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PERSPECTIVE

A new penumbra: transitioning from injury into repair after stroke

Eng H Lo

The penumbra is an area of brain tissue that is damaged but not yet dead after focal ischemia. The existence of a penumbra implies that therapeutic salvage is theoretically possible after stroke. The first decade of penumbral science investigated the ischemic regulation of electrophysiology, cerebral blood flow and metabolism. The second decade advanced our understanding of molecular mechanisms that mediate penumbral cell death. And the third decade saw the rapid development of clinical neuroimaging tools that are now increasingly applied in stroke patients. But how can we look ahead as we move into the fourth decade of penumbra research? This author speculates that a paradigm shift is needed. Most molecular targets for therapy have biphasic roles in stroke pathophysiology. During the acute phase, these targets mediate injury. During the recovery phase, the same mediators contribute to neurovascular remodeling. It is this boundary zone that comprises the new penumbra, and future investigations should dissect where, when and how damaged brain makes the transition from injury into repair.

In 1977, a group of four scientists published what is arguably the most important paper in modern stroke research[1]. Astrup *et al.*[1] showed that after the onset of focal ischemia in a nonhuman primate brain, measurements of electrical activity revealed regions that were dysfunctional but not yet dead. In central areas of the stroke, blood flow deficits were severe and cells died rapidly. But in peripheral areas of the stroke, blood flow deficits were milder. In these areas, the ability of neurons to fire action potentials was lost. However, these neurons retained enough energy to sustain their –70-mV resting membrane potentials. When Astrup *et al.*[1] increased the blood pressure and improved collateral blood flow, these areas recovered, and action potentials were transiently restored. But after prolonged ischemia, these neurons underwent anoxic depolarization and died. Astrup *et al.*[1] called these brain areas the penumbra, presumably named after the astronomical term indicating areas of half-light and half-shadow. Thus, the original definition of the ischemic penumbra referred to areas of brain that were damaged but not yet dead, offering the promise that if proper therapies could be found, one could rescue brain tissue after stroke.

Eng H. Lo is at the Neuroprotection Research Laboratory, Massachusetts General Hospital, and the Program in Neuroscience, Harvard Medical School, Massachusetts General Hospital East 149-2401, Charlestown, Massachusetts 02129, USA.
e-mail: Lo@helix.mgh.harvard.edu
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Since this seminal study, there has been an exponential rise in the number of papers about the penumbra. Looking back over the literature, we can discern three successive decades of conceptual development. The first decade mostly comprised studies that characterized the physiologic profiles of penumbral tissue after stroke. Cerebral blood flow was mapped, and oxygen and glucose consumption rates were quantified to define the metabolic thresholds and fates of these at-risk areas[2–4]. The second decade saw a shift in emphasis as the underlying mechanisms of neuronal death were revealed. Intricate biochemical pathways involving excitotoxicity, oxidative stress and apoptotic-like mediators of programmed cell death were dissected[5,6]. Penumbral science became an active area of molecular biology that rapidly yielded many potential targets for neuroprotection. And, finally, the third decade of research saw a translational leap into clinical applications. Positron emission tomography (PET) scanning can identify brain tissue with reduced blood flow but transiently preserved metabolism[7,8]. Magnetic resonance imaging (MRI) can detect portions of ischemic tissue that have not yet succumbed to cytotoxic swelling and necrosis[9,10]. Together, these PET and MRI techniques allow us to image, in real-time, areas of 'stunned' brain that survive the initial insult after stroke. We can begin to see the penumbra, and thus we may be able find the stroke patients who still have it.

Yet here we are, 30 years later, still desperately searching for a viable neuroprotective therapy in stroke. No doubt, translating experimental leads into effective drug therapies is extremely difficult[11,12]. But if we can understand the physiologic basis of the penumbra, if we can map the detailed molecular mechanisms of neuronal death, and if we can now even image these 'at-risk' areas in patients, why are we still unable to find a neuroprotectant that works? Has the concept of the penumbra failed us?

Perhaps part of the answer lies in the fact that the three characterizations of penumbras thus far (physiologic, mechanistic and imaging) are only measuring a one-dimensional aspect of stroke pathophysiology, that is, a presumed linear progression from initial injury to final infarct. But accumulating data now suggest that after stroke, multiple processes of endogenous repair and remodeling are triggered. Without taking these mechanisms into consideration, it is probable that we are only assessing half of the signals operating in stunned brain tissue after stroke. The penumbra is not just passively dying over time. It is also actively recovering.

Many pathways are triggered after stroke[13]. Evolutionarily conserved responses to brain injury suggest that 'deleterious' mediators may be upregulated for a reason. Signals that mediate cell death during the acute stage of stroke might in fact promote repair during the recovery phase. The *N*-methyl-D-aspartate (NMDA) receptor, perhaps the most intensely studied target in neuroprotection, provides a good example. An enormous amount of literature based in cell and animal models suggests that NMDA antagonists are extremely effective in preventing

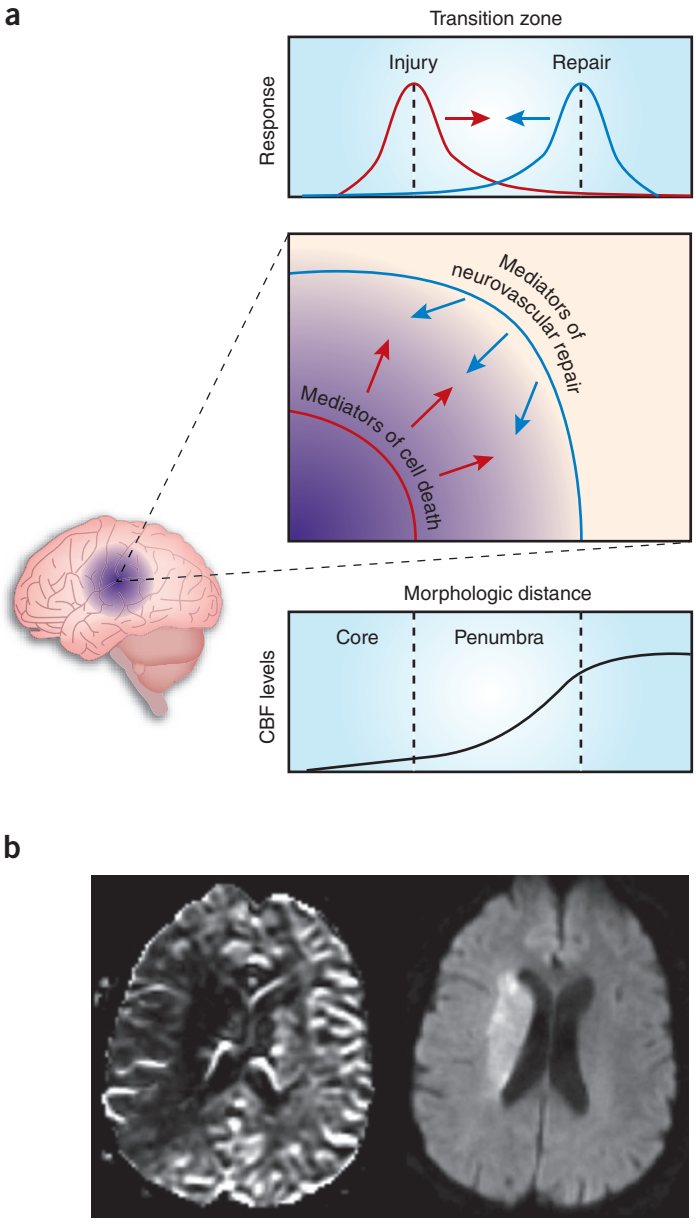

Figure 1 The penumbra. (**a**) The penumbra is traditionally defined as an area with mild to moderate reductions in cerebral blood flow (CBF, bottom graph). Within such areas, spreading waves of death mediators convert at-risk brain tissue into infarction. The new penumbra comprises the transition zone between injury and repair (top graphics). Unknowns to be addressed include the levels of blood flow and initial injury corresponding to these transition zones and the regulatory mechanisms and molecular signals in multiple cell types that mediate the switch from injury into repair. It is also important to note that these phenomena are highly dynamic. CBF thresholds evolve over time, mediators switch from deleterious to beneficial roles and injury-repair transition zones may be anatomically heterogeneous with simultaneous mechanisms of tissue decline and recovery. (**b**) At the present time, the clinically applied imaging penumbra is operationally defined as the mismatch between larger areas of cerebral blood perfusion deficits (left) and smaller lesions detected on diffusion-weighted MRI (right). From a clinical perspective, it will be important to find new imaging techniques which can help distinguish injury versus repair gradients within these areas of perfusion-diffusion mismatch in stroke subjects. MRI images are adapted from Lo *et al.*[13], originally reproduced courtesy of O. Wu and A.G. Sorensen.

neuronal death. After cerebral ischemia, rapid increases in extracellular glutamate overactivate these receptors, leading to an influx of calcium and the subsequent induction of multiple cell death cascades. Thus, blockade of the upstream NMDA receptor should be a logical neuroprotective approach. But clinical stroke trials testing NMDA antagonists have all failed[11,12]. Why? In part, narrow time windows for treatment are to blame. However, when one looks more carefully at the data, one also finds that NMDA signaling may have biphasic roles after stroke. In the early stages, over-activation of NMDA receptors is clearly detrimental. But in the delayed phase, these same NMDA signals may be required for recovery. NMDA receptors mediate the neuroplasticity and protection against apoptosis that is afforded by environmental enrichment[14]. In fact, prolonged use of NMDA receptor antagonists increases cell death in mouse models of traumatic brain injury[15]. Some data also support a role for NMDA signaling in promoting the endogenous neurogenesis that occurs after stroke[16]. Emerging molecular studies now suggest that these beneficial NMDA mechanisms may involve augmentation of protective cyclic AMP response element–binding protein signaling in neurons[17]. Taken together, in retrospect, these analyses suggest that an untitrated and wholesale blockade of NMDA receptors might not have been an optimal strategy for stroke treatment[18].

Another example of the biphasic nature of molecular signals in the penumbra involves extracellular proteases from the matrix metalloproteinase (MMP) family. Accumulating data implicate MMPs in stroke pathophysiology. By degrading neurovascular matrix, MMPs damage the blood-brain barrier and cause edema, hemorrhage and neuronal death[19]. Knockout of genes encoding MMPs or inhibition of MMPs with selected drugs have all proven considerably protective in animal models of stroke[19]. The importance of MMPs is also underscored by the fact that they are upregulated by tissue plasminogen activator, the only US Food and Drug Administration–approved thrombolytic treatment for acute ischemic strokes[20]. Thus, blocking MMPs may also prevent the hemorrhagic complications that currently limit the widespread application of tissue plasminogen activator therapy[21]. But, once again, things are never as simple as we wish them to be. Although MMPs disrupt neurovascular matrix and cause injury during acute stroke, they can promote neurovascular remodeling in peri-infarct cortex during the delayed stages of stroke recovery[22]. MMPs also mediate the movement of neuroblasts during the endogenous neurogenic response that is triggered after brain injury[23]. Whereas the use of inhibitors of MMPs during the first few hours after stroke reduces infarction, the same inhibitors worsen outcomes when applied several days later[24].

Neuroprotective targets such as MMPs probably comprise just part of the overall endogenous response of the brain to cellular stress. So it is not surprising that similar patterns emerge when one looks at other injury mechanisms in stroke. The c-Jun N-terminal kinase (JNK) pathway represents a stress-activated protein kinase cascade that is triggered during neuronal injury. Many studies have shown that select inhibitors of JNK are neuroprotective in animal models of cerebral ischemia[25]. In fact, one particular strength of this approach is thought to be the relatively long therapeutic window that exists for JNK. Inhibitors can be effectively administered even up to six hours after stroke onset[26]. However, emerging data now suggest that JNK also has a biphasic role in terms of injury versus repair. Whereas acute overactivation of this pathway triggers caspases and other cell death mediators, a delayed and regulated role for JNK may contribute to neuronal recovery. During brain development, JNK is a requisite signal

for neural precursor cell migration, microtubule assembly and axonal guidance[27]. An analogous role might exist in adult brain, wherein JNK contributes to dendritic sprouting and axonal regrowth after injury[27]. This is a testable hypothesis—JNK inhibition during the first few hours after stroke should be beneficial, whereas inhibition several days later should worsen outcomes.

If most neuroprotective targets in stroke have biphasic roles in the peripheral zones of ischemic injury, then a new interpretation of the penumbra should take into account this transition between injury and repair (**Fig. 1**). From a clinical perspective, this concept is crucial for two reasons. First, we need to know how and when these injury-to-repair transitions occur so that we can determine how long to continue stroke therapies. Second, we need to know where these transitions take place so that we can assess the balance between injury and repair in individual patients. Two recently completed clinical trials may provide a basis for discussing this idea.

Given the large literature base implicating free radicals and oxidative stress in neuronal injury, the Stroke Acute Ischemic NXY-059 Treatment (SAINT) trials testing NXY-059 as a free-radical spin trap to treat stroke should be a reasonable therapeutic approach. Results from the first SAINT-1 trial were intriguing—subjects receiving the drug did slightly better at 90 days compared to placebo-treated control subjects[28]. However, the larger SAINT-2 trial did not replicate these initial findings[29]. *Post-hoc* analyses will surely reveal many potential reasons for this disappointment[30,31]. But perhaps there may also have been a lack of attention to the complex multifactorial actions of free radicals in ischemic brain. No doubt, overwhelming amounts of free radicals and reactive oxygen species can damage proteins, lipids and DNA substrates. For example, nitric oxide (NO) is deleterious when large amounts are produced by uncontrolled neuronal or inducible nitric oxide synthase isoforms[32,33]. Alternatively, however, homeostatic amounts of NO derived from endothelial nitric oxide synthase are beneficial because they sustain blood flow in the ischemic periphery[34,35]. Baseline levels of reactive species might also participate in other normal cell functions. By oxidizing cysteine residues on phosphatases, free radicals help regulate kinase signaling cascades[36]. Free radicals may also participate in angiogenesis; NO promotes angiogenic sprouting[37], and vascular endothelial growth factor signaling requires the functional production of radicals from the NADPH oxidase pathway[38]. Is it possible that suppressing free radicals for too long interferes with essential triggers that regulate the secondary processes of angiogenesis and repair in the recovering penumbra?

In the SAINT trials, and, indeed, in all clinical stroke trials, a great deal of effort is made to determine the pharmacokinetic and pharmacodynamic basis for dosing. How much drug should be given to reach the target and achieve the desired blockade? Much attention is also increasingly paid to the therapeutic time window. How long after stroke onset is it still possible to administer the drug effectively? Yet it seems that almost no attention is paid to how long the treatment should last. In the SAINT trials, the free-radical spin trap was administered for three days[28,29]. Why? How long are the free radical targets active? During the first few hours after stroke, it is highly likely that molecular events in the penumbra mostly comprise injury, and most would agree that a few weeks later they mainly comprise repair. But what happens in the crucial periods in between—during the first few days, or even within 12 to 24 hours after stroke onset? What are the molecular signals that trigger the transition from injury to repair within damaged but not yet dead brain tissue? When do these complex transitions occur? What cell types are involved? Are there effects on neurovascular coupling[39]? Even during the early stages of cerebral ischemia, a complex network of endogenous neuroprotective responses may be triggered[40–43]. How do our acute therapies affect these repair mechanisms? Is there a continuum between early cellular repair and delayed neuronal plasticity and vascular remodeling? Without a full understanding of how our targets switch from deleterious to beneficial roles over time, we will not be able to maximize neuroprotection without interfering with endogenous recovery.

A staggering number of clinical trials in stroke treatment have failed. Many analyses have raised important limitations, including unanticipated side effects and drug delivery barriers[44]. But a major limitation has perhaps been the unrealistic therapeutic time windows tested—how long after stroke can we still treat damaged brain effectively? An important advance in this regard has been the application of advanced MRI to find people who 'still have a penumbra', that is, people with salvageable tissue[9,10]. This was the strategy used by the Desmoteplase in Acute Ischemic Stroke (DIAS-1) and Dose Escalation of Desmoteplase for Acute Ischemic Stroke (DEDAS) trials of a plasminogen activator derived from vampire bat saliva[45]. These first two trials seemed to validate the approach—subjects with salvageable tissue as defined by advanced MRI seemed to respond to thrombolysis even up to nine hours after stroke, far outside the original three-hour time window thought to be required for safe reperfusion[45,46]. Disappointingly, however, the follow-up trial DIAS-2 failed (W. Hacke *et al.*, personal communication). *Post-hoc* and imaging data analyses of the results from this trial will tell us more. But initial impressions suggested that the placebo group included many subjects who recovered well, even without treatment (W. Hacke *et al.*, personal communication). Is it possible that the imaging parameters used to define eligible subjects for the study in fact not only captured subjects who still had a salvageable injury penumbra, but also inadvertently included subjects with milder strokes and prominent repair penumbras? Optimizing a clinical stroke trial means that tails of the statistical distribution in an admittedly heterogeneous and variable subject population must be eliminated. The imaging penumbra excludes subjects whose strokes are too severe[47]. But is it possible that it does not fully exclude subjects who may simply recover on their own? Not all of the peripherally ischemic brain tissue is doomed to die. Restoration of blood flow is known to reverse acute lesions visualized by diffision-weighted MRI[48]. It may be possible that these acute MRI lesions can also partially recover with spontaneous clot lysis and recanalization. Furthermore, depending on collateral blood supply, penumbral tissue may sometimes persist for surprisingly long periods of time[49], perhaps allowing an opportunity for endogenous repair. And, technically, much also depends on how the MRI perfusion images are quantitatively obtained and what imaging thresholds are used for defining areas of potentially salvageable tissue. Subjects with large injury-repair transition zones will recover anyway, even without therapy, so inclusion of these subsets will dilute the statistical power of any clinical stroke trial. Where do these injury-repair transition zones take place, and in what patient subsets do they exist? Recent MRI methods now allow us to map multiparametric indices of neurovascular remodeling in animal stroke models[50]. If these techniques can be fully validated in the more structurally complex human brain, they might eventually provide new ways to map the gradients of injury versus repair in the new penumbra.

It is now 30 years since the ischemic penumbra was first defined in stroke. How do we move forward as we enter the fourth decade of penumbral science? By only focusing on mechanisms of injury, we may have been seeing only half of the picture. Many treatment targets have biphasic roles where initial deleterious responses transition into beneficial mechanisms for neuronal and vascular recovery. Studies of the new penumbra should comprise investigations into how, when and where stunned brain tissue makes the transition from injury into repair. How do these thresholds and zones correlate with the old characterizations of the penumbra? What levels of ischemia lead to irreversible damage, and what levels of blood flow reduction trigger early cellular repair and delayed neurovascular remodeling? What are the energy and metabolic requirements of such repair and

remodeling phenomena? What molecular mediators are involved, and how is this delicate balance maintained? For each target, when does the switch occur, and what neuronal, glial or vascular cells are involved? We need to understand how this injury-to-repair transition is regulated in stroke so that we can optimize treatments to block the desired target without interfering with endogenous recovery. Perhaps approaches that seek to augment endogenous protective pathways and prepare the ground for plasticity might be more likely to succeed than those that blindly attempt to block targets that ultimately convert into beneficial signals. We must ask how these injury-to-repair transition zones are affected by age, gender and altered baselines in diseased systems. Vascular and metabolic diseases are common correlates of stroke, and these influences on the balance between cell death and recovery must be understood. This new penumbra is also likely to be heterogeneous in space and time, so the constantly shifting boundaries of simultaneous injury and repair may make stroke a very difficult disease to treat. And, finally, we must find a way to image these processes so that we can find the right subjects for our clinical trials. How much of the MRI-defined penumbra is doomed to die, and how much is meant for repair and recovery, even without therapy? How can we identify subjects with salvageable tissue while excluding those subjects with already maximized potential for repair?

The concept of the penumbra has provided a productive intellectual framework for an enormous spectrum of basic and clinical stroke research. Perhaps a new interpretation is now needed to move forward. The transition zones between injury and repair underlie the brain's highly regulated and complex response to stroke. A deeper dissection of this new penumbra may ultimately help us to make the extraordinarily difficult translational leap from experimental models into viable clinical therapies.

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