PATHOPHYSIOLOGY OF ACUTE ISCHEMIC STROKE

Tudor G. Jovin, Andrew M. Demchuk, Rishi Gupta

ABSTRACT

In acute ischemic stroke, abrupt vessel occlusion results in a drop in regional CBF, leading to time-dependent compartmentalization of the ischemic brain into tissue that is irreversibly damaged (ischemic core), tissue that is functionally impaired but structurally intact and thus potentially salvageable (penumbra), and tissue that is hypoperfused but not threatened under normal circumstances (oligemic brain). At a cellular level, neuronal damage occurs through a complex interaction of mechanisms (necrosis, apoptosis, excitotoxicity, inflammation, peri-infarct depolarization, acidosis, and free radical formation) that are characteristic for each compartment. All these mechanisms are potential targets for neuroprotective therapy, which, combined with flow restoration strategies, is likely to improve outcome significantly in human stroke.

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INTRODUCTION

Acute ischemic stroke is characterized by abrupt neurologic dysfunction due to focal brain ischemia resulting in persistent neurologic deficit or accompanied by characteristic abnormalities on brain imaging (Albers et al, 2002).

Until recently, stroke was defined using clinical criteria alone, based on duration of symptoms lasting 24 hours or longer (Ad Hoc Committee, 1975). If the symptoms persisted for less than 24 hours, the condition was termed transient ischemic attack (TIA) However, modern neuroimaging techniques, especially diffusion MRI, have shown that defining stroke or TIA based only on duration of symptoms may not be accurate, since permanent brain damage can occur even when symptoms last only minutes (Ay et al, 1999; Engelter et al, 1999; Kidwell et al, 1999). Therefore, recently proposed definitions of TIA take into account both the duration of symptoms (typically less than an hour) and lack of acute infarction on brain imaging (Albers et al, 2002). Ovbiagele and colleagues (2003) estimated that adopting a definition of TIA based on the above criteria would reduce estimates of the annual incidence of TIA by 33% (currently estimated to be 180,000 annually) and increase annual number of strokes by 7% (currently estimated at 820,000 per year).

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ETIOLOGIC AND PATHOLOGIC ASPECTS

It is important to recognize that ischemic stroke results from a heterogeneous group of disorders whose final common pathway leading to clinical manifestations is interruption of blood flow through vascular occlusion. This results in an infarct of which the size is dependent on extent, duration, and severity of ischemia. Brain infarcts resulting from arterial occlusion are divided based on their macroscopic appearance into white (bland) and red (hemorrhagic) infarcts (Garcia et al, 1998). By gross anatomy, the former are composed of few or no petechiae while the latter are characterized by grossly visible blood. This latter term is equivalent to hemorrhagic transformation, which refers to leaking of red blood cells into a dying and ischemic brain tissue, and should not be confused with parenchymal hematoma, which represents a homogenous collection of blood usually resulting from a ruptured blood vessel. Serial brain imaging studies in patients with acute stroke have demonstrated that hemorrhagic transformation of an initially bland infarct can occur in up to 80% of patients (Hart and Easton, 1986; Lyden and Zivin, 1993; Mayer et al, 2000). The risk of early hemorrhagic transformation and parenchymal hematoma is greatly increased by administration of thrombolytics or anticoagulants in acute ischemic stroke (Larrue et al, 1997).

Gross anatomy studies reveal that arterial infarcts evolve over several stages (Garcia et al, 1998). In the first 12 to 24 hours after the ictus, the lesion is barely visible to the naked eye. Swelling reaches its zenith at days 3 to 5; in large strokes this can become life threatening due to displacement and compression of neighboring structures. Between days 5 and 10, the infarcted brain becomes sharply demarcated from the unaffected brain tissue. The chronic stage, occurring weeks or months after the ictus, features a fluid-filled cavity that results from reabsorption of necrotic debris, hence the name liquefaction necrosis.

STROKE MECHANISMS

Two major mechanisms are responsible for ischemia in acute stroke: thromboembolism and hemodynamic failure. The former usually occurs as a result of embolism or in situ thrombosis and leads to an abrupt fall in regional cerebral blood flow (CBF). The latter usually occurs with arterial occlusion or stenosis, when collateral blood supply maintains CBF at levels that are sufficient for preservation of brain function under normal circumstances. In these cases, cerebral ischemia may be triggered by conditions that decrease perfusion proximally to the arterial lesion (systemic hypotension or low cardiac output) and increase metabolic demands (fever, acidosis) or conditions that lead to ''steal'' of blood from affected to unaffected areas in the brain (carbon dioxide retention) (Alexandrov et al, 2007). Strokes occurring through these mechanisms are located predominantly in the so-called borderzones or watershed regions, which are areas in the brain bordering major vascular territories such as the middle cerebral artery (MCA)/internal carotid artery or MCA/posterior cerebral artery interface (Klijn et al, 1997). Caplan and Hennerici (1998) postulated that embolism and hypoperfusion oftentimes coexist and potentiate each other. They proposed impaired clearance of emboli due to low flow states as a link between these two factors in the pathophysiology of brain infarction.

Embolism

Embolic material formed within the heart or vascular system travels through the arterial system, lodging in a vessel and partially or completely occluding it.

KEY POINTS

- Ischemic stroke results from a heterogeneous group of disorders whose final common pathway is interruption of blood flow through vascular occlusion.
- Two major mechanisms are responsible for ischemia in acute stroke: thromboembolism and hemodynamic failure.
- Embolism and hypoperfusion can coexist and potentiate each other.

KEY POINT

- The most common sources of emboli are the heart and large arteries.

The most common sources of emboli are the heart and large arteries. Other rare sources of emboli are air, fat, cholesterol, bacteria, tumor cells, and particulate matter from injected drugs (Caplan, 2000).

Cardioembolism. Cardioembolism accounts for 20% to 30% of all ischemic stroke (Grau et al, 2001; Kolominsky-Rabas et al, 2001; Petty et al, 2000; Sacco et al, 1995). **Table 2-1** outlines the high risk versus low or uncertain risk conditions for cardioembolic stroke (Ferro, 2003). Conditions considered at high risk for embolization to the brain are atrial fibrillation, sustained atrial flutter, sick sinus syndrome, left atrial thrombus, left atrial appendage thrombus, left atrial myxoma, mitral stenosis, prosthetic valve, infective endocarditis, noninfective endocarditis, left ventricular thrombus, left ventricular myxoma, recent anterior myocardial infarct, and dilated cardiomyopathy. Conditions considered at low or uncertain risk for brain embolization include patent foramen ovale, atrial septal aneurysm, spontaneous atrial contrast, mitral annulus calcification, mitral valve prolapse, calcified aortic stenosis, fibroelastoma, giant Lambl excrescences, akinetic or dyskinetic ventricular wall segment, subaortic hypertrophic cardiomyopathy, and congestive heart failure (Marchal et al, 1999).

Artery-to-artery embolism. Emboli occluding brain arteries can also originate from large vessels situated more proximally, such as the aorta, extracranial carotid, or vertebral arteries or intracranial arteries. In these circumstances, the embolic material is composed of clot, platelet aggregates or plaque debris that usually breaks off from atherosclerotic plaques (Furlan et al, 1996). This is a major mechanism responsible for stroke due to large vessel atherosclerosis, which accounts for 15% to 20% of all ischemic strokes (Grau et al, 2001; Kolominsky-Rabas

TABLE 2-1 High Risk Versus Low or Uncertain Risk Conditions for Cardioembolic Stroke

▶ **High Risk Conditions**
Atrial fibrillation
Sustained atrial flutter
Sick sinus syndrome
Left atrial thrombus
Left atrial appendage thrombus
Left atrial myxoma
Mitral stenosis
Mechanical valve
Infective endocarditis
Noninfective endocarditis
Left ventricular myxoma
Recent anterior myocardial infarct
Dilated cardiomyopathy
▶ **Low or Uncertain Risk Condition**
Patent foramen ovale
Atrial septal aneurysm
Spontaneous atrial contrast
Mitral valve prolapse
Calcified aortic stenosis
Fibroelastoma
Giant Lambel excrescence
Akinetic or dyskinetic ventricular wall segment
Subaortic hypertrophic cardiomyopathy
Congestive heart failure

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et al, 2001; Petty et al, 2000; Sacco et al, 1995).

Thrombosis

Thrombosis represents an obstruction of flow with thrombus formation resulting from an occlusive process initiated within the vessel wall. In the vast majority of cases this is caused by atherosclerotic disease, hence the name atherothrombosis. Less common vascular pathologies leading to vessel stenosis or occlusion include arterial dissection (intracranial or extracranial), fibromuscular dysplasia, vasospasm (drug induced, inflammatory, or infectious), radiation-induced vasculopathy, extrinsic compression such as tumor or other mass lesion, or moyamoya disease.

Small vessel disease. Thrombotic occlusion of the small penetrating arteries in the brain is another important cause of strokes, accounting for another approximately 20% to 30% of all ischemic strokes. This type of vascular lesion is strongly associated with hypertension and is characterized pathologically by lipohyalinosis, microatheroma, fibrinoid necrosis, and Charcot-Bouchard aneurysms (Bamford and Warlow, 1988; Fisher, 1982; Fisher, 1998; Mohr, 1982). Lipohyalinosis is characterized by replacement of the normal vessel wall with fibrin and collagen and is specifically associated with hypertension. Microatheroma represents an atheromatous plaque of the small vessel that may involve the origin of a penetrating artery (Caplan, 1989). This latter mechanism is believed to be responsible for larger subcortical infarcts. Fibrinoid necrosis is usually associated with extremely high blood pressure, leading to necrosis of smooth muscle cells and extravasation of plasma proteins, which appear microscopically as fine granular eosinophilic deposits in the connective tissue of the vessel wall. Charcot-Bouchard aneurysms are areas of focal dilatation in the small vessel wall, which may thrombose, leading to vessel occlusion.

CEREBRAL BLOOD FLOW CHANGES

Introduction

Following vessel occlusion, the main factors ultimately determining tissue outcome are regional CBF and duration of vessel occlusion. A decrease in regional CBF leads to diminished tissue perfusion. In persistent large vessel occlusion, local perfusion pressure, which is the main factor influencing the eventual outcome of tissue (Baron, 2001), depends on several factors such as the presence and extent of collaterals and systemic arterial pressure (due to loss of the ischemic brain's autoregulatory capacity). It is inversely correlated to the local tissue pressure (which is increased by ischemic edema).

Cerebral Blood Flow Thresholds in Acute Cerebral Ischemia

The difference in tissue outcome following arterial occlusion is based on the concept that CBF thresholds exist, below which neuronal integrity and function are differentially affected (**Figure 2-1**). Early human studies performed in the 1950s during carotid artery clamping for carotid endarterectomy using intracarotid xenon 133 injections (Boysen, 1971; Jennett et al, 1966) reported that hemiparesis occurred when regional CBF fell below 50% to 30% of normal, and permanent neurologic deficit occurred if mean CBF fell below 30% of normal. Evidence also indicated that development of permanent neurologic sequelae is a time-dependent process; for any given blood flow level, low CBF values are tolerated only for a short period of time, while higher CBF values require longer time for infarction to occur. Several investigators (Sundt et al, 1974; Trojaborg and Boysen, 1973) have

KEY POINTS

- Thrombosis is associated with thrombus formation as a consequence of an occlusive process initiated within the vessel wall and is usually caused by atherosclerotic disease.
- Occlusive disease of small penetrating arteries in the brain (small vessel disease) accounts for 20% to 30% of ischemic strokes.
- Following vessel occlusion, the main factors ultimately determining tissue outcome are regional cerebral blood flow and duration of vessel occlusion.
- The difference in tissue outcome following arterial occlusion is based on the concept that cerebral blood flow thresholds exist, below which neuronal integrity and function are differentially affected.

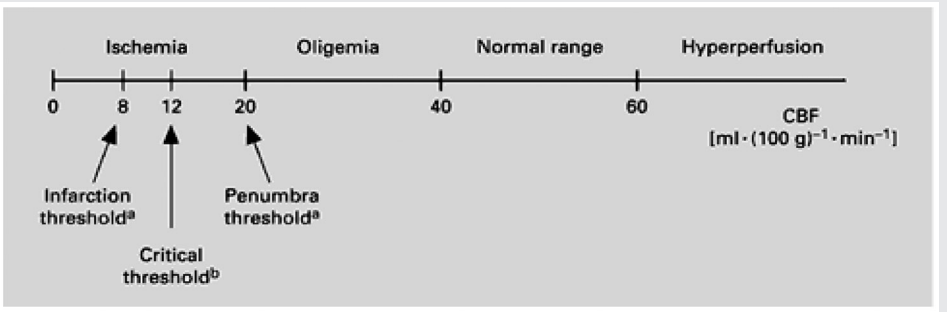

FIGURE 2-1 Schematic drawing of the different cerebral blood flow thresholds in man.

[a]Furlan M, Marchal G, Viader F, et al. Spontaneous neurological recovery after stroke and the fate of the ischemic penumbra. Ann Neurol 1996;40(2):216–226.
Marchal G, Beaudouin V, Rioux P, et al. Prolonged persistence of substantial volumes of potentially viable brain tissue after stroke. Stroke 1996;27(4):599–606.
Marchal G, Benali K, Iglésias S, et al. Voxel-based mapping of irreversible ischaemic damage with PET in acute stroke. Brain 1999;122(pt 12):2387–2400.
[b]Heiss WD, Grond M, Thiel A, et al. Tissue at risk of infarction rescued by early reperfusion: A positron emission tomography study in systemic recombinant tissue plasminogen activator thrombolysis of acute stroke. J Cereb Blood Flow Metab 1998;18(12):1298–1307.
Heiss WD, Thiel A, Grond M, Graf R. Which targets are relevant for therapy of acute ischemic stroke? Stroke 1999;30(7):1486–1489.

Baron JC. Perfusion thresholds in human cerebral ischemia: historical perspective and therapeutic implications. Cerebrovasc Dis 2001;11(suppl 1):2–8. Reprinted with permission from S. Karger, AG, Basel.

studied the relationship between EEG changes and regional CBF during carotid clamping. EEG would slow down when mean CBF fell below 23 mL/100 g/min, while at values below 15 mL/100 g/min the EEG would become flat.

The concept of CBF threshold in focal cerebral ischemia proposed by these early human studies was then reinforced by landmark studies performed by Symon and colleagues (1977), who investigated the relationship between severity of local CBF impairment and degree of neurologic dysfunction at various durations of ischemia in a baboon model of MCA occlusion. Symon and colleagues (1977) demonstrated that brain tissue perfuses between certain CBF values (22 mL/100 mg/min to 8 mL/100 mg/min) even when prolonged hypoperfusion stops functioning, but maintains its structural integrity and, most importantly, can be salvaged with reperfusion. **Figure 2-2** depicts a summary of metabolic and electrophysiologic disturbances according to reductions of cortical blood flow at different thresholds.

The time dependence of ischemic thresholds in producing permanent or transient neurologic damage has been demonstrated by Jones and colleagues (1981) in primate studies using a temporary or permanent MCA occlusion model. These experiments demonstrated that the CBF values below which brain tissue becomes infarcted are dependent on the duration of vessel occlusion. Two hours of continuous MCA occlusion in awake macaque monkeys required CBF values of 5 mL/100 g/min to produce infarction, while 3 hours of continuous occlusion resulted in infarction if CBF values were 12 mL/100 mg/min or less. Permanent occlusion resulted in infarction if flows were 18 mL/100 mg/min or less. It should be noted that 30 minutes of occlusion did not result in infarction even at CBF values below 5 mL/100 mg/min.

It is important to understand that the blood flow thresholds studied in most animal and human experiments refer to ischemic tolerance of the brain cortex. Thresholds for deep white

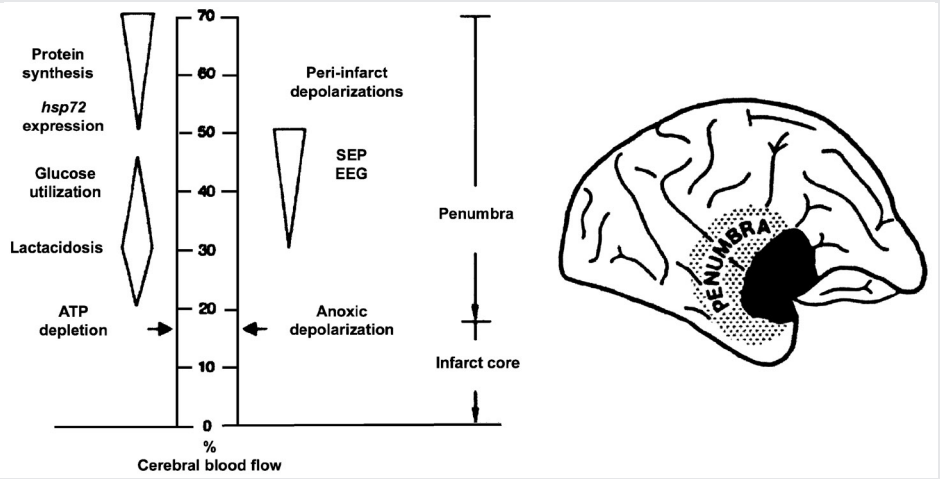

FIGURE 2-2 Thresholds of metabolic (*left*) and electrophysiologic (*right*) disturbances during gradual reduction of cortical blood flow.

SEP = somatosensory evoked potentials; EEG = electroencephalogram; ATP = adenosine triphosphate.

Hossmann KA. Pathophysiology and therapy of experimental stroke. Cell Mol Neurobiol 2006;26(7–8):1057–1083. Reprinted with permission from Springer Science and Business Media.

KEY POINTS

- The ischemic core represents tissue that is irreversibly damaged.
- The ischemic penumbra represents tissue that is functionally impaired but structurally intact and, as such, potentially salvageable. Salvaging this tissue by restoring its flow to nonischemic levels is the aim of reperfusion therapy in acute stroke.

matter or basal ganglia have not been studied rigorously and are simply unknown. It is believed, however, that gray matter is more susceptible to infarction than white matter, and that within the gray matter the basal ganglia have a lower ischemic tolerance than the cortex (Marcouz et al, 1982).

Concept of Ischemic Core and Ischemic Penumbra

The notion that in acute stroke, depending on the extent and duration of hypoperfusion, the tissue supplied by the occluded artery is compartmentalized into areas of irreversibly damaged brain tissue and areas of brain tissue that are hypoperfused but viable led to the concept of ischemic core and ischemic penumbra proposed by Astrup and colleagues (1981). The ischemic core represents tissue that is irreversibly damaged. PET studies in humans suggest that beyond a certain time limit (probably no longer than an hour) the ischemic core corresponds to CBF values of less than 7 mL/100 mg/min (Furlan et al, 1996; Marchal et al, 1996; Marchal et al, 1999) to 12 mL/100 mg/min (Heiss, 2000; Heiss et al, 2001b). The ischemic penumbra represents tissue that is functionally impaired but structurally intact and, as such, potentially salvageable. It corresponds to a high CBF limit of 17 mL/100 mg/min to 22 mL/100 mg/min and a low CBF limit of 7 mL/100 mg/min to 12 mL/100 mg/min. Salvaging this tissue by restoring its flow to nonischemic levels is the aim of acute stroke therapy. Another compartment, termed by Symon and colleagues (1977) *oligemia*, represents mildly hypoperfused tissue from the normal range down to around 22 mL/100 mg/min. It is believed that under normal circumstances this tissue is not at risk of infarction (Baron, 2001). It is conceivable, however, that under certain circumstances, such as hypotension, fever, or acidosis, oligemic tissue can be incorporated into penumbra and subsequently undergo infarction.

Evidence in the literature suggests that there is temporal evolution of the core, which grows at the expense of

penumbra (Ginsberg, 2003; Heiss et al, 2001a; Raichle, 1982) (**Figure 2-3**). This process occurs because of the interaction of a multitude of complex factors acting concomitantly or sequentially (Hossmann, 2006). It is known that the ischemic penumbra represents a dynamic phenomenon that evolves in space and time. If vessel occlusion persists, the penumbra may shrink because of progressive recruitment into the core. Alternatively, it may return to a normal state following vessel recanalization or possibly neuroprotective interventions. It thus appears that the ischemic penumbra represents a transitional state between evolution into permanent ischemia as one possibility and transformation into normal tissue as the other possibility. On the basis of a rat model of MCA occlusion studied in a multimodal fashion assessing CBF, metabolism, and gene expression, Ginsberg (2003) concluded that the penumbra lies within a narrow range of perfusion and thus is precariously dependent on small perfusion pressure changes; that the penumbra is electrophysiologically dynamic and undergoes recurrent depolarizations; and that it is metabolically unstable, being

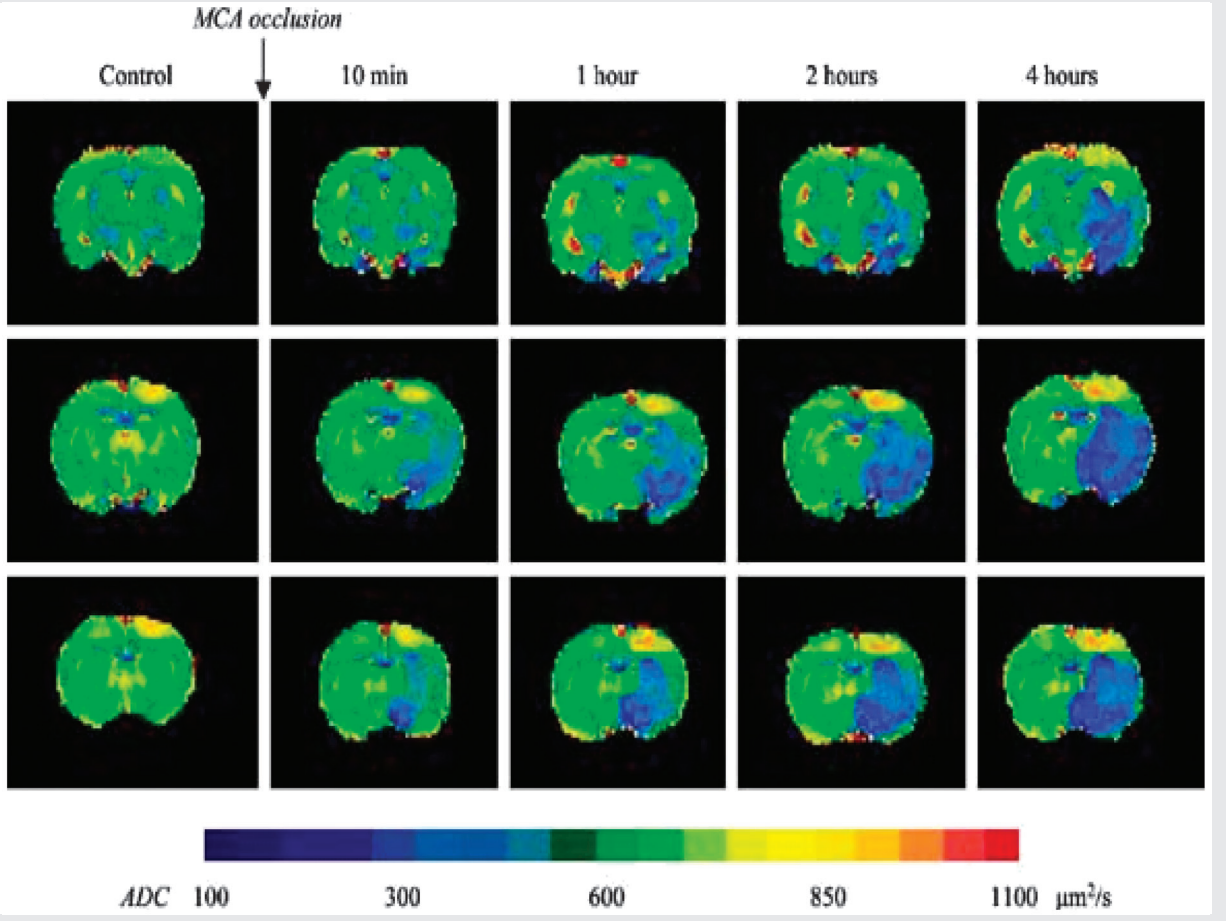

FIGURE 2-3 Experimental model of middle cerebral artery occlusion in rats. Serial MRIs (coronal sections) at three levels in the brain depicting the apparent diffusion coefficient of water (ADC) (*blue*) demonstrating the time-dependent growth of the ischemic core.

Hossmann KA. Pathophysiology and therapy of experimental stroke. Cell Mol Neurobiol 2006;26(7–8):1057–1083. Reprinted with permission from Springer Science and Business Media.

the site of severe metabolism/flow dissociation.

Restriction of acute stroke therapy aimed at vessel recanalization to 3 hours from onset of symptoms for IV thrombolysis and 6 hours for intraarterial thrombolysis is based on the concept that the ischemic penumbra has a short lifespan, being rapidly incorporated into the core within hours of the ictus (Heiss et al, 2001b; Kaufman et al, 1999). Recent evidence suggests, however, that penumbral brain tissue of significant extent is present even beyond 6 hours of stroke onset. PET studies using quantitative CBF assessment (Furlan et al, 1996; Marchal et al, 1999) or markers of tissue hypoxia such as 18F fluoromisonidazole (Read et al, 2000) to assess penumbra, included patients studied within 6 hours to as late as 51 hours after stroke onset and reported the existence of penumbra comprising 30% to 45% of the total ischemic tissue at risk. Several investigators have estimated the penumbra based on diffusion/perfusion MRI (diffusion-weighted imaging [DWI]/perfusion-weighted imaging [PWI]) mismatch in acute stroke (Hjort et al, 2005; Schlaug et al, 1999). Since the diffusion abnormalities are presumed to represent an approximation of the irreversible ischemic lesion and the perfusion abnormalities are presumed to represent the brain territory at risk, the area of mismatch between DWI and PWI is considered a territory still viable but at risk of undergoing infarction and corresponds theoretically to the concept of ischemic penumbra. The major shortcoming of this concept derives from the lack of quantitative data provided by MRI imaging. It has been shown that the DWI lesion is not precise in distinguishing between irreversible and reversible ischemia (Guadagno et al, 2005). It incorporates both types of ischemia and therefore cannot be considered equivalent to the ischemic core (Guadagno et al, 2004; Guadagno et al, 2005; Sobesky et al, 2005). Additionally, the PWI lesion has been shown to incorporate both imminently threatened brain and brain that will not undergo infarction as a consequence of persistent vessel occlusion (Heiss et al, 2004). Since, by definition, penumbra represents tissue that will undergo infarction with continuous vessel occlusion, assessment of penumbral extent based on perfusion MRI is also not precise.

Using MRI technology, Schlaug and colleagues (1999) demonstrated that penumbra comprises about 40% of the total ischemic territory in a cohort of patients that was studied within 24 hours of symptom onset. Similar extent of penumbral volumes has been reported by numerous other investigators (Barber et al, 1999; Rordorf et al, 1998; Schellinger et al, 2001; Staroselskaya et al, 2001). These reports have also described that the presence of diffusion/perfusion mismatch is highly correlated with the presence of large vessel (internal carotid artery, MCA, or major division) occlusion. SPECT studies performed acutely in patients with large vessel occlusion have confirmed these findings (Ogasawara et al, 2000; Ueda et al, 1999). Insights into the pathophysiology of acute stroke as it relates to reversible versus irreversible brain tissue are provided by a study in which a homogenous group of patients with stroke due to angiographically proven M1 MCA occlusion were studied within 6 hours of symptom onset with xenon-CT-CBF technology (Jovin et al, 2003). This study, in which core and penumbra were determined based on established perfusion thresholds, indicated that within this time frame, irrespective of the point in time at which the patients were studied, the ischemic penumbra was consistently present and relatively constant, comprising

KEY POINTS

- At a cellular level, the biochemical and electrophysiologic mechanisms involved in the ischemic brain injury vary according to the extent of cerebral ischemia.
- Neuronal cell death occurs as a result of two main mechanisms: necrosis and apoptosis.
- Necrosis occurs predominantly in the hyperacute stage within the ischemic core. It occurs mainly as a consequence of disruption of cellular homeostasis due to energy failure and is accompanied by cellular swelling, membrane lysis, inflammation, vascular damage, and edema formation.

approximately one-third of the MCA territory. In contrast to the penumbra, the ischemic core was highly variable, ranging from 20% to 70% of cortical MCA territory. The authors found that both in patients who recanalized and in those who did not recanalize, the extent of core and not that of penumbra was correlated with clinical outcome.

CELLULAR MECHANISMS OF ISCHEMIC NEURONAL INJURY IN ACUTE STROKE

Introduction

At a cellular level, the biochemical and electrophysiologic mechanisms involved in the ischemic brain injury vary according to the extent of cerebral ischemia. Neuronal cell death occurs as a result of two main mechanisms: necrosis and apoptosis. Necrosis is a process that is not regulated or programmed and is the predominant mechanism that follows acute permanent focal vascular occlusion. Necrosis occurs mainly as a consequence of disruption of cellular homeostasis due to energy failure and is accompanied by cellular swelling, membrane lysis, inflammation, vascular damage, and edema formation (Bhardwaj et al, 2003) (**Figure 2-4**). Apoptosis, or programmed cell death, is characterized by cell shrinkage, chromatin clumping, and cytoplasmic blebbing and is not associated with inflammation or secondary injury to surrounding brain (Graham and Chen, 2001; Thompson, 1995; Vaux et al, 1994) (**Figure 2-5**). These two distinct types of neuronal death appear to represent opposite poles of a spectrum that coexist within the ischemic brain, with necrosis being the main mechanism of neuronal injury in the ischemic core and apoptosis being the main mechanism of neuronal injury in the penumbra where, because of the milder degree of ischemia, sufficient energy is produced to allow for expression of new proteins that mediate apoptosis (Bhardwaj et al, 2003; Dirnagl et al, 1999; Graham and Chen, 2001).

Acute vascular occlusion triggers a complex sequence of pathophysiologic events that evolve over time and space. Major pathogenic mechanisms of the ischemic cascade leading to neuronal injury constitute active targets for various neuroprotective strategies and include cytotoxicity, peri-infarct depolarization, inflammation, tissue acidosis, nitric oxide, and free radical production, as well as, at a later stage, apoptosis (Barber et al, 2003; Doyle et al, 2008; Dirnagl et al, 1999).

Excitotoxicity, Peri-infarct Depolarizations, Acidosis, Inflammation

The reduction in regional CBF through insufficient delivery of the neuron's main energy substrates, oxygen and glucose, results in inadequate production of energy required to maintain ionic gradients (Martin et al, 1994). Since the transport of calcium from the cell into the extracellular space is an energy-dependent process, this leads to intracellular accumulation of calcium. Calcium influx is further enhanced by impairment in the energy-dependent reuptake of excitatory amino acids, especially glutamate, and by release of excitatory amino acids into the extracellular space. An increase in extracellular glutamate leads to increased calcium influx, through increased stimulation of the NMDA or non-NMDA (mainly α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid [AMPA]) receptor (Budd, 1998). At the same time, sodium and chloride enter the neuron via channels for monovalent ions (Tyson et al, 1996). Water follows osmotic gradients, leading to edema, which is predominantly cytotoxic and can further diminish perfusion in regions surrounding the core, leading to

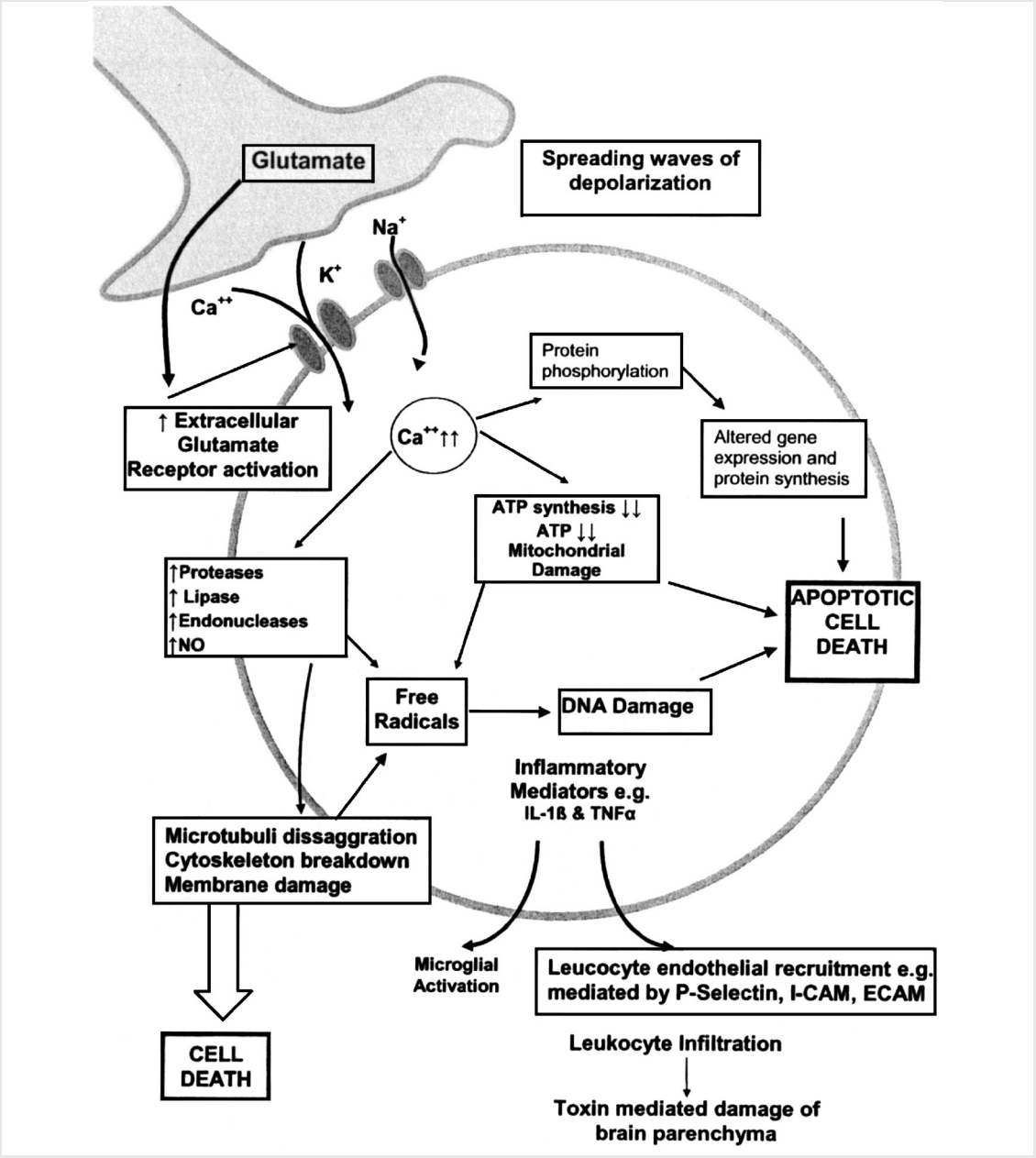

FIGURE 2-4 Cellular mechanisms of ischemic neuronal injury in acute stroke.

IL-1β = interleukin 1β; TNFα = tumor necrosis factor-α; ICAM = intercellular adhesion molecule; ECAM = endothelial cell adhesion molecule.

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recruitment of penumbral areas into the core (Hossmann, 2006; Raichle, 1982). Effects of delayed edema formation (at this stage predominantly vasogenic) include increased intracranial pressure, shift and displacement of brain structures, vascular compression, and herniation (Hossmann, 2006).

The accumulation of intracellular calcium leads to a series of events at both the cytoplasmic and nuclear levels that result in cell death through several

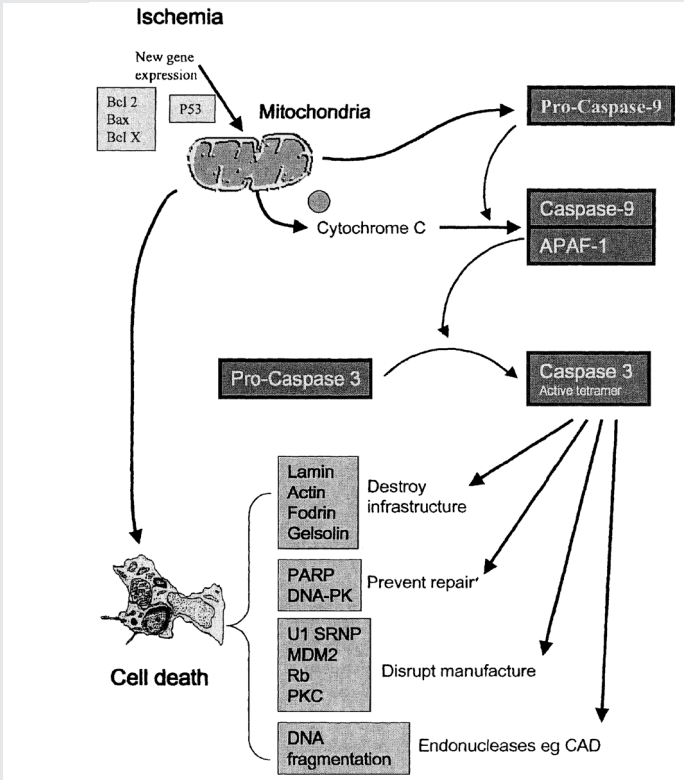

FIGURE 2-5 Overview of molecular events involved in apoptosis. PKC = protein kinase C; CAD = capase activated DNAase.

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mechanisms: activation of enzymes that degrade cytoskeletal proteins (Baudry et al, 1981; Chen and Strickland, 1997), activation of lipoxygenase and cyclooxygenase, xanthine oxydase and nitric oxide synthase with resultant accumulation of highly cytotoxic oxygen free radicals oxygen (O_2•), hydrogen peroxide (H2O2), hydroxyl (OH•), and nitric oxide (NO•). These reactions occur both in the cytoplasm and in the mitochondria. Mitochondria are an important source of reactive oxygen species. As a consequence of free radical–mediated disruption of the inner mitochondrial membrane and the oxidation of the proteins that mediate electron transport (Dugan and Choi, 1994), the mitochondrial membrane becomes leaky through the formation of a so-called mitochondrial permeability transition pore in the mitochondrial membrane (Mergenthaler et al, 2004). This results in mitochondrial swelling, intramitochondrial calcium accumulation, impaired energy production, and reactive oxygen species production (Kristian and Siesjo, 1998). Another consequence of disrupted mitochondrial permeability is the release of proapoptotic molecules, such as cytochrome *c* and caspase-9 (Dirnagl et al, 1999; Doyle et al, 2008).

Following energy loss, membrane potentials cannot be maintained, leading to depolarization of neurons and glia (Dirnagl et al, 1999). While in the core region depolarization may be permanent, in the penumbral area cells can depolarize and then undergo repetitive depolarization, an active energy-requiring

process. This so-called peri-infarct depolarization contributes to the increase in size of the infarct by further depleting energy reserves (Back et al, 1996; Doyle et al, 2008; Hossmann, 1996).

Acidosis, arising during ischemia, enhances brain damage through several mechanisms, such as edema formation, accumulation of hydrogen ions in the cell, inhibition of lactate oxidation, and impairment of mitochondrial respiration (Barber et al, 2003). On the other hand, acidosis appears to have antiexcitotoxic effects, and therefore some authors argue that the role of acidosis in focal cerebral ischemia is complex and poorly understood (Mergenthaler et al, 2004).

Inflammation further exacerbates the ischemic injury. Soon after onset of ischemia, astrocytes, microglia, endothelial cells, and leukocytes are activated. Peripherally derived leukocytes, such as polymorphonuclear leukocytes, T lymphocytes, and natural killer cells, also accumulate in the ischemic tissue (Clark et al, 1993a; Clark et al, 1993b; Ritter et al, 2000).

The accumulation of inflammatory cells in the ischemic lesion occurs as a result of intracellular calcium accumulation, increase in oxygen free radicals, as well as hypoxia itself (Dirnagl et al, 1999) and appears to be mediated through adhesion molecules such as integrins, selectins, and immunoglobulins (Doyle et al, 2008). Activation of inflammatory cells in the ischemic lesion results in the production of cytokines (Rothwell, 1997), such as tumor necrosis factor-α, interleukin-6, and interleukin-1. The last exacerbates the ischemic injury through fever, arachidonic acid release, enhancement of NMDA-mediated excitotoxicity, and stimulation of nitric oxide synthesis (Doyle et al, 2008).

Another deleterious effect of cytokines is the enhanced expression of adhesion molecules on the endothelial cell surface, including intercellular adhesion molecule-1, P selectin, and E selectin (Doyle et al, 2008; Gong et al, 1998; Lindsberg et al, 1991; Mergenthaler et al, 2004; Zhang et al, 1998). As a consequence, more neutrophils, and, later, macrophages and monocytes, adhere to the endothelium, cross the vascular wall, and enter the brain parenchyma. Microvascular obstruction by neutrophils can aggravate the degree of ischemia (del Zoppo et al, 1991) through worsening of microvascular perfusion. This phenomenon is similar to the no-reflow phenomenon known from the cardiology literature and may explain why, in some instances, tissue perfusion fails to improve significantly despite proximal vessel recanalization (Doyle et al, 2008). Other deleterious effects of inflammation on ischemic tissue include production of toxic mediators (oxygen free radicals, toxic prostanoids, tumor necrosis factor-α) by activated inflammatory cells and facilitation of apoptosis (Iadecola et al, 1997).

Apoptosis (Programmed Cell Death)

Apoptosis represents the predominant mechanism of neuronal damage in milder ischemic injury. It is characterized by an ordered and tightly controlled set of changes in gene expression and protein activity that results in neuronal cell death (Graham and Chen, 2001). A central role in apoptosis-mediated mechanisms of ischemic injury is attributed to genes that suppress or promote cell death and to a family of aspartate-specific cysteine proteases called caspases, of which 14 different enzymes have, to date, been described (Graham and Chen, 2001). These enzymes are protein cleaving and ultimately lead to destruction of key intracellular proteins with resultant cell disassembly and death.

Genes that control apoptosis include those that prevent cell death,

KEY POINT

- Apoptosis is the main mechanism of neuronal injury in the penumbra where, because of the milder degree of ischemia, sufficient energy is produced to allow for expression of new proteins that mediate cell death through an ordered and tightly controlled set of changes in gene expression and protein activity.

such as *BCL2*, and genes that promote cell death, such as *BAX* or p53. The main sites at which apoptosis can be initiated are the mitochondria, cell membrane receptors, and chromosomal DNA. Mitochondrial injury may result in release of cytochrome *c*, leading to activation of apoptosis through caspase-dependent mechanisms. However, a caspase-independent mechanism may also initiate apoptosis at the mitochondrial level (Green and Read, 1998). DNA damage can trigger apoptosis by inducing expression of the transcription factor p53 (Miyashita et al, 1994). This leads to alteration of transcription of several genes (including *BAX)* and in the initiation of apoptosis.

Blood-Brain Barrier and the Neurovascular Unit

The integrity of the blood-brain barrier plays an important role in the pathophysiology of acute stroke. Cellular elements that form the blood-brain barrier matrix include endothelial cells and astrocytes. During cerebral ischemia, the normal structure of this matrix and its intercellular signal exchange are affected by the ischemic process. A prominent role in the changes underlying blood-brain barrier dysfunction is attributed to a family of proteases called matrix metalloproteases (Lo, 2008; Mergenthaler et al, 2004). Increased presence of these enzymes, especially metalloproteinase-9, has been correlated with damage to the blood-brain barrier, with an increased risk of hemorrhagic transformation after tissue plasminogen activator (t-PA) administration and with the extent of neuronal damage (Mergenthaler et al, 2004).

An emerging concept in the pathophysiology of acute stroke is that of the neurovascular unit comprising endothelium and astrocytes in addition to the neuron (Lo, 2008; Lo et al, 2005). While, traditionally, stroke has been seen as primarily a neuronal disorder, the interaction between neurons, endothelium, and astrocytes through cell-cell signaling and cell-matrix interactions is regarded as increasingly important in the understanding of stroke and its response to stroke treatments (Lo, 2008).

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