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Blowing up neural repair for stroke recovery:

Pre-clinical and clinical trial considerations

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Abstract

The repair and recovery of the brain after stroke is a field that is emerging in its pre-clinical science and clinical trials. However, recent large, multicenter clinical trials have been negative, and conflicting results emerge on biological targets in pre-clinical studies. The coalescence of negative clinical translation and confusion in pre-clinical studies raises the suggestion that perhaps the field of stroke recovery faces a fate similar to stroke neuroprotection, with interesting science ultimately proving difficult to translate to the clinic. This review highlights improvements in four areas of the stroke neural repair field that should re-orient the field toward successful clinical translation: improvements in rodent genetic models of stroke recovery, consideration of the biological target in stroke recovery, stratification in clinical trials, and the use of appropriate clinical trial endpoints.

Non-standard Abbreviations and Acronyms

AFFINITY	Assessment oF FluoxetINe In sTroke recoverY clinical trial
ApoE	apoliprotein E
CRE	cis-regulatory element (DNA) tyrosine recombinase
CST	corticospinal tract
DARS	Dopamine Augmented Rehabilitation in Stroke clinical trial
DRE	Dre tyrosine recombinase
EFFECTS	Efficacy of Fluoxetine - a Trial in Stroke clinical trial
FLP	flippase site specific DNA recombinase
FOCUS	Fluoxetine Or Control Under Supervision clinical trial
GABAα5	gamma-aminobutyric acid receptor alpha 5 subunit
mRS	modified Rankin Scale
RESTORE BRAIN	Randomized Efficacy and Safety Trial With Oral S 44819 After Recent Ischemic Cerebral Event. International, Multi-centre, Randomized, Doubleblind Placebo-controlled Phase II Study
SHR	spontaneously hypertensive rat
SPARC	secreted protein, acidic, cysteine-rich protein
SRRR	Stroke Recovery and Rehabilitation Roundtable
VEGF	vascular endothelial growth factor

Introduction

The term ‘critical mass’ refers to the smallest amount of radioactive material needed to sustain a nuclear chain reaction. In translational medicine, the aim is for fields to reach critical mass in their

identification of early stage mechanisms of disease, setting off a chain reaction of clinical trials and ultimately an explosion leading to definitive treatments. The example of stroke neuroprotection highlights how the process is not always successful. Despite a critical mass of knowledge in the mechanisms of neuroprotection as nuclear starting material, there was no chain reaction and the expected explosive development of stroke neuroprotective drugs did not materialize[1]. The subsequent loss of scientific and pharmaceutical interest in neuroprotection has left only low-level radioactivity, poisoning the landscape for years. There are some worrying parallels with the field of stroke recovery, in which we have reached a critical mass in our understanding of the cellular and molecular principles of neural repair and activity-induced recovery. Despite this, the first large clinical trials of 'stroke recovery drugs' in humans have been disappointing. The FOCUS[2], AFFINITY[3] and EFFECTS[4] phase III trials investigated nearly 6000 patients between them and showed that prescribing 20mg of fluoxetine for the first 6 months post-stroke had no effect on disability, as illustrated by the adjusted common odds ratio for the FOCUS (OR 0.95 [95% CI 0.84-1.08]), AFFINITY (OR 0.94 [95% CI 0.76-1.15]) and EFFECTS trials (OR 0.94 [95% CI 0.78-1.13]). Similarly, the RESTORE BRAIN[5] trial enrolled 585 patients and demonstrated that prescribing the oral $GABA_A$ α5 receptor antagonist S44819 also had no effect on disability (OR 1·17 [95% CI 0·81-1·67] for 300mg dose compared to placebo). The DARS trial[6] (Ford et al., 2019) enrolled 593 patients and demonstrated that prescribing co-careldopa for 8 weeks early after stroke did not improve independent mobility compared to placebo (OR 0·78 [95% CI 0·53-1·15]).

These results remind us that despite compelling pre-clinical data, navigating the translational pipeline from bench to bedside is difficult and requires careful consideration of a number of factors. Neural repair strategies for stroke recovery represent a major opportunity to reduce the global impact of stroke and we cannot afford to repeat the obvious and well-documented mistakes made in neuroprotection pre-clinical studies and trial design[1]. We must give the next clinical trials in neural repair every chance to succeed, but this will require a critical revaluation of our current approaches.

Rodent Genetic Models and Inference of Disease Mechanisms

Biological targets for stroke recovery drugs are identified in the pre-clinical science. For example, studies with genetically modified mice identify biological targets by testing whether the presence or absence of a candidate molecular system influences stroke recovery through neural repair. Genetically modified rodents also allow the modeling of co-morbid conditions, which are part of

the stroke process in most human cases. However, these two commonly used approaches in rodent genetic models, identification of molecular targets and co-morbid disease modeling, may confound the neural repair field. These confounds appear to be increasing in the literature and require important constraints.

Molecular Target Identification. Constitutive knockout mouse models inactivate a specific gene for the whole life cycle of the mouse. This leads to compensation from related gene systems for the knockout gene. Also, constitutive knockout of a gene inevitably influences all stages of stroke, making specific inferences about that gene/molecule's effect on stroke recovery vs. initial stroke damage impossible. For example, a constitutive knockout of the chemokine receptor CCR5 will have the opportunity for producing compensation in other chemokine signaling systems over the whole lifetime of the mouse[7]. This may explain why CCR5 constitutive knockouts produce a very different picture[8,9] to that obtained when CCR5 blockade is achieved only after stroke[10]. In another example, selectively reducing Ephrin-A5 after stroke results in potent improvement in axonal sprouting and recovery[11]. However, in a constitutive knockout of Ephrin-A5, there is no effect on stroke recovery[12] most likely due to either pre-stroke compensation from other Ephrin systems, as occurs with other axonal growth inhibitors[13], or an effect on the initial stages of cell death in stroke as well as the later stages of neural repair. To understand a gene or molecule's unique role in neural repair, it is important that prior stages, including cell death, are left unaltered from normal, and that the gene manipulation not be present until after stroke. Such approaches are now routinely available with inducible gene knockout or induction (inducible CRE, DRE or FLIP), or viral gene induction or knockout.

Co-morbid disease modeling. A second problem with rodent genetic models in stroke recovery is in off-target gene effects. In the spontaneously hypertensive rat (SHR), the endogenous development of high blood pressure allows modeling of co-morbid conditions in stroke[14] and facilitates white matter ischemic models[15]. However, the SHR is a complex genetic condition, with alterations in growth factor signaling (VEGF), regenerative extracellular matrix production (SPARC) and serum proteins (albumin)[16]). These altered molecular systems are likely to influence mechanisms of neural repair and change the outcome of stroke recovery studies compared to a non-genetically altered rodent species. Similarly, ApoE knockout mice have been used to model hyperlipidemia as a co-morbidity[17,18,19]. However, ApoE plays a critical role in cholesterol trafficking that supports axonal growth cone function[20] and astrocyte and microglial signaling[21] Both SHR and ApoE knockout models change molecular systems that might influence recovery independently of the co-morbidity that they are modelling, making it difficult to make inferences about normal neural repair processes.

In summary, the development of new neural repair therapies in stroke recovery requires changing the pipeline for identification of biological targets. Biological targets are more rapidly identified using genetically modified rodents, but these genetic modifications introduce their own effects, which if not controlled by specific temporal gene manipulation and attention to off-target effects, may mis-identify a molecular target as promising or as ineffective. Rodent stroke models have other limitations, in addition to these concerns regarding genetics, such as in the type of stroke that is modeled, age as a factor in modeling and anatomical constraints in stroke modeling in the rodent brain. These limitations to rodent stroke modeling have been extensively reviewed.[22,23,24].

Recovery from stroke requires behavioral activity

Simply prescribing a drug to influence the identified biological target is unlikely to promote behavioral recovery and cannot be considered a substitute for neurorehabilitation. Consider firstly that brain function is critically dependent on the activity in its circuits. Secondly, the patterns and intensity of cognitive and motor activity change the brain at all levels, from molecules to synapses to circuits to wholesale brain structure and functional connectivity. Stroke recovery is therefore not just a reflection of the static induction or suppression of a certain cell-signaling event, but the process of that cell signaling within the context of behavioral activity. In traditional drug development, the drug has a biological target, such as a T cell checkpoint receptor in a cancer therapy or a cholesterol synthetic enzyme in vascular disease, but in stroke recovery the target is a molecular system within an appropriately active brain circuit - a “pharmaco-activity” target. Put another way, stroke recovery drugs may change the state of the target brain circuit but it is unlikely that behavioral gains will occur unless the circuit is appropriately active. The requirement for a close temporal relationship between drug and activity was recognized by many early pre-clinical and clinical investigators[25,26,27]. Recent trials suggest that recovery drugs given on their own do not improve disability after stroke, but the effect of the drug on appropriate neurobehavioural training has not yet been investigated in humans. Such trials will need to provide specific neurorehabilitation protocols to stimulate activity in the brain circuits targeted by the candidate therapy, e.g. motor control circuits for upper limb recovery. The DARS trial did make a point of giving the drug 45-60 minutes before ‘routine’ motor therapy. However, the dose, scheduling, and specificity of the therapy was likely insufficient to take advantage of any effect co-careldopa may have had on motor circuits governing walking[28]. In general, pharmacological stroke recovery trials have not given enough consideration to the appropriate training required to effect behavioural change. The recent Stroke Recovery and Rehabilitation Roundtable (SRRR) recommended that at the very least, recovery trials need to capture participant activity data for analysis or even stratification[29].

One-size does not fit all

A one-size fits all approach to stroke recovery treatments is probably not going to work. Those conducting thrombolysis trials realized early on how important it was to stratify patients according to likely outcome[30]. Stratification excludes those with little chance of benefitting, based either on prognosis or on the proposed mechanism of action of the intervention. The SRRR consensus group on biomarkers of recovery agreed that there was evidence that both neuroimaging and neurophysiology markers of corticospinal tract (CST) damage had some value in predicting motor outcome and response to therapy after stroke[31] and recommended that measures of CST integrity are used to stratify patients in future motor recovery trials[31]. Stratification of patients based on expected outcome is a key strategy in designing effective clinical trials in stroke recovery[32].

The other way to think about stratification in pharmacological stroke recovery trials is in terms of precision medicine. In cancer medicine, the strategy of targeting individual patients based on the presence of known biological targets is accepted as the rational approach[33]. Although the rationale for both fluoxetine and GABAα5 receptor antagonists is to enhance the potential for experience dependent plasticity, they have very different biological targets. GABAα5 receptor antagonists/inverse agonists block an increase in extra-synaptic (tonic) inhibition that is triggered early after stroke in response to focal brain damage in some preclinical stroke models[34,35]. Fluoxetine on the other hand can reopen critical periods of plasticity in adult brains in stroke and non-stroke models through multiple potential mechanisms including reduced intracortical GABAergic signaling and increased BDNF expression[36]. However, it is likely that the biological processes that these drugs target differ across individuals and with time post-stroke[37]. Distinguishing which biological process in neural repair predominates in subgroups or even individual human stroke patients would help select a more credible strategy for phase III clinical trials. Making the distinction however requires human biomarkers of these underlying biological processes[37], something we currently do not possess. Here, a biomarker is defined as an indicator of disease state that is useful clinically as a substitute measure, reflecting underlying molecular/cellular events that are difficult to measure directly in humans[31]. For example, there is interest in whether changes in the characteristics of neuronal oscillations detected with electro- or magnetoencephalography in humans reflect changes in the type of GABAergic signaling that fluoxetine and GABAα5 antagonists might be targeting[38]. The urgent requirement to develop biomarkers to help identify which patients have the appropriate biological targets to benefit from specific drugs in early clinical trial work has been recognized by the SRRR[31].

Understanding outcome measures

The most commonly used (and most contentious) outcome measure in human stroke recovery trials is the modified Rankin Scale (mRS). The recent SRRR consensus acknowledged the role of the mRS in assessing the overall degree of dependency in activities of daily living but called for a broad range of validated outcome measures to be used in future stroke recovery trials[39]. The consensus focused on upper limb recovery and stressed the importance of including established impairment measures (e.g. Fugl-Meyer) and fine-grained assessments of motor behavior (e.g. kinematics). Implicit in the recommendations was the idea that outcome measures should be aligned with the proposed mechanism of action of the treatment. In pre-clinical stroke studies, recovery is assessed in specific domains such as sensory, motor, spatial and contextual memory, in order to directly test recovery in the specific brain circuits damaged by the stroke. In human trials then, dependency in activities of daily living may not be the appropriate domain to assess when the treatment itself targets specific brain circuits. The mRS is often selected as the primary outcome measure because it is assumed that the goal of all recovery treatments is to reduce overall disability, and that change in impairment is only relevant if it concurrently leads to disability reduction. However, this is not necessarily the case. Firstly, changes in any scale, including impairment measures, are independently important both for individuals and for group studies if they are large enough. Our trials should be designed to achieve at least pre-defined minimum clinically important differences, not simply statistically significant differences. Secondly, changes in disability scales do not correlate with changes in more fine-grained measures of impairment after stroke[40,41] or even patient self-report[42,43], suggesting that they are each measuring different aspects of recovery governed by different underlying mechanisms. Why would we expect a treatment acting at the level of brain circuits to have an immediate effect on disability? Lastly, changes in impairment can open the door for patients to train in more functionally relevant ways, which ultimately improves the chances of reducing disability further down the line if this is the therapeutic goal. It is likely that even minimum clinically important reductions in impairment require additional approaches, most likely physical or behavioral training, to have a large downstream effect on disability. It is time to accept that neurorehabilitation is a complex intervention, one with multiple interacting component parts. Reducing stroke recovery treatment to a single controllable intervention (in this case a drug) makes the design of standard randomized controlled trials easier but is not necessarily suited to the stroke recovery field, where we should make more use of established guidelines for evaluating complex interventions[44].

Conclusions

We have reached a critical mass in our understanding of the cellular and molecular principles of neural repair and activity-induced recovery after stroke. However, there has been no chain reaction to push us towards effective stroke recovery treatments. The emergence of large-scale clinical trials of the most promising approaches is encouraging, but their failure has been all too predictable. Stroke recovery treatments are not simply drug treatments and understanding how they fit into a complex intervention is a prerequisite for designing stroke recovery neural repair trials. We run the risk of extinguishing the field of stroke recovery, as we did with neuroprotection, before it has had a chance to explode into life. With a well-reasoned roadmap of pre-clinical to clinical studies in stroke neural repair, it is time to blow things up into clinical translation.

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