Pathophysiology of Focal Cerebral Ischemia: a Therapeutic Perspective

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The pathophysiology of cerebral ischemia is best understood in animal models of stroke. Within minutes of interrupted blood flow, mitochondria are deprived of substrate, which prevents adenosine triphosphate generation and results in membrane depolarization. This leads to increased intracellular calcium and sodium concentration followed by generation of free radicals and initiation of apoptosis. Glutamate release from ischemic neurons contributes to cellular damage. Each step in this complex, interdependent series of events offers a potential point to intervene and prevent neuronal death. Although many human trials in acute stroke therapy have had disappointing results, many promising therapies are in the pipeline, including hypothermia and free-radical inhibitors. Herein, the author discusses the pathophysiology of focal cerebral ischemia as has been revealed in rodent models and reviews the major human trials according to treatment mechanism.

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Abbreviations: ATP = adenosine triphosphate, MCA = middle cerebral artery, NMDA = N-methyl-d-aspartate, PARP = poly (adenosine diphosphate ribose) polymerase

BRAIN tissue is exquisitely sensitive to ischemia such that even brief ischemia to cerebral neurons can initiate a complex sequence of events that ultimately culminate in cellular death. Ischemia of cerebral tissue and cellular death underlie all forms of stroke, including focal ischemia (as in embolic occlusion of the middle cerebral artery [MCA]), global ischemia (as in cardiac arrest), and, likely, intraparenchymal hemorrhage. In addition, it overlaps with the processes of neuronal damage in closed head injury and subarachnoid hemorrhage. Conversely, there are remarkable differences in the causes of cell death between global ischemia and focal ischemia, and, within focal ischemia, there are important processes that are unique to the tissue that has been reperfused. Understanding these processes better should lead to new therapies for mitigating stroke.

Herein, I focus on focal ischemia pathophysiology because this is most relevant to human ischemic stroke. Important distinctions between this and other forms of ischemia will be discussed where relevant. Data from human neuroprotection and revascularization trials will be discussed to illustrate what has been translated from the laboratory to human stroke. This article is necessarily limited in scope; the interested reader is referred to the comprehensive review of ischemic cell death by Lipton (1) and a review of potential neuroprotective strategies in ischemia and trauma (2). I will not address the fascinating topic of neural regeneration following ischemia, so the interested reader is referred elsewhere (3).

DEFINITION OF TERMS AND CONCEPTS

Ischemia is defined as a reduction in cerebral blood flow sufficient to alter cerebral function. Different brain regions have different thresholds for ischemia, with white matter being more resilient than gray matter. In addition, certain populations of cerebral neurons are *selectively vulnerable* to ischemia, as in hippocampal CA1 cells compared with dentate granule cells and cerebral neurons compared with brain stem neurons. *Infarction* is a histologic finding applied to a region of brain that has been injured by ischemia. The region of infarction appears pale on brain slices stained with hematoxylin and eosin and, at microscopy, shows edema and cellular swelling but initially no loss of cellular elements. The area of stroke in animal models is defined by this region, and the volume of stroke is measured by integrating areas of infarction over multiple brain slices. *Infarct volume reduction* is a reduction in this volume following some intervention. The region of infarction in humans can be visualized with diffusion-weighted magnetic resonance (MR) imaging (4). In focal ischemia, a central region of brain tissue is infarcted rapidly; this region is called the *core* of the infarct. The ischemic region around the core is called the *ischemic penumbra*. The processes of cellular injury and death are remarkably different in these two regions and will be discussed below.

An ischemic neuron does not nec-

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essarily die. The process of *cellular death* happens long after ischemia and infarction, typically 2 to 3 days in rodent models (1). Cellular death has clearly occurred when cytoskeletal breakdown occurs, but a cell may be committed to death long before obvious morphologic change is observable. In focal ischemia, cellular death is often accompanied by *necrosis,* in which cytoskeletal elements, edema, and inflammatory cells are found. Cells may also die by means of programmed cell death or *apoptosis*. Apoptosis is a complex process by which a neuron that has experienced even a transient insult will begin to degrade its nucleus and initiate a self-destruct sequence that happens days later. Although apoptosis likely exists to help the developing brain make appropriate connections, it may also have an important role in mature and degenerating brain and is the subject of intense research.

PATHOPHYSIOLOGY OF FOCAL CEREBRAL ISCHEMIA

The Core and Penumbral Regions of Infarction

There are two major categories of experimental ischemia: (*a*) global hypoxia and/or ischemia models and (*b*) focal ischemia models. In global hypoxia and/or ischemia models, typically two or four cervical vessels to the brain are temporarily interrupted and circulation restored after some delay. In focal ischemia models, the MCA is typically occluded either permanently or temporarily to allow reperfusion (5).

Herein, I will focus on the pathophysiology of brain cell death in focal ischemia models because human ischemic stroke is best modeled with both permanent and reperfusion models of stroke. Following typical embolic vascular occlusion in humans, there is spontaneous thrombolysis and spontaneous recanalization that occurs but at variable times following initial occlusion. Angiographic controlled studies in humans have shown that spontaneous recanalization can occur around 17% of the time within the first 6 to 8 hours of stroke and that approximately half of the vessels will reopen in 3 to 4 days (6). In animal models of focal MCA occlusion, the volume of cerebral infarction becomes equal to that of permanent MCA occlusion when temporary MCA recanalization is allowed beyond 2 to 3 hours. Therefore, in most cases, human MCA occlusion is probably best modeled with permanent cerebral ischemia models. Because the current basis of emergent stroke treatment is vessel recanalization, however, both permanent and reperfusion models are relevant for human stroke therapy.

Brain injury and neuronal death necessitates at least 1 to 2 minutes of focal vascular occlusion. In animal models, blood flow is most greatly reduced in a central region of brain (infarct "core") and in a graded fashion centrifugally from the core ("penumbra"). Cerebral blood flow decreases to less than 15% of baseline within the core, which leads to reductions in adenosine triphosphate (ATP) levels to 25% of baseline. Cerebral blood flow decreases to between 15% and 40% by definition in penumbral regions, and ATP levels decrease to between 50% and 70% of control within minutes of vessel occlusion. All neurons within the core region will infarct despite experiencing an increase in ATP levels to two-thirds of normal and return of normal oxygen partial pressure if the duration of ischemia is 30 minutes or more. Some neurons within the penumbra will die as well; as the duration of focal ischemia lengthens, the size of the infarct will increase so that after 2 to 3 hours of focal ischemia in rodents, the size of the infarct will be equal to that found with permanent vascular occlusion. The process by which the penumbra is destroyed is the focus of most ischemia research, as prevention of this infarct growth with intervention would be expected to salvage neuronal tissue. Several strategies are successful for protecting against penumbral destruction, fostering the concept of "neuroprotection."

Cells die by means of two major methods: necrosis and apoptosis. Necrotic cell death is an energy-passive process independent of protein synthesis that is characterized by loss of cellular architecture and ultimately culminates in cytoskeletal breakdown, with edema formation within 12 to 24 hours of ischemia. The morphologic features of apoptotic cell death are quite different, with DNA laddering and regular clumping of chromatin (apoptotic bodies). This is followed by a stereotypical loss of cellular architecture (which takes several days) that involves the activity of caspases (family of cysteine proteases) and other enzyme systems. Necrotic cell death is more common with more extreme levels of ischemia, whereas apoptotic cell death is more common with less severe insults. Thus, the core tissue of an infarct dies with a necrotic process, and, depending on the location within the penumbra, cells die by means of either method, with apoptosis more common for cells further away from the core.

Process of Cerebral Infarction and Cellular Death

Within minutes of vascular occlusion, brain tissue is deprived of glucose and oxygen and the acidic byproducts of metabolism accumulate. Although the exact sequence of events is debatable, what follows is a likely sequence of events responsible for the early damage to neurons. This sequence of events is summarized in the **Figure**. This loss of substrate and decrease in pH level leads to cessation of the electron transport chain activity within mitochondria, which results in a rapid decline in ATP concentration. Loss of ATP leads to failure of the Na^+,K^+-ATPase, which results in a marked intracellular increase in intracellular Na^+ concentration. Persistent depolarization allows Ca^{2+} entry, and higher intracellular Na^+ levels reduce the efficacy of the $2Na^+$-Ca^{2+} symport, which further increases intracellular Ca^{2+}. Because the membrane potential reaches the electrical threshold for discharge, neurons inside the core infarct exhibit ischemic discharges whereby they fire repetitively, releasing their transmitters locally and at distant targets. These ischemic depolarizations further exacerbate energy needs.

A high intracellular Ca^{2+} level initiates several events, including activation of calpain protease activity that effects the structural integrity of both the intra- and extracellular structure and phospholipase activity that degrades cellular membranes. Increased Ca^{2+} also induces nitric oxide synthase activity and expression, which favors the formation of peroxynitrate.

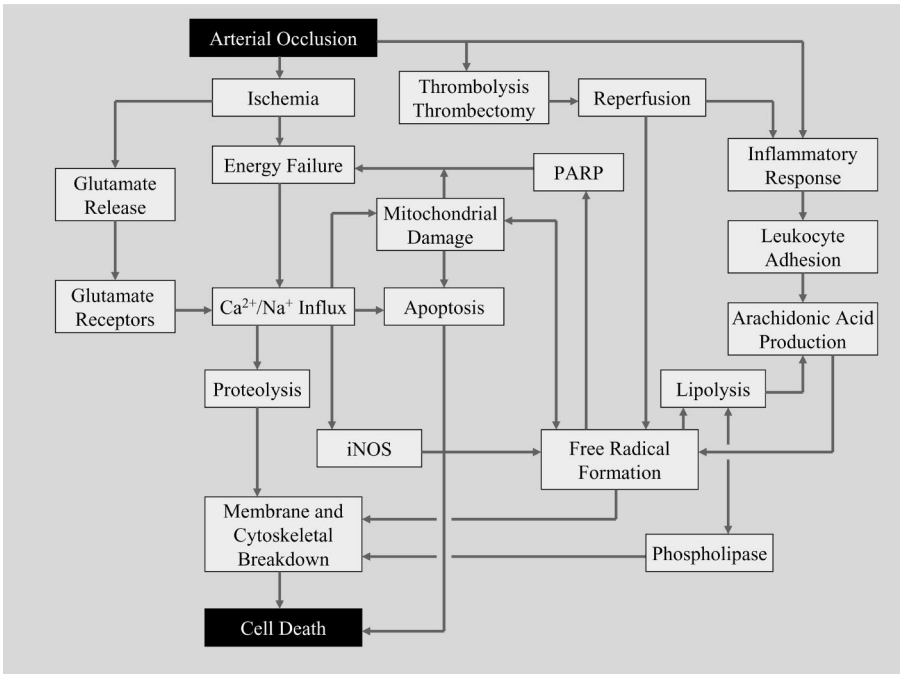

Figure. Major processes and mediators involved in focal cerebral ischemia; see text for explanation. PARP = poly (ADP-ribose) polymerase; iNOS = inducible nitric oxide synthase.

Peroxynitrate is a highly reactive free radical species.

The resulting influx of Ca^{2+} damages the mitochondria, which further exacerbates energy failure. Mitochondrial damage occurs in part by means of direct calcium toxicity as mitochondria attempt to sequester Ca^{2+}. This eventually overwhelms the mitochondria, which leads to mitochondrial depolarization and swelling. As an additional mechanism of damage, the mitochondrial transition pore is opened by high Ca^{2+} levels, free radicals, and Ca^{2+}-dependent calpain activity, which allows mitochondria to release mitochondrial ions and cytochromes—most important cytochrome C—into the cytoplasm. Cytochrome C release likely initiates apoptosis. Mitochondria also produce free radicals that are toxic both to the mitochondria and the cell as a whole, especially if sufficient oxygen is present as during reperfusion.

The amount of cellular damage is highly dependent on the tissue glucose concentration. Perhaps paradoxically, the volume of brain infarction is markedly increased when the tissue glucose level exceeds 16 to 20 mmol/L. This is likely a pH effect as an oversupply of glucose in the setting of oxygen deficit enhances glycolysis, which further reduces the pH level. Hyperglycemia in human stroke is associated with infarct expansion (7) and worse neurologic outcome (8).

Depending on the degree of ischemia and its duration, the above events may be minimal and short-lived, resulting in only temporary cellular dysfunction. This is the likely cause of transient ischemic attacks in which neurologic dysfunction is apparent but full recovery is the rule. For more severe levels of ischemia (ie, less collateral flow) and more sudden decline in blood flow (embolic as opposed to thrombotic), the chemical cascade released owing to ischemia will overcome cellular homeostasis and lead to cellular death.

Neurons within the ischemic core die exclusively by means of a necrotic mechanism initiated and propagated by the mechanisms reviewed earlier. Damage within the penumbra is mediated by different mechanisms. Because ATP levels and blood flow magnitude are only marginally reduced within the penumbra, there is insufficient ischemia to directly cause the cataclysmic processes that happen so quickly within the core. Important insight into the mechanism of penumbral infarction came from the discovery that blockade of the glutamate receptor mitigated penumbral damage and reduced infarct size (9). Glutamate levels within the core and penumbra increase rapidly in ischemia, likely due to synaptic release from core neurons undergoing ischemic depolarization but also directly from cells damaged by ischemia. Glutamate agonizes the *N*-methyl-d-aspartate (NMDA) receptor, a Ca^{2+} membrane channel, which increases calcium influx to the cell. Glutamate also agonizes the amino-hydroxy-methyl-isoxalone propionic acid (AMPA)/kainate receptor, which allows both Na^{+} and Ca^{+} entry, and the metabotropic receptor (quisqualate), which increases intracellular cyclic adenosine monophosphate levels, altering protein kinase activity, proteolysis, and lipolysis. Iontophoresis of glutamate, a glutamate agonist, causes immediate injury to cerebral neurons, and glutamate levels within experimental ischemic brain can reach levels sufficient to be toxic (1). This increase in glutamate is bimodal in focal ischemia, increasing within 1 to 2 minutes of ischemia but also after several hours. NMDA-receptor competitive and noncompetitive antagonists can reduce experimental infarct volume as much as 30%–50% (1). NMDA-receptor antagonists are effective for mitigating damage during this early window of time and are most effective if present within tissue before the induction of ischemia.

Ischemic depolarization of core neurons results in a centrifugal release of glutamate into the penumbra, which leads to direct toxicity of penumbral neurons (10). This may lead to a sufficient increase in intracellular Ca^{2+} levels to be directly toxic to penumbral neurons or, if insufficient, allow these neurons to recover function over time. The cascade of events in the core, however, produces a diffusible cadre of K^{+}, free radicals, and glutamate that can directly damage penumbral neurons.

It is important that free radical production is enhanced within penumbral tissue during reperfusion, and this may account for much of the damage seen in the penumbra (1). Free radicals are produced in high concentration

from partially damaged mitochondria that are exposed to oxygen. Spin-trap free radical inhibitors and uric acid can dramatically reduce infarct volume in reperfusion models, which supports the contribution of free radical damage in reperfusion (11–13). In addition, moderate increases in intracellular Ca^{2+} lead to induction of inducible nitric oxide and subsequent peroxynitrate production, so free radical generation can be delayed and prolonged, leading to secondary damage to neurons hours to days later. Free radicals convert the unsaturated lipids to radical species that both damage membrane lipid and propagate free radical generation. Phospholipase A_2 is activated by lipid peroxidation and degrades membrane lipid, which leads to direct membrane breakdown and loss of fluidity. Free radicals also directly affect proteins, especially membrane channels leading to exacerbation of high intracellular Ca^{2+} and inhibition of mitochondria respiratory chain enzymes. Peroxynitrate and other free radial species produce single-strand DNA breaks within penumbral regions during reperfusion. These breaks induce poly (adenosine diphosphate ribose) polymerase (PARP), a nuclear enzyme that repairs single-strand breaks. PARP activity is fueled by a reduction in nicotinamide-adenine dinucleotide, and it is hypothesized that marked increases in PARP activity deplete the reduced form of nicotinamide-adenine dinucleotide, contributing to cellular energy failure (1). PARP inhibition, or knockout of PARP, markedly reduces infarct volume in reperfusion models but has no effect on permanent ischemia, which supports the role of free radicals in reperfusion injury.

Sublethal injury to neurons favors initiation of apoptosis, causing cells within the penumbra to die by means of this pathway rather than the necrotic pathway, depending on the magnitude of initial injury. How apoptosis is initiated is unclear, but two major contributors are increased cytosolic cytochrome C, which is released through the mitochondrial permeability transition pore from injured mitochondria, and calpain activity from increased intracellular Ca^{2+} concentration. Cytochrome C induces caspase activity, a major apoptotic enzyme system, setting forth the complex, likely irreversible process of apoptosis. Cells undergoing apoptotic cell death exhibit regular, rounded regions of nuclear chromatin clumping followed by formation of apoptotic bodies that contain cytoplasm and clumped chromatin. DNA double-stand breaks occur and DNA laddering is seen. This contrasts with free radical–induced single-strand DNA breaks that induce PARP activity and lead to necrotic cell death. As opposed to necrotic death, cellular cytoplasm does not become eosinophilic and the cells simply appear to shrink; there is no surrounding edema.

A hallmark distinction between necrotic cell death and apoptosis is that energy and protein synthesis are necessary for the latter to proceed (14). Because apoptosis requires further gene transcription and protein synthesis, there must be some integrity of the nucleus and gene transcription processes. This may account for the differential pathways selected for cell death depending on location within the core or penumbra. Another remarkable feature of apoptosis is the time between ischemic insult and eventual death of the neuron. Depending on the severity and duration of ischemia, apoptotic cell death does not begin until the 3rd day and can extend as far as 2 weeks. It is interesting that although the process is inexorable once initiated, it can be halted with proteases. It can be prevented altogether with mild hypothermia (35°C) during the early stages of ischemia, which suggests a potential therapeutic role for hypothermia in penumbra salvage (15). Conversely, the process is accelerated by hyperthermia as are necrotic processes.

Finally, ischemia also damages the brain's capillaries and endothelium and incites an inflammatory response whereby white blood cells infiltrate regions of infarct. The contribution of white blood cells to the process of secondary damage is controversial (16), but white blood cells (chiefly neutrophils) appear within the infarct within 24 hours, at the appropriate time to cause damage. In addition, the prevention of leukocyte accumulation (by blocking cytokines, blocking adhesion molecules like intracellular adhesion molecule 1, or depleting leukocytes) reduces the experimental infarct size—especially in reperfusion models. Neutrophils are neurotoxic in several ways, including generation of free radicals from nicotinamide adenine dinucleotide phosphate oxidase, nitric oxide production from inducible nitric oxidesynthase within neutrophils, and formation of arachidonic acid leading to more free radical formation. It does not appear that intravascular sludging by white blood cells exacerbates ischemia (1).

Cerebral tissue can protect itself from repeated ischemic insults. A rat brain exposed to transient MCA occlusion will be protected against ischemic cell death within the conditioned zone after several days and lasting up to 7 days (17). Preconditioned cells will develop initial morphologic changes of early ischemia but will later recover. This ischemic tolerance of brain is likely mediated by the induction of protective genes, including heat-shock protein and BCL-2 (protein involved in the apoptosis pathway), and an increased ability of cells to sequester intracellular Ca^{2+}. This process may have teleologic importance and can potentially offer a neuroprotective avenue.

POTENTIAL INTERVENTION TO MITIGATE CEREBRAL ISCHEMIA

As reviewed in the previous section, there are several ways to reduce the eventual size of infarction, as follows: (*a*) limit the time of ischemia, (*b*) lower the temperature of the tissue, (*c*) limit the intracellular Ca^{2+} concentration, (*d*) limit the intracellular Na^{+} concentration, (*e*) block the primary or secondary effects of glutamate, (*f*) trap free radicals, (*g*) inhibit PARP activity, (*h*) block caspase activity, (*i*) block white cell adhesion, and (*j*) change membrane fluidity. Many of these major laboratory discoveries have led to phase I–III human clinical trials in an attempt to limit infarct size, especially during the 1990s. Despite these exciting laboratory findings, however, only a few trials have been successful.

Major human trials designed to limit infarct size and, hence, improve clinical outcome are summarized in the **Table**. In the **Table**, each trial or group of trials is organized according to intervention type. The interested reader is referred to an on-line resource that summaries the current status of past and ongoing stroke trials (http://www.strokecenter.org/trials).

Revascularization of cerebral ves-

Table
Major Human Trials in Acute Ischemic Stroke Therapy

Mechanism*	Agent and Trial Name	No. of Patients	Time between Stroke Onset and Treatment†	Efficacy‡
Thrombolysis				
Intravenous	Recombinant tissue-type plasminogen activator			
	NINDS (18)	624	0–3	Effective
	ECASS-I (19)	620	0–6	Ineffective
	ECASS-II (20)	800	0–6	Ineffective
	ATLANTIS (21)	613	3–5	Ineffective
	Streptokinase			
	ASK (51)	340	0–4	Ineffective
	MAST-I (52)	622	0–6	Ineffective (increased mortality)
	MAST-E (53)	310	0–6	Ineffective (increased mortality)
	Ancrod			
	Stroke Study (54)	132	0–6	Ineffective
	STAT (55)	500	0–3	Effective
	ESTAT§	1,222	0–6	Ineffective
Intraarterial	Prourokinase			
	PROACT-I (56)	40	0–6	Effective
	PROACT-II (22)	180	0–6	Effective
Antithrombotics	Heparin			
	IST (57)	19,435	0–48	Ineffective
	CAST (58)	20,000	0–48	Ineffective
	Low-molecular-weight heparin (59)	Meta-analysis: 3,048		Ineffective
	Abciximab§	400	0–6	Effective
	Aspirin			
	IST (57)	20,000	0–48	Effective
	CAST (58)	20,000	0–48	Effective
	MAST-I (52)	622	0–6	Ineffective
Hypothermia	Surface cooling (60)	17	0–12	Phase I study
	Surface and/or endovascular cooling (61)	36	0–54	Ineffective
	COOL-AID (26)	19	0–8	Phase I study
	Endovascular			
	COOL-AID§	50	0–12	Phase III study (ongoing)
NMDA antagonists	Selfotel (62)	567	0–6	Ineffective (increased mortality)
	Aptiganel (38)	626	0–6	Ineffective
	Remacemide (63)	64	0–12	Phase II study
	Dextrorphan (64)	66	0–48	Phase I study
	Magnesium			
	FAST-MAG P§	20	0–12	Phase I study (feasible)
	FAST-MAG§	1,270‖	0–2	Ongoing
	IMAGES§	2,700‖	0–12	Ongoing
	Gavestinel			
	GAIN Americas (65)	1,367	0–6	Ineffective
	GAIN International (66)	1,804	0–6	Ineffective
	AR-R15896AR (67)	175	0–24	Phase II
Glutamate	Fosphenytoin§	462	0–4	Ineffective
Release inhibitors	Lubeluzole			
	International (68)	1,786	0–8	Ineffective
	U.S. and Canadian (69)	721	0–6	Effective
	Sipatrigine (70)	27	0–12	Phase II study
		170	0–12	Phase II/III study (halted)
Calcium antagonists	Nimodipine (28–37)	3,518	0–48	Ineffective even if given <6 h after stroke
	Flunarizine (71)	329	0–24	Ineffective
K^+ channel agonist	BMS-204352§	1,978	0–6	Ineffective

(continued)

Table (Continued)

Mechanism*	Agent and Trial Name	No. of Patients	Time between Stroke Onset and Treatment†	Efficacy‡
GABA agonists	Clomethiazole (72)	1,360	0–12	Ineffective
Serotonin agonist	Repinotan§	240	0–6	Phase II study Phase III study (ongoing)
Free radical scavengers	Tirilazad			
	RANTTAS I (40)	556	0–4	Ineffective
	RANTTAS II (41)	126	0–6	Ineffective
	Ebselen (42)	1,300	0–48	Ineffective
Antiadhesion molecules	Enlimomab (43)	625	0–6	Ineffective (increased mortality)
	HU23F2G§			Ineffective
Membrane stabilization	Citicoline			
	Precursor (45)	272	0–14 d	Effective
	C-001 (46)	259	0–24	Effective
	C-007 (47)	394	0–12	Ineffective
	ECCO-2000 (48)	899	0–24	Ineffective
Opiate antagonists	Cervene (73)	368	0–6	Ineffective
Gangliosides	GM-1			
	IASS (74)	502	0–12	Ineffective
	EST (75)	792	0–4	Ineffective
	SASS (76)	287	0–48	Ineffective
Growth factors	Fiblast (bFGP)§	302	0–6	Ineffective (increased mortality)
Unknown	Cerebrolysin			
	Hungary§	48	0–12	Effective
	Austria§	146	0–24	Effective
	Piracetam			
	Rehab (77)	158	0–3 mo	Ineffective
	PASS (78)	927	0–12	Ineffective
	PASS-II§		0–7	Phase III study (ongoing)

Note.—ASK = Australian Streptokinase; ATLANTIS = Alteplase Thrombolysis for Acute Noninterventional Therapy in Ischemic Stroke; CAST = Chinese Acute Stroke Trial; COOL-AID = Cooling for Acute Ischemic Brain Damage; ECASS = European Cooperative Acute Stroke Study; EST = Early Stroke Trial; ESTAT = European Stroke Treatment with Ancrod Trial; FAST-MAG = Field Administration of Stroke Therapy-Magnesium; FAST-MAG P = Field Administration of Stroke Therapy-Magnesium Pilot Trial; GAIN = Glycine Antagonist in Neuroprotection; IASS = Italian Acute Stroke Study; IMAGES = Intravenous Magnesium Efficacy in Stroke; IST = International Stroke Trial; MAST = Multicentre Acute Stroke Trial; NINDS = National Institute of Neurological Disorders and Stroke; PASS = Piracetam in Acute Stroke Study; PROACT = Prolyse in Acute Cerebral Thromboembolism Trial; RANTTAS = Randomized Trial of Tirilazad Mesylate in Acute Stroke; SASS = Syngen in Acute Stroke Study; STAT = Stroke Treatment with Ancrod Trial.

* Classification of mechanism is by most likely mechanism; some agents may have more than one mechanism of action.

† Except where indicated, the time between stroke and treatment is given in hours.

‡ Efficacy is noted only for phase III trials.

§ As of January 2003, the results of this study were not available in a peer-reviewed publication; additional details can be found at http://www.strokecenter.org/trials.

‖ Target enrollment.

sels during acute ischemic stroke has been the only proved method for improving clinical outcome in human stroke. The positive results of the NINDS trial (18) must be compared with the neutral or marginally positive results of other trials, including ECASS I (19), ECASS II (20), and the ATLANTIS study (21). The chief distinction between these trials is the substantially earlier enrollment time in the NINDS trial, in which half of the enrolled patients received intravenous tissue-type plasminogen activator within 90 minutes of stroke onset. The other trials allowed various time windows, up to 6 hours, and only one showed positive results: the second PROACT trial, in which intraarterial pro-urokinase was used (22). The success of PROACT II and the failure of intravenous trials with longer windows may be explained by the homogeneity of patients in the PROACT trial, in which patients with MCA occlusion were eligible for treatment. Selection of this patient subset—a subset of patients with a markedly poor clinical prognosis if untreated—may have helped select a group of patients best suited to benefit with vascular recanalization. However, benefit of recanalization out to the 8th hour of focal ischemia, as occurred in the PROACT study, contrasts with laboratory models of focal ischemia whereby infarct size in reperfusion models becomes equal to that with permanent occlusion models—typically within 2 to 3

hours. It is unclear whether this reflects a species difference of ischemic tolerance of brains between rodents and humans or a confounding factor of species difference in cerebral vasculature. Regardless of the explanation, the findings from the PROACT study greatly increased the enthusiasm for the use of thrombolytics with intraarterial intervention in stroke. This shifts the focus toward exploring adjuvant neuroprotection and inhibition of free radicals that are generated during reperfusion and likely exacerbate infarction. Newer evidence that intravenous abciximab given within 12 hours may safely improve clinical outcome is an exciting new result, and publication of results in a peer-reviewed journal are awaited.

Rodent models have demonstrated the dependency of stroke on brain temperature, and this has also been found in human stroke. A lower body temperature at presentation is associated with lower neurologic morbidity (23), and even mild fever increases stroke volume and is associated with worse clinical outcome (23,24). To my knowledge, clinical trials in which hypothermia is induced have not yet shown benefit in humans; however, this area of human research is just beginning. Although hypothermia clearly reduces intracranial pressure (25), it may also have the unintended consequence of rebound cerebral edema at rewarming. A well-designed trial is currently under way in which an indwelling inferior vena cava heat exchange catheter is used to induce, maintain, and emerge from moderate (33°C) hypothermia after a pilot study found it to be safe (26). Because even mild hypothermia (35°C) blocks apoptosis, and apoptosis is possibly responsible for a substantial fraction of dying neurons in the penumbra, the clinical benefit of even very mild hypothermia could be substantial and carry a lower risk than moderate hypothermia. Alternatively, because fever is so prevalent in patients with stroke, the simple suppression of fever with a goal of achieving a normal brain temperature may improve outcome. Fever suppression is difficult practically when using antipyretics and uncomfortable when using surface cooling techniques. Perhaps the use of indwelling hypothermia catheters to control fever will improve the consistency of temperature control and be shown to improve clinical outcomes.

Limiting intracellular Ca^{2+} concentration is problematic in humans because drugs used to limit Ca^{2+} entry through voltage-gated channels also reduce systemic blood pressure, and hypotension can markedly increase infarct size by threatening collateral blood flow and worsen the clinical outcome (27). The use of nimodipine has been evaluated in several trials, with mixed results (28–37). Currently, nimodipine is not recommended for focal ischemia except in the prevention of ischemia from subarachnoid hemorrhage–associated vasospasm.

The discovery that glutamate blockade at the receptor or postreceptor level mitigated penumbral infarction led to clinical testing of several drugs in the 1990s. These trials are summarized in the **Table**. Although I focus on phase III trials, I also include important phase I–II trials. Many NMDA antagonists produce either hypotension or substantial psychosis and, thus, have never reached phase III testing. Although some, like lubeluzole, were quite well tolerated, they were not found to be effective despite promising results in animal studies. A careful review of the **Table**, however, shows that most neuroprotective agents in this class and others were tested quite late in the ischemia process. For example, aptiganel was administered as late as 6 hours into ischemia (38). In general, results of animal models indicate a benefit for glutamate only in the 1st hour or two of vascular occlusion (1). Because much of the preclinical data about these drugs are proprietary and unpublished, one can only speculate why such a long time window was allowed in these trials. This decision was likely influenced by the practical difficulty of enrolling patients within the 1st hour of stroke. As stated earlier, however, the enrollment of patients within 90 minutes of stroke onset in the NINDS trial of intravenous thrombolysis probably accounted for that trial's success (39). It is possible that several of the glutamate pathway neuroprotective agents listed in the **Table** could have been proved effective with use of a shorter time window. Unfortunately, drug development was halted on most agents of this class. The ongoing Field Administration of Stroke Therapy-Magnesium trial, in which intravenous magnesium (magnesium blocks the NMDA receptor channel) is given by paramedics, is an exciting trial because of its unprecedented short time window of 2 hours. If this trial proves that paramedic delivery of the drug is feasible, perhaps other neuroprotective agents could be tested with this innovative trial design.

Free-radical inhibition is an exciting target for human stroke therapy because the therapeutic window may be longer than that of glutamatergic pathways. So far, tirilazad, a 21-amino-steroid that works by means of sequestration of free radicals, was not found to be effective in human ischemic stroke (40,41). The second major trial of tirilazad, however, was halted because of the disappointing results obtained in subarachnoid hemorrhage. The data acquired up to that point, however, were suggestive of efficacy. Another free-radical inhibitor, ebselen, was found to have a trend toward efficacy in one trial, and clinical research is continuing in Japan (42). The drug NXY-059, a spin-trap agent, has been shown to reduce stroke volume in animals (11,12) and will likely soon be used in human clinical trials.

The blockade of white cell adhesion at the receptor level by using the anti-intracellular adhesion molecule 1 antibody enlimomab was found to be harmful (43). Although the cause of harm is speculative, it may have been from induction of a febrile response in patients. As mentioned earlier, fever is quite harmful in ischemia, so any future drugs must not induce fever. Other potential methods to reduce the cellular inflammatory response in humans have not been studied in controlled trials.

The **Table** lists additional drugs that work through various known and unknown pathways that reached clinical testing. Most notably, the drug citicoline was designed to stabilize cellular membranes by donating a choline moiety; its mechanism of action, however, is likely more complex (44). The results of initial clinical trials were positive; however, two major follow-up trials reported disappointing results (45–48). As a major contribution to clinical research in stroke, one of these trials included perfusion-diffusion MR imaging in the protocol and

showed that this technology can be used and is a promising surrogate for clinical outcome (49). Although two small cerebrolysin (pig brain homogenates) trials showed a trend toward benefit, the results have not yet been published in a peer-reviewed journal.

Thus, despite intensive clinical research, outside the proved benefit of vascular recanalization, no strategy proved in the laboratory has been effective in human stroke. There are probably several reasons for this discrepancy. There may be species differences between rodent and human brain. Even among rodents there are remarkable intraspecies differences in experimental neuroprotection. It is possible that some drugs found to be ineffective in rodents, and therefore abandoned for further development, could be neuroprotective in humans. Perhaps preclinical testing in primates should be performed before human experimentation, and pilot studies in humans should include a radiographic surrogate to help better decide if phase II–III trials should be pursued (49). A better explanation, however, is the lack of substantial dose-response testing in animal models before testing in humans (50). It is not reasonable to assume that a drug that has only been shown as neuroprotective, if given within a few minutes of experimental ischemia induction—and certainly only if given before induction of ischemia—could be neuroprotective in humans if given near the 6th hour of stroke onset. Therefore, either promising evidence for efficacy within a 4 to 12-hour time window or a clinical trial designed to limit enrollment to the first 1 to 3 hours is necessary before any further human testing is done.

SUMMARY

The pathophysiology of cerebral ischemia is complex, involving multiple ionic, enzymatic, and genetic steps that vary as a function of depth and duration of ischemia. The most complex model of focal cerebral ischemia is the reperfusion model, and that model best applies to patients undergoing therapeutic recanalization of a cerebral vessel during stroke. Initiated by deprivation of energy substrate to mitochondria, the secondary processes that follow are complex and interact at several levels. Clearly, the most damaging agent is high intracellular Ca^{2+}, followed by free radicals. Damage is exacerbated by high glucose concentration and hyperthermia. The initiation of apoptosis provides an additional pathway by which cells become doomed to die over the course of days.

Limiting the duration of ischemia by vascular recanalization is the only clinically proved method that limits stroke size and morbidity in humans. Initial enthusiasm for the blockade of the glutamate receptor and the effects of glutamate has been tempered by lack of efficacy in clinical trials, yet few trials actually tested such drugs very early in the course of stroke. Because it is difficult to intervene early in stroke, focus has shifted toward preventing the process of cellular ischemia and cell death at later times. The principal processes that lead to delayed cellular death are free radical–mediated damage and apoptosis. Free radical inhibitors are highly protective in experimental models and have not been tested as extensively in humans as glutamate pathway agents. These agents will be responsible for the next wave of clinical trials. Although the inhibition of apoptosis has been quite promising in experimental models, this is not yet feasible in humans. The discovery that a substantial amount of cellular death within otherwise viable penumbral cells is due to apoptosis, coupled with the understanding that apoptosis proceeds over several hours and days and is highly dependent on temperature, leads to optimism for future treatments. Early institution of hypothermia in human stroke has not been extensively tested but holds promise. Although not proved clinically, early control of the serum glucose level is likely beneficial, as is elimination of fever.

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