, only hypothesis-driven drug screening experiments were conducted in order to avoid unnecessary studies. Indeed, we examined essentially the effects induced by existing molecular entities such as receptor agonists belonging to families of neurotransmitters already known to be expressed (neurotransmitter transmembrane receptors) in the corresponding area of the spinal cord (e.g., several serotonergic, adrenergic, glutamatergic, dopaminergic and neuropeptidergic receptors have been identified in lumbar segments of the spinal cord where locomotor and ejaculation centers are located) [7,14].

To best compare drug between groups, we standardized as much as possible the conditions of testing. For instance, all tests were conducted in one-week paraplegic mice (allowing enough recovery time post-surgery but prior to spontaneously occurring reflex movements) [14-16]. Each paraplegic animal was tested no more than once (single i.p. or s.c. injection of 1 molecule). Each drug was tested several times (approximately 10 mice) at either low or large doses.

Acute effects induced upon injection on the recovery of a specific spinal cord-mediated function (e.g., locomotor, micturition, ejaculation, etc.) were assessed separately.

To properly assess drug-induced effects, we created reliable assays capable either to quantitatively assess the incidence, amplitude, or frequency of locomotion, ejaculation, or micturition. For assessing locomotor effects (i.e., hindlimb motor and locomotor movement induction), we developed a method called ACOS that reports quantitatively the amount locomotor-like and non-locomotor-like hindlimb movements in previously completely paraplegic mice. Given that these animals are completely low-thoracic (Th9/10) transected, all movements to be found following drug administration (even systemically administered) can only be mediated by sublesional structures (e.g., central pattern generator activation, afferent input modulation, direct motoneuronal depolarization, etc.). For a complete description, see peer-reviewed articles from this laboratory [14-16]. Specific assays to report drug-induced ejaculatory and micturition functions were also developed but have not been yet published.

All in all, remaining extremely focused and conducting directly all tests *in vivo* rather than *in vitro* with known molecular entities has most certainly contributed, using this hypothesis-driven platform, to shorten the time (2-3 year faster) and cost (several million dollars less) of research and development compared with most approaches typically used in the industry (e.g., high-throughput screening, *in vitro* drug screening, computational and conformational chemistry, etc.). In fact, our approach has led to the identification of two (2) different drug candidates for locomotor function and ejaculatory function recovery in less than five year and with less than 3 million U.S. dollars (see details below).

NEW DRUG TREATMENT CANDIDATES

For instance, 5-HT1A, 5-HT2A and 5-HT7 receptor agonists, used without other forms of stimulation, were found to partially induce CPG-mediated locomotor-like movements [reviewed in 14,19]. We found also that dopamine agonists with binding affinity for the D1-like subclass (D1 and D5 receptors) were also relatively potent CPG activators [19]. Combining some of these compounds, for instance L-DOPA (noradrenaline/dopamine precursor) and quipazine (5-HT2A/2C agonist), was reported to synergistically induce spinal stepping-like movements in spinal cord-transected mice [19,22]. Dopamine receptor agonists combined with serotonin receptor agonists were also found recently to synergistically induce outstanding stepping-like movements in complete paraplegic mice [24].

Although locomotor-like movements were induced by some of these drugs, none were found to elicit real walking (weight bearing stepping) but, instead, only crawling. However, some of them, when combined, revealed to display significantly superior effects that including real weight bearing stepping and some plantar foot placement capabilities. Among these few combination products, we chose a combination constituted of a serotonin receptor agonist and an adrenergic/dopaminergic precursor that can acutely induced during approximately 30 min up to 60 min involuntary weight-bearing stepping in completely paraplegic mice [25, 26]. We recently conducted proof-of-

concept experiments aimed at assessing efficacy upon oral administration of our final drug combination therapy for CPG activation and corresponding locomotor movement generation [22]. Co-administration orally (by gavage) of buspirone, levodopa and carbidopa was found to dose-dependently induce episodes of steady weight bearing stepping in low-thoracic (Th9/10) spinal cord-transected mice (with no other form of assistance or training). Robust hindlimb stepping with weight bearing capabilities was induced with the tri-therapy but not with clinically relevant doses of these compounds administered separately [22]. These results provide evidence suggesting that this drug combination may be ideally suited to constitute a *first-in-class* therapy (CPG activator) for locomotor activity induction in chronic SCI individuals given that efficacy was shown using commercially available brain-permeable small molecules already known as safe for the treatment of various neurological indications [22].

Finally, we also recently identified a drug combination capable of acutely eliciting full ejaculation (seminal emission and active seminal expulsion) in non-sensory stimulated and completely paraplegic mice [23, 27, 28]. We are currently conducting optimization experiments using adrenergic compounds for final confirmation of the candidate product most suitable for further development. Final preclinical efficacy data are expected to be obtained in Q2-2011.

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ABBREVIATIONS

NDA	=	New Drug Application
U.S.	=	United States (U.S.)
FDA	=	Food and Drug Administration
IPO	=	Initial public offering
CNS	=	Central nervous system
SCI	=	Spinal cord injury
ACOS	=	Average combined score

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