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Recommendations of the National Heart, Lung, and Blood Institute Working Group on Cerebrovascular Biology and Disease

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Abstract

The National Heart, Lung, and Blood Institute (NHLBI) convened a conference call working group, consisting of experts in stroke and cerebrovascular biology on January 28, 2005. The purpose of this working group was to develop a prioritized set of recommendations for NHLBI to establish a focused and comprehensive set of research activities in cerebrovascular biology and disease. Three thematic areas of research emerged: (1) molecular and cellular neurobiology of cerebral blood vessels, focusing on genomics and proteomics, neurovascular signaling and cerebrovascular embryogenesis, development and plasticity; (2) resource development, involving the development of new methodological approaches for normal and altered function of the neurovascular unit, collaborative research, and training in cerebrovascular pathobiology; and (3) cerebrovascular diseases and translational approaches, addressing vascular mechanisms of disease, the role of risk factors, importance of biomarkers with the ultimate goal of developing new treatments.

Keywords

cerebrovascular disease; microcirculation

Cerebrovascular disorders, such as ischemic and hemorrhagic stroke, vascular cognitive impairment, and subarachnoid hemorrhage, remain a leading cause of death and disability in industrialized countries.¹ However, the repertoire of therapeutic interventions for these devastating conditions has remained remarkably limited. Although the approval by the Food and Drug Administration of recombinant tissue plasminogen activator for the treatment of acute ischemic stroke constitutes a significant step in the right direction, only a limited number of patients can benefit from this therapy and no specific treatment is available for other cerebrovascular pathologies. Therefore, there is a great need to develop new therapeutic approaches that can be used in patients with cerebrovascular diseases.

The development of new therapies for stroke and other cerebrovascular diseases is hampered by a lack of understanding of the fundamental factors governing the blood supply of the brain

in the normal state and in disease, as well as factors influencing injury to vascular structures and responses to vascular injury. Over the past few decades, most efforts have focused on the cellular and molecular events responsible for the susceptibility of neurons to injury, and on the development of strategies aimed at protecting the neurons from the consequences of ischemia and other injurious events.² However, relatively less attention has been devoted to the pathobiology of cerebral blood vessels, and to the interaction between cerebrovascular cells (endothelial and smooth-muscle cells, pericytes and cells of the adventitia) and other brain cells. Thus, there are large gaps in our understanding of: (a) the genetic basis of the unique phenotype and heterogeneity of cerebral blood vessels compared with vessels in other vascular districts; (b) the molecular and cellular events underlying the development of cerebral blood vessels and their relationships to brain development; (c) the factors controlling the functional interplay between vascular cells, neurons and glia, including the vascular effects of neurotransmitter; (d) the interaction of cerebral blood vessels with clotting factors and intravascular cells; and (e) the alterations in these fundamental processes brought about by disease and related risk factors.

The focus on cerebral blood vessels and their relationships with other brain cells is also justified by a growing body of evidence indicating that neurons, glia (astrocytes, microglia, oligodendocytes) and vascular cells (endothelium, smooth-muscle cells/pericytes, adventitial cells) are closely related developmentally, structurally and functionally. The term “neurovascular unit” was introduced to highlight the intimate functional relationships between these cells and their coordinated pattern of reaction to injury.^{2,3,4}

We are now at a crossroad in understanding the pathophysiologic processes that contribute to cerebrovascular diseases, and we are uniquely positioned to use newly developed molecular probes and tools, as well as high resolution imaging approaches, to investigate the underpinnings of these processes at the cellular and molecular levels.

The National Heart, Lung, and Blood Institute (NHLBI) convened a panel of experts on January 28, 2005 to examine these issues in detail and identify specific areas in which the gap of knowledge is most evident, and to provide to NHLBI a prioritized list of recommendations to develop a focused and comprehensive program in cerebrovascular biology and its diseases. The “Report of the Stroke Progress Review Group,”³ which was commissioned by the National Institute of Neurological Disorders and Stroke, provided an invaluable resource for the working group. In view of this comprehensive report and its recommendations, emphasis was placed on how enhanced basic knowledge about the vascular component of the neurovascular unit would promote translational approaches leading to novel treatment strategies and, ultimately, clinical trials. The following recommendations in 3 broad areas emerged:

1. Molecular and Cellular Neurobiology of Cerebral Blood Vessels

Genomics and Proteomics

Studies are needed to investigate the genes and gene products that determine the unique phenotype of cerebral blood vessels, focusing on endothelial cells, smooth-muscle cells, pericytes, as well as the adventitia. Considering the distinctive structural and functional heterogeneity of the brain, examination of the microvascular arrangement (microvascular units) in various brain regions would also be helpful. Investigations of the different segments of the cerebral circulation (extra- and intracranial cerebral arteries, pial arteries and arterioles, capillaries, venules, veins) and comparisons between vessels in gray and white matter would provide insight into the segmental differences in the structure and function of cerebral blood vessels and on their relationships to astrocytes and neurons. Gene expression analysis of perivascular neurons and astrocytes would also be informative.

Neurovascular Signaling

Investigations are needed to elucidate the reciprocal interactions between neurons, astrocytes, smooth-muscle cells, pericytes, endothelial cells and adventitia. Areas of interest include transcriptional and post-transcriptional mechanisms, signaling pathways, and effector molecules through which these cells communicate and coordinate their integrated functional responses. Genetic studies of susceptibility genes for ischemic and hemorrhagic stroke^{5,6} can unveil novel proteins involved in the normal function of the neurovascular unit and provide clues about pathogenic mechanisms.

Cerebrovascular Embryogenesis, Development and Plasticity

Studies on the embryogenesis and development of cerebral blood vessels and their interaction with developing neurons and astrocytes should be encouraged. For example, homeobox genes not only coordinate vascular patterning during development, but are also involved in critical adaptive responses of cerebral capillaries in the adult brain, and mediate the microvascular alterations associated with neurodegeneration.⁷ The potential for differences between cerebrovascular cells in large and small blood vessels supplying gray and white matter structures should be explored. There is evidence that newly formed vessels can influence the fate of neuronal progenitors.⁸ In-depth studies of the relationships between angiogenesis, neurogenesis and gliogenesis would be important for gaining further insight into normal brain development and into the genesis of cerebrovascular malformations as well. Furthermore, circulating progenitor cells, including endothelial progenitors, need to be investigated with respect to their roles in vascular development, normal and abnormal remodeling, and repair processes.⁹ Angiogenesis and vascular remodeling studies should also be extended to the adult brain to examine the plasticity of the vascular systems under different functional demands imposed by brain activity.¹⁰ These investigations would be critical for gaining insight into the microvascular reorganization and remodeling associated with brain plasticity, brain injury, and in abnormal collateral vascular development, as seen in Moya-Moya disease. The study of genes whose mutation leads to diseases of cerebral blood vessels, eg, notch 3 in CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy),¹¹ would be instructive. These data may help with development of therapies to mitigate the effects of vascular injury and may lead to new therapies for regression or reversal of arteriopathies.

2. Resource Development

Central to the investigation of cerebrovascular diseases is the development of new methodological approaches to investigate the normal function of the neurovascular unit, the alterations induced by cerebrovascular diseases and the synergistic pathogenic effect of cerebrovascular risk factors. The cooperation of investigators with complementary expertise would benefit the development of appropriate resources, as well as the conduct of the research. In addition, there is a need for training of new scientists with expertise in cerebrovascular pathobiology to attract new talent to the field and replenish the investigator pool.

New Models to Study Neurovascular Interactions

New experimental approaches to study the function of the neurovascular unit at the cellular and molecular levels are critical for addressing the mechanistic questions posed in the first recommendation of this report. In vitro approaches, such as cocultures of different cells of the neurovascular unit and brain slices, have to be complemented and validated with in vivo approaches. New imaging technologies to assess cerebrovascular function and dysfunction, eg, laser speckle flow-metry, infrared-based technologies, 2-photon confocal microscopy, functional MRI-based approaches, optical imaging, etc, combined with genetically engineered

mice expressing specific cellular markers are needed to provide cellular and molecular resolution to in vivo approaches.

Disease Models

New models of acute focal ischemia applicable not only to rodents, but also to primates, which are phylogenetically closer to humans, are needed. Models of global ischemia, mimicking the brain injury produced by cardiac arrest, are also needed especially in mice and primates. Models of chronic reductions in cerebral blood flow reproducing the hemodynamic alterations that may occur in vascular cognitive impairment are also needed. Current models of intracerebral and subarachnoid hemorrhage have major deficiencies, and new large and small animal models that more faithfully replicate the natural history and lesion topography seen in humans would be desirable. These models have to be applicable to young and aged animals, and to both sexes. Furthermore, these animal models have to incorporate the effect of cerebrovascular risk factors, such as hypertension, diabetes, apolipoprotein E, hyperlipidemias, metabolic syndrome, insulin resistance, hyperhomocysteinemia, vascular amyloid, etc. Models addressing the powerful influence of the ethnic background on the expression of cerebrovascular diseases and on the efficacy of therapeutic approaches need to be developed.¹² Genetically modified mice and in vivo gene transfer approaches mimicking risk factors are powerful investigative tools.

Application of Genomic and Proteomic Techniques

High throughput genomics and proteomics techniques need to be applied to the neurovascular unit and are critically needed for the gene expression profiling studies described in the first recommendation and to provide insight about pathomechanisms (see third recommendation). Expression profiling of patient material or animal models would provide mechanistic clues that could have important therapeutic implications. Genomic and proteomic approaches could also be useful in identifying transcripts that are involved in the phenomenon of ischemic preconditioning, one of the most powerful protective strategies whose neurovascular mechanisms have not been identified.

3. Cerebrovascular Diseases and Translational Approaches

In addition to efforts focusing on the normal function of the neurovascular unit and on new methodological approaches, investigations have to be directed at addressing the mechanisms of cerebrovascular diseases. These studies will build on the knowledge generated by basic studies of the neurovascular unit and would take full advantage of the resources described in the previous sections.

Genomic and Proteomics of Cerebrovascular Diseases

Studies are needed to investigate how cerebrovascular diseases alter gene expression in the components of the neurovascular unit. Monogenic diseases in which a single gene is responsible for the disease phenotype could be used as a starting point (CADASIL, amyloid angiopathy, vascular malformations). Expression profiling of vessels and neurons in models of cerebrovascular diseases, including models of pediatric stroke, would provide insight into pathogenesis. Similarly, analysis of the transcripts generated after subarachnoid hemorrhage can provide insight into the factors causing vasospasm. The modifications induced by aging and risk factors (hypertension, diabetes, apolipoprotein E, amyloid, etc) would be very informative. In addition, the effect of gender needs to be carefully evaluated.

Vascular Mechanisms of Disease

Studies need to address how cerebral ischemia alters the function of the neurovascular unit. Most efforts have focused on neurons and glial cells, and little is known about the effects of

ischemia on cerebrovascular cells. Although in many instances vascular occlusion is the initiating factor in ischemic cerebrovascular diseases, the role of vascular factors in the propagation and amplification of the tissue injury has not been investigated in detail. For example, the pathogenic role of clotting factors and intravascular cells in the evolution of the injury and the vascular mechanisms leading to the disruption of the extracellular matrix induced by ischemia have not been examined in detail. The factors triggering intravascular thrombosis in cerebral arteries need to be explored. Similarly, the mechanisms of the cerebrovascular dysregulation induced by ischemia and their role in the amplification of ischemic injury remains virtually unexplored. Vasospasm after subarachnoid hemorrhage remains a major cause of death in this condition.¹³ The identification of the molecular mechanisms underlying this devastating complication needs to be encouraged. Even less is known about the mechanisms by which sublethal reductions in cerebral blood flow (oligemia) alter the function of the neurovascular unit and how the white matter responds to lethal and sublethal ischemia. These issues are central to understanding the pathogenic mechanisms of vascular cognitive impairment and dementia.¹⁴

Risk Factors

The mechanisms by which risk factors alter the outcome of cerebral ischemia remain poorly understood. Studies investigating the effects of risk factors on cerebrovascular function would provide insight into their effect on the outcome of cerebral ischemia. In addition, studies are needed to assess the effect of intensive cardiovascular risk factor management and control on cerebrovascular pathobiology and gene expression. Furthermore, studies investigating the interplay among risk factors, their combined effect on the outcome of cerebral ischemia, and the interaction with age, gender and ethnic background-race would also be informative.

Biomarkers

Markers of disease are needed for the correct diagnosis of cerebrovascular pathologies, and for monitoring progression of disease and therapeutic interventions. The search and validation of these markers can be performed in experimental models and then tested in the clinical setting. Biochemical markers could be examined in specimens derived from patients (eg, plasma, cerebrospinal fluid, biopsy tissue, brain dialysate), whereas imaging technologies using spectroscopy or tracers could noninvasively detect variables linked to the diseases process. For example, establishing the fraction of the ischemic territory that remains viable after acute stroke would be of great importance for treatment decisions, whereas in vascular dementias assessment of disease progression over time would be an invaluable investigative and management tool. Molecular imaging techniques are particularly attractive because of their potential analytical power and noninvasiveness.

Translational Approaches and Development of New Therapies

The ultimate goal of these investigations is to develop new therapies for cerebrovascular diseases. A better understanding of the cerebral vasculature and its reaction to injury would lead to novel treatment strategies based on protecting cerebral blood vessels and on limiting the component of the damage that is related to blood vessel dysfunction. These vascular protective or “angioprotective” approaches could complement “neuroprotective” or “glioprotective” treatment strategies, thereby targeting all the components of the neurovascular unit. Transposing therapies from the laboratory to the emergency room would be facilitated by using experimental models that more closely reflect human diseases. It will be important to establish the time course of development of various pathophysiologic derangements in various tissue and cell compartments. In addition, pharmacokinetic and pharmacodynamic studies need to be performed on with promising potential therapies. These data will provide invaluable information on the optimal timing and duration of therapy(ies). Lessons learned from the failure

of clinical trials of putative neuroprotective therapies will need to be applied to future research efforts.¹⁵ Furthermore, the implementation of therapeutic approaches has to take into account the clinical reality of the specific diseases being treated. For example, interventions for acute stroke should have a therapeutic window consistent with the delay with which stroke patients reach the emergency room.¹⁶ Similarly, interventions for vascular dementia, a condition in which there is significant white matter pathology, should be tested in animal species in which the ratio between gray and white matter is similar to that of the human brain. The effects of age, sex and race on therapeutic efficacy should be thoroughly investigated, as an increasing number of preclinical therapeutic interventions are effective in males but not in females.¹⁷ Combination therapies should be carefully tested in a preclinical stroke setting to determine whether the effects of protective agents are truly additive.

Conclusions

The development of specific therapeutic approaches for cerebrovascular diseases would benefit from a deeper understanding of the developmental biology of cerebral blood vessels, their functional interaction with neurons and glia, and their pattern of reaction to injury and susceptibility to risk factors. Genomic and proteomics techniques offer the prospect of identifying novel therapeutic targets based on angioprotection. New clinically-relevant models of acute and chronic cerebrovascular diseases that take into account the contribution of risk factors and the powerful effects of age and sex are needed. Molecular, biochemical or imaging biomarkers would be critical for accurate diagnosis, acute management, and assessment of outcome. Ultimately, well-designed clinical trials are needed to validate the therapeutic approaches developed in animal models and assure their translation into clinical practice.

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