Translational Medicine for Stroke Drug Discovery
The Pharmaceutical Industry Perspective

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Abstract—Over the past 20 years, an estimated $1 billion has been spent in research and development of stroke therapeutics; however, this huge investment has failed to produce a clinically efficacious drug with the exception of the thrombolytic agent Activase (tPA). This sobering reality has been the subject of numerous reflections by renowned leaders in stroke research with special focus on the most recent failed clinical trials. The validity of the neuroprotection strategy has been questioned and efforts to substantially modify the quality of stroke research have been examined. The consistent failures of the pharmaceutical industry to develop a neuroprotective drug for ischemic stroke have had a major impact on the assessment of stroke as an attractive therapeutic area for drug discovery. Many pharmaceutical companies have scaled down their stroke programs, reflecting skepticism about the prospect of contemporary stroke drug discovery strategy based on neuroprotective agents. In this article, we present a Translational Medicine perspective on critical issues that the pharmaceutical industry and the academic community encounter but often ignore during stroke therapeutic development. This Translational Medicine framework offers a systematic analysis of the possible deficiencies that likely underwrote the colossal failure of clinical trials with neuroprotective drugs. In addition, we offer a biomarker-based system that aims at providing “proof of concept” along the discovery and development pipeline, which if implemented along early preclinical and clinical development phases, might significantly reduce risks and enable success. (Stroke. 2009;40[suppl 1]:S121-S125.)

Key Words: stroke ■ translational medicine ■ biomarkers ■ penumbra ■ stroke models ■ drug discovery

In recent years, all major pharmaceutical companies have implemented Translational Medicine activities in tandem with their traditional drug discovery and development schemes. The mission of this new entity, which is different from Translational Medicine as an academic discipline, is simply to improve predictability of the potential success of compounds as they transition through the different stages of drug development toward fulfilling a medical benefit. It also provides cues about the safety and tolerability profile of drugs as tests in humans are initiated. Translational Medicine activities have been focused on discovery, validation, and implementation of biomarkers throughout the various stages of drug development ranging from target and compound identification all the way to proof of safety (phase I) and efficacy (phase II) clinical studies (Figure 1). Furthermore, Translational Medicine activities are built on a principle of congruency (vide infra) between preclinical models and the human disease (Figure 2).8–11

Biomarkers: A Utilitarian Classification

Biomarkers are cornerstones of the modern drug discovery and development process and are equally important during preclinical and clinical studies. These markers are defined as biological, biochemical, or biophysical parameters that can be monitored objectively and reproducibly in animal models or humans. This broad definition is, however, of little utility because it does not qualify a biomarker for its significance and applicability during the drug development process. Thus, a utilitarian classification (Figure 1) is essential for the purpose of rational implementation of a biomarker strategy in support of promising drug candidates. For this purpose, we offer the following classes and definitions:

Target Validation Biomarkers
Biomarkers aimed at providing evidence that the target identified for drug discovery and development plays a significant role in the human disease condition. Modulation of such molecular target is expected to provide medical benefits.

Biomarkers of Target–Drug Interaction
Biomarkers that confirm and substantiate the direct physical interaction of the compound with the target selected for pharmacological manipulation. Information on direct compound-target interaction is of particular importance in drugs designed for neurological and psychiatric disorders because affirmation of compound penetration into the brain, its association with the target at the expected locale, and its residency time on the target are paramount to assess likeli-
hood of compound effects, dosing regimens, and predicted medical success.

**Pharmacokinetic and Pharmacodynamic Biomarkers**

Biomarkers that define the relationships of the compound and the biological target selected for therapeutic intervention. The pharmacokinetic (PK)/pharmacodynamic (PD) relationships must include not only the efficacy but also the safety biomarkers, primarily those that are mechanism-based (e.g., adverse effects that could be the result of the very mechanism of action that is hoped to provide benefits). Monitoring PK/PD in stroke drug discovery and development is particularly challenging because the milieu of the stroke site changes substantially over time. Factors like alterations in blood–brain barrier permeability, edema, reduced or lack of blood flow, and metabolic status have direct impact on compound distribution and activity, which are especially difficult to monitor in the rapidly changing ischemic tissue.

**Disease Biomarkers**

Biomarkers that define molecular, biochemical, and physiological or pathological features of the disease. Disease biomarkers must correlate (statistically) with any of the clinical disease features such as initiation, progression, regression, severity scales, relapses, or remissions. Furthermore, disease biomarkers could also serve as “disease modifying biomarkers” which in turn could serve as “surrogate biomarkers” and might be useful for drug registration before clinical outcome studies.

**Biomarkers for Patient Selection and Stratification**

Biomarkers that allow patient selection for clinical trials have become a centerpiece in the modern pharmacological clinical trials. These biomarkers define patients who are likely to respond, or not to respond, to the treatment. Patient stratification biomarkers underwrite the “personalized medicine” era in drug discovery and development. Such biomarkers are often driven by genetic and genomic biomarkers such as single nucleotide polymorphisms (SNP) or biochemical information on drug metabolizing enzyme (such as Cytochrome P450 variations).

In addition to these 5 biomarker categories described above, the Translational Medicine discipline in the Pharmaceutical Industry is centered around a principle of congruency that aims at increasing the fidelity of compound translation from preclinical to clinical development (see Figure 2). This principle covers three critical domains:

- **Congruency in PK/PD relationship between preclinical models and the expected exposure of the compound in humans.** This relationship ensures that the mechanism of action whereby PD responses are generated in the preclinical phase of the program is the same or similar to the mechanism that drives the PD responses in humans.
- **Congruency between biomarkers measured in preclinical models and in the clinical condition.** This means that what can and should be monitored in the clinical phases should be monitored in the preclinical (including animal models) phases. This is the “bed to bench” activity that helps predict congruency between disease models and the human condition.
- **Congruency between end points measured in preclinical models and those monitored in clinical studies (including genomic, biochemical, physiological and anatomic).**

In the following section, this set of translational medicine criteria will be examined in light of drug discovery and development for acute ischemic focal stroke in the adult/elderly population.

**Target Validation for Acute Ischemic Stroke**

The molecular targets selected for therapeutic intervention for stroke can be grossly summarized as belonging to either 1 of the following 4 major classes: (1) Ion channels; (2) “Excitotoxic” neurotransmitters/mediators; (3) Reactive radicals (oxygen and other molecular species); (4) Inflammatory modulators. The scope of this commentary does not allow careful and comprehensive review of the vast work published over the past 25 years of stroke research. Rather, we summarize
our view of these targets in the context of human stroke pathophysiology as discussed in many recent reviews.4,6

**Ion Channels**

Key ion channels (Na⁺, Ca²⁺, Cl⁻, K⁻) have been claimed to play a detrimental role in neuronal (and other brain cells) function and survival in the acute phase of ischemia. Research efforts have been primarily focused on channel mediated Ca²⁺/Na⁺ overloads.11 These events are triggered in a matter of minutes after an ischemic insult. In this context, the onset of ion derangements and the subsequent electric demise of neurons most likely occur several hours before there is a realistic opportunity to intervene pharmacologically. More important, the onset of these Na⁺/Ca²⁺ ion perturbations and their residual role in patients at time of treatment is unknown. Although it is possible and maybe likely that some regions within the ischemic penumbra are still subject to fluctuations in extracellular and intracellular ionic changes, such residual events are neither defined in humans nor is their impact on clinical outcome established. One can adopt the position that treatment with Na⁺/Ca²⁺ channel blockers has never been validated against the role these ions play in stroke patients at a realistic time frame of their availability for treatment.

**Reactive Oxygen Radicals**

Many compounds that were claimed to be “radical scavengers” have been tried without success in clinical trials. Claims for the “oxidative stress condition” as a therapeutic target for stroke have been largely derived from in vitro (cell-based) and in vivo models of ischemia with various classes of compounds.12 However, the “radical target” is a rather elusive one. The precise species of radicals that are “the target” have never been sufficiently characterized. The diversity of radicals, their molecular origin (O, N, Cl), their time frame of generation and “life span,” and their actions (on lipids, proteins, nucleic acid) remain obscure. No “antioxidant” agent has been shown to prevent (or reverse) the “oxidative fingerprint” (ie, modified proteins, lipids, nucleic acids, etc), and considering the very early time frame of radical generation, reversal of presumed damage is likely to be futile. The latest trial with NXY-059, a highly publicized program, is a case study in which none of the “target validation” criteria were actually achieved.5,14 More importantly, the conditions in which the “reactive oxygen radicals theory” emerged, reperfusion after short ischemic insults in animal models or acute deprivation/restoration of glucose and oxygen in cultured cells or brain slices, are remote and nonrelevant to the human condition.

**Excitotoxic Mediators**

Glutamate, glycine, and bioactive lipids (eicosanoids, platelet-activating factor, and others) have been the subject of intense preclinical research and clinical trials. The most promising studies were those focused on glutamate/glycine, which emanated from “the compelling preclinical demonstration” that glutamate and its receptors and agonists play a major role in neuronal cell death in vitro and in vivo. From the Translational Medicine perspective, the excitotoxic actions of such mediators, especially “the glutamate surge,” occur very early beginning at the onset of ischemia. Various experimental models confirmed the “minutes-time frame” of the release and activity of these preformed mediators. Thus, in a realistic and clinically meaningful time frame it is likely that the release of these mediators, their actions, and their subsequent detrimental effects are largely exercised. Furthermore, as indicated for RORs, conditions that triggered a robust release of these mediators have never been confirmed in humans. In this scenario, it is not surprising that within the clinical window of treatment, drugs aimed at preventing excitotoxicity showed little benefit.

**Inflammation Modulators**

Over the past two decades numerous reports have pointed out that inflammation is a consistent reaction to focal brain ischemia. The inflammatory response to ischemia has been documented in animal models as well as in stroke patients. Several inflammation targets have been studied including cytokines (interleukin [IL]-6, IL-1beta) and adhesion molecules (anti-intercellular adhesion molecule-1 [ICAM-1], anti-CD11, and neutrophil inhibitory factor). These biological targets are thought to promote migration of inflammatory cells into the injured brain. Several clinical trials using antiinflammatory agents have also been conducted, yet with negative results. From our perspective, none of the inflammation targets have been validated with respect to their role in the human condition. The robustness and redundancies of the inflammatory reaction as described in numerous models, its time course in reference to the ischemic event, and its potential role in the repair and regeneration process have never been investigated in relation to the inflammation targets selected for trials. The naive approach to “test the hypothesis” in clinical outcome trials with single agents that inhibit a narrow aspect of the inflammation process, without understanding the spatial and temporal relationships of the target to the overall scheme of events, has clearly turned counterproductive.15,16

In summary, there is a need to better validate the molecular targets that are chosen for drug discovery and development. This must be done considering the realistic time frames in which patients access evaluation and treatment centers. In this regard, the historical classes of targets selected for therapeutic intervention in acute ischemic stroke are most likely to continue to carry high risk of failure (Figure 3).1,2,3,5

**Target Compound Interaction in the Ischemic Brain**

The need to study the direct interaction between the molecular target and the candidate compound is critical in neurological and neuropsychiatric diseases. The reasons are multiple, including the need to demonstrate compound penetration into the brain (BBB), compound interaction with its target (denoting access to proper locale), the residency time of the compound on the target (to secure efficacy throughout the dosing period), and its metabolic fate. Such evaluations are difficult in the context of an acute neurological disease such as stroke but are much more feasible in experimental animals. However, such exploratory preclinical work is rarely done and never completely.
PK and PD Factors

Understanding the relationships between the PK and PD of the drug candidate are paramount to success. Often, dosing regimens are proposed for human studies based on limited PK/PD information in animal models and after a doubtful allometric scaling. Evidence that the compound engaged its biological target and generated a biochemical or physiological response and that it confirmed specific target engagement is virtually absent. While such studies in humans (healthy volunteer and patients) are difficult or unachievable based on the current state of technology, much more can be done in the experimental (preclinical) phase. For instance, the most recent and promising stroke program, Cerovive/NXY-059, failed to provide evidence of compound modulation of its proposed targets (reactive oxygen radicals) in the ischemic brain.14

Disease Biomarkers

Diagnosis of stroke is largely a straightforward medical task facilitated by imaging with computed tomography that allows accurate distinction of hemorrhagic from focal ischemic infarction. Acute ischemic stroke is a highly variable disease at various levels including type of lesion, time of presentation, neurological deficits, and disease outcome, and these variables are likely influenced by genomic variability in the population. Nevertheless, a critical disease biomarker that defines the topography, biochemistry, and physiology of the “salvable tissue” or “ischemic penumbra” could be theoretically implemented in preclinical and clinical studies. In this regard, technologies that allow for these types of measurements are vastly underdeveloped.

Ideally, new technologies for “bed side” assessment of the dynamic evolution and viability of the ischemic penumbra20 could have a major impact on selection and implementation of optimal strategies to manage each stroke patient based on his particular stroke condition. The current state of the art in neuroimaging shows promising results particularly in magnetic resonance imaging that allows monitoring of penumbra in preclinical models and stroke patients.17–19

Patient Selection and Stratification

The variability of stroke patients in respect to the initiation, progression, and response to thrombolysis is well established. Furthermore, a large number of confounding risk factors are usually associated with stroke as it is a disease of the elderly. However, the most important issue that has not as yet been addressed to clinical trials with neuroprotective agents is the knowledge of “who stands to benefit from the compound.” The premise of the neuroprotection framework requires that
the drugs are supposed to “protect” the neurons (and other cells) located in brain tissue that is “salvageable.” That means that the neuroprotective agent merely provides temporary cytoprotection while blood flow is restored to deliver oxygen and glucose allowing replenishment of energy in the form of ATP. This time frame is not well defined, yet clearly it is limited and short. Patients in which “penumbra” is absent or those who have limited penumbra could not be expected to benefit from neuroprotective agents because no salvage effect can be expected on restoration of a viable cellular environment. In contrast, patients who display a significant proportion of penumbral tissue are more likely to benefit from therapy if most of this tissue, or a meaningful part of it, recovers function within a proper time frame. Preliminary efforts to monitor the penumbra using multimodal magnetic resonance imaging that includes diffusion- and perfusion-weighted imaging (DWI/PWI mismatch) have been slow to evolve.20 There is a critical need to better define the parameters of the “mismatch” and to begin studying patients prospectively using this technology. This effort is likely to make a significant impact in current efforts aimed at developing drugs for ischemic stroke.

Summary

In this brief commentary, we have described a generic pharmaceutical translational medicine criterion (Figure 4) that we feel could guide future stroke drug discovery and development efforts. This system is broad and not restricted to stroke, yet it reveals the vast gaps of knowledge and fundamental errors in preclinical stroke research and clinical trials. The key areas of progress must include target validation, PK/PD relationships, and patient selection and stratification. In this regard, the role of imaging is likely to take a central role. The advancements in technology (CT, MRI, molecular imaging) that emerge in the 21st century hold the promise of allowing relevant patient selections for future neuroprotection as well as neurogenerative treatment modalities.

Disclosures

None.

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