Comments and Opinions

Found in Translation
Preclinical Stroke Research Predicts Human Pathophysiology, Clinical Phenotypes, and Therapeutic Outcomes

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Stroke presents a major global burden to patients, their relatives, and whole economies.1 Worldwide intense efforts are being undertaken to understand the pathobiology of stroke and to develop novel, effective treatments. This has led to the discovery of a plethora of preclinically promising treatment strategies,2 but intravenous thrombolysis remains the only specific pharmacological treatment with proven efficacy in acute ischemic stroke.3 Apparently, of several hundred controlled clinical trials4 aiming to protect the brain against ischemia (neuroprotection) with compounds previously found effective in animal experiments, not a single one has led to the regulatory approval of a drug for this indication. Many pharmaceutical companies have, therefore, stopped developing treatments for this devastating disease, and nihilism is spreading in the field.

A roadblock seems to exist between bench and bedside, preventing the translation of highly effective preclinical neuroprotectants into the clinical realm.5 Despite the prevailing mantra of translational medicine,6 other fields report similar problems when making the preclinical-to-clinical transition. Much has been speculated about the reasons underlying the translational roadblock, and the phrase lost in translation has entered the titles of numerous biomedical publications.

In this article, we propose that contrary to current opinion, preclinical stroke research can indeed predict clinical outcomes and phenotypes. Numerous neuroprotectants were reported as lost in translation (Figure 1A), but there is reason to argue that the clinical trials were based on rather weak preclinical evidence, and that many pathophysiological principles and therapies were actually discovered in translation (Figure 1B). We propose that there is little reason for nihilism. We present a concise list of potential improvements that may help to reduce the current attrition rate of bench-to-bedside translation, even in neuroprotection. Stroke may even serve as a model disease because our analysis may apply to other fields where development of novel, clinically effective therapies lags behind the breakthroughs of basic research.

Stroke: A Global Problem With Unmet Medical Needs

Stroke accounts for a substantial and growing share of overall mortality and disease burden worldwide.7 Comprehensive specialized stroke care (including stroke units) that incorporates rehabilitation has demonstrated clear benefit for patients, and treatment of acute ischemic stroke with thrombolysis with intravenous recombinant tissue-type plasminogen activator (rtPA) within 3 to 4.5 hours can be highly effective.8 However, because of contraindications or lack of access, only a minor portion of stroke victims worldwide benefit from these therapies. Currently, no neuroprotective or neurorestorative therapy has demonstrated efficacy in large, randomized controlled clinical trials. Pharmacological protection for the brain against focal cerebral ischemia or hemorrhage and fostering functional recovery through induced plasticity and repair remain the leitmotifs of stroke research.

Unraveling Stroke Pathophysiology Through Basic Research: A Success Story

Research in the past few decades has partially unraveled a bewildering complexity of pathobiological events after deprivation of blood supply in brain tissue.2,8-11 Although many issues remain open, basic and preclinical stroke research have provided an elementary understanding of stroke pathophysiology (Figure 2A and 2B) and unveiled a myriad of promising therapeutic targets. Numerous experimental stroke studies, mostly in rodents, have demonstrated therapeutic infarct volume reductions of 30% to 50%, accompanied by substantial improvements in functional outcome. Some experimental treatments were effective in improving outcome even when given after full maturation of the ischemic damage; in other words, they may have promoted some form of tissue regeneration.12,14

Protecting the Brain Against the Consequences of Ischemia: Is the Road to Translation Blocked?

Despite the apparent insights into stroke pathophysiology, an abundance of promising targets in preclinical development, several underpowered phase I/II clinical trials that have detected signals of effectiveness,15-17 and all large phase II/III randomized controlled clinical trials of neuroprotectants...
has failed to deliver evidence for a clinical benefit of any of the neuroprotectants tested. Recent examples include erythropoietin,
NXY-095, and granulocyte-colony-stimulating factor. This translational roadblock seems so overwhelming and costly that it has been generalized to the predictiveness of experimental stroke research and even basic stroke research in general (Figure 1A), leading to almost universal nihilism on preclinical investigation of stroke. Most major drug companies have terminated their stroke programs, and academic scientists have started to refute the existence of pharmacological neuroprotection in humans.

Unfortunately, because of the complexities of the preclinical and clinical literature, a general meta-analytical approach to the question whether preclinical modeling is not predictive for human stroke is not feasible. Therefore, we propose an alternative, much more selective, and potentially anecdotal and, therefore, biased approach: we have selected key areas of stroke research (acute therapy, pathophysiological mechanisms, neuropsychiatric, and medical complications) where preclinical and parallel clinical evidence exist, and ask whether preclinical research has predictive value for human pathophysiology. We pose either the question of whether preclinical studies were predictive for clinical phenotypes or study results, or the reverse question, which is for all practical purposes identical: How predictive is clinical stroke research for animal experiments of the disease? The latter, reverse approach is informed by the frequent scenarios where experimental stroke studies have been conducted after a corresponding clinical study was published. Although the translational challenge is a prospective one, we intentionally use a retrospective approach using the 20/20 vision of hindsight.

Penumbra and Evolution of Infarct
Occlusion of a main artery of the brain leads to severe ischemia in its central supply territory, in which severe energy failure within minutes initiates the rapid demise of all cellular elements. Within hours this results in infarction. The core area is surrounded by tissue in which energy failure has led to electric silence (and hence loss of function), but because of residual perfusion in this region, vascular, cellular, and molecular processes may either lead to rescue of function or to their own recruitment into the ischemic core (ie, infarction). This so-called penumbra is the main target of putative neuroprotection therapies. It was first described in a monkey model of stroke by Symon et al and Astrup et al and has over decades of electrophysiological, biochemical, molecular, and imaging research in animal models evolved to form the basis of the tissue at risk concept. Through imaging studies using positron emission tomography and later also MRI, the penumbra concept was firmly established in humans. Currently, several clinical trials are investigating the usefulness of the tissue at risk concept for treating patients with stroke and thrombolysis who would not normally receive tPA because of advanced age or because symptom onset to treatment time extends beyond the 4.5-hour time window.

Cortical Spreading Depression and Depolarizations
Waves of cortical spreading electrophysiological excitation followed by silence (depression) were first described by Leão after experimental mechanical irritation of rabbit brains and then further characterized in rodent models.
Figure 2. A. Complexity of cellular and molecular mechanisms of ischemic cell death in the brain (adapted from Dirnagl12 with permission of the publisher. Copyright ©2012, New York Academy of Sciences. Authorization for this adaptation has been obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation). B. Simplified temporal sequence of destructive and protective or restorative mechanisms in stroke (adapted from Dirnagl et al8 with permission of the publisher. Copyright ©1999, Elsevier Ltd. Authorization for this adaptation has been obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation). AGE indicates advanced glycosylation product; BBB, blood brain barrier; EC, extracellular; ECM, extracellular matrix; HMGB, high mobility group box protein; HSP, heat shock proteins; MMP, matrix metalloproteinase; PCD, programmed cell death; PDGF, platelet-derived growth factor; and TGF, transforming growth factor.
Experimental studies found cortical spreading depolarization-like depolarizations after occlusion of the middle cerebral artery (MCA) in animals. In rodent stroke models, the concept was further developed that cortical spreading depolarizations propagate the expansion of the ischemic core into the penumbra, making them an attractive therapeutic target. Only recently and through the use of invasive electrophysiological recordings was ischemic stroke in human beings shown to induce spreading depolarizations of electric activity, which in turn enlarge focal ischemic brain regions in patients with stroke.

**Exofocal Postischemic Neuronal Damage**

During the past several years, it was increasingly recognized that after focal ischemic lesions, delayed exofocal postischemic neuronal damage (EPND) develops, which may contribute to outcome and even serve as a novel target for neuroprotection. Typically, EPND affects regions that are synaptically connected with the ischemic lesion (eg, the striatum and cortex within the MCA territory) and often include the ipsilateral thalamus and the ipsilateral substantia nigra. Experimental models in rodents have systematically investigated this phenomenon and have implicated a number of pathophysiological mechanisms. Faithfully reflecting evidence from animals, computed tomography and MRI have systematically demonstrated EPND in the thalamus and substantia nigra in patients having anterior circulation territory strokes, along with Wallerian degeneration of the pyramidal tracts.

Although the functional significance of EPND and its potential contribution to long-term outcome is not entirely clear, recent preclinical studies demonstrate that EPND can be prevented and hence may serve as a (secondary) target for neuroprotection.

**Thrombolysis**

Intravenous thrombolysis with rtPA is the only pharmacological treatment for acute ischemic stroke whose clinical use has been proven. rtPA-induced brain reperfusion after clot occlusion was first demonstrated in a rabbit model and was later found effective in humans in the seminal National Institutes of Neurological Disorders and Stroke trial. Although the clinical use of intravenous rtPA to induce reperfusion after myocardial infarction may have been the main stimulus for the NINDS trial, it nonetheless must be noted that current therapy of ischemic stroke with rtPA is the result of successful bench (rabbit) to bedside (man) translation. Importantly, the time window for the efficacy of rtPA in clot models of stroke in rodents is virtually identical to intravenous thrombolysis in patients with ischemic stroke and ranges between 3 and 4.5 hours, questioning the notion that the temporal dynamics of the evolution of ischemic damage differ in principle between rodents and humans. Interestingly, the apparent negative interaction of tPA and erythropoetin, which led to a significantly higher mortality in patients who received thrombolysis and rtPA than in those receiving rtPA or erythropoetin alone, was reflected in a stroke mouse model in which the combination promoted vascular permeability and extracellular matrix breakdown.

**Hypothermia**

Hypothermic protection against ischemia was discovered, then mechanistically evaluated, and effective treatment schemes were developed (timing, duration, target temperature) in animal models. After several successful safety and feasibility studies, the efficacy of hypothermia is now under investigation in stroke in 2 large international trials (Eurohyp-1, NCT01833312 and ICTuS2/3, NCT01123161). Hypothermia has already been demonstrated to be effective in protecting the human brain after global ischemia. However, a recent study questions the effectiveness of cooling after cardiac arrest. How this will affect international guidelines for the treatment of cardiac arrest (class I, level of evidence B8), which recommend hypothermia in this indication, is presently unclear.

**Mouse Studies Predict Outcomes of Human Antiplatelet Trials**

Inhibitors of platelet function, anticoagulants, and thrombolytics are established therapies for both prevention and acute treatment of ischemic stroke, but they all come at the cost of increased bleeding rates. Animal experiments have clearly predicted the risk associated with glycoprotein IIb/IIIa platelet inhibitors, which block the crucial downstream pathway for irreversible platelet aggregation and thrombus formation. Anti-GPIIb/IIIaF(ab)2 fragments did not have protective effects on lesion size or functional outcome but rather increased hemorrhage risk and mortality—in contrast, for example, to GPIIb/IIIa inhibition. Unfortunately, this did not inform the Abciximab in Emergency Treatment of Stroke Trial (AbEST) phase III clinical trial testing the efficacy of the GPIIb/IIIa blocker abciximab in patients with acute ischemic stroke, which had to be stopped prematurely because of increased intracranial bleeding risk in the abciximab arm.

**Statin Use During Stroke Is Protective, Abrupt Discontinuation Is Harmful in Mouse and in Man**

Statins (inhibitors of 3-hydroxy-3-methyl-glutaryl-CoA [HMG-CoA] reductase) are cholesterol-lowering agents and reduce vascular risk in patients with coronary heart disease and stroke. In addition to lowering cholesterol, statins exert anti-inflammatory, immunomodulatory, and endothelial effects. Experimental evidence has clearly demonstrated that statins may protect animals from brain ischemia, increase cerebral blood flow, decrease cerebral lesion sizes, and improve functional outcome. In addition, statins may promote vascular thrombolysis by upregulating endogenous rtPA production and downregulating tissue factor, von Willebrand factor, and plasminogen activator-inhibitor 1. In fact, in animal models, statin cotreatment enhanced the efficacy and extended the series of experiments were performed in mouse stroke models that discovered hypothermic protection against ischemia. However, this rebound effect after sudden discontinuation of statin treatment has functional consequences in vivo because withdrawal of statin medication abrogates stroke protection in mice. Importantly, all of these experimental findings have been replicated in randomized clinical trials. There is ample evidence of
Inflammation and Immunity
Anti-inflammatory therapy with an isogenic antibody targeted at the intercellular adhesion molecule (ICAM)-1 was shown to be effective in stroke models in the rat. The Enlimomab trial then evaluated without success the efficacy of a heterologous, murine IgG2a monoclonal antibody directed against extracellular domain 2 of human ICAM-1 in patients with stroke, and even found clinically worse outcomes in patients receiving this heterologous antibody. This trial is often quoted as a prime example for the translational failures in this field. What is less often noted is that in a subsequent bed-to-bench translation, the Hallenbeck group found that this failure could have been predicted because heterologous murine antirat anti-ICAM-1 was immunogenic and produced adverse effects in rats similar to those observed by Enlimomab in humans.

It is well known that patients with stroke are highly susceptible to infection, in particular pneumonia and urinary tract infection, and that infections are a major cause of morbidity and mortality. Prass et al demonstrated in a mouse model of stroke that MCA-occlusion leads within hours to a substantial and longlasting downregulation of innate and adaptive immune systems. They further demonstrated a causal link between downregulation of the immune system and susceptibility both to infection and to outcome. Meanwhile, a number of groups have investigated changes in the peripheral immune system of patients with stroke, including tonsils. These clinical studies reveal a strong congruence between rodent and human data with respect to temporal dynamics, as well as cellularity and immune cell function. As in rodents, preventive treatment with antibiotics was able to reduce the rate of infections in randomized controlled stroke trials.

Neuropsychiatric Complications
In addition to sensory-motor dysfunction, neuropsychological and even neuropsychiatric sequelae of stroke can be studied in rodents. For example, after mild focal cerebral ischemia, mice develop deficits in strategy-switching resembling the dysexecutive syndromes typically observed in patients with subcortical vascular disease. Moreover, rodents develop differential behavioral and biochemical phenotypes after left versus right MCA infarcts. Several groups have since then described a poststroke phenotype in animals with evidence of hyperactivity, anxiety, despair, and anhedonia, which may in fact resemble poststroke depression (PSD) in patients with stroke. PSD in mice was induced by left but not right MCA-occlusion and was associated with delayed degeneration of dopaminergic neurons in ipsilateral midbrain and reduced dopamine concentrations in ischemic striatum and nucleus accumbens. Importantly, delayed chronic antidepressant treatment with the selective serotonin reuptake inhibitor citalopram not only prevented PSD but also protected from EPND and chronic lesion maturation, supporting the notion that prevention of secondary neurodegeneration by antidepressants may provide a novel target for subacute stroke therapy. Similarly, citalopram enhances neurovascular regeneration and sensorimotor functional recovery after ischemic stroke in mice.

Again, all of these findings from studies in animal models were highly predictive for several important observations in patients with stroke. PSD is an important complication after stroke affecting at least 30% of all patients with significant effect on functional outcome and even mortality. Although the exact pathophysiological mechanisms are not yet clear, alterations of the mesolimbic reward system and EPND in the ipsilateral...
midbrain may play a role.100,102 In humans, a potential association of lesion location with the development of PSD is a matter of ongoing debate.103 However, several clinical studies demonstrate that left, that is, dominant, frontal, and basal ganglia lesions, are linked to major PSD.104,105 The most important finding, however, is the fact that early antidepressant treatment with selective serotonin reuptake inhibitors may not only prevent the development of PSD but may also positively affect long-term cognition, functional motor scores, and global functional outcome at 3 months.106–109

The Roadblock to Neuroprotection: Saying It Ain’t So

As stated above, there is broad sentiment in academia and industry that bench-to-bedside translation has failed in neuroprotection research. In fact, translating preclinical results that have demonstrated cell protection against ischemia/reperfusion injury to successful therapies in patients is challenging in all fields of medicine (eg, cardiology).110 However, it has been argued that the preclinical literature is confounded by false-positives and the clinical studies by false-negatives.111,112 Systematic reviews of preclinical neuroprotection studies have tried to quantify the bias introduced by the factors listed in Table 1. According to some meta-analyses, the lack of randomization, blinding, confounding pathology or advanced age, as well as publication bias, taken individually each lead to a 10% to 30% overestimation of true effect sizes.120,121 Because of a lack of multivariate analyses (because of low n-numbers in the primary literature), it is unclear at present whether these effects are linearly additive. It is likely, however, that preclinical studies have grossly overestimated the true effect sizes of putative neuroproteoctants, and many false-positives may populate the literature. Other research fields, which are equally troubled by translational failures, have recently reported shockingly low reproducibility of preclinical study findings (between 5% and 15% successful reproduction).122–124 Factoring in bias and low predictive value,111,112 in many cases the preclinical evidence for the efficacy of neuroprotocants which later failed in clinical testing, may not have been robust.

As stated above, true effect sizes in preclinical neuroprotection trials may have been overestimated, whereas most clinical neuroprotection trials were not powered to detect small or even moderate effects. This may partially explain the conundrum over why the translational bottleneck is particularly obstructive in neuroprotection, whereas in other areas, such as pathophysiological mechanisms (eg, penumbra, spreading depolarization, and immunodepression), translation was more successful despite the fact that most of the biases listed in Table 1 apply to preclinical stroke research in general. Remarkably, however, bench-to-bedside translation was successful in intravenous thrombosis, the only currently available pharmacological treatment of acute ischemic stroke, possibly because of the substantial effect size of thrombosis when administered within the first hours after onset of ischemia.125

Summary and Recommendations

Preclinical stroke researchers seem to be much more successful in treating stroke in rodents than clinicians in humans. The discrepancy between our apparently advanced knowledge about stroke pathophysiology as derived from experimental models and the large number of failed neuroprotection trials in patients with stroke has frustrated researchers, clinicians, industry, funding agencies, and patients. Failure to protect the human brain has led to the notion that there is a general disconnect between the preclinical and the clinical realms. As judged by the plethora of articles lamenting a loss in translation and the omnipresent but unfulfilled mantra of translational medicine, other fields of medicine also seem to be struggling to bridge bench and bedside. We here argue that in stroke research, contrary to the current view, there is ample evidence that preclinical research can indeed inform clinical medicine, and that in fact some of the apparent failures were based on biased and underpowered preclinical research—and hence may have faithfully reproduced small or even nonexistent effects in stroke models. We have not covered other potentially important reasons for the hiatus between bench and bedside, such as those related to clinical trials (small trials, treatment too late, etc.) because they have been treated elsewhere.114,126–130 In any case, even if one accepts that translation has failed in neuroprotection, we maintain that this should not be uncritically generalized to other aspects of stroke pathophysiology. Table 2 lists recommendations, which all aim at reducing bias and increasing the power and positive prediction of preclinical research. Of note, none of these are specific to stroke research. Although most of these recommendations address researchers, it is clear that many are difficult to implement for the individual scientist. For example, the academic system and publishers reward spectacular original findings, but robust and potentially neutral findings are as unpopular as replications. Researchers, funding agencies, academic institutions, publishers, and professional societies should work together to harness the tremendous potential of basic and preclinical research, in stroke research, and other fields of medicine.

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Table 2. Improving the Predictiveness of Preclinical Stroke Research

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<th>Recommendations</th>
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<tr>
<td>Improve internal validity: randomization, blinding treatment allocation and outcome assessment, predefined inclusion/exclusion criteria</td>
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<tr>
<td>Improve external validity: study stroke pathophysiology and test treatments in animals of both sexes, old age, and with comorbidities</td>
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<td>Increase statistical power (increase n, decrease variance) and positive predictive value</td>
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<td>Replicate pivotal findings</td>
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<td>Publish negative findings</td>
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<td>Emphasize long-term, functional outcomes over structural outcomes (infarct size)</td>
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<td>Use meta-analysis of preclinical studies</td>
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<td>Establish international multicenter phase III preclinical trials</td>
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<td>Establish registries of preclinical studies</td>
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Disclosures
None.

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