Targeting the Ischemic Penumbra

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Background—In the last 3 decades, and from a therapeutic point of view, the classical concept of ischemic penumbra based on hemodynamic and electrophysiological parameters has loosened up the rigidity of therapeutic windows in acute stroke management. Thirty years later, the ischemic penumbra is an evolved concept that presents more applications. Thus, the ischemic penumbra is a diagnostic target, allowing the extension of therapeutic windows; it is also a biochemical target, in which an intermittent bioenergetic compromise takes place, and it is a target for brain plasticity, neuroprotection, and neurorepair.

Summary of Review—In this work, we review how the concept of ischemic penumbra has been evolving from its purely electrophysiological/ hemodynamic based definition to the wider metabolic–cellular–therapeutic concept that is managed today by neuroscientists. (Stroke. 2011;42[suppl 1]:S7-S11.)

Key Words: ischemic penumbra • neuroprotection • neurorepair • stroke

The introduction of the concept of ischemic penumbra by Astrup,1 and its subsequent development, has been the key for a change in the consideration of ischemic stroke from a “preventable catastrophe” to a “treatable disease.” The “time is brain” aphorism, and the consideration that stroke is a neurological emergency, as a result of the ischemic penumbra concept, has allowed patients to profit from quick treatment by trained medical staff and transfer to specialized stroke units.

The penumbra was classically defined as the hypoperfused tissue surrounding the ischemic core in which blood flow is too low to maintain electric activity but sufficient to preserve ion channels. However, this area is subjected to a wave of deleterious metabolic processes propagated from the core to the neighboring tissue, including excitotoxicity, spreading depression, oxidative stress, and inflammatory response, which lead to the expansion of the ischemic core and the subsequent worsening clinical outcome.

From a therapeutic point of view, the concept of ischemic penumbra has loosened up the rigidity of therapeutic windows, but 33 years from its establishment, the ischemic penumbra presents more practical applications that we review in the following sections: (1) the ischemic penumbra as a diagnostic target (to extend the therapeutic windows); (2) the ischemic penumbra as a biochemical target (intermittent bioenergetic compromise); (3) the ischemic penumbra as a target for brain plasticity; and (4) the ischemic penumbra as a target for neuroprotective and neurorepair treatments.

The Ischemic Penumbra as a Diagnostic Target

The most relevant definition of ischemic penumbra for clinical practice is based on neuroimaging techniques. It is widely accepted that brain tissue with reduced blood perfusion, as seen in MR perfusion-weighted imaging (PWI) but not included into the lesion core, as seen in MR diffusion-weighted imaging (DWI), indicates potentially salvageable tissue. Thus, the combination of PWI and DWI images has led to the PWI/DWI mismatch concept.2

Although it is generally accepted that hyperintense signal on DWI represents the lesion core, quantitative measurements of apparent diffusion coefficients reveal that during ischemia, apparent diffusion coefficient declines before energy metabolism fails, indicating that the increase in DWI signal intensity is not restricted to the infarct core.2 Therefore, the discrimination between penumbra and infarct core by DWI is not always clear, because DWI signal increases in both sides.3

In addition, PWI-detectable flow decreases are pathophysiologically relevant only when they interfere with adequate oxygen supply to the tissue. Thus, the PWI/DWI mismatch includes not only the penumbra periphery, but also surrounding intact tissue.2

Today, neuroscientists are able to delineate the ischemic penumbra using alternatives to this hemodynamic concept. Among others, tissue hypoxia can be determined by MR spectroscopy; the autoregulatory adjustment of the cerebral vasculature to reduced perfusion can be estimated by cerebral blood volume assessment, blood oxygen level-dependent imaging allows the detection of areas of increased oxygen extraction, anoxic depolarization is depicted by manganese-enhanced MRI, tissue acidosis is shown by pH-weighted imaging, and so on. However, none of these methodologies is able to provide clear-cut threshold values to differentiate among ischemic core, penumbra, and surrounding intact brain.

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tissue by themselves, and a multimodality approach is recommended.\textsuperscript{2,3}

Most of these MRI methods are unfortunately restricted to experimental models and state-of-the-art brain imaging and must be fully validated for the human brain before translation into clinical practice. Nonetheless, these methods might provide the neuroscientist new opportunities to better map and understand the gradients of injury versus repair zones in the penumbra.\textsuperscript{4}

Other concepts technologically more available have been developed, like the clinical–DWI mismatch. This concept is based on the fact that most clinical symptoms of stroke, evaluated by the National Institutes of Health Stroke Scale, correlate better with the abnormal PWI volume than with DWI volumes.\textsuperscript{5} Likewise, the penumbra can be also imaged using technologies such as perfusion CT\textsuperscript{6} and positron emission tomography/single photon emission CT.\textsuperscript{7} Positron emission tomography/single photon emission CT allows the measurement of cerebral blood flow and glucose metabolism. Positron emission tomography is considered the “gold standard” technique to identify the penumbra in humans. However, important limitations, including cost, availability, and spatial resolution, make them less useful than CT and MRI in daily clinical practice.

The Ischemic Penumbra as a Biochemical Target
Considering the problematic definition of the penumbra by imaging techniques, one could consider its identification based on a different concept: the cascade of molecular events that recruits penumbral tissue toward the lesion core, providing in this way a variety of definitions of the ischemic penumbra in molecular terms.\textsuperscript{8}

The major responsibility for the evolution of the ischemic penumbra is the status of local cerebral blood flow. A decrease in cerebral blood flow yields reduced adenosine 5’-triphosphate and failure of Na\textsuperscript{+}/K\textsuperscript{+} pumps, increasing extracellular glutamate and activating glutamate-mediated channels (N-methyl-D-aspartate and \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid), ending in an increase of intracellular calcium.\textsuperscript{9} Intracellular calcium participates in the formation of free radicals through the activation of nitric oxide synthase, which promotes nitric oxide formation and the subsequent synthesis of the highly toxic peroxynitrite radicals.\textsuperscript{10}

Thus, the spread of glutamate from the ischemic core to its periphery is a mechanism that induces irreversible damage to tissue. Glutamate acts as a mediator of peri-infarct depolarizations, or spreading depression, which originates in a marked disruption of ionic homeostasis and causes acidosis and increases energy demand and neurotransmitter effluxes, all starting from the core and propagating through its periphery to increase infarct volumes (Figure 1).\textsuperscript{11,12}

Deleterious processes propagating from the core to the penumbra can induce additional mechanisms of damage, contributing to the evolution of the ischemic lesion. Such mechanisms include oxidative stress, nitric oxide overproduction, release of inflammatory cytokines (eg, tumor necrosis factor-\(\alpha\) and interleukin-6), expression of adhesion molecules (eg, intercellular molecule adhesion-1 and vascular cellular adhesion molecule), and production of matrix metalloproteinases.\textsuperscript{13}

Protein synthesis and apoptosis are also considered active processes on the penumbra. A reduction on protein synthesis is one of the earliest and most sensitive metabolic conse-
quences of ischemia, although stress proteins such as heat shock protein 70 are upregulated. Correlations have been found between regions of expression of heat shock proteins and the penumbra, defined as the region of suppressed synthesis of proteins with preservation of adenosine 5'-triphosphate levels.

Apoptosis also contributes to the recruitment of penumbral tissue to the core lesion. In fact, apoptosis is considered the prototypical form of cell death in the penumbra.

All these events demonstrate that, from a molecular point of view, the penumbra is well-defined in respect to the ischemic core and healthy tissue. Therefore, the term penumbra may be referred as “peri-infarct tissue” and defined by the tissue affected by these molecular processes and based on molecular markers of them. This concept, very useful for the development of neuroprotective therapies, is difficult to apply in daily clinical practice, so the “classical penumbra” remains as standard in the clinics.

The Ischemic Penumbra as a Target for Brain Plasticity

In the acute phase, stroke treatment focuses on saving as much penumbral tissue as possible. However, many patients with still viable penumbra are not treated by reperfusion therapies. It is expected that the development of effective therapies based not on recanalization, but on the enhancement of brain plasticity will have a significant impact on clinical applications in the future.

Altman demonstrated in 1962 that new neurons are produced during adulthood, so neurogenesis continues postnatally for some mammals, specifically in the subgranular zone of the hippocampus and the forebrain subventricular zone of the lateral ventricle (subventricular zone). Since then, neurogenesis is considered a new target for the treatment of stroke.

Growth factors such as transforming growth factor-β or fibroblast growth factor-2 and the chemokine stromal cell-derived factor-1 are overexpressed in the penumbra, being involved in the recruitment of bone marrow-derived cells and neural stem cells to sites of ischemic injury. Stromal cell-derived factor-1 is expressed up to 30 days after stroke in the penumbra, and even later in the ischemic core, when new blood vessels appear. Indeed, stromal cell-derived factor-1 expression in the penumbra was associated with reactive perivascular astrocytes, suggesting that stromal cell-derived factor-1 may play a role in enhancing plasticity after ischemia. The administration of vascular endothelial growth factor has also been related to the promotion of neurogenesis in the subventricular zone and angiogenesis in the ischemic penumbra after stroke.

Recently, Lo has speculated about the need for a shift on the penumbra paradigm, because most molecular targets for therapy present biphasic roles in stroke pathophysiology. During the acute phase, molecules such as matrix metalloproteinases or c-Jun N-terminal kinase mediate in injury, whereas during the recovery phase, they contribute to neurovascular remodeling. This contradictory phenomenon takes

Figure 2. Gd-doped liposomes used for target delivery of drugs (PEG indicates polyethylene glycol). Liposomes are visible in vivo, on T1-weighted MR images (hyperintense signals), and ex vivo in fluorescence microscopy. Liposomes were prepared and monitored at our laboratory using a 9.4-Tesla scanner.
place at the ischemic penumbra. Thus, Lo defines a “new penumbra” as the boundary zone between dead and healthy tissue, proposing that new research lines are needed to address how to dissect where, when, and how the damaged tissue of this penumbra makes the transition from injury to repair. It is therefore tempting to postulate that the ischemic penumbra could be defined as the target region for brain plasticity and neurorepair.

The Ischemic Penumbra as a Target for Neuroprotective and Neurorepair Treatments

Accepting that peri-infarct tissue (called penumbra or not) is a target for neurorepair and neuroprotective therapies, a challenge arises to reach this area with therapeutic agents. A widely accepted strategy for this purpose is to use drug carriers such as liposomes. The incorporation of liposome-encapsulated therapeutic agents into the brain has been already described.

Liposomes can carry drugs and keep them stable for long residence times in the bloodstream until they reach the brain parenchyma through the blood–brain-barrier, a process facilitated by their lipophilic nature.

Furthermore, today it is possible to visualize the process of drug delivery to the brain by noninvasive means. Liposomes can include gadolinium in their structure, allowing their location in vivo using MRI. In Figure 2 we show a MR image of a rat brain in which Gd liposomes can be located in the brain as hyperintense areas. Liposomes also include rhodamine in their structure, making possible to correlate in vivo MR images with ex vivo fluorescence microscopy.

Encapsulation of neuroreparative or neuroprotective agents in liposomes enhances their therapeutic effect by proportioning a safe environment to the carried agent increasing its stability, its residence time in the bloodstream, and its bioavailability to the brain tissue. As an example, we report the results of an in vivo study on an animal model of stroke (Figure 3), in which we used MRI to state that citicoline, a well-known therapeutic agent for stroke, is more effective when it is encapsulated in liposomes.

Liposomes can be additionally modified by vectorization of its surface with antibodies specific to molecules overexpressed in target regions. The design of immunofunctionalized carriers allows to target the ischemic penumbra for a more effective therapeutic effect. As discussed before, the penumbra can be defined by a series of molecular targets. Those could be used for the vectorization of liposomes. Advances in this field will lead to the increase of safety and activity of neuroprotective and neuroreparative agents by reducing the required dose, increasing their stability in the body and their circulating time in the blood stream, facilitating their bioavailability in the brain by enhancing the crossing of drugs through the blood–brain barrier and, finally, increasing the local concentration of therapeutic agents in the target tissue (the penumbra) by molecular recognition processes between cells in the penumbra and immunofunctionalized liposomes.

Conclusions

The concept of ischemic penumbra has been evolving since its introduction 3 decades ago. The practical implications of such evolution, from a blood hemodynamics-based definition to a concept of peri-infarct tissue, defined on the basis of molecular events that take place on it, and as the target tissue for processes triggered after an ischemic insult such as neurorepair have been reviewed in this work.

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