2013 Thomas Willis Award Lecture
Causation and Collaboration for Stroke Research

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Abstract—The pathophysiology of stroke is complex. Adaptive and maladaptive signalling occurs between multiple cell types in the brain. There is crosstalk between central and systemic responses. And there are overlapping pathways during initial injury and subsequent repair. These numerous feed-forward and feed-back interactions have made it difficult to translate experimental discoveries into clinical applications. An emerging hypothesis in biomedical research now suggests that contrary to a traditional model, translation may not be efficiently obtained without a rigorous understanding of mechanisms. Hence, to optimize diagnostics and therapeutics for stroke patients, it is necessary to identify and define causal mechanisms. Mirroring the multi-compartment interactions in stroke pathophysiology, bench-to-bedside, and bedside-back-to-bench advances in stroke may be best achieved with inter-disciplinary collaborations between basic research, neuroimaging, and broadly based clinical science. Causation can then be two-fold, ie, dissecting mechanisms and targets, as well as developing future scientists who can blur the boundaries between basic, translational, and clinical research. In systems theory, a critical goal is to distinguish causation from correlation. In stroke research, causation may perhaps be found through a collaborative search for mechanisms. (Stroke. 2014;45:305-308.)

Key Words: brain injuries ■ cerebrovascular disorders ■ translational research

Translational Challenges

There have been numerous failures in clinical stroke trials. Why is it so difficult to translate basic science knowledge into clinical applications? Of course, for such a complex challenge, there are many reasons involved. However, during the past few years, it is increasingly recognized that 3 major issues may be worth discussing.

First, there is now an important movement to improve quality controls in animal model experiments.12 Meta-analyses of a wide spectrum of targets and drugs suggest that experimental design in some of the published literature may not be optimal.3 Is it possible that some of the false-positives were because of inadequate attention to key aspects of preclinical drug development, including proper randomization, blinding, and statistical powering of study design? Although this is surely not the only rate-limiting step in our translational process, more careful attention to these basic aspects of research design and execution should improve overall data quality not only in stroke, but also broadly across all of biomedical science.4

Potential limitations in study design may not be exclusive to the domain of experimental models. A second critical issue in stroke translation may pertain to the need to optimize clinical trial design as well. Because patient populations in stroke are heterogeneous, it is unlikely that any single target should be equally effective for all patients. Is it possible that a take-all-comers approach to power through all variations with large numbers and a single dichotomized end point may not necessarily be the most sensitive way to find effective therapeutics?5

Perhaps clinical trials may need to be restructured so that the emphasis is not on the sheer accumulation of large numbers per se but instead on the rigorous definition of target mechanisms and clinical biology in humans so that we can separate responders from nonresponders.

Beyond challenges in preclinical and clinical experimental design, the third and perhaps most important issue may be related to the relevance of mechanisms in translational research. The old standard model of translation was predicated on the principle of so long as it works, we do not need to know how and why. In retrospect, such an approach may not be useful because it is based on the assumption that one is lucky enough to have accidental discoveries. If one has a drug that works, then of course it is unnecessary to understand the how and why. But if an effective diagnostic or therapeutic does not yet exist, then relying on such a translational principle would be somewhat akin to just hoping for good luck. The power of serendipity should never be underestimated. But an emerging hypothesis in biomedical research now suggests that contrary to a traditional model, translation may not be efficiently obtained without a rigorous understanding of mechanisms.6,7 This may be especially true in a complex disease such as stroke.

Complex Mechanisms

Ischemic stroke is caused by a lack of blood flow. Hemorrhagic stroke is caused by a leak in blood vessels. For intracerebral hemorrhage, currently available therapeutic options comprise medical and surgical management of the
hematoma. For the early stages of cerebral ischemia, recanalization with tissue-type plasminogen activator or mechanical devices may be efficacious in properly selected patients. However, treatment effects may sometimes be modest, and narrow time windows limit the number of patients who can be treated. Therefore, it is logical for ongoing research to focus on strategies that can amplify reperfusion or develop new biomarker methods for finding patients who are most responsive to therapy. But beyond these efforts to normalize blood flow or restore blood vessel integrity, it has been difficult to find effective treatments that target fundamental cell death processes in injured neurons.

The initial triggers in stroke may be deceptively simple. Loss of blood flow and energy supply or the traumatic stress of an expanding hematoma leads to rapid neuronal cell death in severely damaged core regions. Yet, belying these relatively straightforward early events, the subsequent pathophysiology is highly complex because surrounding penumbral areas continue to succumb. It is now clear that stroke-induced brain injury is not a purely neuronal disease. Multiple signals are induced in all neuronal, glial, and vascular cells. The penumbra decays over time not just because cell death programs are activated in susceptible neurons, but also because cell–cell signaling in the entire neurovascular unit becomes dysfunctional after stroke onset. Perturbations in astrocytic glutamate reuptake mechanisms may exacerbate excitotoxicity. Alterations in pericyte regulation may affect perfusion and blood–brain barrier function. And the blood vessel itself may not just be inert plumbing. Instead, the entire cerebrovascular network may act as a trophic organ embedded within the brain itself. Thus, dysfunctional microvessels may lead to dysfunctional parenchyma even in the absence of immediate infarction. Furthermore, vascular signals in stroke may not be unique to the brain itself, and crosstalk between central and systemic responses is beginning to be revealed. The utility of targeting these pathways may begin to emerge because efforts are now underway to map vascular transcriptome and proteome signatures onto gene databases of human disease.

Beyond the complexity of multiple signals in multiple cell types, another emerging concept in central nervous system disease postulates that there are no sharp boundaries between injury and repair. For stroke, this implies that the penumbra is not only actively dying, but may also be actively trying to recover. The same mediators that contribute to injury in the acute phase may surprisingly provide the substrates for endogenous repair and remodeling in the delayed phase. For example, overactivation of N-methyl-D-aspartate (NMDA) signaling leads to excitotoxic neuron death. But without appropriately regulated NMDA signaling, neuroplasticity cannot take place during recovery. It may not be possible to develop effective therapies without understanding these overlapping signals for injury and repair. As stroke mechanisms are further dissected for causality, might it be possible for future targets to decrease acute cell death and also simultaneously promote neurorecovery in the delayed phase after stroke?

Finally, the entire network of cells and signals will be influenced by a whole host of modifying risk factors, including aging, hypertension, hyperlipidemia, diabetes mellitus, metabolic disease, and overall vascular inflammation. For example, in diabetic brains, upregulation of vascular proteases may degrade trophic signaling in neurons. Cardiovascular disease may augment negative feedback loops among the brain, heart, and diseased vessels. In the aged neurovascular niche, inflammatory microglia begin to suppress neurogenesis. In aging white matter, oligodendrocyte precursors may lose their endogenous abilities for repair. Defining causal mechanisms for stroke cannot be accomplished without the context of all these important stroke comorbidities.

Translation in stroke is difficult because the underlying mechanisms are highly complex. There are adaptive and maladaptive interactions between multiple cell types in the central nervous system, crosstalk between central and systemic responses, and changes in the neurovascular unit. The entire network of cells and signals will be influenced by a wide spectrum of modifying factors that include aging, hypertension, hyperlipidemia, diabetes mellitus, obesity, metabolic disease, and overall inflammation because of genetic, physiological, and lifestyle factors.
and overlapping cascades during initial injury and subsequent repair. Layered over all this interactive and recursive signaling is the influence of multiple factors that modify risk of disease, progression of injury, and response to treatments (Figure 1). Is it possible that our failure to translate may be, in part, to the intense pressure to jump into clinical trials before the causality of complex mechanisms in stroke is fully elucidated?

Causation via Collaboration

Precisely because the science is so challenging, laboratories have become increasingly specialized. There are many advanced tools to explore causal mechanisms at the molecular, cellular, and systems levels. The convergence of inflammatory mechanisms in disease may be investigated using techniques of molecular evolution. Manipulation of neuronal signaling cascades can be performed with precise optogenetic methods. Cellular and animal models now span the range from Caenorhabditis elegans and drosophila to rats and mice. Transgenic nonhuman primates are being developed. In vivo imaging tools can map neurobiology in real time. Advances in molecular MRI may eventually allow the investigation of stroke pathophysiology in humans. These are all sophisticated and difficult tools. No one single laboratory can effectively use all these powerful technologies. Collaboration is necessary.

Mirroring the multicompartment interactions in stroke pathophysiology, bench-to-bedside and bedside-back-to-bench collaborations may perhaps be attempted with interdisciplinary networks that connect basic neurovascular research, neuroimaging, and broadly based clinical science (Figure 2). To find clinically effective solutions, causality in the underlying mechanisms of stroke must be defined and targeted. In systems theory, a key goal is to separate causation from correlation. In translational stroke research, it is likely that causation cannot be determined without extensive collaboration to define mechanisms.

Opportunities

Translation in stroke research is challenging not because the fundamental biology is incorrect or irrelevant. Translation is difficult because the underlying mechanisms of stroke are highly complex. The risk of stroke, the transitions between injury and remodeling after stroke, and the overall response to potential treatments are mediated by recursive interactions between multiple cell types in central and systemic compartments, all of which are further influenced by an ever-widening array of risk factors. Because of this complexity, the dissection of mechanisms requires a broad approach that can only be achieved with interdisciplinary collaborations. It may be important to also recognize that the most effective collaborations are those that are encouraged but not designed. There is no master narrative that can be centrally directed. Committees and consortia tend to be less nimble. Top-down structures lead to homogeneity of ideas. In stroke research, as in any other intellectual endeavor, accumulated knowledge and the discovery of new knowledge may be a dispersed social phenomenon. Hence, to some degree, science will be personal and intuitive. The best opportunities for progress in stroke research will come in new ideas about new mechanisms from new people working together. By encouraging interdisciplinary collaborations that span the molecular gradients from cells and animals to systems and humans, we should strive to define causal mechanisms. These collaborations may also have a beneficial side effect. In pursuit of causation, we may also have the opportunity to train and inspire future scientists who will eventually blur the artificial boundaries between what is currently and incorrectly segregated as basic, clinical, and translational research.

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References


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卒中研究的困境

近年来，卒中临床试验大都以失败告终。人们不禁要问：为什么将基础研究成果转化到临床应用如此困难？当然，其中原因很多。然而就过去几年来看，有三个问题非常值得我们关注。

第一，动物实验质量控制越来越重要。研究范围包含靶点和药物的各种Meta分析表明，一些研究在实验设计上存在缺陷。由于未正确关注临床前药物研发的重要方面，如随机化、盲法、统计效能等，是否可能使一些研究出现假阳性结果？尽管并不确定这是限制转化医学进程的唯一因素，但是我们需要更加关注研究设计及研究执行的基础性方面。这对于卒中领域乃至范围更广的所有生物科学的研究质量提高都非常重要。

第二，与基础实验研究设计缺陷相似，临床试验研究设计同样也待改进。由于卒中患者的异质性，任何一个靶终点不可能对所有患者都有效。通过大量变量和一个二分化终点来找到对所有受试者有效的靶终点，未必是发现有效治疗措施的最敏感方法。临床试验的重点可能需要重新调整，不应是患者数量上的单纯累积，而应严格定义人类机体靶点作用机制及临床生物学，以区分应答者和非应答者。

除了临床前和临床试验研究设计存在缺陷之外，第三个或许是最重要的问题可能是转化医学的相关作用机制研究。转化医学以往的标准是定格在“只要它有效，不必知道它如何有效和为什么有效”（how and why）的原则上。以往这种靠运气的偶然发现可能最有效的方法。如果某种药物有效，我们当然就没必要去明白它如何有效和为什么有效。如果没有一种有效的诊断或治疗方法，那么这种传统研究方法就有些像在那里“撞大运”。显然，偶然的科学发现有时很重要。但是，现在大家越来越认识到，未来转化医学应不同于传统研究模式，需要充分了解相关作用机制才能有效推进。而这点对于复杂疾病如卒中尤为重要。

卒中机制之复杂

顾名思义，缺血性卒中由缺血导致，而出血性卒中由出血导致。对于颅内出血，目前的治疗措施包括对血肿的药物治疗和手术治疗。在出血性卒中的早期阶段，采用组织纤溶酶原激活物（tPA）或机械装置实现再通对于部分病人可能有效，但治疗效果有时并不明显，而且过窄的时间窗限制了可治疗的患者数量。因此，一些进行中的研究正致力于寻找能够增强再通效果的新策略。然而，我们努力恢复血流和血管完整性之外，却很难发现以损伤神经元细胞凋亡为靶点的有效治疗方法。

卒中机制之复杂

卒中研究的因果关系与协作

Causation and Collaboration for Stroke Research

Translation of Medicine

近年来，卒中临床试验大都以失败告终。人们不禁要问：为什么将基础研究成果转化到临床应用如此困难？当然，其中原因很多。然而就过去几年来看，有三个问题非常值得我们关注。

第一，动物实验质量控制越来越重要。研究范围包含靶点和药物的各种Meta分析表明，一些研究在实验设计上存在缺陷。由于未正确关注临床前药物研发的重要方面，如随机化、盲法、统计效能等，是否可能使一些研究出现假阳性结果？尽管并不确定这是限制转化医学进程的唯一因素，但是我们需要更加关注研究设计及研究执行的基础性方面。这对于卒中领域乃至范围更广的所有生物科学的研究质量提高都非常重要。

第二，与基础实验研究设计缺陷相似，临床试验研究设计同样也待改进。由于卒中患者的异质性，任何一个靶终点不可能对所有患者都有效。通过大量变量和一个二分化终点来找到对所有受试者有效的靶终点，未必是发现有效治疗措施的最敏感方法。临床试验的重点可能需要重新调整，不应是患者数量上的单纯累积，而应严格定义人类机体靶点作用机制及临床生物学，以区分应答者和非应答者。

除了临床前和临床试验研究设计存在缺陷之外，第三个或许是最重要的问题可能是转化医学的相关作用机制研究。转化医学以往的标准是定格在“只要它有效，不必知道它如何有效和为什么有效”（how and why）的原则上。以往这种靠运气的偶然发现可能最有效的方法。如果某种药物有效，我们当然就没必要去明白它如何有效和为什么有效。如果没有一种有效的诊断或治疗方法，那么这种传统研究方法就有些像在那里“撞大运”。显然，偶然的科学发现有时很重要。但是，现在大家越来越认识到，未来转化医学应不同于传统研究模式，需要充分了解相关作用机制才能有效推进。而这点对于复杂疾病如卒中尤为重要。
利用这些信号通路作为靶目标将成为可能。

除了各种细胞间复杂的信号转导机制外，在中枢神经系统疾病正出现的另一概念是假定损伤和修复之间没有明显的界限。对于卒中，半暗带不仅意味着正在发生死亡，同时也预示着机体正在修复。导致急性期损伤的介质可能成为后期内源性修复和重构的底物。例如，N-甲基-D-天冬氨酸（NMDA）信号通路过度激活导致神经元兴奋毒性死亡。然而如果没有正确调控的NMDA信号通路，恢复期的神经修复就无法进行。如果没有明确损伤与修复之间的交叉性信号，我们就无法找到有效的治疗方法。随着卒中因果作用机制的进一步阐明，未来是否能发现既降低卒中急性期死亡又促进随后神经修复的靶点？

最后，很多可改变的危险因素，包括高龄、高血压、高血脂、糖尿病，代谢综合征以及血管炎症，都可对整个细胞和信号网络产生影响。例如，糖尿病患者的脑组织，血管蛋白酶上调可能抑制神经元营养信号传递；心血管疾病可能增大动脉硬化、心脏以及血管疾病的风险循环。在老年人神经血管单元，炎性小胶质细胞开始抑制神经再生；老年人的胰岛素，少突胶质细胞前体可能丧失了内在修复能力。如果没有这些卒中伴随疾病的治疗线索，也无法明确卒中的因果作用机制。

由于潜在作用机制的复杂性，使得卒中转化医学研究更加困难。中枢神经系统多种细胞间存在着各种适应性相互作用，中枢和全身应答之间存在着对话交流，卒中和继发修复过程中存在交叉性级联反应。另一方面，疾病危险因素改变、疾病进展和治疗措施可能影响这些重叠的交叉式和循环式信号通路。图1展示卒中机制的复杂性包括神经元-神经胶质细胞-血管各部分之间的循环式相互作用，中枢神经系统和全身应答之间的对话，以及原发损伤和继发修复的交叉级联反应。这些级联机制进一步受到各种危险因素的影响，例如高龄、高血压、高血脂、糖尿病、肥胖、代谢综合征以及由于遗传因素、生理因素和生活方式导致的其他种类的疾病。图2展示来自麻省总医院的包括基础研究（白圈）、临床研究（黑圈）和神经影像（灰圈）的多学科合作研究网络。左图：2001-2006年的合作研究网络；右图：2007-2013年的合作研究网络；后者的合作性发表论文数量显著高于前者。

图1. 广义上的神经血管单元。卒中机制的复杂性包括神经元-神经胶质细胞-血管各部分之间的循环式相互作用，中枢神经系统和全身应答之间的对话，以及原发损伤和继发修复的交叉级联反应。这些级联机制进一步受到各种危险因素的影响，例如高龄、高血压、高血脂、糖尿病、肥胖、代谢综合征以及由于遗传因素、生理因素和生活方式导致的其他种类的疾病。

图2. 来自麻省总医院的包括基础研究（白圈）、临床研究（黑圈）和神经影像（灰圈）的多学科合作研究网络。
其潜在作用机制具有高度复杂性。卒中风险、卒中后损伤和修复之间的转变以及治疗效果反应，都受中枢和全身各部分细胞相互作用的介导，而这些相互作用又进一步受各种危险因素的影响。由于这种复杂性，只有通过多学科合作才能阐明这些作用机制。很重要的一点，我们应该认识到，最有效的合作是那些受激励而不是被指定安排的。卒中研究合作，没有中央导向性主体机构陈述，委员会和联盟的权力将变弱，因而自上而下的体制容易导致观点同化。

同其他科学领域一样，卒中研究领域累积的旧知识与新知识的发现可能是一种传播的社会现象。因此，在某种程度上，科学具有个性化和自觉化。卒中研究进展的最好机会，是关于卒中作用机制的新发现，应鼓励多学科协作，包括从细胞、动物模型到系统、人群的广泛研究领域，以努力发现卒中的因果作用机制。这种多学科协作可能会产生一种有益的附带效应：探究卒中因果关系时，可能出现新的科学发现，最终破除当前在基础医学、临床医学和转化医学研究之间的不合适人为界限，我们将可能提供机会培训和鼓励这些研究者，从而推进三者更广泛深入的结合和发展。

参考文献