Molecular Identification of the Ischemic Penumbra

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Abstract—Review of results of experimental and clinical studies indicates that the penumbra of physiologically impaired but potentially salvageable tissue surrounding the central core of focal cerebral ischemia that develops shortly after onset of major conducting vessel occlusion is complex and dynamic with severity and duration thresholds for hypoxic stress and injury that are specific to tissue site, cell type, molecular pathway or gene expression investigated and efficiency of collateral or residual flow and reperfusion. Imaging methods that have been utilized in vivo to identify penumbra and predict response to reperfusion and other protective therapies include magnetic resonance spectroscopy, diffusion- and perfusion-MRI as well as positron emission tomography. However, resolution of focal lesions characterized by lactic acidosis or cellular edema does not predict tissue survival, and imaging thresholds for resuscitation after reperfusion have not been determined experimentally. HSP-70 stress protein induction represents an endogenous protective mechanism that occurs in penumbra but not core neurons. A robust protective effect has been demonstrated during focal ischemia in transgenic mice overexpressing HSP-70 perhaps by suppressing early cytochrome c release. Delayed manganese mediated striatal neurodegeneration can be detected with T1 MRI after brief episodes of transient focal ischemia. Future studies may define endogenous cytotoxic and cytoprotective molecular penumbras that can be exploited to improve outcome after temporary focal ischemia. (Stroke. 2004;35[suppl I]:2666-2670.)

Key Words: brain edema □ blood flow □ energy metabolism □ magnetic resonance imaging □ protein synthesis □ stroke, ischemic

The concept of penumbra during focal cerebral ischemia refers to the regions of brain tissue, usually peripheral in location, where blood flow is sufficiently reduced to result in hypoxia severe enough to arrest physiological function, but not so complete as to cause irreversible failure of energy metabolism and cellular necrosis. These are the cells that can be rescued and resuscitated by restoration of perfusion and other protective therapies that have been the subject of intensive investigation in an effort to prevent paralysis caused by stroke. To better understand and exploit the endogenous cytotoxic and cytoprotective mechanisms at work in focal ischemia, molecular and genetic events have been examined in detail. This article reviews studies that describe some of the multiple cell-specific molecular penumbras that may exist after different durations of vascular occlusion and in various locations during focal ischemia and reperfusion, with special emphasis on those for which some correlation with imaging parameters is available. These penumbras may depend on the severity of ischemia and the efficiency of collateral blood flow. More recently, DNA microarray studies have identified large numbers of genes that are either upregulated or down-regulated after temporary middle cerebral artery (MCA) occlusion in rats. These results, which are not reviewed in detail here, document the complexity of molecular events that characterize brain tissue response to incomplete focal ischemia.

In addition to measurement of cerebral blood flow (CBF) reduction, energy metabolite depletion has been extensively assessed to document the metabolic consequences of ischemia and characterize the penumbra during focal ischemia. Tissue oxygen and glucose metabolic rates can be determined clinically with positron emission tomography scanning. ATP, phosphocreatine, pH, lactate, and n-acetyl aspartate (NAA) concentrations can be estimated experimentally and clinically with magnetic resonance spectroscopy (MRS) to distinguish ischemic core from penumbra. MRS imaging studies demonstrated differential elevation of intracellular lactate in rat striatum and MCA cortex during 1-hour temporary occlusion that resolved completely during 1-hour reperfusion using the endovascular internal carotid artery suture model. However, repeat imaging after 24 hours and 72 hours of reperfusion defined recurrent lactate elevation consistent with delayed injury that occurred despite restoration of blood flow (Figure 1). Although NAA levels are easily detected with 1-hour MRS imaging, and NAA is predominantly intracellular and neuronal in location, it was not found to be a useful marker to identify penumbra acutely because depletion was not evident until 2 hours or more after onset of
ischemia in these studies. Clinical application of MRS imaging has been limited in acute stroke patients by complexity and duration of data acquisition and processing required and subsequent availability of more rapid diffusion and perfusion imaging methodology.

Protein synthesis reduction or arrest is one of the earliest and most sensitive metabolic responses to ischemia that may be reversible in penumbra but not in the core. It occurs after CBF reductions of only 50% and is not caused by failure of energy metabolism, because ATP depletion is not observed until CBF decreases to 20%.\(^8\) Inactivation of initiation, elongation, and exchange factors such as elF-2, GEF, and eEF may be responsible.\(^9,10\)

Stress proteins such as heat shock protein 70 (HSP-70) are upregulated and have been studied extensively as potentially useful markers of cellular metabolic deprivation that might distinguish sublethal reversible ischemia from irreversible injury in cells that are destined for delayed necrosis or apoptosis.\(^11\) Presence of proteins denatured by heat, ischemia, or prolonged cellular depolarization induces HSP-70 mRNA and protein transcription. Focal ischemia studies have identified a penumbra pattern for induction of HSP-70, which is thought to function as an endogenous protective mechanism that facilitates renaturation of proteins after reversal of metabolic stress.\(^12\) Absence of HSP-70 in neurons and glia may distinguish core from penumbra, although the protein may be present in endothelial cells of infarcts. Heme oxygenase-1 is another stress protein that is induced during focal ischemia, mainly in vessels in the infarct core and in microglia, astrocytes, and scattered neurons in the periphery.\(^13\) It may also be induced in cingulate and other cortical areas beyond the ischemic region, perhaps as a response to spreading depression.

Hypoxia-inducible factor (HIF-1) is a transcription factor induced by reduction in levels of molecular oxygen\(^14\) but not by inhibition of mitochondrial respiration. It may be activated by reduction of a heme protein.\(^15\) HIF-1-alpha is induced after internal carotid artery–MCA occlusion with the endovascular suture model in the same penumbra regions as HSP-70, but also in more peripheral areas with presumably less severe hypoxia but persistent CBF reduction such as the cingulate cortex in the anterior cerebral artery (ACA) territory.\(^16\) The role of HIF-1 remains to be determined because both beneficial and deleterious effects have been demonstrated that vary in different cell types.

Immediate early genes (IEGs) such as c-fos are induced diffusely throughout the nonischemic regions of the ipsilateral hemisphere in rats subjected to MCA occlusion.\(^17\) Such responses are thought to be caused by occurrence of spreading depression because they can also be caused by cortical potassium chloride application and blocked by administration of NMDA antagonists. IEGs are also induced in the contralateral hemisphere after focal ischemia.\(^17\)–\(^19\) The role of IEG induction remains conjectural, although enhancement of brain plasticity and behavioral recovery after stroke have been suggested.\(^2\) COX-2, which catalyzes metabolism of arachi-
Donic acid to prostaglandins, is also induced during focal ischemia and spreading depression in a pattern similar to that of c-fos and other IEGs. It was also expressed in regions where transient or brief apparent diffusion coefficient (ADC) reduction was observed with diffusion-weighted MRI (DMRI) presumably caused by ischemic depolarization during 30 minutes and 60 minutes of MCA occlusion.

A variety of other gene-regulated responses to focal cerebral ischemia that are thought to effect outcome and could have therapeutic implications have been investigated, including adhesion molecules, cytokines, chemokines, matrix metalloproteinases, apoptosis-inducing genes, DNA repair genes, NOS, TNF, NFκB, interleukins, and growth factors. Our studies have focused on mapping of HSP-70, c-fos, and COX-2 induction and patterns of protein synthesis alteration for comparison with in vivo MRI to determine whether imaging parameters can be calibrated to identify ischemic penumbra. Previous studies in other laboratories indicated that HSP-72 mRNA was induced in penumbral cortex but not in core or normal brain 3 hour after MCA occlusion in mice. Expression of c-fos mRNA was observed in penumbra and normal cortex but not in the ischemic core. Penumbra defined as the area of cerebral protein synthesis suppression associated with preservation of ATP levels was found to correlate with the region of HSP expression after permanent MCA occlusion. The penumbra had disappeared 24 or 72 hours later.

The ADC of water as measured by DMRI has been used as an indirect metabolic marker of ischemia because it reflects cellular edema caused by reduced blood flow and energy metabolism that may be associated with cell membrane depolarization after onset of ischemia. Thus, in our initial studies correlating ADC maps with CBF autoradiographs, DMRI identified the penumbra after 0.5 hour or 1.5 hours of permanent MCA occlusion in rats (Figure 2). The DMRI lesion expands and intensifies (edema worsens), and the penumbra disappears over time. However, the area of significant ADC reduction defined only 53% of the region at risk for infarction determined by CBF after 0.5-hour MCA occlusion and 74% of the irreversibly ischemic region after 1.5

Figure 3. ADC maps and histology for 2 representative rats subjected to 30 minutes of MCA occlusion and for 1 representative rat subjected to 60 minutes of MCA occlusion.
Reversal of ADC lesions in both core and penumbra occurred not to predict tissue recovery after reperfusion. Observation of MRS imaging study results, resolution of ADC lesions does sequentially DMRI studies. However, as suggested by the stress has occurred.

Identify penumbra regions where sublethal and reversible metabolic reductions and c-fos induction occurred. These results suggest that CBF decrease. HSP-70 was not induced where transient ADC reduction reversed only in penumbra were present on histological sections, as well. ADC reductions as low as 45% of the contralateral region in penumbra reversed initially after reperfusion but subsequently were correlated with delayed infarction. No ADC threshold for irreversible cellular edema in penumbra destined for infarction was established experimentally.

DMRI-derived ADC maps distinguished penumbra from core at 36 minutes but not at 48 or more minutes after 1-hour temporary MCA occlusion in rats (Figure 4). Delayed injury was again seen in striatum on ADC maps after 24-hour reperfusion despite resolution at 1 hour. ADC levels were significantly different in core and penumbra at 24 hours. Although HSP-70 was induced in the periphery, Neu-N and GFAP staining were reduced, indicating that separate penumbras may exist for different cells, with some sustaining sublethal metabolic stress and others sustaining irreversible injury in areas surrounding the core after MCA occlusion in the rat suture model. A trend toward greater reduction of protein synthesis in core than penumbra was also observed in this study. In a subsequent study not correlated with imaging, protein synthesis recovery was less at 72 hours than at 48 hours and 24 hours after 90-minute temporary MCA occlusion. Again, HSP-70 expression was seen in cortical penumbra neurons, but only in endothelial and some glial cells in striatum.

Using transgenic mice overexpressing HSP-70, it has been possible to demonstrate a cytoprotective effect with reduction during reperfusion after 30 minutes or 60 minutes occlusion. Temporary MCA occlusion was followed by histological demonstration of lesions in the MCA distribution 72 hours after onset of reperfusion, indicating that infarction had occurred (Figure 3). After 90 minutes, MCA occlusion lesions that reversed only in penumbra were present on histological sections, as well.

In subsequent studies, serial echo-planar DMRI at 30-second intervals demonstrated transient (3 minutes) ADC decrease similar to spreading depression that was associated with c-fos but not HSP mRNA induction during brief MCA occlusion. Longer temporary (10 minutes) episodes of ADC reduction during 0.5-hour or 1-hour permanent MCA were associated with peripheral HSP-70 mRNA induction. Perfusion-weighted MRI confirmed CBF reduction in the entire MCA territory, and HSP induction was greatest where perfusion reduction was less severe. HSP-70 was induced mostly in the periphery and not in the core of regions with persistent ADC reduction. HSP-70 was induced where ADC reduction reversed after 10 minutes, and perfusion-weighted MRI showed less severe CBF decrease. HSP-70 was not induced where transient ADC reductions and c-fos induction occurred. These results suggest that dynamic imaging early during focal ischemia may be useful to identify penumbra regions where sublethal and reversible metabolic stress has occurred.

Delayed ischemic injury has also been demonstrated with sequential DMRI studies. However, as suggested by the MRS imaging study results, resolution of ADC lesions does not predict tissue recovery after reperfusion. Observation of reversal of ADC lesions in both core and penumbra occurred hours. Thus, ADC mapping may misrepresent the distribution of core and penumbra at early time points after onset of focal ischemia.

In cortical perfusion values during occlusion and for 30 minutes after reperfusion. There were no significant decreases between HSP-70 transgenic and wild-type mice at any time point. B, Infarction volumes 24 hours after 30 minutes of MCA occlusion. The infarction volume in HSP-70 transgenic mice at any time point. C and D, Representative photographs of coronal sections, with cresyl violet staining, after transient focal ischemia in wild-type (C) and HSP-70 transgenic (D) mice. Scale bar = 2 mm.
of neuronal damage or infarction in comparison to wild-type controls\textsuperscript{20} (Figure 5). One possible protective mechanism demonstrated after permanent MCA occlusion in these mice is reduction of apoptosis by suppressing early mitochondrial cytochrome c release, which in turn initiates the caspase cascade, leading to apoptotic cell death.\textsuperscript{30}

An example of another ischemic molecular penumbra that can be detected with in vivo early T2 MRI and late T1 MRI and appears to play a role in delayed cell damage is manganese (Mn)-mediated striatal neurodegeneration, which developed between 5 and 28 days after 15-minute temporary MCA occlusion in rats.\textsuperscript{31} Mn, MnSOD, glutamine synthetase, and amyloid precursor protein accumulated, GFAP reactivity increased, and neuronal necrosis increased in dorsolateral striatum in association with deterioration in motor control and choice memory activity progressively up to 16 weeks after ischemia. Similar changes were demonstrated by MRI in 6 patients after brief focal ischemia.

In summary, results of our studies and those of many other investigators suggest that a complex interaction of multiple molecular events characterizes the survival response of brain tissue to focal ischemia and reperfusion. These are cellubulary, subcellularly, biochemically, and anatomically specific. They depend on the location severity and duration of ischemia. They may be responsible for immediate or substantially delayed cell damage and loss of neurological function. Thus, some forms of inflammatory and degenerative neuronal loss may be initiated by brief focal ischemia.\textsuperscript{2,31}

References

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