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Poised for Success: Implementation of Sound Conditioning Strategies to Promote Endogenous Protective Responses to Stroke in Patients

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INTRODUCTION

The following Perspective represents our summary of questions, ideas, concerns, and recommendations expressed by speakers and discussants at the 2nd Biennial Translational Preconditioning Workshop held in Miami in December 2011. Presentations by a diverse group of clinicians, clinician-scientists, and scientists, some of which are provided elsewhere in this issue, served to anchor many fruitful discussions about the current state of the field, and its future evolution with respect to promoting the best basic science research on this topic, and to optimizing this therapeutic approach for clinical use.

In this review, we attempt to synthesize the essence of many of these relatively unstructured exchanges utilizing an ‘Opportunities and Needs’ format that we hope will be of value for both those experienced and working in the field, as well as those relatively new to it. The reader is also referred to previous reviews on the theme of translating endogenous cerebroprotective responses to clinical trials (1–3).

Throughout this Perspective, we defer to the use of the broader term “conditioning” to represent the intentional promotion of endogenous protective responses, whether the stimulus is presented before, during, or after stroke. This was done in part because many of the points raised are inclusive of all treatments, and in part because the semantics regarding pre-, per-, and post-conditioning may limit, to some extent, a more encompassing translational acceptance of this therapeutic approach. We first focus on opportunities and needs related to the preclinical efforts in the field, then move to clinical considerations, and biomarker identification as well as discuss the need to promote discourse through these kinds of forums to ensure rational trial design and advocate for enhanced funding from multiple mechanisms.

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PRECLINICAL CONSIDERATIONS

Opportunities

A general consensus exists that leveraging a conditioning-based protocol for patients presenting with stroke, or for patients at high risk for stroke, has the potential to provide a host of benefits to the field of stroke therapeutics and our patients who currently have no FDA-approved neuroprotective agents available to them to mitigate stroke-induced cell death. While the general opinion of the attendees was that any therapeutic would be beneficial if it augmented or evoked endogenous cytoprotective pathways, there was also the sense that conditioning-based interventions are uniquely poised to offer a safe, low-cost and potentially highly effective means to preserve CNS function.

A wide variety of conditioning treatments have proven efficacious in preclinical models of ischemic injury to brain as well as to other tissues. Thus, the bench-based foundation for translation to the clinic would seem to be both broad, and in some cases, quite deep. Some pharmacologic-based conditioning stimuli utilize drugs that have already gained clinical approval for other disorders (4, 5), and some of the physiologic/mechanical interventions (intermittent ischemia of the upper or lower limb by a blood pressure cuff) that promote cerebral tolerance in animals (and endothelial tolerance in the peripheral vasculature of humans) are already being implemented in human trials at UCLA, The University of Miami, and Aarhus University in Denmark (6). Many favorable elements that support the likelihood of successful, conditioning-based clinical trials as well as the opportunity to reinvigorate interest in stroke neuroprotection were readily acknowledged by the group.

Based on the insights gained from over two decades of laboratory investigations of conditioning-induced ischemic tolerance, the innate ischemic resistance of the neonatal brain, and comparative physiology-based studies of hypoxia-tolerant species, hibernators, and even invertebrates, the epigenetic basis of the responses underlying the stroke-tolerant phenotype is becoming clearer.

One major advantage that conditioning has over other therapeutic approaches is that preclinical literature strongly supports the notion that the protection exhibited by the tolerant cell/tissue is multifactorial across a host of injury phenotypes (apoptotic, excitotoxic/necrotic, oxidative, immune, inflammatory) and is manifest across all resident brain cells as well – perhaps even including the cells circulating through the brain's vasculature. Thus, conditioning-based treatment approaches goes well beyond the 'one drug-one mechanism' approach to neuroprotection and clearly meet the widely recognized need for a pleiotropic stroke therapeutic (7). Triggering these multi-cellular adaptive responses can be accomplished using a number of different physiologic and pharmacologic approaches with wide safety margins. Therefore, concerns regarding treatment side effects and related sequelae should be allayed relative to the clinical testing of a new synthetic pharmaceutical. That said, much more work in the preclinical arena is still needed to accelerate progress of the field towards clinical trials. These needs are developed more fully here and elsewhere in this Perspective.

Needs

Despite over twenty years of preclinical studies and the translational excitement these studies have raised, many at the workshop sympathized with the point that more bench work is needed prior to the testing of conditioning-based therapies on stroke patients. In particular, given that the majority of animal conditioning studies to date are characterized by conclusions based on morphologic or other molecular indices of injury/protection, there remains an ongoing need for more preclinical studies founded on any number of functional endpoints. These would include neuro-sensory, -motor, and/or -cognitive. Future studies should optimize the use of clinically-relevant metrics, although a case can be made for a relative lack of similar high quality data defining the clinical study spectrum as well. The most widely used tool to rapidly assess functional outcome following stroke is the Modified Rankin, which is a 0–6 point scale for measuring the degree of disability or dependence in daily activities 90 days post-stroke. With ‘0’ being no deficits and ‘6’ used to denote ‘death’, there is clearly a great deal of improvement needed in assessing functional outcomes clinically.

There are also an admittedly sparse number of neurovascular unit-focused investigations on conditioning, as well as studies examining glial and white matter injury/protection endpoints. Similarly, long-term outcome measures where protection is confirmed at recovery time points distant from the stroke event are also underrepresented in the literature. There are many examples of promising therapeutics that demonstrated robust protection when assessed early after stroke, only to discover that such treatments simply delayed the infarct maturation rate, but not its magnitude. It is therefore essential to establish the long-term benefits of conditioning in our animal models, and certify that conditioning does not simply delay the rate at which the same poor outcome is achieved. Functional studies over protracted recovery periods could address both of these key needs. Facing many of the same set of problems as other areas of research, the growth and excitement within the field of neuroprotection in general, and endogenous neuroprotection in particular, seems to have slowed in the last decade. Participants raised concerns about the dependability of ongoing investments in this kind of research at both the federal and industry level. Without question, enhanced funding and innovation is essential to support the studies needed to strengthen the case for conditioning-based clinical trials. Finally, the translational attractiveness of the field of ‘post-conditioning’ could be advanced by demonstrating the efficacy of such treatments when initiated many hours after stroke, given the temporal disconnect between early treatment times in animals and delayed treatment options in patients presenting with stroke. Indeed, failure to address this fundamental temporal shift has been considered responsible, in part, for some of the failures of other putative neuroprotectants in clinical settings.

If conditioning therapeutics are viewed with the same careful eye that we assess other potential treatments, then the impetus to undertake clinical trials for any particular conditioning stimulus will benefit from STAIR-based (8) and/or RIGOR-based studies (9) that support the efficacy of that stimulus across both gender and age, and in the presence of common co-morbidities like hypertension, diabetes, and obesity (not to mention the regular use of common therapies like beta blockers, statins, etc.). In fact, some preclinical studies have revealed distinct sex-dependent tolerance phenotypes (10) and others have hinted at a

potential loss of potency in older animals (11). In short, while we know that gender, age, and these common co-morbid characteristics define well the patient population we expect to treat, we still continue to overlook them in our modeling.

It seems intuitively obvious that further movement of the field along a translational trajectory would benefit from investigations based on achieving these underlying objectives. If the efficacy of conditioning is significantly reduced in animal models with morbidities and risk factors that parallel the human stroke population, then it is vitally important to understand the nature and scope of these limitations prior to embarking on clinical trials. However, many in attendance at the workshop also expressed concerns that the co-morbidity testing recommendations in STAIR, in all their possible combinations, may be impossible to meet with respect to both time and available funding

Without question, preclinical studies going forward must adhere to good laboratory practice (GLP) with regard to randomization, sample size calculations, blinding, appropriate controls, proper statistical analyses, transparent reporting, and other study design issues (12). These efforts will strengthen the translational potential for any given conditioning stimulus. Of wide appeal was the suggestion that a multi-site, NIH-supported consortium, modeled on the CAESAR cardioprotection model (13), could be established for the testing of our most promising conditioning therapeutics, to help streamline the collection of the most convincing data needed to support their clinical testing.

Even if the existing volume of preclinical studies on conditioning-induced stroke tolerance are found under closer scrutiny to have not met the STAIR criteria in an appropriately comprehensive fashion, the issue going forward is how best to hold future preclinical conditioning studies to these standards so as to maximize their translational relevance? The criteria of note include the aforementioned GLP records and the idea of demonstrating efficacy in multiple strains and species (which implies the value of diverse models, not limiting studies to a select few). An important secondary consideration is that potential age and gender effects of a putative neuroprotective therapy be elucidated, and that conditioning therapies ultimately be tested in animal models with comorbidities (hypertension, diabetes, etc.) that parallel those common to human stroke.

While all of these guidelines have merit, questions raised at the workshop were not substantially different from those asked of any other putative therapeutic being considered at the bench-to-bedside interface: What is the inherent predictive value of studies performed to date that did not meet some of these criteria, and, going forward, is it even possible, or a good use of time and resources, to meet every STAIR criterion for a given conditioning therapeutic to be considered 'ready' for clinical trials? Are the STAIR criteria appropriate as written for a preconditioning therapy vis-à-vis a more standard post-stroke treatment? GLP-based criteria can be more easily and more quickly incorporated into our preclinical efforts than studies in animals with co-morbidities. In fact, potential combinations of gender, age, and the aforementioned co-morbidity variables are practically unlimited, and thus, some argued, unachievable. So, should the goal be to rank these variables in some way, and identify those that are the most critical to meet first and foremost? Perhaps more important, and also more difficult to answer, is whether any of these comorbidity criteria are a

necessary “translational bridge” and absolutely have to be met in our collective preclinical efforts, without exception, before moving forward with a trial? It would be hard to argue against the notion that the efficacy of a particular conditioning-based treatment should be unequivocally demonstrated in females, but would failure in an aged mouse model, or a hypertensive and diabetic rat study from one laboratory, end further clinical consideration of that particular therapy? Similarly, studies in nonhuman primates are “recommended” in STAIR, but should such studies be a precondition (no pun intended) to initiating clinical trials of a promising conditioning-based therapy? At present, these are sticky questions with no clear answers; given the hundred-plus list of failed clinical trials for stroke, it is not surprising that the metrics for evaluating the translational potential of a given therapeutic – including conditioning-based therapeutics – have evolved to this point, but moving from theory to practice to incorporate these ‘recommendations’ will be challenging

It is also from these criteria, in part, that an argument for studies of stroke tolerance in gyrencephalic species was questioned, particularly given the paucity of gyrencephalic brain models in use in many countries (that said, we are aware of several conditioning studies conducted in pigs (6, 14)). Other preclinical needs discussed included demonstrating in animals that a conditioning stimulus destined for clinical trials has no adverse effects on the efficacy of thrombolytic therapy, as well as implementing models that test whether a conditioning therapy expands the therapeutic window of tPA. A fundamental issue related to stroke modeling and tPA is that most animal models of focal stroke have been optimized to have reproducible spheres of injury and mechanisms of action as a result of relying on impeding brain blood flow by physically occluding the middle cerebral artery either temporarily or permanently. Ischemic strokes, however, are most commonly caused by thrombosis or embolism, wherein tPA administration could promote recovery. Thus, studies of conditioning in embolic stroke models should be expanded and also consider common post stroke pharmacotherapies such as aspirin based regimes and (vaso) pressors to control blood pressure and clotting (15).

All of these considerations for optimizing preclinical studies ultimately lead us back to the sticky issue of the extent to which our current animal models of stroke are predictive of human outcomes. Arguments for and against the faithfulness of rodent intraluminal suture models, cardiac arrest models, and others, to human stroke are just as valid with respect to assessing the efficacy of conditioning-based neurotherapeutics as any other treatment. The need for continued improvement in stroke models and more diverse and rigorous monitoring of physiologic variables remains a given. In addition, the effects of anesthesia, species- and strain-specific collateral perfusion, and other confounders require greater scrutiny, not only for their role in stroke-induced brain injury but also the extent to which these factors positively or negatively contribute, inadvertently, to the observed effects of treatment (16). The question of whether, going forward, we as a sub discipline should simplify, standardize, and/or adopt more uniform preclinical models of stroke across laboratories, not to mention focus on just a handful of conditioning stimuli, remains unanswered.

It would appear the conditioning-and-tolerance scientific community have collectively identified so many different ways to condition the brain, and also so many resultant genotypes and phenotypes that define its protective effects that we have proven this to be a

‘real’ phenomenon, and thus very likely to be efficacious in humans. In other words, past and future successes across so many models and species should ultimately, in theory, predict success in the clinic. Moreover, differences in dosing and other translationally-relevant caveats will be better revealed when utilizing a broad and diverse preclinical testing foundation. On the other hand, will the failure to establish isoflurane preconditioning-induced tolerance against transient MCAO in an aged female rat, for example, mean that isoflurane preconditioning will not likely work in aged females subjected to permanent MCAO, or in an embolic model with tPA, or in a cardiac arrest model? Or that isoflurane preconditioning should not be attempted under any conditions in human stroke? In turn, it could also be argued that continuing to move forward with a variety of conditioning treatments and stroke models may be disadvantageous in that we will spread ourselves too thin to define a logical translational ‘next step’, and that trying to promote more standardized models and limiting the conditioning stimuli we test may prevent fundamentally important insights and fortuitous discoveries that could have huge translational payoffs. Such are a snapshot of the questions we are currently asking among ourselves regarding the translational relevance of our animal models.

Finally, given that dozens of stimuli promote stroke tolerance in animal and cell culture studies, many in attendance shared the thought that in future preclinical studies, stronger emphasis should be placed on investigations of conditioning stimuli with reasonable safety/toxicity profiles. For example, some conditioning treatments used in preclinical models (e.g., anesthetics, herbs, ‘nutriceuticals’ and other natural products, brief limb ischemia) are already clinically approved; others (e.g., LPS, ion channel modulators) may never obtain such approval. Moreover, if we have conditioning therapies with proven safety profiles in humans, be they physiologic, pharmacologic, or “mechanical”, some attendants advocated that we should begin more robust testing for their safety in Phase I trials. On a somewhat related note, while a variety of mechanism-oriented preclinical investigations will likely continue into the future, consensus was mixed on the issue of whether it is really necessary to ‘understand’ the detailed molecular/genetic mechanisms, and the “omic” changes by which a given conditioning stimulus promotes stroke tolerance in our animal models before embarking on a clinical trial using that stimulus. This is particularly relevant for the aforementioned ‘safe’ conditioning treatments; if they afford reproducibly robust protection in our preclinical models, do we need to understand the mechanisms responsible for that effect prior to launching Phase 1 trials?

BIOMARKERS

There was a general consensus that one of the greatest areas of unmet need was that of developing more robust metrics and pairings of modalities that correlate with outcomes both in preclinical and clinical settings. These areas, generally termed ‘biomarkers’ of efficacy and outcomes, are essential to better coordinate clinical medicine and basic science efforts in the field of conditioning and stroke biology.

Thus, more emphasis should be placed in our future animal studies on defining blood or imaging biomarkers that reflect the efficacy of conditioning (prior to the stroke for preconditioning-based studies) and that predict the latent cerebroprotective phenotype of the

conditioned brain. It is quite likely that not all animals (or patients) respond to a particular conditioning stimulus in the same way, with respect to the degree of tolerance so induced. This issue is important because the development of any conditioning-centered therapy will be accelerated if physicians can come to rely on inexpensive, relatively easy-to-measure biomarkers that reliably reflect a 'successful' conditioning treatment. These metrics will likely vary based on patient profiles, but clarity in objectives and measures will go a long way to ensuring successful collaborations with medical staff who will implement trials both in academic centers as well as those in the field.

Opportunities

From a clinical standpoint, there are abundant opportunities to conduct trials and procure specimens both clinically and preclinically. The number of tools we have to perform genomic, metabolomic, and proteomic analysis of peripheral samples is boundless. Moreover, we have improved our ability to identify a number of patient subpopulations who are at the highest immediate risk for stroke. For example, patients who suffer one or more transient ischemic attacks (TIAs), and can be identified secondary to ABCDD or other scoring criteria, are at a 10 times risk for subsequent stroke than matched controls (17). With the prevalence of TIAs in the United States estimated at 5,000,000/year, an abundant clinical pool of patients is available to draw upon for conditioning clinical trials.

Indeed, none of the features of the ABCDD criteria fall into the traditional categories of 'biomarkers' whose definitions resonate with bench scientists. Advancing age, hypertension, duration of ischemic stress, obesity, diabetes, and other clinical co-morbidities are all features that are largely controlled for within our pre-clinical models through the use of inbred strains of rodents and highly stereotyped methodologies. The ability to identify populations of animals which can be induced to undergo periods of permissive hypertension, diabetic crisis, or periods of high coagulability would add a much needed parallel to the clinical reality of stroke care.

Even beyond the patients who have TIAs prior to stroke, the sheer number of primary strokes, and the number of patients who are placed on controlled periods of hypoxia and reperfusion during cardiac and neurovascular surgery, over any given year is staggering.

Recognizing the need to build national resources for trials, the NINDS has provided a clearer mechanism to implement preclinical studies and small scale trials via the establishment of the NeuroNEXT program. Investigators now have an unprecedented opportunity to access over 25 major medical centers with the infrastructure to facilitate rapid development and implementation of protocols in neurological disorders. Applications to NeuroNEXT <http://www.neuronext.org/> are short, rapidly screened and, if selected for secondary screening, require only one IRB across universities. Moreover, funding via the Network and home resources such as GCRC pilot awards offers a first of its kind mechanism to draw basic scientists together with teams of clinicians, and to access a more diverse patient population than could be achieved at a single site.

These kinds of investments have only been seeded in the last two years but offer the potential to attract pools of researchers who do not have the means to fund or drive clinical

studies with the same power that an entire network provides. An additional advantage of the NeuroNEXT program is that applicants need not be affiliated with the primary research site of the network to propose or implement a study.

Needs

The needs for biomarker development are many, a sentiment which was shared by all conference attendees. While some molecular markers of conditioning, such as activation of protein kinases, production of reactive oxygen species, upregulation of hypoxia inducible factor (HIF) and chaperone production, are well appreciated and even considered to be hallmarks in establishing new models (18–23), the ability to translate these characteristic responses into bona fide ‘biomarkers’ is debatable.

Oxidative modification of proteins, DNA, and lipids occurs in both central and peripheral forms of conditioning, and blocking redox stress impedes cytoprotection. The identification of changes in oxidized substrates within the CNS that predicts cellular outcome has been elusive. Trials spearheaded by the NIEHS at NIH have provided strong evidence that the most sensitive and reliable metrics of oxidative stress are formation of the bio inert arachidonic acid derivatives F_{2t}IsoPs (24, 25).

With no NIH driven initiative to create a central repository for brain banking of tissue exposed to global ischemia, local infarction, intermittent ischemia from sleep apnea or even post mortem tissue from stroke patients, the ability to measure lipid oxidation or other pathways relevant to conditioning is greatly hindered. Indeed, federally- and privately-funded brain banks exist for all manner of neurodegenerative diseases from Alzheimer’s to rare metabolic dysfunctions. NINDS has not, however, invested in maintaining a bank of postmortem samples from patients with stroke. Given that stroke is the fourth leading cause of death in the United States and the leading cause of long-term disability, the underrepresentation of stroke research infrastructure is enormous compared to Alzheimer’s and Parkinson’s disease. Brain banking, IRB assistance, and translational research assistance is spearheaded by 27 university-based Centers for Excellence both for Alzheimer’s and Parkinson’s. Without clinical specimens, researchers in the conditioning field are forced to run their own trials for procuring such specimens, or develop collaborations with clinical colleagues who have the resources to obtain IRB consent. An informal poll of attendees cited insufficient funding and lack of clinical colleagues with shared expertise in conditioning as the major reason they were not directly engaged in trials with patients.

CLINICAL MANAGEMENT ISSUES

Opportunities

The field of neurointervention emerged in the shadow of over 100 unsuccessful small molecule therapies for stroke neuroprotection, and has expanded the opportunities for improving stroke morbidity and mortality by treating patients with cerebrovascular stenosis and emboli that would have previously been monitored and placed on an aspirin based anticoagulation-based therapy after passing the approved 3.0–4.5 h window for TPA administration. Those with broader training in neurology, neuroradiology, neurosurgery, and endovascular surgery can now offer a small arsenal of stents and deployable clot retrieval

devices equipped with the ability to locally infuse infarcted tissue with drugs, as well as to sample blood immediately pre- and post-reperfusion, in a highly controlled manner. The concern with these device based strategies is that they are not subject to the rigorous clinical trials needed to proof efficacy for drugs like tPA. Regulated by the FDA, devices, such as the MERCI and PENUMBRA capture methods need only show that they are safe, not necessarily effective. Given the plethora of capture methods available, trials has lagged greatly behind device utilization (26, 27) (28)

These interventions have been coupled with a progressively more powerful series of imaging tools that offer the potential to provide 3-dimensional assessments of regional infarction and tissue survival; in fact, these advances in imaging provide a common platform for assessing injury status ranging from small and large animal models, to humans.

From a procedural standpoint, the larger proportion of those in attendance agreed that the therapeutic potential of pre-conditioning was greater than that of post- conditioning, but that both of these forms of conditioning present their own unique set of clinical parallels and their own specific needs and opportunities. The blood pressure cuff model of remote conditioning is hugely enticing as a low-cost means of providing protection. Moreover, blood pressure cuff ischemia and reperfusion offers the opportunity to place first responders such as EMS workers and emergency room staff in the position of beginning therapies minutes, and in some instances, hours before neurologists and intensivists can assess patients. While these interventions are actually extremely inexpensive to implement, the burden of establishing trials comes at a time when the field of neurostroke medicine is under increasing institutional pressure as a revenue stream; thus, this limits the ability of academic facilities to implement even low-cost trials for this already very cost-burdensome disease.

The importance of aggressive management and monitoring of individuals at highest risk for stroke intersects the field of ‘conditioning’ biology when the clinical features of patients who have had prior TIAs are more closely examined. We know that those who have had a TIA are at high risk of having a stroke; indeed 40 percent of all people who have experienced a TIA will go on to have an actual stroke. Most studies show that 5% of patients with TIA will have a stroke within two days and 10–15% of patients with TIA will have a stroke within 3 months of having had a TIA (29). The question arises is if the individuals who have had a prior ischemic insult without evidence of functional loss represent a unique, and possibly innately protected, group of individuals (30–34)? The notion that TIAs are neuroprotective is not without controversy (17).

That is, patients with previous TIAs have a more favorable outcome following a subsequent ischemic event than those without TIAs. Even TIAs lasting 10 to 20 min are still associated with a more favorable outcome when confounding variables, such as diabetes, are taken into account. Decreases in infarct volumes were more pronounced among patients where TIAs occurred fewer than 4 weeks prior to the onset of stroke compared with patients without prodromal TIAs. Patients with prodromal TIAs also had milder final clinical deficits than those without; in fact, prodromal TIA was the only predictive parameter of smaller final infarct volumes in some studies (32, 35).

Recent imaging studies suggest that the protection and enhanced functional outcomes associated with TIAs preceding stroke are not dependent on variations in blood flow and collateral development. That is, no observable changes between individuals with or without a history of TIA were observed on T2-weighted lesion volumes, apparent diffusion coefficient maps, or perfusion maps in imaging performed within the first 12 h after stroke (36). Therefore, despite having equivalent severities of ischemia initially, the final stroke lesion volume was smaller in patients with a prodromal TIA, implicating the important contribution of activated neurovascular cytoprotective mechanisms.

An important caveat to these observations is that conditions such as diabetes or genetic risk of Alzheimer's disease can dramatically alter the predicted 'neuroprotective' outcome trajectory of prodromal TIA (37, 38). These data speak to the need to rigorously assess individual risk factors and to expand genetic testing of patients, before surgical intervention, whenever possible.

Needs

Today, physicians and physician-scientists interested in neurostroke as a discipline have a number of choices for additional training and sub-specialization; such individuals remain underrepresented across the country and thus are highly sought after. We now generally take for granted the fact that tPA and interventional strategies offer the potential to provide patients with brain-saving alternatives that were not available 15 years ago. However, major gaps still exist in the very foundations of the practice of stroke medicine. Particularly at smaller community-based hospitals that lack access to specialized care in neurology and neurosurgery, training in diagnosis on-site and via real-time teleconferencing can decrease the need to route patients to Level 1 facilities by > 80% in a single year (O'Duffy and McLaughlin, unpublished data). Moreover, for many reasons, a 2010 report from the Centers for Disease Control and Prevention stated fewer than 10% of patients eligible for tPA receive it. Thus, there remains an urgent need for medical personnel to recognize stroke as a brain emergency, and for implementing rapid deployment in the first hours as well as transport of patients who have salvageable injuries.

It is worth noting that, in contrast to primary stroke, the diagnosis of TIA is much more difficult because the classical definition is often a diagnosis of exclusion, wherein stroke-like symptoms are present for < 1 h and fully resolve within 24 h, with no damage evident on imaging. However, as imaging tools become increasingly more robust, the 'lack of changes in imaging' criterion has come under increasing scrutiny, creating the quagmire of a potential 'moving target' of injury. That said, such advances in imaging may help us classify those TIA patients who go on to suffer a major stroke according to outcome.

TRIAL CONSIDERATIONS

Opportunities

As alluded to above, many workshop participants argued that conditioning-based therapeutics are ripe for clinical trials, including a strong preclinical literature that features a myriad of conditioning stimuli, protection endpoints, and animal models, as well as mechanistic studies that support an inherent multi-modal 'cocktail' approach to

cytoprotection. Moreover, the identification and successful bench testing of several harmless conditioning stimuli in multiple laboratories and in multiple models of ischemic and hemorrhagic stroke provide a critical foundation on which to anchor clinical trial proposals in the near term; that some of these stimuli are already FDA-approved for clinical use puts several hurdles behind us with respect to moving along the track to Phase II clinical trials. Of help in this effort could be the recently developed Blueprint Therapeutics Program at NIH to assist academicians in navigating FDA and IND guidelines and regulations and thereby help move a promising compound to Phase I testing. (see: http://www.ninds.nih.gov/funding/areas/translational_research/bpn_program_info.htm)

As highlighted earlier for TIAs, that many highly regarded retrospective and prospective studies have identified patient subgroups with well-defined, significantly higher-than-normal risks for stroke provides several distinct target populations for small-scale testing of endogenous cerebroprotective approaches. Although not without caveats, many attendees were of the strong opinion that assessing the efficacy of a conditioning treatment in such defined subpopulations is inherently advantageous relative to the testing of a standard therapeutic in a larger, more heterogeneous stroke population. As a result of its potential ability to broaden the therapeutic window for a given stroke therapy (be it a thrombolytic or others), or enhance its efficacy, it was proposed that specific conditioning approaches should also receive clinical consideration as adjunct therapies. Clearly, positive, proof-of-concept phase trial findings for a conditioning-based therapeutic could re-energize the field of stroke neuroprotection, which, given the long list of failed trials currently languishes in the eyes of both clinicians and industry alike (39).

Despite this apparent ‘readiness’ and many valid reasons for translational optimism, workshop participants are also of the mindset that it would still behoove the field to address other, as yet unmet, needs to better set the stage, and increase the odds, for successful clinical trial outcomes.

Needs

Considerable attention is being given to the practical aspects of implementing safe and rational clinical trials by NIH and clinician-scientists. This is due in part to struggles with ongoing clinical trial failures, to improving preclinical study quality, as advanced in the STAIR (initially established in 1999 and updated a decade later) and RIGOR reports, the 2012 NIH Stroke Progress Review Group (http://www.ninds.nih.gov/find_people/groups/stroke_prg/01-2012-stroke-prg-report.htm) report (12), and others; while not singled out, it is not unlikely that many of these study design concerns apply to a significant fraction of conditioning-based preclinical stroke tolerance studies in the literature. This in turn implies that perhaps the efficacy of some conditioning treatments may not be as robust as initially reported, and may therefore, from a collective standpoint, inherently hinder the degree of enthusiasm for clinical translation. Retrospective meta analyses examining the extent to which the stroke conditioning literature has, or has not, met these criteria, are not yet available to confirm or deny this possibility.

Then there is the question of what specific conditioning treatment, of known efficacy in our animal models, should be advanced to clinical trials first? As mentioned earlier, general

safety margins for some physiologic, and some pharmacologic, interventions that serve as conditioning stimuli in animals are already established in humans. Nevertheless, in moving from the bench to the bedside, because of a dearth of relevant biomarkers, it remains unclear whether the ‘dose’ chosen for any such stimulus will be far enough up the human dose-response curve to induce stroke tolerance – or, in turn, on the far side of the dose-response curve, thereby carrying the potential for injury in addition to ineffectiveness. Initial progress in this regard has recently been reported in a study of human subarachnoid hemorrhage patients (40). Another issue, addressed earlier, is whether it is reasonable to assume that some conditioning stimuli are more ‘safe’ than others (e.g., cycles of blood pressure cuff inflation/deflation versus a pharmacologic agent), particularly vis-à-vis the attendant risks of the conditioning protocol in the specific subpopulation being treated. Certainly some stimuli may face fewer clinical challenges than others in terms of implementation, all other things being equal; again, remote conditioning by repeated limb cuff inflation/deflation fits this bill. A very recent prospective and randomized study demonstrated that bilateral upper limb ischemia in patients with symptomatic atherosclerotic intracranial arterial stenosis. After receiving almost a year of bilateral forearm ischemia five times a day by blood pressure cuff inflation, patients in the preconditioning group experienced improved cerebral perfusion and reduced stroke recurrence (41).

Other US trials discussed at the meeting have moved away from forearm ischemia/reperfusion to restricting blood flow to a leg for ease of access. Limb ischemic preconditioning has the added benefit that analysis of metrics like antioxidant responses, lipid peroxidation, and age-dependent changes in acute stress have been reported in young (age 20–33) and older (age 62–81) patients subject to brief repeated periods of ischemia/reperfusion by blood pressure cuff inflation around the forearm. These data demonstrate that lipid peroxidation detection by F_2 -Isoprostane measures in urine and plasma are highly sensitive indices of oxidative stress, that older adults have impairments in their ability to repair and/or prevent the oxidative modification of substrates in response to acute ischemic insults, and that antioxidant supplementation can augment endogenous antioxidant responses in older individuals (42).

We have seldom actively looked for evidence of injurious side effects in our preclinical investigations, and, admittedly, we still do not really understand the cellular basis of our conditioning ‘targets’, be they on endothelium, glia, neurons, and/or, given the intriguing aspects of remote conditioning, circulating in the blood. Another question remaining unanswered among workshop attendees is whether it is appropriate, lacking such target validation studies, additional biomarker-based effectiveness assays, pharmacokinetics, mechanism of action, and to some extent safety data, to advocate advancing a given conditioning therapy to a clinical trial?

Independent of the conditioning stimulus, initiating clinical trials of even the best-tested and most efficacious conditioning treatments deriving from our animal studies will require the need to ‘recalibrate’ that stimulus in the patient, and, again, that depends heavily on as-of-yet unavailable biomarkers that reflect efficacy and can perhaps even separate ‘responders’ from ‘nonresponses’. Moreover, this dose-response may shift with age or other patient subgroup characteristics. If it is an inescapable fact that the conditioning therapy *du jour* will

be expected to show real and tangible clinical benefit, then it is imperative that trials beyond Phase I proceed judiciously through proof-of-concept studies to surrogate injury markers to authentic cerebroprotective endpoints in large, appropriately-powered multicenter trials.

As highlighted earlier, trials in a specific patient subpopulation already identified as being at high-risk for a stroke or ischemic event may comprise a better cohort for testing a conditioning treatment than those experiencing unpredictable, first strokes. In addition to those with TIA, groups in the former include patients undergoing carotid endarterectomy, those undergoing coronary bypass surgery, and those with prior stroke; in all of these populations, the 7-, 30-, and 90-day risks for stroke have been epidemiologically determined as significantly higher than controls, and thus represent authentic target populations. How to maintain a more chronic stroke-tolerant state in such patients is an attendant challenge; however, a recent preclinical study revealed that repeated hypoxic conditioning in mice extended the period of tolerance from days to months, suggesting that, as with exercise training, a long-lasting ischemia-resistant phenotype may be attainable with intermittent conditioning (43). Patients scheduled for aneurysm surgery, where the risk of ischemia is known prior to surgery, would seem to be an ideal subgroup for standard short-term preconditioning. And those surviving subarachnoid hemorrhage, at high risk for vasospasm and delayed cerebral ischemia represent suitable subjects for post conditioning.

Questions also remain among workshop participants about what endpoints clinicians should employ for assessing injury/protection with the greatest of sensitivity, to maximize the chances of finding a statistically significant effect and thus being the most informative with respect to efficacy of preconditioning-based prophylactic cerebroprotection strategies. Endpoints for smaller proof-of-concept studies may be different from those larger studies designed to show reductions in surrogate markers of clinical endpoints or true clinical outcomes reflecting cerebroprotection. Large sample sizes will ultimately be needed to power the latter.

Phase I and II clinical trials of conditioning-based therapeutics for stroke, subarachnoid hemorrhage, or other clinical conditions related to a high probability of cerebral ischemia have already been initiated in many countries (see: <http://clinicaltrials.gov>), and encouraging results from some completed trials can be found in the literature (40) (2) (44). There is, however, a risk in proceeding too quickly with an inadequately designed trial that leads to evidence of untoward effects and/or fails to show efficacy. For those involved in promoting endogenous cerebroprotection for stroke, the repercussions of such failed studies would be significant and multi-dimensional across all levels of the field – even if subsequent analyses revealed that the failure was secondary to inadequate powering, faulty patient selection criteria, the endpoints targeted for measurement, or other factors not related specifically to the conditioning treatment. Moreover, if the conditioning stimulus chosen is simply not strong enough to trigger the epigenetic responses needed to establish a more ischemia-resistant phenotype in that subpopulation...and that stimulus is then ‘written off’ as ineffective when in fact is just a question of not having the proper dose, then this would be most unfortunate for the field - and of course for the patients who could ultimately benefit. Thus, appropriate titration of the conditioning stimulus likely depends on reliable, yet-to-be-identified biomarkers, and may vary considerably depending on the subpopulation under

study; thus, such studies represent a critically-needed foundation for strengthening the likelihood of successful trials of the next phase.

Of note, a much larger number of clinical trials have been conducted in the field of cardiac ischemia – particularly for remote conditioning (45) – and yet, despite an order of magnitude greater collection of supportive preclinical myocardial conditioning studies, and despite some reported successes (46–48), many of these trials have still failed (49). Whether these failures were the result of not optimizing the conditioning stimulus, or were unsuccessful secondary to inappropriate patient selection or other poor study designs, etc., it would seem there are many lessons in the field of neurological conditioning to be gleaned from tracking the collective experience of our cardiology partners.

Regarding the funding of conditioning-based clinical trials, the downfall of the field could be the simplicity with which many physiologic and/or mechanical conditioning treatments can be administered, because that very fact makes it unlikely that pharmaceutical companies would financially underwrite such trials, particularly those considered to date to be the safest and most easily delivered (e.g., remote intermittent limb ischemia by blood pressure cuff, or the breathing of hypoxic or hyperoxic gas mixtures). This conclusion assumes no patentable pharmaceutical mimetic will emerge from the identification of a conditioning ‘sensor’ capable of providing the industry a potential for profit. Workshop participants expressed the bottom-line concern that, unless financial support for the necessary trial infrastructure is forthcoming from the NIH and other national health agencies, clinical trials for conditioning may be slow in progressing. Down the road, if successful trial data emerges and continues to snowball, the long-term value of supporting such studies may finally become tangible, and more centralized funding for them may grow.

Finally, launching clinical trials of conditioning-based therapeutics depends, to some extent, on the enthusiasm of emergency medicine physicians and neurologists to initiate them, and that requires familiarity with the field of endogenous protection, and its potential. That cells and tissues mount a multi-cellular adaptive response to stress would not likely be dismissed by these physicians at a conceptual level, but, at the same time, how a therapy based on leveraging this response can be brought to the bedside still seems to confound most physicians and critical care staff not intimately familiar with this field. While this conundrum could be abrogated to a significant extent by ‘marketing’ the concept more effectively, some of the skepticism and/or hesitation may stem from semantics – that the field was born and bred largely on the term “preconditioning”, and the notion that some kind of stroke pretreatment is being advocated. In turn, this limits objective considerations of the concept by those who fail to grasp the implications of leveraging endogenous, pleiotropic cerebroprotective responses for therapeutic gain. The same applies to the rapidly emerging field of post conditioning and how well the latter dovetails with commonly understood therapeutic windows. Thus, considerable energy still needs to be devoted to selling the potential of the idea of innate neurovascular protection to the broader clinical stroke community.

In sum, while there seems to be a consensus that carefully designed, risk-stratified clinical trials are highly desirable for the testing of attractive conditioning agents with apparent wide

safety margins and a successful preclinical track record, there was also agreement that many unmet needs and questions must still be addressed in the near term to help accelerate our journey across this translational bridge.

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